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SCORING ABERRANT $4^+ : 4^-$ ASCI IN *SORDARIA BREVICOLLIS*

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Abstract : Frequencies of aberrant $4^+ : 4^-$ asci are useful in checking on predictions of recombination models. In *Sordaria brevicollis* counting of aberrant $4^+ : 4^-$s is complicated by the occurrence of third division spindle overlap. A method is indicated where this snag could be overcome. It involves counting particular sequences of aberrant $4^+ : 4^-$s that are not masked by third division spindle overlap and then introducing a correction to obtain the true frequency of aberrant $4^+ : 4^-$s.

An aberrant $4^+ : 4^-$ octad or ascus is an ascus where 2 of the 4 spore pairs are heterozygous (see figure 1). This means that the particular ascus shows post-meiotic segregation. Post-meiotic segregation is segregation of genes at the 3rd or mitotic division that comes after the 2 meiotic divisions. An ascus showing post meiotic segregation is an aberrant ascus. Hence an aberrant $4^+ : 4^-$ ascus is so called because it shows $4^+ : 4^-$ (+ stands for wild type; - stands for mutant) segregation, but is aberrant.

An aberrant $4^+ : 4^-$ is produced by the formation of symmetric hybrid DNA formed during recombination between 2 chromatids, followed by no repair of mispaired bases. (see figure 2).

Symmetric hybrid DNA implies the occurrence of hybrid DNA on both the recombining chromatids (figure 2) whilst asymmetric hybrid DNA implies the occurrence of hybrid DNA on only one of the recombining chromatids (see figure 3).

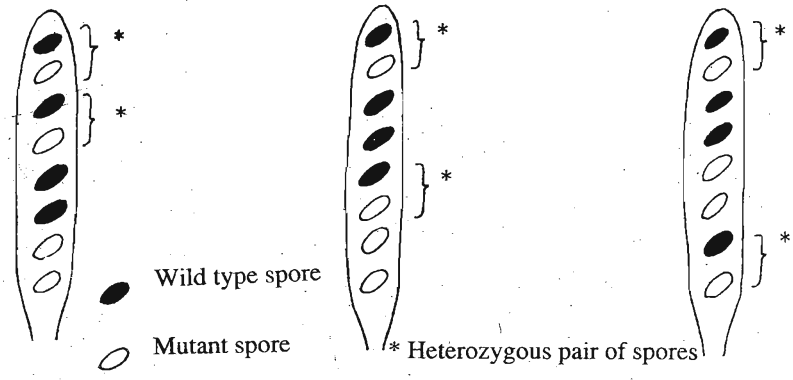

Figure 1 : Examples of aberrant $4^+ : 4^-$ asci from a cross between the wild type and a spore colour mutant

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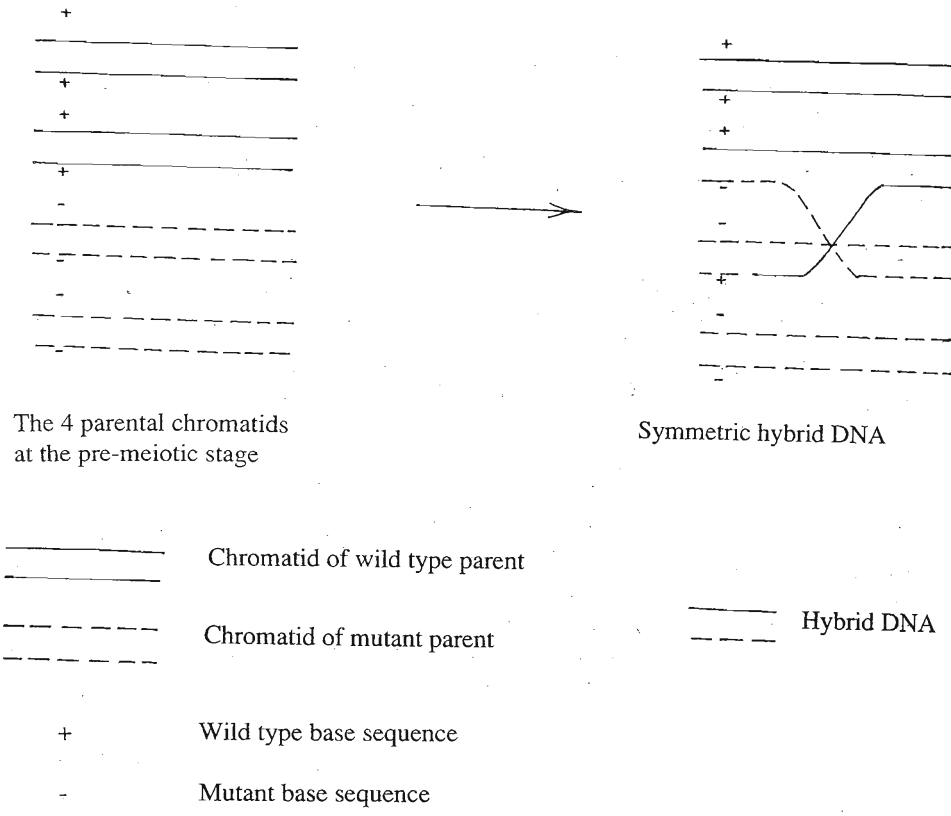

