Genet. Res., Camb. (1964), **5**, *pp.* 366–378
Printed in Great Britain

Recombination frequency and wild-type ancestry in linkage group I of *Neurospora crassa*

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(*Received* 20 *December* 1963)

1. INTRODUCTION

It is now widely recognized that the wild-type ancestry of strains of *Neurospora crassa* influences the frequency of recombination. Barratt (1954) reported heterogeneity in recombination frequencies from crosses between Lindegren and hybrid Abbott Lindegren stocks and also that the centromere distances of several mutant loci were significantly reduced in crosses between Abbott 4 and Lindegren stocks compared with those in crosses between two Lindegren stocks.

An extensive survey of data on centromere distances of strains with known wild-type ancestries was made by Frost (1955*a*, 1955*b*, 1961) who found that in crosses of Abbott 4 × Lindegren ancestry the centromere distances of all but distal loci were significantly lower than in crosses of Lindegren × Lindegren ancestry. Abbott 12 × Lindegren crosses gave consistently reduced centromere distances but they were not significantly lower than in Lindegren × Lindegren crosses. Groups of crosses with the same wild-type ancestry gave homogeneous centromere distances for any one locus with the exception of groups which were more complex backcrosses or intercrosses involving both Abbott 4 or Abbott 12 and Lindegren wild-types in their ancestry.

The wild-type ancestries of present-day mutant strains are usually complex or unknown. Theoretically much of the mixed ancestry and consequent heterogeneity in recombination frequencies in these existing strains could be removed by backcrossing repetitively to a single wild-type as suggested by Frost (1961).

The aims of this investigation were to determine if mutant strains repetitively backcrossed to Abbott 4*A*, Abbott 12*a* or Lindegren 1*A* wild-types would give rise to homogeneous recombination frequencies and to determine the effect of various wild-type backcross ancestries on the frequency of recombination.

2. MATERIALS AND METHODS

(i) *Strains*

The nomenclature of Barratt, Newmeyer, Perkins & Garnjobst (1954) has been followed for mutant strains except that the aurescent strain (34508) is termed *aur*

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https://doi.org/10.1017/S0016672300034819 Published online by Cambridge University Press

instead of *al-1*. The wild-types have been abbreviated according to Beadle & Tatum (1945).

The original strains used in this work are listed below:

Wild-types: Abb 4*A*, Abbott; Abb 12*a*, Abbott; L 1*A*, Lindegren; L 25*a*, Lindegren } Supplied by R. W. Barratt

Mutants: *cr* F.945, 56, crisp

me-6 (35809), methionine

al (G2), albino; *lys-3* (4545), lysine } Supplied by B. M. Elliott

aur (34508), aurescent; *nic-1* (3416), nicotinic acid } Supplied by D. G. Catcheside

Single and double mutant strains were isolated from the original strains and then backcrossed repetitively to the wild-types as shown in Table 1.

All mutant markers are situated on the right arm of linkage group I. Their relative order (proximal to distal) is: *cr*, *me-6*, *aur*, *lys-3*, *nic-1*, (Perkins, 1959). The locus *al* (G2) lies between *al-2* (15300) and *nic-1*, and is probably allelic with *aur*.

The techniques used were based on those of Beadle and Tatum (1945). Modifications and additional methods are outlined below.

All crosses were made under standard conditions on minimal reproductive medium (Westergaard & Mitchell, 1947) supplemented, to support the growth of nutritional mutants, with the appropriate vitamin or amino acid to give a concentration of 0·2 mg. per ml. There may have been small environmental variations among the crosses, but it is unlikely that these would affect the recombination frequency significantly since Perkins (1959) found that recombination frequencies were similar from repeated crosses between the same two parents.

