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Variability of Allelic Recombination Frequencies

An Analysis, and Interpretation in Terms of Variable Frequency and Direction of Repair Phenomena

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Summary. Estimates of allelic recombination frequencies are shown to have coefficients of variation of between 20 and 40%. In *Coprinus* this is true of both high and low recombination frequencies and is also true when the alleles involved show marker effect. This variability is not confined to *Coprinus* but is a general feature of both meiotic and mitotic allelic recombination. Experimental errors do not make a major contribution to the observed variation although it has the nature of a sampling variation. It is suggested that the variation arises from the diversity of ways in which the initial errors introduced by hybrid DNA formation can be resolved during the excision-repair stages of recombination. If the enzymes responsible for these processes are present in low concentrations then much latitude can be anticipated in the way the same errors are dealt with by separate, though isogenic, diploid or dikaryotic organisms. Each separate cross is thus interpreted as providing an estimate of the recombination frequency which is but a sample from a varied population of possible estimates of the same recombination frequency. Each pair of alleles exhibits a recombination frequency which, within the statistical boundaries of the general variation, is sufficiently reproducible to be described as a characteristic of them. Combinations of allelic recombination frequencies derived from pair-wise crosses fall into patterns that are sufficiently consistent for allele maps to be drawn; and, providing a sufficient number of replicate crosses have been analysed, the allele map can be shown to be statistically soundly based. Two marker effect situations are examined. One causes reduction of recombination frequency and is probably intrinsic to the mutant site itself, the other causes enhancement of recombination frequency and is due to a factor or factors distinct from the allelic mutant site in the strain in which it was first identified. When intercrossed the two effects counteract one another.

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Introduction

The *ftr* cistron of the Basidiomycete *Coprinus cinereus* is the structural gene for a binding protein involved in transport of hexose sugars into the cell. Alleles of the gene can be obtained by selecting for resistance to any of a number of growth-inhibiting sugar analogues (Moore and Stewart, 1971; Moore, 1973b); but wild-type recombinants arising from crosses made between *ftr* alleles can also be selectively identified since wild-type growth on fructose is much better than the growth made by the mutants on this sugar (Moore and Stewart, 1971). We have used *ftr* alleles to investigate fine structure mapping and marker effect (Moore, 1972), and some indications of functional differentiation within the cistron have been obtained from comparisons of mutant phenotypes with map positions (Moore, 1972) and from the differential effects of different selection media (Moore and Devadatham, 1975) and mutagenic treatments (Moore, 1975). Central to all of these studies is a reliable allele map. Since the *ftr* cistron is, so far, the only known representative of its linkage group, the allele map can only be constructed from "recombination frequencies" which are measured in terms of the numbers of wild-type recombinants obtained from pair-wise crosses of *ftr* alleles, but such recombination frequencies are very variable.

Variability of fine structure recombination frequencies is expressed in two ways. Variation is found when the strains used have heterogeneous backgrounds. Careful analysis of this sort of situation resulted in the recognition of recombination control genes (Jessop and Catcheside, 1965; Catcheside, 1975). Considerable variability is still found, though, even where this type of effect can

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be excluded; as when inbred or other homogeneous strains are used (Murray, 1963; Millington-Ward, 1970) or where the comparison is between repeated attempts of the same cross (Day, Wellman and Martin, 1972; Moore, 1973a). The determination of allelic recombination frequencies is clearly subject to error but this is not sufficient to account for all of the variability encountered. At least among fungi, this variation is the rule rather than the exception. From the very earliest studies examples can be found of disparate results being obtained from crosses between the same pair of alleles. Pateman (1960) reported the results of 7 measurements of the recombination frequency between am^2 and am^3 in *Neurospora crassa*, the coefficient of variation (i.e. standard deviation/mean) of which was 0.33. Radford's (1968) data for the *pdx* −1 locus of *Neurospora* had a coefficient of variation of 0.42, while data reported by Moore and Sherman (1975) show that X-ray induced mitotic recombination in yeast is similarly variable, the coefficient of variation averaging 0.34.

In the study reported here, 5 alleles of the *ftr* cistron have been crossed in all possible heteroallelic combinations; between 11 and 53 replicates of each cross being analysed, each cross being fruited from an independent dikaryon. The different technical factors likely to disturb the measurements are assessed and the nature and extent of the variation examined. Some marker effect crosses are analysed in like manner.

Materials and Methods

The methods employed have been described before (Moore, 1972).

Results

The results of the crosses performed are displayed in Figures 1 and 2, and Table 1. Figure 1 shows the results of each cross separately as plots in which the numbers of wild-type recombinants are compared with the numbers of viable progeny tested; the slopes of these graphs are measures of the average recombination frequency. The scatter of points around the regression lines gives an immediate visual indication of the degree of variability, and this is shown more formally by the numerical data in Table 1. The coefficient of variation for these crosses averages 0.37. Errors due to the manipulative procedures were estimated by making replicated measurements of the recombination frequency from a single spore suspension (Table 2). Twelve separate estimates were made of (*a*) the number of colonies formed on glucose-medium, (*b*) the number of wild-types selected on fructose + sorbose medium, and (*c*) the number of wild-types selected on fructose-medium. The 36 separate sampling events from the master suspension allow a number of points to be considered. Variation in the colony counts on a single type of medium gives an estimate of sampling error which, in the case of the glucose-medium counts, is also potentially compounded by errors involved in making dilutions. As can be seen from Table 2, the coefficients of variation do not exceed 0.056. The merits of the two test media can be compared, and it emerges that the difference

