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Recombination events that span sites within neighbouring gene loci of *Neurospora**

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SUMMARY

Evidence is given that *me-7* and *me-9* are separate but contiguous gene loci.

The pattern of polarized recombination throughout the *me-7 me-9* region indicates the location of a recombinational discontinuity between the known *me-7* alleles and the known *me-9* alleles.

Recombination events may include sites in both genes but such events are not preferentially associated with parental combinations of flanking markers.

Recombination events extending into both genes provide an extra criterion for the ordering of the sites within the *me-7* locus. The order so obtained confirms that deduced from the flanking markers of me$^+$ recombinants from *me-7* × *me-7* crosses.

In *me-7* × *me-9* crosses but *not* in *me-7* × *me-7* crosses, the map order derived on the assumption that single exchanges are more frequent than apparent triple exchanges is the reverse of that derived from prototroph frequencies. It is concluded that the former criterion is more likely to have provided the correct order and the anomalous prototroph frequencies reflect the polarity of gene conversion within *me-7*.

1. INTRODUCTION

Genetic analyses of recombination in fungi generally use either markers that are at least a few map units apart, or markers that are alleles at a single gene locus. The linkage maps of fungi indicate relatively few regions where markers throughout two or more contiguous genes may be used in rigorous genetic analyses. One possible such region comprises *me-7* and *me-9* of *Neurospora crassa* which are in all probability contiguous but separate genes.

Analyses of allelic recombination have demonstrated the phenomenon of polarized recombination (e.g. Lissouba & Rizet, 1960; Murray, 1961; Siddiqi, 1961) and have led to the suggestion that recombination events are discontinuously distri-

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buted. On a hybrid-DNA model the discontinuities may be visualized as specific DNA sequences at which cleavage of one of the two strands of the double helix takes place prior to the exchange of strands and the formation of a hybrid (heteroduplex) region (Holliday, 1964; Hastings & Whitehouse, 1964). If a hybrid-DNA region contains a mismatched base pair, or base pairs, as a consequence of the genetic nonidentity of the parental homologues it may be the substrate for an excision and repair process. Allelic recombinants would result from repair (correction) and this is assumed to be the mechanism of gene conversion.

The extension of fine structure analyses to include both the *me-7* and *me-9* genes as described in the present paper locates a postulated discontinuity with closely linked markers on both sides of it, and thus provides a system for testing the following basic feature of one of the hybrid-DNA models. According to this model (Hastings & Whitehouse, 1964, or Whitehouse, 1966), allelic recombinants having parental arrangements of flanking markers result from a 'double crossover' structure. The first 'crossover' requires hybrid DNA formation to one side of the point of initial DNA breakage (i.e. the discontinuity) and the second (the 'reverse crossover') requires hybrid DNA formation to the opposite side of the initial breakage point. This structure leads to the prediction that allelic recombination (gene conversion) in the hybrid region to the left of the postulated discontinuity should be associated with recombination in the hybrid region to the right of the discontinuity, provided that the flanking markers are maintained in parental arrangement. Such a test system in which closely linked genetic markers are present on both sides of the postulated discontinuity, is described and used in the following experiments. The data are analysed in terms of hybrid-DNA models.

2. MATERIALS AND METHODS

me-7 is located close to the centromere of linkage group VII approximately 2 to 3 units right of *thi-3* (thiamine-3) and 1 to 2 units left of *wc* (white collar). *me-9* is very close to, but right of, *me-7* (see Table 1). *ars-1* (aryl sulphataseless) was shown by R. Metzenberg (personal communication) to be very closely linked to *me-7* and in these studies it is placed less than one unit left of *me-7*. The order of genes in this region is therefore *thi-3 ars-1 me-7 me-9 wc*, but the position of the centromere is unknown.

The *me-7* strains and the temperature-sensitive *me-9* strain NM43t were isolated following ultraviolet irradiation of the wild-type strain Emerson a. The other *me-9* allele (C124) was described by Dubes (1953).

