"SELFING" AND OTHER FORMS OF ABERRANT RECOMBINATION IN ISOLEUCINE-VALINE MUTANTS OF NEUROSPORA¹

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THE appearance of wild-type segregants from material homozygous for certain mutant alleles, termed "selfing" by DEMEREC (1962), has been reported on several occasions. A number of mechanisms have been invoked, in different systems, to explain such occurrences. Apparent reversion in Bar homozygotes in *Drosophila* was found by STURTEVANT (1925) to result from unequal crossing over. The Bar eye mutation represents a tandem repeat within the genetic material, and crossing over involving nonhomologous breakpoints can occasionally restore the normal unduplicated condition. Several further cases of this nature have subsequently come to light (LEWIS 1941; SCHULTZ, in MORGAN, SCHULTZ and CURRY 1941; LAUGHAN 1949; JUDD 1964). The occurrence of apparent spontaneous reversions in homozygous mutants of yeast was shown by MAGNI and VON BORSTEL (1962) to be several times more frequent at meiosis than at mitosis. The proposed interpretation (MAGNI 1963) resembles that of STURTEVANT in invoking unequal crossing over. Here, however, the mutations undergoing such apparent reversion are thought to represent additions or deletions of single nucleotides and to exert their phenotypic effect principally by throwing out of phase the reading of the triplet code by which nucleotide sequence information is translated into protein amino acid sequence. Unequal crossing over can presumably restore to the affected triplet its normal complement of three nucleotides, and thus reinstate normal reading of the code. Such a mechanism was considered also by DEMEREC (1962) for "selfing" observed as homologous phage transduction in *Salmonella*. More recent, unexpected, findings have led him (DEMEREC 1963) to propose a quite different mechanism. According to this hypothesis, an undefined instability is induced in certain alleles by some aspect of the transduction process, and the wild types observed are the products of reverse mutation.

KIRITANI (1962) reported the occasional occurrence of the "selfing" phenomenon among a large group of crosses between a series of isoleucine-valine (*iv*) mutants of *Neurospora*. In addition, in a small number of crosses involving non-identical alleles, prototrophs were encountered at frequencies in excess of 5%,

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despite the fact that the bulk of the data clearly indicated close linkage of all the alleles studied. Both the excessive apparent recombination between alleles and the "selfing" behavior were restricted to crosses in which at least one allele of a particular group, designated group 2, was involved. In both cases, the anomalous prototrophs represented predominantly a single recombinant type with respect to the outside markers. Lastly, evidence was obtained that these exceptional results could not be ascribed to the formation of disomics (pseudowild types; MITCHELL, PITTINGER, and MITCHELL 1952).

In the present paper, further studies on this system are described. Analysis of ordered tetrads has led the authors to favor interpretations based on unequal crossing over, though further study is needed before sound conclusions as to mechanism will be possible.

MATERIALS AND METHODS

The following *iv* mutants were used in this study: Group 1—T319, T322, T324; Group 2—T304, T311, T318, 16117; Group 3—T327, T328, T329. Mutants of Groups 2 and 3 are leaky, i.e., grow slowly in the absence of isoleucine and valine. Mutants of Group 1 are not. Other strains employed were lysine-1 (33933) (*lys*) and spray (B132) (*sp*). Mutants T304 through T329 (*Tiv* mutants) were derived in this laboratory following ultraviolet (UV) irradiation of the wild-type strain EMERSON 5256A by WAGNER, SOMERS, and BERGQUIST (1960). Mutant 16117 was described by BONNER, TATUM, and BEADLE (1943), and was obtained by X-irradiation of a standard wild-type strain.

Mutants T304 through T329 represent a group of 19 *Tiv* alleles that, together with 16117, were studied by KIRITANI (1962). He found them all to be closely linked and to lie in linkage group V between the loci of lysine-1 and spray, the region in which 16117 had been previously placed by the data of STRICKLAND, PERKINS, and VEATCH (1959). The locus of the *Tiv* mutations was then mapped by KIRITANI (1962) through the use of strains marked with one or the other of the linked markers, lysine-1 and spray. On the basis of recombination frequencies and outside-marker asymmetries, three groups were defined as shown above. Ordering of mutant sites within groups was not possible, as clear asymmetries were not obtained. Map relations are shown in Figure 1. The lysine marker lies quite close to the centromere. The mutant groups defined by genetic mapping correspond well to those indicated by biochemical and growth-response studies (WAGNER, KIRITANI, and BERGQUIST 1962; WAGNER, BERGQUIST, BARBEE, and KIRITANI 1964).

All crosses were performed on the crossing medium of WESTERGAARD and MITCHELL (1947) adjusted to pH 6.5 and supplemented with 50 μ g/ml of the L isomers of lysine, isoleucine, and valine. Crosses were incubated at 24°C for three to four weeks. The percentage of wild types among germinated random spores was determined by the plating method of NEWMAYER (1954), using the minimal medium of VOGEL (1956). These values were not doubled, but are presented in the tables simply as percent *iv*⁺ prototrophs among total viable spores tested.

Dissection of asci was performed upon 6% agar using fine glass needles. The spores were allowed to germinate on minimal agar containing lysine, isoleucine, valine, 2% sorbose, 0.05% glucose, and 0.05% fructose (BROCKMAN and DESERRES 1963).

FIGURE 1.—Spatial arrangement of isoleucine-valine and marker loci and of mutant groups within the *iv* locus. Figures represent percent recombination, and each is an average of many measurements.

Isolates were tested for growth capabilities by transfer to minimal agar plates lacking the appropriate amino acids. They were tested for leakiness by incubation for 12 days in minimal liquid containing lysine (20 ml per 75 ml flask at 24°C).

Ability of an *iv* strain to produce reversions in response to UV was tested as follows. Distilled water suspensions of conidia were prepared from 7-day-old cultures growing on complete agar. The conidia were filtered and washed, using Millipore membrane filters. They were then soaked in 2% yeast extract for 60 minutes at 24°C and irradiated in distilled water. Following a 2-hour soak in 2% yeast extract, the material was washed, suspended, and diluted in distilled water. Aliquots were then spread on the surface of lysine minimal agar containing 2% sorbose, 0.05% glucose, and 0.05% fructose, with and without isoleucine and valine.

RESULTS

Occurrence of unexpected isoleucine-valine prototrophs: The data in Table 1 are primarily from crosses involving Group 2 strains and are quite comparable to the corresponding data of KIRITANI (1962). Unexpected prototrophs were encountered in at least 13 of the crosses, one of them a selfing cross (see also Table 2). All classes of crosses involving Group 2 mutants were represented among the aberrant results: 2×1 , 2×2 , and 2×3 . Among the Group $2 \times$ Group 1 crosses, where only moderately close linkage is indicated, it is not possible to separate clearly one class of crosses as presenting elevated percentages of apparent recombination. Elsewhere, where the indicated linkage is quite close, the aberrant crosses stand out as a discrete class.