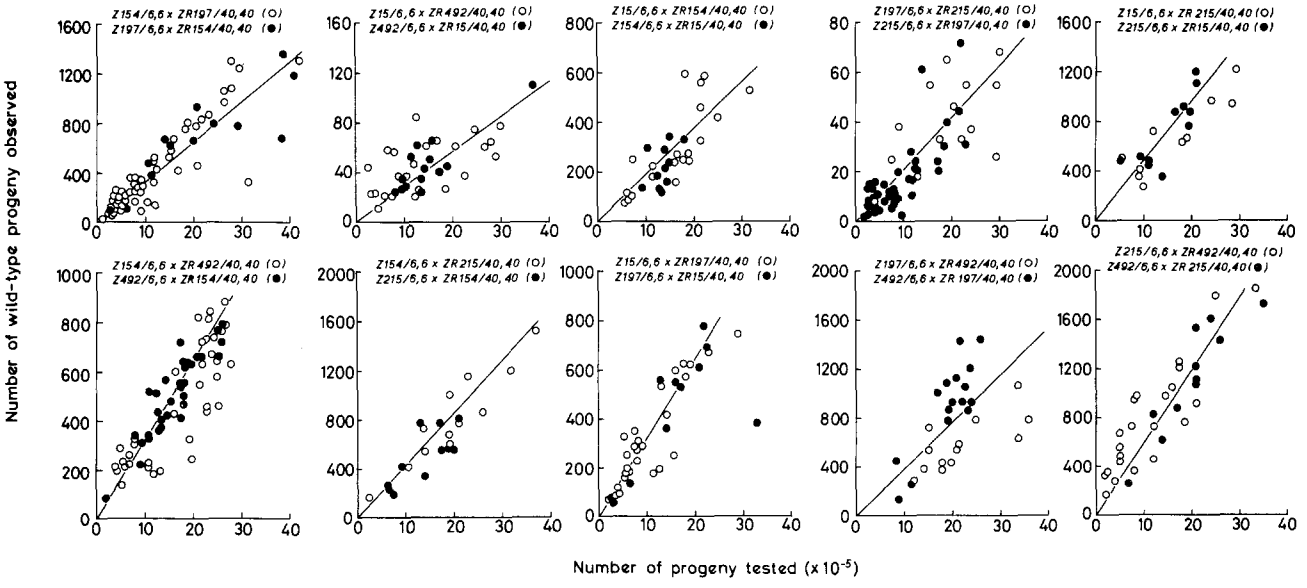

Fig. 1. Results of crosses used to assess variability of allelic recombination. Each point gives the result of a single cross made between the indicated alleles. The lines drawn are lines of regression through the origin calculated for all the displayed data although in two cases (see Table 1) results of reciprocal crosses differed significantly. We are indebted to Miss Shiela Ellis who first suggested we might use this type plot to display these results

Table 1. Results of crosses undertaken to investigate the variability of allelic recombination frequencies

Cross	Number of trials	Total progeny tested ($\times 10^{-5}$)	Wild types observed	Estimates of the frequency of wild-type recombinants ($\times 10^5$)			
				Weighted mean	Mean and S.D. of separate trials		Slope[a]
					Mean	S.D.	
Z15/6,6 × ZR492/40,40	24	341.265	1,081	3.2	4.3	3.4	–
Z492/6,6 × ZR15/40,40	14	206.950	648	3.1	3.2	0.8	–
combined:	38	548.215	1,729	3.2	3.9	2.8	2.8
Z15/6,6 × ZR154/40,40	22	388.200	7,435	19.2	18.6	6.3	–
Z154/6,6 × ZR15/40,40	11	150.400	2,415	16.1	16.1	6.0	–
combined:	33	538.600	9,850	18.3	17.8	6.3	18.9
Z154/6,6 × ZR197/40,40	53	726.020	24,715	34.0	34.2	11.6	–
Z197/6,6 × ZR154/40,40	12	271.950	8,388	30.8	32.4	9.5	–
combined:	65	997.970	33,103	33.2	33.9	11.2	32.3
Z197/6,6 × ZR215/40,40	14	261.500	566	2.2	2.4	1.1	–
Z215/6,6 × ZR197/40,40	42	391.850	869	2.2	2.2	2.3	–
combined:	56	653.350	1,435	2.2	2.3	2.1	2.1
Z154/6,6 × ZR492/40,40	37	614.330	17,620	28.7	32.0	6.2	–
Z492/6,6 × ZR154/40,40	36	900.040	29,043	32.3	32.8	7.6	–
combined:	73	1514.370	46,663	30.8	31.6	8.6	32.0
Z154/6,6 × ZR215/40,40	13	267.800	12,004	44.8	45.3	13.3	–
Z215/6,6 × ZR154/40,40	11	151.550	5,424	35.8	36.1	11.0	–
combined:	24	419.350	17,428	41.6	41.1	13.0	42.8
Z15/6,6 × ZR197/40,40	29	338.575	11,316	33.4	32.9	10.9	–
Z197/6,6 × ZR15/40,40	14	217.150	6,619	30.5	29.7	10.6	–
combined:	43	555.725	17,935	32.3	31.9	10.8	32.0
Z197/6,6 × ZR492/40,40	13	284,450	7,506	26.4	27.2	7.4	–
Z492/6,6 × ZR197/40,40	17	334.960	16,168	48.3	46.4	13.8	–
combined:	30[b]	619.410[b]	23,674[b]	38.2[b]	38.1[b]	14.9[b]	37.6[b]
Z15/6,6 × ZR215/40,40	11	203.100	9,608	47.3	47.6	19.5	–
Z215/6,6 × ZR15/40,40	12	209.200	10,187	48.7	50.6	15.6	–
combined:	23	412.300	19,795	48.0	49.2	17.2	49.4
Z215/6,6 × ZR492/40,40	22	251.125	17,428	69.4	84.7	34.8	–
Z492/6,6 × ZR215/40,40	11	218.020	12,348	56.6	55.8	10.7	–
combined:	33[b]	469.145[b]	29,776[b]	63.5[b]	75.1[b]	31.9[b]	59.2[b]

