EXISTENCE OF HOMOGENEOUS CATEGORIES OF MUTANTS EXHIBITING VARIOUS CONVERSION PATTERNS IN GENE *75* OF *ASCOBOLUS IMMERSUS*

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ONE characteristic of intragenic recombination in Ascomycetes is nonreciprocal segregation (MITCHELL 1955). This results in ratios other than 2:2 for the four products of meiosis at one heterozygous site. The nonreciprocal segregation may either be meiotic, giving a ratio of 3:1 (3 mutant spore pairs : 1 wild-type pair in an eight-spored ascus) or 1:3 (1 mutant : 3 wild type) or post-meiotic, yielding 3:5 or 5:3 ratios (KITANI, OLIVE and EL ANI 1962). These ratios represent gene conversion in both directions (wild type to mutant and mutant to wild type).

It has been clear for several years that the frequencies of these unusual segregations are not the same from one site to another, even within the same gene. The attempt to correlate that variation with other possible variables is one approach to an understanding of the intragenic recombination process. We now have some indications about what factors influence conversion frequencies. Several studies have shown that site localization inside the gene may play a strong role, at least in some segments of genetic material. A relative polarity has been shown in crosses between pairs of allelic mutants in several genes. The most extreme case was found among ascospore color mutants of gene *46* in Ascobolus. In each cross, recombinant asci result from a conversion for the mutant located the furthest to the right (LISSOUBA 1961; ROSSIGNOL 1964). Similar results were found in Neurospora by MURRAY (1963) for mutants at the *me-2* locus and by STADLER and TOWE (1963) for *cys* mutants. There are also indications of polarity in Saccharomyces (FOGEL and HURST 1967). In some cases, polarity has been more directly established by measuring the frequency of conversion in single-mutant × wild type crosses. In gene *46*, the conversion frequency increases gradually from the left to the right end of the gene (LISSOUBA 1961; ROSSIGNOL 1964). The ascospore color mutants of gene *19* show a more complex polarity, since the conversion frequency is maximal at each end of the gene and minimal in the middle (MOUSSEAU 1966).

In the above cases, the position of a mutant site appears to determine its conversion frequency. In each case at least one end of the gene shows a maximum frequency. However, this result may not be general in all systems. KRUSZEWSKA and GAJEWSKI (1967) studied mutants of the *Y* gene of Ascobolus (also controlling ascospore color) and failed to find any correlation between position of the

mutant site and conversion frequency. Furthermore, they found that two of their mutants which did not recombine with each other gave very different frequencies of conversion. This suggests that factors other than the position of a mutant site must have an important role in conversion. Hypotheses have been proposed whereby gene conversion results from a process in which regions of hybrid DNA are formed (HASTINGS and WHITEHOUSE 1964; HOLLIDAY 1964). According to these hypotheses, one may conceive that the specific base sequences at a heterozygous site should influence the overall frequency of odd segregations at this site. If this is true, both the chemical nature and the position of a heterozygous site may be independent factors of variation.

The base sequences of a heterozygous site may also affect the *relative* frequencies of the different types of unusual segregation. These frequencies would depend upon the likelihood of correction along each strand of the hybrid DNA, and this might be a consequence of the type of base mispairing. One might expect that mutant sites which had the same type of mispairing would give the same relative proportions of the different segregation ratios. If a polarity based on position also occurred, it could be demonstrated clearly only among mutants belonging to the same mispairing type.

The results presented here appear to fit such an interpretation. Each of 15 colorless ascospore mutants of gene *75* of *Ascobolus immersus* has been crossed to wild type, and the frequencies of the various segregation ratios have been determined. For comparison, frequencies of conversion have been estimated in various mutant × mutant crosses.

MATERIALS AND METHODS

Gene 75: More than 200 spontaneous mutants with colorless ascospores have been localized within gene *75* (LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL 1962). This study involves the central part of gene *75*. Most of the 15 mutants studied are regularly spaced along this segment. One of those mutants (*w 141*) was furnished by Dr. GAJEWSKI. All of the mutants are stable; no wild-type spores have occurred among over 10^5 asci examined from each of the self-crosses.

