Current Genetics 1, 103–112 (1980)

Current Genetics

Characterization of Genes which Influence Allelic Recombination in *Coprinus cinereus*

Najah S. O. Katy and David Moore

Department of Botany, The University, Manchester M13 9PL, Great Britain

Summary. Previous work has shown that recombination between alleles of the *ftr* cistron is disturbed by two "marker effects", one reduces the recombination frequency and the other increases it. These effects have been studied further. The results show that both enhancement and reduction are controlled by single genes which seem to be independent of one another. The genes are symbolized *recE* (for recombination enhancement) and *recR* (recombination reduction). Both genes are fully dominant, non-additive, and segregate readily from one another and from the *ftr* cistron. Recombination between any pair of *ftr* alleles is increased when *recE* is part of the genotype, but *recE* has no effect on recombination between alleles of the *me*-5 locus. On the other hand, the reduction of recombination caused by *recR* in the *ftr* cistron is polarised and allele-specific, but *recR increases* the frequency of recombination between *me*-5 alleles. The data are interpreted on the basis that the *rec*-gene products may be involved in chromatid pairing and that polymorphic variants of them cause differences in pairing which, by altering the opportunities for recombination, are observed as differences in allelic recombination frequency.

Key words: Meiotic recombination – Marker effects – *Rec* genes.

Introduction

The *ftr* cistron of the Basidiomycete *Coprinus cinereus* is the structural gene for a carrier molecule involved in sugar transport. Because of the way in which sugar transport is organized in this fungus (Moore and Devadatham, 1979) mutants of this gene have phenotypic characteristics which make them ideal for purely genetic studies. The *ftr* transport defect interferes with the entry of growth-inhibiting sugar analogues so the mutants are able to grow on media which inhibit growth of wild-type. The defect also seriously limits uptake of fructose so that the mutants grow much less well than the wild-type on media containing this sugar as sole carbon source (Moore and Stewart, 1971). A consequence of the pleiotropic phenotype is that selection techniques can be designed for selection of genetic change in both directions; either mutant to wild-type (select for ability to grow on fructose) or wild-type to mutant (select for resistance to sugar analogues like sorbose or 2-deoxy-D-glucose).

A fine-structure map of the cistron has been constructed (Moore, 1972) and mutational peculiarities caused by differential effects of selection media (Moore and Devadatham, 1975) and chemical mutagens (Moore, 1975) have been investigated. Attention has also been given to the characteristics of the processes which give rise to recombination between allelic mutants. The recombination frequencies obtained in crosses between *ftr* alleles (expressed in terms of the frequency of wild-type recombinants in a large random sample of basidiospores) are sufficiently reproducible for a reliable allele map to be made (Moore, 1972). Nevertheless such recombination frequencies are variable in the sense that results from repeated attempts of the same cross (using the same parental strains) are more disperse than expected from a binomial distribution. The variability has been studied in detail (Moore, 1973; Moore and Katy, 1978) and shown to be an intrinsic biological phenomenon. It has been interpreted as an expression of the latitude available to the excision-repair mechanisms which are widely considered to be responsible for formation of the allelically-recombinant genotype as the last step in a

Offprint requests to: D. Moore at the above address

0172-8083/80/0001/0103/$02.00

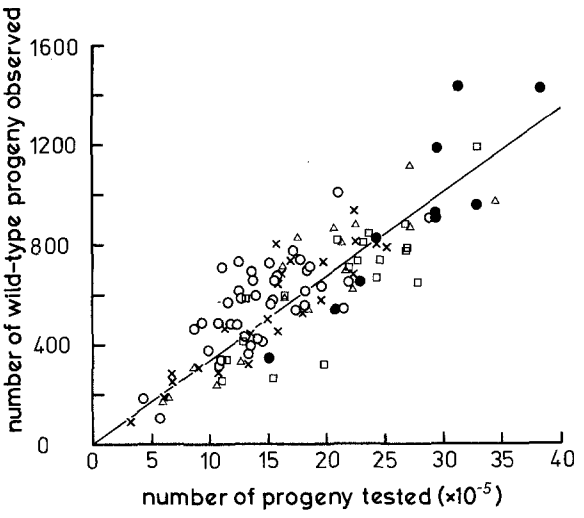

Fig. 1. Results of 114 crosses made between strains carrying alleles 154 and 492. Each point shows the result of a single cross. The line drawn is the line of regression through the origin. Five crosses involving different strains are represented: Z492/6,6 x ZR154/40,40 (○); ZR492/40,40 x Z154/6,6 (□); ZJ492/5,5 x Z154/6,6 (△); ZR492/40,40 x ZJ154/5,5 (x); and ZJ492/5,5 x ZR154/40,40 (●)

recombination process initially involving formation of hybrid DNA.

Despite the difficulties inherent in allele mapping, some *ftr* alleles have been identified which show patterns of recombination frequencies which are inconsistent with their map positions. Such "marker effect" alleles have been identified before in other organisms (Kitani and Olive, 1967; Kruszewska and Gajewski, 1967; Esposito, 1971; Gutz, 1971; Leblon, 1972a and b). In this paper we report a detailed analysis of the marker effect alleles of the *Coprinus ftr* cistron and compare their characteristics with those of similar phenomena in other organisms.