[a] Slope = the slope of the line of regression through the origin for the combined data of both reciprocal crosses in the plots of Figure 1
[b] The results of these reciprocal crosses differ significantly at the 5% level

between the mean colony counts on the two media is not significant ($t = 0.995$, with 22 degrees of freedom, $P = 0.3$–0.4). Finally, since a normal test involves one set of glucose plates, one set of fructose plates and one set of fructose + sorbose plates, these data can be combined in 12^3 different ways to yield 1728 estimates of the recombination frequency between the two alleles in this one cross. The mean of these was 32.6×10^{-5} and the standard deviation 1.86×10^{-5}. The coefficient of variation (0.057) is comparable with those of the original colony counts. Because replicate samples were analysed the standard deviation of the mean can be computed in this case. This is usually not possible because in most experiments on allelic recombination few, if any, replicates are made.

The most frequently used measure of variation is the standard error (S.E.). In the particular cross under discussion here the S.E. was 0.323×10^{-5} (as a coefficient of variation this = 0.01). Including the replicated cross discussed above, the strains Z492/6,6 and ZR154/40,40 were crossed together 36 times. The average S.E. was 1.5×10^{-5}, the mean recombination frequency was 32.8×10^{-5} but the standard deviation was 7.6×10^{-5}.

From this and similar analyses using data such as those in Table 3 it can be concluded that the errors introduced by the technical procedures contribute

Table 2. Estimate of sampling errors

	Number of colonies observed on medium containing		
	10 mM glucose	5 mM fructose	5 mM fructose +2 mM sorbose
	484	444	402
	522	433	441
	535	437	423
	482	445	367
	545	408	403
	528	425	412
	542	420	447
	482	400	437
	502	417	417
	508	426	419
	522	425	390
	555	407	435
Mean	517.25	423.92	416.08
Standard deviation	25.57	14.41	23.17
Coefficient of variation	0.049	0.034	0.056

A single spore suspension was prepared from one of the crosses made between Z492/6,6 and ZR154/40,40. 36 1 ml samples were removed and treated as follows: 12 were diluted by a factor of 10^3 and then 0.4 ml quantities were plated in glucose medium; 12 were plated without dilution in fructose medium; and 12 were plated without dilution in fructose + sorbose medium

Table 4. Dependence of recombination frequency on basidiospore plating density and viability

Statistic	Identity of parameter x	
	Number of viable spores plated	viability of the plated spores
Correlation coefficient between x and y[a]	−0.052	−0.119
Regression of x on y:		
slope	−0.002	−0.002
intercept	1.041	1.113
Mean of x	17.85×10^5	52.9%
Number of paired variables	266	266

[a] The parameter y represents the recombination frequency. To bring together the results from replicated crosses with different recombination frequencies the individual results were converted to a ratio by the equation

$$y = \frac{\text{recombination frequency of each separate cross}}{\text{weighted mean recombination frequency of all reciprocal crosses}}$$

Table 3. Estimates of sampling errors in two additional crosses

Sample number	Number of colonies observed on medium containing					
	Cross: Z154/6,6 × ZR492/40,40			Cross: Z412/6,6 × ZR98/40,40		
	10 mM glucose	5 mM fructose	5 mM fructose +2 mM sorbose	10 mM glucose	5 mM fructose	5 mM fructose +2 mM sorbose
1	247	318	295	466	211	180
2	244	325	304	385	216	172
3	226	298	269	411	234	161
4	211	298	308	393	240	218
5	228	270	322	445	199	195
6	221	301	311	376	182	201
7	190	269	278	394	222	218
8	231	306	291	409	229	183
9	231	263	278	424	186	212
10	–	–	–	389	213	218
Mean	225.44	294.22	295.11	409.20	213.20	195.80
Standard deviation	17.18	22.15	17.69	28.52	19.45	20.98
Coefficient of variation	0.076	0.075	0.060	0.069	0.091	0.107
Number of estimates of the recombination frequency		729			1000	
Mean recombination frequency		52.53×10^{-5}			20.07×10^{-5}	
Standard deviation		4.72×10^{-5}			1.84×10^{-5}	
Coefficient of variation		0.089			0.092	

only a small part of the variation seen between different crosses. Furthermore, it is clear that while the S.E. gives a reasonable indication of the sampling errors for individual crosses it is, in the contect of allele mapping, an irrelevant statistic as it gives no indication of the amount by which the results of independent crosses can be shown to differ.

Two other potential sources of variation considered are the numbers of viable spores plated in each cross and the percentage viability of the spores (Table 4). Neither of the two correlation coefficients shown differ significantly from zero.

