POLARIZED RECOMBINATION AND FINE STRUCTURE WITHIN THE *me-2* GENE OF NEUROSPORA CRASSA[1]

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Received April 5, 1963

THIS study of interallelic recombination was originally initiated to compare the recombination and complementation maps of the *me-2* gene of *Neurospora crassa* (MURRAY 1960b). Methionine-independent progeny from crosses between *me-2* alleles were classified with respect to the markers which were present on both sides of the *me-2* gene. One of the two classes of methionine prototrophs having markers recombined occurred in excess of the other, and when the markers entered the cross in the opposite phase, a similar excess was found in the reciprocal class. In this respect the results resembled those obtained for the *pan-2* gene (CASE and GILES 1958), and on the basis of these asymmetries the *me-2* alleles were ordered linearly. The order determined agreed with that based on additive prototroph frequencies.

In another respect, however, the results differed strikingly from most studies of intragenic recombination such as those of CASE and GILES (1958), FREESE (1957), and ST. LAWRENCE (1956); pronounced differences were observed in the numbers of the two *parentally* marked classes of prototrophs. These asymmetries were reversed when the markers entered the cross in the opposite phase. On the assumption that recombination was the result of both reciprocal crossing over and conversion, the asymmetries between the two classes of parentally marked prototrophs were at first described in terms of possible differences in conversion frequency between the *me-2* alleles (MURRAY 1960b); this interpretation is disproved by the present data.

The low frequency of recombination between *me-2* alleles has prevented any investigation involving tetrads, and therefore no distinction has been possible between reciprocal and nonreciprocal recombinations. Other studies have shown intragenic recombination in Neurospora to be primarily nonreciprocal (MITCHELL 1955; CASE and GILES 1958; STADLER 1959a; SUYAMA, MUNKRES and WOODWARD 1959). In spite of the lack of information from tetrads, further investigation of recombination within the *me-2* gene seemed desirable, because the data suggested a polarization of intragenic recombination (MURRAY 1961). Polarized recombination has been reported for *Aspergillus nidulans* (SIDDIQI 1961, 1962) and, earlier, on the basis of a different criterion, it was demonstrated for the Ascomycete *Ascobolus immersus* (LISSOUBA and RIZET 1960).

[1] Supported by research grant AI-01462 from the Public Health Service.

Two basic types of mechanism have been suggested that would result in polarized recombination. In the first, it is assumed that chromosome replication is an essential condition for recombination and that replication itself is polarized (RIZET, LISSOUBA and MOUSSEAU 1960; SIDDIQI 1961). (The hypothesis of RIZET and his co-workers has the additional requisite of a discontinuous organization of the genetic material). In the alternative, replication may not be a necessary condition for recombination, but the genetic material must be discontinuous; either complete pairing between predetermined homologous subunits of the genetic material (STAHL 1961), or nonrandom pairing enforced only in those regions adjacent to discontinuities (MURRAY 1961), is invoked. An understanding of polarized recombination is important, therefore, when considering both the organization of the genetic material and the mechanism of intragenic recombination.

This paper presents in full the data on recombination between 19 *me-2* alleles. The results are considered with reference to possible mechanisms of intragenic recombination.

MATERIALS AND METHODS

The *me-2* strains were isolated following ultraviolet irradiation of the wild-type stock Emerson *a* (5297), except for H98, K5 and K23 which were induced in different strains and subsequently backcrossed three or four times to the standard wild type (Emerson *a* or *A*). Further details of the origin of the mutant strains were given in an earlier paper (MURRAY 1960a).

The present analysis used *tryp-4* (Y2198) and *pan-1* (5531) as unselected markers. Data from many crosses indicate that *me-2* is approximately six units distal to *tryp-4* and four units proximal to *pan-1*. Cultures of the required genotypes were extracted from crosses of the *me-2* alleles to the stock *tryp-4* (Y2198) *pan-1* (5531). (The *tryp-4* and *pan-1* markers were induced in Lindegren wild-type strains, but had been crossed to a St. Lawrence wild-type to a limited extent.) In some cases, the marked *me-2* strains were backcrossed to Emerson wild type, and stocks of increased fertility were selected. Crosses were made of the type *tryp-4* me^x *pan-1*$^+$ $\times$ *tryp-4*$^+$ me^y *pan-1*, where me^x and me^y are *me-2* alleles of independent origin.

Marker genes which had been induced in the Emerson *a* strain (5297) also were incorporated into some *me-2* strains. These markers were *tryp-4* (A35) and *hist-4* (P143h); in the present studies the former was eight units proximal to *me-2* and the latter was less than two units distal to *pan-1*.

The methods were in general as described previously (MURRAY 1960a,b). The following slight modifications were made to the layer plating technique: 1) The selective medium was supplemented with indole (10 mg/l) rather than tryptophan, plus either calcium pantothenate (10 mg/l) or L-histidine (0.5 g/l) according to the requirements of the unselected marker genes. 2) Samples (1 ml) of ascospore suspensions were dispensed to tubes containing 1.5 ml of molten 0.5 percent agar medium containing the required supplements, 0.1 percent

sucrose, but no sorbose. These tubes were subsequently subjected to heat-shock before the contents of each tube were distributed to form the upper layer of a plate. 3) The number of viable spores per Petri plate was increased from 2,000 to between 5,000 and 10,000.

The plates were inspected after approximately two, three and four days incubation at 25°C. The majority of colonies were detected at the first inspection, but in every experiment all the true wild-type colonies were isolated, and all were tested for their marker-gene combinations. The colonies were isolated following microscopic examination, and with rare exceptions it was readily confirmed that a colony resulted from the growth of a germinated ascospore. Few additional colonies were observed at the third inspection unless the methionine alleles complemented each other, in which case pseudowild-type (PWT) colonies (presumed to originate as heterozygous disomics) were always found. In four crosses not expected to give PWT's one, or a few, slow-growing colonies were obtained. Such isolates were stimulated by methionine and the progeny from a backcross to wild type included methionine-dependent isolates carrying the marker gene combination of the slow-growing parent. All the observations from random spores in these four cases were consistent with the occurrence of a suppressor mutation. In crosses where PWT's grew sufficiently well to be morphologically difficult to distinguish from true wilds (e.g. crosses of P81 × H98 and P133 × P81), all isolates were backcrossed to a strain containing an *me-2* allele (H98). Half of the progeny from such a cross should be methionine-independent if the original isolate was a true wild type that resulted from recombination between the two *me-2* alleles. Where this was the case, a sample of methionine-independent progeny was isolated and tested with respect to the outside marker genes.

Many of the earlier crosses gave approximately forty percent germination of ascospores, but the majority of the results were collected from crosses giving between sixty and eighty percent germination. Crosses giving abnormally low germination or a high frequency of colorless spores were discarded.

The increase in density of plating, to 10,000 spores per plate, had no, or little, apparent adverse effect such as might be anticipated as the result of heterocaryosis between a methionine-prototrophic colony and the germ tubes of methionine-dependent spores. The distribution of methionine prototrophs with respect to the marker-gene combinations was independent of the number of spores per plate and of the length of the incubation period prior to isolation of the prototrophic colonies. The only possible indication of heterocaryosis was that an occasional methionine prototroph scored ambiguously with respect to a marker gene, e.g. a methionine prototroph which was initially scored as *tryp*⁻ or *pan*⁻ after one day of incubation at 34°, showed growth in the absence of the respective supplement on the following day. Such isolates were backcrossed to an *me-2* strain and methionine-independent progeny were isolated and scored with respect to the marker genes. The scoring of the first day was always substantiated. This leakiness could have been due to infrequent heterocaryon formation with background spores to give heterocaryons having extremely disproportionate nuclear ratios.