A considerable number of crosses have been performed using isolates requiring isoleucine plus valine from certain of the anomalous crosses. These have been crossed to each other, to the parent strains, and to other *iv* strains. Representative results, presented in Table 3, reveal that elevated recombination frequencies are of very common occurrence among such crosses, including simple backcrosses (crosses 130, 132, 141–225).

Factors determining the appearance of unexpected prototrophs: Several obser-

TABLE 1
*Crosses of isoleucine-valine mutant strains, grouped according
to percent apparent recombination*

<i>iv</i> groups represented	Nature of result	Number of crosses	Percent <i>iv</i> ⁺ ascospores
2×1	Typical	11	0.45 — 4.8
	Aberrant	5	7.25 — 15.8
2×3	Typical	17	0.003 — 0.06
	Aberrant	4	7.1 — 10.0
2×2	Typical	10	0.00 — 0.005
	Aberrant	3	9.25 — 13.5
2×2 (selfing)	Typical	7	0.0 — 0.000
	Aberrant	1	1.35 —
3×3 (selfing)	Typical	3	0.0 — 0.000
	Aberrant	0	. . . —

TABLE 2

Crosses displaying elevated recombination or "selfing"

Cross No.	Parents	Viable spores	Percent germination	<i>iv</i> ⁺ spores	Percent <i>iv</i> ⁺
Group 2 × Group 3					
1	327 <i>sp A</i> × 304 <i>lys a</i>	1.6×10^4	17.2	1.6×10^3	10.0
12	327 <i>sp a</i> × 318 <i>lys A</i>	9.4×10^3	107	8.8×10^2	9.35
15	328 <i>sp a</i> × 318 <i>lys A</i>	8.3×10^3	73.7	7.7×10^2	9.2
18	329 <i>sp a</i> × 318 <i>lys A</i>	2.8×10^4	25.5	2.0×10^3	7.1
Group 2 × Group 2					
26	304 <i>sp a</i> × 318 <i>lys A</i>	6.7×10^2	70.0	7.8×10	11.6
32	311 <i>sp a</i> × 318 <i>lys A</i>	8.1×10^2	107	7.5×10	9.25
34	16117 <i>sp a</i> × 318 <i>lys A</i>	6.1×10^2	90.3	8.2×10	13.5
Group 2 × Group 1					
44	324 <i>sp A</i> × 311 <i>lys a</i>	3.3×10^3	31.7	3.9×10^2	11.8
46	318 <i>sp A</i> × 322 <i>lys a</i>	1.0×10^4	96.3	7.5×10^2	7.5
49	322 <i>sp a</i> × 318 <i>lys A</i>	1.8×10^3	55.5	2.4×10^2	13.3
53	16117 <i>sp a</i> × 322 <i>lys A</i>	9.5×10^3	78.6	1.5×10^3	15.8
54	16117 <i>sp A</i> × 322 <i>lys a</i>	6.4×10^3	59.8	4.6×10^2	7.25
Group 2 × Group 2 (selfing)					
67	318 <i>sp a</i> × 318 <i>lys A</i>	2.9×10^4	44.0	4.0×10^2	1.35

vations pertinent to the appearance of unexpected prototrophs may be made. Recombinational results of this nature occur sporadically throughout all classes of crosses involving Group 2 alleles. Nevertheless, repetitions of the aberrant crosses of Table 2 have shown that almost without exception comparable results are obtained when crosses are repeated, or when fresh harvests are made from the same crossing bottles. Aberrant results are not dependent upon heterozygosity at either of the outside-marker loci, lysine-1 and spray, (cf. Table 3, crosses 106–129, 141–143, 190, 212, 234, and others). Nor are such results related to germination percentages. Further, it was found that certain strains, which may be referred to as "efficient", are associated with such behavior with a regularity unapproached by other strains. This fact, observable also in KIRITANI's (1962) data, is best exemplified by the strain T318 *lys A* (cf. crosses 12, 15, 18, 26, 32, 34, 49, and 67). One may also identify "inefficient" strains, e.g., T318 *sp a*, that, used in several crosses, were never associated with anomalous behavior unless crossed to a strain of the former description.

From the selfing cross 67, between the efficient strain T318 *lys A* and the inefficient strain T318 *sp a*, a number of *iv* progeny were isolated and their outside marker constitutions determined. Each was then backcrossed to the parent of opposite mating type, and the backcrosses were tested for the occurrence and frequency of *iv* recombination ("selfing"). Representative crosses are included in Table 3, while the data as a whole are summarized in Table 4. It is evident that efficiency occurred more frequently among the *lys sp*⁺ than among the *lys*⁺ *sp* segregants. The former commonly exhibited "selfing" behavior when crossed to either parent; the latter rarely, unless crossed to the efficient parent,

TABLE 3

Representative backcrosses, outcrosses, and inter se crosses of iv isolates from aberrant crosses No. 49 (T318 lys A × T322 sp a) and No. 67 (T318 lys A × T318 sp a).

Isolate designations include the Tiv allele presumed to be present and, in parentheses, the cross from which the isolate was derived and the isolate number

