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The detection, in unordered octads, of 6+:2*m* and 2+:6*m* ratios with postmeiotic segregation, and of aberrant 4:4s, and their use in corresponding-site interference studies

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SUMMARY

Systems for the detection of postmeiotic segregation in unordered octads are described for 4:4(2), 6:2(2), 2:6(2), 5:3(3), 3:5(3) and 4:4(4) segregations, where the number in parentheses is the number of pairs of non-identical sister-spores. Linkage relations, centromere distances and phenotypic detectability of suitable combinations of markers for initial selective identification of such segregations, and subsequent confirmation tests, are discussed; correction formulae are given to allow for non-detection of certain classes in particular circumstances. Using one such system in *Ascobolus immersus*, we detected 4:4(2), 6:2(2) and 2:6(2) segregations at the *w*-78 site: 2:6(2)s were previously unknown. Initial visual detection in this system was from octad phenotype ratios for *w*-78 (white ascospore) and *gr*-3 (granular ascospore pigmentation), with mating type and parafluorophenylalanine resistance as confirmatory markers after germination. Corresponding-site interference for wider ratio and – for the first time – narrower ratio unique classes was determined: it was moderately positive or moderately negative, depending on the cross and segregation class involved.

1. INTRODUCTION

The study of aberrant segregation ratios in fungal tetrads and octads has been of great value in understanding recombination and gene conversion. In wild-type (+) × mutant (*m*) crosses, the ratios 6+:2*m*, 2:6, 5:3 and 3:5 are well known from several fungi and have been termed 'narrower ratios' as opposed to the 'wider ratios' 8:0, 0:8, 7:1 and 1:7 (Lamb, 1972). Lamb & Wickramaratne (1973) reported the occurrence of genuine wider ratio octads 8+:0*m* and 7+:1*m* in the Pasadena strains of *Ascobolus immersus* and pointed out that such segregations required that both pairs of non-sister chromatids of a bivalent must be involved in hybrid DNA formation at exactly the same point, although narrower ratio segregations only require one pair of non-sister chromatids to pair at a point. As well as the wider ratio octads, they predicted the occurrence of other 'unique classes' (those requiring hybrid DNA formation in both pairs of chromatids at a particular site), namely 6:2(2), 2:6(2), 5:3(3), 3:5(3) and 4:4(4), where the number in parentheses is the number of pairs of non-identical sister-spores; that is,

tetrad products showing post-meiotic segregation when the octad stage is reached.

One aim of the present work was to devise methods for identifying as many unique classes with postmeiotic segregation as possible, and to identify aberrant 4:4(2) segregations, even in unordered octads where sister-spores are not identifiable just from their physical arrangement in an ascus as they are in ordered octads. Even with ordered octads, as in *Neurospora crassa*, *Sordaria fimicola* and *Sordaria brevicollis*, additional markers are advisable for checking that apparent postmeiotic segregation has not arisen by spindle overlap (partial or complete) at the third division in the ascus, or nuclear passing or spore slippage. The systems described here for unordered octads can also be used with ordered octads, especially as a safeguard against spurious postmeiotic segregations.

The second aim was to use segregation types such as 6:2(2) and 2:6(2) in *Ascobolus immersus* for studying corresponding-site interference, for comparison with the data on 8:0 and 7:1 classes obtained by Lamb & Wickramaratne (1973). They defined corresponding-site interference as: 'interference between the two pairs of non-sister chromatids of a bivalent in hybrid-DNA formation at exactly corresponding sites', and derived formulae for expected frequencies of all 'unique classes' in the absence of corresponding-site interference. In the present work, correction factors have been deduced to allow for the expected non-detection of certain proportions of postmeiotic segregation events in the special systems used here with unordered octads. Information on 4:4(2) frequencies was required for these corresponding-site interference calculations and also for the interpretation of conversion results (Wickramaratne & Lamb, 1975) for the *w*-10 and *w*-78 sites in *Ascobolus immersus*, where a factor closely linked to the two sites determines high or low conversion frequencies (HCF or LCF, respectively) at the two sites, which are themselves closely linked.

The possibility of detecting 4:4(2) segregations in unordered octads was mentioned by Emerson (1966), who used the method of two visual ascospore mutants plus other marker genes in *Ascobolus* (Emerson, 1969), but although he gave 4:4(2) data, the method itself was not described.

2. THE DETECTION SYSTEM IN UNORDERED OCTADS

(i) *Number and linkage relations of markers*

In theory, nearly all postmeiotic segregations should be detectable in suitably multiply-marked crosses, where various pairs of markers can show tetratype segregations that define the spore-pairs and thus enable postmeiotic segregation – giving non-identical sister-spores – to be recognized. Almost any kinds of marker gene could be used but in practice the detection of rare postmeiotic segregation classes can be greatly facilitated by using a system with visual markers, such as ones for ascospore colour production or distribution, or ascospore size or shape, which can be scored directly in the octad, without the need for germinating and testing each spore before even preliminary assessments can be made. If a suffi-

cient number of visual markers is not available in an organism, a compromise is to use perhaps two visual markers for an initial detection of octads possibly having the relevant postmeiotic segregations for one of the visual markers, then isolate that octad, germinate the spores and make confirmatory tests with other, non-visual markers.

