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Genetic control of recombination and the incompatibility system in *Schizophyllum commune*

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1. INTRODUCTION

Although linkage between two genes is known to reflect their presence on the same chromosome, the frequency of recombination does not depend exclusively on the physical distance between them. Among the other factors influencing recombination, the 'genetic background' has received much attention but relatively little designed experimentation. We shall not try to review the literature concerning the genetic control of recombination as this has been done by Bodmer & Parsons (1962); additional and more recent reports are presented by Day & Anderson (1961), Lawrence (1963), Griffing & Langridge (1963), Lavigne & Frost (1964), Jessop & Catcheside (1965), Scott-Emuakpor (1965), Griffiths & Threlkeld (1966) and Smith (1965, 1966). However, a most striking and originally rather puzzling instance of heterogeneity in recombination values was found in different strains of *Schizophyllum commune* for the linked sub-units of the *A* incompatibility factor (Raper *et al.*, 1958*a*, 1960). The present study attempts to analyse further the variation reported by Raper and co-workers.

The system with which we are dealing here consists of two naturally occurring markers, the α and β loci, which operate together in determining mating ability; the relationship between these two is presumably directly subjected to natural selection. Hence this is an exceptionally interesting situation where the usual role of recombination in releasing heritable variation (see Bodmer & Parsons, 1962) is only secondary. Or in other words, while the significance of linkage and crossing-over between any two artificially induced markers, say in *Neurospora*, can only be a matter of unfounded speculation and requires a detailed study of the polygenic systems in this particular chromosomal segment, the consequences of recombination within the *A* factor of *Schizophyllum* can be directly evaluated in terms of its effect on breeding behaviour. Knowledge of the genetic control of recombination within the *A* factor may help us to understand the peculiar structure of the incompatibility factors and ultimately the process by which they have evolved, as the latter should be reflected in the controlling gene system.

2. BACKGROUND, MATERIALS AND METHODS

Incompatibility in *S. commune* is determined by two multiple allelomorphic factors, *A* and *B*, for which paired monokaryons must have different specificities

in order to form dikaryotic mycelium and fertile fruit bodies. *A* and *B* are not linked and reassort independently at meiosis. It is commonly found that new *A* specificities, and less frequently new *B*s, arise which are thought to be the result of recombination within the factors. Tetrad analysis has shown that the two non-parental *A* factors arising from a dikaryon are the reciprocal products of conventional crossing-over between two of the four strands in meiosis, and that a dikaryon resulting from a cross between the two new *A* factors has yielded the two original *A*s in a similar manner (Papazian, 1951). Further studies have shown that the *A* factor consists of two loci, α and β, and that non-identity in one of these loci determines the functional compatibility of any two *A* factors (Raper *et al.*, 1958*a*, 1960). The number of specificities in the α and β loci is estimated to be nine and fifty respectively (nine and twenty-five have been recovered from the wild by Raper *et al.*, 1960).

The material used for the experiments to be reported here originated from Isolate 4 in our collection (Simchen, 1966), which was isolated as a dikaryon from a fruit body collected by Professor J. R. Raper in November 1963, near Cambridge, Massachusetts. The mating type specificities of Isolate 4 were designated (*A*7*B*7 + *A*8*B*8), but these do not correspond to numbers of mating types originating from Professor Raper's collection in Harvard University. Fruiting of this and other dikaryons was carried out on inverted Petri dishes containing 20 ml. SF medium (Simchen & Jinks, 1964) at 18°C., under continuous illumination by 'daylight' fluorescent tubes (250–400 lm./sq. ft.). Prints of basidiospores were obtained on dry Petri dishes under the same conditions, and were suspended in 'Tween 80' (1:100,000). The spores were then diluted and spread on Migration Complete medium (Snider & Raper, 1958—this medium was used throughout the experiments except for fruiting) to give 30–50 minute colonies after 2 days of incubation at 25°C. These were isolated under the dissecting microscope, and transferred to fresh Petri dishes at the rate of five colonies per plate. The Petri dishes were incubated (at 25°C.) for a further 2-day period, and then stored in the refrigerator (5°C.) for a few days until examination of the mating types could be made.

Mating types were determined by mating each unknown monokaryon with two testers, *A*7*B*4 and *A*8*B*4, on the opposite edges of the same Petri dish, the original monokaryon being placed in the centre as control. Following 3–4 days of incubation, the specificity of the *A* factor was determined as follows (Raper *et al.*, 1958*a*):

		Unknown monokaryons					
		*A*7*B*7	*A*7*B*8	*A*8*B*7	*A*8*B*8	A_*B7	A_*B8
Testers	*A*7*B*4	F	F	+	+	+	+
	*A*8*B*4	+	+	F	F	+	+

where + stands for dikaryon formation, F for 'flat' mycelium ('common-*A* heterokaryon'), and A_* stands for a non-parental (i.e. recombinant) *A*. Thus an efficient method of scoring the frequency of recombination within the *A* factor

was achieved, where the A specificity of each monokaryon could be determined at a glance (doubtful cases were of course checked microscopically, and sometimes the matings were repeated). Admittedly, the B factor was ignored, and no distinction was made between the two possible recombinant As which arose from every $A7 \times A8$ cross (see Raper *et al.*, 1958*a*, 1960).