Analysis by rankit diagrams (Ipsen and Jerne, 1944) has shown that the individual estimates of each recombination frequency for each cross described here are normally distributed. Yet these estimates are far more disperse than expected from a binomial distribution. The impression, therefore, is that the variation observed between the results of replicated crosses has some of the characteristics of a sampling variation, but since experimental sampling error has too small an effect to account for the degree of variation observed, the nature of the sampling event remains to be identified (see Discussion).

The Allele Map. Allelic crosses are undertaken primarily to allow construction of allele maps. Two of the maps which can be drawn from the data given here are shown in Figure 2. The essential procedure in allele mapping is the comparison of a trio of three recombination frequencies so that, for example, the alleles *a, b* and *c* can be placed in that order because the *ac* "distance" is greater than either *ab* or *bc* (Tessman, 1965). If the map is additive then $ab+bc=ac$ will hold, but strict additivity is not essential for map construction (though if the departure from additivity is too great the procedure will be invalidated). The recombination frequencies shown in Fig. 2b are the group means of the replicated crosses; these can be subjected to statistical scrutiny rather more easily than the weighted means of Fig. 2a. Two means are shown for the intervals between alleles 492 and 197 and 492 and 215 because the reciprocal crosses for these pairs of alleles gave significantly different results. In other cases results of reciprocal crosses are bulked together.

The reliability of the data can be determined by showing that there is some statistically valid approximation to the equation $ab+bc=ac$. If the *t*-test indicates that there is no significant difference between the means which represent estimates of the three recombination frequencies symbolised by *ab*, *bc* and *ac* then the data are inadequate and the trio of alleles cannot be mapped. Note that intervals *ab* and *bc* can be identical (when allele *b* is midway between *a* and *c*); the critical point is that interval *ac must* be greater than *both ab* and *bc*. The reliability of the whole map is judged from the compatibility of the orders derived for these trios of alleles.

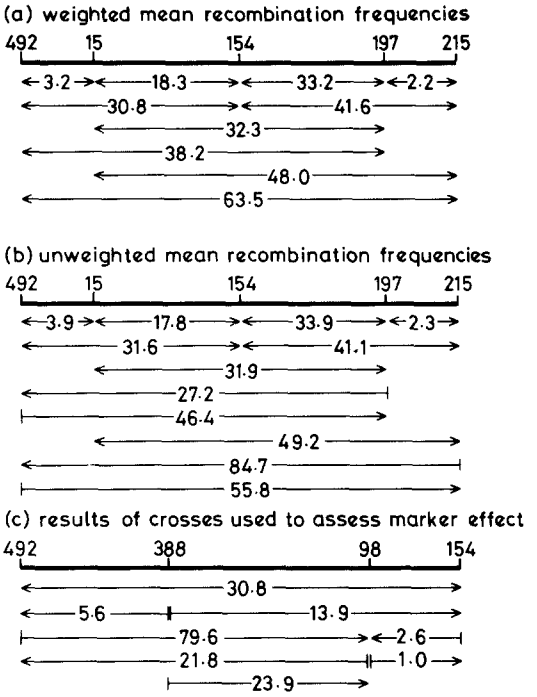

Fig. 2. Allele maps of the *ftr* cistron drawn with data reported here. (a) and (b) use weighted and unweighted mean recombination frequencies ($\times 10^5$) derived from Table 1. Double headed arrows denote data obtained from bulking reciprocal crosses together, single headed arrows point towards alleles used with $A_{40}B_{40}$ mating type specificities. Most of the data for (c) come from Table 6

Such an analysis (Table 5) shows that the allele order in Figure 2 is supported by all but one of the possible trios; the exception is a set which does not indicate any particular order. Thus although the map shown is not perfectly additive it seems to satisfy all of the available data. This is not to say that the map is necessarily true, nor yet that is has any relation to physical reality. The statistical analyses point to a map which is a valid interpretation of the data to hand; other data and other considerations may justify belief in a different allele order. The map shown in Figure 2 is not sufficiently extensive for additivity to be assessed by a map expansion plot (Holliday, 1964). Nevertheless, inspection reveals examples of map expansion (e.g. 492:15:154), map contraction (15:154:197) and fair additivity (154:197:215). The absence of any clear trend for map expansion in this cistron has been indicated previously (Moore, 1972).

Marker Effect. Previous studies (Moore, 1972; 1973a) have identified two aspects of marker effect. Alleles in the vicinity of allele 15 reduced the recombination frequency, while an allele close to 154 enhanced

Table 5. Use of the *t*-test to determine the significance of differences between mean recombination frequencies used to map trios of alleles

