An entirely new pattern of gene segregation, aberrant 4:4 segregation, was discovered as early as in 1962; at that time it was astonishing. The acceptance of this gene segregation pattern into the implication of the mechanism of gene conversion and recombination was done readily. Nevertheless, no presentation of actual photograph of this ascus type has yet been done. Therefore, it has long been desired by many researchers in the related fields to see a colour picture of aberrant 4:4 ascus to acquire an appropriate understanding on this pattern of gene segregation. By this reason, to give a clear distinction of aberrant 4:4 ascus from normal 4:4 asci in colour plate is the prime objective of this presentation.

Accounting correctly or not of aberrant 4:4 type in 2-chromatid conversion event makes difference in the understanding of ascus types in 4-chromatid conversion event. Therefore, to introduce a clear new method of separating the fundamental ascus types of the involved two sets of chromatid pairs (chromatids 2, 3 and chromatids 1, 4) for all the ascus types expected in 4-chromatid conversion event is the second objective of this presentation.

I. Analysis of aberrant asci particularly regarding aberrant 4:4

The asci subjected in the colour exhibition (Fig. 1) are from the one-point cross of g, analyzed in Kitani and Whitehouse (1974), and they are sister asci in a single perithecium. For the general practice of gene conversion analysis, refer to Materials and Methods of Kitani and Olive (1967, 1969), Kitani and Whitehouse (1974) and Kitani (1978). For the general information of all the aberrant asci in Sordaria fimicola, please refer to Appendix of Kitani (1978). The relation of the g-locus alleles and the outside markers can be seen in Fig. 1 of Kitani (1978).

II. Means for the recognition of aberrant 4:4 segregation

The discovery of aberrant 4:4 segregation like the one shown in Fig. 1f was done by a combination of curiosity and luck in the gene alignment of the cross then in examination. In Sordaria fimicola, the frequency of asci with spore displacement (called as "slippage" or "spindle overlap") is low (about 6%). However, when a spore displacement occurs between the second (white) spore pair and the third (black) in the ascus.

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shown at the upper-right corner of Fig. 2 (normal 4:4 conversion), the spore arrangement becomes the same as the one in the ascus at the centre of the figure (aberrant 4:4).

Since spore displacement usually occurs only between the adjacent spore pairs, it looked curious when an ascus of spore arrangement g, g+, g+, g+, g+, g, g, g was discovered. After the dissection of this ascus it was found that the spore colours of the following generation were just the same as the dissected spores. But surprisingly indeed, the colonial genotypes of the upper four and the lower four spores were \( sp \text{ mat}^+ \text{ cor} \) and \( sp^+ \text{ mat} \text{ cor}^+ \), respectively, without displacement.

The presence of the entirely new mode of gene segregation, aberrant 4:4 segregation, was first reported simultaneously in two papers (Kitani 1962 and Kitani et al. 1962). Then, readily incorporated into the explanations of the origin of gene conversion by Whitehouse (1964) and Emerson (1966). However, to rely the recognition of aberrant 4:4 type only on the morphological flanking makers is inefficient because the recognition can be done only after the troublesome process of ascus dissection, spore isolation and cultivation. To get rid of this inconvenience and to improve the accuracy drastically, an unlinked spore colour marker \textit{indigo} (i) was employed in the system for the \( g \)-locus conversion analysis. All the \( g \)-locus asci reported in Kitani and Olive (1967) and thereafter are marked with \textit{indigo}. However, no colour photographs of aberrant 4:4 asci were clear enough in the distinction of \textit{indigo} character until the photomicrograph of Fig. 1 was taken in 1974 in the one-point cross of \( g_7 \).

As shown in the colour plate (Fig. 1), the \( g-g^+ \) spore pairs (customarily called “odd-spore-pairs”) of the aberrant 4:4 ascus (f) can be distinguished from the displaced spore pairs of the normal 4:4 ascus (e). The \textit{indigo} phenotypes of the \( g^+ \) (darkly pigmented) spores of two odd-spore-pairs are different from each other in aberrant 4:4 asci, but the same (either i or i+) in normal 4:4 asci with spore displacement. Spore pairs 2nd and 3rd (spores 3, 4 and 5, 6) in Fig. 1e and 1st and 2nd (spores 1, 2 and 3, 4) in Fig. 1f are the odd-spore-pairs. In the case of Fig. 1f (Ab. 4:4), spores No. 2 and No. 3 are darkly pigmented \( g^+ \), but regarding the \textit{indigo} (i) locus No. 2 spore is i+ (therefore, brownish) and No. 3 spore is i (therefore, bluish). Spore No. 1 is yellowish grey (genotype \( g \text{ i}^+ \)) and spore No. 4 is bluish grey (genotype \( g \text{ i} \)) as clear in the figure. While in Fig. 1e (N. 4:4), both of the \( g^+ \) spores (Nos. 4 and 6) of the odd-spore-pairs are the same i (bluish).

