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# Variability of recombination frequencies in the *ftr* cistron of *Coprinus* and its influence on the identification of marker effect alleles

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SUMMARY

The variability of recombination frequencies between alleles of the *ftr* cistron is assessed and shown to be normally in the range of ± 25 % though occasionally replicates differed by a factor of two and in rare cases even more extremely. It is shown that the variation results from biological differences between separate attempts at the same cross and cannot be ascribed to sampling error or any other controllable cause. In the light of this variability the marker effect alleles already recognized in this cistron are re-examined with a new series of crosses. It is confirmed that marker effect may act to enhance or reduce the recombination frequency but it is shown that enhancement can be expressed in two entirely different ways. There is one type of site which causes a uniform doubling of the recombination frequency in all crosses no matter where the second site is placed in the map. The second type of marker effect enhancement site causes a very great increase in recombination frequency in crosses against relatively closely linked mutants, but its effect diminishes as the distance between the marker effect site and the second site is increased.

## 1. INTRODUCTION

Recombination frequencies from crosses between mutants of *Coprinus* which are alleles of the *ftr* cistron (a component of the sugar transport system) have been used to construct a detailed allele map of the gene (Fig. 1). Two potentially important points arise from the allele map. First, some alleles can be recognized which, though accurately positioned, produce patterns of recombination frequencies which are not entirely consistent with their position. These so-called marker-effect sites are thought to influence recombination itself so that an examination of them could yield information about the process. Secondly, a degree of correlation between map position and physiological function was recognized. While inconclusive at present, the correlations observed encourage the view that a detailed determination of mutant function could eventually contribute to an understanding of the exact function of the polypeptide specified by the cistron (Moore, 1972). Work is proceeding along these lines, but important foundations for both are the assumptions that the allele map is a true representation of the physical disposition of mutant sites within the DNA and that recombination frequencies are reliable

measures of distance between mutant sites. If such significance is to be attached to individual recombination frequencies it is essential to have some measure of their reliability.

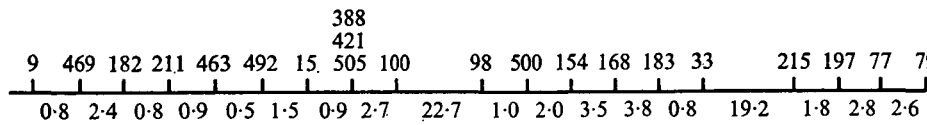


Fig. 1. Simplified allele map of the *ftr* cistron; it includes only those alleles referred to in the text. The map is not drawn to scale but the best estimates of the distances between the sites are indicated below the line.

There is occasionally cause to doubt the ability to construct reliable allele maps (Kruszewska & Gajewski, 1967; Stadler & Kariya, 1969; Norkin, 1970), but on the other hand allele orders derived from recombination frequencies are often confirmed by analyses involving outside markers (Siddiqi, 1962; Fincham, 1967), while there are some studies, unfortunately few in number, in which allele maps have been shown to compare favourably with polypeptide maps (Yanofsky *et al.* 1964; Sherman *et al.* 1970). The impression gained from the literature is that allele maps are generally reliable but that the reliability of a particular system must in some way be demonstrated if the map is to be used as a basis for further experiment. This paper presents an assessment of the variability of recombination frequencies between *ftr* alleles and attempts a critical appraisal of the allele map and the interpretation of the marker effect sites.

## 2. MATERIALS AND METHODS

The organism, mutant strains, media and techniques used in the experiments described here were exactly the same as those used previously (Moore, 1972). All of the mutants used were from the set selected after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

## 3. RESULTS AND DISCUSSION

Table 1 presents the recombination frequencies obtained from repeat crosses involving the same parental strains. It is clear that while the characteristic range of variation is about $\pm 25\%$, different estimations of the recombination frequency between the same pair of alleles can differ by as much as a factor of two. This extreme range of variation has also been demonstrated by Radford (1968) for prototroph frequencies in different crosses involving the same alleles of the *pdx*-1 locus of *Neurospora*.

