FINE STRUCTURE ANALYSIS AT THE *td* LOCUS OF NEUROSPORA CRASSA

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WITHIN the past several years, genetic fine structure analyses have been extended to a number of loci in both phage and bacteria (DEMEREC, GOLDMAN and LAHR 1958; YANOFSKY and LENNOX 1959; HARTMAN, LOPER and SERMAN 1960; BENZER 1961; BALBINDER 1962). A number of loci have also been used for fine structure genetic analysis in Neurospora (CASE and GILES 1958; DESERRES 1958; MURRAY 1960; ISHIKAWA 1962; WOODWARD 1962). From these studies and those cited above, we see the gene to be composed of a large number of elementary units, arranged in a linear sequence.

Previous communications (BONNER, SUYAMA and DEMOSS 1960; SUYAMA 1960; YANOFSKY 1960; LACY and BONNER 1961; SUYAMA, LACY and BONNER 1964) have discussed the effects of mutations at the *td* locus of Neurospora. Mutants are isolated because they lack the ability to convert indole glycerol phosphate to L-tryptophan. Such mutants are initially divided into two groups based upon the presence or absence of a protein serologically related to tryptophan synthetase (SUSKIND, YANOFSKY and BONNER 1955). Mutations which result in the loss of a protein serologically related to the wild-type enzyme (phenotypic designation, CRM^-) are scattered throughout the genetic map. Mutants having residual CRM activity may be further grouped on the basis of residual enzymatic activity. CRM^+ mutants having similar phenotypes have been found to occupy similar regions within the genetic map as determined by two-point crosses.

With the exception of the *rII* region of phage T4, intragenic three-point crosses have not been extensively used in genetic fine structure analyses. This is now possible as the result of our ability to isolate a series of intragenic double mutants (KAPLAN, SUYAMA, and BONNER 1963) at the *td* locus of Neurospora. These mutants have been derived from an indole utilizing mutant isolated by AHMAD and CATCHESIDE (1960) designated A78, and characterized by RACHMELER and YANOFSKY (1961), who redesignated it *td*201.

When the double mutants are used in three-point crosses, it is possible to isolate either prototrophs and/or indole utilizers. The recombinants may result from

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either a single or double crossover or both. Therefore, in comparison to other systems, we are able to isolate two crossover types from these intragenic three-point crosses.

Through the use of these double mutants we have been able to confirm and extend the observed clustering of similar CRM+ mutant phenotypes within the genetic map. Additionally, we have been able to locate, with a high degree of accuracy, those mutations responsible for a CRM- phenotype within the map of the *td* locus, heretofore a difficulty (SUYAMA, LACY and BONNER 1964). The use of intragenic three-point crosses has also enabled us to observe levels of negative interference previously unobserved.

MATERIALS AND METHODS

Strains: All of the mutants used in this study have been described elsewhere (SUYAMA, LACY and BONNER 1964). Prior to use in these experiments *td*201 and *td*2 were backcrossed to the ST. LAWRENCE wild type. The origin of the double mutants has been described in detail (KAPLAN, SUYAMA and BONNER 1963). The double mutants were isolated from the indole utilizing point mutant *td*201 (original designation A78) after ultraviolet irradiation and selection. Strains specifically requiring L-tryptophan for growth, in contrast to the parental strain *td*201 which could grow on either tryptophan or indole supplemented media, were designated as presumptive double mutants. The second mutations of the double mutants were placed in the genetic map by crossing the presumptive double mutants to a standard *td* marker allele and determining the proportion and type (prototroph and/or indole utilizer) of recombinants. In this manner, the newly introduced mutations of the double mutants were positioned in the genetic map relative to the parental strain *td*201 and the *td* marker used. The double mutants have been designated 78-101, 78-109, 78-110, 78-137, and 78-174. The prefix 78 is after the original designation of the *td*201 mutation (AHMAD and CATCHESIDE 1960). The second mutations of the double mutants, 101, 109, 110, 137 and 174 have not been prefixed by *td* when cited in the text; this has been done to distinguish these markers from those primary *td* isolates of independent origin, which *have* been prefixed by *td*, e.g., *td*101. Table 1 is a summary of the mutants used in this study. The table indicates which mutants are CRM- and CRM+. The CRM+ mutants have been further described with respect to the nature of the tryptophan synthetase activity remaining. The table also lists the types of revertants obtainable from each mutant.

Media and crossing: Mutants were maintained on slants of the minimal medium of VOGEL (1956) supplemented with 150 μg L-tryptophan per ml.