Materials and Methods

Most of the methods and strains employed were described by Moore (1972). Tetrads were isolated using the "on-glass" technique described earlier (Moore, 1966). A major departure in some crosses has been the investigation of allelic recombination in two genes simultaneously. This was possible because the selection methods for the two genes concerned (*ftr* and a methionine auxotroph, *me*-5) proved to be compatible. Recombinants between *ftr* alleles are selected on medium containing fructose or fructose + sorbose, total progeny being enumerated on a medium containing glucose. Recombinants between *me*-5 alleles are selected on a methionine-free medium, while total progeny are enumerated on one containing methionine supplementation. Tests showed that the inclusion of methionine had no effect on results of crosses between *ftr* mutants, while results obtained from crosses between *me*-5 alleles were unaffected by the nature of the carbon source in the medium. It was thus possible to assemble double-mutants carrying different alleles of both *ftr* and *me*-5 and, by intercrossing them, to study allelic recombination in both cistrons at the same time. As far as we are aware this is the first time that intragenic recombination has been studied simultaneously in two unlinked genes.

Results

Variability of Allelic Recombination. The variability encountered in replicated crosses made between *ftr* alleles is illustrated in Fig. 1 which shows the results of 114 separate crosses made between alleles 154 and 492.

Each point in Fig. 1 represents the result of a single cross, the number of wild-type recombinants observed being plotted against the number of progeny analysed. Six strains differing in mating type were crossed in five different combinations to produce these results: there were 41 replicates of the cross between 154 $A_{40}B_{40}$ and 492 A_6B_6, 24 replicates of the cross between 154 A_5B_5 and 492 $A_{40}B_{40}$, 20 replicates of the cross between 154 A_6B_6 and 492 $A_{40}B_{40}$, 19 replicates of the cross between 154 A_6B_6 and 492 A_5B_5, and 10 replicates of the cross between 154 $A_{40}B_{40}$ and 492 A_5B_5. A grand total of 1.9991×10^8 progeny were analysed and 68620 wild-types were observed. The overall mean recombination frequency from the individual crosses was 34.62 wild-type recombinants per 10^5 progeny tested, and the standard deviation was 7.95 wild types per 10^5 progeny tested. The coefficient of variation (standard deviation/mean) of 0.23 is a numerical measure of the dispersion evident in Fig. 1. It is important to note that the scatter of points was about the same for each combination of strains and that each combination gave about the same mean recombination frequency. This shows that the variability was intrinsic to the recombination process and was independent of genetic background. Variability has been studied in detail before (Moore and Katy, 1978) and for present purposes the more important point is that Fig. 1 also shows that the recombination frequency was a character of the pair of alleles involved, being independent of both variability and genetic background.

The proposition that the recombination frequency (measured as the frequency of wild-type recombinants among a random sample of basidiospores) is intrinsic to the allele pair involved in the cross is the basis of the use of such measures for the construction of an allele map. The variability of the measurement does not jeopardise this approach, but it does impose the requirement that crosses must be replicated if reliable results are to be obtained. It is for this reason that the rest of the results reported here were obtained by carrying out at least six and usually rather more replicated trials of each cross.

The Allele Map. Although a map of this cistron has been published (Moore, 1972) it does not include all of the alleles used in the present work and was not based on replicated crosses. A map of the non-marker effect alleles used here is shown in Fig. 2. Departures from additivity were not great. As reported before (Moore, 1972), there was no evidence for map-expansion.

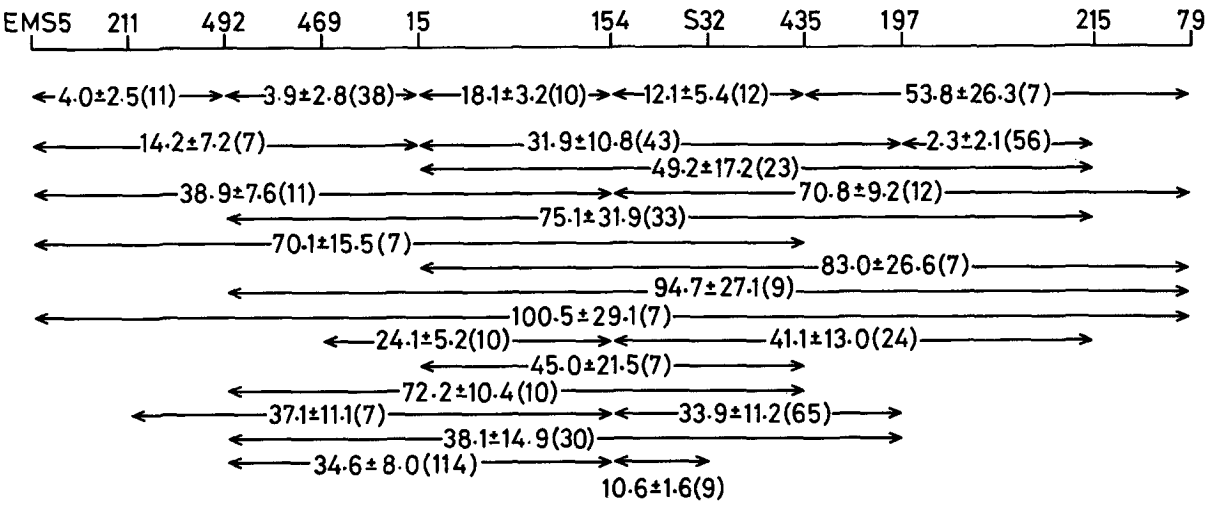

Fig. 2. Partial allele map of the *ftr* cistron. Recombination frequencies are shown as the mean and standard deviation of the numbers of replicate crosses shown in brackets. Note that alleles EMS5 and 79 represent the currently known boundaries of the cistron