Statistical transformation of recombinant frequencies

For the analyses, the frequencies (p) were transformed into angles (ϕ) by $p = \sin^2\phi$ (angle values obtained in degrees). The need for the transformation was primarily statistical as frequencies are not normally distributed; it also provided us with a theoretical error variance, $820{\cdot}7/\bar{n}$ (referred to throughout the tables as error variance) where $\bar{n}$ is the harmonic mean of the numbers of progenies from which the frequencies were calculated. χ^2 in Tables 2–4 is obtained as sum of squares (S.S.) divided by error variance.

3. RESULTS AND ANALYSES

(i) *Original dikaryon*

Progeny of Isolate 4 were obtained on four different occasions, the first of which was eighteen months before the beginning of the main experiments in this study while the other three ran co-parallel with various stages in the experiments. The proportion of monokaryons with recombinant A factors did not show any signs of heterogeneity between the four samples ($\chi^2_{(3)} = 1{\cdot}55$), and therefore the results were pooled giving $16/330 = 4{\cdot}85 \pm 1{\cdot}40\%$ recombination.

(ii) *Multiple crosses programme*

Among the second set of monokaryotic progeny of the original dikaryon, six were randomly chosen of mating type $A7B7$ and six of $A8B8$. These were mated in all possible combinations to give thirty-six progeny dikaryons. The recombination value for each dikaryon was determined by examining the mating types of 100 of its progeny, hence 3,600 mycelia were tested in order to complete the 6×6 table of frequencies (Table 1—Although 100 were tested for each dikaryon, only monokaryotic mycelia were taken into account when the frequencies of recombinants were calculated, and therefore some of the values are based on slightly smaller samples. The smallest samples are 92—for *dk* and *fl*). In order to minimize environmental effects, the dikaryons were split into six groups according to a Latin Square design (Fisher & Yates, 1963) so that each group contained one representative of each row and each column in the table. The dikaryons of each group were fruited at the same time and progeny were isolated and tested together. This precaution was, however, found to be unnecessary, as the different groups did not show any sign of heterogeneity. In the orthogonal analysis of variance (Table 2) the interaction item includes also the (non-significant)

variation between groups. The two main items in the analysis are significant while the interaction is not. It seems, therefore, that the significant variation between genotypes in their frequencies of recombinants could be wholly accounted for by additive differences between single arrays. Close inspection of Table 1

Table 1. *Percentage of recombinant* A *factors among the progenies of thirty-six dikaryons*

Mono-karyons	Monokaryons *A8B8*						
A7B7	*g*	*h*	*i*	*j*	*k*	*l*	Totals
a	1·03	9·00	8·16	13·00	4·00	7·07	7·07
b	4·04	4·00	10·42	19·00	4·26	5·05	7·82
c	6·06	4·17	8·60	12·00	0·00	4·21	5·83
d	1·02	6·06	4·08	9·09	2·17	5·21	4·64
e	7·07	6·06	7·07	14·00	7·22	9·18	8·28
f	4·00	3·00	4·17	12·24	4·04	7·61	5·81
Totals	3·89	5·39	6·90	13·23	3·61	6·39	6·58

The marginal values are based on the total number of progeny in each array.

Table 2. *Analysis of variance of angles (in degrees) corresponding to the percentages in Table* 1

	d.f.	S.S.	χ^2	*P*	M.S.	F	*P*
Total	35	785·17	93·57	< 0·001			
Error variance		8·39					
Orthogonal analysis:							
Between *a–f*	5	93·56	11·15	0·05–0·02	18·71	2·11	0·10–0·05
Between *g-l*	5	469·55	55·95	< 0·001	93·91	10·57	< 0·001
Interaction	25	222·06	26·46	0·50–0·30	8·88		
Hierarchical analysis:							
Array *j* vs. rest	1	353·42	42·12	< 0·001	353·42	27·83	< 0·001
Within	34	431·75	51·45	0·05–0·02	12·70		
Within *j*	5	36·83	4·39	0·50–0·30			
Within rest	29	394·924	47·06	0·02–0·01			

Before the transformation into angles, the zero value for dikaryon *ck* (see Table 1) was substituted by $\frac{1}{4}/n$, as proposed by Bartlett (1947).

suggests that an important source of variation is the contrast between array *j* and the rest of the table; but the appropriate comparisons in the analysis of variance (the hierarchical breakdown in Table 2) did not exclude other sources of variation. We shall return to the interpretation of the genetic constitution of the various mycelia at a later stage.

(iii) *Selection*

The last group in the multiple crosses programme included the dikaryons *bj* and *ck*, which gave the most extreme recombination values among the thirty-six genotypes examined. From these two dikaryons, the 'High' and 'Low' selections were derived as follows. Among the 100 monokaryotic progeny of each dikaryon,

