Genet. Res., Camb. (1973), **22**, *pp.* 101–111
With 4 text-figures
Printed in Great Britain

Genetic control of recombination in *Schizophyllum commune*: evidence for a new type of regulatory site

By JUDITH STAMBERG and Y. KOLTIN
Genetics Unit, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

(*Received* 27 *February* 1973)

SUMMARY

Two strains of *S. commune* characterized by different levels of high recombination frequency in a particular region of the genome (between the two subunits, α and β, of the *B* incompatibility factor) were crossed, and their progeny tested for recombination frequency in the same region. The difference between the strains in recombination frequency is found to be due to some factor located within the recombining region itself.

The segregation among the progeny indicates that the factor consists of a number of sites, with additive effects. This and the dominance relationships suggest that these sites may be recognition sites which comprise a part of the fine control of recombination.

1. INTRODUCTION

In higher organisms, frequencies of recombination are controlled differentially in short, specific regions of the genome. Simchen & Stamberg (1969*a*) proposed a model for the genetic fine control of recombination which involves two types of elements. The first element includes a number of genes which regulate the amount of recombination permitted throughout the genome, each gene acting at a limited number of regions. These fine-control genes may be unlinked or loosely linked to the regions whose recombination they control. Each region thus controlled possesses the second element in the fine control, a recognition site specific for the product of its controlling gene. Schaap (1971) suggested that recognition sites may play an active role in determining recombination frequencies by competing for the controlling-gene product. The number and distribution of identical recognition sites would influence the number and distribution of recombination events.

Recent work with *Neurospora crassa* and *Schizophyllum commune* has verified the existence of fine-control genes. Catcheside and co-workers (see review, 1968) and Landner (1971) have identified a series of *rec* genes which control recombination within small segments of the *Neurospora* genome. In *Schizophyllum*, recombination in a number of unlinked regions, including the component loci of the incompatibility factors, *A* and *B*, is genetically controlled by separate fine-control elements (Simchen, 1967; Stamberg, 1968; Stamberg, 1969; Simchen & Stamberg, 1969*b*). All of the fine-control genes so far identified in both organisms are outside

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

of the region whose recombination they control; and in all cases low frequency of recombination is dominant to high. The existence of the second type of element in the fine control of recombination has been demonstrated by Angel, Austin & Catcheside (1970), who identified a recognition site ('*cog*'). The *cog* site is located within a region responding to a fine-control gene. Two allelic forms of *cog* are known, the allele for high frequency of recombination being dominant to the allele for low.

Koltin & Stamberg (1973) identified a gene in *Schizophyllum*, *B-rec-1*, which controls recombination frequency between the two component loci, α and β, of the *B* incompatibility factor. These two loci function jointly but are separable by recombination, and at least one gene is known to map between them (Koltin & Stamberg, 1972). *B-rec-1* was located 9 map units from $B\beta$, and the allele for low frequency of recombination was found to be dominant to the allele for high frequency of recombination. We now report evidence for the segregation of elements between $B\alpha$ and $B\beta$ which affect recombination frequency in this region. The data obtained suggest that these elements may be the recognition sites which respond to the fine-control genes.

2. MATERIALS AND METHODS

Strains. The strains used in this study, and their incompatibility factors, are the following: 991, *A97 B97* ($\alpha 6$-$\beta 7$); 14, *A4 B4* ($\alpha 2$-$\beta 6$); 699, *A41 B41* ($\alpha 3$-$\beta 2$). All are characterized by a high frequency of recombination between the α and β of the *B* factor (Stamberg, 1969).

(i) *Media and culture conditions*

Crosses and test matings were made on complete-agar medium (Koltin, Raper & Simchen, 1967). Fruiting and meiosis occurred at 20 °C. Procedures for performing crosses, isolation of spores, and test matings are described in previous publications (Koltin *et al.* 1967; Stamberg, 1968).