RESULTS

Control crosses: Ascospores from crosses which were homozygous with respect to the *me-2* alleles, but heterozygous for the marker genes, were screened for reversion to methionine prototrophy. The results (Table 1) showed that prototroph formation in crosses between alleles of independent origin was not usually the result of reversion: the highest reversion frequency observed was 0.1 in 10^5 viable spores.

Allele-specific behavior: The early crosses studied involved the *me-2* alleles K23, K44, P81 and H98. When either of the alleles K23 or K44 was crossed to either P81 or H98, a very consistent pattern was observed when the prototrophic recombinants were classified into the four possible marker-gene combinations (Table 2). Distinct inequalities were observed both between the two parentally marked classes and between the two classes having marker genes recombined. The intensity and the direction of these two asymmetries were correlated. The reversal of the asymmetries, when the crosses were made in the opposite phase, suggested that the observed patterns were determined by the specific *me-2* alleles rather than by any differences in genetic background. This was supported by the finding that the observed recombination pattern was independent of the background in which the particular *me-2* alleles were induced (K23 and H98 were induced in genetic backgrounds which differed from each other and from the Emerson *a*).

TABLE 1

Control crosses with me-2 *alleles homozygous*

Isolation numbers of *me-2* alleles	Number of viable spores plated $\times 10^{-5}$	Number of methionine prototrophs
H98	3.8	0
K5	15.8	1†
K23	3.9	0
K43	7.8	0
K44	14.4	0
K86*	. .	. .
K98	5.1	1‡
P2	9.9	1†
P24	4.3	0
P78	3.4	0
P81	4.5	0
P99	5.8	0
P133 × P145§	9.6	0
P140	3.3	0
P143m	11.0	0
P159	2.4	0
P169	2.6	0
P174	0.5	0

* Attempts to make homozygous cross were unsuccessful.
† Prototrophs grew well and were not tested for suppressor mutations.
‡ Prototroph grew slowly in the absence of methionine and was the result of a suppressor mutation.
§ Homozygous crosses omitted for these alleles because no prototrophs were obtained from this intercross.

TABLE 2

Analysis of methionine prototrophs demonstrating the allele-specific determination of interallelic recombination

Genotype of parents: $tryp^-me^xpan^+$ × $tryp^+me^ypan^-$	Number of methionine prototrophs	Percent *me* prototrophs in the four classes of marker-gene combinations: Parental		Recombinant	
		$tryp^-pan^+$	$tryp^+pan^-$	$tryp^-pan^-$	$tryp^+pan^+$
— K44* + × + H98† —	114	11	**44**	9	**36**
— H98 + × + K44 —	91	**43**	7	**44**	7
— K44 + × + P81 —	84	14	**36**	13	**37**
— P81 + × + K44 —	78	**44**	12	**36**	9
— K23 + × + H98 —	103	13	**41**	17	**29**
— H98 + × + K23 —	109	**35**	16	**34**	16
— K23 + × + P81 —	92	15	**41**	9	**35**
— P81 + × + K23 —	95	**47**	7	**29**	17

* Isolation number of *me-2* allele entering cross with $tryp^-$ marker.
† Isolation number of *me-2* allele entering cross with pan^- marker.

Allele-specific behavior as a function of the positions of the mutant sites: On the basis of genetic analysis, the 12 *me-2* alleles which exhibited interallelic complementation (MURRAY 1960b) were divided into two groups. One group (comprising alleles K23, K44, K98, P133, P140 and P145; this group is later referred to as the α cluster) was located in the proximal region of the map of the *me-2* gene, whilst the other group (comprising alleles H98, K86, P81, P99, P169 and P174; this group is later referred to as the γ cluster) was located in the distal region. Any cross between representatives of the two groups gave results similar to those exemplified by the cross of K44 × P81 (see Table 2). Thirteen of the possible 36 pairwise combinations were made and examined, such that each of the 12 alleles was tested in at least one cross (Table 4, crosses of type $\alpha \times \gamma$). Pronounced asymmetries were always observed between the two classes of prototrophs which were parental with respect to the marker genes. If these asymmetries reflected differences in frequencies of conversion, then the *me-2* alleles in the distal group were always converted to prototrophy more frequently than were the alleles in the proximal group. This finding suggested that the recombination pattern was a function of the positions of the mutant sites within the *me-2* gene.

The possibility remained that the asymmetries were induced by some closely linked factor which had been incorporated when the marked *me-2* strains were extracted from the cross of *me-2* × *tryp-4* (Y2198) *pan-1* (5531). Marker genes which had been induced in the Emerson *a* strain were therefore incorporated into some of the *me-2* strains. A *tryp-4* allele (A35) served as proximal marker and a *hist-4* allele (P143h) as distal marker. The use of these new markers did not significantly change the frequencies with which the prototrophs were distributed amongst the four marker-gene combinations (see Table 3). It was concluded that the observed recombination patterns reflected properties of the specific *me-2* alleles.

TABLE 3

Analysis of methionine prototrophs from crosses between me-2 *strains carrying markers which had been induced in the same wild-type strain*

Genotypes of parents: *tryp⁻ me^x hist⁺* × *tryp⁺ me^y hist⁻*	Methionine prototrophs in the four classes: Parental *tryp⁻ hist⁺*	Parental *tryp⁺ hist⁻*	Recombinant *tryp⁻ hist⁻*	Recombinant *tryp⁺ hist⁺*
— K44* + × + P81† —	4 (8%)	**19** (37%)	3 (6%)	**26** (50%)
— P81 + × + K44 —	**27** (47%)	6 (10%)	**18** (30%)	8 (14%)
— P133 + × + P81 —	5 (8%)	**27** (41%)	6 (9%)	**27** (41%)

* Isolation number of *me-2* allele entering cross with *tryp⁻* parent.
† Isolation number of *me-2* allele entering cross with *hist⁻* parent.

Allele-specific asymmetries can be explained by a hypothesis invoking conversion, where conversion is a mutational type of event in which some alleles are more unstable than others. Alternatively, asymmetries could result from a mechanism involving multiple exchanges within paired segments of the genomes if the two mutated sites were of different sizes. The latter hypothesis has been formulated by EPHRUSSI-TAYLOR (1961) to explain the nonequivalent recombination frequencies within the amylomaltase locus of *Diplococcus pneumonii* (LACKS and HOTCHKISS 1960). Neither of these mechanisms is a likely mode of origin of the present asymmetries, because the asymmetries appeared to be determined solely by the positions of the mutant sites within the *me-2* region. A more critical test of the determination of recombination behavior by the position of mutant sites within the *me-2* gene required mutant sites to be located at points other than within the two clusters. Two new points were found when seven noncomplementing alleles were included in the analysis.