Strains used as protoperithecial parents were inoculated onto crossing medium: 2 percent corn meal agar (Difco) supplemented with 0.2 percent dextrose and 500 μg L-tryptophan per ml. After five days incubation at 25°C a 0.5 ml suspension of the conidial parent was introduced over the surface of the agar layer. All crosses were performed reciprocally.

After four weeks at 25°C the liberated ascospores were suspended in 5 ml of VOGEL minimal medium (1956) supplemented with 0.05 per cent dextrose, 1 percent sorbose, and when needed, 80 μg indole per ml. The suspension was added to 25 ml of molten agar medium (1.5 percent Difco agar) and held at 60°C for 35 min to effect heat shocking of the ascospores. Five aliquots of 5 ml each were then overlaid on Petri plates containing 20 ml of the same medium.

After 48 hours incubation at 30°C the plates were scored for recombinants. When indole utilizing recombinants could be obtained, the ascospore suspension was plated on indole supplemented medium; otherwise minimal medium was used. Colonies from indole plates were transferred to minimal medium and the number of prototrophs determined. The number of indole utilizing recombinants was obtained by difference. Selfings were made with more than one half of the strains used. No colonies were ever observed under such conditions. When pseudowild types (MITCHELL, PITTENGER and MITCHELL 1952) occurred, they were clearly distinguishable

TABLE 1

Mutant strains used in this investigation

Strain	Cross reacting material	Reaction catalyzed 1 2 3	Revert to: wild type	Revert to: indole utilizer
*td*201	+	2	+	—
*td*3	+	.	+	—
*td*2	+	3	+	—
*td*100	+	3	+	—
*td*101	+	3	+	—
*td*6	+	.	+	—
*td*99	+	3	+	—
*td*97	+	3	+	—
*td*102	+	3	+	—
*td*133	—	.	—	+
*td*48	—	.	+	+
*td*132R	+	2	+	—
*td*140	—	.	+	—
*td*37	—	.	+	+
*td*138	—	.	+	+
*td*128	—	.	+	—
*td*139	—	.	+	—
*td*1	—	.	+	—
*td*120	—	.	—	—
Double mutants:				
78–101†	—	.	—	+
78–109	—	.	—	+
78–110	—	.	—	+
78–137	+	.	—	+
78–174	—	.	—	+

Reaction (1) Indole glycerol phosphate→ tryptophan
Reaction (2) Indole→ tryptophan
Reaction (3) Indole glycerol phosphate→ indole
† *td*201 and 78 are synonymous. 101 is the second component of the double mutant 78–101 and should not be confused with *td*101, which is an independent point mutant.

from true recombinants by the morphology of their growth (LACY 1959). Pseudowilds did not appear until 24 to 48 hours after the recombinants.

The total number of plated spores was used in determining the recombination frequency. To determine the number of ascospores, two 0.25 cm^2 areas were counted on each plate of a cross, and the total number of plated spores was calculated. Viability was estimated from the number of germinated and nongerminated ascospores in a total of 300 counted spores. The $t = 0.05$ confidence limits for two-point crosses are ± 40 percent and for three-point crosses ± 25 percent.

RESULTS

Table 2 lists the results obtained in a series of two-point crosses involving a number of CRM^+ *td* mutants. These crosses have been shown diagrammatically in Figure 1. When the data are examined, it can be seen that the mutants fall into two clusters relative to *td*201 (SUYAMA, LACY and BONNER 1964). One cluster is situated between 0.020 and 0.030 percent recombination (prototroph frequency) from *td*201. This cluster consists of mutants *td*2, *td*3, *td*100, *td*101 and *td*6. The

TABLE 2

CRM+ × CRM+ crosses

Cross	Prototroph frequency*	Spores × 10^3	Wild-type Recombinants	Percent viability
†*td*201 × *td*3	.019	55	11	71
*td*201 × *td*2	.019	113	21	75
*td*201 × *td*100	.019	248	47	74
*td*201 × *td*101	.027	62	17	70
*td*201 × *td*6	.047	147	69	64
*td*201 × *td*99	.045	170	76	75
*td*201 × *td*97	.060	62	39	93
*td*201 × *td*102	.060	61	36	65
*td*100 × *td*3	.001	71	1	53
*td*100 × *td*99	.035	71	25	56
*td*100 × *td*97	.038	40	15	75
*td*100 × *td*2	. . .	. .	. .	. .
*td*100 × *td*101	<.002	44	0	48
*td*100 × *td*6	.004	26	1	52
*td*100 × *td*102	.039	15	6	58
*td*101 × *td*97	.026	23	6	89
*td*101 × *td*2	<.002	64	0	68
*td*101 × *td*99	.021	19	4	71
*td*101 × *td*6	.006	31	2	48
*td*101 × *td*102	.028	18	5	51
*td*101 × *td*3	<.004	25	0	70
*td*6 × *td*102	.068	50	34	51
*td*6 × *td*3	<.006	18	0	64
*td*6 × *td*97	.035	23	8	90
*td*2 × *td*97	.006	72	4	85
*td*2 × *td*99	.004	53	2	60
*td*2 × *td*6	<.001	87	0	54
*td*2 × *td*102	.007	29	2	60
*td*2 × *td*3	.002	60	1	67
*td*102 × *td*3	.040	40	16	60
*td*102 × *td*97	<.003	37	0	97
*td*3 × *td*97	.036	55	20	91
*td*99 × *td*97	.012	171	21	88
*td*99 × *td*102	.017	100	17	63