Cross No.	Parents		Viable spores	Percent germination	<i>iv</i> ⁺ spores	Percent <i>iv</i> ⁺
Crosses of isolates from cross 49						
106	318(49-14) <i>A</i>	× 318 <i>sp a</i>	7.5 × 10	55.1	2.0 × 10	26.7
108	322(49-24) <i>A</i>	× 318 <i>sp a</i>	5.5 × 10	53.9	0.9 × 10	16.4
110	318(49-6) <i>a</i>	× 318 <i>sp A</i>	7.5 × 10	81.7	1.8 × 10	24.0
111	318(49-7) <i>a</i>	× 318 <i>sp A</i>	1.3 × 10 ²	74.9	3.5 × 10	26.1
121	318(49-12) <i>a</i>	× 322 <i>lys A</i>	1.4 × 10 ³	76.0	1.6 × 10 ²	11.3
123	318(49-20) <i>a</i>	× 322 <i>lys A</i>	3.0 × 10 ²	79.4	4.6 × 10	15.3
126	318(49-14) <i>A</i>	× 322 <i>lys a</i>	1.1 × 10 ³	93.1	1.1 × 10 ²	10.0
129	318(49-41) <i>A</i>	× 322 <i>lys a</i>	2.1 × 10 ²	74.8	3.2 × 10	15.4
130	318(49-2) <i>lys A</i>	× 318 <i>sp a</i>	2.0 × 10 ³	35.0	1.5 × 10 ²	7.5
131	318(49-8) <i>lys a</i>	× 318 <i>sp A</i>	9.0 × 10 ²	17.8	1.9 × 10 ²	21.0
132	318(49-21) <i>lys A</i>	× 318 <i>sp a</i>	2.0 × 10 ³	47	1.6 × 10 ²	8.0
133	318(40-28) <i>lys a</i>	× 318 <i>sp A</i>	8.0 × 10 ²	77	1.6 × 10 ²	20.0
134	318(49-49) <i>lys a</i>	× 318 <i>sp A</i>	1.5 × 10 ³	79	2.6 × 10 ²	17.3
135	318(49-65) <i>lys a</i>	× 318 <i>sp A</i>	1.0 × 10 ³	88	2.0 × 10 ²	20.0
136	322(49-7) <i>lys sp A</i>	× 318(49-12) <i>a</i>	2.5 × 10 ²	36	3.9 × 10	15.6
137	322(49-7) <i>lys sp A</i>	× 318(49-19) <i>a</i>	2.8 × 10 ²	66	7.2 × 10	25.3
138	322(49-10) <i>lys sp a</i>	× 318(49-40) <i>A</i>	3.5 × 10 ²	44	5.7 × 10	16.3
139	322(49-10) <i>lys sp a</i>	× 318(49-41) <i>A</i>	3.5 × 10 ²	54.0	5.5 × 10	15.7
140	322(49-7) <i>lys sp A</i>	× 318(49-59) <i>a</i>	3.0 × 10 ²	75.2	7.6 × 10	25.3
Crosses of isolates from cross 67						
141	318(67-1) <i>lys a</i>	× 318 <i>lys A</i>	2.5 × 10 ⁴	55.6	1.0 × 10 ⁴	40.0
142	318(67-20) <i>lys a</i>	× 318 <i>lys A</i>	1.4 × 10 ⁴	35.4	5.0 × 10 ²	3.6
143	318(67-29) <i>lys a</i>	× 318 <i>lys A</i>	3.6 × 10 ⁴	80.0	5.0 × 10 ²	1.4
144	318(67-39) <i>lys a</i>	× 318 <i>lys A</i>	3.0 × 10 ⁴	60.0	0	0.00
145	318(67-35) <i>lys a</i>	× 318 <i>lys A</i>	1.4 × 10 ⁴	66.9	0	0.00
151	318(67-2) <i>lys A</i>	× 318 <i>sp a</i>	6.8 × 10 ³	100	2.0 × 10 ²	2.9
156	318(67-13) <i>lys A</i>	× 318 <i>sp a</i>	5.6 × 10 ³	80.0	1.0 × 10 ²	1.8
161	318(67-28) <i>lys A</i>	× 318 <i>sp a</i>	1.8 × 10 ⁴	83.3	1.0 × 10 ³	5.6
171	318(67-124) <i>sp a</i>	× 318 <i>lys A</i>	3.5 × 10 ⁴	97.5	1.4 × 10 ³	4.0
175	318(67-118) <i>sp a</i>	× 318 <i>lys A</i>	9.0 × 10 ⁴	89.3	1.1 × 10 ³	1.2
177	318(67-127) <i>sp a</i>	× 318 <i>lys A</i>	2.1 × 10 ⁴	100	3.0 × 10 ³	14.3
185	318(67-16) <i>sp a</i>	× 318 <i>lys A</i>	8.9 × 10 ²	56.1	1.2 × 10 ²	13.5
187	318(67-22) <i>sp a</i>	× 318 <i>lys A</i>	1.9 × 10 ³	57.4	1.4 × 10 ²	7.4
190	318(67-106) <i>sp A</i>	× 318 <i>sp a</i>	2.0 × 10 ⁴	55.6	2.5 × 10 ²	1.25
191	318(67-116) <i>sp A</i>	× 318 <i>sp a</i>	5.0 × 10 ⁴	66.7	0	0.00
192	318(67-128) <i>sp A</i>	× 318 <i>sp a</i>	8.0 × 10 ³	9.5	0	0.0
198	318(67-117) <i>sp A</i>	× 318 <i>sp a</i>	3.0 × 10 ³	57.7	0	0.0
202	318(67-126) <i>sp A</i>	× 318 <i>sp a</i>	4.0 × 10 ²	100	0	0.0
206	318(67-57) <i>a</i>	× 318 <i>lys A</i>	5.0 × 10 ⁴	100	0	0.00
212	318(67-71) <i>a</i>	× 318 <i>lys A</i>	2.0 × 10 ⁵	60.7	5.0 × 10 ³	2.5
215	318(67-40) <i>a</i>	× 318 <i>lys A</i>	8.0 × 10 ³	79.3	0	0.0
216	318(67-43) <i>a</i>	× 318 <i>lys A</i>	1.7 × 10 ³	60.1	2.5 × 10	1.5
219	318(67-61) <i>A</i>	× 318 <i>sp a</i>	3.5 × 10 ³	35.0	0	0.0
222	318(67-73) <i>A</i>	× 318 <i>sp a</i>	9.6 × 10 ⁴	69.6	1.0 × 10 ³	1.0
224	318(67-81) <i>lys sp A</i>	× 318 <i>sp a</i>	1.5 × 10 ⁴	43.5	1.5 × 10 ²	1.0
225	318(67-84) <i>lys sp A</i>	× 318 <i>sp a</i>	1.5 × 10 ³	25.0	0	0.0
227	318(67-32) <i>lys A</i>	× 304 <i>sp a</i>	1 × 10 ³	37.1	1.1 × 10 ²	11
228	318(67-38) <i>lys A</i>	× 304 <i>sp a</i>	1 × 10 ³	46.9	1.5 × 10 ²	15
232	318(67-26) <i>sp A</i>	× 311 <i>sp a</i>	1.6 × 10 ⁴	28.6	0	0.00
233	318(67-36) <i>lys A</i>	× 16117 <i>sp a</i>	2.6 × 10 ²		4.3 × 10	16.5
234	318(67-37) <i>A</i>	× 16117 <i>sp a</i>		28.6		5
235	318(67-42) <i>lys A</i>	× 327 <i>sp a</i>	2.3 × 10 ²	48.0	2.5 × 10	10.8
236	318(67-J1) <i>lys sp a</i>	× 324 <i>A</i>	1.9 × 10 ⁴	51.8	1.5 × 10	0.079
238	318(67-J1) <i>lys sp a</i>	× 318(67-37) <i>A</i>	6.8 × 10 ³	20.0	5.1 × 10	0.75
239	318(67-J3) <i>lys sp a</i>	× 318(67-37) <i>A</i>	5.8 × 10 ³	21.4	2.1 × 10	0.36