Genetic map: The map of the *me-2* gene (Figure 1) was constructed from the data listed in Tables 4 and 5. The order of two mutant sites with respect to the marker genes (*tryp-4* and *pan-1*) was inferred from the more frequent of the two classes having marker genes recombined. On this criterion, a "cluster map" of the mutated sites was readily constructed. This simplified map is represented by four points designated α, β, γ and δ. The α point represents the proximal cluster of seven alleles (six complementing and one noncomplementing). The highest observed prototroph frequency amongst crosses involving six combinations between alleles within the α cluster was 1.4 in 10^5 viable ascospores (Table 5). A single noncomplementing allele, P2, was located at point β between the proximal cluster (α) and the distal cluster (γ). The latter cluster comprises nine alleles (six complementing and three noncomplementing) and is more diffuse than the proximal cluster. The maximum prototroph frequency observed, in crosses between two γ alleles, was 6 in 10^5 (Table 5). Two closely linked noncomplementing alleles were represented by the point δ, which was located distal to the γ cluster. These alleles recombined to give prototrophs with a frequency of 0.3 in 10^5 viable ascospores.

The distances between points on the map of the *me-2* gene (Figure 1) are

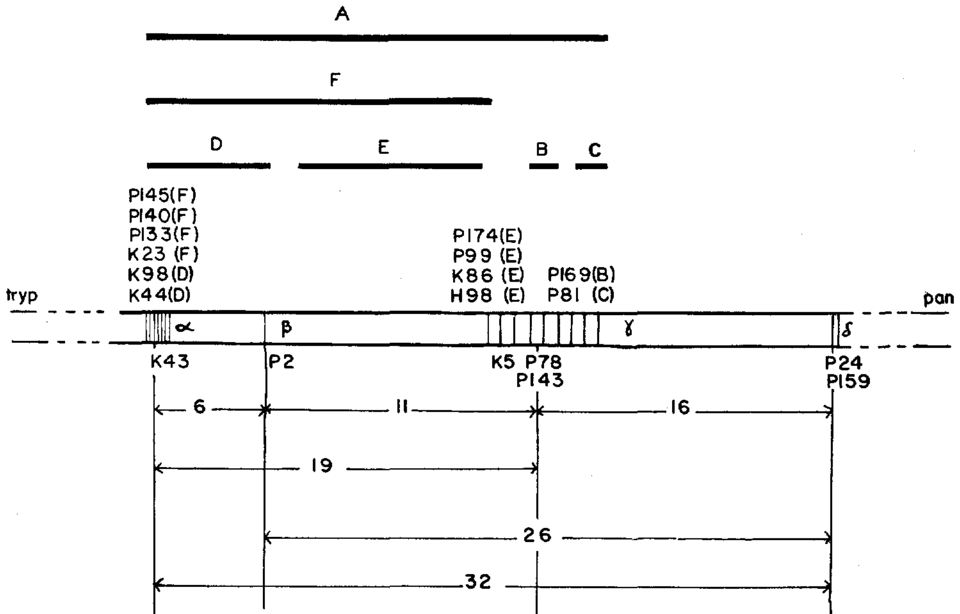


FIGURE 1.—Genetic map of the methionine-2 locus showing the order and spacing of the four major regions. The distances are expressed as prototroph frequencies per 10^5 viable ascospores. Isolation numbers below the map are those of the noncomplementing alleles (i.e., complementation group A). The complementing alleles are listed above the map, and behind each isolation number is indicated the complementation group it represents. A complementation map, drawn according to the usual conventions, is given above the genetic map.

expressed in terms of the weighted mean frequencies of prototrophs from all crosses of a particular type (see Table 8). The linear arrangement of sites derived from the majority class of those prototrophs having markers recombined is consistent with that determined by the prototroph frequencies. However, the good additivity of prototroph frequencies indicated in Figure 1 is misleading in one respect: the frequency of prototroph formation between a given pair of *me-2* alleles is very susceptible to variations induced either by changes in ascospore viability or by differences in genetic background (see Table 4). As has been found by other workers (e.g. CASE 1957), a general decrease in the prototroph frequency per viable spore accompanies an increase in germination frequency. (The percent germination of ascospores was not included in Table 4 because pooled data from a number of crosses were often used.) In early studies (MURRAY 1960b) prototroph frequencies from crosses of the type $\alpha \times \gamma$ approximated 40 in 10^5 viable ascospores. Subsequently, some of the marked *me-2* strains were backcrossed to the standard wild type, and strains were selected which gave higher ascospore germination. The use of these strains yielded prototroph frequencies approximating 20 in 10^5 for crosses of the same type. In crosses involving the strains incorporating markers which had been induced in the same wild-type background, the prototroph frequency in an $\alpha \times \gamma$ cross was reduced to 4

TABLE 4

Analysis of methionine prototrophs from crosses between alleles representing the four major "points" in the map of the methionine-2 gene

Genotypes of parents: *tryp⁻ me^x pan⁺* × *tryp⁺ me^y pan⁻*	Positions of *me-2* alleles on genetic map (Figure 1)	Methionine prototrophs: Number	Methionine prototrophs: Frequency per 10^5 viable spores	Classification of methionine prototrophs, Parental: *tryp⁻ pan⁺*	Parental: *tryp⁺ pan⁻*	Recombinant: *tryp⁻ pan⁻*	Recombinant: *tryp⁺ pan⁺*
— K44* + × + P2† —	α × β	157	8	**26** (17%)	**59** (38%)	16 (10%)	**56** (36%)
— P2 + × + K44 —	β × α	209	5	**84** (40%)	23 (11%)	**87** (42%)	15 (7%)
— K23 + × + P2 —	α × β	63	4	3 (5%)	38 (60%)	6 (10%)	**16** (26%)
— P2 + × + K23 —	β × α	113	7	**54** (48%)	12 (11%)	**32** (28%)	15 (13%)
— K44 + × + H98 —	α × γ	114	15	13 (11%)	**50** (44%)	10 (9%)	**41** (36%)
‡— H98 + × + K44 —	γ × α	91	45	**39** (43%)	6 (7%)	**40** (44%)	6 (7%)
‡— K44 + × + P81 —	α × γ	84	46	12 (14%)	**30** (36%)	11 (13%)	**31** (37%)
‡— P81 + × + K44 —	γ × α	78	58	**34** (44%)	9 (12%)	**28** (36%)	7 (9%)
— K44 + × + P169 —	α × γ	81	41	7 (9%)	**38** (47%)	10 (12%)	**26** (32%)
— K44 + × + P174 —	α × γ	242	21	44 (18%)	**97** (40%)	33 (14%)	**68** (28%)
— K86 + × + K44 —	γ × α	186	13	**85** (46%)	15 (8%)	**71** (38%)	15 (8%)
— K44 + × + K5 —	α × γ	114	19	11 (10%)	**50** (44%)	12 (11%)	**41** (36%)
— K5 + × + K44 —	γ × α	211	17	**94** (45%)	17 (8%)	**82** (39%)	18 (9%)
— P78 + × + K44 —	γ × α	97	11	**49** (51%)	2 (2%)	**38** (39%)	8 (8%)
— K44 + × + P143 —	α × γ	53	4	8 (15%)	**23** (43%)	6 (11%)	**16** (30%)
‡— K23 + × + H98 —	α × γ	103	43	13 (13%)	**42** (41%)	18 (17%)	**30** (29%)
‡— H98 + × + K23 —	γ × α	109	33	**38** (35%)	17 (16%)	**37** (39%)	17 (16%)
— K23 + × + P81 —	α × γ	92	18	14 (15%)	**38** (41%)	8 (9%)	**32** (35%)
— P81 + × + K23 —	γ × α	95	24	**45** (47%)	7 (7%)	**27** (29%)	16 (17%)
— P99 + × + K23 —	γ × α	143	19	**62** (43%)	7 (5%)	**56** (39%)	18 (13%)
— K23 + × + K5 —	α × γ	94	73	7 (7%)	**42** (45%)	15 (16%)	**30** (32%)
— K5 + × + K23 —	γ × α	120	15	**54** (45%)	15 (13%)	**42** (35%)	9 (8%)
— P133 + × + H98 —	α × γ	141	26	14 (10%)	**61** (43%)	15 (11%)	**51** (36%)
— P140 + × + H98 —	α × γ	67	11	12 (18%)	**24** (36%)	8 (12%)	**23** (34%)
— P145 + × + H98 —	α × γ	237	54	26 (11%)	**86** (36%)	37 (16%)	**88** (37%)
— K98 + × + H98 —	α × γ	112	19	14 (13%)	**51** (46%)	13 (12%)	**34** (30%)
— K43 + × + H98 —	α × γ	66	13	9 (14%)	**28** (42%)	8 (12%)	**21** (32%)
— P81 + × + K98 —	γ × α	170	19	**49** (29%)	32 (19%)	**71** (42%)	18 (11%)
— K44 + × + P24 —	α × δ	252	48	29 (12%)	**139** (55%)	31 (12%)	**53** (21%)
— P24 + × + K44 —	δ × α	192	33	**117** (61%)	17 (9%)	**36** (19%)	22 (11%)
— P159 + × + K44 —	δ × α	213	48	**125** (59%)	18 (8%)	**46** (22%)	24 (11%)

