GENE CONVERSION IN THE PASADENA STRAIN OF *ASCOBOLUS IMMERSUS*[1]

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STUDIES on a strain of *Ascobolus immersus* collected in Pasadena, California (YU-SUN 1964) have shown unusually high rates of gene conversion at a number of sites of white-spored mutations. In most such examples conversion ratios 5+:3w (wild type:white) and especially 3+:5w have been common. Closely linked sites frequently have had very similar distributions of the different conversion ratios. In some instances mutants recovered following gene conversion (e.g., two of the six white segregants in 2+:6w asci) have characteristics considerably altered from those of the parental mutant, and from mutants recovered following regular segregation. Some of the data to be reported have a bearing on the lengths of regions undergoing repair—assuming that repair is the mechanism actually responsible for gene conversion (HOLLIDAY 1964; WHITEHOUSE and HASTINGS 1965; and others).

MATERIALS AND METHODS

Two standard wild-type stocks of opposite mating type, $K_5(+)$ and $P_5(-)$, were isolated from different asci occurring on the same sample of dung collected in the Arroyo Seco at Pasadena, California. These two stocks have turned out to differ in a number of respects other than mating type. Of importance to these studies is a difference in conversion rates of some white-spored mutants in crosses to these two wild types. All white-spored mutants to be discussed arose spontaneously in crosses between $K_5(+)$ and $P_5(-)$ and hence are of somewhat mixed ancestry. Because of difficulties in establishing allelism among mutants with a common phenotype the white-spored mutants to be discussed are referred to by their isolation numbers. Closely linked mutants *w*-79 and *w*-80 are near *clo*-6 in linkage group II (YU-SUN 1964); closely linked *w*-72 and *w*-87 in the same linkage group, but at a considerable distance; *w*-62 is in linkage group VI at a considerable distance from *clo*-4; closely linked *w*-10 and *w*-78 are less closely linked to *w*-75, and independent of all other established markers; and closely linked *w*-6 and *w*-68 are in a further independent linkage group at some distance from another white-spored mutant, *w*-74 (YU-SUN, unpublished).

In order to test mutant segregants of the (+) mating type with a wild type having the characteristics of $K_5(+)$ with respect to conversion rates and, similarly, to test mating type (—) mutants with wild types resembling $P_5(-)$, special wild-type stocks were derived from crosses with the particular mutants concerned. For convenience in summarizing data from different crosses which are essentially similar except for the mating types of the parents, the following symbols are used:

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P, any wild-type stock irrespective of mating type, which has the characteristics of $P_5(-)$ in relation to the mutant concerned.

K, any wild-type stock having the characteristics of $K_5(+)$ with respect to the mutant involved.

w-x, either the original mutant stock or a stock which is opposite in mating type and still retains the conversion characteristics of the original in crosses to P and K.

w-x(P) and *w*-x(K), stocks which have arisen by the introduction through gene conversion of the *w*-x mutant allele into chromatids of $P_5(-)$ and $K_5(+)$, respectively.

RESULTS

Variations in frequencies and types of conversion: Many of our white-spored mutants have not been sufficiently studied to determine their rates of gene conversion, and it is probable that a number of them have low rates comparable to those reported for Neurospora, Sordaria, etc.—usually not much more than 0.1% (literature reviews: HOLLIDAY 1964; WHITEHOUSE and HASTINGS 1965; EMERSON