* Based on results pooled from reciprocal crosses.
† Redesignation for the original isolate A78.

second cluster consists of mutants *td*99, *td*97 and *td*102 lying between 0.045 and 0.070 percent recombination from *td*201. Within the clusters, the designated order of mutational sites fits the data well. Two exceptions are noted when strains *td*2 or *td*6 are used. In many crosses, *td*2 gives very low recombination frequencies. On the other hand, *td*6 gives a high recombination frequency with nearly all other markers. The ambiguities associated with *td*2 may be due to the fact that it has been derived from a different wild type, in spite of having been extensively backcrossed.

Since the above ordering of mutational sites was based upon a series of two-

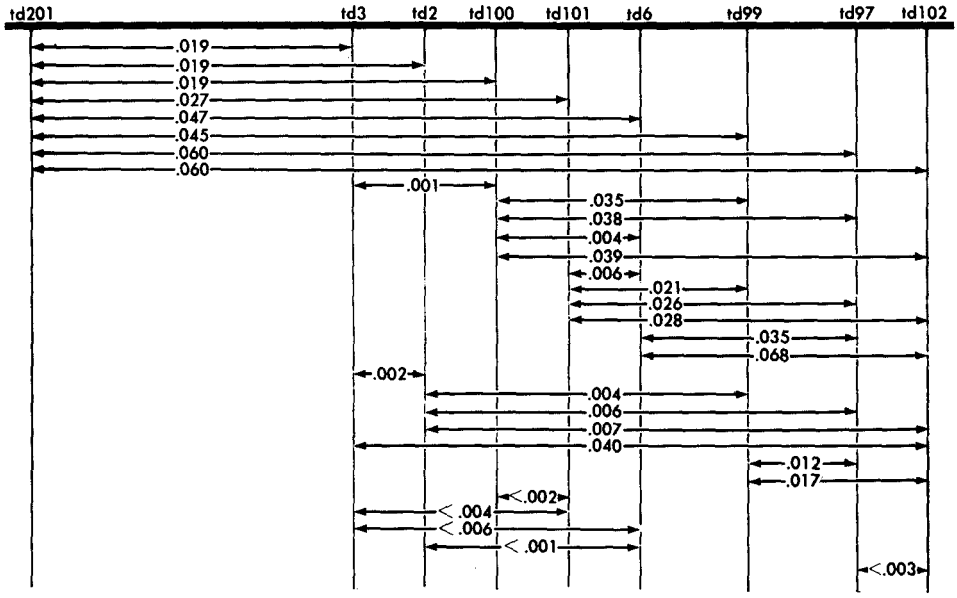

FIGURE 1.—Results of CRM+ × CRM+ crosses. *td* markers have been listed above the line. Prototroph frequencies between markers have been listed by arrows.

point crosses, it was felt that a higher degree of reliability could be placed upon the order and clusters observed by employing these mutants in a series of three-point crosses. For positioning of the double mutants see KAPLAN, SUYAMA and BONNER (1963).

Table 3 and Figure 2 give the results of three-point crosses using the double mutants and the above listed markers. First, it is seen that the clustering effect noted in two-point crosses is maintained. The general order of markers within each cluster is similar to that previously observed, although there is some indication that certain orders may be inverted, e.g., *td*100 and *td*101, *td*97 and *td*102. As noted previously, *td*2 and *td*6 show a tendency towards low and high recombination frequencies, respectively, but retain their approximate position within the genetic map.

Two further features of these three-point crosses are apparent. The distance measured from *td*201 to markers to the right is enlarged in certain crosses. This increase in recombination frequency is generally by a factor of two, when these crosses are compared to the previous set. In most instances, the recombination frequencies are very much like those observed for two-factor crosses.

Upon examination of crosses involving double mutants 78-110 and 78-137, it may be seen that a number of double crossover types are observed. With 78-110, the double-crossover class is an indole utilizer, and with 78-137 this class is wild-type. The last column of Tables 3 and 5 give the calculated frequency of double crossovers. As seen, values from 30 to 500 times the calculated frequency are observed.