Media, methods of crossing, ascospore isolation and scoring were as described by Perkins (1959); interallelic crosses were made and the ascospores plated according to the procedures outlined by Murray (1969). *ars-1* was scored according to the protocol of R. Metzenberg (personal communication). This was to grow the cultures on 1 ml of neural liquid medium in which cysteic acid replaced the inorganic sulphur source. After 3 days at 34°, one drop of 0·05 M *p*-nitrophenyl sulphate in 0·05 M Tris-HCl at pH 7·5 was added to each culture, and the medium of *ars*$^+$ cultures turned yellow.

3. RESULTS

(i) me-7 me-9—*two genes or one*?

The *me-7* alleles are blocked in the conversion of cysteine to cystathionine and lack the enzyme cystathionine-γ-synthase (Kerr & Flavin, 1969). The *me-9* alleles are blocked in the conversion of homocysteine to methionine but they have both been shown to produce the *me-7* enzyme cystathionine-γ-synthase (Kerr & Flavin, 1969). Both the *me-7* and *me-9* loci have a temperature sensitive allele and under nonpermissive conditions (high temperature) the temperature-sensitive *me-9* strain retains the *me-7* function (Kerr & Flavin, 1969) while the temperature-sensitive *me-7* strain is still able to convert homocysteine to methionine.

In the present analysis both complementation tests and genetic mapping divide the *me-7 me-9* region into two discrete loci comprising 12 *me-7* alleles and two *me-9* alleles. The paucity of *me-9* alleles may be explained by the finding that the two known isolates are leaky.

No mutation which removes both the *me-7* and *me-9* functions has been isolated. If the *me-7 me-9* region coded for a single bifunctional polypeptide chain then both functions could be lost as the result of a single nonsense or frame-shift mutation. On this interpretation, if transcription and translation were initiated from the *me-9* end, a nonsense or a frame-shift mutation in the *me-9* region should impair both functions and the mutant strain would not be leaky. Many mutant hunts have failed to provide such mutations, Alternatively, if transcription or translation were initiated from the *me-7* end a nonsense or frame-shift mutation in the *me-7* region should remove both functions. One *me-7* allele (NM251) behaves like a nonsense mutant of the amber type since it is suppressible by a supersuppressor known to suppress specific CRM-less, non-complementing alleles at the *am* (amination) locus (Seale, 1968) and certain polar mutations within the *arom* (aromatic) cluster (Case & Giles, 1968).

A further *me-7* strain (K79) has a translocation (I; VII) and it is possible that the *me-7* lesion results from the location of one of the break-points within the *me-7* region. In this case such a mutation would be expected to prevent the formation of the *me-7* (or *me-7 me-9*) gene product since part of the gene would be in linkage group I and part in linkage group VII. To test whether the *me-7* lesion is separable from the rearrangement, *me-7* (K79) was crossed to *me-9* (NM43t) and the progeny were screened for prototrophs. In this way it was possible to screen for gene conversion of the *me-7* lesion. None of the 27 methionine prototrophs selected from approximately $2{\cdot}5 \times 10^5$ viable ascospores carried the aberration and it therefore seems probable that the *me-7* (K79) lesion is inseparable from the aberration. Although *me-7* (K79) lacks the *me-7* function, like other *me-7* alleles, it is not blocked in the *me-9* function.

All available evidence is thus consistent with the idea that *me-7* and *me-9* are two gene loci.

(ii) me-7 *by* me-9 *crosses*

The recombinants from allelic crosses in Neurospora are generally distributed among all four flanking marker combinations, and the presence of the minority *non*parental combination provides evidence that the two allelic mutations may fall within the region spanned by a recombination event (pairing region). On a multiple exchange hypothesis the minority nonparental combination demands, in addition to the selected event, one exchange to the left and another to the right of the selected region. On a hybrid-DNA model both sites must fall within the hybrid-DNA region, and independent correction of each heterozygous region is postulated. If pairing regions were discrete, crosses between closely linked mutants in contiguous pairing regions would be characterized by the absence of recombinants having flanking markers of the minority nonparental combination, as was found for crosses involving the closely linked genes *cys*-1 and *cys*-2 of Neurospora (Murray, 1965; Stadler & Towe, 1968).