In the following discussion, it will always be aberrant segregation at marker *1* that is being studied, with markers *2*, *3*, *4* and so on as aids for its detection. It is essential that marker *1* should not regularly co-convert with other key markers being used. A minimum of two markers, *1* and *2*, is required for the initial detection of postmeiotic segregation but there should preferably be other markers present for confirmation. The situation where only two markers, *1* and *2*, are required is where marker *2* has been shown in other crosses to have no conversion of its own, so that any unusual mutual segregation pattern for *1* and *2* can be attributed solely to aberrant ratios at *1*. If, however, both markers show appreciable conversion (the term is used here to include allele-interaction events giving meiotic or postmeiotic segregation), at least one further marker, preferably more, is required to determine which site, *1* or *2*, underwent aberrant segregation. Where groups of eight spores are scored after ascal dehiscence, as is usually the case with *Ascobolus*, the segregation patterns of the additional markers provide a useful check against false clusters; that is, octads formed of spores from more than one ascus and simulating the product of a single ascus.

If two markers are used for the initial, visual detection, provided they do not co-convert, they can be non-synnemal ('non-syntenic') or synnemal, unlinked or linked, though the linkage relations can affect the efficiency (in a calculable way) of detection of particular segregation patterns. For example, in detecting 4:4(2)s for site *1*, all such segregations should be detected when *1* and *2* give parental ditype (*PD*) or non-parental ditype (*NPD*) segregations but only half are detectable in tetratype (*T*) octads. For 6:2(2)s from a 3:1 event in both pairs of chromatids, such segregations are not detected in *PD* or *NPD* combinations of *1* and *2*, but are in all *T* octads. The tetratype frequencies, which depend on centromere distances for non-synnemal loci and on recombination frequencies for synnemal loci, affect in a predictable way the detection efficiencies. Different suitable marker combinations could therefore be chosen for maximum detection of different segregations. When further markers are used, it is best if they show high tetratype frequencies with each other, to assist identification of spore-pairs, so some markers should be far from their centomeres.

In the following account of individual segregations, genotypes will be given for repulsion-phase crosses, *1*, $+ \times +$, *2*, and when details are given for linked markers, only single crossovers will be considered, but the basic analysis can easily be applied to include coupling crosses and multiple crossovers. With linked markers, accurate assessments would require knowledge of any interference between crossovers and the possibility of conversion-associated crossovers should be considered.

(ii) *4:4(2)*

The 'singly aberrant' 4:4, 4:4(2), is not one of the 'unique classes' mentioned earlier. It is detectable in octads *PD* and *NPD* for *1* and *2*, giving in repulsion crosses genotype ratios of 1 + +, 3 *1*+, 3 +*2*, 1 *12* and 3 + +, 1 *1*+, 1 +*2*, 3 *12*, respectively. In *T* octads, three arrangements are possible: 3 + +, 1 *1*+, 1 +*2*, 3 *12*; 2+ +, 2 *1*+, 2 +*2*, 2 *12*; 1 + +, 3 *1*+, 3 +*2*, 1 *12*. For non-synnemal markers, with independent assortment, these will occur in the ratio 1:2:1 respectively, where the second element is not different in unordered octads from a normal tetratype. so those particular 4:4(2)s would go undetected. The other two kinds will be phenotypically distinct, so for non-synnemal markers, only half the 4:4(2)s in *T* octads will be detected, with the over-all ratio of 4:4(2) events detected to those occurring being $(PD+NPD+\frac{1}{2}T)/(PD+NPD+T)$, with detection being from octad ratios 1 + +, 3 *1*+, 3 +*2*, 1 *12* and 3 + +, 1 *1*+, 1 +*2*, 3 *12*.

For synnemal markers, one has to consider various crossover and non-crossover possibilities between sites *1* and *2*. In the repulsion cross, the absence of a crossover in this region gives 1 + +, 3 *1*+, 3 +*2*, 1 *12*, as does a crossover between the two converting chromatids, while a crossover between the two non-converting chromatids gives 3 + +, 1 *1*+, 1 +*2*, 3 *12*: all these would be recognizable as a 4:4(2) segregation. A crossover between one converting and one non-converting chromatid would give 2 + + 2, *1*+, 2 +*2*, 2 *12*, which in unordered tetrads is the same as from an ordinary *T* octad, so that particular proportion of 4:4(2) segregations would not be detected, but would need to be calculated and allowed for.