Figure 2 : Formation of symmetric hybrid DNA during genetic recombination

Aberrant $4^+ : 4^-$ octads form a fairly crucial aberrant ascus class important in studies into the mechanism of genetic recombination. An aberrant $4^+ : 4^-$ that is produced by asymmetric hybrid DNA would require two independent recombination events, namely a $5^+ : 3^-$ event and a $3^+ : 5^-$ event at corresponding sites.[2] Hence by and large, aberrant $4^+ : 4^-$s are good evidence for the occurence of symmetrical hybrid DNA. This fact was made use of by Paquette & Rossignol[7] to test a prediction of the Meselson - Radding model that asymmetric hybrid DNA was more frequent closer to the site of recombinant initiation.

Studies in aberrant $4^+ : 4^-$s ought to be attempted mainly with spore mutants, for obvious reasons. Of all the fungal organisms that are normally used in genetic recombination studies, the only one in which aberrant $4^+ : 4^-$s can be

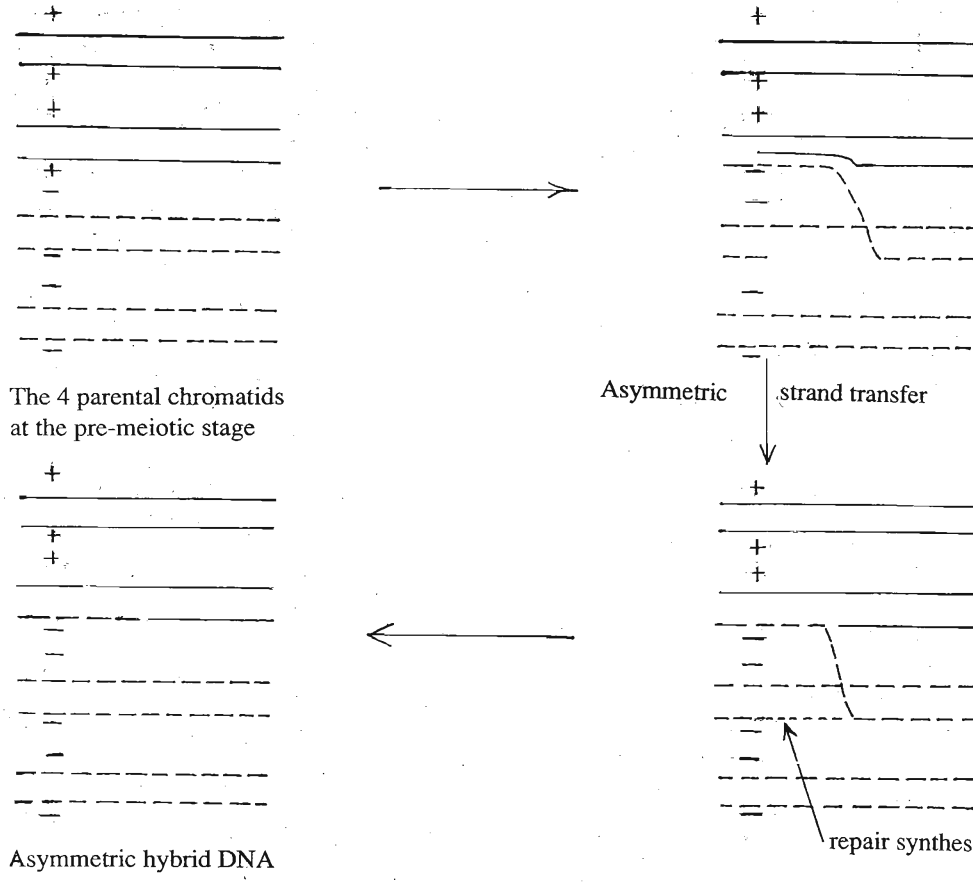

Figure 3 : Formation of asymmetric hybrid DNA during genetic recombination

directly and unambiguously scored in a single site system, and using only a single spore marker at a time, is *Sordaria fimicola*.[4,5] In *Ascobolus immersus*, where octads are unordered, aberrant $4^+ : 4^-$s cannot be detected in a system having only a single spore marker. Ghikas & Lamb[3] used a system involving two spore markers, a white *(w)* ascospore colour mutant and a granular *(gr)* ascospore mutant to detect aberrant $4^+ : 4^-$s, while Paquette[6] used systems involving both two spore markers (white ascospore colour and round ascospore shape ; white ascospore colour and granular spore) and three spore markers (white spore colour, round spore shape and granular spore) to detect large numbers of aberrant $4^+ : 4^-$s.

In *Sordaria brevicollis* the octads are relatively well ordered but the detection of aberrant $4^+ : 4^-$s is complicated by the occurrence of third division spindle overlap. In this organism third division spindle overlap occurs to the extent of 2% - 5%[1] whereas aberrant $4^+ : 4^-$s may be at least two orders of magnitude lower in frequency.