Complementation tests with heterocaryotic spores (MOUSSEAU 1963; ROSSIGNOL 1964) and with diploid spores (JUPIN 1965) always give negative results for mutants within gene *75*. Therefore, we conclude that the locus corresponds to a single functional unit.

The linkage map of the segment under analysis is shown in Figure 1. Twelve mutants of gene *75* were previously mapped in a preliminary study by RIZET and ROSSIGNOL (1966). They concluded that three of the mutants (*1303*, *w 141* and *1168*) behaved like deficiencies in recombination studies: they did not give any recombinants with several contiguous mutants. This conclusion has been confirmed for *1303* and *w 141*, but *1168* shows very peculiar characteristics: it gives a very low frequency of 6:2 (6 mutant white : 2 wild dark) recombinant asci (0.05 to 0.4×10^{-3}) in crosses with any of seven contiguous mutants (*302–1573* in Figure 1), while much higher frequencies are shown in crosses among these mutants (Figure 1). As it is impossible to localize this mutant on the map, it is represented by a dashed line overlapping the seven sites with which it shows this special behavior (Figure 1). The three new mutants utilized in the present study are *1245* (located between *2029* and *147*) and *1848* and *1983* which are situated on the right side of the gene near *1472*. The relative order of the three sites *1472*, *1848* and *1983* has not yet been established because crosses among them give a very low frequency of recombination (about 1×10^{-4}). In addition to verifying the frequencies of total 6:2 asci in all of the two-point crosses among these 15 mutants (RIZET and ROSSIGNOL 1966; ROSSIGNOL 1967), the accuracy

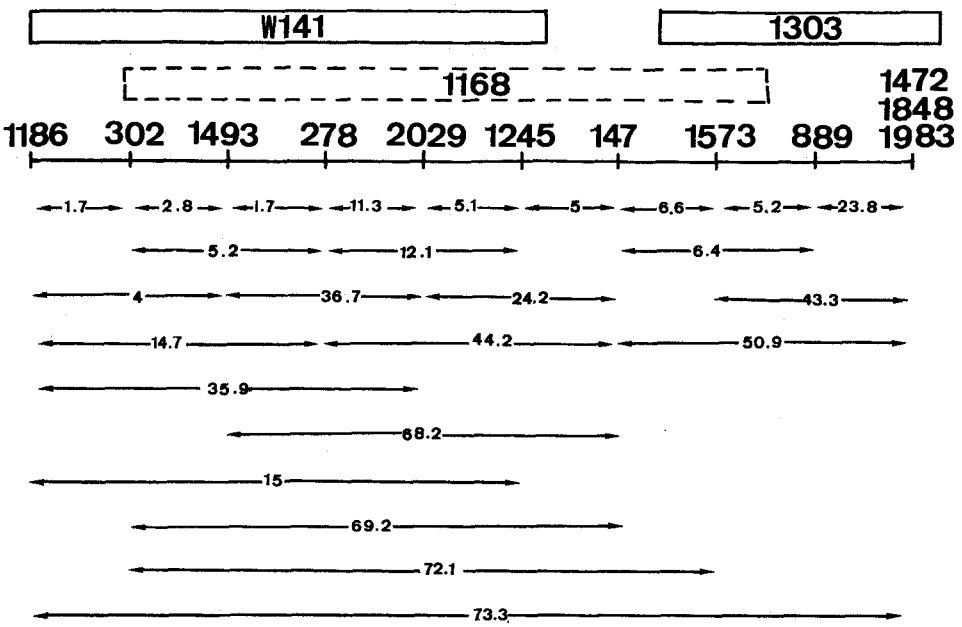

FIGURE 1.—Map of gene 75. Only the middle part of the gene (which contains all the mutants employed in this study) is shown. The frequencies of 6:2 recombinant asci are given per thousand asci.

of the genetic map has been also verified by crosses between these mutants and many other alleles as well as by the results of several three-point crosses.

Determination of conversion frequencies: Each of the 15 mutants was crossed to wild type. In each cross the frequency of spore color segregations other than 4:4 was determined. These segregations may have four different explanations: Nonpenetrance or phenocopying (accidental pigmentation of mutant spores or pigmentation failure of wild-type spores); new mutation to colorless spores; accidental clustering of spores from two different asci during spore projection; and gene conversion.