(iii) *6:2(2)*

These have two possible origins: either by a 3:1 ratio in each pair of non-sister chromatids, or by a 4:0 event in one pair of chromatids and 2:2(2) in the other pair. With the first of these origins, 6:2(2)s are not usually detectable as such in unordered *PD* or *NPD* octads because they give genotype ratios identical to those from ordinary 6:2s (without postmeiotic segregation) – that is, 2 + +, 2 *1*+, 4 +*2* and 4 + +, 2 +*2*, 2 *12*, respectively. The 6:2(2)s of this origin are all detectable in *T* octads, giving 3 + +, 1 *1*+, 3 +*2*, 1 *12*, unlike normal 6:2s which would give 4 + +, 2 +*2*, 2 *12* or 2 + +, 2 *1*+, 4 +*2*. For 6:2(2) segregations from two 3:1 events, the ratio of detected segregations to those occurring is therefore $T/(PD+NPD+T)$ for non-synnemal genes. For synnemal genes, such 6:2(2) events with no crossover between sites *1* and *2* will be undetected as the 2 + +, 2 *1*+, 4 +*2* genotype could come from ordinary 6:2s. A crossover between the sites, in either pair of non-sister chromatids, gives 3 + +, 1 *1*+, 3 +*2*, 1 *12*, which is recognizable as a 6:2(2).

6:2(2)s from a 4:0 event plus a 2:2(2) event should be detected in all *PD* and *NPD* octads, giving 3 + +, 1 *1*+, 3 +*2*, 1 *12*. In *T* octads, there are three possible genotypes: 4 + +, 2 +*2*, 2 *12*; 3 + +, 1 *1*+, 3 +*2*, 1 *12* and 2 + +, 2 *1*+, 4 +*2*. For non-synnemal markers, these will occur in the ratio 1:2:1, with the first and

third elements not distinguishable from ordinary 6:2s. For 6:2(2)s from a 4:0 plus a 2:2(2) event, the ratio of detected segregations to those occurring is thus $(PD+NPD+\frac{1}{2}T)/(PD+NPD+T)$. For synnemal markers, 6:2(2)s of this origin with no crossovers will be detectable as 6:2(2)s, giving 3 + +, 1 *1*+, 3 +*2*, 1 *12*, and of those with crossovers between *1* and *2*, half (those with a crossover between the two chromatids involved in the conversion, or the two not involved) will be detected, giving 3 + +, 1 *1*+, 1 +*2*, 3 *12*, and half (those with a crossover between an involved and non-involved chromatid) will be undetected, giving 2 + +, 2 *1*+, 4 *12* or 4 *1*+, 2 +*2*, 2 *12*.

The 6:2(2)s from the two origins (3:1+3:1, or 4:0+2:2(2)) could not be distinguished from each other using genotype ratios for *1* and *2* alone, but they could be distinguished if outside markers were used for site *1*. The same applies to 2:6(2)s of two different origins.

(iv) *2:6(2)*

This class comes either from a 1:3 ratio in each pair of chromatids or from a 0:4 in one pair and 2:2(2) in the other pair of chromatids. With the first of these origins, 2:6(2)s are not detected as such in unordered *PD* or *NPD* octads because they give identical genotype ratios to those from ordinary 2:6s, that is, 4 *1*+, 2 +*2*, 2 *12* and 2 + +, 2 *1*+, 4 *12*, respectively. 2:6(2)s of this origin are all detectable in *T* octads, giving 1 + +, 3 *1*+, 1 +*2*, 3 *12*. For 2:6(2)s from two 1:3 events, the ratio of detected segregations to those occurring is therefore

$$T/(PD+NPD+T)$$

for non-synnemal genes. For synnemal genes, such 2:6(2)s with no crossover between sites *1* and *2* will go undetected as the 4 *1*+, 2 +*2*, 2 *12* genotype could come from ordinary 2:6s. A crossover between the sites gives 1 + +, 3 *1*+, 1 +*2*, 3 *12*, which would be recognised as a 2:6(2).

2:6(2)s from a 0:4 event plus a 2:2(2) event should be detected in all *PD* and *NPD* octads, giving 1 + +, 3 *1*+, 1 +*2*, 3 *12*. In *T* octads, there are three possible genotypes: 2 + +, 2 *1*+, 4 *12*; 1 + +, 3 *1*+, 1 +*2*, 3 *12*; 4 *1*+, 2 +*2*, 2 *12*. For non-synnemal markers, these will occur in the ratio 1:2:1, with the first and third elements not distinguishable from normal 2:6s. For 2:6(2)s from a 0:4 plus a 2:2(2) event, the ratio of detected segregations to those occurring is thus $(PD+NPD+\frac{1}{2}T)/(PD+NPD+T)$. With synnemal genes, 2:6(2)s of this origin with no crossovers will be detectable, giving 1 + +, 3 *1*+, 1 +*2*, 3 *12*, and of those with crossovers between *1* and *2*, half will be detected, giving 1 + +, 3 *1*+, 1 +*2*, 3 *12* and half will be undetected, giving 2 + +, 2 *1*+, 4 *12* or 4 *1*+, 2 +*2*, 2 *12*.