(ii) *Experimental design*

The two high-*B*-recombining strains 991 and 14 were crossed, and a number of their progeny were characterized for incompatibility gentoype. Each of the progeny was then characterized for recombination frequency in the *B* factor by crossing it to the tester strain 699 (which is high-*B*-recombining and therefore recessive for this characteristic [Stamberg, 1969]) and determining the frequency of *B*-factor recombinants arising from each cross. The progeny of 991×14 tested were of four *B*-factor genotypes: 21 carried one of the parental *B* factors (*B4* $\alpha 2$-$\beta 6$ or *B97* $\alpha 6$-$\beta 7$), and 28 carried a recombinant *B* factor ($B\alpha 2$-$\beta 7$ or $B\alpha 6$-$\beta 6$). The high proportion of recombinant-*B* progeny tested does not reflect their actual frequency of occurrence; a large number of progeny of 991×14 were tested and the recombinant-*B* progeny were purposely selected for the analysis, since they proved to be the most informative (see below).

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

(iii) *Statistical analysis*

The variance in recombination frequencies among groups of progeny from the cross 991 × 14 was compared with the variance obtained from replicate crosses of 991 × tester 699, and of 14 × tester 699, by means of the 'F ratio'. Frequencies of recombination were transformed into angles, ϕ, for the analysis. Another estimate of the significance of a variance was obtained by calculating the theoretical error variance (T.E.V.), which is defined as 820·7 divided by the mean sample size, and which has an infinite number of degrees of freedom. The t test was used for comparison of any two means.

3. RESULTS AND DISCUSSION

Repeated crosses of the high-B-recombining strains 991 and 14 to the tester strain 699 have mean B-factor recombination frequencies of 7·9% and 5·8% respectively, with no significant variance within each group of repeats (Table 1).

Table 1. *Recombination frequencies in replicate crosses of the parental strains with tester strain 699*

Cross	Sample	Recomb. B %	Recomb. B ϕ	Mean % rec.†	V	Theoretical error variance	F‡
991	198	7·07	15·45				
	108	7·41	15·79				
	106	7·55	15·94				
	105	7·62	16·02				
	157	7·64	16·05	7·9	1·65	6·70	§
	103	7·77	16·22				
	97	9·28	17·76				
	106	10·38	18·81				
14	112	2.68	9·46				
	101	3·96	11·54				
	173	5·78	13·94				
	174	6·32	14·54	5·8	6·99	6·69	1·05 (N.S.)
	102	6·86	15·23				
	93	7·53	15·89				
	103	8·74	17·15				

Weighted parental variance $(V_p) = \dfrac{7(1{\cdot}65) + 6(6{\cdot}99)}{13} = 4{\cdot}11.$

† t test to compare parental means: $t = 2{\cdot}32$, d.f. $= 13$, $P < 0{\cdot}05$.

‡ The F ratio is defined as the ratio of two variances, in this case V and T.E.V. (theoretical error variance).

§ The actual variance is smaller than the theoretical error variance and therefore not significant (N.S.).

These two mean recombination frequencies do, however, differ significantly from each other (as shown by a t test, Table 1). To determine the factor responsible for this difference, the two strains were crossed and their progeny were characterized

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

Table 2. *Recombination frequencies of progeny from a cross between 991* (Bα6-β7) *and 14* (Bα2-β6). *All progeny were crossed to the tester strain 699*

Non-recombinant progeny	Sample	Recomb. B %	Recomb. B ϕ	Recombinant progeny	Sample	Recomb. B %	Recomb. B ϕ
B97 (α6–β7)	137	5·84	13·99	*Bα2–β7*	145	0·00	2·36†
	144	6·94	15·27		147	0·68	4·73
	142	7·04	15·38		147	2·72	9·49
	159	8·18	16·62		140	3·57	10·89
	133	8·27	16·71		146	4·11	11·70
	131	8·40	16·85		120	4·17	11·79
	140	8·57	17·02		150	4·67	12·48
	141	9·22	17·68		150	4·67	12·48
	76	10·53	18·94		119	5·04	12·97
	55	10·91	19·29		98	5·10	13·05
					154	5·20	13·18
B4 (α2–β6)	140	2·86	9·74		150	6·00	14·18
	136	2·94	9·88		137	6·57	14·85
	127	3·94	11·45		133	12·03	20·30
	119	4·20	11·83				
	89	4·49	12·24	*Bα6–β6*	156	1·92	7·96
	105	4·76	12·60		147	2·72	9·49
	124	4·84	12·71		131	3·82	11·27
	118	5·93	14·10		102	3·92	11·42
	109	6·42	14·67		113	4·43	12·15
	115	6·96	15·30		79	5·06	13·00
	129	10·08	18·51		97	5·16	13·13
					200	5·50	13·56
					107	5·61	13·69
					168	5·95	14·12
					145	6·21	14·43
					109	6·42	14·67
					138	6·52	14·79
					137	12·41	20·63

† For the 0·0 % value, Bartlett's correction was applied.

