important staphylococcal characteristics such as hemolysin and coagulase production will become available for analysis, either directly through selection or by means of linkage to other selectable markers. More details of the above and the results of further experiments will be reported elsewhere.

Summary.—Genetic transduction by staphylococcal bacteriophage has been demonstrated. About one phage particle in $10^7$–$10^8$ transfers the genes for resistance to streptomycin and novobiocin.

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2 National Collection of Type Cultures (NCTC), London, received in lyophilized form, of the following phages have been examined: 9300(42F); 8429(55); 8413(29); 9788(80); 8406(53). Excepting NCTC 8406, these phages were either difficult to grow, or non-lysogenizing (lytic) phages.


6 Symbols are $N$, Novobiocin; $S$, Streptomycin; $E$, erythromycin; $Lac$, lactose fermentation; $Man$, mannitol fermentation; $Ly$, lysogenicity. Superscripted: $r, s$, resistant and sensitive respectively; $+,-$, ability to ferment and inability to ferment carbohydrates, except in the case of $Ly$, where $+$ signifies lysogenic, $-$ signifies nonlysogenic and sensitive to exogenous phage. The parent staphylococcal strain is inhibited by 1 mcg/ml streptomycin and 0.05 mcg/ml novobiocin. The resistant strains are not inhibited by 25 mcg/ml and 2 mcg/ml, respectively. The concentration of the drugs in the overlay agar were: $S$, 100 mcg/ml; $N$, 20 mcg/ml.

7 Unpublished observations and a personal communication from Dr. P. M. Rountree.

8 Unpublished observations, John Mann, this laboratory.


ABERRANT TETRADS IN SORDARIA FIMICOLA*

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In an earlier paper1 describing three ascospore color mutants in the homothallic pyrenomyecete *Sordaria fimicola*, the rare occurrence of unusual tetrads with unexpected ratios of wild-type and mutant ascospores was noted for all three loci. In the course of further studies of two of these mutant loci, a number of ascii showing aberrant tetrads were isolated and analyzed in an effort to obtain a clearer idea of the mechanism responsible for their occurrence. Such tetrads in yeast had previously been explained by Lindegren2 as resulting from "gene conversion" in a heterozygote, usually involving a change of the dominant gene to its recessive allele. Later, Mitchell3 reported similar abnormal segregations for the pyridoxine locus in *Neurospora*, which she explained as probably resulting from double replication.
A similar explanation is offered by Case and Giles in an interpretation of irregular segregation at the pan-2 locus in N. crassa. These authors use the term "copy choice" suggested earlier by Lederberg. Glass refers to the phenomenon as "transreplication," a term that seems adequate for purposes of this discussion.

Procedure.—All asci described here were derived from crosses between cultures with the wild-type allele for the production of dark ascospores and either of two different ascospore color mutants, hyaline-spored (h) or gray-spored (g). All crosses were also heterozygous for one other mutant factor. Perithecia from the line of contact between paired cultures were pressed under a cover slip, after which the hybrid clusters of asci were removed to an adjacent drop of water on the slide and flattened out under a cover slip. These asci were then examined for aberrant tetrads showing disproportionate numbers of wild-type and mutant spores. A cluster containing such an ascus was removed to a dish of agar and the desired ascus isolated and dissected.

Results.—An analysis of 23 abnormal asci was made (Table 1). The first 12 asci were derived from crosses heterozygous for the h locus, which is situated at about 32.5 crossover units from the centromere, and for one other locus—d1, d2, or a-2. Unpublished data on the last three loci show that they are unlinked, but that d1 is linked to h with a distance of about 28 crossover units between them. Both d1 and d2 cause dwarfness of cultures, while a-2 is recessive for ascus abortion. In asci heterozygous for the a-2 locus, the a-2 ascospores develop normally. The d1 locus is only a few crossover units from the centromere, while d2 is about 14.5 units out on its chromosome arm, and a-2 is far out on its chromosome arm. The h factor, in addition to causing hyaline spore color, is lethal for ascospore germination.

Asc1–7, 10, and 11 have a ratio of 6h+:2h ascospores. With respect to the

<table>
<thead>
<tr>
<th>Cross</th>
<th>Spore Color Ratio</th>
<th>Spore No. and Genotype</th>
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<tbody>
<tr>
<td>h+d1+Xh1d1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h+a-2Xh2a-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g+alpha-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xg+alpha-1</td>
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* Unrecovered progeny in parentheses. The h allele is lethal for ascospore germination. The aberrant alleles, whose identifiable (in 3:3 asci), are indicated in bold type. The symbols + and − signify wild type and mutant alleles, respectively; the first symbol under each spore genotype refers to a spore color locus.
second locus involved in each cross, two pairs of dark spores carry the mutant allele and one pair the wild-type, or two pairs carry the wild-type and one pair the mutant allele. In other words, one pair of spores which would be expected to contain the \( h \) allele, has instead the wild-type allele at that locus. It is not possible to determine which of the three pairs is the aberrant one. Since \( h \) and \( d_1 \) are linked and \( d_1 \) is near the centromere, it is likely that in a majority of the first seven asci, the converted pair is the one adjacent to the hyaline pair. Asci 8, 9, and 12 contain ascospores in the surprising ratio of 5\( h^+ : 3h \). In each of them it is possible to identify the spore carrying the transreplicated locus, since its adjacent partner carries the \( h \) allele and is hyaline.