(v) *5:3(3)*

5:3(3)s arise by a combination of a 3:1 event in one pair of chromatids with a 2:2(2) in the other pair. If the detection system only has markers *1* and *2*, 5:3(3)s cannot usually be distinguished from ordinary 5:3(1)s in unordered tetrads as the same genotype ratios are produced: 3 + +, 1 *1*+, 2 +*2*, 2 *12* and

2 + +, 2 *1*+, 3 +*2*, 1 *12* (5:3(1)s also give other ratios distinct from those of 5:3(3)s). There is one situation in which a system with just markers *1* and *2* could be used for initial detection of 5:3(3)s. This is where the two markers, if non-synnemal, are very close to their centromeres, showing almost entirely first division segregation at each locus and hence almost no tetratype segregations. *PD* and *NPD* for *1* and *2* give 2 + +, 2 *1*+, 3 +*2*, 1 *12* and 3 + +, 1 *1*+, 2 +*2*, 2 *12* with a 5:3(3) for *1*, and *T* gives either of these, whereas with a 5:3(1), *PD* gives 1 + +, 3 *1*+, 4 +*2*, *NPD* gives 4 *1*+, 1 +*2*, 3 *12*, and only the tetratypes for 5:3(1), giving 3 + +, 1 *1*+, 2 +*2*, 2 *12* and 2 + +, 2 *1*+, 3 +*2*, 1 *12*, could cause confusion with the 5:3(3)s in unordered octads.

If markers *1* and *2* gave no *T*s, then 5:3(3)s would be the only class giving the above two genotypes. Such a system would be usable with *1* and *2* as visual markers for the initial detection, with other markers being used after isolation, germination and testing, to confirm that this was a 5:3(3), not a rare tetratype for *1* and *2* with a 5:3(1) segregation. It could also be used, with more labour, where *1* and *2* had a low frequency of *T* octads, with many octads of appropriate genotype ratio for *1* and *2* being isolated and tested to distinguish ordinary 5:3(1)s from rare 5:3(3)s. For linked markers, this system could also be used if the loci were close enough to give only a low frequency of crossovers and hence of tetratypes.

If such special 'very low or no *T*' systems cannot be used, 5:3(3)s are not distinguishable from 5:3(1)s just by using markers *1* and *2* in unordered tetrads, though they could be in ordered tetrads. Further markers would have to be used, to give tetratypes defining the four spore-pairs, so the postmeiotic segregations of *1* in 5:3(3)s could be detected. Ideally, such a system would have three markers, including *1*, scoreable visually for the initial detection, with further tests for other markers to confirm that it is marker *1*, not *2* or *3*, with the aberrant segregation. Without visual markers or alternative selection systems, the finding of such rare classes as 5:3(3) in unordered tetrads would be extremely laborious.

(vi) *3:5(3)*

These could be identified under the same circumstances as 5:3(3)s. The crucial genotypes are: 1 + +, 3 *1*+, 2 +*2*, 2 *12* (from *PD* or *T* with 3:5(3)) and 2 + +, 2 *1*+, 1 +*2*, 3 *12* (from *NPD* or *T* with 3:5(3)).

(vii) *4:4(4)*

Irrespective of whether the octad type for *1* and *2* would have been *PD*, *NPD* or *T*, 4:4(4)s give genotype ratios of 2 + +, 2 *1*+, 2 +*2*, 2 *12*, not usually distinguishable from regular 4:4 segregation in *T* octads when unordered. The only reported 'doubly aberrant 4:4' is one detected visually from patterns seen in the ordered asci of *Sordaria fimicola* (Kitani & Whitehouse, 1974).

As well as regular 4:4s from *T* octads, half the 4:4(2)s (see (ii) above) from *T* octads give the same ratio, 2 + +, 2 *1*+, 2 +*2*, 2 *12* as 4:4(4)s. If markers *1* and *2* were such as to give no or very few tetratypes (as discussed in v above) all or a large proportion of octads with this genotype ratio would come from

originally *PD* or *NPD* 4:4(2)s, as there would be no or few *T*s with 4:4 or 4:4(2) segregations to give that ratio. If 'very low or no *T*' systems cannot be used, further markers must be employed to provide identifiable *T*s, so 4:4(4)s can then be recognized by their four pairs of non-identical sister-spores.

(viii) *Wider ratios*

Wider ratio octads, with 8:0, 0:8, 7:1 or 1:7 segregations, are detected in both unordered and ordered octads simply from the allele ratios observed.