TABLE 4

Segregation of "efficiency", as revealed by backcrosses of iv random spores from a "selfing" cross (No. 67)

Genotypes of isolates	Parent strain used in backcross	Backcrosses yielding <i>iv</i> ⁺	Backcrosses not yielding <i>iv</i> ⁺	Total isolates crossed
<i>lys sp</i> ⁺	318 <i>lys A</i>	3	2	5
	318 <i>sp a</i>	19	2	21
<i>lys</i> ⁺ <i>sp</i>	318 <i>lys A</i>	20	2	22
	318 <i>sp a</i>	2	14	16
<i>lys sp</i>	318 <i>sp a</i>	1	2	3
<i>lys</i> ⁺ <i>sp</i> ⁺	318 <i>lys A</i>	4	9	13
	318 <i>sp a</i>	3	4	7

T318 *lys A*. It should be noted that the outside marker constitution of the more efficient class of segregants is the same as that of the efficient parent and vice versa. This may be taken as evidence that there exist within the *lys-iv-sp* region of linkage group V important genetic factors conducive to the selfing behavior of T318. Crossover isolates (*lys sp* and *lys*⁺ *sp*⁺) were more evenly divided with respect to this property.

Relation to recombination of outside markers: In each aberrant cross represented in Table 2, and in some of those in Table 3, a small random sample of about 30 to 40 *iv*⁺ segregants were tested for their constitution with respect to the outside markers *lys* and *sp*. The results are given in Table 5. Accompanying these data, in most cases, are corresponding figures for the segregation of markers in random samples of unselected spores. The data, especially those pertaining to crosses of the original marked stocks, are in agreement with the observation of KIRITANI (1962) that in general the predominant outside marker genotype among the *iv*⁺ segregants is *lys sp*. This result was obtained also in the course of ordered tetrad analysis, as will be discussed below. For many of the crosses, wide disparities were encountered in the frequencies of the various genotypes in the unselected spores. Such variation must presumably be ascribed to disproportionate germination, but in any case it speaks for caution in drawing conclusions based on frequencies of marker combinations among *iv*⁺ spores.

In most crosses, the sense of the outside marker asymmetry among *iv*⁺ segregants is that predicted by the known order and linkage of alleles in the cross. It should be recalled that the data of KIRITANI (1962) do not permit the ordering of alleles within groups. In two of the crosses, the asymmetry encountered among *iv*⁺ segregants is the reverse of that expected, a situation paralleled in KIRITANI's data. In one of these, cross 1, a sample of 108 unselected spores were found to be uniformly *sp*⁺. In the other, cross 49, between T318 *lys A* and T322 *sp a*, this reversal of the expected asymmetry finds no explanation in the ratios for unselected spores from the same cross. Furthermore, as will shortly be discussed, the reversed asymmetry reappeared when ordered tetrad analysis was performed.

In order to determine whether a tendency exists for elevated apparent *iv* recombination to be accompanied by elevated total recombination between *lys*

TABLE 5
Genotypes of iv⁺ and of unselected spores in aberrant crosses

Cross No.	Parents	Percent <i>iv</i> ⁺	Genotypes* of <i>iv</i> ⁺			Total	Genotypes of unselected spores			Total
			<i>sp</i> ⁺ <i>lys</i> ⁺	<i>sp</i> <i>lys</i> ⁺	<i>sp</i> <i>lys</i>		<i>sp</i> ⁺ <i>lys</i> ⁺	<i>sp</i> <i>lys</i> ⁺	<i>sp</i> <i>lys</i>	
1	327 <i>sp</i> A	10.0	36	4	0	40	(108 <i>sp</i> ⁺ ; 0 <i>sp</i>)			108
12	327 <i>sp</i> a	9.35	0	1	4	28	27	53	46	33
15	328 <i>sp</i> a	9.2	1	4	0	23	29	117	16	33
18	329 <i>sp</i> a	7.1	2	11	3	23	39			204
26	304 <i>sp</i> a	11.6	0	6	2	20	28			144
32	311 <i>sp</i> a	9.25	1	1	1	29	32	20	71	25
34	16117 <i>sp</i> a	13.5	0	4	2	24	30	26	53	23
44	324 <i>sp</i> A	11.8	34	4	2	0	40	14	65	43
46	318 <i>sp</i> A	7.7	0	2	1	37	40	12	24	1
49	322 <i>sp</i> a	13.3	1	8	2	29	40	2	26	5
53	16117 <i>sp</i> a	15.8	0	2	2	24	28	27	12	46
54	16117 <i>sp</i> A	7.25	0	2	2	24	40	22	70	21
67	318 <i>sp</i> a	13.5	0	1	3	36	40	18	74	21
130	318(49-2) <i>lys</i> A	7.7	7	2	6	0	15	36	58	66
131	318(49-8) <i>lys</i> a	21.0	3	2	1	10	16	24	37	27
132	318(49-21) <i>lys</i> A	7.95	4	2	12	0	18	13	35	24
133	318(49-28) <i>lys</i> a	20.25	1	2	1	8	12	22	35	37
134	318(49-49) <i>lys</i> a	17.3	0	1	3	5	9	12	50	23
135	318(49-65) <i>lys</i> a	19.7	0	5	3	14	22	9	46	14
136	322(49-7) <i>lys</i> <i>sp</i> A	15.6	7	9	0	1	17	10	41	23
137	322(49-7) <i>lys</i> <i>sp</i> A	25.3	18	26	0	2	46	31	19	6
138	322(49-10) <i>lys</i> <i>sp</i> a	16.3	11	14	1	0	26	36	30	9
139	322(49-10) <i>lys</i> <i>sp</i> a	15.7	16	10	0	2	28	41	16	6
140	322(49-7) <i>lys</i> <i>sp</i> A	25.3	11	13	4	3	31	26	17	7
234	318(67-37) A	5	2	4	5	29	40	27	26	15
227	318(67-32) <i>lys</i> A	11	0	6	6	28	40			
228	318(67-38) <i>lys</i> A	15	0	5	6	29	40			
233	318(67-36) <i>lys</i> A	17.9	2	3	5	30	40			
235	318(67-42) <i>lys</i> A	10.8	7	32	0	1	40	4	28	4
216	318(67-43) a	1.5	(15 <i>lys</i> ⁺ ; 13 <i>lys</i>)			28				0
185	318(67-16) <i>sp</i> a	13.5	0	2	9	29	40			
187	318(67-22) <i>sp</i> a	7.4	1	13	3	23	40			
239	318(67-13) <i>lys</i> <i>sp</i> a	0.36	10	1	14	10	35			
238	318(67-11) <i>lys</i> <i>sp</i> a	0.75	7	0	5	7	19			