In order to distinguish gene conversion from these other kinds of events, we have analyzed a sample of each category of aberrant asci from each cross. We can easily identify phenocopying or nonpenetrance and new mutation by back-crosses. In order to reduce the chance of false clusters, the recovered asci were kept far apart by frequent replacement of the collecting surface. Mating type segregation furnished a supplementary check against false clusters.

In mutant × mutant crosses, 6:2 recombinant asci result either from a crossover or from a conversion to wild type. The frequency of conversion for each of the two mutant sites has been determined in each cross by analyzing a sample of 6:2 recombinant asci by the method of MITCHELL (1955).

Culture media and crossing techniques: Crosses were carried out at 23–24°C. The media and the techniques of culture were those described previously by LISSOUBA (1961) and by LISSOUBA *et al.* (1962). The minimal medium of YU SUN (1964) was used for vegetative growth.

RESULTS

Mutant × wild type crosses: All possible aberrant segregations (8:0, 7:1, 6:2, 5:3, 3:5, 2:6, 1:7, 0:8) occurred in one or another of the crosses of mutants to

TABLE 1

Frequencies of 3:1 and 1:3 segregations in single-mutant × wild type crosses

Mutant	Number of asci			Number 3:1 seg. in analyzed sample of 6:2 asci	Number 1:3 seg. in analyzed sample of 2:6 asci	Computed frequencies of segregations		Frequencies of conversions
	Total	6:2	2:6			3:1	1:3	
1186	23274	205	17	33/44	4/5	6.6 (5.1–8.8)	0.6 (0.3–1.6)	7.2
302	9797	200	167	34/43	39/43	16.1 (12.6–19.5)	15.5 (12.4–18.1)	31.6
1493	8027	226	176	32/43	29/29	20.9 (16.2–25.3)	21.9 (17.7–24.4)	42.8
278	9242	165	137	20/35	23/26	10.2 (7–13.5)	13.1 (9.8–15.7)	23.3
2029	9549	151	204	37/47	72/80	12.4 (9.8–15.2)	19.2 (16.1–22.3)	31.6
1245	6294	127	11	40/66	2/5	12 (9.1–15.5)	0.7 (0.1–1.9)	12.7
147	10378	315	260	54/61	58/58	26.8 (22.6–30.5)	25 (21.5–27.6)	51.8
1573	6630	182	183	30/39	30/31	21.1 (16.2–25.5)	26.7 (21.5–30.1)	47.8
889	7981	134	17	59/69	9/10	14.4 (11.5–17.3)	1.9 (0.7–2.2)	16.3
1983	6332	148	14	48/52	6/11	21.6 (16.9–25.2)	1.2 (0.4–2.4)	22.8
1472	3308	123	9	41/65	3/5	23.4 (17.8–30)	1.6 (0.4–3.7)	25
1848	1771	55	83	9/10	14/17	27.9 (15.1–34.3)	38.6 (23.1–48.3)	66.5
w141	11066	73	71	1/33	20/20	0.2 (0.03–1.1)	6.4 (3.9–7.6)	6.6
1303	4704	40	92	1/9	16/16	0.9 (0.16–6.1)	19.6 (11.4–22.2)	20.5
1168	6978	380	141	25/29	25/26	46.9 (34.2–53.2)	19.4 (13.7–22.1)	62.9

Spore ratios (e.g. 6:2 and 2:6) are given for observed phenotypic segregations, spore pair ratios (e.g., 3:1 and 1:3) indicate actual segregation for the mutant site. Frequencies are given $\times 10^{-3}$; 95% confidence limits are shown in parentheses (the confidence limits take in account both sets of observed and analyzed samples).

wild type (Table 1). However, all except 8:0 (8 white : 0 dark), 6:2 and 2:6 are extremely rare. Furthermore, the analysis of the rare types shows them to result from phenocopies or, occasionally, from false clusters (as revealed by aberrant segregation for mating type). After subtracting these spurious cases, the remaining frequencies of these rare classes are either zero or extremely low for all 15 mutants. The 8:0 asci also prove to result from causes other than gene conversion: all of those analyzed are explained by phenocopies or by new mutations to colorless.