3. MATERIALS AND METHODS

General methods, materials and the Pasadena strains of *Ascobolus immersus* were usually as described by Lamb & Wickramaratne (1973) and Wickramaratne (1974). One difference was that in these experiments, 0·7 g/l of methyl-*p*-hydroxybenzoate was used as an *in situ* germination inhibitor in the spore collection agar. Crosses were incubated at 17·5 °C and length of heat-shock at 50 °C given to ascospores before germination was 30 min for white spores, 1–2 h for red, non-granular spores; granular spores were not heat-shocked.

The markers used were:

white(*w*)-*78*, a spontaneous white ascospore mutation (Emerson & Yu-Sun, 1967) which, depending on closely linked genetic controlling factors, gives high conversion frequencies (HCF) or low conversion frequencies (LCF);

granular (*gr*)-*3*, a UV-induced mutation giving ascospore pigment in prominent granules outside the spore wall instead of the wild-type uniform distribution over the wall;

pfr-1, an NMG-induced mutation, parafluorophenylalanine resistance, conferring the ability to grow in the presence of 100 mg/l of this chemical.

mating type, (*mt*), + or −.

These markers are all unlinked to each other: the relations between our *gr*-3 and Emerson's (1969) *gr*-1, or Yu-Sun's (1966) *gr-2*, and between our *pfr-1* and Stadler, Towe & Rossignol's (1970) *fpr* are unknown. From tetratype frequencies (method of Whitehouse, 1957), centromere distances have been calculated for *w*-78, *gr*-3 and *pfr*-1 as 30, 1·5 and 23·5 recombination units respectively, and Yu-Sun (1966) gave 2 as the value for mating-type.

Dehisced octads were scored visually for segregation of *w*-78, corresponding to marker *1* in the previous section, and *gr*-3, acting as marker *2*, with isolation, germination and subsequent testing for *pfr* and *mt* in octads of interest. *w*-78 is epistatic to *gr*-3, so *w*-78$^-$, *gr*$^+$ and *w*-78$^-$, *gr*$^-$ have the same phenotype, white: these correspond to *1*+ and *12*. As can be seen from Table 1, this epistasis of *1* over *2* does not affect detection of the desired segregations for *1*, although it would affect detection of certain aberrant ratios for *2* if these were being simultaneously studied. With this kind of system, the marker whose segregation is being primarily studied may be epistatic but should not be hypostatic to any of the other markers being used.

In control crosses of *w*$^+$, *gr*$^+ \times w^+$, *gr*$^-$, the granular site showed extremely low

conversion, only 0·004 %. This is so much lower than the conversion frequency of *w*-78 (3–14 % in different crosses, LCF and HCF) that nearly all unusual genotypic ratios for *w*-78, *gr* segregations are likely to be from aberrant ratios at the *w*-78 site: in the present experiments, all such unusual ratios were shown, with aid of *pfr* and *mt* where necessary, to be due to events at the *w*-78 site.

Table 1. *Genotype and phenotype ratios and their origins for narrower-ratio unique and non-unique classes in a repulsion cross*, $1+ \times +2$

Genotype ratios*				Phenotype ratios with *1* epistatic to $2^+/2^-$			Origin: allele ratio at site *1* and unordered octad type for *1*, *2* (with 4:4 for site *2*)
++	*1+*	*+2*	*12*	++ R	*+2* G	*1+/12* W†	
4	.	2	2	4	2	2	6:2(2), NPD, T; 6:2, NPD, T
4	.	1	3	4	1	3	5: 3, NPD
4	.	.	4	4	.	4	4:4, NPD
‡3	1	3	1	3	3	2	6:2(2), PD, NPD, T
3	1	2	2	3	2	3	5:3(3), NPD, T; 5:3, T
§3	1	1	3	3	1	4	4:4(2), NPD, T
3	1	.	4	3	.	5	3:5, NPD
2	2	4	.	2	4	2	6:2(2), PD, T; 6:2, PD, T
2	2	3	1	2	3	3	5:3(3), PD, T; 5:3, T
2	2	2	2	2	2	4	4:4(4), PD, NPD, T; 4:4(2), T; 4:4, T
2	2	1	3	2	1	5	3:5(3), NPD, T; 3:5, T
2	2	.	4	2	.	6	2:6(2), NPD, T; 2:6, NPD, T
1	3	4	.	1	4	3	5:3, PD
§1	3	3	1	1	3	4	4:4(2), PD, T
1	3	2	2	1	2	5	3:5(3), PD, T; 3:5, T
‡1	3	1	3	1	1	6	2:6(2), PD, NPD, T
.	4	4	.	.	4	4	4:4, PD
.	4	3	1	.	3	5	3:5, PD
.	4	2	2	.	2	6	2:6(2), PD, T; 2:6, PD, T

* With no epistasis, and if *1* and *2* give different phenotypes, these genotype ratios will also be the phenotype ratios.

† R, red, non-granular ascospores, w^+, gr^+; G, red granular, w^+, gr^-; W, white ascospores, w^-, gr^+ and w^-, gr^-.