and *sp*, all crosses for which data on both *iv* recombination and outside marker recombination were available have been collected and are shown in Figure 2. Most of these data are included in Table 5. Plotting percent *lys-sp* recombination

against percent apparent *iv* recombination (percent *iv*⁺ spores $\times$ 2), a scatter diagram was prepared consisting of one point for each cross. The two variables were shown to be dependent at the 99% level of confidence ($r = 0.48$; $N = 30$). The slope of the line is approximately 0.12. This positive correlation between outside marker recombination and apparent recombination at the *iv* locus, finds a parallel in the data of DEMEREC (1928) dealing with reversion of the reddish-alpha mutation in *Drosophila virilis*. It bears on the question whether the anomalous prototrophs result from a recombinational or a mutational event.

Nature of the event responsible for the occurrence of unexpected prototrophs: The isoleucine-valine alleles under study are quite stable. They have not been observed to revert in vegetative culture; furthermore, prototrophs are not detected in most selfing crosses and intragroup crosses, among samples of the order of 10^5 to 10^6 ascospores. In order to test this stability further, the highly efficient strain T318 *lys A* was subjected to UV as was a derivative of cross 32 that also bears the T318 allele. For each of several dose levels employed, a sample of about 10^8 or 10^9 conidia were tested by plating in lysine minimal agar. No revertant colonies were recovered.

It was desired to ascertain whether the event studied might be occurring at some point other than the *iv* locus, i.e., in response to a suppressor mutation or to the creation, by recombination, of a complex capable of acting as a suppressor of *iv* alleles. Thirteen *iv*⁺ recombinants, representing ten anomalous crosses and bearing various combinations of the outside markers, were crossed to the wild-type strains KJT 1960A or KJT 1960a. From these crosses random samples of unselected ascospores were tested for isoleucine-valine dependence. The sample sizes ranged from 16 to 116, with a total of 773 isolates tested in all. No *iv* segre-

FIGURE 2.—Relation of outside marker exchange to apparent recombination at the *iv* region. Data from 30 crosses, most of them included in Table 5, are presented as a scatter diagram. The indicated regression of outside marker exchange on *iv* recombination has a slope of 0.12. The two variables are positively correlated at the 99% level of confidence ($r = 0.48$; $N = 30$).

gants were found, indicating that recombination between the *iv* locus and suppressor locus does not occur or occurs so rarely as to place the suppressor within the *iv* region. One of the anomalous *iv*⁺ tested in this way represented a selfing cross (of the allele T318). From the cross of this isolate to KJT 1960a, 79 progeny were tested.

It was deemed of paramount importance to determine whether there arises, simultaneously with the *iv*⁺ segregant, another bearing both mutant alleles, i.e., whether the event is reciprocal or nonreciprocal. Small numbers of ordered tetrads containing *iv*⁺ spore pairs were obtained in eight aberrant crosses. Segregation in representative tetrads containing *iv*⁺ segregants forms the material of Table 6. The number of asci of each type encountered in each cross is presented in Table 7, as are overall recombination percentages, and numbers of asci possessing certain features discussed below.

A preponderance of the *lys sp* recombinant type among *iv*⁺ segregants is encountered in every cross. In several of the crosses, 12, 15, 26, 32, and the selfing cross 177, both parents are leaky. Among the ascus-mates of *iv*⁺ segregants from such crosses, there appears characteristically (29 asci out of 34) one pair of spores that is decidedly less leaky than the other two pairs. This spore pair

TABLE 6

Segregation of markers and leakiness with respect to isoleucine-valine requirement in asci containing iv⁺ spores from aberrant crosses

Crosses of two leaky parents:

Cross No. 12 (T 318 *lys A* × T 327 *sp a*); 3 asci.

<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	+	<i>sp</i>
<i>lys</i>	+	<i>sp</i>	<i>lys</i>	<i>iv</i> ^{sl}	<i>sp</i>	<i>lys</i>	<i>iv</i> ^{lk}	<i>sp</i>
+	<i>iv</i> ^{sl}	+	+	<i>iv</i> ^{lk}	+	+	<i>iv</i> ^{sl}	+
+	<i>iv</i> ^{lk}	<i>sp</i>	+	+	<i>sp</i>	+	<i>iv</i> ^{lk}	+

(1 ascus)

(1 ascus)

(1 ascus)

Cross No. 15 (T 318 *lys A* × T 328 *sp a*); 9 asci.

<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	<i>iv</i> ^{sl}	+	<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	+	+
<i>lys</i>	+	<i>sp</i>	<i>lys</i>	+	<i>sp</i>	<i>lys</i>	<i>iv</i> ^{sl}	<i>sp</i>	<i>lys</i>	<i>iv</i>	+
+	<i>iv</i> ^{sl}	+	+	<i>iv</i> ^{lk}	+	+	+	+	+	<i>iv</i>	<i>sp</i>
+	<i>iv</i> ^{lk}	<i>sp</i>	+	<i>iv</i> ^{lk}	<i>sp</i>	+	<i>iv</i> ^{lk}	<i>sp</i>	+	<i>iv</i>	<i>sp</i>

(5 asci)

(2 asci)

(1 ascus)

(1 ascus)

Cross No. 26 (T 318 *lys A* × T 304 *sp a*); 6 asci.

<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	<i>iv</i> ^{sl}	+	<i>lys</i>	+	+
<i>lys</i>	+	<i>sp</i>	<i>lys</i>	+	<i>sp</i>	<i>lys</i>	<i>iv</i> ^{lk}	+
+	<i>iv</i> ^{sl}	+	+	<i>iv</i> ^{sl}	+	+	<i>iv</i> ^{lk}	<i>sp</i>
+	<i>iv</i> ^{lk}	<i>sp</i>	+	<i>iv</i> ^{lk}	<i>sp</i>	+	<i>iv</i> ^{lk}	<i>sp</i>

(4 asci)

(1 ascus)

(1 ascus)

Cross No. 32 (T 318 *lys A* × T 311 *sp a*); 5 asci.