Table 1. *The mutant and wild-type parents of several backcross series each of which involved at least four successive backcrosses of the mutant to wild-type*

Mutant parent	Wild-type parent
cr(f1L)1*a*	Abb 4*A*
aur-CU-2*a*	Abb 4*A*
me-6, *aur*-SL-5*a*	Abb 4*A*
aur, *lys-3*-SL-17*a*	Abb 4*A*
aur, *nic-1*-SL-30*a*	Abb 4*A*
cr-SL-4*A*	Abb 12*a*
aur-CU-3*A*	Abb 12*a*
al(G2), *nic-1*-CU-24*A*	Abb 12*a*
cr(f1)1*a*	L 1*A*
aur-CU-2*a*	L 1*A*
me-6, *al*(G2)-SL-4*a*	L 1*A*
aur, *lys-3*-SL-17*a*	L 1*A*
aur, *nic-1*-SL-30*a*	L 1*A*

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(ii) *Procedure for backcrossing mutants to wild-types*

The wild-type was made the protoperithecial or maternal parent and was fertilized by conidia of a mutant in the first cross and each successive backcross. The mutant conidia were suspended in 5 ml. of sterile distilled water containing any required nutrients (0·2 mg. per ml.) and poured over a week-old culture of the wild-type. These fertilized cultures were incubated for four or more weeks before ascospores were analysed. At least four successive backcrosses were made to each wild-type and mutant progeny of both mating types were taken at random from the final backcross.

Selection for high fertility among the backcrosses was necessary since fertility deteriorated with continual inbreeding, especially with Abb 4*A* and Abb 12*a* wild-types, which showed segregation in each backcross of one or more ascospore abortion factors as first reported by Emerson & Cushing (1946). A few backcrosses to Abb 12*a* had to be made by inoculating wild-type and mutant together because fertilization of Abb 12*a* protoperithecia by mutant conidia failed.

(iii) *A selective method for determining recombination frequency between crisp and linked markers*

The choice of crisp and albino morphological markers together with one or two nutritional markers has enabled a large number of progeny to be analysed easily and quickly (Lavigne, 1962). Using a selective medium, half parental and half recombinant types were recovered and used to calculate the frequency of recombination. The accuracy of this method was checked statistically by comparison with the frequency of recombinants in 100 random spore isolates for all crosses except some involving crisp progeny from the fifth backcross to Abb 4*A*, Abb 12*a* and L 1*A*.

Samples of progeny from a few crosses were fully analysed, but only the numbers of parental and recombinant types which would have been scored by the selective plating technique were used for calculating the recombination frequency, and for comparison with the numbers of parental and recombinant types obtained by the selective technique in other crosses.

(iv) *Terminology and designation of strains used in crosses*

Lindegren (1933) used 'f1' to denote the first filial generation of *Neurospora crassa* because it is a haploid organism. Thus 'r' will be used to denote the backcross generations in this work.

The ancestry of a mutant has been expressed by the number of the backcross generation from which it was isolated followed by the abbreviation for the wild-type parent, e.g., *aur* (r6L)3*a* = *aur* mutant, isolate no. 3 of *a* mating type from the r6 generation of which Lindegren 1*A* was the wild-type parent. Double mutant strains have been designated in the following manner. If two single mutants were

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crossed and the double mutant isolated and then backcrossed to a wild-type, this was denoted as, e.g., *aur*, *nic-1*(r6L)7*A*. Alternatively, a double mutant isolated from a cross between two single mutants, both of which had been backcrossed to a wild-type, was designated, e.g., *aur*(r6L), *nic-1*(r7L)67*A*. The wild strain (r6s2L)12*a* was obtained by crossing L 1*A* and L25*a*, then backcrossing isolates of *a* mating type to the L 1*A* parent until the r6 generation was reached. Two strains of the r6 generation were self-crossed to give the (r6s1) generation and two strains from this generation were self-crossed to give the (r6s2L) generation.

The symbol (♀) has been placed after the protoperithecial parent of a cross. There have been no symbols assigned to the conidial (or paternal) strains, or to strains of a cross which were inoculated together.