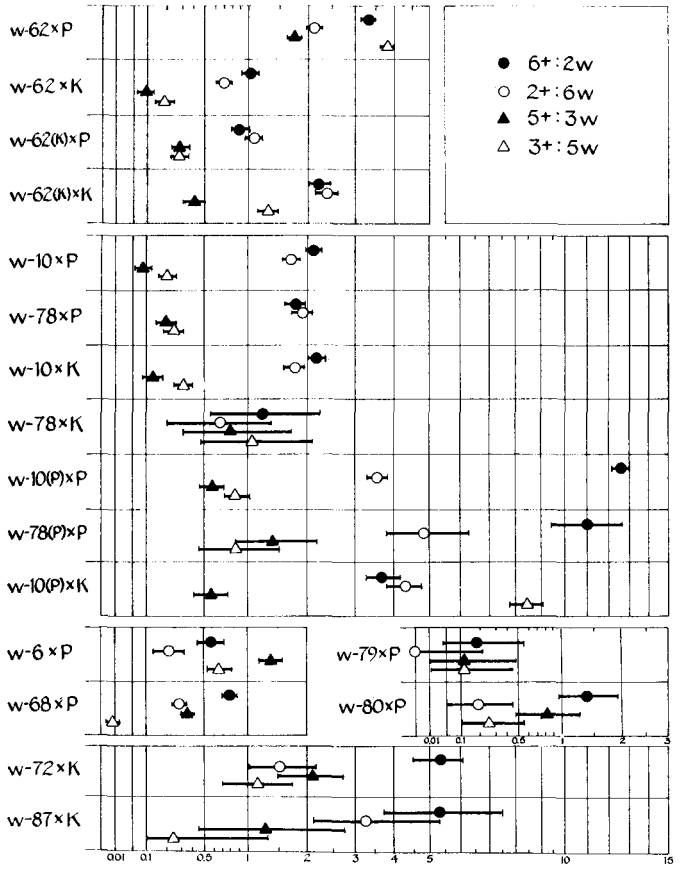

FIGURE 1.—Frequencies, in percent of total asci, of asci with different conversion ratios in crosses of different white-spored mutants to wild types. Horizontal bars designate the interval between 95% confidence limits.

1966). The lowest conversion frequency among our more thoroughly studied mutants is 0.43% (95% confidence limits +0.44/−0.25%) in the cross *w*-79 × P; and the highest frequencies are 17.7 ± 1.5% in *w*-10(P) × P, and 18.2 ± 2.0% in *w*-78(P) × P.

In addition to the considerable variation between mutants in total frequencies of gene conversion there are also, as can be seen in Figure 1, marked differences in the proportions of asci exhibiting the different ratios characteristic of gene conversion. For example the relative frequencies of asci with ratios 6+:2w, 2+:6w, 5+:3w and 3+:5w are respectively: 52%, 41%, 2% and 5% of 1,378 converted asci in the cross *w*-10 × P; 30%, 19%, 16% and 35% of 7,953 converted asci in the cross *w*-62 × P; and 20%, 8%, 48% and 24% of 325 converted asci in the cross *w*-6 × P.

Closely linked mutant sites: Observations of four pairs of closely linked mutant sites, *w*-10 and *w*-78, *w*-6 and *w*-68, *w*-79 and *w*-80, and *w*-72 and *w*-87, are summarized in Figure 1. The relative frequencies of different conversion ratios are very similar in the two members of each pair in crosses to the same wild type. The absolute frequencies are also similar except in the pair *w*-79 and *w*-80. Although there is greater similarity between pairs of closely linked mutants than between random pairs of unlinked mutants, larger clusters of closely linked mutants must be studied before any general tendency in this regard can be established.

Available data relative to linkage relationships between the pairs of mutants just referred to are summarized in Table 1. Functional tests for allelism, such as complementation between alleles in occasional heterokaryotic spores as described by ROSSIGNOL (1964), have not been attempted. On the other hand, two purely genetic observations may give some indication of allelic relationships between pairs of linked mutant sites.

(1) The meagre data from genotypic tests of 2+:6w asci produced in intercrosses indicate that reciprocal recombination is common between *w*-10 and *w*-75, which are less closely linked than others, and possibly also between *w*-79 and *w*-80. On the other hand, recombination between *w*-6 and *w*-68 is nearly always nonreciprocal, and less significantly so between *w*-72 and *w*-87. A low frequency of reciprocal relative to nonreciprocal exchange is a characteristic of heteroalleles in instances in which allelism has been established by functional criteria (for example, CASE and GILES 1964).