A large fraction of the 6:2 and 2:6 asci do result from conversion, as can be

seen in Table 1. Among the 6 white : 2 dark asci which are not the result of conversion, nearly all represent a tetratype segregation for the parent mutant and a new mutation to colorless. Analysis shows that a few of the 2:6 asci do not result from conversion, but from nonpenetrance (two of the pigmented spores are genetically mutant). These results of the analysis of the 6:2 and 2:6 asci are similar to those found for the mutants of locus *46* (LISSOUBA 1961; ROSSIGNOL 1964) and locus *19* (MOUSSEAU 1967).

Table 1 reveals that gene conversion may result in either a 3:1 or a 1:3 segregation at each of the 15 mutant sites. It is notable that this is true for mutant *1168*, the peculiar behavior of which was already pointed out, as well as for the apparent deletion mutants *w 141* and *1303*.

The order of the twelve point mutants in Table 1 corresponds to their location on the genetic map. The frequency of conversion may be very different from one mutant site to the next, but the observed variations do not show any simple relationship to the map order of the mutant sites. This is true whether we compare 3:1 or 1:3 segregation frequencies or their sums.

For a given mutant site, the frequency of 3:1 segregation may be significantly different from the frequency of 1:3. In some cases the 3:1 segregations are more numerous, while in others the reverse is true. This dissymmetry between the two types of odd meiotic segregations for each mutant can be represented by the ratio of its 3:1 to 1:3 segregation frequencies. Thus the dissymmetry coefficient (DC) for mutant *1186*, for example, is equal to $6.6/0.6 = 11$. The DC for *w141* on the contrary, is equal to $0.2/6.4 = 0.03$.

The criterion of dissymmetry coefficient permits one to place the mutants in five homogeneous categories, as shown in Table 2. In the α category DC varies from

TABLE 2

Separation of the mutants into five categories according to their dissymmetry coefficients

DC category	Mutant	DC	Average DC	Conversion frequency
	1186	11		7.2
	1245	17		12.7
α	*889*	8	14	16.3
	1983	18		22.8
	1472	19		25
	302	1		31.6
β	*1493*	0.96	1	42.8
	147	1.1		51.8
	278	0.78		23.3
	2029	0.65		31.6
γ	*1573*	0.79	0.73	41.8
	1848	0.72		66.5
δ	*w 141*	0.03	0.04	6.6
	1303	0.05		20.5
ε	*1168*	2.2	2.2	62.9

TABLE 3

Frequencies of 1:3 segregations in mutant × mutant crosses

	1186	*302*	*1493*	*278*	*2029*	*1245*	*147*	*1573*	*889*	*1472*
1186		1.7— 5/5	3.6— 8/9	13.6—102/110	27.2—25/33	0.8— 1/19	22.4—30/42	38.4— 5/7	1.8— 1/28	0.9— 1/85
302	0— 0/5		1— 6/17	2.6— 9/18	13.9— 8/20	0— 0/13	29.9— 5/13	22.2— 8/19	0— 0/18	— —
1493	0— 0/9	1.8—11/17		0.6— 1/3	17.6— 8/20	0— 0/11	44.1—11/17	15.2— 4/14	0— 0/19	— —
278	0— 0/110	2.6— 9/18	1.1— 2/3		6.4—18/32	0— 0/30	27.9—12/19	8.1— 4/13	0.7— 1/43	0— 0/33
2029	3.3— 3/33	13.9— 8/20	24.2—11/20	3.5—10/32		0.2— 1/22	9.8—18/33	8.8— 5/19	2.9— 1/13	1.9— 1/31
1245	0.8— 1/19	11.6— 8/13	17.4— 8/11	8.1—20/30	4.8—21/22		4.6—24/26	12 —10/11	0.3— 1/21	2.4— 1/17
147	0— 0/42	10 — 2/13	12 — 3/17	9.3— 4/19	13.6—13/33	0— 0/26		2.3— 5/14	0— 0/15	0— 0/15
1573	0— 0/7	13.9— 5/19	22.8— 6/14	6.1— 3/13	22.9—13/19	0— 0/11	3.8— 8/14		0.4— 1/12	— —
889	0— 0/28	10.3— 4/18	37.9— 9/19	17.1—24/43	29.9—10/13	0.6— 2/21	6.4—15/15	4.8—11/12		3.4— 2/14
1472	0.9— 1/85	— —	— —	24 —16/33	34.9—18/31	0— 0/17	27.2— 8/15	— —	1.7— 1/14	
wild type	0.6	15.5	21.9	13.1	19.2	0.7	25	26.7	1.9	1.6