Table 2(*a*). *Grouping of crosses from Table 2 on the basis of* B *factor recombination frequencies*

Crosses	Mean % recomb.	V	D.F.	T.E.V.	V *v.* T.E.V.	V *v.* V_p
All progeny	5·4	12·34	48	6·35	1·94**	3·00*
All progeny carrying *B4*	5·1	6·46	10	6·88	†	1·57
All progeny carrying *B97*	8·3	2·65	9	6·52	†	†
All progeny carrying *Bα6–β6*	5·2	8·60	13	6·28	1·37	2·09
All progeny carrying *Bα2–β7*	4·1	18·32	13	5·94	3·08**	4·46**
All *Bα2–β7* progeny with > 2 % recomb.	5·2	7·13	11	5·99	1·19	1·73

* $P = 0{\cdot}05 - 0{\cdot}01$; ** $P < 0{\cdot}01$. Unstarred values, $P > 0{\cdot}05$.

† The actual variance is smaller than the theoretical error variance and therefore not significant.

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

Table 2(*b*). *Comparison of progeny and parental means*† *by the* t *test*

Progeny	Parents	
	B4	*B97*
B4	0·71	3·37**
B97	2·71*	0·61
Bα6–β6	0·37	1·70
Bα2–β7 with > 2% recomb.	0·60	3·11*
Bα2–β7 with < 1% recomb.	5·13**	13·12**

*, ** Significance levels for *t* values indicated by asterisks as in Table 2(*a*).
† Parental means are taken from Table 1; progeny means from Table 2(*a*).

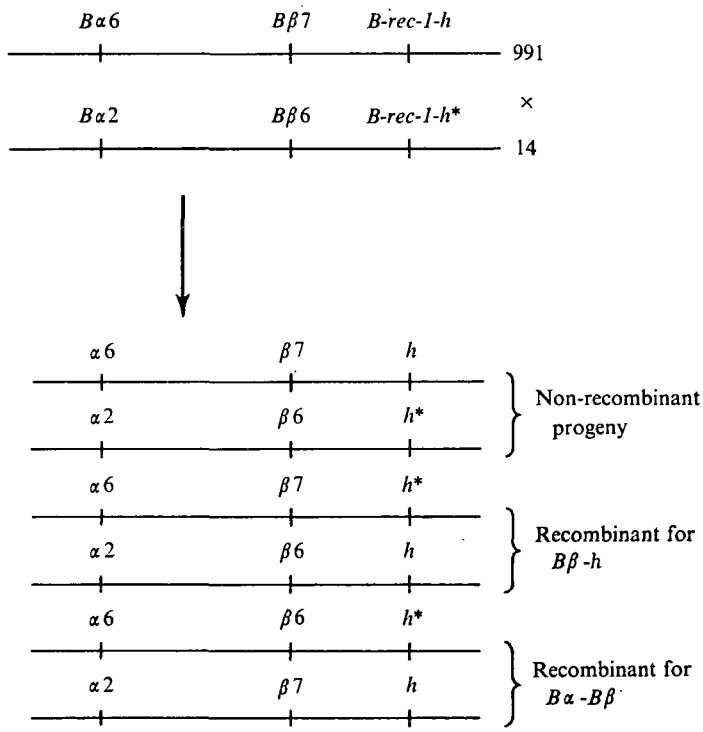

Fig. 1. Expected progeny genotypes based on the hypothesis of segregation of *B-rec-1* alleles.

for *B*-factor recombination frequency by crossing them with the tester strain 699. The progeny carrying *B97* form a homogeneous group with respect to recombination frequency, with a mean of 8·3% (not significantly different from the mean, 7·9%, of the parent carrying *B97*). Likewise, the progeny carrying *B4* are homogeneous, with a mean recombination frequency of 5·1%, which is not significantly different from the mean, 5·8%, of the parent carrying *B4* (see Tables 2, 2*a*, 2*b*). Thus, the controlling element for *B*-factor recombination frequency segregates together with the *B* factor. We know from previous crosses (Koltin & Stamberg,

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

1973) that 991 has a gene, *B-rec-1*, which is closely linked to *Bβ* and which controls *B*-factor recombination. The fact that the progeny carrying *B97* have the same recombinational characteristic as the parent that carried *B97*, and the progeny carrying *B4* are like the parent that carried *B4*, could be interpreted in either of the two following ways.