TABLE 4—Continued

Genotypes of parents: $tryp^-$ me^x pan^+ × $tryp^+$ me^y pan^-	Positions of of *me-2* alleles on genetic map (Figure 1)	Methionine prototrophs		Classification of methionine prototrophs			
				Parental		Recombinant	
		Number	Frequency per 10^5 viable spores	$tryp^-$ pan^+	$tryp^+$ pan^-	$tryp^-$ pan^-	$tryp^+$ pan^+
− K23 + × + P24 −	α × δ	109	10	10 (9%)	**56** (51%)	15 (14%)	**28** (26%)
− P24 + × + K23 −	δ × α	171	32	**87** (51%)	14 (8%)	**46** (27%)	24 (14%)
− P133 + × + P24 −	α × δ	136	110	17 (13%)	**55** (40%)	26 (19%)	**38** (28%)
− P133 + × + P159 −	α × δ	108	29	12 (11%)	**56** (52%)	14 (13%)	**26** (24%)
− P2 + × + H98 −	β × γ	372	12	82 (22%)	**131** (35%)	52 (14%)	**107** (29%)
− H98 + × + P2 −	γ × β	114	10	**37** (32%)	26 (22%)	**36** (32%)	15 (13%)
− P2 + × + K5 −	β × γ	286	9	55 (19%)	**91** (32%)	51 (18%)	**89** (31%)
− K5 + × + P2 −	γ × β	299	9	**114** (38%)	66 (22%)	**83** (28%)	36 (12%)
− P2 + × + P78 −	β × γ	105	16	20 (19%)	**38** (36%)	17 (16%)	**30** (29%)
− P78 + × + P2 −	γ × β	170	19	**61** (36%)	38 (22%)	**49** (29%)	32 (13%)
− P2 + × + P169 −	β × γ	140	12	30 (21%)	**57** (41%)	13 (9%)	**40** (29%)
− P2 + × + P143 −	β × γ	78	7	18 (23%)	**30** (38%)	9 (12%)	**21** (27%)
− P2 + × + P24 −	β × δ	188	30	37 (20%)	**80** (43%)	30 (16%)	**41** (22%)
− P24 + × + P2 −	δ × β	103	31	**53** (51%)	19 (18%)	**18** (17%)	13 (13%)
− P2 + × + P159 −	β × δ	155	18	21 (14%)	**69** (45%)	27 (17%)	**38** (25%)
− P159 + × + P2 −	δ × β	109	32	**47** (43%)	21 (19%)	**31** (28%)	10 (9%)
− H98 + × + P24 −	γ × δ	141	10	32 (23%)	**74** (52%)	15 (11%)	**20** (14%)
− P24 + × + H98 −	δ × γ	284	14	**125** (44%)	49 (17%)	**64** (23%)	46 (16%)
− P159 + × + H98 −	δ × γ	206	37	**107** (52%)	34 (17%)	**36** (17%)	29 (14%)
− K5 + × + P24 −	γ × δ	263	16	47 (18%)	**116** (44%)	34 (13%)	**66** (25%)
− P24 + × + K5 −	δ × γ	124	13	**67** (54%)	20 (16%)	**24** (19%)	13 (11%)
− P159 + × + K5 −	δ × γ	136	24	**66** (49%)	27 (20%)	**25** (18%)	18 (13%)

* Isolation number of *me-2* allele entering cross with *tryp*− (protoperithecial) parent.
† Isolation number of *me-2* allele entering cross with *pan*− parent.
‡ Results published in MURRAY 1960b.

in 10^5, although there was no further increase in germination frequency. This latter finding is consistent with the observation of DE SERRES (1962) that decrease in isogenicity, in the immediate region in which recombination is being studied, leads to an increase in recombination frequency.

The variation in prototroph frequencies due to differences in the genetic background was sufficiently great to suggest that prototroph frequencies be used cautiously in the construction of a genic map. However, wide variations in proto-

TABLE 5

Prototroph frequencies from crosses between alleles located in the same cluster

me-2 alleles represented in cross*	Location on map of *me-2* gene	Number of methionine prototrophs	Number of viable spores × 10^{-5}	Frequency of *me-2* prototrophs per 10^5 viable spores
†K23 × K44	α	17	33	0.5
K23 × K98	α	1	7	0.2
K43 × K44	α	6	5	1.1
K44 × P140	α	50	35	1.4
P133 × P145	α	0	10	. .
P133 × K98	α	4	13	0.3
H98 × K86	γ	92	49	1.9
H98 × P99	γ	0	2	. .
H98 × P78	γ	1	3	0.3
H98 × P81	γ	33	9	3.8
H98 × K5	γ	141	130	1.1
K5 × P143	γ	3	14	0.2
K5 × P78	γ	6	7	0.8
K5 × P81	γ	317	79	4.0
K5 × P169	γ	301	49	6.1
P78 × P169	γ	128	33	3.9
†P81 × P169	γ	16	8	1.9
†P169 × P99	γ	20	4	5.6
†P99 × P174	γ	18	9	2.0
P24 × P159	δ	7	22	0.3

The data from all crosses between a given pair of alleles have been summed.

* Marker genes were present, but are ignored in this table.
† These results include those from early crosses (MURRAY 1960b) before backcrossed strains were isolated; prototroph frequencies may therefore be magnified.

troph frequencies were not accompanied by any marked change in the distribution of the prototrophs amongst the four possible marker classes.

Crosses involving the *me-2* allele, P143m, gave abnormally low recombination frequencies, but the pattern of distribution of prototrophs, with respect to the marker genes, was consistent with its inferred position in the γ cluster.

At the present time there is no evidence to suggest that any of the mutants are multisite mutations which would be useful in constructing a topographical map as BENZER (1959) has done for the *r*II cistrons in T4 phage.

On a two-process hypothesis, where an excess of methionine prototrophs in one of the classes with markers recombined is attributed to reciprocal crossing over, whilst the asymmetries between the classes which are parental with respect to the marker genes are assumed to reflect differences in the frequencies with which *me-2* alleles are converted to prototrophy, there is a polarized increase with respect to conversion frequency, with progression from the proximal to the distal end of the gene. An examination of Table 4 will confirm that the conversion frequencies from mutant to wild are K44 (α) < P2 (β) < H98 (γ) < P24 (δ).