The data from crosses of *me-7* by *me-9* are presented in Table 1. Although all 365 prototrophic isolates from one cross were backcrossed and reisolated before confirming the flanking marker combinations, in other crosses only those isolates that were either difficult to score or were thi^+ wc^+ (i.e. possible pseudowilds–heterozygous disomics) were backcrossed. In every cross recombinants with *all* four flanking marker combinations were detected, suggesting that the recombination event can span sites within closely linked genes. The data like those from crosses between *me-7* alleles (Murray, 1969) are remarkable in that approximately 75 % of the prototrophic recombinants carried parental combinations of flanking markers.

Table 1. *Classification of methionine prototrophs from crosses of* me-7 *alleles by* me-9 *alleles*

Cross. Genotypes of parents							Classification of methionine prototrophs			
thi	*me-7*	*wc*	×	*thi*	*me-9*	*wc*	p^{me-7}	p^{me-9}	R^1	R^2
+	271	−	×	−	43t	+	53	27	15	3
−	271	+	×	+	43t	−	90	57	32	8
−	73	+	×	+	43t	−	107	56	35	6
*+	73	−	×	−	43t	+	162	92	93	18
+	73	+	×	−	43t	−	31	19	6	1
+	73	−	×	−	C124	+	81	53	10	2
+	21	−	×	−	43t	+	37	49	13	3
−	56	+	×	+	43t	−	40	44	22	2
+	56	−	×	−	43t	+	34	52	32	7

* All isolates from the fourth cross were backcrossed, me^+ progeny isolated and flanking markers confirmed.

p^{me-7} Parental combination of flanking markers entering with the $me\text{-}7^-$ allele.

p^{me-9} Parental combination of flanking markers entering with the $me\text{-}9^-$ allele.

R^1 Majority nonparental combination of flanking markers indicating the order *thi me-7 me-9 wc*

R^2 Minority nonparental combination of flanking markers.

(iii) *The polarity pattern within the* me-7 *and* me-9 *region*

The data from crosses of *me-7* by *me-9* (Table 1) show that a recombination event can span sites within these two genes and this finding would be expected if there is no recombinational discontinuity between the genes. Is this interpretation consistent with the polarity pattern?