Marker Effect Alleles. The generally good additivity of a map such as is shown in Fig. 2 is the background against which marker effect alleles have been identified (Moore and Katy, 1978). Allele 98 (in strain no. ZR98/40,40) showed a recombination frequency of $2.7 \pm 0.8 \times 10^{-5}$ wild-types in 11 crosses made against allele 154. Reference to Fig. 2 would lead to the prediction that the recombination frequency in crosses between alleles 98 and 492 should be about 35×10^{-5}, but when crosses between ZR98/40,40 and Z492/6,6 were carried out the mean and standard deviation of 44 replicates proved to be $70.33 \pm 17.39 \times 10^{-5}$ wild-types. As this behaviour was consistently observed in crosses involving ZR98/40,40 it was characterised as a strain showing marker effect enhancement of recombination.

The reverse situation was encountered with strains carrying allele 505. Eight crosses against allele 492 gave a mean recombination frequency of $6.34 \pm 3.16 \times 10^{-5}$ wild-types. So by reference to Fig. 2 one would expect a minimum recombination frequency of about 28×10^{-5} in crosses with allele 154. However, the mean and standard deviation of 47 crosses made between alleles 154 and 505 were $11.62 \pm 4.21 \times 10^{-5}$ wild-types. Since this behaviour was again consistently observed with allele 505 it was characterised as showing marker effect reduction of recombination.

Extent of Marker Effect Expression within the Cistron. The extent of the marker effects was assessed by crossing ZR98/40,40 or ZR505/40,40 against a selection of alleles which was representative of the different parts of the cistron. These results were compared with control crosses using non-marker effect alleles. Allele 154 was used as control for investigation of marker effect enhancement caused by ZR98/40,40, and allele 492 was the control for crosses studying marker effect reduction caused by ZR505/40,40. The results (Table 1) showed that ZR98/40,40 exerted its influence over the entire cistron although the magnitude of the effect varied from about 2 to about 4 times the control value. The situation with regard to ZR505/40,40 was different (Table 2). A considerable reduction in recombination frequency was evident in crosses placed to the right of site 505 in the allele map, but when allowance is made for the separation from the control allele very little, if any, effect can be seen to the left even though allele EMS5 is the most distant allele available on the left-hand side. These data thus seem to confirm the suggestion that the effect of allele 505 is polarised (Moore, 1973), but they do show that the magnitude of the reduction in recombination is maintained over a greater distance to the right than was previously supposed.

Isoalleles of Allele 505. Although allele 98 is the only representative of its mutant site which is known, allele 505 was the first of 7 isoalleles which mapped at the site referred to as the "site 505 hotspot" (Moore, 1972). A specific search for further representatives of this mutant site increased the number of known isoalleles to 18, of which 16 were isolated after nitrosoguanidine mutagenesis and one after ethyl methanesulphonate mutagenesis, the remaining one being a spontaneous mutant. All 18 isoalleles were crossed against allele 154 (Table 3a) and while all showed considerable reduction in recombination frequency from the "expected" value of about 28×10^{-5}, the extent of the reduction varied greatly. The most extreme expression (allele 432) gave a greater than 10-fold reduction, while alleles 382 and 456 gave only about $\frac{1}{3}$-reduction.

Table 1. Results of crosses undertaken to investigate the extent of marker effect enhancement

Cross[a]	Number of trials	Mean frequency of wild-type recombinants x 10^5	Standard deviation x 10^5	Enhancement factor[b]
Z79/6,6 x ZR98/40,40	17	143.74	26.42	2.03
Z79/6,6 x ZR154/40,40	12	70.78	9.23	
Z197/6,6 x ZR98/40,40	13	84.23	15.05	2.11
Z197/6,6 x ZR154/40,40	9	39.99	8.33	
Z435/6,6 x ZR98/40,40	13	34.03	7.72	2.82
Z435/6,6 x ZR154/40,40	12	12.07	5.41	
S32/6,6 x ZR98/40,40	12	38.47	8.08	3.62
S32/6,6 x ZR154/40,40	9	10.63	1.57	
ZJ154/5,5 x ZR98/40,40	9	2.97	1.29	3.81
ZJ154/5,5 x Z98/6,6	8	0.78	0.23	
Z15/6,6 x ZR98/40,40	12	37.38	8.33	2.07
Z15/6,6 x ZR154/40,40	10	18.05	3.24	
Z469/6,6 x ZR98/40,40	12	85.37	20.93	3.54
Z469/6,6 x ZR154/40,40	10	24.09	5.16	
Z211/6,6 x ZR98/40,40	11	107.47	20.87	2.90
Z211/6,6 x ZR154/40,40	7	37.07	11.10	
EMS5/6,6 x ZR98/40,40	13	136.19	24.92	3.50
EMS5/6,6 x ZR154/40,40	11	38.92	7.57	

[a] ZR98/40,40 carries *recE*, all of the other strains are *recE*$^+$ (see text)
[b] The enhancement factor is the ratio of the two mean frequencies of wild-type recombinants

Table 2. Results of crosses undertaken to investigate the extent of marker effect reduction of allelic recombination frequency