Recombination between alleles within the same cluster: The low frequencies of methionine prototrophs, particularly from crosses between alleles within the

proximal cluster, and the presence of strong complementation between certain representatives of the γ cluster, made the analysis of crosses between alleles of one cluster very laborious. A complete ordering of sites within the clusters has not, therefore, been attempted, but analysis of recombination within clusters was undertaken to ascertain whether the characteristic pattern of asymmetries would be obtained.

Analysis of recombination between two alleles of the α cluster was restricted to the cross (P140 × K44) which gave the highest prototroph frequency [(1.4 in 10^5); see Table 5]. Although only 84 prototrophs were isolated, significant asymmetries were observed between both the two parentally marked classes and the two classes having marker genes recombined (Table 6).

Analysis of recombination within the γ cluster was more favorable in that prototroph frequencies were higher. However, the number of crosses which could be efficiently studied was limited by the strong complementation between many of the possible combinations of alleles. Initially, the aim was to analyze crosses between representative alleles of different complementation groups for the purpose of correlating genetic and complementation maps. On the basis of dry-weight assays of heterocaryons (MURRAY 1960b) it was predicted that, in addition to the cross of P81 (complementation group C) × P169 (group B), the cross of either P81 (group C) or P169 (group B) × H98 (group E) would be suitable for analysis. The heterocaryon P81 (or P169) + H98 gave only one quarter as much growth as was found from combinations of P81 (or P169) with any of the three other alleles of complementation group E (see Figure 1). Unexpectedly the progeny from the cross of P81 × H98 included a high frequency of PWT's which

TABLE 6

Analysis of methionine prototrophs from crosses between alleles located in the same cluster

Genotypes of parents: *tryp*⁻ *me*^{x} *pan*⁺ × *tryp*⁺ *me*^{y} *pan*⁻	Cluster represented	Methionine prototrophs: Number	Methionine prototrophs: Frequency per 10^5 viable spores	Parental: *tryp*⁻ *pan*⁺	Parental: *tryp*⁺ *pan*⁻	Recombinant: *tryp*⁻ *pan*⁻	Recombinant: *tryp*⁺ *pan*⁺
— P140* + × + K44† —	α	84	1.4‡	**36** (42%)	8 (10%)	**27** (32%)	13 (15%)
— K86 + × + H98 —	γ	92	1.9	12 (13%)	**44** (48%)	3 (3%)	**33** (36%)
— K5 + × + H98 —	γ	130	1.1	23 (18%)	**43** (33%)	17 (13%)	**47** (36%)
— K5 + × + P169 —	γ	108	4.9	29 (27%)	**49** (45%)	7 (6%)	**23** (21%)
— P169 + × + K5 —	γ	193	7.0	**79** (41%)	36 (19%)	**53** (27%)	25 (13%)
§— K5 + × + P81 —	γ	205	4.2	33 (16%)	**64** (31%)	31 (15%)	**77** (38%)
— P81 + × + H98 —	γ	33	3.8	**15** (46%)	7 (21%)	**7** (21%)	4 (12%)
— P78 + × + P169 —	γ	128	3.9	29 (23%)	**54** (42%)	14 (11%)	**31** (24%)

Only those crosses, which in one phase, have yielded more than thirty methionine prototrophs are included in this analysis.
* Isolation number of *me-2* allele entering cross with *tryp*⁻ marker.
† Isolation number of *me-2* allele entering cross with *pan*⁻ marker.
‡ This estimate is based on the first 50 prototrophs isolated (see Table 5).
§ The reciprocal cross which is included in Table 5 is an anomalous result described in detail in Table 7.

were morphologically indistinguishable from true wild types. The slow growth rate of heterocaryons involving H98 may therefore be due to the presence of a minor incompatibility factor rather than to an inherently weaker ability to complement. Thus any cross of P81 (group C) × a group E representative (and probably of B (P169) × E) necessitates that each and every methionine prototroph isolated be backcrossed to ascertain whether it is a true or a pseudowild type. Only 33 of 800 methionine prototrophs isolated from the cross of P81 × H98, and tested individually by backcrossing, proved to be true wild types. Analysis of crosses such as this one, which gave PWT's that were difficult to distinguish from true wilds, were so laborious that they were discontinued in favor of using a noncomplementing allele located in the γ cluster, or crosses between alleles of the same complementation group.

The noncomplementing allele, K5, was crossed to a sample of the complementing alleles of the γ cluster. The results were consistent with the location of K5 close to the proximal border of the cluster, and in all crosses analysed (Table 6) asymmetries were obtained between both the two parentally marked classes and the two classes having marker genes recombined. Similar asymmetries were obtained in a cross involving a second noncomplementing allele (P78) and in a cross between two alleles of complementation group E.

A preliminary map of the γ cluster has been constructed (Figure 2) from the data listed in Tables 5 and 6. Wherever sufficient prototrophs were isolated from an intracluster cross, the order of the two *me-2* sites could be inferred. The suggested order is generally consistent with the observed prototroph frequencies. The tentative order of alleles P81 and P169 is based on earlier results (MURRAY 1960b).

Comparison of complementation and genetic maps: In the absence of further overlapping groups, the complementation map (Figure 1) is not sufficiently limiting to justify a critical comparison of the complementation and genetic maps, but three points of interest are apparent. First, the 12 complementing alleles are located in two clusters, suggesting that mutations within each of two regions of the genetic map are particularly likely to give an altered protein capable of complementation. As all the mutants, with the exception of H98, were isolated following ultraviolet irradiation, the above interpretation assumes that the α and γ clusters are not solely the result of a pronounced susceptibility of these two regions to ultraviolet-induced mutation. Second, members of the γ cluster are extremely closely linked, but a number of combinations between mutants of the γ cluster gave maximum complementation. Third, members of the same complementation group are located within very small regions on the recombination map of the *me-2* gene. Analysis of crosses between representatives of the γ cluster places the alleles P169 and P81 (of complementation groups B and C respectively) distal to the group E alleles (Figure 2). This is the order to be expected if there is colinearity of genetic and complementation maps.

Anomalous results: A few crosses involving the allele P81 (γ cluster) have not shown the expected inequalities between the numbers in the two classes of methionine prototrophs which are parental with respect to the marker genes (Table 7).