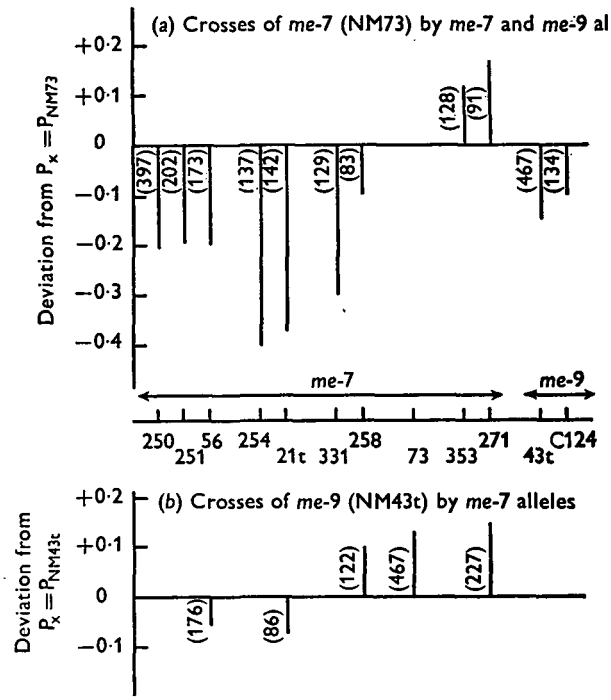

Fig. 1. Map showing order of *me-9* and *me-7* mutants and the polarity relationships. The site order is deduced from the more frequent class having flanking markers recombined. The heights of the bars indicate deviations from equality of the two parentally marked classes of prototrophs (see text for more detailed explanation). The numbers in parentheses are the numbers of prototrophs used to estimate the deviations. In (*a*) the data are from crosses involving *me-7* (NM73) and in (*b*) the data are from crosses involving *me-9* (NM43t). Alleles NM250, NM251 and NM56 comprise a tight cluster within which the order is unknown; no recombination has been obtained between alleles NM254 and NM21t; NM43t and C124 are very closely linked; NM258 has recently been located between NM21t and NM73 but is has not been crossed to NM331.

A detailed analysis of recombination between *me-7* alleles has been published (Murray, 1969) and these data supplemented by a few additional crosses between *me-7* alleles and the data from crosses of *me-7* by *me-9* (Table 1) are used to define the polarity pattern in the *me-7 me-9* region (Fig.1 *a*). The map order of the *me-7* alleles was deduced from the more frequent class with flanking markers recombined although a few uncertainties remain where alleles are tightly clustered. The data in Table 1 place *me-9* to the right of *me-7*. Polarity of allelic recombination is inferred

from the inequality of the classes that have parental combinations of flanking markers, i.e. when P_1 deviates from P_2. The polarity pattern throughout the region is most readily seen when allele NM73, in the right-hand half of the locus, is crossed to other *me-7* alleles and to the *me-9* alleles. Figure 1*a* summarizes these data; the bars on the histogram represent deviations of the two parentally marked classes from equality (i.e. a value on the ordinate is the fraction of all parentally marked prototrophs that were of the P_x type, minus 0·5). For an allele, x, a positive entry indicates that $P_x > P_{NM73}$ and conversely a negative entry indicates that $P_x < P_{NM73}$. The resultant curve has two inflexions, one with a minimum within *me-7* and the second with a maximum marked by the extreme right-hand *me-7* allele (NM271). On a hybrid DNA model this pattern demands a discontinuity close to the right-most *me-7* allele. The two right-most *me-7* alleles show marked polarity when intercrossed (i.e. $1P^{353}$: $19P^{271}$). This suggests that the discontinuity is to the right of all the known *me-7* alleles.

In Fig. 1*b* data from crosses of *me-9* (NM43t) by *me-7* alleles are plotted in the same way.

The preceding conclusion (section ii) that *me-7* and *me-9* sites can be included within a recombinational event may be accommodated if hybrid-DNA initiated to the right of the *me-9* mutation may sometimes extend through *me-9* into the neighbouring *me-7* gene. In general terms this would mean that the region of hybrid DNA can extend from the discontinuity where it originates, through and beyond a neighbouring discontinuity.

(iv) *Coincident recombination within* me-7 *and adjoining genes*

The hybrid DNA model of Hastings & Whitehouse (1964) postulates that allelic recombinants having parental arrangements of flanking markers result from a 'double crossover event' (see Fig. 2) in which the first crossover requires hybrid DNA formation to one side of the discontinuity (point of initial DNA breakage) and the second (the 'reverse crossover') requires hybrid DNA formation to the opposite side of the initial breakage point. The pattern of polarity for the *me-7 me-9* region supports the concept of *me-7* and *me-9* as contiguous genes with a discontinuity located within the region—possibly between the genes. If therefore we make a cross of the sort shown in Fig. 3 and select for recombination between *me-7* alleles, i.e. hybrid DNA formation to the left of the discontinuity, we may ask whether there is evidence for a reverse crossover event that would include the *me-9* marker. Providing that the *me-9* marker is close enough to the discontinuity, the above model predicts a high frequency of coincident recombination associated with the parental arrangements of flanking markers.