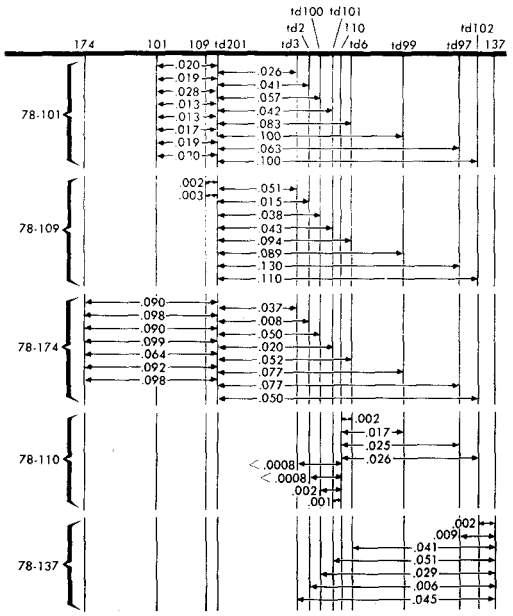

FIGURE 2.—Results of CRM$^+$ × double mutants. *td* markers have been listed above the line and prefixed by *td*. Double mutant markers have not been prefixed with *td*. Prototroph frequencis between markers have been listed between arrows. It should be remembered that *td*201 and 78 are synonymous.

Table 4 gives the results of double mutant × double mutant crosses. The values bear out the conclusion that each mutation of the double mutants maps as a point and, from the values listed in Table 3, in a predictable fashion. The validity of the three-point crosses is also justified because the recombinant type from a double mutant × double mutant cross is an indole utilizer; whereas the overall distance from the previous set of three-point crosses is derived from both wild-type and indole utilizing recombinants.

The double mutants were crossed to a number of CRM$^-$ primary isolates in the last series of crosses. As has been previously reported (KAPLAN, SUYUMA and BONNER 1963) CRM$^-$ mutants map as extended mutations or multisite lesions in two-point crosses. It was of interest to determine whether or not three-point crosses would be more effective in determining the extent of the mutation occurring in CRM$^-$ mutants. Table 5 and Figure 3 give the results of three-factor crosses involving primary CRM$^-$ isolates and, in the case of *td*132R, an indole utilizing revertant of the CRM$^-$ strain, *td*132.

From these data, it can be concluded that in three-point crosses, the CRM$^-$ mutants may be mapped without difficulty. Each CRM$^-$ mutant used in the present study can be placed as a point mutation and each may be placed relative to all of the markers used. We have listed *td*1 and *td*133, and *td*139 and *td*140 as separate points. Mutants *td*1 and *td*133 show different reversion properties (see Table 1).