(1) The strains 991 and 14 carry different *B-rec-1* alleles, the one carried by 991 permitting recombination to occur at a higher frequency than that carried by 14. On this hypothesis approximately 9% of the progeny with parental *B* factors should have the recombinational characteristic of the other parent (since *B-rec-1*

Table 3. *Recombination frequencies in replicate crosses of 991 × 14*

Sample	Recomb. B %	Recomb. B ϕ	Mean % recomb.	V	T.E.V.	V *v.* T.E.V.
148	13·51	21·56				
104	10·57	18·97				
171	8·18	16·62	10·00	3·77	5·68	†
120	9·16	17·62				
179	8·94	17·40				

Mean % recomb. vs. mean of 14 × 699 (from table 1): $t = 3{\cdot}21$, df = 10, $P = < 0{\cdot}01$.
† The actual variance is smaller than the theoretical error variance and therefore not significant.

is about 9 map units from *Bβ*); all recombinant progeny of type *Bα2-β7* should be high-recombining, like 991, whereas the *Bα6-β6* recombinant progeny should have the recombination characteristic of strain 14 (Fig. 1). In addition, the recombination frequency in crosses between 14 and 991 should be approximately 5·8%, equivalent to the value characteristic of strain 14, since at the *B-rec-1* locus low recombination is dominant to high.

(2) Both parental strains, 991 and 14, have the same *B-rec-1* allele (which therefore does not display segregation among the progeny) but differ in some factor located between *Bα* and *Bβ*, and which therefore does not segregate among progeny with parental *B* factors but would be expected to segregate among progeny with recombinant *B* factors.

Neither of the predictions made by the first possibility is fulfilled (Tables 2, 2*a*). The *B97* progeny and the *B4* progeny each form a homogeneous group, with means equivalent to the respective parental means. There is some evidence for a spread in recombination frequency within the groups, although this tendency is not statistically significant; however, a more serious deviation from the predictions of the first hypothesis occurs among the recombinant progeny. The *Bα6-β6* recombinants recombined, as predicted, at an average value of 5·2% (like parent 14) with no significant heterogeneity, but with a spread of 2–12%. But the *Bα2-β7* progeny formed a heterogeneous group, consisting of 12 progeny having recombination frequencies equivalent to that of parent 14, and two progeny with recombination frequencies significantly lower than that of either parent. The

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

second prediction, based on the first possibility, is that the recombination frequency in crosses between 991 and 14 should be equivalent to those obtained in crosses of 14 × 699 (Fig. 1). The strains 991 and 14 were crossed and recombination frequencies of five replicates are listed in Table 3. The mean recombination frequency is 10·0 %, which is significantly higher than the 5·8 % recombination obtained in crosses of 14 × 699. Thus the first possibility mentioned above does not adequately explain the data, and one must look for causes other than the *B-rec-1* gene to explain the difference between 14 and 991.