Cross[a]	Number of trials	Mean frequency of wild-type recombinants x10^5	Standard deviation x 10^5	Reduction factor[b]
Z79/6,6 x ZR505/40,40	9	62.18	15.28	0.70
Z79/6,6 x ZR492/40,40	9	94.67	27.13	
Z435/6,6 x ZR505/40,40	9	39.59	6.96	0.60
Z435/6,6 x ZR492/40,40	10	72.21	10.36	
S32/6,6 x ZR505/40,40	9	26.56	11.66	0.65
S32/6,6 x ZR492/40,40	8	47.25	15.35	
EMS5/6,6 x ZR505/40,40	10	8.93	5.65	0.86
EMS5/6,6 x ZR492/40,40	11	4.03	2.50	
ZJ492/5,5 x ZR505/40,40	8	6.34	3.16	

[a] ZR505/40,40 carries *recR* (see text) which causes the marker effect reduction of allelic recombination frequency; all of the other strains are normal (*recR*$^+$)
[b] The reduction factor is the ratio of the two mean frequencies of wild-type recombinants, but calculated after allowing for the distance between sites 505 and 492

Interaction between Marker Effects. In earlier studies when ZR98/40,40 was crossed with either 505 or 388 the marker effects seemed to cancel one another since approximately normal recombination frequencies were obtained from such crosses even though they included both marker effects (Moore, 1972; Moore and Katy, 1978). Further examination of this point with a wider selection of isoalleles of 505 confirmed this point but made it clear that in such crosses both marker effects were still being expressed (Table 3b). The very much reduced recombination frequency obtained in crosses between allele 432 (the most effective marker effect reducer) and

Table 3. Characteristics of isoalleles of the site-505 mutational hotspot

Allele under test	Crosses against allele 154			Crosses against strain ZR98/40,40		
	Number of trials	Recombination frequency (x 10^5)		Number of trials	Recombination frequency (x 10^5)	
		Mean	Standard deviation		Mean	Standard deviation
432	12	2.26	1.16	10	3.87	1.56
495	12	5.05	1.29			
409	11	10.12	5.85			
390	10	10.53	4.54			
376	14	11.39	3.79			
505	47	11.62	4.21	14	26.18	7.72
EMS30	11	11.93	3.03	6	22.20	9.93
S26	18	12.97	4.19	8	28.09	4.95
494	10	13.82	3.24			
388	8	14.07	5.13	19	26.00	6.20
412	20	14.38	4.06	9	26.63	6.55
421	20	14.70	5.84	8	28.99	7.14
445	17	15.11	5.29	9	25.99	6.29
358	13	16.52	6.20			
496	46	16.67	4.95	10	38.90	10.60
499	11	18.01	4.63			
382	10	18.71	4.85			
456	34	18.71	5.41	9	41.57	12.01

ZR98/40,40 clearly demonstrated that the reducing effect was active despite the presence of strain ZR98/40,40 marker effect enhancement. Similarly, the enhanced recombination frequencies seen in crosses between ZR98/40,40 and some of the least effective reducers (alleles 496 and 456) demonstrated the continued action of marker effect enhancement despite the presence of marker effect reduction. The apparently normal recombination frequencies observed in the other crosses is presumed to be due to the arithmetical coincidence that for these alleles marker effect reduction reduced the recombination frequency by about the same amount that strain ZR98/40,40 enhanced it.

Segregation of the Marker Effect Enhancement Phenotype. Segregation was studied by means of tetrad analysis of a cross made between ZR98/40,40 and Z492/6,6. A total of 13 tetrads were isolated from this cross; the way in which the spores were characterized can be understood by consideration of some examples. Excepting those arising from intragenic recombination, all the spores from such a cross will carry *ftr* mutations, but two spores of each tetrad must carry allele 98 while the other two carry allele 492. The identity of these was established by crosses between the progeny spores and appropriate tester strains. For example, a progeny spore carrying allele 492 will not form recombinants when crossed against a tester known to carry 492; however, a progeny spore carrying allele 98 is expected to show a recombination frequency of at least 30 x 10^{-5} when crossed with such a tester. Conversely, when crossed against a tester known to carry allele 154 a progeny spore carrying allele 98 will give a recombination frequency of not more than about 3 x 10^{-5} but one carrying allele 492 will give a recombination frequency of at least 30 x 10^{-5}. The presence of the marker effect phenotype was detected by replicated crosses against appropriate testers also. For example, a progeny spore carrying allele 492 when crossed against a 154 tester will give a recombination frequency of about 30 x 10^{-5} in the absence of marker effect enhancement and one of about 60 x 10^{-5} in its presence. A similar distinction between results is expected when progeny spores carrying allele 98 are crossed against a tester carrying allele 492. Progeny mating types were determined by scoring dikaryon formation in confrontations between inocula from the progeny and inocula from strains of known mating type.

After determination of the spore genotypes the tetrads were classified as illustrated in Fig. 3. It is clear that the marker effect enhancement phenotype segregated as though it were controlled by a single major gene. We designate this gene *recE* (*rec*ombination *e*nhancement in the *ftr* cistron). In Table 4 the combined tetrad data are presented and although the number of tetrads examined was small it is clear that no linkage was evident between any gene pair involved.