TABLE 3

Crosses of CRM+ × double mutant

Cross	Prototroph frequency‡	Spores $\times 10^3$	Colonies	Percent viability	Wild type	Indole utilizer	Frequency wild type	Frequency indole utilizer	Calculated frequency of double
†78–101 × *td*3	.045	143	65	22	38	27	.026	.019	
78–101 × *td*2	.061	15	9	42	6	3	.041	.020	
78–101 × *td*100	.085	163	138	50	92	46	.057	.028	
78–101 × *td*101	.056	83	46	39	35	11	.042	.013	
78–101 × *td*6	.096	82	79	20	68	11	.083	.013	
78–101 × *td*99	.117	65	76	34	65	11	.100	.017	
78–101 × *td*97	.083	87	72	39	56	16	.063	.019	
78–101 × *td*102	.120	168	202	53	169	33	.100	.020	
78–109 × *td*3	.051	238	121	25	121	0	.051	. . .	
78–109 × *td*2	.015	19	3	44	3	0	.015	. . .	
78–109 × *td*100	.040	104	44	10	42	2	.038	.002	
78–109 × *td*101	.046	37	17	52	16	1	.043	.003	
78–109 × *td*6	.094	158	149	18	149	0	.094	. . .	
78–109 × *td*99	.089	64	57	49	57	0	.089	. . .	
78–109 × *td*97	.129	21	27	50	27	0	.129	. . .	
78–109 × *td*102	.113	111	126	20	126	0	.113	. . .	
78–174 × *td*3	.135	115	155	72	42	113	.037	.098	
78–174 × *td*2	.015	182	27	38	14	13	.008	.007	
78–174 × *td*100	.140	109	153	40	55	98	.050	.090	
78–174 × *td*101	.119	55	65	51	10	55	.020	.099	
78–174 × *td*6	.116	185	214	49	96	118	.052	.064	
78–174 × *td*99	.169	82	138	60	63	75	.077	.092	
78–174 × *td*97	.175	187	327	83	144	183	.077	.098	
78–174 × *td*102	.140	109	153	40	55	98	.050	.090	
78–110 × *td*3	<.0008	130	0	22	0	0	. . .	. . .	. . . .
78–110 × *td*2	<.0008	127	0	47	0	0	. . .	. . .	. . . .
78–110 × *td*100	<.002	63	0	12	0	0	. . .	. . .	. . . .
78–110 × *td*101	<.001	90	0	36	0	0	. . .	. . .	. . . .
78–110 × *td*6	.003	130	4	17	3	1	.0023	**.0007***	1.2×10^{-6}
78–110 × *td*99	.020	41	8	50	7	1	.017	**.003**	1×10^{-5}
78–110 × *td*97	.038	8	3	41	2	1	.025	**.013**	2.2×10^{-5}
78–110 × *td*102	.026	51	13	20	13	0	.026	. . .	. . . .
78–137 × *td*3	.045	192	86	70	1	85	**.0005**	.0445	1.6×10^{-5}
78–137 × *td*2	.006	128	8	30	1	7	**.0008**	.006	8×10^{-6}
78–137 × *td*100	.034	48	16	30	2	14	**.005**	.029	1.6×10^{-5}
78–137 × *td*101	.053	55	29	60	1	28	**.002**	.051	1.5×10^{-5}
78–137 × *td*6	.046	126	58	69	6	52	**.005**	.041	2.5×10^{-5}
78–137 × *td*97	.009	106	10	82	0	10	. . .	.009	. . . .
78–137 × *td*102	.002	145	3	40	0	3	. . .	.002	. . . .

* Values in bold face are frequencies for observed double crossovers.
† The 78 allele is synonymous with *td*201.
‡ Results pooled from reciprocal crosses.

Strains *td*139 and *td*140 appear to represent different points but the evidence is not conclusive.

Mutant *td*120 in three-point crosses, as in two-point crosses, maps as a deletion. Unlike all the other CRM⁻ mutants used in this study, *td*120 is the only one which

TABLE 4

Crosses of double × double

Cross	Prototroph frequency*	Spores $\times 10^3$	Indole utilizer (recombinants)	Percent viability
†78–101 × 78–109	.0198	232	46	83
78–101 × 78–137	.111	277	308	89
78–109 × 78–137	.094	385	361	86
78–101 × 78–110	.064	360	230	82
78–110 × 78–137	.044	231	102	80
78–109 × 78–110	.025	321	81	82
78–109 × 78–174	.083	352	293	76
78–137 × 78–174	.170	90	150	75
78–110 × 78–174	.150	299	436	76
78–101 × 78–174	.057	136	77	78

* Results pooled from reciprocal crosses.
† The 78 allele is synonymous with *td*201.

is not revertible in repeated attempts, suggesting that *td*120 is a deletion. In two-point crosses, *td*120 will not show recombination with any of the markers used in the previous section with the exception of *td*201. These results lead us to conclude that all of the CRM⁻ strains used (except *td*120) consist of point mutations.

It must be mentioned that in a few crosses, recombination frequencies larger than expected were obtained using certain marker combinations (e.g., 78-101 × *td*139 and *td*140). As in the three-point crosses used above, when a larger than normal recombination frequency is obtained, it is generally twice the expected frequency (based on two-point crosses).

DISCUSSION

In the present communication, the results of over 100 crosses involving mutations at the *td* locus of Neurospora have been summarized. We have shown that a linear sequence of mutational sites could be arranged on the basis of recombination frequencies alone, using two-point crosses.

Using three-point crosses, the positions of a large number of mutations have been fixed relative to markers on either side. The determination of the order of mutational sites within regions must still rely upon the relative frequencies of recombination. Yet, the results of two- and three-point crosses shown here agree. In addition the clustering effect of certain CRM⁺ types is maintained.