Closer inspection shows that this snag of the occurrence of third division spindle overlap could be overcome.

Figure 4 sets out all possible spore sequences of aberrant $4^+ : 4^-$s. There are 24 in all. It will be seen that only 12 of these sequences are masked by third division spindle overlap. These sequences however will not be all equally frequent.

In an ascomycete when segregation of a marker is studied in asci, one can normally distinguish three classes of spore arrangements[8] in non-aberrant asci, namely the 4 : 4, 2:2:2:2 and 2:4:2. The frequency of each of these three classes will be the nett result of the percentage of second division segregation and second division spindle overlap, but what is relevant to the present discussion are the frequencies of the three 'spore arrangements'. Each of the spore sequences shown in Figure 4 could belong to either of two spore arrangements, e.g. spore sequence 1 could be a 2:2:2:2 or a 2:4:2 depending on whether the chromatid that was initially wild type homoduplex and later became hybird, occupied the topmost position in the sequence or the second from top position in the sequence, respectively. Any ascus in any of the sequences 9 to 16 could similarly be 4:4 or a 2:4:2, while any ascus in any of the sequences 17 to 24 could be a 4:4 or a 2:2:2:2.

The position in the asci of the two chomatids, that were initially homoduplex and later became hybrid, will be eventually determined by both second division segregation and second division spindle overlap. Hence e.g. in the sequences 9-16 the ratio of asci showing the 4:4 spore arrangement to those asci showing the 2:4:2 spore arrangement will be the same whether one considered the total populaton of aberrant $4^+ : 4^-$ asci not masked by third divison spindle overlap and obtain the true frequency of aberrant $4^+ : 4^-$s by introducing a correction.