It is already well established that there is no correlation in the *ftr* system between recombination frequencies and either viability or number of spores plated (Moore, 1972), so the cause of this variability must be sought elsewhere. There are three obvious points at which error or variation may be introduced:

(*a*) variation between different attempts of the same cross; (*b*) variation between different sporophores from the one dikaryon; (*c*) simple sample variation caused by errors in the techniques. Table 1 shows that differences between independent crosses can be quite considerable, but the other possibilities are testable with this system since sporophores can be easily separated from one another, while the

Table 1. *Variability in recombination frequencies in replicate crosses*

| Cross parental alleles | No. of progeny tested ($\times 10^{-5}$) | Wild-types observed | Recombination frequency ($\times 10^5$) | Mean | S.D. | S.D. as % of mean |
|---|---|---|---|---|---|---|
| 154 × 197 | 3·50 | 67 | 19·14 | | | |
| 154 × 197 | 23·35 | 876 | 37·52 | | | |
| 154 × 197 | 91·85 | 3019 | 32·87 | 28·70 | 7·99 | 27·8 |
| 154 × 197 | 6·25 | 119 | 19·04 | | | |
| 154 × 197 | 15·05 | 526 | 34·95 | | | |
| 98 × 197 | 12·05 | 382 | 31·70 | | | |
| 98 × 197 | 11·90 | 698 | 58·66 | | | |
| 98 × 197 | 4·30 | 167 | 38·84 | 43·02 | 12·19 | 28·3 |
| 98 × 197 | 7·75 | 230 | 29·68 | | | |
| 98 × 197 | 25·80 | 1451 | 56·24 | | | |
| 182 × 98 | 16·95 | 838 | 49·44 | | | |
| 182 × 98 | 19·95 | 1435 | 71·93 | 58·29 | 13·35 | 22·9 |
| 182 × 98 | 13·15 | 545 | 41·45 | | | |
| 182 × 98 | 22·80 | 1604 | 70·35 | | | |

Table 2. *Variability in recombination frequencies obtained from repeated tests of spore samples from separate sporophores from the cross* 154 × 197

| Sporophore no. | Sample no. | Recombination frequency ($\times 10^5$) | Means | S.D. | S.D. as % of mean |
|---|---|---|---|---|---|
| 1 | 1 | 35·78 | | | |
| 1 | 2 | 33·27 | 34·44 | 1·03 | 2·9 |
| 1 | 3 | 34·27 | | | |
| 2 | 1 | 35·30 | | | |
| 2 | 2 | 28·50 | 30·97 | 3·07 | 9·9 |
| 2 | 3 | 29·09 | | | |
| 3 | 1 | 34·13 | | | |
| 3 | 2 | 34·21 | 33·02 | 1·63 | 5·0 |
| 3 | 3 | 30·72 | | | |

Mean overall = $32{\cdot}74 \times 10^{-5}$; standard deviation = $1{\cdot}47 \times 10^{-5}$ (= 4·0 %).

numbers of spores available from single sporophores are sufficient for repeated tests of samples from the same spore suspension. Table 2 illustrates such a test, where spore suspensions prepared from three separate sporophores from a single fruiting culture were each tested three times. These data show that sampling errors and differences between individual sporophores only contribute about one fifth of the normally observed variation in recombination frequencies. The major part of this variation must therefore be caused by differences between independent

dikaryons. This occurs despite the fact that every effort is made to ensure uniformity in preparation, culturing and test conditions.

In the data obtained in constructing the allele map there are no consistent indications of any effect which could be correlated with the variability in recombination frequencies. Occasionally reciprocal crosses have given very different recombination frequencies (differing by up to a factor of four), but, except in the case of allele 9 marker effect previously alluded to (Moore, 1972), this has been observed too infrequently for any clear influence of the background genome to be identified. Another interesting effect which has sometimes been observed is that crosses which take a very long time to fruit often (but not always) produce unusual recombination frequencies. Crosses between *ftr* alleles usually form mature sporophores in about 8–12 days. Rarely the fruiting period is extended to up to 40 days. Where this extension of the fruiting period has occurred in one of a pair of replicate crosses it has often been seen that the one with the long fruiting period gives an inflated recombination frequency which, again, can be as much as four times greater than its replicate. These extreme differences have only rarely been observed and no effort has yet been made to study them systematically, but they do suggest that some connexion exists between the overall background metabolism and the great variation seen in recombination values.