Allele isolation numbers			Intervals compared[a]			Probable order	Mean recombination frequencies			Additivity quotient[c]
a	*b*	*c*	*ab/bc*	*ab/ac*	*bc/ac*		*a–b*	*b–c*	*a–c*	
492	15	154	12.6(69)	16.8(83)	7.3(78)	492–15–154	3.9	17.8	32.1	1.5
492	15	197[b]	15.8(79)	16.6(49)	*1.5*(54)	[492/15]–197	3.9	31.9	27.2	–
492	15	197[b]	15.8(79)	18.5(53)	4.4(58)	492–15–197	3.9	31.9	46.4	1.3
492	15	215[b]	16.1(59)	14.3(58)	4.4(43)	492–15–215	3.9	49.2	84.7	1.6
492	15	215[b]	16.1(59)	27.7(47)	*1.2*(32)	[492/15]–215	3.9	49.2	55.8	–
492	154	197[b]	*0.8*(110)	*1.6*(58)	2.1(76)	?	32.1	33.9	27.2	–
492	154	197[b]	*0.8*(110)	4.5(62)	3.9(80)	492–154–197	32.1	33.9	46.4	0.7
492	154	215[b]	3.3(69)	9.6(67)	5.8(44)	492–154–215	32.1	49.2	84.7	1.0
492	154	215[b]	3.3(69)	6.9(56)	3.5(33)	492–154–215	32.1	49.2	55.8	0.7
492	197	215[b]	22.2(67)	5.9(33)	17.8(76)	492–197–215	27.2	2.3	84.7	2.9
492	197	215[b]	22.2(67)	7.7(22)	35.4(65)	492–197–215	27.2	2.3	55.8	1.9
492	197	215[b]	23.5(71)	4.3(37)	17.8(76)	492–197–215	46.4	2.3	84.7	1.7
492	197	215[b]	23.5(71)	*1.9*(26)	35.4(65)	492–[197/215]	46.4	2.3	55.8	–
15	154	197	7.7(96)	6.8(74)	*0.9*(106)	[15/154]–197	17.8	33.9	31.9	–
15	154	215	9.6(55)	9.7(54)	*1.9*(45)	[15/154]–215	17.8	41.1	49.2	–
15	197	215	20.4(97)	5.1(64)	20.3(77)	15–197–215	31.9	2.3	49.2	1.4
154	197	215	20.9(119)	2.7(87)	23.5(78)	154–197–215	33.9	2.3	41.1	1.1

[a] Entries in this part of the table are values of *t* with (in brackets) the number of degrees of freedom. Non significant differences are shown in *italics*

[b] Trios which involve either of the intervals 492–197 or 492–215 are calculated twice since the reciprocal crosses between these pairs of alleles gave significantly different results

[c] Additivity quotient = recombination frequency *a–c*/ sum of recombination frequencies *b–c* and *a–b*

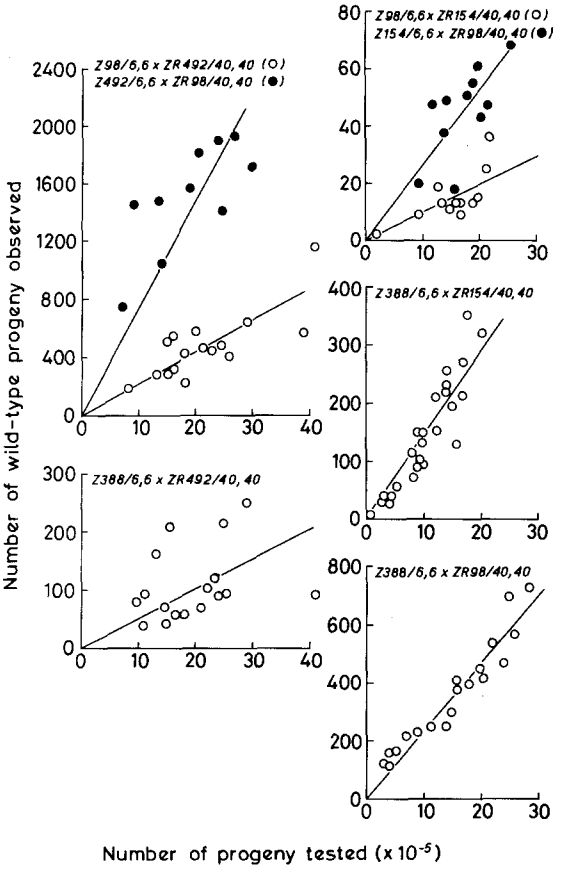

Fig. 3. Results of crosses used to assess marker effect caused by strains Z388/6,6 and ZR98/40,40. Lines of regression through the origin are drawn separately for each set of results

recombination frequencies. These points have been confirmed by replicate crosses involving allele 388 (expected to reduce recombination frequencies) and allele 98 (expected to enhance them). Allele 98 was available in both mating type specificities and reciprocal crosses were carried out against 154 and 492; allele 388 was available only in the A_6B_6 mating type and was crossed against 492, 154 and 98. Results are shown graphically in Figure 3, with numerical data in Table 6 and the appropriate allele map in Figure 2c. The reference interval for the marker effect crosses is the weighted mean recombination frequency between 492 and 154 (30.8×10^{-5}) shown in Table 1. Allele 388 gives an under-estimated view of recombination with 154, the observed frequency being 13.9×10^{-5} compared with an expected value (assuming perfect additivity) of 25.2×10^{-5}, and is thus confirmed as a reducer of recombination frequencies measured, as here, as the frequency of wild-type recombinants. The $A_{40}B_{40}$ strain of allele 98, used for most of the previous work done with this allele, is similarly confirmed as one which enhances the frequency of recombination; a frequency of 79.6×10^{-5} being obtained in crosses against Z492/6,6 instead of the expected value of about 29×10^{-5}. Also confirmed is the fact that apparently normal frequencies are obtained from crosses made between the two marker effect alleles. The cross between Z388/6,6 and ZR98/40,40 yielding a recombination frequency of 23.9×10^{-5} compared with the best es-