‡ Narrower-ratio unique classes distinguishable as such. Unique classes are those requiring both pairs of non-sister chromatids of a bivalent to pair at the segregating site.

§ Aberrant 4:4s, i.e. 4:4(2)s, are not a unique ratio class but their postmeiotic segregation can be detected here.

4. RESULTS

Octads with phenotype ratios expected for 4:4(2), 6:2(2) and 2:6(2) segregations at *w*-78 were detected visually; after germination and further tests, including crossing to check *w*-78 and *gr* genotypes, all proved genuine, with genotypes corresponding to visual phenotypes and *gr*, *mt* and *pfr* showing regular 4:4 segregation. Table 2 shows data for high and low conversion frequency crosses of $w\text{-}78^+$, $gr\text{-}3^-$, pfr^R, $mt^+ \times w\text{-}78^-$, $gr\text{-}3^+$, pfr^S, mt^-.

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Table 2. *Segregation classes for* w-*78, and unordered tetrad types*

Cross	6+:2*w*	5:3	3:5	2:6	4:4(2) 3R:1G:4W	4:4(2) 1R:3G:4W	6:2(2)	2:6(2)
HCF*								
No.	2511	378	258	1016	28	26	7	4
%	7·04	1·06	0·72	2·56	0·08	0·07	0·02	0·01
LCF*								
No.	406	94	129	344	22	20	2	4
%	1·47	0·34	0·47	1·25	0·08	0·07	0·007	0·01

Cross	8:0	7:1	1:7	0:8	4:4 T	4:4 PD+NPD	Total
HCF							
No.	12	4	5	6	21040	10373	35668
%	0·03	0·01	0·01	0·02	58·99	29·08	
LCF							
No.	2	1	1	8	17818	8750	27596
%	0·007	0·004	0·004	0·03	64·55	31·71	

* HCF, high conversion frequency crosses, total narrower ratio conversion frequency, 11·86%; LCF, low conversion frequency crosses, total narrower ratio conversion frequency 3·56%.

Table 3. *Specimen 4:4(2), 6:2(2) and 2:6(2) octads to show confirmed genotypes and spore-pairs with postmeiotic segregation for* w-78

No. of spores	*w*-78	*gr*	*pfr*	*mt*	No. of spores	*w*-78	*gr*	*pfr*	*mt*
	4:4(2), 3R: 1G: 4W					4:4(2), 1R: 3G: 4W			
2	+	+	S	+	2	+	+	S	+
2	−	−	R	−	2	−	−	R	−
1	+	+	S	+	1	+	+	S	+
1	−	+	S	+	1	−	+	S	+
1	+	−	R	−	1	−	−	R	−
1	−	−	R	−	1	+	−	R	−
	6:2(2), 3R: 3G: 2W					2:6(2), 1R:1G:6W			
2	+	+	R	+	2	−	+	R	+
2	+	−	R	−	2	−	−	S	−
1	+	+	S	−	1	+	+	R	+
1	−	+	S	−	1	−	+	R	+
1	+	−	S	+	1	+	−	S	−
1	−	−	S	+	1	−	−	S	−

* The brackets show pairs of spores with postmeiotic segregation for *w*-78. In the 6:2(2), the segregations of *gr*, *pfr* and *mt* identify unambiguously which spores are sisters, especially as *pfr* and *mt* showed tetratype segregation. In the 4:4(2), 3R, 1G, 4W, one spore (−, +, S, +, and +, −, R, −) of each pair of non-identical sister-spores is unique, clearly belonging to a pair of non-identical sister-spores, but the other spore in each case (+, +, S, +, and −, −, R, −) has two similar spores in the octad and it is not known which of the three belongs to the non-identical sister-spore pairs.

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4:4(2)s can give two phenotype ratios, 3R:1G:4W or 1R:3G:4W, depending on whether they arose in *NPD* or *T*, or *PD* or *T* octads (see Table 1) and, as expected, the two types were about equally frequent. Because of the high frequency of tetratypes, 5:3(3), 3:5(3) and 4:4(4) segregations could not be detected. Of ten 4:4(2) octads tested, all nine fully analysable octads proved genuine: in the tenth octad, not all spores germinated. All three 6:2(2) and 2:6(2) octads tested were fully analysable and genuine.

Table 4. *Corresponding-site interference data*

Frequencies of events per pair of chromatids: $a(=4{:}0) = \frac{1}{2}6+:2m+8{:}0+\frac{1}{2}7{:}1$; $b(=0{:}4) = \frac{1}{2}2{:}6+0{:}8+\frac{1}{2}1{:}7$; $c(=3{:}1) = \frac{1}{2}5{:}3+\frac{1}{2}7{:}1$; $d(=1{:}3) = \frac{1}{2}3{:}5+\frac{1}{2}1{:}7$; $e(=2{:}2(2)) = \frac{1}{2}4{:}4(2)$. The above formulae have been adapted from the 'partly corrected' formulae of Lamb & Wickramaratne (1973).