(2) Frequencies of 2+:6w recombinant asci in intercrosses between closely linked mutants are less than the sums of 6+:2w convertant asci in crosses of each mutant singly to wild type—2.6% instead of 10.5% in *w*-72 × *w*-87, 0.52% instead of 1.35% in *w*-6 × *w*-68, 0.24% instead of 3.96% in *w*-10 × *w*-78, and 3.8% instead of 23.7% in *w*-10(P) × *w*-78(P). There must either (a) be very much less conversion occurring when two closely linked sites are heterozygous than when only one is, or (b) conversions resulting in 6+:2*w*-x segregation at one site is very frequently accompanied by conversion resulting in 2+:6*w*-y at the other site to produce asci with eight white spores, six of which are + *w*-y and two *w*-x +.

TABLE 1

Frequencies of conversion to wild type in crosses of closely linked white-spored mutants in crosses to wild type, and frequencies of wild-type recombinants in intercrosses between mutants

	Crosses to wild type			Intercrosses between linked mutants						Tests of 2+:6w asci		
	Conversion ratios			Segregation ratios							Converted	
Cross	6+:2w (%)	5+:3w (%)	Total asci	1+:7w (%)	2+:6w (%)	3+:5w (%)	4+:4w (%)	Total asci	Map distance*	Reciprocal	to + at	
w-10 × P	2.18	0.09	33,071	0.06	6.4	0	0.01	8,549	3.2	11	*w*-10	*w*-75
w-75 × P	1.87	1.27	6,469								3	0
w-79 × P	0.19	0.12	1,617	0	0.87	0	0	5,131	0.44	1	*w*-79	*w*-80
w-80 × P	1.89	1.27	2,265								1	1
w-72 × K	5.28	2.08	1,899	0	2.6	0	0	1,021	1.3	0	*w*-72	*w*-87
w-87 × K	5.25	1.25	265								3	1
w-6 × P	0.56	1.37	11,563	0.02	0.52	0	0	9,862	0.27	0	*w*-6	*w*-68
w-68 × P	0.79	0.36	46,482								6	4
w-10 × P	2.18	0.09	33,071	0.18	0.24	0	0.01	8,755	0.18	0	*w*-10	*w*-78
w-78 × P	1.78	0.20	17,858								4	1
w-10(P) × P	12.70	0.57	15,038	0.8	3.8	0.1	0	791	2.2	. .	. .	. .
w-78(P) × P	11.0	1.38	1,373								. .	. .

* Map distance calculated as twice the number of wild-type spores among total spores, expressed as percent.

Alternative (a) resembles interpretations of the 'depressor' or 'marker' effect observed in pneumococcal transformations (ROTHEIM 1962), and possibly receives some support from observations to be reported in the following section. Alternative (b) has been demonstrated to be of frequent occurrence in interallelic recombination at the *pan-2* locus of Neurospora. Of 1457 asci from the cross *23* + *35* × + *72* + analyzed by CASE and GILES (1964, see review by EMERSON 1966), 11 exhibited nonreciprocal interallelic recombination. In one of these (ascus 78), segregation was 2+:6*m* at site *23* and 6+:2m at site *72*; in two asci (565 and 581) segregation was 6+:2m at site *23*, 2m:6m at site *72*, and 6+:2m at site *36*; and in a fourth ascus (529), segregation was 5+:3m at site *72* and 3+:5m at site *36*. Such results are expected on the repair interpretation (Figure 2) whenever two heterozygous sites are included in a common heteroduplex region in which excision of one strand extends over both sites. An analogous situation exists in bacterial transformation when the length of the donor DNA fragment incorporated into the bacterial genome extends over two heterozygous sites, and a quantitative treatment has been made by HOTCHKISS (1958).