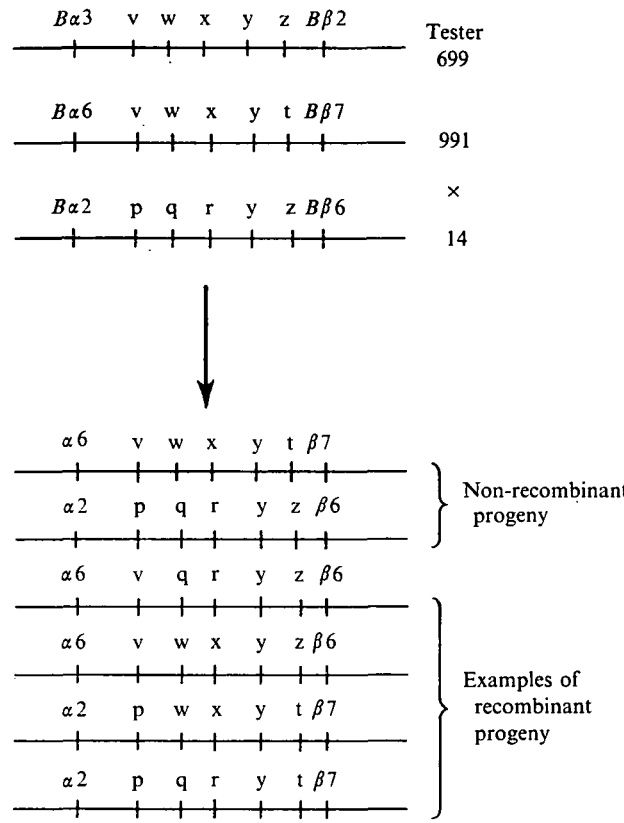

Fig. 2. Expected progeny genotypes based on the hypothesis of differences in structural homology. Homologous regions are represented by the same letter.

According to the second possibility, the cause of the difference in recombinational behaviour between 14 and 991 lies within the *B* factor itself. The prediction from this hypothesis, that only progeny with recombinant *B* factors should segregate for the recombination characteristic, is fulfilled. However, the type of segregation displayed is not reciprocal. One interpretation of such results is that there is a difference in the degree of structural homology for this region. On this assumption, 991 and the tester strain 699 have a high degree of homology for the region between *Bα* and *Bβ* and therefore can undergo complete pairing and recombination; 14 and

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

699 have a lower degree of homology and this is reflected in a lower recombination frequency. Among the progeny of a cross between 991 and 14, all progeny with non-recombinant *B* factors have exactly the same chromosomal structure in the *B* factor region as their like parent, and therefore the same degree of homology with 699; progeny with recombinant *B* factors could have a variable degree of homology with 699, depending on the exact position of the recombinational event with respect to the regions of homology (Fig. 2). This type of variation was in fact found among the progeny.

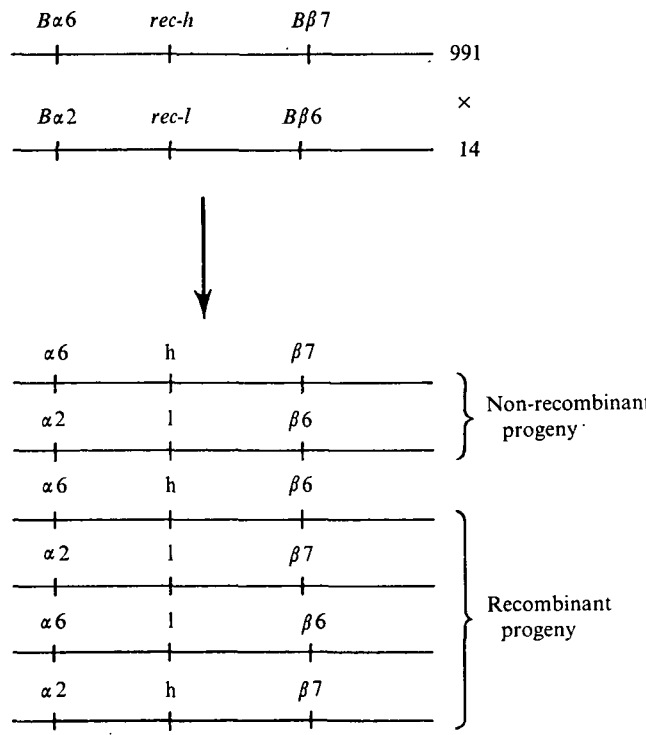

Fig. 3. Expected progeny genotypes based on the hypothesis of segregation of a fine-control gene located between *Bα* and *Bβ*.