Frequencies are given per 10^{-3} tetrads. Each column refers to the same mutant site. The associated mutant site in the cross is mentioned on the corresponding line. The numbers of 1:3 segregations in the analyzed sample of 6:2 recombinant asci are shown beside each corresponding frequency. Frequencies of 1:3 segregations in mutant × wild type crosses are recalled in the last line.

8 to 18. It is about 1 in the β category and about 0.7 in the γ category. Category δ has a DC close to 0. The DC is about 2 for *1168*, the lone mutant in category ε. Except for the categories β and γ, which have very similar DC values, all classes are significantly different from each other. It is notable that the two deficiences constitute one class (δ) and that this class has a DC ratio which is extremely different from the others. It should also be noted that this mutant *1168*, of which the peculiar and unique recombinational behavior was previously pointed out, has a DC value which is significantly different from all the others.

Total conversion frequencies (corresponding to the sum of 3:1 and 1:3 segregations) are indicated for each mutant in Table 2. The comparison of these frequencies within each DC category reveals a striking feature. Conversion frequencies increase progressively with the location of mutant sites from the left to the right end of the map. Within the γ category alone, the probability of a consistent polarity occurring by chance in either the right or left direction is only 0.08. The same is true for category α. When we consider all four categories which each contain more than one mutant (α, β, δ and γ) the polarity appears to be highly significant.

Mutant $\times$ *mutant crosses:* Frequencies of 1:3 segregations observed for ten mutants crossed to one another are presented in Table 3. Mutants belonging to category β and γ show, at least in some crosses, high frequencies of 1:3 segregation (often exceeding 10×10^{-3}). These frequencies vary from cross to cross for any given mutant site. When the distance to the second mutant site is low, the frequency is lower than expected on the basis of the results from mutant $\times$ wild type crosses. The frequency increases with distance and can be greater than that observed in the one-point cross. The reduction of detected 1:3 segregations for these mutants when distance to the second mutant site decreases may either be apparent, resulting from the existence of concomitant conversion of wild type to mutant which would not lead to a detectable wild-type product, or it may result from an actual diminution of frequency caused by the proximity of the second mutant site. We know from further studies that at least the first factor should have a strong influence (ROSSIGNOL 1967), but it is not possible to rule out the second factor whose existence was pointed out by KRUSZEWSKA and GAJEWSKI (1966).

The four mutants which constitute the α DC category show a distinct behavior: in all crosses they give a low frequency of 1:3 segregation.

Since 3:1 segregation cannot be detected in mutant $\times$ mutant crosses, we cannot draw conclusions about the dissymmetry coefficient. Nevertheless, there is a clear difference of behavior between α mutants and β and γ mutants which is consistent with the conversion pattern which would be expected from mutant $\times$ wild type crosses.

It should be pointed out that there is no evidence of strong relative polarity in these two-point crosses. Conversion can affect the left side (lower part of Table 3) as well as the right side site (upper part). In crosses involving an α site and a β or γ site, almost all the conversions occur at the β or γ site. This is not surprising in view of the properties of α sites, and cannot be accounted for by any simple

polarity relationship since this result is found regardless of the respective location of the two crossed mutants.

DISCUSSION

Previous investigators of intragenic recombination have not grouped their mutants according to dissymmetry coefficients. Dissymmetries between 3:1 and 1:3 segregation frequencies have often been observed, in particular in Ascobolus (ROSSIGNOL 1964, MOUSSEAU 1966; KRUSZEWSKA and GAJEWSKI 1967), but the low conversion frequencies or the small numbers of mutants studied would have made this type of analysis difficult. Nevertheless, some analogies should be pointed out. Thus, in gene *46* (ROSSIGNOL 1964), mutant *63* (DC = 30) behaves like the α mutants of the present study, and this is probably true also for mutant *1604*. Mutant *137* (DC = 0.95) comes under the β category. At the *Y* locus (KRUSZEWSKA and GAJEWSKI 1967), among the five mutants for which both 3:1 and 1:3 segregations were observed, three fall in the α category (*Y*, *183* and *73*) with DC values of 13; 10 and 7, and two are in the γ category (*77* and *775*, DC values of 0.72 and 0.73, respectively). It seems that, in Ascobolus at least, there is a very precise range of conversion dissymmetries.