The data from crosses of the type shown in Fig. 3 are analysed in Table 2. The selected recombination event is within the *me-7* gene but those isolates which, on a multiple exchange hypothesis, also demand an exchange between the *me-9* marker and the right flanking marker are identified as instances of coincident recombination. On a hybrid-DNA model these recombinants require that the *me-9* marker is included in the region of hybrid DNA. The data are presented for only those crosses

from which a minimum of twenty examples of coincident recombination were detected (Table 2) but all crosses support the same conclusions. Both of these crosses involve an *me-7 me-9* double mutant that was obtained following ultraviolet irradiation of the temperature-sensitive *me-9* strain, NM43t.

Attempts were made to construct *me-7 me-9* strains by recombination but two doubly mutant strains, each isolated from a different cross, carried an *me-7* allele different from the one entering the cross. It was not possible therefore to make

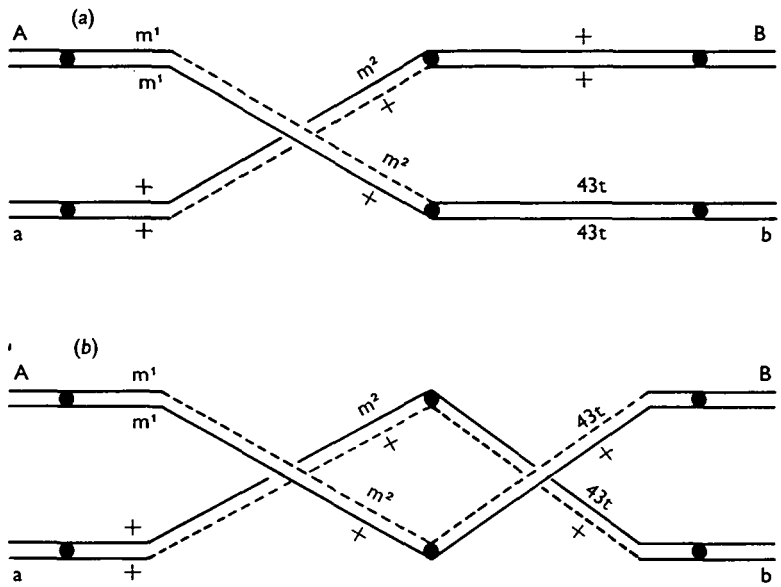

Fig. 2. Diagrams to illustrate the nature of hybrid DNA formation according to the model of Whitehouse & Hastings (1965). The broken lines indicate newly-synthesized nucleotide chains. Regions where one strand is continuous the other broken are hybrid in the sense that the sequence represented by a broken line is specified by one parent and that represented by a continuous line by the other parent. A solid circle indicates a fixed point of primary nucleotide breakage. (*a*) Diagrams the origins of conversion with crossing over, and (*b*) conversion in the absence of crossing over of flanking markers.

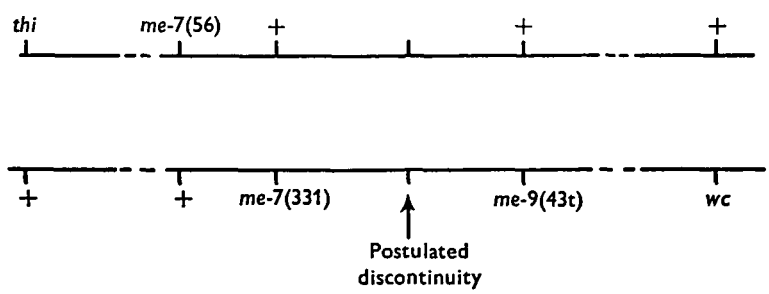

Fig. 3. The type of cross used to test for coincident recombination in the *me-7* and *me-9* genes. NM43t is a nonselective (temperature sensitive) marker in *me-9*. NM331 and NM56 are *me-7* alleles.

crosses in which the *me-9* marker entered first with one and then with the other *me-7* allele.