Thus the marker effect enhancement was caused by a single major gene, *recE* which was unlinked to the *ftr* cistron on which it exerted its effect. Since *recE* was

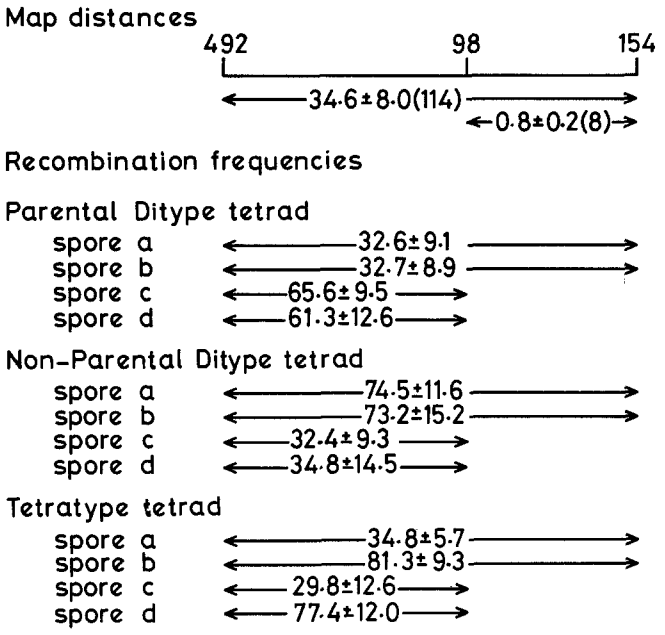

Fig. 3. Tetrad types observed in a cross between Z492/6,6 and ZR98/40,40 undertaken to determine whether marker effect enhancement of recombination frequency could segregate. In each case spores a and b were shown by other tests to carry allele 492 and the results of crosses against a tester carrying allele 154 are illustrated here, while spores c and d were shown to carry allele 98 and are here crossed with a 492 tester. Recombination frequencies are given as the mean and standard deviation of 6 replicate crosses. In the parental ditype tetrad progeny spores carrying allele 98 are associated with enhanced recombination frequency, as in the parental strain ZR98/40,40. In the non-parental ditype tetrad the recombination enhancement phenotype is found in progeny spores carrying allele 492, and in the tetratype tetrad all four combinations are found

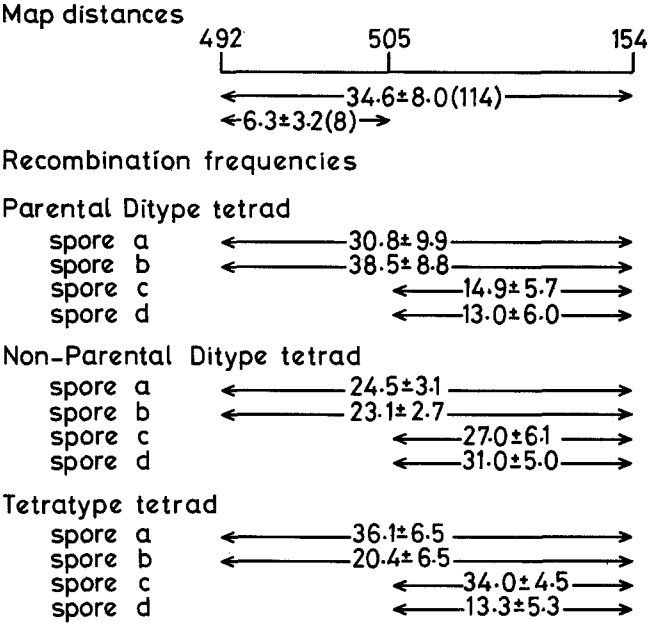

Fig. 4. Tetrad types observed in a cross between Z505/6,6 and ZR154/40,40 undertaken to demonstrate segregation of marker effect reduction of recombination frequency. Other tests showed that in each case spores a and b carried allele 154, and c and d carried allele 505. Recombination frequencies are shown as the means and standard deviations of 6 replicate crosses. In the parental ditype tetrad the reduction in recombination frequency is associated with allele 505 in progeny spores c and d, as it was in the parental strain Z505/6,6. In the non-parental ditype tetrad the recombination reduction is seen to be associated with allele 154 in progeny spores a and b. The tetratype tetrad shows all four combinations. In each case progeny spores a and b were crossed against a tester strain carrying allele 492, and spores c and d were crossed against a tester carrying allele 154

expressed when any strain carrying it was crossed against one carrying the normal recombination system (designated $recE^+$), we assume that *recE* is dominant to $recE^+$. It is also important to emphasise that *recE* expression was independent of allele 98. Enhanced recombination frequencies were obtained in crosses between the 154 tester and progeny carrying *recE* together with allele 492. Thus allele 98 need not be present in the genotype for *recE* to be expressed and the presence of this allele in the strain in which enhancement was first identified was fortuitous.

Segregation of the Marker Effect Reduction Phenotype. Similar strategy was used in the analysis of 13 tetrads from a cross between Z505/6,6 and ZR154/40,40. After isolation the progeny were tested for recombination in crosses against testers carrying alleles 492 and 154. Illustrations of the types of tetrad observed are given in Fig 4. A difficulty apparent in these tests was the imperfect expression of the recombination reduction phenotype in crosses involving progeny carrying allele 154. The problem is illustrated by the tetratype tetrad shown in Fig. 4; here it can be seen that recombination frequency was reduced to less than 40% of the normal value in the spore carrying allele 505, but in that carrying allele 154 the reduction was to 55% of normal. Taken together with the different degrees of expression observed with different isoalleles of allele 505, we believe that this reveals some dependence on the existence and nature of a mutant site at the position occupied by allele 505. This did not interfere with characterization of progeny genotypes and it was clear that recombination reduction was a phenotype that segregated as though controlled by a single major gene. We designate that gene *recR* (for *rec*ombination *r*eduction in the *ftr* cistron). Combined tetrad data (Table 5) showed that *recR* was independent of *ftr* and the mating type factors. Since *recR* was expressed when the strain