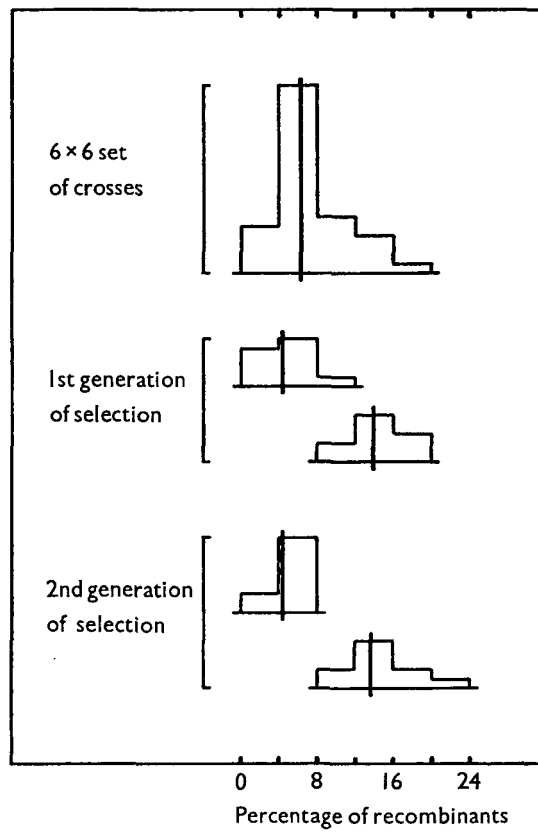

Fig. 1. Frequency distributions of recombination values of individual dikaryons. The bars indicate the means of the distributions. See Tables 2 and 3 for the analyses of variance of these data.

twenty-five randomly chosen mycelia of mating type $A7$ were mated with twenty-five $A8$ monokaryons. About half of these matings develop into dikaryons, since they were compatible in respect of the B factor. Ten such dikaryons among the progeny of *bj* gave the first generation of the 'High' selection and similarly ten dikaryotic progeny of *ck* gave the 'Low' selection. The same procedure was applied to the dikaryons which had the highest and lowest recombination values in the 'High' and 'Low' lines respectively, thus producing the second generation of selection which also consisted of twenty dikaryons.

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The individual frequencies of intra-*A* recombinants for both generations of selection are not given in detail, but only diagrammatically (Fig. 1). The analysis of variance of the angles which correspond to the frequencies is given in Table 3. No evidence was found for variation between individual dikaryons within either selection line in both generations. And indeed, there was no response to the second cycle of selection.

Table 3. *Analysis of variance of angles (in degrees) corresponding to the proportion of intra-*A *recombinants among the progenies of individual dikaryons in the selections*

	d.f.	S.S.	χ^2	*P*	M.S.	F	*P*
First generation							
'High' vs. 'Low'	1	559·15	67·61	< 0·001	559·15	69·09	< 0·001
Within	18	145·67	17·62	0·50–0·30	8·09		
Within 'High'	9	44·80	5·42	0·80–0·70			
Within 'Low'	9	100·88	12·20	0·30–0·20			
Error variance		8·27					
Second generation							
'High' vs. 'Low'	1	449·64	54·37	< 0·001	449·64	68·35	< 0·001
Within	18	118·41	14·32	0·80–0·70	6·58		
Within 'High'	9	87·27	10·55	0·40–0·30			
Within 'Low'	9	31·14	3·77	0·95–0·90			
Error variance		8·27					

(iv) *Crosses between the selection lines*

Eighteen dikaryons were produced by mating monokaryotic progeny from the extreme 'High' with progeny of the extreme 'Low' dikaryons of the second generation of selection (recombination values of 21·00% and 2·04%, respectively). This is comparable to raising an F_1 generation in diploid organisms. As we had no previous knowledge about the role the incompatibility factors themselves played in the determination of recombination values, nine of the eighteen dikaryons had *A*7 monokaryons from the 'High' and *A*8 from the 'Low' parent, and the converse was true for the other nine dikaryons. Again, the linkage values which are given in Table 4 were determined on samples of 100 progeny per dikaryon. The linkage values proved to be heterogeneous, thus suggesting that at least one of the two selection lines was not homogeneous in respect of genes affecting recombination within the *A* factor. A comparison could, however, be made between the two 'reciprocal' groups of crosses, i.e. *A*7 'High' × *A*8 'Low' and *A*7 'Low' × *A*8 'High', by which all the heterogeneity among the F_1 crosses could be explained.

Table 4. *Crosses between the 'High' and 'Low' selections:* (1) *percentages of intra-*A *recombinants of individual dikaryons, and* (2) *analysis of variance of angles* (*in degrees*) *corresponding to these percentages*

(1)	(*a*) 'High' *A*7 × 'Low' *A*8				(*b*) 'Low' *A*7 × 'High' *A*8		
	4·00	5·00	7·14		6·67*	11·00	12·12
	4·04	6·06	8·16		9·47	11·00	14·29
	4·17	6·45	10·31*		10·31	11·11	20·20

(2)	d.f.	S.S.	χ^2	*P*	M.S.	F	*P*
Total	17	279·22	35·30	0·01–0·001			
(*a*) vs. (*b*)	1	147·40	18·63	< 0·001	147·40	17·89	< 0·001
Within	16	131·82	16·66	0·50–0·30	8·24		
Within (*a*)	8	49·54	6·26	0·70–0·50			
Within (*b*)	8	82·28	10·40	0·30–0·20			
Error variance		7·91					

* Based on two homogeneous samples of progeny.

(v) *Genetic interpretation*

The results which were described in some detail in the foregoing sections are summarized in Table 5. Data were pooled within each set of dikaryons in order to enable us to compare means of generations. Heterogeneity in recombination values within generations is indicated when present. The chi-squares for the meaningful comparisons of the various generations are given in Table 6.