However, the conclusions are not biased by any inequality in the direction of correction since in the first cross correction of *me-9* in the absence of exchange of flanking markers is from *me-9* (43t)$^-$ to *me-9*$^+$ whereas in the second cross it is from *me-9*$^+$ to 43t$^-$. The test system used will not identify *all* instances in which in addition to correction of heterozygosity in *me-7* to give *me-7*$^+$ there was correction of heterozygosity in *me-9*. This point will be considered in the Discussion.

If the data from all crosses are summed then among 1705 *me-7* prototrophs with parental flanking markers 43 (2·5 %) instances of coincident recombination were detected while among 300 *me-7* prototrophs with flanking markers recombined 36 (12 %) instances of coincident recombination were detected. Therefore coincident recombination as detected in this experiment is not preferentially associated with the parental combinations of flanking markers and no support is obtained

Table 2. me-7$^+$ *recombinants scored for* me-9(43t) *and flanking markers*

Cross					Classification of *me-7*$^+$ isolates				
						Parental		Recombinant	
					me-9 genotype	*thi*$^-$*wc*$^+$	*thi*$^+$*wc*$^-$	*thi*$^-$*wc*$^-$	*thi*$^+$*wc*$^+$
thi	*me-7*(56)	+	+	+	+	245	9*	6*	74
+	+	*me-7*(331)	*me-9*(43t)	*wc*	−	0	523	15	6*
Frequency of coincident recombination (total = 21/878 = 2·4 %)						9/777 = 1·2 %		12/101 = 11·9 %	
+	+	*me-7*(271)	+	*wc*	+	0	291	12	7*
thi	*me-7*(331)	+	*me-9*(43t)	+	−	78	16*	0	44
Frequency of coincident recombination (total = 23/448 = 5·1 %)						16/385 = 4·2 %		7/63 = 11·6 %	

* Scored as instances of coincident recombination (on a multiple exchange hypothesis an exchange would be required between *me-9* and *wc*).

for the type of structure shown in Fig. 2, but it must be emphasized that a 'reverse crossover' could be sufficiently short that it would not include the *me-9* marker. The data are most readily understood if coincident recombination results from hybrid DNA spreading into the *me-7* gene from a point of origin to the right of the *me-9* marker.

Lack of coincident recombination for ars-1 *and* me-7. Since coincident recombination was demonstrated for *me-7* and its closest neighbour to the right an attempt was made to detect coincident recombination for *me-7* and its closest neighbour to the left. *ars-1* was shown to be less than one crossover unit to the left of *me-7* and crosses were analysed in which recombination between *me-7* alleles was selected and *ars-1* was used as a non-selective marker in addition to the flanking markers. The

me-7 alleles used were located in the left-hand end of the *me-7* gene (i.e. the end closer to *ars-1*). No evidence for coincident recombination was found.

(v) *Coincident recombination and map order*

The data in Table 1 were used to order the *me-7* and *me-9* genes with respect to each other and their flanking markers. In each cross the more frequent class of prototrophs with flanking markers recombined is consistent with the order *thi me-7 me-9 wc*. Similar data (Murray, 1969) from crosses between *me-7* alleles were used to construct the map shown in Fig. 1.

The finding that recombinational events between *me-7* alleles may include the *me-9* marker provides two novel ways of ordering the *me-7* alleles with respect to *me-9*. First, one would predict that the frequency of coincident recombination would increase the closer the selected exchange is to the *me-9* gene. The data in Table 2 indicate a coincident recombination frequency of only 2 % for a cross of *me-7* (NM 56) × *me-7* (NM 331) *me-9* (NM 43t) but of 5 % for a cross of *me-7* (NM 271) × *me-7* (NM 331) *me-9* (NM 43t). This agrees with NM 271 being closer to *me-9* than is *me-7* (NM 56). Second, it has been suggested that the excision and repair process extends over a distance (Holliday, 1968). Data supporting this view are to be found from tetrad analyses to both locus 46 of *Ascobolus* (Rossignol, 1964) and the *pan-2* locus of *Neurospora* (Case & Giles, 1964) and more recently from the extensive tetrad analyses in yeast (Fogel & Mortimer, 1969). The present data suggest that the repair process can include both *me-7* and *me-9* mutant sites and if repair is preceded by a joint excision then the *me-7* allele involved must be the one that is closer to *me-9*. For each cross in Table 2 the instances of coincident recombination associated with parental combinations of flanking markers may be explained as linked correction of adjacent sites. From these data 25 isolates can be explained by correction of adjacent sites if the map order is as postulated and none requires the alternative order. For crosses of *me-7* (NM 56) by *me-7* (NM 331) the allele NM 331 is more frequently involved and for the cross of *me-7* (NM 331) by *me-7* (NM 271) the allele NM 271 is more frequently involved. This confirms the order of these sites shown in Fig. 1, i.e. NM 56–NM 331–NM 271–*me-9*.