<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	<i>iv</i> ^{lk}	+	<i>lys</i>	+	+
<i>lys</i>	+	<i>sp</i>	<i>lys</i>	+	<i>sp</i>	<i>lys</i>	<i>iv</i> ^{sl}	<i>sp</i>	<i>lys</i>	<i>iv</i> ^{lk}	+
+	<i>iv</i> ^{sl}	+	+	<i>iv</i> ^{lk}	+	+	<i>iv</i> ^{sl}	+	+	<i>iv</i> ^{sl}	<i>sp</i>
+	<i>iv</i> ^{lk}	<i>sp</i>	+	<i>iv</i> ^{sl}	<i>sp</i>	+	+	<i>sp</i>	+	<i>iv</i> ^{lk}	<i>sp</i>

(2 asci)

(1 ascus)

(1 ascus)

(1 ascus)

TABLE 6—Continued

Segregation of markers and leakiness with respect to isoleucine-valine requirement in asci containing iv⁺ spores from aberrant crosses

Cross No. 177 (T 318 <i>lys A</i> × T 318(67-127) <i>sp a</i>); 12 asci.								
<i>lys</i>	<i>iv^{lk}</i>	+	<i>lys</i>	<i>iv^{lk}</i>	+	<i>lys</i>	<i>iv^{lk}</i>	+
<i>lys</i>	+	<i>sp</i>	<i>lys</i>	+	<i>sp</i>	<i>lys</i>	<i>iv^{lk}</i>	<i>sp</i>
+	<i>iv^{sl}</i>	+	+	<i>iv^{lk}</i>	+	+	+	+
+	<i>iv^{lk}</i>	<i>sp</i>	+	<i>iv^{lk}</i>	<i>sp</i>	+	<i>iv^{sl}</i>	<i>sp</i>
(7 asci)			(2 asci)			(1 ascus)		
	<i>lys</i>	+	+	<i>lys</i>	+	+		
	<i>lys</i>	<i>iv^{lk}</i>	<i>sp</i>	<i>lys</i>	<i>iv^{lk}</i>	+		
	+	<i>iv^{sl}</i>	+	+	<i>iv^{sl}</i>	<i>sp</i>		
	+	<i>iv^{lk}</i>	<i>sp</i>	+	<i>iv^{lk}</i>	<i>sp</i>		
(1 ascus)			(1 ascus)					

Crosses of one leaky and one nonleaky parent:

Cross No. 49 (T 318 <i>lys A</i> × T 322 <i>sp a</i>); 5 asci.								
<i>lys</i>	<i>iv^{lk}</i>	+	<i>lys</i>	<i>iv^{lk}</i>	+	<i>lys</i>	+	+
<i>lys</i>	+	<i>sp</i>	<i>lys</i>	+	<i>sp</i>	<i>lys</i>	<i>iv^{lk}</i>	+
+	<i>ivⁿ</i>	+	+	<i>ivⁿ</i>	<i>sp</i>	+	<i>ivⁿ</i>	<i>sp</i>
+	<i>ivⁿ</i>	<i>sp</i>	+	<i>iv^{lk}</i>	<i>sp</i>	+	<i>ivⁿ</i>	<i>sp</i>
(3 asci)			(1 ascus)			(1 ascus)		

Cross No. 53 (16117 <i>sp a</i> × T 322 <i>lys A</i>); 5 asci.								
	<i>lys</i>	<i>ivⁿ</i>	+					
	<i>lys</i>	+	<i>sp</i>					
	+	<i>ivⁿ</i>	+					
	+	<i>iv^{lk}</i>	<i>sp</i>					
(5 asci)								

Cross No. 54 (16117 <i>sp A</i> × T 322 <i>lys a</i>); 7 asci.								
<i>lys</i>	<i>ivⁿ</i>	+	<i>lys</i>	<i>ivⁿ</i>	+			
<i>lys</i>	+	<i>sp</i>	<i>lys</i>	<i>ivⁿ</i>	+			
+	<i>ivⁿ</i>	+	+	+	<i>sp</i>			
+	<i>iv^{lk}</i>	<i>sp</i>	+	<i>iv^{lk}</i>	<i>sp</i>			
(6 asci)*			(1 ascus)					

iv^{lk} = leaky; *iv^{sl}* = slightly leaky; *ivⁿ* = nonleaky.

* Leakiness tested in only five asci of this type.

usually (23 out of 29) bears a marker combination reciprocal to that of the *iv⁺* pair. It seems probable that this reciprocal recombinant, being significantly less leaky than either parental strand, possesses both *iv* alleles present in the cross, i.e., is a double mutant.

In the remaining crosses, Nos. 49, 53, and 54, one parent, bearing the group 1 allele T322, is nonleaky. Here the ascus-mates of *iv⁺* spores are seen to include (in 15 of 16 asci) one leaky pair and two nonleaky pairs. The leaky pair in every case represents the outside marker constitution of the leaky, Group 2, parent, T318 or 16117. The two nonleaky pairs thus characteristically include one pair that bears the marker alleles present in the T322 parent and one that possesses a recombinant genotype, reciprocal to that of the *iv⁺* spore pair. In these asci, then, the reciprocal recombinant may be assumed to bear the T322 (nonleaky) allele.

In order to determine whether these reciprocals contain the leaky (Group 2)

TABLE 7

Summary of data from ordered tetrad analyses

Cross No.	Asci tested	Percent lys-sp recombination	Percent <i>iv</i> ⁺	Asci with <i>iv</i> ⁺	Asci <i>iv</i> ⁺ and recombinant for <i>lys-sp</i>	Asci containing <i>lys iv</i> ⁺ <i>sp</i>	Asci tested for leakiness	Asci with 2 lk:1 sl	Asci with 2 n:1 lk	Asci with sl or n reciprocal to <i>iv</i> ⁺
Crosses of two leaky strains										
12	6	33.3	12.5	3	3	2	3	3	.	2
15	17	44.5	17.7	12	11	9	8	8	.	6
26	25	22.0	8.0	8	7	7	6	4	.	5
32	13	30.8	15.4	8	7	6	5	4	.	3
177	24	35.2	12.5	12	11	9	12	10	.	8
Total	91	...	...	44	39	33	34	29	.	24
Crosses of one leaky and one nonleaky strain										
49	15	23.3	8.4	5	4	4	5	.	4	5
53	35	37.1	3.6	5	5	5	5	.	5	5
54	24	33.3	7.3	7	6	6	6	.	6	6
Total	74	...	...	17	15	15	16	.	15	16

lk = leaky with respect to isoleucine-valine requirement; *sl* = slightly leaky; *n* = nonleaky.