Table 4. Tetrad ratios from the cross between ZR98/40,40 and Z492/6,6

Gene pair	Number of tetrads of each type		
	Parental ditype	Non-parental ditype	Tetratype
recE and *ftr*	1	6	6
recE and *A*	4	2	7
recE and *B*	2	1	10
ftr and *A*	4	4	5
ftr and *B*	1	4	8
A and *B*	4	4	5

Table 5. Tetrad ratios from the cross between Z505/6,6 and ZR154/40,40

Gene pair	Number of tetrads of each type		
	Parental ditype	Non-parental ditype	Tetratype
recR and *ftr*	4	1	8
recR and *A*	6	1	6
recR and *B*	4	1	8
ftr and *B*	2	4	7
A and *B*	1	3	9

None of the PD:NPD ratios differ significantly from 1:1

Table 6. Comparison of heterozygous and homozygous combinations of *rec*-genes

Parental genotypes	Number of trials	Recombination frequency $\times 10^5$	Standard deviation $\times 10^5$
ftr^{98}, $recE^+ \times ftr^{492}$, $recE^+$	9	38.3	10.1
ftr^{98}, $recE^+ \times ftr^{492}$, *recE*	9	76.4	15.0
ftr^{98}, *recE* $\times ftr^{492}$, *recE*	8	82.4	16.2
ftr^{505}, $recR^+ \times ftr^{154}$, $recR^+$	12	32.5	9.4
ftr^{505}, $recR^+ \times ftr^{154}$, *recR*	12	13.9	3.9
ftr^{505}, *recR* $\times ftr^{154}$ *recR*	16	11.2	2.2

carrying it was crossed against a tester known to show normal recombination frequencies we conclude that *recR* is dominant to the normal allele, $recR^+$.

Characteristics of recE *and* recR. As pointed out earlier, crosses between ZR98/40,40 (which carried *recE*) and Z505/6,6 (which carried *recR*) yielded approximately normal recombination frequencies. A single tetrad was isolated from such a cross in order to examine the possible allelism of *recE* and *recR*. Spore *a* of this tetrad was found to have the genotype (ftr^{98}, *recE*) and spore *b* genotype (ftr^{98}, *recR*). Spores *c* and *d* both carried *ftr* allele 505 and both gave normal recombination frequencies in crosses against a 154 tester. To determine whether the latter phenotype was due to the genotype *recR-recE* or to $recR^+$-$recE^+$ further crosses were carried out between progeny spores *c* and *d* and the tester carrying allele 154 (which was itself $recR^+$-$recE^+$). Progeny from these crosses were individually isolated and tested for their recombination phenotype. All of the progeny from the cross involving tetrad spore *d* showed normal recombination frequencies; on the other hand the progeny of spore *c* segregated normal, reducing and enhancing recombination phenotypes.We concluded that in the original tetrad spore *c* had the genotype (ftr^{505}, *recR*, *recE*) and spore *d* the genotype (ftr^{505}, $recR^+$, $recE^+$). If *recR* and *recE* were alleles a tetrad of this sort could only have arisen by reciprocal allelic recombination within the *rec*-gene. The liklihood of this being observed is vanishingly small, so we conclude that *recE* and *recR* represent different genes.

Both *recE* and *recR* are dominant since their effects are evident in crosses in which only one of the parents carries them. Strains have been constructed which allow crosses to be done in which both parents carry one or other of the *rec*-genes. The results (Table 6) suggest that in each case the homozygous configuration marginally amplified the *rec*-gene effect, but on these data differences between heterozygotes and homozygotes were not statistically significant so the genes cannot be described as additive.

As there was no evidence for linkage between the *ftr* cistron and either *recE* or *recR* these two genes must exert their effects through the formation of gene products which are released at least into the nucleoplasm.
This raised the possibility that the *rec*-genes may affect recombination in other cistrons than *ftr*. To test this we constructed double mutants which carried alleles both of the *ftr* cistron and of the unlinked *me*-5 locus. By crossing double mutants together it was possible to monitor the effects of the *rec*-genes by estimating recombination frequencies between *ftr* alleles and to study their effect on *me*-5 by measuring recombination frequencies between alleles of that gene. Results, detailed in Table 7, showed that *recE* had no significant effect on recombination between *me*-5 alleles. Paradoxically, although *recR* reduced recombination frequencies between *ftr* alleles, it actually *enhanced* recombination frequencies between *me*-5 alleles.