Genetic changes accompanying conversion: After both (+) and (−) stocks of mutant *w*-62 had been established it was observed that the frequency of conver-

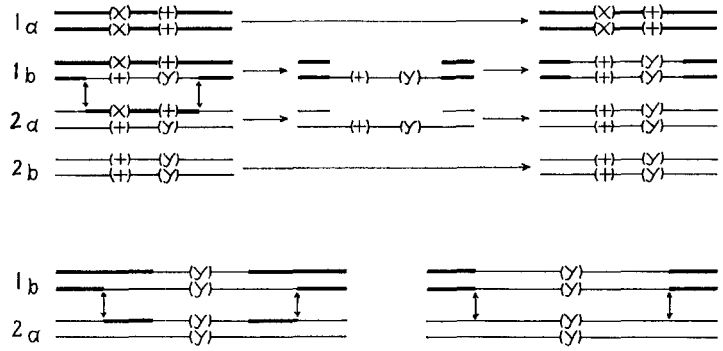

FIGURE 2.—*Upper part.* Repair model showing the origin of an ascus segregating 2 *w*-x+ : 6+ *w*-y. 1a and 1b, sister chromatids of one chromosome diagrammed as the two strands of a DNA double helix; 2a and 2b, sister chromatids of the homologous chromosome. An exchange between one base chain of 1b (heavy line) and one of 2a (light line) to form complementary heteroduplexes is shown in the left column: (x), the base in either chain corresponding to allele *w*-x; (y), that corresponding to *w*-y; (+), bases corresponding to wild-type alleles at either site. Center column: excision of bases in one chain of each heteroduplex, removing both *w*-x and the + allele of *w*-y in each. Right column: resynthesis of excised regions by the incorporation of bases complementary to the intact chains. Each of the four chromatids illustrated replicates once before spore formation.—Asci segregating 3 *w*-x + : 5 + *w*-y would result from failure of excision in one of the two heteroduplexes. *Lower part.* The two chromatids which have been involved in conversion in the cross + × *w*-y to give a 2+:6*w*-y ratio. (Diagrams correspond to those for the equivalent chromatids in the rightmost figure above.) At the left, excision has removed a segment shorter than the entire heteroduplex region—all four base chains differ somewhat in parentage as shown by positions of heavy and light sectors of the lines representing them. At the right, excision includes the entire heteroduplex region—both chains in each chromatid have identical parentage.

sion was much greater in the cross *w*-62(+) × P_5(−) than in the cross *w*-62(−) × K_5(+). A summary of all data collected to date from these and similar crosses is presented in the first two items at the top of Figure 1. From the cross *w*-62(+) × P_5(−), 23 randomly isolated wild-type segregants each gave a high frequency of conversion in crosses with *w*-62; and from the cross *w*-62(−) × K_5(+), each of nine randomly isolated wild types gave a low conversion frequency in crosses with *w*-62. These results suggested linkage between *w*-62 and a factor controlling rate of conversion, by which the two wild types differ.

To avoid the tedium of testing for a possibly rare crossover, use was made of a 2+:6w ascus from the cross *w*-62(−) × K_5(+), in which white-spored segregants arising from conversion should have the mutant allele associated with genetic material from K_5 on one or both sides. As shown in Figure 3, two of the six white-spored segregants from this ascus, 207, did give significantly lower frequencies in crosses to P than did the remaining four segregants which retained the characteristics of the original *w*-62 mutant. Unexpectedly, as shown in the second entry in Figure 3, the two isolates with lowered conversion frequencies in crosses with P gave heightened frequencies, especially in 6+:2w and 2+:6w classes, when crossed to K wild types. Additional data from crosses between *w*-62(K) and the two kinds of wild types, P and K, are summarized in Figure 1. Apparently P and K differ in some gene or genes closely linked to *w*-62 which result in lower conversion frequencies at the site of *w*-62 when heterozygous than when homozygous for either the P or K component. This is the observation referred in the previous section as resembling the 'marker' effect in pneumococcal transformation.