The immediate response to selection in the first generation and the lack of any further response suggest that a small number of genes is responsible for the variation disclosed. Let us postulate one locus in which the recessive allele *rec*, when homozygous, determines high recombination frequency. Now this hypothetical locus may also be responsible for the heterogeneity in the F_1 crosses, but then it ought to be linked to the *A* factor. On this model, the original dikaryon was *A*7 *rec*/*A*8 +, the monokaryons *a–f* were *A*7 *rec* and the monokaryons *g–l* were *A*8 +, except for *j* which was *A*8 *rec*. By selecting the 'High' and 'Low' lines we picked and maintained *A*7 *rec*/*A*8 *rec* and *A*7 *rec*/*A*8 + dikaryons respectively, the former being homogeneous in respect of the major effect of *rec* and the latter not segregating because of the tight linkage between *A* and *rec*. This linkage would be expected to be even tighter in the 'Low' selection if the effect on the crossing-over process is not specific to the chromosomal region within the *A* factor only, but also extends to the neighbouring regions, notably between *A* and *rec*.

The *rec* locus accounts for a major part of the heritable variation disclosed, but not for all of it, as is shown by the significant variation between the *rec*/+ dikaryons in the second analysis of Table 2 ('Within rest'). Nor does it account for the last two significant comparisons in Table 6, although these could partly be explained by crossing-overs between *rec* and *A*. It seems, therefore, reasonable to assume that other genetic factors affecting intra-*A* recombination also segregated among the progeny of the original dikaryon.

Table 5. *Pooled data over generations, and recombination frequencies derived from them*

	A factors in monokaryotic progeny		Percentage recombination
	Recombinant	Parental	
(1) Original dikaryon	16	314	4·85 ± 1·40
(2) Multiple crosses programme (heterogeneous)	232	3292	6·58 ± 0·42
(2*a*) array *j* only	79	518	13·23 ± 1·39
(2*b*) without array *j**	153	2774	5·23 ± 0·41
(3) Selection: first generation			
(3*a*) 'High'	141	853	14·19 ± 1·11
(3*b*) 'Low'	42	949	4·24 ± 0·64
(4) Selection: second generation			
(4*a*) 'High'	134	860	13·47 ± 1·08
(4*b*) 'Low'	43	948	4·34 ± 0·64
(5) Combined selections			
(5*a*) 'High'	275	1713	13·83 ± 0·77
(5*b*) 'Low'	85	1897	4·29 ± 0·46
(6) Crosses between 'High' and 'Low' (heterogeneous)	175	1784	8·93 ± 0·64
(6*a*) 'High' *A*7 × 'Low' *A*8	64	913	6·55 ± 0·79
(6*b*) 'Low' *A*7 × 'High' *A*8	111	871	11·30 ± 1·01

* Also heterogeneous (see Table 2).

Table 6. *Comparisons between generations of Table 5 which were not given before*

Comparisons*	Heterogeneity χ^2 (1 d.f.)	P†
(1) vs. (2*a*)	16·24	< 0·001
(1) vs. (2*b*)	0·09	> 0·10
(3*a*) vs. (4*a*)	0·21	> 0·10
(3*b*) vs. (4*b*)	0·01	> 0·10
(2*a*) vs. (5*a*)	0·14	> 0·10 ($\frac{1}{2}$)
(2*b*) vs. (5*b*)	2·26	0·10–0·05 ($\frac{1}{2}$)
(2*a*) vs. (6*b*)	1·31	> 0·10
(2*b*) vs. (6*a*)	2·44	> 0·10
(5*a*) vs. (6*b*)	3·72	0·05–0·025 ($\frac{1}{2}$)
(5*b*) vs. (6*a*)	7·00	0·005–0·0005 ($\frac{1}{2}$)

* Notation corresponds to Table 5.

† The probabilities were halved ($\frac{1}{2}$) when change of frequency in one direction only was expected.

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It should be noted that although we postulate the *rec* allele as being recessive to +, we have not so far obtained a dikaryon with a +/+ genotype. But this could not have a much lower frequency of recombinant *A* factors than *rec*/+ has. In any case the phenotypes of +/+ and *rec*/+ will be more similar to each other than the latter to *rec*/*rec*, and therefore the dominance relationships as argued above should hold. In spite of the presence of dominance in the system, it was not detected in the analysis of variance (Table 2); this is a direct result of one set of monokaryons (*a*–*f*) being all of the same recessive genotype, *rec*.

4. DISCUSSION

The postulated gene system, which consists of one locus with a major effect on recombination and several loci with minor effects, explains sufficiently the results obtained. More wide-ranged data such as given by Raper *et al.* (1958*a*, 1960)

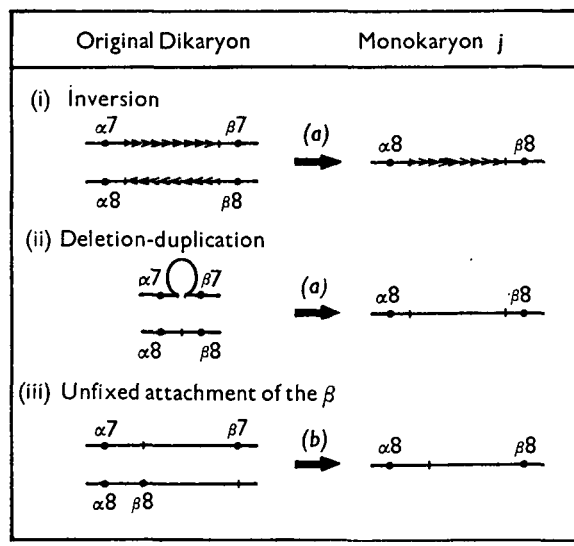

Fig. 2. Alternative interpretations to the *rec*/+ model. The letter above the arrow stands for the change required to give rise to the *j* monokaryon and the 'High' selection line: (*a*) double crossing-over between α and β, (*b*) change of position of β8.