Previously (SUYAMA, LACY and BONNER 1964), it had been impossible to locate and order many of the mutations resulting in a CRM⁻ phenotype. The difficulties encountered in CRM⁻ two-point crosses are unexplainable. The ambiguities of such crosses involve the presence of those alleles resulting in a CRM⁻ phenotype; two-point crosses not involving CRM⁻ mutants show excellent placement of the marker alleles. When three-point crosses are used, CRM⁻ mutants no longer map as extended lesions; instead each can be placed so as to occupy a point on the genetic map. Strain *td*120 in both two-and three-point crosses maps as a deletion at

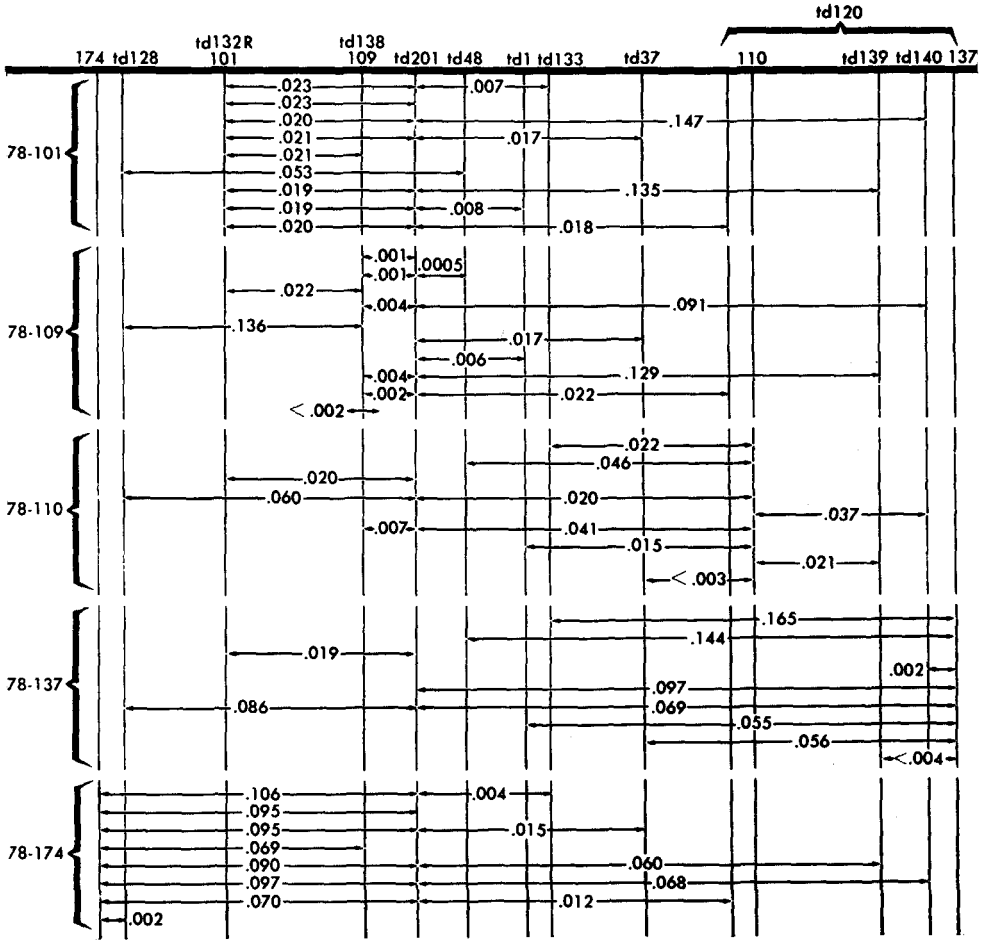

FIGURE 3.—Results of CRM⁻ × double mutants. *td* markers have been listed above the line and prefixed by *td*. Double mutant markers have not been prefixed with *td*. Prototroph frequencies between markers have been listed by arrows. It should be remembered *td*201 and 78 are synonymous.

the *td* locus extending from markers *td*3 through 137. Unlike any of the CRM⁻ mutants used in this study, *td*120 is incapable of reverting to the wild-type state. With this evidence *td*120 has been designated a deletion of approximately 25 percent of the *td* locus.

Of particular interest in the three-point crosses, and especially those CRM⁺ three-point crosses involving strains 78-101 and 78-109, is the twofold increase in recombination frequency with markers to the right of the *td*201 allele, but not to the left. This observation, taken with the evidence presented below, leads to difficulties in estimating the size of the locus and the amount of information coded therein.

The present series of three-point crosses is of particular advantage in examining