Table 6. Results of crosses undertaken to investigate marker effect

Cross	Number of trials	Total progeny tested ($\times 10^{-5}$)	Wild types observed	Estimates of the frequency of wild-type recombinants ($\times 10^5$)			
				Weighted mean	Mean and S.D. of separate trials		Slope[a]
					Mean	S.D.	
Z98/6,6 × ZR154/40,40	12	184.475	178	1.0	1.0	0.3	1.0
Z154/6,6 × ZR98/40,40	11	191.850	503	2.6	2.7	0.8	2.6
Z98/6,6 × ZR492/40,40	16	343.000	7,482	21.8	22.2	6.3	21.5
Z492/6,6 × ZR98/40,40	10	189.510	15,078	79.6	88.6	31.1	73.5
Z388/6,6 × ZR492/40,40	17	334.532	1,863	5.6	6.0	3.4	5.1
Z388/6,6 × ZR154/40,40	25	264.600	3,666	13.9	12.9	3.9	14.4
Z388/6,6 × ZR98/40,40	19	288.180	6,903	23.9	26.0	6.2	23.5

[a] Slope = the slope of the line of regression through the origin for the plots of Figure 3

Table 7. Results of crosses undertaken to investigate the behaviour of isoalleles of allele 388 when crossed with allele 154

Cross	Number of trials	Total progeny tested ($\times 10^{-5}$)	Wild types observed	Estimates of the frequency of wild-type recombinants ($\times 10^5$)		
				Weighted mean	Mean and S.D. of separate trials	
					Mean	S.D.
Z505/6,6 × ZR154/40,40	16	215.31	2,249	10.4	9.7	3.1
Z154/6,6 × ZR505/40,40	14	250.63	3,011	12.0	12.1	4.4
combined:	30	465.94	5,260	11.3	10.8	3.9
Z496/6,6 × ZR154/40,40	16	275.35	4,700	17.1	16.6	4.3
Z154/6,6 × ZR496/40,40	16	401.10	6,663	16.6	16.6	4.4
combined:	32	676.45	11,363	16.8	16.6	4.3
Z456/6,6 × ZR154/40,40	14	388.40	6,683	17.2	17.6	4.1
Z154/6,6 × ZR456/40,40	16	450.80	9,063	20.1	20.4	5.7
combined:	30	839.20	15,746	18.8	19.1	5.2
Z445/6,6 × ZR154/40,40	13	250.45	4,236	16.9	15.7	5.0
Z421/6,6 × ZR154/40,40	15	207.05	2,809	13.4	14.7	5.7
Z412/6,6 × ZR154/40,40	16	310,90	4,794	15.4	15.1	3.0
S 26/6,6 × ZR154/40,40	14	208.55	2,762	13.2	12.9	3.8

Table 8. Results of crosses made between isoalleles of allele 388 and the marker effect strain ZR98/40,40

Cross	Number of trials	Total progeny tested ($\times 10^{-5}$)	Wild types observed	Estimates of the frequency of wild-type recombinants ($\times 10^5$)		
				Weighted mean	Mean and S.D. of separate trials	
					Mean	S.D.
Z505/6,6 × ZR98/40,40	6	135.10	3,018	22.3	23.7	7.3
Z496/6,6 × ZR98/40,40	6	117.90	4,068	34.5	37.1	10.9
Z456/6,6 × ZR98/40,40	6	126.60	5,743	45.4	43.7	7.1
Z445/6,6 × ZR98/40,40	5	108.70	2,917	26.8	27.5	6.1
Z421/6,6 × ZR98/40,40	5	105.05	3,064	29.2	29.4	6.8
Z412/6,6 × ZR98/40,40	5	139.70	3,569	25.2	26.9	6.2
S 26/6,6 × ZR98/40,40	4	76.30	2,248	29.5	28.1	7.2
Z492/6,6 × ZR98/40,40[a]	5	90.85	5,885	64.8	66.5	7.1

[a] Crosses made between 492 and 98 are the control crosses; they show the marker effect enhancement of recombination frequency which is characteristic of strain ZR98/40,40. In the allele map site 492 is less then 6 units distal to the site at while allele 388 and its isoalleles are located

timate of the expected value of 23.4×10^{-5}. Two new features emerge from these data. Firstly, the marker effect character is not the result of the readily observed variation in recombination frequency. The results of marker effect crosses had an average coefficient of variation of 0.34. Secondly, allele 98 marker effect is shown to depend on genetic background. The results of both pairs of reciprocal crosses in Table 6 differ significantly with strain Z98/6,6 seeming to behave almost normally. Thus allele 98 marker effect *is not* intrinsic to the mutant site itself.

Allele 388 is one of 7 alleles categorised as isoalleles of allele 505 (Moore, 1972). A total of 22 crosses made between various members of this group of isoalleles yielded only 19 wild types from 4.58×10^7 progeny. The overall frequency of wild-types (4.15×10^{-7}) is of the same order of magnitude as the reversion frequency (Moore, 1972) so it is assumed that alleles 505, 496, 456, 445, 421, 412, 388 and S26 were caused by independent recurrences of mutation at the same site. All of these isoalleles gave unexpectedly low recombination frequencies in crosses with allele 154, and this was true whether they were used with the $A_6 B_6$ or $A_{40} B_{40}$ mating type specificity (Table 7). Similarly, all seemed to have the ability to restore the expected recombination frequency when crossed with strain ZR98/40,40. Although all 8 alleles share these general characteristics, they do not have identical effects. A gradient of decreasing ability to counteract the allele 98 enhancement of recombination frequency (Table 8) is paralleled by a gradient of decreasing ability to reduce recombination frequency in crosses with allele 154. The coincidence of these gradients in quite independent experiments encourages the view that they may be real. However, a larger number of replicate crosses of a larger number of isoalleles will have to be analysed before a statistically satisfactory subclassification of these marker effect alleles can be accomplished.