could also be explained in a similar way. It should be remembered that the studies reported here deal only with loci at which Isolate 4, our original dikaryon, was heterokaryotic. But other loci affecting recombination, for which Isolate 4 was homokaryotic, could also vary in the wild, thus resulting in a wider range of genotypes. These, of course, could respond differently to changes in the environment, resulting in genotype-temperature interaction as observed by Raper *et al.* (1958*a*, Table 1—our calculation for the interaction $\chi^2_{(8)} = 23{\cdot}64$, $P = 0{\cdot}01$–$0{\cdot}001$).

Alternative interpretations may be suggested for our results, whereby the recombination frequency *is* in fact directly related to the chromosomal distance

between the two loci (α and β) or its availability for crossing-over, the latter varying from one A factor to another. Three such models are shown in Fig. 2, together with the supposed change which must have occurred in $A8$ to give rise to monokaryon j and its derivative, the 'High' selection. On all three models, low frequencies of recombination will appear to be dominant to high frequencies, since only crossing-over in the short homologous segments will give rise to viable recombinant A factors. However, a double crossover within the A is required in order to explain the response to selection on either of the two chromosomal rearrangements. The third alternative, in which at least one of the sub-units of A can change its place on the chromosome, is of the same pattern of 'episomal' behaviour as the one suggested already by Ellingboe (1963, 1965) for the incompatibility factors of *S. commune*. However, it will be rather difficult to explain on this model the stability of the incompatibility factors and the remarkable consistency of recombination values throughout the experiments (from one generation to the next and over the arrays of the multiple crosses programme).

All three alternative interpretations become very elaborate once the variation for recombination frequencies between a whole set of wild isolates has to be explained (see, for example, Table 1 in Raper *et al.*, 1960). Similarly, the interaction between genotype and temperature in respect of intra-A recombination could not be explained either. We are, therefore, of the opinion that the variation disclosed by Raper *et al.* and here reflects variability in the gene system controlling the crossing-over process, and not structural variations within the A factor itself.

The system revealed here may prove to be an opening to further studies on recombination. Immediate questions which may be answered experimentally are: What is the effect of the *rec*/+ locus (and other genes) in different environments, say the whole range of temperatures at which *Schizophyllum* will fruit? Is the difference between *rec*/*rec* and *rec*/+ specific to the A factor only, or does it extend to adjacent chromosomal regions or even to markers on other chromosomes? It is hoped that studies along these lines will provide useful and relevant information to the understanding of the process of crossing-over and the genes taking part in its control. It is also likely that the genic, or alternatively the 'structural', interpretations of the variation in intra-A recombination will be proved unambiguously by such further studies (for instance, the 'structural' interpretations will require the recombination differences to be confined mainly to the α–β interval). Further experiments and test crosses are in progress in our laboratory.

The experiments reported presently suggest that genes determining low frequency of recombination are dominant over genes which increase the recombination frequencies. Genotypes with a high frequency of recombination were selected and established among the progeny of a wild isolate with low recombination. We have got similar results from studies on growth rate of monokaryons among progeny of a different and unrelated wild dikaryon, Isolate 2 (Simchen, 1966; V. Connolly, unpublished). Crosses within and between lines selected for high and low growth-rates, which were derived by nine to sixteen generations of haploid sib-mating (equivalent to selfing in diploids). have shown a significantly higher frequency of

A factor recombinants than the original dikaryon. From these two sets of results one can argue that since genes which increase recombination within the *A* factor of *S. commune* tend to be recessive, they are probably selected against in nature (by doing this we follow Fisher's 'evolution of dominance' theory; see, for example, Fisher (1958) and Mather (1966)). This reasoning makes sense once the effects of intra-*A* recombination on the organism and its breeding system are considered (see Appendix which follows this section). Thus the out-breeding potential of the population increases as a result of the two-locus structure of the incompatibility factors, particularly when the number of specificities in the population is not very large. However, high recombination within the incompatibility factors is beneficial only when a dikaryon is in isolation, otherwise it increases the inbreeding potential and is therefore presumably disadvantageous. With low recombination the large number of incompatibility factors can be maintained together with high out-breeding potential, while inbreeding is kept very low and near to its minimum. That very low recombination within the incompatibility factors is sufficient to maintain flexible two-locus systems can be seen in *Coprinus lagopus* where Day (1963) found different associations of the same αs and βs to give nine different *A* factors; the frequency of recombination within the *A* factor in this fungus is only 0·07% (Day & Anderson, 1961). In *Schizophyllum*, dikaryons can be derived which will have up to 20–25% *A* recombinants, but they are expected to be selected for in nature only under special circumstances such as isolation or colonization of a new habitus. Otherwise low recombination within the *A* factor will be maintained throughout the population by virtue of dominance over high recombination.