Although a negligible proportion of aberrant 4:4 asci would still be overlooked (detailed explanation is given in Kitani and Whitehouse 1974), the \textit{indigo} spore colour character facilitated recognition of aberrant 4:4 ascis from spore displacement under the microscope. The \( g \)-locus—i-marker research system in \textit{Sordaria fimicola} has long been the only effective system allowing full recognition of this ascus type. Recently, Paquette (in press) established an effective system to recognize aberrant 4:4 ascis with two spore morphology mutants (\textit{round-spored} and \textit{granular-spored}) and two mycelial markers (\textit{brown} and \textit{wave}).

III. Origin of aberrant 4:4 and five other fundamental ascus types

Aberrant 4:4 is one of the six gene conversion patterns characteristic to the octads of one-point-cross conversion events of eukaryotic organisms. These are shown in Fig. 2
Fig. 1. Asci showing segregations of normal 4:4 and aberrant 4:4.
Fig. 2. Origin of six fundamental ascus types of one-point cross gene conversion. Chromatid 2 and chromatid 3 carried mutant type allele and wild type allele, respectively, before they were involved into hybrid DNA (DNA heteroduplex) formation. For classes Ra and Rp, and subclasses -1 and 2, refer to Kitani and Olive (1967) and the legend of Fig. 3. (r) and (s) indicate that the respective ascus types belong to restoration group and substitution group, respectively (refer to Kitani and Olive 1969).

Fig. 3. Hypothetical steps of heteroduplex (hybrid) DNA formation. These steps are followed with base correction depicted in Fig. 2. The characteristic features of these models are the involvement of entire region of a cistron in heteroduplex DNA formation and the differentiation of classes Ra and Rp (flanking marker recombination absent and present) at the first step. Through Model I, conversion event is not associated with flanking marker recombination (class Ra), but through Model II, conversion event is associated with flanking marker recombination (class Rp).
with the possible origin of ascus genotypes based on the concept of heteroduplex DNA formation and base correction. The ascus type "aberrant 4:4 segregation" is shown here as resulted by a combination of the mode "no correction" of DNA bases in both of the chromatids (chromatids 2 and 3) involved in the heteroduplex DNA formation. This figure itself is a modified version of the one appeared in Kitani and Olive (1967), but originally based on Whitehouse (1963, 1964) and Emerson (1965).

According to the concept precipitated as Fig. 2, gene conversion ascus types are determined by the following steps:

1) Heteroduplex (hybrid) DNA formation at a gene locus. The real mechanism of hybrid DNA formation in eukaryotes is not at all known even though numerous hypotheses are in flourish (Sobell 1972; Meselson and Radding 1975, etc.).

2) Base correction at the base-unmatched portion of the heteroduplex DNA in the two chromatids involved. Choice of the three correction modes is independent from each other in the respective two chromatids. From different combinations of base correction modes, different ascus types are developed in the octads.

Aberrant 4:4 type is, therefore, the product of a combination of "no correction" mode in both chromatids 2 and 3 as shown in Figs. 2 and 3 (Fig. 3 is to show the process of heteroduplex DNA formation). Since this type is the direct product of the intact (base uncorrected) DNA heteroduplex, this is the basic type of all the other conversion ascus types. Whatever the mechanism of initiation of heteroduplex DNA was, the process and the ultimate situation of the chromatids involved are like shown in Fig. 3.

IV. Method to separate a 4-chromatid conversion ascus type into two 2-chromatid fundamental ascus types

Only two of the four chromatids of a meiotic bivalent are involved in almost absolutely all the aberrant asci found in Sordaria and Neurospora in both one-point and interallelic crosses. This fact was confirmed repeatedly (Kitani and Olive 1967, 1969, 1970), and there was no ambiguity at all in aberrant 4:4 asci in determining the number of chromatids involved. Only a few conversion asci exceptionally involving more than two chromatids are shown in some detail, including one double aberrant 4:4 same as the one at the centre of Fig. 4 and a 7+ :1 m ascus, in Appendix of Kitani (1978). These asci can be explained by the involvement of four chromatids in a single conversion event. Quite different from Sordaria, octads of so called "wider ratio" (8:0, 0:8, 7:1 and and 1:7) frequently occur in one-point crosses of Ascobolus immersus. These asci were explained by Lamb and Wickramaratne (1973) as resulted from two pairs of heteroduplex DNA, which means that all four chromatids of a bivalent were involved in a single gene conversion event.