Discussion

The techniques employed in allele mapping are always tailored to the requirements of the organism and genetic system used, but adequate replication of crosses is rare. In the strict context of allele mapping this lack of replication is relatively unimportant because many of the errors which might be introduced are compensated by the combination of results from many different crosses (Moore, 1973a). It is more difficult to understand adherence to this conventional lack of adequate replication in studies where a small number of allelic intervals forms the basis of the investigation. In their study of the relations between genetic and physical distances Moore and Sherman (1975) used only 2 to 7 replicate diploids (made from various haploid strains) to determine mean recombination frequencies between 4 alleles of the *cycl* gene of yeast. Assessment of the differences between the means in related sets (with the *t*-test) shows many of the comparisons to be invalid. Only 14 of the 30 pairwise comparisons show significant differences at the 5% level (6 at the 1% level) which means that only 3 of the 10 trios of recombination frequencies can be used to sequence the alleles concerned. This severly limits the value of the study. Instances such as this are not unusual; too much weight is too often given to individual recombination frequencies.

Assuming a coefficient of variation of 0.35, the minimum sample sizes required to demonstrate statistically significant differences between mean recombination frequencies (at the 5% level of significance and with a 95% probability of success) can be calculated by the Neyman method (Snedecor and Cochran, 1967). A single sample of each cross would be sufficient to reveal a difference between two means which truly differed by 200%, 5 samples of each are required if the true difference is 100%, 14 samples for a true difference of 50%, 52 when the two actually differ by 25%, and 320 replicates of each cross are required if the two means differ by only 10%.

This degree of variation obviously has considerable implication for the design of experiments but since it appears to have the nature of a sampling variation despite the fact that technical sources of error can be excluded, it is pertinent to inquire into its origin. Current models of recombination, disregarding their finer details, envisage a two-stage process in which regions of hybrid DNA are first established in non-sister chromatids and, secondly, the base mismatching resulting from inclusion of heterozygous mutant sites in the hybrid DNA is repaired. The first stage is responsible, but not inevitably, for the formation of chiasmata, while the second produces the allelically recombinant genotype. It is the production of hybrid DNA which can most easily be seen as having some (even if imperfect) relationship to physical distances; so it is the frequency of occurrence of this process which must be measured if a meaningful allele map is to be produced. Paradoxically, only the products of the repair process can be enumerated genetically by random spore analysis. Since it is not inevitable that the repair will be made at all and since, if it is made, repair in any particular direction cannot be guaranteed the repair mechanisms are likely to deal with independent mismatches in a variable manner.

The basic events involved in the generation of random spore recombinants are shown in Table 9. To develop a numerical account some assumptions must be made about the relative liklihood of the various alternatives. First, the base mismatching may or may not be repaired. This seems to depend on the organism, as the frequency of asci showing a 4:4 spore pattern with post-meiotic segregation can be as high as 30% in *Sordaria* (Kitani and Olive, 1967) while such asci are rare in *Neurospora* (Case and Giles, 1964). For the sake of simplicity we assume that all base mismatches are repaired. It is also necessary to define whether the hybrid DNA will include both mutant sites involved in the cross or only one, and whether each allele is equally likely to be included in single-site hybrid regions. The former depends on the relation between the physical separation of the sites and the average length of the hybrid DNA segment (Fogel and Mortimer, 1969) the latter

Table 9. Catalogue of excision-repair options in heterozygous hybrid-DNA

Original Non-Sister Chromatids			
m_1+ m_1+	DNA helix, chromatid 1		
$+\ m_2$ $+\ m_2$	DNA helix chromatid 2		
Hybrid DNA is Generated			
Hybridity at site m_1 only	Hybridity encompasses both sites	Hybridity at site m_2 only	Strand designation
m_1+	m_1+	m_1+	a
$+\ +$	$+\ m_2$	m_1m_2	b
m_1m_2	m_1+	$+\ +$	c
$+\ m_2$	$+\ m_2$	$+\ m_2$	d
Frequency of wild-type strands if no repair accomplished			
0.25	0	0.25	
Possible Single Repair Events and Products			
Hybridity at site m_1 only	repair strand (a) $m_1\rightarrow+$, $=++$ strand repair strand (b) $+\rightarrow m_1$, $=m+$ strand repair strand (c) $m_1\rightarrow+$ $=+m$ strand repair strand (d) $+\rightarrow m_1$ $=mm$ strand		
Hybridity encompasses both sites	repair strand (a) $m_1\rightarrow+$ $=++$ strand repair strand (a) $+\rightarrow m_2=mm$ strand repair strand (b) $+\rightarrow m_1=mm$ strand repair strand (b) $m_2\rightarrow+$ $=++$ strand repair strand (c) $+\rightarrow m_2=mm$ strand repair strand (c) $m_1\rightarrow+$ $=++$ strand repair strand (d) $+\rightarrow m_1=mm$ strand repair strand (d) $m_2\rightarrow+$ $=++$ strand		
Hybridity at site m_2 only	repair strand (a) $+\rightarrow m_2=mm$ strand repair strand (b) $m_2\rightarrow+$ $=m+$ strand repair strand (c) $+\rightarrow m_2=+m$ strand repair strand (d) $m_2\rightarrow+$ $=++$ strand		

depends on whether there is any marked polarity in the formation of hybrid DNA (Whitehouse and Hastings, 1965). We will assume, again for initial simplicity, that all three types of hybrid segment are equally likely. Finally we will assume that both directions of repair (mutant→wild-type and wildtype→mutant) occur with equal frequency and that, where the opportunity exists, repairs at the mutant sites occur independently of one another (this last assumption may be the least realistic).