Asci containing an excess of \( h \) spores in proportion to \( h^+ \) were also observed, but it was not feasible to dissect them for further analysis in view of the failure of \( h \) spores to germinate. However, an examination of 2,700 hybrid asci was made in order to obtain some idea of the relative frequency of the different types of abnormal tetrads. Of this number 11 asci, or about 0.4 per cent, contained visibly abnormal tetrads. Six showed a 6\( h^+ : 2h \) segregation, five of these having the six dark spores in a series and one having the arrangement 2\( h^+ : 2h : 4h^+ \); five asci showed a ratio of 2\( h^+ : 6h \), four of these having all six hyaline spores in an uninterrupted series and one having them in the order 2\( h : 2h : 4h \). No 5:3 segregations were observed in this group, and in general they appear to be less common than 6:2 asci. It would appear from the data that transreplication of the \( h \) locus, if that is the phenomenon involved, is about equally common in either direction. However, in view of the fact that rare hyaline spores are produced probably as the result of other aberrations in nuclear division, it is not possible to be sure that all extra hyaline spores have developed in like manner.

The gray-spored locus (\( g \)) offers a better approach to this problem, since gray and wild-type spores are equally viable. Asci 13–23 resulted from crosses heterozygous for the \( g \) locus, which is situated far out on its chromosome arm, and for one additional factor either \( stm \) or \( a-1 \). The loci appear to be unlinked. The crosses involving \( stm \) are all homozygous for another mutant locus \( st-1 \), which causes near sterility in cultures carrying it. The \( stm \) allele converts \( st-1 \) cultures into completely self-sterile ones.\(^7\) The \( a-1 \) allele has an effect exactly like \( a-2 \) in causing ascus abortion. There appears to be no linkage of \( g \) with either \( stm \) or \( a-1 \). The \( stm \) locus is about 6 crossover units from the centromere, while \( a-1 \) is far out on its chromosome arm. Asci 16–20 show a segregation of 6\( g^+ : 2g \), and in all of them the second locus segregated in a 4:4 ratio. Again it is not possible to identify any of the three pairs as the one which has resulted from transreplication. Figure 1 shows an ascus of this type and Figure 2, the eight cultures derived from spores of the same ascus (Ascus 19, Table 1). All of these crosses are heterozygous for unselected factors affecting growth rate and perithecial distribution. This is of some advantage to the analysis, since it offers another way of recognizing spore pairs resulting from the third division in the ascus and insures against any misinterpretation due to disorderly spore arrangement.

Asci 13–15 and 21, which show a ratio of 5\( g^+ : 3g \), are among the most revealing ones obtained. In all of them it is possible to identify the spore carrying the transreplicated locus, since it is a dark spore and its sister spore is gray. Figure 3 shows such an ascus and Figure 4, the eight cultures obtained from it (Ascus 21, Table 1).
Figs. 1–4.—Aberrant tetrads in Sordaria fimicola. Fig. 1, 6g+ : 2g ascus (Ascus 19, Table 1); Fig. 2, progeny on corn meal agar plates; Fig. 3, 5g+ : 3g ascus (Ascus 21, Table 1); Fig. 4, progeny on corn meal agar plates. (Spores in asci are numbered from left to right.)
It is obvious that the second spore in this ascus is the odd one. The cultures demonstrate another interesting feature, i.e., the culture derived from the odd dark spore is conspicuously darker than that derived from the gray sister spore. Our original gray-spored mutant produces a light, opaque mycelium, whereas, the wild-type produces a dark pigment, probably melanin. The difference is usually more pronounced in test tube cultures than on agar plates. In all of the $5g^+ : 3g$ ascis the unexpected dark spore gives rise to a pigmented mycelium. Also the dark spores in ascis with a ratio of $6g^+ : 2g$ produce pigmented colonies. Mycelial pigmentation is in part a pleiotropic effect of the $g$ locus. Some of our unpublished data indicate that one or more closely linked loci may also be involved in mycelial pigmentation, but since this character is not a sharply defined one and since other unselected loci also modify the degree of pigmentation, we have not been able definitely to establish this. If such loci do exist, the data indicate that they may also undergo transreplication simultaneously with the $g$ locus. However, it is not likely that this could be confirmed without the aid of closely linked markers with more clear-cut phenotypic effects.