Discussion

The genes affecting recombination which are described here differ in one or other respect from those described

Table 7. Results of crosses undertaken to investigate the effect of *rec*-genes on the *me-5* cistron

Parental genotypes	Number of trials	Recombination frequency (mean ± SD) x 10^5	
		Between *ftr* alleles	Between *me-5* alleles
ftr^{505}, *me-5*^{G1905}, *recR*$^+$ x *ftr*^{154}, *me-5*^{M23}, *recR*$^+$	6	28.8 ± 8.5	27.4 ± 6.5
ftr^{505}, *me-5*^{G1905}*recR* x *ftr*^{154}, *me-5*^{M23}, *recR*$^+$	24[a]	15.4 ± 4.7	50.1 ± 12.6
ftr^{98}, *me-5*^{M37}, *recE*$^+$ x *ftr*^{492}, *me-5*^{M23}, *recE*$^+$	28[a]	31.3 ± 10.4	134.1 ± 36.7
ftr^{98}, *me-5*^{M37}, *recE* x *ftr*^{492}, *me-5*^{M23}, *recE*$^+$	7	59.3 ± 14.3	147.5 ± 52.3

[a] bulked data from crosses involving strains of different ancestry though sharing the indicated genotype

previously in other organisms. The most obvious quantitative difference was in the magnitude of their effect. Generally, *recE* increased recombination frequencies by factors of about 2 to 4, and although *recR* shows variable effect depending on the 505-isoallele involved in the cross, the majority of alleles yielded recombination frequencies reduced by about a factor of 2. In contrast, the *rec-1*, *rec-2* and *rec-3* genes of *Neurospora* caused 10 to 30-fold reduction of allelic recombination (Catcheside, 1977); the M26 mutant of the *ade6* locus in *Schizosaccharomyces pombe* increased recombination in that locus by up to 20 times (Gutz, 1971); and the *rec* and *pop* genes of *Aspergillus nidulans* altered mitotic recombination frequencies by factors of about 100 (Parag and Parag, 1975; Parag, 1977). In terms of the scale of their effects *recE* and *recR* were more similar to the *cv* factors found in *Ascobolus immersus* (Girard and Rossignol, 1974), but *recE* and *recR* were fully dominant whereas *cv* heterozygotes differed from both homozygotes. Moreover, most of the *cv* factors were closely linked to their target genes; the *Coprinus rec*-genes were not. Other controlling factors of recombination events in *Ascobolus* (Lamb and Helmi, 1978; Wickramaratne and Lamb, 1978) were also near to the target site. The closest comparison seems to be with some of the control elements which influence recombination between the mating type subunits in *Schizophyllum commune* (Simchen, 1967; Stamberg, 1968). There are differences even in this case though, for in *Schizophyllum* low recombination frequency was dominant to high (Simchen, 1967) whereas in *Coprinus* both *rec*-genes exhibited a dominant high-recombination phenotype, but in different cistrons.

It seems, therefore, that this *Coprinus* system is unique in its details, though it has a clear relation to systems described in other organisms. In attempting to interpret the mechanism of action it is worth recalling first the origin of the *rec*-genes. No attempt has ever been made to induce *rec*-mutants in *Coprinus*; both *recE* and *recR* were simply identified as being present in the "genetic background". The basis of the identification was their tendency to cause recombination frequencies between *ftr* alleles which were inconsistent with the bulk of such values. All *ftr* alleles were selected using the same haploid parental wild-type, strain number BC9/6,6 (Moore and Stewart, 1971), although a number of chemical mutagens and selection procedures have been used (Moore, 1975; Moore and Devadatham, 1975). Thus apart from coincident mutation in genes other than *ftr* all original *ftr* mutant strains are expected to have the same genotype. The *recE* phenotype became evident after particular strains were outcrossed to a different wild-type to prepare mating type recombinants. This wild-type, strain number ZBw601/40,40, originated in Czechoslovakia (Moore and Stewart, 1972). Strain BC9/6,6 was derived from wild-types collected in England, so it seems reasonable to suggest that *recE* is a polymorphic variant which was present in the Czechoslovakian strain but not in the English one. In contrast to this the effects of *recR* were observed in crosses involving the original *ftr* mutant strains and must therefore have been present in the BC9/6,6 genome. Only *ftr* alleles mapping at site 505 have shown the recombination reduction phenotype to a sufficient extent for their recombination frequencies to be inconsistent with the rest of the allele map; yet there are too many isoalleles of site 505 for it to be reasonable to suggest that the situation arose through coincident mutation at both *recR* and a particular site in the *ftr* cistron. We much prefer the interpretation that *recR* existed as a population polymorphism in the BC9/6,6 genome but that its existence only became clear when particular mutations in the *ftr* cistron created a specific DNA coding pattern to which it could respond. Since these unlinked *rec*-genes must function through the formation of some product which is released into the nucleoplasm it is likely that mutation in the vicinity of site 505 in the *ftr* cistron changed the normal coding pattern to one which was recognised as a binding site by the *recR* gene product. This would explain the localization of the *recR* response as well as the variation in response between different isoalleles. Presumably for

some types of mutation (exemplified by alleles 432 and 495 – Table 3) the binding site similarity was extremely close, for others it was weak (e.g. 382 and 456), and for the most usual type of mutation (e.g. 388 and 505) it was close enough for the level of phenotypic expression used in the analyses described here. We do not believe that this region of the *ftr* cistron provides the normal binding site for the *recR* product, rather that a mutated version of the region was wrongly identified as a binding site. If this interpretation is correct then it rises the question of whether recombination reduction is the true phenotype of *recR*. It is significant that *recR* enhanced recombination in the *me*-5 cistron and it may well be that both genes are responsible for elevating recombination frequencies.