It is thus clear that recombination frequencies are subject to a variation which is rarely less than 25 % and sometimes considerably more. It would also seem that there is a purely biological cause for this variation but its nature is completely obscure.

An extensive allele map (i.e. one containing more than 20 sites) inevitably requires a large number of crosses for its construction so it is reasonable to expect the majority of these errors to compensate for one another. An extensive map will therefore be an accurate representation of the gene despite the variation to which recombination frequencies are prone even though no attempt is made to perform the optimum number of replicate crosses (Table 1 suggests that five replicates would be barely sufficient) to ensure the greatest accuracy for each individual recombination value. Continuous refinement of distances and even ordering of closely adjacent sites may be necessary but gross corrections should not be required. (In the *ftr* cistron the map was ambiguous until about 10–12 sites were positioned; no gross alterations have been needed since the 15th site was mapped.) It is when individual recombination frequencies are considered in isolation from the overall map that the extent of this variation is most significant. No allele can be said to be unusual unless the effect ascribed to it is consistently expressed across a number of crosses. Some measure is obviously required which can be used to assess this consistency. In order to maintain a connexion with the overall fine structure map the best procedure seems to be to relate the recombination frequency experimentally obtained between a particular pair of alleles to the value expected from the previously constructed allele map. This is formally equivalent to the calculation of the values used to construct map expansion plots (Holliday, 1964) except that the stress is placed on the individual values rather

than their combination. The term 'coefficient of marker effect' (CME) is proposed for the decimal fraction obtained from the relation recombination frequency/map distance. A CME of more than one indicates marker effect enhancement of the recombination frequency, but the variability of individual frequencies suggests that it must exceed 1·25 to be an effective indicator of this effect. The most sensible way of using this coefficient is to cross the alleged marker effect allele with a range of other alleles in the map. The spectrum of CME values obtained from these crosses will both indicate the overall marker effect status of the allele under

Table 3. *Coefficient of marker effect values for crosses between presumed marker effect alleles and various reference alleles*

| Test allele | Reference allele 9 | 469 | 182 | 211 | 463 | 492 | 15 | 505 | 100 | 98 | 500 | 154 | 168 | 183 | 33 | 215 | 197 | 77 | 79 | Av. CME value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Testing alleles 9, 469 and 182 as marker effect alleles with allele 463 as a control | | | | | | | | | | | | | | | | | | | | |
| 9 | — | 1·0 | 1·0 | 22·1 | 14·4 | — | 4·2 | 5·1 | | 1·8 | 1·4 | 0·3 | — | — | 0·7 | 0·8 | 0·5 | — | — | 4·4 |
| 469 | 1·0 | — | — | 0·5 | 0·1 | — | 0·4 | 3·0 | | 1·5 | 0·9 | 0·6 | — | — | — | 1·0 | 0·5 | — | — | 1·0 |
| 182 | 1·0 | — | — | 5·3 | 1·0 | — | 0·7 | 3·1 | | 1·9 | 0·8 | 0·4 | — | — | — | 1·0 | 0·9 | — | — | 1·6 |
| 463 | — | 0·1 | 1·0 | 1·0 | — | — | 1·9 | 2·2 | | 1·5 | 0·7 | 1·0 | — | — | — | 0·9 | 1·1 | — | — | 1·1 |
| (*b*) Testing allele 98 as a marker effect allele, using allele 500 as a control | | | | | | | | | | | | | | | | | | | | |
| 98 | 1·8 | 1·5 | 1·9 | 2·2 | | 1·9 | 1·3 | 0·7 | 2·1 | — | 2·6 | 1·2 | 0·9 | 3·3 | 1·5 | 2·3 | 1·5 | 2·0 | 2·3 | 1·8 |
| 500 | 1·4 | 0·9 | 0·8 | 1·5 | | 0·8 | 1·1 | 0·5 | 0·9 | 2·6 | — | 1·8 | 0·9 | 0·7 | 0·8 | 1·1 | 0·9 | 1·1 | 1·0 | 1·1 |
| (*c*) Testing alleles 505, 421 and 388 as marker effect alleles, using allele 15 as a control | | | | | | | | | | | | | | | | | | | | |
| 505 | 5·1 | 3·0 | 3·1 | — | 2·2 | 2·1 | 0·7 | — | 1·0 | 0·7 | 0·5 | 0·3 | — | — | — | 1·0 | 0·5 | — | — | 1·7 |
| 421 | — | — | — | — | 1·6 | — | 0·9 | — | — | 0·8 | 0·2 | 0·2 | — | — | — | 0·8 | 0·5 | 0·8 | — | 0·7 |
| 388 | — | — | — | — | — | — | 1·0 | — | — | 1·1 | 0·2 | 0·2 | — | — | — | 0·6 | 0·3 | 0·9 | — | 0·6 |
| 15 | 1·8 | — | — | — | 1·9 | 1·4 | — | 0·7 | 0·8 | 1·3 | 1·1 | 0·9 | — | — | — | 1·0 | 0·6 | 1·1 | — | 1·1 |