A prediction following from the 'structural homology' hypothesis is that 991 and 14, having different degrees of homology with the tester strain 699, must have regions of incomplete homology with each other, which would be reflected by a lower recombination frequency in crosses between the strains 991 and 14. The recombination frequency should be no higher than that obtained in crosses between 14 and 699. As mentioned above, the recombination frequency in crosses between 991 and 14 is characteristically high (Table 3), and there is no evidence for interference in pairing. Areas of structural dissimilarity causing pairing disturbances can be rearranged in a number of ways other than that illustrated in Fig. 2, but none of these arrangements explains both the high frequency of recombination in crosses of 991 × 14, and the large variation in recombination when progeny of 991 × 14 carrying recombinant *B* factors are crossed to strain 699.

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

A variation on the second possibility is that the element located within the *B* factor and affecting recombination there is a fine-control gene similar to *B-rec-1*. If 991 carried an allele for high frequency of recombination at this locus and 14 an allele for lower frequency, then from the progeny of a cross between 991 and 14, all those carrying the *B* factor of 991 should carry the high allele and those carrying the *B* factor of 14 the low allele. Progeny with recombinant *B*'s might carry either of the two alleles, and from the relative frequencies of the classes *Bα6-β6-high*, *Bα6-β6-low*, *Bα2-β7-high*, and *Bα2-β7-low*, the position of the controlling gene,

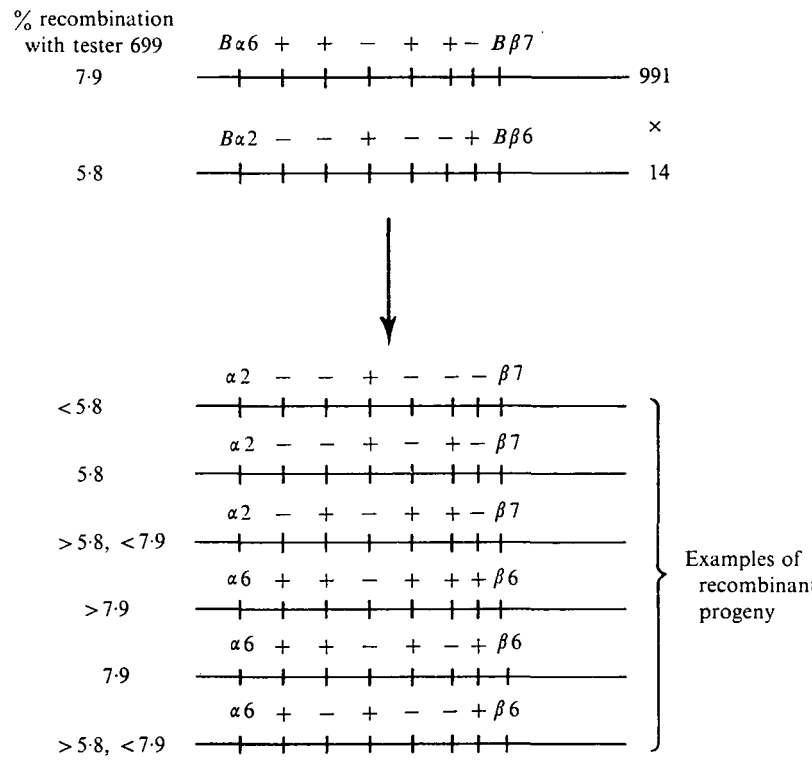

Fig. 4. Expected progeny genotypes based on the hypothesis of segregation of a number of recognition sites located between *Bα* and *Bβ*. + indicates the dominant, active form of a recognition site and − the recessive, inactive form.

with respect to *Bα* and *Bβ*, could be estimated (Fig. 3). However, the recombinant progeny did not form the four predicted classes. The *Bα6-β6* recombinants were uniformly like the *B4* parent; and most of the *Bα2-β7* recombinants were also similar to *B4*, with two recombinants being significantly lower than either parent. The simple hypothesis of a one-gene difference within the *B*-factor region does not fit these results. In addition, low frequency of recombination in such a gene would be expected to be dominant to high, since in all fine-control genes thus far identified low recombination is dominant. Crosses between 991 and 14 should give the frequency of recombination characteristic of 14, and this result was not obtained.