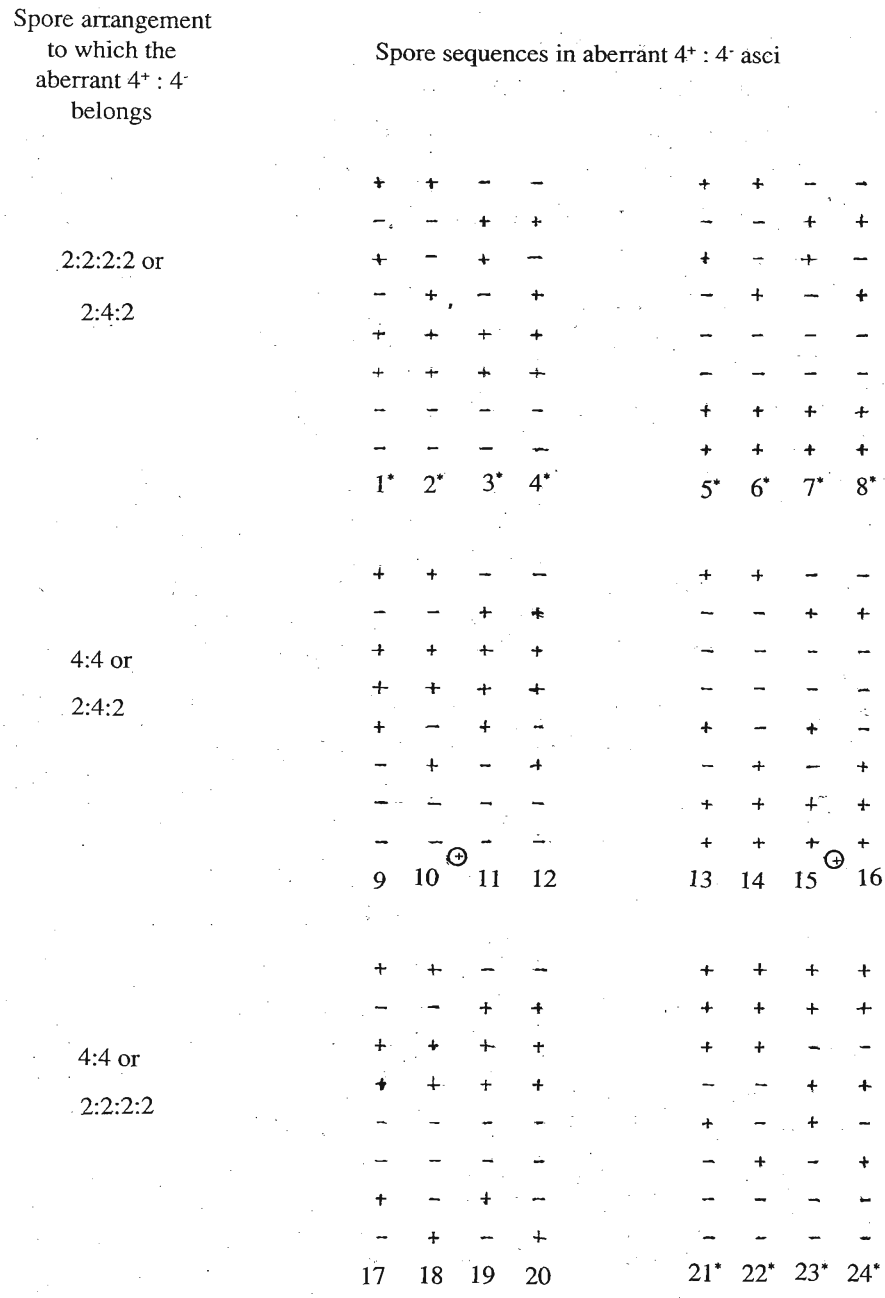

Figure 4: 24 spore sequences each of which could amount to an aberrant $4^+ : 4^-$. 12 of these are masked by 3rd division spindle overlap.

* Masked by 3rd division spindle overlap.

(+) Masked by 3rd division spindle overlap occurring (extremely rarely) simultaneously at the centre and at one of the poles. This sequence is therefore counted as a genuine aberrant $4^+ : 4^-$

Let t = Total frequency of aberrant $4^+ : 4^-$s

Let r = Frequency of 2:2:2:2 spore arrangement

Let s = Frequency of 2:4:2 spore arrangement

Then 1-r-s = Frequency of 4:4 spore arrangement

Then frequency of aberrant $4^+ : 4^-$s that belong to the 4:4 spore arrangement = t (1-r-s).....(1)

Let total frequency of sequences 9 - 16 = x

Then frequency of sequences 9 - 16 that belong to the 4:4 spore arrangement = x (1-r-s).

and the frequency of sequences 9 - 16 that belong to the 2:4:2 spore arrangement = xs.