TABLE 5

Crosses of CRM⁻ × double mutant

Cross	Prototroph frequency*	Spores $\times 10^8$	Colonies	Percent viability	Wild type	Indole utilizer	Frequency wild type	Frequency indole utilizer	Calculated frequency of double
‡78–110 × *td*133	.022	297	66	53	0	66	. .	.022	
78–110 × *td*48	.046	581	268	57	0	268	. .	.046	
78–110 × *td*132R	.020	285	57	60	57	0	.02	. . .	
78–110 × *td*128	.080	30	24	73	18	6	.06	.02	
78–110 × *td*140	.037	38	14	88	14	0	.037	. . .	
78–110 × *td*138	.048	27	13	80	2	11	.007	.041	
78–110 × *td*37	<.003	32	0	71	0	0	. . .	. . .	
78–110 × *td*1	.015	26	4	73	0	4	. . .	.015	
78–110 × *td*139	.021	39	8	81	8	0	.021	. . .	
78–110 × *td*120	<.0003	359	0	90	0	0	. . .	. . .	
78–137 × *td*133	.166	148	246	51	1	245	**.0007**†	.165	1.7×10^{-5}
78–137 × *td*48	.144	134	193	57	0	193	. . .	.144	
78–137 × *td*132R	.019	345	67	60	67	0	.019	. . .	
78–137 × *td*140	.002	45	1	88	0	1	. . .	.002	
78–137 × *td*138	.097	54	52	74	0	52	. . .	.097	
78–137 × *td*128	.155	58	90	87	50	40	.086	.069	
78–137 × *td*1	.055	22	12	71	0	12	. . .	.055	
78–137 × *td*120	<.0005	211	0	82	0	0	. . .	. . .	
78–137 × *td*139	<.004	26	0	77	0	0	. . .	. . .	
78–137 × *td*37	.056	27	15	80	0	15	. . .	.056	
78–174 × *td*133	.110	415	443	61	12	431	.004	.106	
78–174 × *td*48	.095	520	493	64	0	493	. . .	.095	
78–174 × *td*132R	.00098	405	4	68	4	0	**.00098**	. . .	3×10^{-5}
78–174 × *td*37	.110	19	21	80	3	18	.015	.095	
78–174 × *td*138	.069	99	68	83	0	68	. . .	.069	
78–174 × *td*128	<.002	48	0	80	0	0	. . .	. . .	
78–174 × *td*139	.150	22	33	77	11	22	.06	.09	
78–174 × *td*140	.165	42	70	82	29	41	.068	.097	
78–174 × *td*120	.082	257	211	76	30	181	.012	.070	
78–101 × *td*133	.030	172	52	48	13	39	.007	.023	
78–101 × *td*48	.023	194	44	32	0	44	. . .	.023	
78–101 × *td*132R	<.0004	256	0	64	. .	. .	. . .	. . .	
78–101 × *td*140	.167	36	60	64	53	7	.147	.020	
78–101 × *td*37	.038	40	15	48	7	8	.017	.021	
78–101 × *td*138	.021	83	17	88	0	17	. . .	.021	
78–101 × *td*128	.053	38	20	92	20	0	.053	. . .	
78–101 × *td*139	.154	37	57	83	50	7	.135	.019	
78–101 × *td*1	.027	37	10	91	3	7	.008	.019	
78–101 × *td*120	.038	327	125	83	59	66	.018	.020	
78–109 × *td*133	.0014	511	7	48	0	7	. . .	.0014	
78–109 × *td*48	.0013	456	6	60	2	4	.0005	.0008	
78–109 × *td*132R	.022	393	88	60	88	0	.022	. . .	
78–109 × *td*140	.095	60	57	73	55	2	.091	.004	
78–109 × *td*138	<.002	61	0	82	0	0	. . .	. . .	
78–109 × *td*128	.136	43	58	77	58	0	.136	. . .	
78–109 × *td*37	.017	24	4	81	4	0	.017	. . .	
78–109 × *td*1	.006	36	2	80	2	0	.006	. . .	
78–109 × *td*139	.133	51	68	77	66	2	.129	.004	
78–109 × *td*120	.024	472	114	81	105	9	.022	.002	

* Results pooled from reciprocal crosses.
† Values in bold face are observed double crossover frequencies.
‡ The 78 allele is synonymous with *td*201.

the phenomenon of high localized negative interference. Contrasted to a number of systems currently under investigation, the present system has enabled us to study high localized negative interference entirely within the confines of a single gene.

In crosses giving rise to only one double crossover it was necessary to rule out the possibility of a reversion. (1) Each colony was shown to have arisen from a single ascospore. (2) The parental strains were selfed. No colonies resembling the double crossover classes obtained were ever observed. (3) Each isolate was tested for mating type. Of the 16 wild-type isolates, ten were *A* mating type, and six were *a* mating type. Of the three indole utilizing isolates, two were *A* mating type, and one was *a* mating type.

Although the occurrence of double crossovers was rare, it can be seen that the observed number of double crossovers occurs at a frequency 30 to 500 times the expected frequency. This is similar to the results observed with phage by CHASE and DOERMANN (1958); in Salmonella by DEMEREC, GOLDMAN and LAHR (1958); in Aspergillus by PRITCHARD (1960) and in Neurospora by MURRAY (1960, 1963). However, unlike most systems, the double-crossover type selected for in this instance may be either a mutant or wild type, and either class may occur with equal frequency. DEMEREC, GOLDMAN and LAHR (1958) have suggested the possibility that by a copy-choice mechanism, the wild-type allele may be copied in preference to the mutant form of the allele.