6:2(2) formed $= 2ae+c^2$; 2:6(2) formed $= 2be+d^2$.

$$6{:}2(2) \text{ detected here} = 2ae\times\frac{PD+NPD+\frac{1}{2}T}{PD+NPD+T}+c^2\times\frac{T}{PD+NPD+T}.$$

$$2{:}6(2) \text{ detected here} = 2be\times\frac{PD+NPD+\frac{1}{2}T}{PD+NPD+T}+d^2\times\frac{T}{PD+NPD+T}.$$

$$\text{Proportion of 4:4(2)s detected here} = \frac{PD+NDP+\frac{1}{2}T.}{PD+NPD+T}$$

Cross ...	HCF		LCF		HCF		LCF	
	No.	%	No.	%	No.	%	No.	%
	Class: 6:2(2)				Class: 2:6(2)			
Observed*	7	0·02	2	0·007	4	0·01	4	0·01
Expected*	2·5	0·007	0·4	0·001	1·1	0·003	0·4	0·001
CSICC†	2·8		5·0		3·6		10	
	Class: 8:0				Class: 7:1			
Observed	12	0·03	2	0·007	4	0·01	1	0·004
Expected	46	0·13	1·5	0·006	14	0·04	0·71	0·003
CSICC	0·3		1·3		0·3		1·4	
	Class: 1:7				Class: 0:8			
Observed	5	0·01	1	0·004	6	0·02	8	0·03
Expected	3·8	0·01	0·9	0·003	7·5	0·02	1·2	0·004
CSICC	1·3		1·1		0·8		6·7	

* These are directly comparable as the expected values (expected in the absence of corresponding-site interference) allow for the non-detection of a proportion of 6:2(2) and 2:6(2) events in accordance with the above formulae.

† CSICC Corresponding-site interference coincidence coefficient, observed/expected values.

Typical tested octads, giving segregations for *w*-78, *gr*, *pfr* and *mt*, are given in Table 3 to show how postmeiotic segregation could be recognized in 4:4(2), 6:2(2) and 2:6(2) octads.

Formulae (Lamb & Wickramaratne, 1973), modifying factors for the detection

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system used here (see section 2), and observed and expected values for unique narrower ratio classes are given in Table 4 for corresponding-site interference estimation: wider-ratio classes are also considered. Amongst the 6:2(2) and 2:6(2) classes in LCF and HCF crosses, there were in each of the four cases more octads of these types observed than would have been expected in the absence of corresponding-site interference. The excess is about 3- to 10-fold, though the small numbers make an accurate assessment difficult. Observed and expected values agree fairly well for: 8:0s, LCF; 7:1, LCF; 1:7 HCF and LCF; and 0:8 HCF. The interesting wider ratio values are the HCF 8:0s and the HCF 7:1s, where fewer were observed than expected ($\chi^2 = 19{\cdot}9$, $P = < 1\%$ and $\chi^2 = 4{\cdot}9$, $P = 2$–5%, respectively). Lamb & Wickramaratne (1973) found that nearly all 8:0s and 7:1s in *Ascobolus* crosses (many of which used *w*-78, as here) were genuine but that 0:8s often arose by mutation, so the 0:8 data here may not be entirely reliable as the 0:8s were not isolated for checking.

5. DISCUSSION

These results show that unordered octads can be used to detect events giving rise to certain postmeiotic segregation classes such as 6:2(2), 2:6(2) and 4:4(2), even when they are very rare, providing suitable marker combinations, especially of visual markers, are available. Genetic analysis for rare unique narrower ratio classes can therefore be carried out in fungi with unordered octads. Even where there was uncertainty as to which of three spores belonged to a pair of non-identical sister spores, the postmeiotic segregation was still recognizable (examples in Table 3).

Leblon & Rossignol (1973), with the European strains of *Ascobolus immersus*, detected 4:4(2)s in unordered octads using two-point coupling crosses between heteroallelic intragenic suppressors at the b_2 ascospore colour locus. A disadvantage of such closely linked and co-converting sites is that simultaneous 4:4(2)s, from hybrid DNA formation at both sites without correction, are not detected.

Of all possible segregation patterns for a pair of alleles, the non-unique narrower ratio classes 4:4, 6:2, 2:6, 5:3, 3:5 and 4:4(2) are well known from a number of organisms. The work of Kitani (e.g. Kitani & Whitehouse, 1974) with *Sordaria fimicola*, Lamb (1972), Lamb & Wickramaratne (1973) and Leblon (1972) with *Ascobolus immersus*, and of Yu-Sun, Wickramaratne & Whitehouse (1977) with *Sordaria brevicollis*, has firmly established the existence of all wider ratios, 8:0, 0:8, 7:1 and 1:7. Of the unique narrower ratios, one 4:4(4) was reported by Kitani & Whitehouse (1974); they also found one 6:2(2) and we (Table 2, present work) found nine of these; we also found eight 2:6(2)s, the first such report. Of the remaining possible classes predicted by Lamb & Wickramaratne (1973) only the 5:3(3) and 3:5(3)s await discovery.