test and reveal any localization in expression of the effect. An important additional requirement, though, is a similar range of crosses in which an unexceptional allele closely linked to the one thought to show marker effect is tested. This provides a series of control CME values which can be used to evaluate the influence of extraneous factors like variability and slight errors in the map itself.

Three different marker effect situations have been recognized in the *ftr* cistron (Moore, 1972). Allele 9, at the extreme left-hand end of the map, has been indicated as a mutant which enhances recombination frequencies, a property apparently shared by some closely adjacent sites like 469 and 182. Allele 98, more centrally placed in the map, has also been identified as an allele that increases recombination frequencies, whereas the members of the mutational hotspot characterized as site 505 seemed to have the ability to reduce recombination frequencies. These features have been re-examined using the approach outlined above by performing a new series of crosses and relating their results to the existing map. The CME values so obtained are shown in Table 3. The average CME values give an overall indication of the divergence from normality. Significantly, the averages for the three control alleles, 463, 500 and 15, are all very close to unity, thus supporting the view that variation in recombination frequency can be compensated by a large

number of crosses. By far the clearest expression of marker effect is shown by allele 98, which regularly gives recombination frequencies of about twice the expected value. Allele 9 is also clearly revealed as a definite enhancer of recombination frequency, but it must be concluded that the sites adjacent to 9, namely 469 and 182, are not established as marker effect alleles by these data; so the earlier suggestion that allele 9 marker effect extends over some distance at the left-hand end of the cistron cannot be supported.

The average CME value for site 505 does not vindicate its description as a site which reduces recombination frequencies, though its isoalleles 388 and 421 are notable as the only ones with an average CME much less than one. However, examination of the spectrum of CME values obtained shows quite clearly that in all three cases there was a drastic reduction in the recombination frequencies obtained in crosses against alleles in the centre of the cistron.

Broadly speaking, then, this analysis confirms the major conclusions about marker effect in this cistron which were presented in an earlier report (Moore, 1972). They can be summarized thus: (*a*) allele 9 and allele 98 are alike in being able to enhance recombination frequencies, but the activity of allele 9 is probably not shared by the adjacent sites; (*b*) mutants at the position characterized by allele 505 are able to reduce recombination frequencies but this activity is unidirectional (being expressed to their right), reaches its maximum expression about 30 units from site 505 and is considerably reduced, if not absent at a distance of 60–70 units; (*c*) the marker effect reduction of recombination frequency caused by isoalleles at site 505 is cancelled by the enhancement caused by site 98. In addition, a significant new feature is evident from these data. Although alleles 9 and 98 both increase recombination frequencies the spectra of their activities are completely different. Allele 98 simply doubles the expected value and seems to exert this effect uniformly over the entire cistron, on the other hand allele 9 shows a massive but localized surge of activity that reaches a maximum with alleles placed about 5 units away, its severity gradually decreasing until it is hardly apparent in crosses with alleles some 30–40 units distant.

Thus it is demonstrated that marker effect phenomena are complicated in yet another way in this cistron. Not only can they be expressed as both reduction and enhancements of recombination, but the latter may also take two different forms. This must have significance in the interpretation of marker effect, for it implies either that there are two separate stages in recombination which can generate this phenomenon, or one stage which responds in two different ways. The former is considered the most likely possibility and in the context of the model of recombination previously proposed (Moore, 1972) it might be that one type of marker effect is produced by an allele which influences unwinding of the DNA helix while the other results from an effect on the excision – repair process.

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