One could suppose that the DC value would be determined by the location of the mutant site, but the observations are not consistent with such a scheme. KRUSZEWSKA and GAJEWSKI (1967) reported two mutants at the *Y* locus which appear to occupy the same site but which give completely different dissymmetry patterns. In the present study within the gene *75*, there is no evidence of clustering of mutants in the same DC class. The point mutants show the following sequence on the map: α–β–β–γ–γ–α–β–γ–α–(α, α, γ). Furthermore, it is not possible to locate a distinct δ region for *1303* and *w 141*, since they overlap sites of two (*1303*) or each of the three (*w 141*) previous categories. On the contrary, the similar recombinational behavior of these two deficiencies suggests that they involve the same kind of mutational damage (deletion or alteration of a considerable segment of gene *75*). The fact that these mutants show the same DC which is one very different from that of other point mutants indicates therefore that the relation between the frequencies of 1:3 and 3:1 segregations could be a consequence of the chemical nature of the mutation. Such an hypothesis can be generalized to all 15 of the mutants which have been studied. On a hybrid DNA hypothesis the dissymmetry pattern is a result of the probability of correction in each direction. We could conceive that this probability is determined either by the identity of mispaired bases at the mutant site, or by an interaction between the neighboring bases and the mispaired site. The finding in the present study that the mutants fall into a small number of homogeneous and distinct categories seems to favor the idea that the chemical specificity of the mispaired site itself has at least a predominant influence. Under the other assumption, the determination of the DC value would be more complex and a small number of distinct classes would not be expected. A more complete answer to this question will await the study of dissymmetry coefficients at mutant sites for which the mutational alteration is known at the DNA level.

Considering the frequencies of total conversion at each site, two points may be made. First, there is a polarity such that these frequencies increase from the left to the right. Secondly, this polarity can only be detected within each DC category. The polarity here is more distinct than that observed in genes *46* and *19*. In gene *46*, only three of the mutants have been examined in the same manner as in the present study (ROSSIGNOL 1964). In gene *19*, the variation in conversion frequencies is not regular (MOUSSEAU 1966), but the DC categories are not known.

The present data show that at least two factors determine conversion frequencies. If position were the only important factor, polarity might be detected directly among the whole set of mutants under study. If the only important factor were the chemical nature of the mutant site, we might not detect any polarity. Since polarity is detected, but only within each DC category, we conclude that total conversion frequency is determined both by the position and by the nature of the mutant site. As stated above, the latter factor is believed to determine also the actual dissymmetry coefficient.

These data may be interpreted in terms of a recombinational event that has two sequential steps. The first step would originate at a fixed position to the right of the studied area, and would extend varying distances toward the left. The nearer a segregating site was to the origin, the higher its probability of being included in the segment affected by this first step. The first step would be necessary but not sufficient for the occurrence of aberrant meiotic segregations. These would require the subsequent step, which, according to the kind of wild type/mutant heterozygosity, would have various probabilities of producing a 2:2 or 3:1 or 1:3 segregation.

The diagram in Figure 2 provides an explanation of the data obtained for point mutants of α, β and γ categories, according to the two-step hypothesis. The probability of the first step at each point is symbolized by the straight line XO. For the α category, the probability that the second step will reestablish a 2:2 segregation (after occurrence of the first step) is equal to XA/XZ; the probability of an aberrant meiotic segregation is AZ/XZ. The probability at each point of an aberrant meiotic segregation for an α site is indicated by the straight line AO. The same reasoning accounts for the conversion frequencies at β and γ sites (Figure 2). The dissymmetries between 3:1 and 1:3 segregation result from the relative probabilities of getting one or the other from the second step.