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

Thus, neither explanations based on a gene controlling *B*-factor recombination and located either outside or inside of the *B* factor, nor explanations based on incomplete structural homology, can account for the results obtained. The unusual properties of the present case are (1) that the source of variation is located between the two loci of the *B* factor; (2) high frequency of recombination determined by this factor is dominant to low; (3) it does not segregate as one gene. Together, these properties suggest that we are dealing with a component of the recombinational fine control other than the controlling genes themselves. The three properties listed above would be anticipated, from the model for fine control, to be characteristic of recognition sites. The *cog* site identified in *Neurospora* possesses the first two characteristics; and if one assumes that, in the present case, several recognition sites with additive effects are located between $B\alpha$ and $B\beta$, then all of the data can be accounted for.

Depending on the exact position of a break between $B\alpha$ and $B\beta$, progeny with varying numbers of recognition sites in this region could be formed, and this would be reflected in the frequency of recombination exhibited by $B\alpha 2\text{-}\beta 7$ and $B\alpha 6\text{-}\beta 6$ progeny (see Fig. 4).

Indications that recombinational events in higher organisms may occur preferentially at specific sites within genes have been obtained by the work of Giles (1952), Pritchard (1955), and recently by Gutz (1971). Such sites may correspond to the recognition sites which comprise part of the fine control of recombination. In the case reported here, recombination between genes was studied. The distance between $B\alpha$ and $B\beta$ could well be large enough to include a number of such recognition sites.

We thank Dr M. J. Lawrence of the University of Birmingham, England, for advice on the analysis of results. The technical assistance of Mrs Sarah Levi is gratefully acknowledged.

REFERENCES

ANGEL, T., AUSTIN, B. & CATCHESIDE, D. G. (1970). Regulation of recombination at the *his-3* locus in *Neurospora crassa*. *Australian Journal of Biological Sciences* **23**, 1229–1240.

CATCHESIDE, D. G. (1968). The control of genetic recombination in *Neurospora crassa*. In *Replication and Recombination of Genetic Material* (ed. W. J. Peacock and R. D. Brock), pp. 216–226. Australian Academy of Science.

GILES, N. H. (1952). Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa*. *Cold Spring Harbor Symposia on Quantitative Biology* **16**, 283–313.

GUTZ, H. (1971). Site specific induction of gene conversion in *Schizosaccharomyces pombe*. *Genetics* **69**, 317–337.

KOLTIN, Y., RAPER, J. R. & SIMCHEN, G. (1967). The genetic structure of the incompatibility factors of *Schizophyllum commune*: The *B* factor. *Proceedings of the National Academy of Sciences of the U.S.A.* **57**, 55–62.

KOLTIN, Y. & STAMBERG, J. (1972). Suppression of a mutation disruptive to nuclear migration in *Schizophyllum* by a gene linked to the *B* incompatibilty factor. *Journal of Bacteriology* **109**, 594–598.

KOLTIN, Y. & STAMBERG, J. (1973). Genetic control of recombination in *Schizophyllum commune*: Location of a gene controlling *B*-factor recombination. *Genetics* (in the Press).

LANDNER, L. (1971). Genetic control of recombination in *Neurospora crassa*: Correlated regulation in unlinked chromosome intervals. *Heredity* **27**, 385–392.

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press

PRITCHARD, R. H. (1955). The linear arrangement of a series of alleles of *Aspergillus nidulans*. *Heredity* **9**, 343–371.

SCHAAP, T. (1971). Recognition sites and coordinate control of recombination in *Schizophyllum*. *Genetica* **42**, 219–230.

SIMCHEN, G. (1967). Genetic control of recombination and the incompatibility system in *Schizophyllum commune*. *Genetical Research* **9**, 195–210.

SIMCHEN, G. & STAMBERG, J. (1969*a*). Fine and coarse controls of genetic recombination. *Nature* **222**, 329–332.

SIMCHEN, G. & STAMBERG, J. (1969*b*). Genetic control of recombination in *Schizophyllum commune*: Specific and independent regulation of adjacent and non-adjacent chromosomal regions. *Heredity* **24**, 369–381.

STAMBERG, J. (1968). Two independent gene systems controlling recombination in *Schizophyllum commune*. *Molecular and General Genetics* **102**, 221–228.

STAMBERG, J. (1969). Genetic control of recombination in *Schizophyllum commune*: The occurrence and significance of natural variation. *Heredity* **24**, 361–368.

https://doi.org/10.1017/S0016672300012878 Published online by Cambridge University Press