Somewhat different results have been obtained with mutant *w*-10 (asci 413 through 421 in Figure 3). In each of five 2+:6w asci from the cross *w*-10(+) × P_5(−), two of the white-spored segregants had greatly increased conversion frequencies in crosses to P, especially in the 6+:2w class. The remaining four white-spored segregants retained the characteristics of the original *w*-10. Crosses to K in this series were poor, and few counts were made. A summary of available information on such crosses is included in Figure 1 (cross *w*-10(P) × K), together with more extensive data from other related crosses. Frequencies and patterns of conversion are nearly identical in crosses *w*-10 × P and *w*-10 × K. In this example, segregants arising from conversion, *w*-10(P), have increased conversion frequencies in crosses to K as well as to P, but relative frequencies of classes 6+:2w and 3+:5w are extremly dissimilar. In contradistinction to *w*-62(P), the 'gene' obtained from P in *w*-10(P) would seem to have a dominant effect, enhancement of conversion, when in the *cis* position which it lacks when in the *trans* position. (P × *w*-10 and K × *w*-10 both give low conversion frequencies; *w*-10(P) × P and *w*-10(P) × K both give high conversion frequencies.) It should be noted that all white-spored segregants were tested for allelism with the original *w*-10, and all tests were positive.

Two points of possible theoretical interest are evident in these instances in which conversion has apparently altered the characteristics of segregants so arising. The two altered segregants from single asci are of a single mating type. In