5. APPENDIX: EVALUATION OF THE TWO-LOCUS STRUCTURE OF THE INCOMPATIBILITY FACTORS

Tetrapolarity in the Basidiomycetes has been assessed in terms of mating-fertility and inbreeding by several authors (Mather, 1942; Whitehouse, 1949; Papazian, 1951; Raper *et al.*, 1958*b*). However, the two-locus structure of the incompatibility factors has not been given detailed consideration except by Day (1960), who assumes the α and β loci to have arisen by duplication (Raper *et al.*, 1958*a*; Day & Holliday, 1959) and to consist of the same number of specificities. Although experiments were designed to prove this hypothesis (Raper *et al.*, 1960; Day, 1963), they failed to do so.

We shall deal with the loci constituting the *A* factor only, although the same treatment could be applied to the *B* factor as well, providing the same two-locus structure holds there (Koltin *et al.*, 1967).

*Intra-*A *recombination in the population*

Suppose the number of alleles at the α locus is n_α and the number of alleles at the β locus is n_β. There are $n_\alpha n_\beta$ different potential *A* factors, all of which are compatible with each other. Suppose all possible *A* factors are equally frequent in the population, each with frequency of $1/n_\alpha n_\beta$. Recombination within the *A*

factor is effective only when both α and β are different in the dikaryotic combination, although different specificities in one locus only are sufficient for compatibility. From the $(n_\alpha n_\beta - 1)$ different A factors that any monokaryon is compatible with, $(n_\beta - 1)$ will have the same α, and $(n_\alpha - 1)$ will have the same β. Thus intra-A recombination can occur only in the following proportion of dikaryons,

$$\frac{(n_\alpha n_\beta - 1) - (n_\alpha - 1) - (n_\beta - 1)}{(n_\alpha n_\beta - 1)} = \frac{(n_\alpha - 1)(n_\beta - 1)}{(n_\alpha n_\beta - 1)}$$

which becomes $(n-1)/(n+1)$ when $n_\alpha = n_\beta = n$. From the estimates available for n_α and n_β in the world-wide population of *S. commune* (Raper *et al.* (1960)), this proportion is 0·873.

Merits of the two-locus structure of the incompatibility factors

1. *The increase in the number of* A *factor specificities in the population and consequently the increase in the proportion of compatible matings between non-sister monokaryons, i.e. increase in out-breeding potential.* If we had all the α and β specificities at one locus, each monokaryon would have been compatible with a proportion of $(n_\alpha + n_\beta - 1)/(n_\alpha + n_\beta)$ of the monokaryons in the population. With the two-locus structure the proportion is $(n_\alpha n_\beta - 1)/n_\alpha n_\beta$. The latter proportion is always larger than the former, and the difference between the two is

$$R = \frac{1}{n_\alpha + n_\beta} - \frac{1}{n_\alpha n_\beta}$$

and measures the advantage of the two-locus structure in terms of potential out-breeding in a population. When is this advantage maximal?

$$\frac{dR}{dn_\alpha} = (n_\alpha^2 n_\beta)^{-1} - (n_\alpha + n_\beta)^{-2} = 0$$

$$\frac{dR}{dn_\beta} = (n_\alpha n_\beta^2)^{-1} - (n_\alpha + n_\beta)^{-2} = 0$$

$$\therefore \quad n_\alpha n_\beta^2 = n_\alpha^2 n_\beta$$

$$\therefore \quad n_\alpha = n_\beta$$

If at R maximum $n_\alpha = n_\beta = n$

$$R = \frac{1}{2n} - \frac{1}{n^2}$$

$$\frac{dR}{dn} = 2n^{-3} - \tfrac{1}{2}n^{-2} = 0$$

$$\therefore \quad n = 4$$

Thus in a population where only four specificities exist at each of the two loci, the increase in fertility and out-breeding potential is maximal when compared to a situation where all eight specificities exist at the same locus: an increase of

6·25% (from 87·5% to 93·75%). There is therefore reason to believe that the two-locus system has evolved from a single locus situation in populations with a small number of specificities, since the former's advantage in these circumstances is maximal. In the world-wide population of *S. commune*, where Raper *et al.* (1960) estimated $n_\alpha = 9$ and $n_\beta = 50$, it is only 1·47%.

The advantage referred to in the foregoing section is the *absolute* difference in out-breeding potential between the two-locus and the one-locus systems. The *relative* increase in out-breeding at the time of establishment of the second series of specificities, say β, is $\frac{1}{2}(n_\alpha + 1)$. This value, which is inversely related to n_α, also suggests that the second series evolved in a situation where the number of specificities in the first series was low.