The literature pertaining to high localized negative interference is abundant. The phenomena of negative interference and nonreciprocal recombination appear to involve a similar mechanism dependent upon an apparent reciprocal exchange (MITCHELL 1955; DESERRES 1958, CASE and GILES 1958; FREESE 1959) of outside markers.

PRITCHARD (1960; see also SIDDIQI 1962) has proposed a model which can account for both phenomena. By this model a nonsynchronous switch during replication of the DNA would result in a 3:1 ratio for the allele under observation and still be recombinant for the outside markers. Multiple switches would be responsible for high negative interference.

Evidence recently accumulated for phage (MESELSON and WEIGLE 1961; KELLENBERGER, ZICHICHI and WEIGLE 1961) and for bacteria (LACKS 1962) has shown that the recombination of markers may involve the physical exchange of homologous genetic material. The model proposed by PRITCHARD does not accommodate these data.

The physical exchange of genetic material accompanied by a small amount of DNA replication may also explain both gene conversion and high negative interference. If chromatid breaks involve breaks within DNA molecules, then requisite to the annealing of nonsister or sister chromatid breaks may well be a small amount of DNA synthesis (TAYLOR, HAUT and TUNG 1962) covering the region of the break. The broken ends of DNA strands would act as the trigger for DNA synthesis (RICHARDSON, SCHILDKRAUT, APOSHIAN, KORNBERG, BODMER and LEDERBERG 1963) and thus complete the healing of broken ends. Owing to the proximity of strands during exchange, either strand could be used as template for DNA synthesis. This process could then lead to the loss of an allele or an apparent ex-

cess of multiple exchanges. Such a mechanism may also be used to explain the increased recombination frequencies observed in ultraviolet-irradiated phage crosses and the induction of prophage in lysogenic cultures of bacteria by assuming ultraviolet to cause breaks in the DNA which trigger its synthesis.

The occurrence of double-crossover types in crosses involving double mutants 78-110 and 78-137 illustrate one further point of interest in connection with the miscopying of alleles during the exchange process. Our observations are consistent with the premise that the alleles present in a cross determine which strand is copied during the healing of broken strands. Those crosses involving double mutant 78-110 indicate that the ratio of observed to expected indole-utilizing double-crossover types remains constant despite the use of different *td* alleles as markers. The converse is true in crosses of 78-137 where the ratio of oberved to expected prototrophs varies with the *td* marker used. In the former instance, we would imagine the 110 allele to be miscopied, and in the latter case the *td*-marker alleles are miscopied.

Figure 4 is a map of the *td* locus drawn to scale with each of the mutants used in this study included. Prior to this investigation, no mutants from the nearly 200 *td* mutants isolated in this laboratory were found which mapped to the left of *td*201. The isolation of double mutants 78-109, 78-101, and in particular 78-174 showed that not only a region of the locus existed to the left of *td*201, but in fact its existence more than doubled the previous size of the *td* locus. Only through careful analyses were we able to isolate the three independent point mutants (*td*128, *td*132 and *td*138) also occurring within this region. This type of result may indicate a basic difficulty in arriving at an accurate estimate of gene size by mutation.

This scale drawing also illustrates the tendency of mutant isolates to map in clusters. Such clustering of mutational sites has been found by SIDDIQI (1962) in the *paba*$_1$ region of Aspergillus and by YANOFSKY and LENNOX (1959) for the *A* gene of *E. coli*. In the present system, the clustering of CRM$^+$ mutants is understandable since these probably reflect those sites on the peptide chain involved in catalysis. It remains to be determined whether a similar effect will be observed for CRM$^-$ mutants.

By isolating intragenic double or triple mutants, it may be possible to fill in the large void of mutational sites between *td*201 and 174.

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FIGURE 4.—Map of *td* region, drawn to scale. The position of each marker within the scale drawing has been derived from the prototroph frequencies given in the text.

SUMMARY

Recombination data have been obtained from over 100 crosses of *td* mutants. Previous fine structure genetic analyses at this locus have been based solely on two-point crosses owing to the lack of closely linked outside markers. By isolating a series of multisite mutants at the *td* locus we have been able to compare the results obtained from two- and three-point crosses. The results obtained from two- and three-point crosses employing CRM^+ marker mutations agree. For CRM^- marker mutations the two sets of crossing data do not agree.

Interference was highly negative with particular combinations of markers.

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