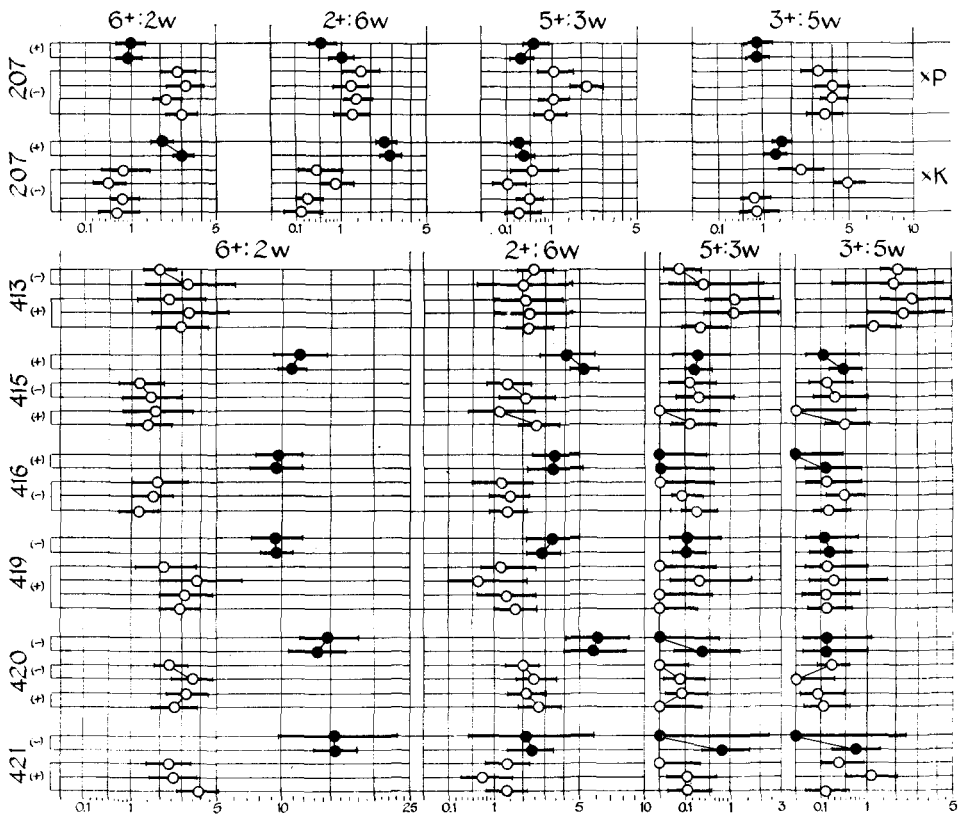

FIGURE 3.—Altered frequencies of conversion in white-spored segregants presumed to have arisen by conversion. 207, the six white segregants of a 2+:6w ascus from the cross w-62 × K_5(+) —frequencies (as percent of total asci) of asci with different conversion ratios in crosses of each segregant to wild types P and K. 413 to 421, tested white segregants of six 2+:6w asci from the cross w-10 × P_5(—)—frequencies of conversion asci in crosses of each segregant to wild type P. (+) and (—), mating types of the segregants. ○, segregants resembling the original parent mutant in conversion characteristics in crosses to wild types P and K; ●, segregants with conversion characteristics differing from the original white mutant, dots connected by diagonal lines indicate known pairs of sister isolates. 95% confidence limits are shown by the horizontal bars. *Note:* Crosses with one white-spored isolate of ascus 413, and one of 421, failed to fruit properly at the time of testing. Crosses with one isolate of ascus 416 produced aborted spores in a large fraction of the asci. These three isolates are omitted from this summary.

the four asci in which the remaining white-spored isolates were of mating type opposite to that of the altered ones it is evident that both altered segregants were derived from a single nucleus of the meiotic tetrad. The two altered segregants from single asci are extremely similar in conversion characteristics. The probability of heterogeneity as great or greater than observed between members of pairs of altered isolates is .99 for 6+:2w frequencies and .91 for 2+:6w frequencies in crosses of w-10(P) to P. On the other hand, there is considerable variation between altered isolates from different asci. (The probability of heterogeneity as

great or greater than observed between pairs from different asci is about .01 and .05 for 6+:2w and 2+:6w frequencies, respectively.)

That two sister spores (products of a single meiotic chromatid) should acquire identical properties during conversion could be readily understood on a model by which whole chromatids were converted by some single event, such as copy choice occurring during the last but one premeiotic replication of DNA. Restrictive assumptions are necessary in accounting for this situation by repair of mispaired bases in heteroduplex DNA molecules whether the heteroduplexes arose by an exchange of base chains during meiosis or by copy choice at the last premeiotic DNA replication. As shown in Figure 2, excision and resynthesis over the entire region of hybrid DNA is required for the production of strands completely identical in parentage; and the strand replaced must be that which had not previously undergone alteration through strand exchange or by copy choice. Repair must include the whole heteroduplex region, but could be more extensive without altering homologies between sister spores. If the difference between pairs of *w*-10(P) segregants in different asci proves to be significant, the differences might be accounted for by differences in length or position of the heteroduplexes. Ascus 413, in which none of the five white-spored isolates had the characteristics of *w*-10(P) could represent an extreme variation of that sort.

We have recently obtained *w*-78(P) segregants in a 2+6w ascus from the cross *w*-78 × P. Conversion characteristics (Figure 1) of this segregant are similar to those of *w*-10(P), with which it is closely linked (Table 1). On the other hand, we have so far failed to obtain altered segregants of the closely linked mutants *w*-6 and *w*-68 in 2+:6w asci from crosses to wild types.

Statistical validity: The use of 95% confidence limits in connection with the data that have been presented is misleading to some extent because variables other than those related to sampling are present. Scoring genotypes on the basis of phenotypic expression of spore color is not always completely accurate, depending to a considerable extent upon the particular stocks crossed and upon environmental conditions during fruiting. It is our belief that errors of this sort are not too serious in the crosses we have reported. The differences in conversion characteristics here reported have been qualitatively consistent throughout; and the quantitative differences observed in crosses of different mutants to wild types are usually so large that we are not too disturbed by the considerable heterogeneity encountered in repetitions of individual crosses.

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SUMMARY

Unusually high frequencies of gene conversion, and a wide range of frequency distribution among different conversion ratios, were observed. Closely linked white-spored mutants (presumptive alleles) have similar conversion characteristics. Certain mutant segregants presumably arising from conversion have con-

version characteristics in crosses to wild type which differ considerably from the parent mutants. The relation of these observations to DNA-repair models for gene conversion is discussed.

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