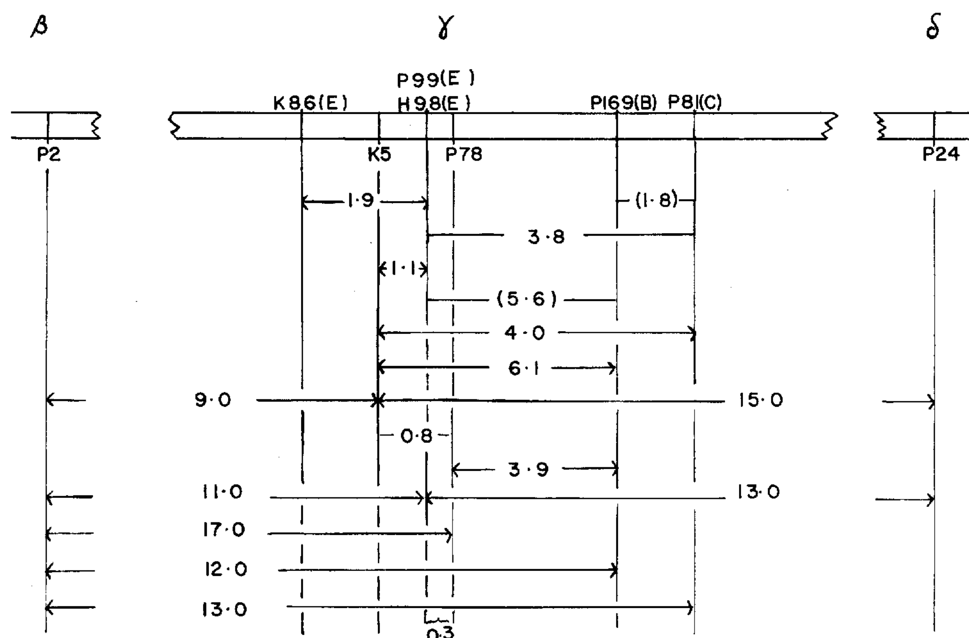


FIGURE 2.—Preliminary map of the γ cluster of the methionine-2 gene. Arrows between two sites indicate that their order was inferred from the significant excess of the more frequent class with marker genes recombined. Omission of arrowheads indicates that the order is tentative because the number of methionine-prototrophic isolates was insufficient to give a significant excess in one of the classes with marker genes recombined. Results from all crosses between any two alleles were used to calculate the prototroph frequencies which were then expressed as the weighted means. The distances are expressed as the prototrophic frequencies per 10^5 viable spores. Values given in parentheses are from early crosses and may be overestimates.

TABLE 7

Analysis of methionine prototrophs from anomalous crosses

Genotypes of parents: *tryp*⁻ *me*^{x} *pan*⁺ × *tryp*⁺ *me*^{y} *pan*⁻	Type of Cross	Methionine prototrophs: Number	Methionine prototrophs: Frequency per 10^5 viable spores	Parental: *tryp*⁻ *pan*⁺	Parental: *tryp*⁺ *pan*⁻	Recombinant: *tryp*⁻ *pan*⁻	Recombinant: *tryp*⁺ *pan*⁺
— P2* + × + P81† —	$\beta \times \gamma$	258	13	67 (26%)	67 (26%)	46 (18%)	78 (30%)
— P81 + × + P2 —	$\gamma \times \beta$	417	29	101 (24%)	109 (26%)	132 (32%)	75 (18%)
— P81 + × + K5 —	$\gamma \times \gamma$	112	4	29 (26%)	42 (38%)	27 (24%)	14 (13%)

* Isolation number of *me-2* allele entering cross with *tryp*– marker.
† Isolation number of *me-2* allele entering cross with *pan*– marker.

The same strain of P81 which failed to show the inequalities in crosses of P2 (β) × P81 (γ) behaved in the more usual way when crossed to any of the three α alleles (see Table 4). In all the exceptional crosses, asymmetries were observed between the two classes of methionine prototrophs having marker genes recom-

bined; the orders inferred from these asymmetries are consistent with those inferred from the remainder of the results. No explanation is attempted for the few exceptional findings.

ANALYSIS AND DISCUSSION

Is the conversion frequency of an allele constant? Preliminary data on recombination within the *me-2* gene (MURRAY 1960b) were consistent with the interpretation that intragenic recombination resulted from both conversion and reciprocal crossing over. The excess of methionine prototrophs in one of the classes having outside markers recombined was attributed to crossing over, whilst the asymmetries between the two parentally marked classes of prototrophs were assumed to reflect differences in the conversion frequencies of the two *me-2* alleles. If alleles had constant conversion frequencies, it was predicted that in a cross between two alleles of low conversion frequency the methionine prototrophs should be predominantly of one class—a class that had markers recombined—whilst in a cross between two alleles of high conversion frequency the prototrophs should be predominantly of the two classes that had markers of parental combinations. In either cross, asymmetries between the two parentally marked classes of prototrophs should be absent or much reduced.

A test of the hypothesis of allele-specific conversion frequencies had to be based on crosses between pairs of *me-2* alleles that were located in the same cluster, because the asymmetries between the two parentally marked classes of prototrophs indicated that the conversion frequency of an *me-2* allele was a function of its location within the gene. The results of such crosses refute any hypothesis in which alleles have a constant conversion frequency. First, crosses either between two alleles of apparent low conversion frequencies ($\alpha \times \alpha$) or two alleles of apparent high conversion frequencies ($\gamma \times \gamma$) gave pronounced asymmetries between the two parentally marked classes of prototrophs. Second, in the case of crosses between two α alleles the methionine prototrophs were not predominantly recombinant with respect to marker genes, nor in the case of crosses between two γ alleles were the prototrophs predominantly parental with respect to the marker genes. Third, in a cross between two γ alleles the frequency of prototroph formation apparently due to conversion is much less than twice the frequency of prototrophs attributed to conversion of the γ allele in an $\alpha \times \gamma$ cross. The latter observation demonstrates that the apparent conversion frequency is influenced by the distance between the two mutant sites (see PRITCHARD 1960).

Intragenic recombination by a mechanism other than crossing over. Interpretation of the present data is handicapped by the absence of a tetrad analysis which would provide direct information on the problem of reciprocal versus nonreciprocal recombination. The evidence from tetrad analyses suggests that in Neurospora intragenic recombination is predominantly nonreciprocal (see MITCHELL 1955; CASE and GILES 1958; STADLER 1959a; SUYAMA, MUNKRES and WOODWARD 1959). This is true even though recombination in three of the genes so analysed is tenfold more frequent than it is for *me-2*.

If a substantial portion of the methionine prototrophs resulted from classical

crossing over (i.e. a single reciprocal exchange), then the frequency of the majority class having markers recombined should increase as the distance between the *me-2* sites increases. This is not the general rule, and when a cross of type $\alpha \times \delta$ is compared with one of the type $\alpha \times \gamma$, not only is the proportion of prototrophs in the possible single "crossover" class decreased in relation to the other three classes, but the absolute frequency of prototrophs of this marker combination is also decreased. It is concluded that even those *me-2* prototrophs that have the markers recombined usually originate by a mechanism other than a single reciprocal exchange.

Hypothesis of multiple exchanges: The results for *me-2* may be conveniently interpreted in terms of multiple exchanges within a short segment of the genome. The data are examined in these terms in Table 8. Methionine prototrophs are assumed to result from an exchange, or exchanges, in the region between the sites of the two *me-2* alleles (region 2 in Table 8). This selected fraction of the progeny is then classified according to the marker-gene combinations, from which are deduced the additional exchanges required to give the observed genotypes. The methionine prototrophs are further classified in the two rightmost columns of Table 8 to indicate the percentage of prototrophs having an additional exchange in the adjacent proximal region (designated region 1), and the percentage having an additional exchange in the adjacent distal region (designated region 3).

In any cross in Table 8 the frequency of exchange in region 1 is much less than that in region 3; the maximum coincident exchange frequency in region 1 is 36 percent whereas the minimum coincident exchange frequency in region 3 is 49 percent. This difference is the cause of the observed asymmetries.

The coincident exchange frequency is a function of the location of the *me-2* sites. The frequency of coincident exchanges in the proximal interval (region 1) is apparently influenced by the position of the more proximal of the *me-2* sites. Approximately 20 to 25 percent of the methionine prototrophs from any cross involving an allele located at point α have an exchange in region 1, compared with 30 to 35 percent from any cross involving either a β or γ allele as the more proximal *me-2* site (see Table 8). The present restriction of methionine alleles to effectively four points on the genetic map severely limits the ability to test the correlation between exchange frequency in region 1 and the position of the more proximal *me-2* site.