2. *The maintenance of the incompatibility factors in small populations.* Random extinction of an A factor can easily happen at any time, but in due course this factor will be formed again by recombination, providing its specific α and β still exist in the population (in association with other specificities). The extinction of an α or β specificity, which exists in a higher proportion of members of the population, requires the elimination forces to be of a much larger scale. In a population of N monokaryons and $n_\alpha n_\beta$ A factor specificities, the mean number of monokaryons carrying any A will be $N/n_\alpha n_\beta$. Each A factor has the probability of $e^{-N/n_\alpha n_\beta}$ of not being carried by any monokaryon (Poisson distribution). The probability that any of the A factors is lost is $n_\alpha n_\beta e^{-N/n_\alpha n_\beta}$. Let this probability be 1%,

$$\tfrac{1}{100} = n_\alpha n_\beta e^{-N/n_\alpha n_\beta}.$$

Then

$$N = n_\alpha n_\beta \log_e (100\, n_\alpha n_\beta).$$

The probability of losing any α specificity is $n_\alpha e^{-N/n_\alpha}$ and losing any β specificity $n_\beta e^{-N/n_\beta}$. Thus reduction of the number of specificities in the two-locus system in 1% of the populations of size N gives

$$\tfrac{1}{100} = n_\alpha e^{-N/n_\alpha} + n_\beta e^{-N/n_\beta}.$$

For simplicity we shall replace n_α and n_β by their geometric mean, $\tilde{n}$

$$\tfrac{1}{100} = 2\tilde{n} e^{-N/\tilde{n}}.$$

Then

$$N = \tilde{n} \log_e 200\tilde{n}.$$

Thus for the 450 A factors of *S. commune*, for instance, a bottle-neck population of 178 monokaryons can still maintain all potential specificities in 99% of the cases ($= 21 \cdot 213 \log_e 4242 \cdot 6$), while 4822 ($= 450 \log_e 45000$) monokaryons will be required in a single locus system. Hence the two-locus structure permits a much higher number of factor specificities (and higher out-breeding level) to be maintained in the population than the latter's size and size fluctuations otherwise determine.

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3. *The increase in the proportion of fertile matings among monokaryons derived from the same fruit-body.* This is an advantage when the fruit body is isolated from other, non-related, fruit bodies, but is of course an increase in the inbreeding potential (mainly so in small populations).

If p_A is the frequency of recombinant A factors, then from a single dikaryotic fruit-body $\frac{1}{2}(1-p_A)$ of the monokaryotic progeny will have each of the two non-recombinant factors and $\frac{1}{2}p_A$ will have each of the two recombinant A factors.

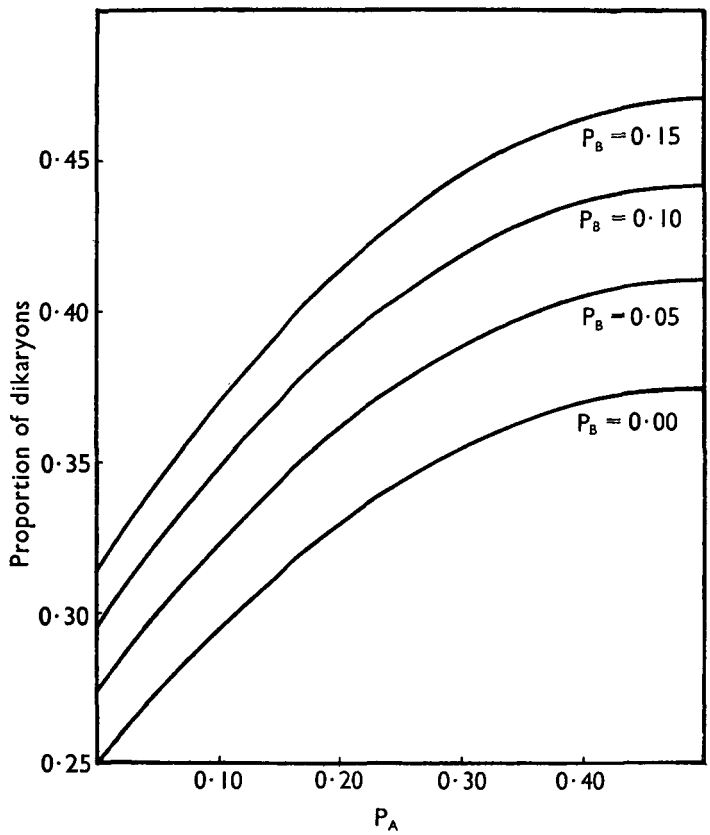

Fig. 3. Proportion of dikaryotic matings among progeny of a single dikaryon, as a function of the recombination values within the incompatibility factors.

The proportion of dikaryons among the random pair matings of monokaryons from a single fruit-body will be $\frac{1}{4}(1+2p_A-2p_A^2)$. This formula differs slightly from the one derived by Papazian (1951) as he considers every 'mutant' (=recombinant) A factor to be compatible with all other monokaryons carrying 'mutant' factors, while we assume here that every recombinant will only be compatible with *half* the other recombinant mycelia (which carry the reciprocal combination). Considering recombination within the B factor as well, (p_B), the proportion of dikaryotic combinations among the progeny of a single fruit-body becomes $\frac{1}{4}(1+2p_A-2p_A^2)\,(1+2p_B-2p_B^2)$. Fig. 3 shows this relationship for four p_B values.

SUMMARY

Crossing-over between the α and the β loci constituting the A incompatibility factor gives rise to two new specificities which are compatible with both parental As and with each other. The frequency of monokaryotic mycelia carrying recombinant A factors is shown to be under genotypic control in a multiple crosses programme (6×6), selection for high and low recombination frequencies (two generations), and crosses between the selection lines. The recombination values based on samples of 100 monokaryons, range from 0 to 21%; however, the more accurately estimated values of the 'High' and 'Low' selections are 14% and 4%, each being based on approximately 2,000 mycelia.