Only two ascis with a greater than normal number of gray spores were isolated from these crosses. They appear to be less common than the foregoing types. Ascus 22 shows a $2g^+ : 6g$ segregation and ascus 23, a $3g^+ : 5g$ ratio. The third spore of the latter ascus, which is obviously the odd one in the series and has apparently resulted from transreplication of the $g$ allele instead of the normal duplication of the $g^+$ allele, produced a mycelium that was lighter than that derived from the fourth, or sister, spore carrying the $g^+$ allele. Otherwise the two cultures were alike in growth rate and appearance, being slower than any of the others. Abnormal tetrads resulting from crosses heterozygous for the $g$ locus are distinctly less common than those from crosses heterozygous for the $h$ locus. Although our observations do not at this time permit an accurate statement regarding their frequency, it is estimated that not more than one ascus in 800–1,000 show such aberrant tetrads.

The genotypes of all spore progeny from abnormal tetrads, when examined at maturity or in crossing tests, proved to carry the color factor assigned to them in direct examination of the ascis. A cross between the culture derived from the odd dark spore (the fourth) in ascus 13 and a $g$ culture resulted in further transreplication among the hybrid ascis. Therefore, the transreplicated locus behaves in every way like the typical wild-type locus.

Discussion.—In an earlier paper it was reported that an examination of many thousands of ascis of both ultraviolet irradiated as well as non-irradiated cultures of each of the spore color mutants failed to produce any evidence of back mutation at the color loci. Although a much larger number of ascis have now been observed over an additional 4-year period, there is still no evidence of back mutation at these loci in pure mutant cultures. It is also clear from the evidence that the aberrant ascis described here cannot be the result of typical crossing over, since there are no reciprocal products of crossing over among the progeny. It is equally obvious that the odd ratios have not resulted from nuclear degeneration in the ascus followed by compensating nuclear divisions among the surviving genotypes, since the spores may be readily paired off by means of a second genetic marker in each cross and by unselected factors affecting growth rate. The most logical explanation would
appear to be one based on a transreplication or copy choice mechanism occurring at meiotic prophase while the chromatin strands are duplicating.

The most significant asci found are those with a 5:3 ratio for spore color. While it is possible to explain 6:2 asci on the basis of transreplication within the conventional 4-strand model at meiotic prophase, this is not feasible with the 5:3 asci. Ris\textsuperscript{8} has obtained evidence from electron microscopy that chromatids are further subdivided into half-chromatids or quarter-chromatids, depending upon the organism and varying states of the chromosomes in the same organism. Taylor\textsuperscript{9} proposes that each chromatid is composed of two functional subunits. He has anticipated the transreplication mechanism that would lead to the production of a 5:3 ratio (see his Fig. 7, B). The 6:2 ratio is explained on the basis of at least one breakage among the substrands in conjunction with transreplication off both substrands of the chromatid being copied at a particular locus (see his Fig. 7, C–E). This interpretation would readily account for the results which we have obtained. Our 5:3 asci support the concept of the chromatid as being composed of two functional subunits.

The aberrant asci reported here could actually be more accurately referred to as octads. Certainly, the 5:3 pattern would not be readily detected in an ordinary tetrad of four meiospores, nor in an 8-spored ascus if the ascospores were isolated in pairs. The third division in the ascus is required for the separation of the transreplicated substrand from its normal partner. In a 4-spored yeast, for example, a spore carrying a heterogeneous chromatid would most likely give rise to mixed progeny and the data interpreted as resulting from a binucleate, heterokaryotic spore, back mutation in the developing colony, or contamination. The phenomenon might also contribute interesting effects elsewhere. For example, some of the reports of self-fertility among single-spore isolates of heterothallic ascomycetes and of spontaneous dikaryotization in single-spore cultures of basidiomycetes might have resulted from this mechanism.

Eight different ascospore color mutants of \textit{S. fimicola} have now been produced in our laboratory. It would be ideal to obtain other mutant loci closely linked on either side of a color locus so that more detailed information could be obtained on the area transreplicated.

\textit{Summary}.—A study of 23 asci showing aberrant segregation for ascospore color revealed that some mechanism other than crossing over, back mutation, or irregularities in nuclear survival is responsible for their occurrence. It is believed they have resulted from transreplication, by which a locus is copied more than the normal number of times during replication at meiotic prophase. The occurrence of 5:3 ratios in some asci lends confirmation to the hypothesis that each chromatid is composed of two functional subunits.

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* Supported by NSF Grant G-2808 and NIH Grant E-2326.
2 Lindegren, C. C., J. Genet., 51, 625–637 (1953).