Postulation of the manner in which recombination frequency is affected must take into account that the method of analysis equates the frequency of wild-type progeny with the allelic recombination frequency. The *rec*-genes could influence excision-repair, since any tendency towards preferential correction of base mismatches to the wild-type configuration would be detected here as recombination enhancement. This would be realistic, however, only if it were accepted that the repair system was unusual in being able to promote one type of repair (say an AC mismatch to a GC match, in preference to the alternative AC to AT) which *by chance* represented correction to the wild-type coding pattern. It would be unrealistic to ascribe to the system any "knowledge" of what represents the wild-type genotype. The corollary must therefore be that in some allelic combinations the preferred direction of repair would *by chance* represent repair to the mutant coding pattern because of the chemical nature of the mutant sites involved. This would be identified as reduction of recombination frequency by virtue of its causing reduction in the yield of wild-type recombinants. This, of course, is "marker effect" in the strict sense, where the phenotype depends on the nature of the mutant site. In this and previous work (Moore, 1972, 1973; Moore and Katy, 1978) *recE* has been involved in crosses between 35 different allele pairs and in every instance recombination enhancement was observed. The uniformity of the phenotype argues against any dependence on the mutant site. A similar conclusion is reached in respect of *recR* (which has been involved in crosses between 14 allele pairs) although this case is complicated by the polarity of effect and isoallele dependence described above. Although we have previously suggested that marker effects in the *ftr* cistron might originate in excision-repair phenomena (Moore and Katy, 1978), we now consider it more likely that the *rec*-genes in *Coprinus* are involved in earlier stages of recombination.

Lamb (1977) has shown that conversion frequencies in fungi imply that an appreciable proportion of the DNA is intimately paired in a considerable proportion of meioses. If this is the case then a fairly large number of genes may be involved in providing the structures which achieve and regulate this pairing. Catcheside (1977) has argued that interference with chromosome pairing can influence recombination frequencies in localised regions. The phenotypes of the *Coprinus rec*-genes can be understood if it is assumed that their gene products form part of the architecture which is responsible for chromatid pairing during meiosis. As a working hypothesis we suggest the following: (*a*) *recE* and *recE*$^+$, and *recR* and *recR*$^+$ are polymorphic variants of two genes whose products are involved in chromatid pairing at meiosis, *recR* and *recE*$^+$ originated in strain BC9/6,6 and *recE* and *recR*$^+$ in strain ZBw601/40,40; (*b*) crosses involving only *recE*$^+$ and *recR*$^+$ yield recombination frequencies between *ftr* and *me*-5 alleles which are arbitrarily described as "normal"; (*c*) inclusion of *recE* in the cross so improves pairing in the region including the *ftr* cistron that opportunities for allelic recombination are increased above normal and enhanced allelic recombination is observed in that locus; (*d*) *recR* has the same effect in the vicinity of the *me*-5 locus; (*e*) all *rec*-genes function by the formation of gene products which interact with binding sites (which we designate *bindE* and *bindR*) on the chromatids; (*f*) mutations in the region of site 505 in the *ftr* cistron can, because of the specific coding pattern of the gene, create an analogue of *bindR* to which the *recR* product attaches; (*g*) the physical presence of *recR* product at this illegitimate binding site interferes with and reduces recombination between *ftr* alleles.

If it is the case that *recE* and *recR* are involved in chromatid pairing, then one might expect their effects to be evident at the level of intergenic recombination. We intend to pursue this aspect in a later study.

Acknowledgement. N. S. O. K. gratefully acknowledges receipt of a Scholarship from the Ministry of Higher Education and Research of the Government of Iraq.

References

Catcheside, D. G.: The Genetics of Recombination. London: Eward Arnold 1977
Esposito, M. S.: Mol. Gen. Genet. 111, 297–299 (1971)
Girard, J., Rossignol, J. L.: Genetics 76, 221–243 (1974)
Gutz, H.: Genetics 69, 317–337 (1971)
Kitani, Y., Olive, L. S.: Genetics 57, 767–782 (1967)
Kruszewska, A., Gajewski, W.: Genet. Res. 9, 159–177 (1967)
Lamb, B. C.: Mol. Gen. Genet. 157, 31–37 (1977)
Lamb, B. C., Helmi, S.: Genet. Res. 32, 67–78 (1978)
Leblon, G.: Mol. Gen. Genet. 115, 36–48 (1972a)
Leblon, G.: Mol. Gen. Genet. 116, 322–335 (1972b)
Moore, D.: Nature 209, 1157–1158 (1966)
Moore, D.: Genet. Res. 19, 281–303 (1972)
Moore, D.: Genet. Res. 22, 187–193 (1973)
Moore, D.: Mutat. Res. 28, 455–458 (1975)

Moore, D., Devadatham, M. S.: Mol. Gen. Genet. **138**, 81–84 (1975)
Moore, D., Devadatham, M. S.: Biochim. Biophys. Acta **550**, 515–526 (1979)
Moore, D., Katy, N. S. O.: Mol. Gen. Genet. **159**, 311–320 (1978)
Moore, D., Stewart, G. R.: Genet. Res. **18**, 341–352 (1971)
Moore, D., Stewart, G. R.: J. Gen. Microbiol. **71**, 333–342 (1972)
Parag, Y.: Mol. Gen. Genet. **155**, 319–327 (1977)
Parag, Y., Parag, G.: Mol. Gen. Genet. **137**, 109–123 (1975)
Simchen, G.: Genet. Res. **9**, 195–210 (1967)
Stamberg, J.: Mol. Gen. Genet. **102**, 221–228 (1968)
Wickramaratne, M. R. T., Lamb, B. C.: Mol. Gen. Genet. **159**, 63–73 (1978)

Communicated by M. Leupold

Received August 6, 1979