The data are compatible with a gene-system consisting of the postulated locus *rec* which has a major effect on recombination and which is linked to the A factor, and several minor effects by other loci. Alternative interpretations are presented and discussed. The apparent dominance of low frequencies of recombination on high frequencies can be related to the breeding behaviour of *S. commune*. Thus close linkage between α and β allows a high number of A specificities to be maintained in a population as well as high out-breeding potential, while the inbreeding potential (i.e. dikaryotic combinations between monokaryons originating from a single fruit-body) is kept low and near its minimum.

The significance of the two-locus structure of the incompatibility factors is examined theoretically in an Appendix at the end of the Discussion section.

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REFERENCES

Bartlett, M. S. (1947). The use of transformation. *Biometrics* **3**, 39–52.

Bodmer, W. F. & Parsons, P. A. (1962). Linkage and recombination in evolution. *Adv. Genet.* **11**, 1–100.

Day, P. R. (1960). The structure of the A mating type locus in *Coprinus lagopus*. *Genetics*, **45**, 641–650.

Day, P. R. (1963). The structure of the A mating type factor in *Coprinus lagopus*: wild alleles. *Genet. Res.* **4**, 323–325.

Day, P. R. & Anderson, G. E. (1961). Two linkage groups in *Coprinus lagopus*. *Genet. Res.* **2**, 414–423.

Day, P. R. & Holliday, R. (1959). Incompatibility in *Schizophyllum commune*. *Am. Nat.* **93**, 387–388.

Ellingboe, A. H. (1963). Illegitimacy and specific factor transfer in *Schizophyllum commune*. *Proc. natn. Acad. Sci., U.S.A.* **49**, 286–292.

Ellingboe, A. H. (1965). Somatic recombination in Basidiomycetes. In *Incompatibility in Fungi* (K. Esser & J. R. Raper, eds.), pp. 36–48. Berlin: Springer-Verlag.

Fisher, R. A. (1958). *The Genetical Theory of Natural Selection*, 2nd ed. New York: Dover.

FISHER, R. A. & YATES, F. (1963). *Statistical Tables*, 6th ed. Edinburgh and London: Oliver & Boyd.

GRIFFING, B. & LANGRIDGE, J. (1963). Factors affecting crossing-over in the tomato. *Aust. J. biol. Sci.* **16**, 826–837.

GRIFFITHS, A. J. F. & THRELKELD, S. F. H. (1966). Internuclear effects on prototroph frequencies of some crosses in *Neurospora crassa*. *Genetics*, **54**, 77–87.

JESSOP, A. P. & CATCHESIDE, D. G. (1965). Interallelic recombination at the *his*-1 locus in *Neurospora crassa* and its genetic control. *Heredity, Lond.* **20**, 237–256.

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

LAVIGNE, S. & FROST, L. C. (1964). Recombination frequency and wild-type ancestry in linkage group I of *Neurospora crassa*. *Genet. Res.* **5**, 366–378.

LAWRENCE, M. J. (1963). The control of crossing-over in the X-chromosome of *Drosophila melanogaster*. *Heredity, Lond.* **18**, 27–46.

MATHER, K. (1942). Heterothally as an out-breeding mechanism in fungi. *Nature, Lond.* **149**, 54–56.

MATHER, K. (1966). Variability and selection. *Proc. R. Soc.* B, **164**, 328–340.

PAPAZIAN, H. P. (1951). The incompatibility factors and a related gene in *Schizophyllum commune*. *Genetics*, **36**, 441–459.

RAPER, J. R., BAXTER, M. G. & MIDDLETON, R. B. (1958*a*). The genetic structure of the incompatibility factors in *Schizophyllum commune*. *Proc. natn. Acad. Sci. U.S.A.* **44**, 889–900.

RAPER, J. R., KRONGELB, G. S. & BAXTER, M. G. (1958*b*). The number and distribution of incompatibility factors in *Schizophyllum*. *Am. Nat.* **92**, 221–232.

RAPER, J. R., BAXTER, M. G. & ELLINGBOE, A. H. (1960). The genetic structure of the incompatibility factors of *Schizophyllum commune*. The *A* factor. *Proc. natn. Acad. Sci. U.S.A.* **46**, 833–842.

SCOTT-EMUAKPOR, M. B. (1965). Genetic recombination in *Neurospora crassa* and *N. sitophila*. *Genet. Res.* **6**, 216–225.

SIMCHEN, G. (1966). Monokaryotic variation and haploid selection in *Schizophyllum commune*. *Heredity, Lond.* **21**, 241–263.

SIMCHEN, G. & JINKS, J. L. (1964). The determination of dikaryotic growth rate in the basidiomycete *Schizophyllum commune*: a biometrical analysis. *Heredity, Lond.* **19**, 629–649.

SMITH, B. R. (1965). Interallelic recombination at the *his*-5 locus in *Neurospora crassa*. *Heredity, Lond.* **20**, 257–276.

SMITH, B. R. (1966). Genetic controls of recombination. I. The recombination-2 gene of *Neurospora crassa*. *Heredity, Lond.* **21**, 481–498.

SNIDER, P. J. & RAPER, J. R. (1958). Nuclear migration in the basidiomycete *Schizophyllum commune*. *Am. J. Bot.* **45**, 538–546.

WHITEHOUSE, H. L. K. (1949). Heterothalism and sex in fungi. *Biol. Rev.* **24**, 411–447.