Applying all of these constraints it emerges that about 31% of available base mismatches will be repaired so as to form a wild-type strand. The potential for variability can be demonstrated by recalculating this factor after altering one or more of the assumptions. If all mismatches are repaired to the mutant coding pattern, then no wild type strands will be formed. Conversely, if all mismatches are repaired to the wild-type configuration, the frequency of wild type strands will be increased to about 66%. Change in other parameters also affects the frequency of wild type strands. If only 50% of the mismatches are repaired then, other factors being unchanged, the frequency of wild type strands formed will reduce to about 24%. Another obvious source of variability is variation in the rate of formation of hybrid DNA; variation in this factor will be compounded by the considerable variability which must be a consequence of the large number of options open to the repair systems. Most of the separate mechanisms which make up the process of recombination must be under enzymic control, and since it is reasonable to expect enzyme activities to differ, perhaps dramatically, even between individuals of identical genotype, it is equally reasonable to presume that this is the major cause of the observed variation in the frequencies of wild-type recombinants.

What we are considering here is variation between different attempts of the same cross and interpreting this as being due to random differences in the intracellular environment which result in the enzymes responsible for recombination showing altered activities. Thus each separate dikaryon must be seen as expressing its individuality in the sense discussed by Spudich and Koshland (1976). As recombination is an extremely rare event (Moore, 1974) enzymes specifically assigned to recombination may be present in extremely low concentrations. This would exacerbate the tendency for random events to greatly affect the expression of their activity.

An interesting pattern of marker effects is revealed by the data presented here. These data underline the fact that marker effect expression depends on the activities of enzymes and that those activities may differ in different cellular environments. It must be presumed that during the cross which was undertaken to prepare the mating type recombinant of the original Z98 mutant strain (Z98/6,6 × ZBw601/40,40) some other factor(s) segregated into the progeny spore which was to become strain ZR98/40,40 along with the *ftr* allele and selected mating type loci. That factor may determine a recombination enzyme or it may be a control element but it is certainly separate from the Z98 mutant site. A better candidate for a marker effect which is intrinsic to the mutant site is that shown by allele 388 and its isoalleles. The marker effect here is seen in the original mutant strains and in their mating type recombinants. So, lacking evidence to the contrary, we may assume that the marker effect of this mutant site results from the ability of the site to so interfere with the process of recombination as to reduce the number of wild-type recombinants obtained.

That these two types of marker effect appear to counteract each other when strains are intercrossed can be accounted for by a model which assumes that marker effects operate at the excision-repair level (Hastings, 1975). If we assume that in crosses between 154 and 492 (the 'normal' control cross) (*a*) there are no double-site hybrid fragments but each site is equally likely to be included in a hybrid region, (*b*) all base mismatches are repaired and (*c*) all repair events are equally likely, then 25% of all available mismatches will be converted to wild-type strands.

Assume next that as the result of some change in its system of recombination enzymes any dikaryon/diploid having ZR98/40,40 as a parent fails to repair *any* mismatch to the mutant con-

figuration. So in a cross between Z492/6,6 and ZR98/40,40 half of the base mismatches at each site are repaired so as to form wild-type strands (although the other half are also repaired to wild-type the repair is done on a chromatid which already carries the mutant version of the other site so fully wild type strands are not formed); thus 50% of available base mismatches are converted to wild type strands and marker effect enhancement of recombination will be observed.

Assume now that because of some peculiarity of its coding pattern site 388 is never repaired to wild-type, but that it is a site-specific effect and other sites are not influenced. In a cross with 154 all mismatches involving 388 (and that accounts for 50% of the total mismatches) are repaired to mutant; but among the other mismatches at site 154 there is the usual 25% conversion to form wild-type strands. Thus the overall frequency of wild-type strands will be 12.5% and marker effect reduction of recombination frequency will be seen.

Consider finally a cross between Z388/6,6 and ZR98/40,40 in which each continues to express its pecularity. Again at site 388 all repairs are to the mutant configuration. But 50% of the base mismatches are at site 98 and none of these will be repaired to mutant; so as before half will emerge as wild-type strands. Hence the frequency of wild-type strands will be 25%, giving an apparently normal recombination frequency.

Although this is a totally speculative scheme the assumptions made are not implausible. Inequality of conversion in the two directions has often been observed (being normal in *Ascobolus immersus*, Leblon and Rossignol, 1973) and has been related to the genetic background (Kitani and Olive, 1967) and to the mutagenic origin (and by implication the coding pattern) of the mutant site (Leblon, 1972a, b). The additional assumptions, that site 388 marker effect reduction is epistatic or dominant to whatever factor causes site 98 marker effect enhancement, and that the latter may segregate from *ftr*, are predictions that are open to test.

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