All the data presented to date support the same map order and when coincident recombination results from joint excision and repair then the latter criterion provides a mapping method akin to deletion mapping.

(vi) *Prototroph frequencies from* me-7 *by* me-9 *crosses*

A fourth means of ordering the *me-7* mutations with respect to each other is to use the prototroph frequencies from crosses of the *me-7* alleles by *me-9* (NM 43t). Recombination frequency is expected to increase with the physical distance. The prototrophs from such crosses have been screened to eliminate pseudowild types and the data for true wild-type recombinants are given in detail in Table 3. χ^2 tests show no heterogeneity of prototroph frequencies for any *me-7* allele but for different *me-7* alleles the prototroph frequencies are significantly different. In summary the

prototroph frequencies for crosses of *me-7* alleles by *me-9* (NM 43t) may be arranged in the following order: NM 271 > NM 73 > NM 21t or NM 56.

Clearly the map order based on the assumption that single exchanges are more frequent than apparent triple exchanges contradicts that based on the assumption that the prototroph (recombination) frequency increases as the physical distance increases. One is forced to conclude that one of these two genetic rules is violated in crosses of *me-7* by *me-9* although not in crosses between *me-7* alleles.

The *me-7 me-9* region is at present unique in that although the data suggest that there is a recombinational discontinuity within the region, crosses between markers spanning the discontinuity yield recombinants most of which are parental with

Table 3. *Prototroph frequencies from crosses of* me-9 NM 43t *by* me-7 *alleles*

Cross. Genotypes of parents							Prototrophic recombinants		
thi	*me-7*	*wc*	×	*thi*	*me-9*	*wc*	Number	Frequency per 10^5 viable spores	Weighted means and standard errors
−	271	+	×	+	43t	−	187	21·62	23·29 ± 1·37
+	271	−	×	−	43t	+	98	26·38	
+	271	+	×	−	43t	−	15	24·00	
−	73	+	×	+	43t	−	111	16·69	19·44 ± 1·03
+	73	−	×	−	43t	+	308	20·14	
+	73	−	×	−	43t	+	52	22·13	
+	73	+	×	−	43t	−	57	18·59	
+	21	−	×	−	43t	+	102	11·52	11·52
−	56	+	×	+	43t	−	108	10·27	10·68 ± 0·65
+	56	−	×	−	43t	+	77	8·51	
+	56	−	×	−	43t	+	48	11·57	
+	56	+	×	−	43t	−	15	10·87	

NOTE. The map order is *thi* 56 21t 73 271 43t *wc*.

respect to flanking markers. This implies that the selected recombinants are *not* predominantly the result of crossover events completed between the mutant sites, but rather that recombination is predominantly by conversion, i.e. correction of heterozygous regions within the hybrid DNA. Essentially therefore the prototroph frequencies reflect the sums of the conversion frequencies of the two mutations and the pronounced polarity of conversion frequency within *me-7* is assumed to be responsible for the anomalous prototroph frequencies.