These will belong to the 4:4 and 2:4:2 arrangements in the ratio 1-r-s : s

Therefore, the proportion of sequences 9 - 16 that belong to the 4:4

$$\text{arrangement} = \frac{1\text{-}r\text{-}s}{1\text{-}r\text{-}s+s} = \frac{1\text{-}r\text{-}s}{1\text{-}r}$$

Therefore the frequency of aberrant $4^+ : 4^-$s that belong to the sequences 9-16 and belong to the spore arrangement

$$4{:}4 = x \frac{1\text{-}r\text{-}s}{1\text{-}r} \quad \text{.....(2)}$$

Let total frequency of sequences 17 - 20 = q

Let total frequency of sequences 17 - 24 = y

Each of the sequences 17 - 24 would be equally frequent.

Therefore y = 2q

Then total frequency of sequences 17 - 20 that belong to the 4:4 spore arrangement = q (1-r-s)

and the total frequency of sequences 17 - 20 that belong to the 2:2:2:2 spore arrangement = qr.

These will belong to the 4:4 and 2:2:2:2 arrangements in the ratio 1-r-s : r

Therefore the proportion of sequences 17 - 20 that belong

$$\text{to the 4 : 4 arrangement} = \frac{1\text{-}r\text{-}s}{1\text{-}r\text{-}s+r} = \frac{1\text{-}r\text{-}s}{1\text{-}s}$$

Therefore the frequency of aberrant $4^+ : 4^-$s that belong to the sequences 17-24

and belong to the spore arrangement 4:4 = $y \frac{1\text{-}r\text{-}s}{1\text{-}s}$ .....(3)

Now, aberrant $4^+ : 4^-$s that belong to the spore arrangement 4:4 belong to only the sequences 9 - 16 and 17 - 24 (see figure 4).

Therefore from equation (2) and (3) the total frequency of aberrant

$4^+ : 4^-$s in the 4:4 arrangement = $x \frac{1\text{-}r\text{-}s}{1\text{-}r} + y \frac{1\text{-}r\text{-}s}{1\text{-}r}$

Now $y = 2q$

therefore total frequency of aberrant $4^+ : 4^-$s in the 4:4 spore

arrangement = $x \frac{1\text{-}r\text{-}s}{1\text{-}r} + 2q \frac{1\text{-}r\text{-}s}{1\text{-}s}$ .....(4)

Therefore from equation (1) and (4)

$$t(1\text{-}r\text{-}s) = x \frac{(1\text{-}r\text{-}s)}{(1\text{-}r)} + 2q \frac{(1\text{-}r\text{-}s)}{(1\text{-}s)}$$

Therefore $t = \frac{x}{1\text{-}r} + \frac{2q}{1\text{-}s}$

Since x, q, r and s are known,

t, the total frequency of aberrant $4^+ : 4^-$s is known.

Hence by counting the sequences 9 to 20 in figure 4 and using the frequencies of the 2:2:2:2 and 2:4:2 spore arrangements in the total sample of asci, the true frequency of aberrant $4^+ : 4^-$s can be evaluated.

Three of the sites, s_{27}, s_{214} and s_{150} in the *ylo-9* spore colour locus in *Sordaria brevicollis* studied gave rather low frequencies ($3.1/10^4$, $1.3/10^4$ and $0.4/10^4$ respectively, raw counts; these were made before the above method for correcting for the true frequency was worked out) of aberrant $4^+ : 4^-$s at 25°C. It is possible that other spore colour mutant sites give higher frequencies and that this method of obtaining the true frequencies of aberrant $4^+ : 4^-$s in *S. brevicollis* may prove useful in obtaining data for checking predictions of recombination models.

Acknowledgement

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