TABLE 8

Crosses to iv-2 strains of nonleaky ascus-mates of iv⁺ segregants from Cross No. 54

Cross No.	Ascus	Spore pair	Spore	Genotype	Tester strain	Viable spores	Percent germination	Number <i>iv</i> ⁺	Percent <i>iv</i> ⁺
451	1	2	3	<i>lys A</i>	16117 <i>sp a</i>	1.2×10^3	23	11	0.92
452	.	.	.		16117 <i>a</i>	2.1×10^5	60	163	0.078
453	.	.	.		318 <i>a</i>	6.0×10^4	31.9	164	0.27
454	.	.	4	<i>lys A</i>	16117 <i>sp a</i>	2.0×10^3	40	100	5.0
455	.	.	.		16117 <i>a</i>	7.5×10^4	50	160	0.21
456	.	.	.		318 <i>a</i>	6.0×10^4	50.0	99	0.16
457	.	3	5	<i>a</i>	16117 <i>sp A</i>	9.5×10^4	19	0	0.000
458	.	.	.		16117 <i>A</i>	3.0×10^5	60	0	0.000
459	.	.	6	<i>a</i>	16117 <i>sp A</i>	1.0×10^5	50	0	0.000
460	.	.	.		16117 <i>A</i>	3.0×10^5	80	0	0.000
461	2	1	1	<i>a</i>	16117 <i>sp A</i>	1.8×10^5	35	0	0.000
462	.	.	.		16117 <i>A</i>	2.0×10^5	40	0	0.000
463	.	.	2	<i>a</i>	16117 <i>sp A</i>	5.5×10^4	35	0	0.00
464	.	.	.		16117 <i>A</i>	9.0×10^4	28	1	0.001
465	.	3	6	<i>lys A</i>	16117 <i>sp a</i>	2.0×10^3	40	274	13.7
466	.	.	.		16117 <i>a</i>	1.9×10^5	50	419	0.22
467	.	.	.		318 <i>a</i>	7.0×10^4	59.1	136	0.19

TABLE 8—Continued

Crosses to iv-2 strains of nonleaky ascus-mates of iv+ segregants from Cross No. 54

Cross No.	Ascus	Spore pair	Spore	Genotype	Tester strain	Viable spores	Percent germination	Number <i>iv</i> ⁺	Percent <i>iv</i> ⁺
468	3	3	5	<i>lys a</i>	16117 <i>sp A</i>	1.3×10^5	25.5	0	0.000
469	.	4	7	<i>lys A</i>	16117 <i>sp a</i>	3.0×10^3	60	300	10.0
470	.	.	.	.	16117 <i>a</i>	2.5×10^3	50	5	0.2
471	.	.	.	.	318 <i>a</i>	8.0×10^4	86.7	186	0.23
472	.	.	8	<i>lys A</i>	16117 <i>sp a</i>	2.0×10^3	40	124	6.2
473	.	.	.	.	16117 <i>a</i>	5.0×10^3	100	5	0.1
474	.	.	.	.	318 <i>a</i>	5.0×10^4	54.6	83	0.17
475	4	2	3	<i>lys A</i>	16117 <i>sp a</i>	1.2×10^3	24	80	6.7
476	.	.	.	.	16117 <i>a</i>	1.5×10^4	100	21	0.14
477	.	.	.	.	318 <i>a</i>	7.5×10^4	33.3	100	0.13
478	.	.	4	<i>lys A</i>	16117 <i>sp a</i>	2.0×10^3	40	150	7.5
479	.	.	.	.	16117 <i>a</i>	5.0×10^4	100	83	0.17
480	.	.	.	.	318 <i>a</i>	9.5×10^4	68.2	19	2.0
481	.	4	7	<i>a</i>	16117 <i>sp A</i>	9.5×10^4	19	0	0.00
482	.	.	.	.	16117 <i>A</i>	1.4×10^5	20	0	0.000
483	5	1	2	<i>a</i>	16117 <i>sp A</i>	1.5×10^5	30	0	0.000
484	.	.	.	.	16117 <i>A</i>	1.0×10^5	100	0	0.000
485	.	3	5	<i>lys A</i>	16117 <i>sp a</i>	4.0×10^3	80	283	7.8
486	.	.	.	.	16117 <i>a</i>	5.0×10^4	100	12	0.024
487	.	.	.	.	318 <i>a</i>	1.8×10^5	79.5	115	0.064
488	.	.	6	<i>lys A</i>	16117 <i>sp a</i>	1.5×10^3	30	121	8.1
490	.	.	.	.	318 <i>a</i>	1.3×10^5	74.1	63	0.048
491	6	2	3	<i>lys A</i>	16117 <i>sp a</i>	1.2×10^5	60	1	0.001
492	.	.	.	.	16117 <i>a</i>	4.0×10^4	100	0	0.00
493	.	.	.	.	318 <i>a</i>	1.0×10^5	52.6	0	0.000
494	.	.	4	<i>lys A</i>	16117 <i>sp a</i>	1.8×10^5	35	0	0.000
495	.	.	.	.	16117 <i>a</i>	1.2×10^3	44.0	750	62.5
496	.	.	.	.	318 <i>a</i>	2.0×10^3	64.3	0	0.0
497	.	3	5	<i>a</i>	16117 <i>sp A</i>	2.8×10^3	55	91	3.25
498	.	.	.	.	16117 <i>A</i>	1.5×10^3	30	19	1.3
499	.	.	6	<i>a</i>	16117 <i>sp A</i>	1.3×10^3	25	74	0.57
500	.	.	.	.	16117 <i>A</i>	3.0×10^3	60	18	0.60
501	7	1	2	<i>lys A</i>	16117 <i>sp a</i>	2.0×10^3	40	246	12.3
502	.	.	.	.	318 <i>a</i>	3.2×10^4	5.9	78	0.24
503	.	4	7	<i>a</i>	16117 <i>sp A</i>	1.3×10^5	26	0	0.000
504	.	.	.	.	16117 <i>A</i>	2.5×10^5	100	0	0.000
505	.	.	8	<i>a</i>	16117 <i>sp A</i>	3.0×10^5	60	0	0.000
506	.	.	.	.	16117 <i>A</i>	2.7×10^5	60	0	0.000

allele as well, i.e., are double mutants at the *iv* locus, all the nonleaky ascus-mates of prototrophs in cross 54 were crossed to stock 16117 and T318 strains (Table 8). Of the seven tetrads tested in this way, each was found to possess one nonleaky component that yielded *iv*⁺ when crossed to Group 2 strains and one that did not. Those that were incapable of recombination with Group 2 alleles must themselves

contain a Group 2 allele, and thus be double mutants, *iv*-1, *iv*-2. In six of the seven tetrads, the double-mutant spore pair, as thus determined, possessed a marker combination reciprocal to that of the *iv*⁺ segregant. This is the only cross in which a backcross test for the presence of the double mutant (CASE and GILES 1958) has yielded an unambiguous result. Success of the test depended on the failure of certain crosses to yield prototrophs by "selfing" or other aberrant recombination, despite the presence of Group 2 mutations, and despite the fact that elevated frequencies of recombination did occur in certain other crosses. The very exceptional result seen in cross 495 may be ascribed either to contamination or to an unexpected "selfing" event the quantitative aspect of which is due in part to disproportionate germination.