Paquette (in press) widened the range of one-point cross ascus types by the helps of spore morphology mutants; he could identify aberrant 6:2, ab. 5:3 and double ab. 4:4 in addition to the fundamental ascus types and the wider ratios. These secondary ascus types can easily be explained as the products of 4-chromatid involvement in a conversion event. The origins of all ascus types of such event can be figured out by the combinations of the three modes of base correction in all four chromatids as shown in Fig. 4. This is an extended application of the concept precipitated as Fig. 2 for
2-chromatid events. The application of Fig. 4 in an actual practice of classifying 4-chromatid conversion ascis in the term of the fundamental 2-chromatid conversion ascus types includes the following steps: a) The respective locations of the ascus type subjected in the analysis are traced in Fig. 4. b) When the location of an ascus type is only one in Fig. 4, the fundamental ascus type for both pairs of chromatids is immediately found through Fig. 2. c) When an ascus type takes more than one location in Fig. 4, to pinpoint the appropriate location depends on the recombination class (and subclass) determined through the crossover relation of outside markers to the conversion locus. For the details of recombination classes and their relation to the ascus genotypes, refer to Kitani and Olive (1969) and Kitani (1978). For the handling of Fig. 4, some modification is necessary from the one for interallelic crosses (Fig. 3 of Kitani 1978).

**DISCUSSION**

Aberrant 4:4 ascus type is the undisturbed intracistron gene segregation pattern after the formation of heteroduplex DNA (for intercistron reciprocal recombination, heteroduplex DNA may not be formed; consequently no aberrants 4:4 segregation). However, the conditions required for the effective recognition of this type are very rigid limiting the working systems allowing analysis of this ascus type only to the g-locus in *Sordaria* and Paquette's one in *Ascobolus*. It should be clearly understood that the
conversion frequency of any mutant allele is not solid when aberrant 4:4 type and normal 4:4 conversion type are overlooked (refer to Fig. 2 and Kitani and Olive 1969). Since it is known that the proportions of these customarily overlooked ascus types can vary from near nil to exceeding 50% cross to cross (Kitani and Olive 1967, 1969), discussions handling conversion frequencies in excluding these ascus types should be weighed with reservations.

There have been two sorts of theories on the mechanism of recombination different regarding the mode and extent of heteroduplex DNA formation. One of them is based on the involvement of only a part of cistron and the uneven participation of chromatid complements. Theories of this sort, such as Whitehouse and Hastings (1965), Fogel and Hurst (1967), Meselson and Radding (1975), require DNA synthesis during the process of recombination (such as the late DNA of lily; a vogue is present to connect it with recombination) and the difference in conversion frequencies among the alleles respective to their locations in a cistron (called as polarity). The other sort is based on the involvement of entire cistron and the even participation of chromatid complements. Theories of this sort, such as Whitehouse (1964), Emerson (1969), Holliday (1964), etc., require no DNA synthesis nor difference in conversion frequencies between alleles in a cistron. A schematic process of hybrid DNA formation to represent this sort is shown in Fig. 3. This figure explains also the origin of flanking marker recombination as an integrated part of gene conversion event.

The data of conversion frequencies gathered in the g-locus system, in which aberrant 4:4 type is fully accounted in the total conversion frequencies, showed a constant value of 0.2% for all the alleles in all kinds of crosses (for this value, refer to Kitani and Olive 1967 and 1969 and Table 3 of Kitani and Whitehouse 1974; for the proportions of ascus types aberrant 4:4 and normal 4:4 conversion to the total frequencies, refer to Table 3 of Kitani and Olive 1967, and Tables 2 and 3 of Kitani and Olive 1969; and for the method to estimate the proportion of normal 4:4 conversion ascus type in the total conversion frequency, refer to Table 3 of Kitani and Olive 1967). This fact and the absence of interference in association with gene conversion events (Kitani 1978) jointly select out one sort of theories with total involvement of a cistron in a hybrid DNA, because the characteristic feature of this sort is the absence of polarity in conversion frequencies among the alleles in a cistron; polarity is the feature in the other sort.

CONCLUSION

Aberrant 4:4 segregation played a key role to select out one sort from the two different sorts of theories on the mechanism of gene recombination. It is therefore hoped that the features of this gene segregation pattern are understood, and means to recognize this kind of octads are organized in many eukaryotic organisms in addition to the present Sordaria and Ascobolus.
SUMMARY

A presentation of an aberrant 4:4 ascus was made in colour photomicrograph. Means for the recognition of aberrant 4:4 asci and the possible origin of conversion ascus types were explained. A new method was introduced to extract the fundamental ascus types of the two pairs of chromatid complements from the ascus types of 4-chromatid conversion. A discussion was done on the role of aberrant 4:4 type in choosing one kind of theories from the two which were different in the manner of hybrid DNA formation.

LITERATURE CITED