The present corresponding-site interference data, although puzzling, are in keeping with the earlier findings for *w*-78 and *w*-10 (Lamb & Wickramaratne, 1973) that coincidence coefficients, and even whether corresponding-site interference was positive or negative, differed for different segregation classes and between HCF and LCF crosses. Thus the previous results gave corresponding-site

interference coincidence coefficients (CSICCs) of 0·5 for 8:0, HCF, but 2·6 for 8:0, LCF, and of 1·5 for 7:1, HCF, but 5·1 for 7:1, LCF. The present values of 0·3, 1·3, 0·3 and 1·4 respectively give fair agreement with the previous ones for 8:0s, taking into account sample sizes, but the 7:1 CSICCs were much lower than before, although the HCF/LCF difference was proportionately maintained. The *Sordaria brevicollis* results of Yu-Sun *et al.* (1977) are equally puzzling, with CSICCs often varying widely between different wider ratio classes for the same mutant, for the same classes between different mutants at the same locus (e.g. 3·9–17·5 for 8:0s at *grey*-5) and between different loci (e.g. 3·3–28·8 for 1:7s at *grey*-3 and *grey*-5). In the *S. brevicollis* data, most CSICCs were between 1·1 and 28·8, so corresponding-site interference varied from almost no interference to quite strong negative interference. By contrast, this interference in the Pasadena strains of *Ascobolus immersus* varied from moderately negative to moderately positive (CSICCs 5·1–0·3 for the larger samples, both of these being significantly different from no interference at $P = 1\%$).

The present *Ascobolus* data thus confirm the previous findings that corresponding-site interference can be negative or positive. These first assessments of corresponding-site interference for the 6:2(2) and 2:6(2) segregations gave moderate but consistent negative interference in both HCF and LCF crosses.

REFERENCES

EMERSON, S. (1966). Quantitative implications of the DNA-repair model of gene conversion. *Genetics* **53**, 475–485.

EMERSON, S. (1969). Linkage and recombination at the chromosomal level. In *Genetic Organisation*, vol. I (ed. by E. W. Caspari and A. W. Ravin), p. 289. New York: Academic Press.

EMERSON, S. & YU-SUN, C. C. C. (1967). Gene conversion in the Pasadena strain of *Ascobolus immersus*. *Genetics* **55**, 39–47.

KITANI, Y. & WHITEHOUSE, H. L. K. (1974). Aberrant ascus genotypes from crosses involving mutants at the *g* locus in *Sordaria fimicola*. *Genetical Research* **24**, 229–250.

LAMB, B. C. (1972). 8:0, 0:8, 7:1 and 1:7 conversion ratios in octads from wild-type × mutant crosses of *Ascobolus immersus*. *Heredity* **29**, 397.

LAMB, B. C. & WICKRAMARATNE, M. R. T. (1973). Corresponding-site interference, synaptinemal complex structure, and 8+:0*m* and 7+:1*m* octads from wild-type × mutant crosses of *Ascobolus immersus*. *Genetical Research* **22**, 113–124.

LEBLON, G. (1972). Mechanism of gene conversion in *Ascobolus immersus*. I. Existence of a correlation between the origin of mutants induced by different mutagens and their conversion spectrum. *Molecular and General Genetics* **115**, 36–48.

LEBLON, G. & ROSSIGNOL, J.-L. (1973). Mechanism of gene conversion in *Ascobolus immersus*. III. The interaction of heteroalleles in the conversion process. *Molecular and General Genetics* **122**, 165–182.

STADLER, D. R., TOWE, A. M. & ROSSIGNOL, J.-L. (1970). Intragenic recombination of ascospore color mutants in *Ascobolus* and its relationship to the segregation of outside markers. *Genetics* **66**, 429–447.

WHITEHOUSE, H. L. K. (1957). Mapping chromosome centromeres from tetratype frequencies. *Journal of Genetics* **55**, 348–360.

WICKRAMARATNE, M. R. T. (1974). Gene conversion in *Ascobolus immersus* and the control of recombination in *Neurospora crassa*. Ph.D. thesis, Imperial College, London.

WICKRAMARATNE, M. R. T. & LAMB, B. C. (1975). The determinants of gene conversion properties in *Ascobolus immersus*. *Heredity* **35**, 428.

YU-SUN, C. C. C. (1966). Linkage groups in *Ascobolus immersus*. *Genetica* **37**, 569–580.

YU-SUN, C. C. C., WICKRAMARATNE, M. R. T. & WHITEHOUSE, H. L. K. (1977). Mutagen specificity in conversion pattern in *Sordaria brevicollis*. *Genetical Research* **29**, 65–81.