It is unfortunate that the only cross in which a rigorous test for the double mutant has thus far been performed is a cross involving a Group 1 strain. An average of about 4% recombination between this and the other two groups was obtained by KIRITANI (1962), while the actual figures ranged from 0.19 to 9.3% with one exceptional value of 38%. Thus cross 54, on which the backcross test was performed, although *iv* recombination in the tetrad data was 14.6%, and although more than 90% of dissected asci were successfully germinated, represents little more than one end of a wide spectrum of recombination frequencies. An unknown proportion of the *iv* recombination in this cross must have resulted from purely classical, distance-dependent exchanges, and such recombination would of course yield the observed double mutant products. In Group 2 $\times$ Group 1 crosses, elevations of recombination frequencies above those expected are not of the same order of magnitude as those encountered in crosses involving very closely linked alleles.

DISCUSSION

This paper presents some preliminary results of the study of two parallel forms of aberrant recombination: (a) the appearance of prototrophs, often at very high frequencies, in crosses where both parents bear the same mutant allele ("selfing") and (b) the appearance of prototrophs at unexpectedly high frequency in crosses of mutant strains bearing nonidentical but closely linked alleles.

Anomalous results of the latter group may be classified according to whether they occur in crosses of a Group 2 and a Group 1 strain, representing a distance of about four recombination units (Figure 1), or in crosses of much more closely linked alleles (less than 0.1% recombination). In either case, but particularly the former, the simplest assumption would be that of a mere elevation in the frequency of classical, distance-dependent, reciprocal exchange. Various physical, chemical, and cytogenetic factors, for example involvement with or proximity to tandem repeats (GREEN 1962), have been shown to increase crossover frequencies. Nevertheless because of the extreme frequency of prototrophic progeny from crosses of very closely linked alleles, and because of the occasional reversal, in Group 2 $\times$ Group 1 crosses, of the expected marker asymmetry among *iv*⁺ progeny (Tables 5 and 8; also KIRITANI 1962) it is felt that something other than classical recombination is taking place here.

Anomalous results in selfing and interallele crosses have in common the following features: their restriction, so far as is known, to crosses involving at least one Group 2 allele (KIRITANI 1962); the very high frequency with which they occur; the fact that they occur or do not occur in response to factors other than the identity of the input alleles; their repeatability with respect to the crosses in which they do occur; and finally the fact that the unexpected prototrophs are predominantly recombinant for the outside markers and exhibit a marked asymmetry, the double mutant being, in most cases, the favored genotype. So far as is known at present, these two types of recombinational events do not differ in any significant respect.

Therefore, for reasons of economy, the authors have tentatively assumed that the mechanisms involved, at least in their more basic aspects, are the same. Existing data bearing on mechanism are primarily from studies on interallele crosses (Tables 6 to 8), and therefore much of what follows must rest on the foregoing assumption.

Strong indication was obtained that, for interallele crosses, the aberrant results stem from a purely reciprocal event. It is not necessary to assume the same with regard to the selfing results, since it is probable that reciprocal and nonreciprocal recombinational events can arise in response to the same underlying situation (FREESE 1957).

Should the selfing results prove to be nonreciprocal, the possibility of reversion by mutation would gain in favor (cf. DEMEREC 1963). Here, however, it becomes more difficult to maintain the assumption of like mechanisms, at the most basic level, for the selfing and interallele results.

The authors prefer, at the present time, a recombinational model, and feel that what is required of such a model is not nonreciprocity but unequal crossing over (nonhomologous breakpoints). Two types of mutational alterations of the genetic material would appear to be capable of "selfing" behavior based on unequal crossing over. One of these would be a gene altered by the addition or deletion of a single nucleotide. Such an event could throw out of phase the reading of the triplet code by which nucleotide sequence information is translated into protein amino acid sequence. Unequal crossing over might restore to the affected triplet its normal complement of three nucleotides. Such a model was considered for "selfing" behavior in *Salmonella* by DEMEREC (1962) and for elevated frequencies of apparent spontaneous mutation at meiosis in yeast by MAGNI (1963). The second type of mutation capable of "selfing" by unequal crossing over is the tandem repeat (STURTEVANT 1925; LEWIS 1941; LAUGHNAN 1949; JUDD 1964). In this case, a tandem triplication, which might be a more extreme allele phenotypically, would be the anticipated reciprocal product.

Interesting aspects of the system about which speculation would seem premature include the following: (1) In most aberrant crosses, the unexpected prototrophs represent predominantly a single recombinant class with respect to outside markers, regardless of which recombinant type was predicted on the basis of the known order of alleles and markers in the cross. (2) The anomalous events occur at very high frequency. (3) The occurrence of the event, or its failure to

occur, cannot be predicted from the identity of the input alleles but must rest on some as yet unknown determining factor or factors. Furthermore, the repeatability of the aberrant results in those crosses in which they do occur would seem to rule out their determination by chance environmental variations. (4) Certain *iv* Group 2 strains (efficient strains) possess the property that all crosses in which they are involved produce aberrant results. There are indications (Table 4) that this property may be heritable and that the genetic factors involved may lie in the *lys-iv-sp* region.

It is felt that the existence of "selfing" and related phenomena under conditions where ordered tetrad analysis is possible places in our hands a tool which may prove of value in elucidating certain aspects of the problem of genetic recombination.

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SUMMARY

Preliminary study has been made of the occurrence of prototrophs at high frequency in crosses involving two identical or closely linked isoleucine-valine (*iv*) alleles. The results could not be ascribed to the action of suppressors external to the *iv* region. The frequency of *iv* prototrophs was shown to be positively correlated with the frequency of exchange between outside markers. Ordered tetrad analysis, mainly on interallele crosses, indicated that the unexpected prototrophs in such crosses are the product of a purely reciprocal recombinational event. This finding has led the authors to favor interpretations based on unequal crossing over, at least with respect to anomalous results in interallele crosses. The capacity of a strain to engage in "selfing" behavior appears to be genetically determined.

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