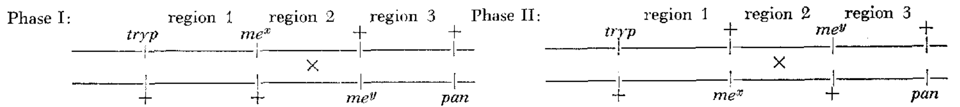
The frequency of coincident exchanges in region 1 appears to be independent of the distance between two *me-2* sites. This can be tested critically by comparing crosses between pairs of alleles within the *same* cluster, with crosses between alleles of *different* clusters (i.e. $\alpha \times \alpha$ with $\alpha \times \beta$, $\alpha \times \gamma$ etc.).

$\alpha \times \alpha$ crosses: Of 84 prototrophs, 21 (25 percent) had an apparent exchange in region 1; compared with the 22 to 23 percent obtained from crosses between an α allele and an allele at another point on the cluster map (Table 8).

$\gamma \times \gamma$ crosses: Summation of the results from all the $\gamma \times \gamma$ crosses, listed in Table 6, indicates a 30 percent recombination frequency in region 1, compared with 31 percent derived by summation of the results of all crosses of type $\gamma \times \delta$ (see Table 8).

TABLE 8

Summation of data from Tables 4 and 6, and analysis of methionine prototrophs in terms of reciprocal exchanges. The methionine prototrophs were assumed to arise from an exchange between the two me-2 *sites (region 2) and on the basis of the marker genes the prototrophs were classified according to additional exchanges in the adjacent marked regions*



Phase of cross	Position of *me-2* alleles on genetic map	Methionine prototrophs: Number	Methionine prototrophs: Frequency per 10^5 viable spores	Classification of *me* prototrophs according to exchanges: Double Regs 1+2	Double Regs 2+3	Triple Regs 1+2+3	Single Reg 2	Percentage *me* prototrophs: With exchange in 1 (1,2's+ 1,2,3's)	With exchange in 3 (2,3's+ 1,2,3's)
I)	$\alpha \times \beta$	220	7	29 (13%)	97 (44%)	22 (10%)	72 (33%)	23	54
II)	$\beta \times \alpha$	325	6	35 (11%)	138 (43%)	30 (9%)	122 (38%)	20	52
Pooled		545	6	64 (12%)	235 (43%)	52 (10%)	194 (36%)	22±4	53±5
I)	$\alpha \times \gamma$	1,600	20	204 (13%)	660 (41%)	204 (13%)	532 (33%)	26	54
II)	$\gamma \times \alpha$	1,300	19	127 (10%)	548 (42%)	132 (10%)	493 (38%)	20	52
Pooled		2,900	19	331 (11%)	1208 (42%)	336 (12%)	1025 (35%)	23±2	53±2
I)	$\alpha \times \delta$	605	28	68 (11%)	306 (51%)	86 (14%)	145 (24%)	25	65
II)	$\delta \times \alpha$	576	37	49 (9%)	329 (57%)	70 (12%)	128 (22%)	21	69
Pooled		1,181	32	117 (10%)	635 (53%)	156 (13%)	273 (23%)	23±2	67±3
I)	$\beta \times \gamma$	981	12	205 (21%)	347 (35%)	142 (15%)	287 (29%)	36	51
II)	$\gamma \times \beta$	583	11	130 (22%)	202 (35%)	83 (14%)	168 (29%)	36	49
Pooled		1,564	11	335 (22%)	549 (35%)	225 (14%)	455 (29%)	36±3	49±3
I)	$\beta \times \delta$	343	23	58 (17%)	149 (43%)	57 (17%)	79 (23%)	34	60
II)	$\delta \times \beta$	212	32	40 (19%)	100 (47%)	23 (11%)	49 (23%)	30	58
Pooled		555	26	98 (18%)	249 (45%)	80 (14%)	128 (23%)	32±4	59±4
I)	$\gamma \times \gamma$	664	1–5	126 (19%)	255 (39%)	72 (11%)	211 (32%)	30	50
II)	$\gamma \times \gamma$	226	4–7	43 (19%)	94 (42%)	29 (13%)	60 (27%)	32	55
Pooled		890	1–6	169 (19%)	349 (39%)	101 (11%)	271 (31%)	30±3	51±3
I)	$\gamma \times \delta$	404	13	79 (20%)	190 (47%)	49 (12%)	86 (21%)	32	59
II)	$\delta \times \gamma$	750	18	130 (17%)	365 (49%)	106 (14%)	149 (20%)	31	63
Pooled		1,154	16	209 (18%)	555 (48%)	155 (13%)	235 (20%)	31±3	61±3

The coincident exchange frequency in the distal interval (region 3) is close to 50 percent in all crosses except those involving a δ allele; the increase in coincident exchange frequency in region 3, associated with δ alleles, will be discussed later.

Common origin of asymmetries: In general, both the direction and intensity of the asymmetries between the two parentally marked classes of methionine prototrophs are correlated with the asymmetries between the two classes having markers recombined; this suggests that both asymmetries have a common origin.

One inference is that all four classes of prototrophs result primarily from a *single* mechanism—a complex of exchanges within short, effectively paired regions of the genomes (as suggested by FREESE 1957). In agreement with the tetrad data from Neurospora, the switches would be nonreciprocal within paired regions, although reciprocal at the ends of the intimately paired regions. Some of the complexes of exchanges within the *me-2* gene would recombine the marker genes, but such a mechanism of interallelic recombination need not be the primary mechanism of intergenic recombination, and would have quite a different basis from classical crossing over. A number of investigations (STADLER 1959b; LISSOUBA and RIZET 1960; SHERMAN and ROMAN 1963) provide evidence, based on criteria other than nonreciprocality of some exchanges, that there is more than one mechanism of recombination. Alternatively the apparent multiple recombinational events may result from a dual mechanism of two distinctly different, but coincident, events. If the conversional event is a double nonreciprocal switch, both classes of methionine prototrophs having marker genes recombined require both conversion and an adjacent reciprocal crossover. The high correlation between the two events would depend on the facilitation of both events within the region of intimate pairing.

Neither the single nor the dual mechanism (and particularly the latter) necessarily predict good additivity of prototroph frequencies. The present results indicate a superficially good additivity (see Figure 1), but also demonstrate that the prototroph frequencies are markedly influenced by factors other than the specific pair of methionine alleles crossed (see Table 4).

If reciprocal intergenic recombination results from clusters of exchange events within a short region of the genome, then only two chromatids may be involved in these events (PERKINS 1962).

Hypothesis of a discontinuity: Either mechanism—dual or single—could result in asymmetries, interpreted as polarized recombination, if some factor reduces the chance of the region of intimate pairing extending proximally from the region between two selected *me-2* sites. It is suggested that a discontinuity exists within the genetic material immediately proximal to the *me-2* region. When selection is made for exchanges between two *me-2* alleles such a discontinuity would impose nonrandomness of coincident exchanges if the region within which associated exchanges occur cannot extend proximal to the discontinuity (MURRAY 1961). A discontinuity could be the junction between contiguous DNA molecules.

Two aspects of the data are not directly accounted for by the hypothesis of a discontinuity. The first concerns the frequency of coincident exchanges in the proximal marked region (region 1, Table 8) and the position of the proximal *me-2* allele. The hypothesis would predict that the coincident recombination frequency in region 1 should increase with an increase in the distance between the discontinuity and the more proximal *me-2* site. The recombination frequency in region 1 was indeed lowest when an α allele was involved, but there was no expected increase when a γ allele rather than the β allele was the more proximal. It should be emphasized, however, that only one β allele has been available for analysis.