This explanation may be related, at the molecular level, to the recombination models of HOLLIDAY (1964) and HASTINGS and WHITEHOUSE (1964). The first step corresponds to the hybridization of complementary DNA strands from two chromatids for a variable distance, from a fixed starting point. The second step corresponds to the correction of base mispairing at heterozygous sites within the hybrid DNA region. This interpretation is not inconsistent with the results of two-point crosses. Absence of any drastic relative polarity in gene *75* may reflect the occurrence of frequent independent correction events when the two mutant sites are involved in hybrid DNA.

It has been seen that the existence of α mutant sites giving very few 1:3 segregations has a consequence on mutant $\times$ mutant crosses. All crosses between

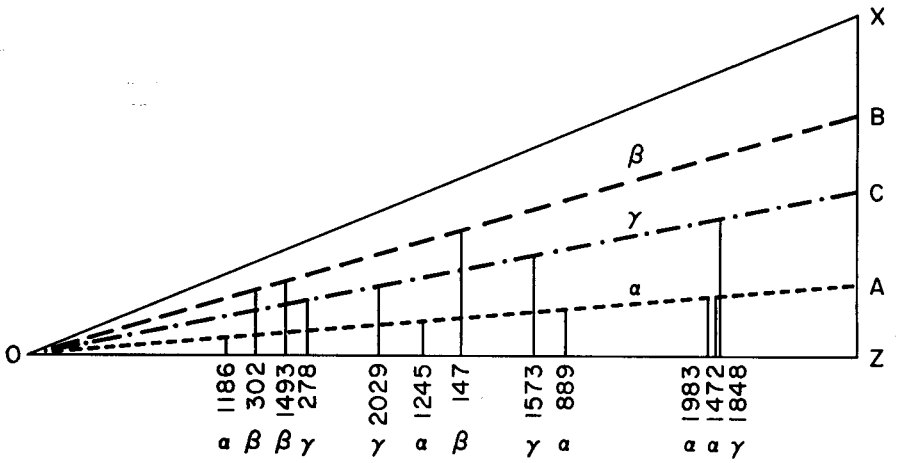

FIGURE 2.—Conversion frequencies as a function of the location and the nature of the mutant sites. The chance at each point of being included in the first step decreases from right to left (XO). Within each DC category the chance of restoring a 2:2 segregation in the second step is constant: lowest for β (XD/XZ), highest for α (XA/XZ), in between for γ (XC/XZ). Therefore, the chance of conversion at any point is represented by the lines BO, AO and CO, for categories β, α and γ, respectively.

a mutant α and a second β or γ mutant give a marked excess of conversion for the second site, regardless of its location. When this phenomenon was observed, for mutants *1186*, *889* and *1472*, it was suggested that this observation might be explained by the existence of several polarized units within gene *75*, with those mutant sites which failed to show 1:3 segregations corresponding to the minimum frequency regions of distinct polarons (RIZET and ROSSIGNOL 1966). This would be in agreement with the more recent hypothesis of HOLLIDAY (1968), which assumes that starting points for hybrid DNA may be located inside of functional genes. The further data of the present study reveal that these three peculiar mutants belong to the same category (α). The behavior of these mutants is no longer considered to be a consequence of their location within different polarons; it now seems more reasonable that this behavior is determined by the chemical nature of the heterozygosity formed between these mutants and the wild-type allele, as was suggested above. If such an interpretation is correct, none of the present findings requires that the starting point for hybrid DNA formation be located within gene *75*.

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SUMMARY

The conversion frequencies were studied in single-mutant × wild type and in mutant × mutant crosses of 15 colorless ascospore mutants of gene *75*. In single-mutant × wild type crosses, significant differences were observed among the mutants by comparing their dissymmetry coefficients (DC), defined by the ratio

of frequencies of 3:1 to 1:3 segregations. The values found for this coefficient permit one to place the mutants in five categories: α (11), β (1), γ (0.7), δ (0.04) and ε (2). Within each DC category, there is a regular increase of conversion frequency from the left to the right end of the map. In crosses between mutants of categories α, β and γ, α mutants are clearly distinguishable from β or γ mutants by their low frequency of 1:3 segregation, as expected from their dissymmetry coefficient.—A two-step process of recombination with 1) DNA-hybrid formation from a fixed starting point and 2) correction of mispaired bases at heterozygous sites, could account for existence of DC categories and polarization within each of these categories.

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