Prototroph frequencies for *me-7* allelic crosses are generally below 10 per 10^5 viable ascospores (although no figure is available for the extreme markers) whereas prototroph frequencies from crosses of *me-7* by *me-9* are between 10 and 25 per 10^5 viable ascospores. This is explained if hybrid DNA is initiated at the discontinuity between the known *me-7* and *me-9* markers and spreads to the left to include one or both *me-7* alleles. In *me-7* by *me-7* crosses where the hybrid DNA

may include both *me-7* sites, both mismatched regions may be excised and corrected together but such an event will not produce a prototroph. Many of the recombinational events will therefore remain undetected in random-spore analyses. This restriction will not be present in *me-7* by *me-9* crosses except for those infrequent events initiated to the right of *me-9* which proceed to the left and include both the *me-9* and the *me-7* sites.

4. DISCUSSION

Other possible examples of contiguous genes that have been used in recombination analyses are the *cys-1* and *cys-2* loci of *Neurospora* and the *ad-9* and *paba-1* loci of *Aspergillus nidulans*. In the former example no evidence was found for recombination events spanning sites within the two loci (Murray, 1965; Stadler & Towe, 1968). In the case of the *ad-9* and *paba-1* genes, Putrament (1967) has used mitotic recombination to test for coincident recombination. Evidence was obtained that a recombination event can extend from one gene into the other. Paszewski (1967) has also presented evidence for conversion events including nonallelic but closely-linked genes.

The data in the present paper support the concept of the *me-7 me-9* region as two contiguous genes with a recombinational discontinuity located within the region, possibly between the genes. Crosses either between *me-7* alleles (Murray, 1969) or of *me-7* by *me-9* (i.e. across the discontinuity) are remarkable in that the majority of the prototrophs have parental combinations of flanking markers. In summary, recombinational events initiated within this region are rare (i.e. recombination frequency is low) and furthermore the majority of these events do not lead to recombination of the flanking markers. The analyses of coincident recombination show that an appreciable proportion of those recombinational events that are associated with exchange of flanking markers span an *me-9* marker and could be initiated to the right of this marker. The simplest assumption on a hybrid-DNA model is that coincident recombination results when a region of hybrid-DNA extends from a neighbouring discontinuity through *me-9* to include at least one *me-7* mutation.

A closer analysis of the data in Table 2 reveals that the detected instances of coincident recombination associated with parental combinations of flanking markers require correction of the *me-9* allele from the same polynucleotide chain as is involved in the correction of the *me-7* allele, i.e. they could result from joint excision of the *me-7* (271) and *me-9* ($43t^+$) strand followed by repair. In contrast the detected instances of coincident recombination that have the more frequent class of exchanged flanking markers require independent excision and correction of the two heteroduplex sites—linked correction of 271 to 271^+ and $43t^+$ to $43t^-$ will not be distinguishable from a cross-over event initiated between the *me-7* and *me-9* alleles in which correction of only 271 to 271^+ is required. In the cross of *me-7* (331) *me-9* (43t) by *me-7* (271) 11·1 % of the *me-7*$^+$ recombinants with flanking markers exchanged were identified as instances of coincident recombination (see Table 2), but many more instances of coincident recombination may have gone undetected,

i.e. both those in which there was independent excision and correction from the same polynucleotide chain and those in which there was linked excision and correction would be scored as events confined to the *me-7* gene.

Most of the recombination events in the *me-7 me-9* region are not associated with crossing over of flanking markers and, of those that are, an appreciable frequency may be initiated to the right of *me-9* and involve a hybrid region that spans both genes. This prompts the question of whether there has been a selective advantage acting to minimize crossing over in the *me-7 me-9* region.

This region is involved in at least two steps in the biosynthetic pathway of methionine and it would be of interest to know whether there is a selective advantage in evolution to maintaining the parental combinations of the *me-7* and *me-9* genes (see Stahl & Murray, 1966).

An alternative explanation for the excess of parentally marked recombinants might be proximity to the centromere. *me-7* is very close to a centromere and if an appropriate chromosomal rearrangement were available to move the locus to a position remote from a centromere then a direct test of this explanation would be possible.

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