A second aspect not accounted for in terms of a discontinuity is the characteristic influence on recombination frequency in the distal marked region (region 3) observed in any cross involving a δ allele (see Table 8). In crosses of $\alpha \times \delta$, $\beta \times \delta$, and $\gamma \times \delta$, the recombination frequency in region 3 is significantly higher than in the respective crosses $\alpha \times \gamma$, $\beta \times \gamma$, and $\gamma \times \gamma$. The two δ alleles, isolated in different filtration enrichment experiments, both behave in the same way, although they are probably not mutations at the same site (Table 5). Possibly recombination is influenced by some configuration of the genetic material in the δ region which increases the likelihood of a return exchange in region 3 (see BALBINDER 1962).

A model invoking discontinuities in the genetic material has been considered by STAHL (1961). In this model a few randomly distributed exchanges occur within predetermined regions of pairing, i.e. within segments of the genome between two discontinuities. Correlated asymmetries are a consequence of such a model, but it is difficult to account for the high frequencies of parentally marked prototrophs (60 to 70 percent) encountered in crosses involving a δ allele.

EPHRUSSI-TAYLOR (1961) has discussed recombination mechanisms with particular reference to DNA mediated transformation, where the process is readily visualized in molecular terms, and pointed out that "the position of a mutant site with respect to the ends of a DNA molecule will very much influence its recombination behavior." OZEKI (1959) presented experimental results, from studies of transduction and abortive transduction in Salmonella, which he explained on the hypothesis that the transducing fragments of the donor chromosome were of fixed compositions and that the probability of complete transduction for any particular marker varied according to the position of the marker with respect to the ends of the fragment carrying it. Mapping studies of the tryptophan genes in *Bacillus subtilis* (ANAGNOSTOPOULOS and CRAWFORD 1961) demonstrated discrepancies between the recombination frequencies of reciprocal crosses between closely linked genes. Results from a number of experiments were consistent with the hypothesis that the less frequently transformed gene is located close to the end of a transforming fragment of fixed length; an alternative explanation invokes polarized replication.

Polarized replication and intragenic recombination: Fine structure analysis involving tetrads from *Ascobolus immersus* led RIZET and coworkers (LISSOUBA and RIZET 1960; RIZET, LISSOUBA and MOUSSEAU 1960) to postulate that polarized nonreciprocal recombination occurs within a unit they have called the *polaron*; and that reciprocal recombination occurs only at the linkage structure between two polarons. The demonstration of polarity depends on the determination of which allele of any pair is converted to wild type. On an arbitrary designation, the rightmost site of any pair of alleles within a polaron is always the one to be converted. The results may be explained by a copy-choice mechanism if replication is polarized and if the points of switching are predetermined in such a way that the switch between sites is always the first.

SIDDIQI (1961, 1962) has reported the existence of polarized recombination within the *paba* region of *Aspergillus nidulans*. Generally, he found that the

coincident exchange frequency in the proximal region was less than that in the distal region, but the exchange frequency in the proximal region increased as the length of the selected interval (i.e. the distance between the *paba* sites) was decreased, and when the two *paba* sites were sufficiently close together the polarization could not be demonstrated. SIDDIQI suggests that chromosome replication (which is assumed to be an essential condition for recombination) is polarized from the distal to the proximal end of the chromosome arm, and that recombination takes place within discontinuously distributed regions of effective pairing. Given these conditions, he predicts that the greater the distance within which selection for an exchange is made, the smaller will be the chance that the region of effective pairing will extend to the proximal side of the selected region.

An examination of those investigations of intragenic recombination which have used outside marker genes revealed that asymmetries between the two classes of parentally marked prototrophs may be characteristic of three systems in Aspergillus (CALEF 1957; PRITCHARD 1960; SIDDIQI 1962), two in Neurospora (STADLER 1959a; MURRAY 1960b) and possibly one in maize (NELSON 1962). In all of these examples, the asymmetries could result from a reduced coincident exchange frequency in the proximal region. Further consideration reveals no correlation between the presence or absence of a polarity effect and the position of the gene with respect to the centromere.

The results for *me-2* differ, in at least three ways, from those obtained by SIDDIQI. 1) The apparent frequency of multiple exchanges within the *me-2* region exceeds that in the *paba* region of Aspergillus; in the *me-2* system the majority class of parentally marked prototrophs is at least as frequent as the majority class of prototrophs having markers recombined, whilst in the *paba* analysis the predicted "crossover" class is always larger. 2) The distance between the two *me-2* sites, within which selection is made for an exchange, does not influence the intensity of polarization (i.e. the frequency of coincident exchanges in the proximal marked region). 3) The intensity of polarization is, however, influenced by the position of the more proximal of the two *me-2* sites (from 22 to 25 percent of the prototrophs from crosses in which an α allele is involved have shown exchanges in the proximal marked region, whilst from 30 to 36 percent of the methionine prototrophs from crosses in which either a β or γ allele is the more proximal have exchanges in this region).

Polarized replication of the genetic material is inadequate to explain the findings for the *me-2* gene. Even in the presence of polarized replication it appears necessary to invoke the presence of a discontinuity.

Conclusion: The data presented here for the *me-2* gene of Neurospora demonstrate a consistent geometrical pattern of intragenic recombination. A speculative interpretation is that the genetic material within a chromosome arm is discontinuous. If the genetic material consists of subunits (e.g. DNA molecules) it can be visualized that the recombination patterns for particular intragenic markers will be influenced by the positions of these markers relative to the ends of the subunit. This interpretation not only accounts for the *me-2* data but seems tenable in view of results with other organisms. There is no evidence of polarized

recombination in some investigations, e.g. those of the '*q*' (*nic-1*) locus (ST. LAWRENCE 1956) and the *pan-2* locus (CASE and GILES 1958) of Neurospora. These could be explained by the assumption that the nearest discontinuity is further from the mapped sites of these genes than the length of the region of effective pairing.

SUMMARY

Methionine-independent progeny from crosses between many pairs of combinations involving alleles at the *me-2* locus were classified with respect to the outside markers (*tryp-4* and *pan-1*). One of the two classes of methionine prototrophs having outside markers recombined occurred in excess of the other. When the markers entered the cross in the opposite phase, a similar excess was found in the reciprocal class. The order of the *me-2* sites determined from these asymmetries was consistent with the observed prototroph frequencies. Within the limits of the test, there is a striking correlation between location on the complementation and on the genetic maps.

Pronounced asymmetries were also observed in the numbers of the two parentally marked classes; when the markers entered the cross in the opposite phase, these asymmetries were reversed. The asymmetries between the two parentally marked classes were correlated in direction with the asymmetries between the two classes having markers recombined.

The results may be interpreted in terms of multiple exchanges within small, discontinuously distributed, regions of effective pairing. The asymmetries could result from a reduction of coincident exchanges in the region proximal to the selected interval, if the *me-2* region is situated immediately distal to some discontinuity in the genetic material.

ACKNOWLEDGMENTS

The author is indebted to DR. DAVID D. PERKINS and DR. DOROTHY NEWMEYER for their discussions and criticisms during the course of this work; to DR. D. G. CATCHESIDE, F.R.S. for his continued interest and for the donation of stocks; and to the Wellcome Foundation for the award of a Research Travel Grant.

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