3. EXPERIMENTAL RESULTS

(i) *Homogeneity of recombination frequency in the* cr-me-6 *or* cr-nic-1 *intervals in crosses involving crisp progeny from the fifth backcross to the wild-type Abb 4*A, *Abb 12*a *or L 1*A

Crisp progeny of the same wild-type ancestry and mating type from the r6 backcross generation were each crossed with the same nutritional mutant strain also from the r6 generation. All crosses produced a 1:1 ratio of crisp to non-crisp progeny. The numbers of progeny with crisp and wild-type morphology were obtained by observing, under low power, germinated ascospores plated on supplemented medium. The crisp hyphae were finer and more branched than the wild-type hyphae. Also, counts were made of viable, normal-sized, black ascospores which had germinated and those which were presumably not viable and had failed to germinate.

Seven *cr*(r6Abb 4) strains of *a* mating type were each crossed with *me-6*(r7L)9*A*, and five *cr*(r6Abb 4) strains of *A* mating type were each crossed with *me-6* (r7L)8*a*. The crisp strains were made the protoperithecial parents and all crosses were highly fertile. Recombination frequencies between *cr* and *me-6* were compared by the heterogeneity χ^2 test and the values are given under Table 2. In crosses involving *cr*(r6Abb 4) strains of *a* mating type, recombination frequencies were heterogeneous; while in crosses involving *cr*(r6Abb 4) strains of *A* mating type they were not significantly heterogeneous. This suggests that one or more factors influencing recombination frequency might be linked to the *A* mating-type locus of the Abb 4 wild-type. However, selection for high fertility, which is not a feature of the Abb 4 wild-type, may have reduced the transfer of the wild-type genome to mutant strains so that heterogeneity due to mixed ancestry persisted even after five successive backcrosses.

Similar crosses were made with *cr*(r6Abb 12) strains but only three fertile crosses were obtained even when these strains were inoculated together with *me-6*(r7L) strains. The data are given in Table 3 where it can be seen that viability was high and recombination frequencies were homogeneous.

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Table 2. *Recombination frequencies between* cr *and* me-6 *in crosses of Abbott 4 × Lindegren ancestry*

Cross*	Crisp parent of cross	No. of progeny: Parental *cr*,+	No. of progeny: Recombinant +,+	% Recombination	% Germination of ascospores
1	*cr*(r6Abb 4)1*a*	658	28	4·1	96·7
2	*cr*(r6Abb 4)2*a*	262	33	11·2	96·6
3	*cr*(r6Abb 4)3*a*	213	18	7·8	96·5
4	*cr*(r6Abb 4)4*a*	255	10	3·8	95·0
5	*cr*(r6Abb 4)5*a*	238	6	2·5	96·2
6	*cr*(r6Abb 4)6*a*	228	23	9·2	97·0
7	*cr*(r6Abb 4)10*a*	268	14	5·0	96·3
8	*cr*(r6Abb 4)7*A*	188	12	6·0	96·2
9	*cr*(r6Abb 4)8*A*	157	6	3·7	94·6
10	*cr*(r6Abb 4)9*A*	216	3	1·4	98·2
11	*cr*(r6Abb 4)11*A*	179	7	3·8	96·3
12	*cr*(r6Abb 4)12*A*	187	13	6·5	94·6

Heterogeneity between crosses in recombination frequency

	χ^2	D.F.	*P*
Crosses 1–7	33·2	6	< 0·001
Crosses 8–12	8·7	4	> 0·05

* In crosses 1–7, crisp strains were maternal and crossed to *me-6*(r7L)9*A*. In crosses 8–12, crisp strains were maternal and crossed to *me-6*(r7L)8*a*.

Table 3. *Recombination frequencies between* cr *and* me-6 *in crosses of Abbott 12 × Lindegren ancestry*

Cross*	Crisp parent of cross	No. of progeny: Parental *cr*,+	No. of progeny: Recombinant +,+	% Recombination	% Germination of ascospores
1	*cr*(r6Abb 12)6*a*	170	21	11·0	92·5
2	*cr*(r6Abb 12)7*a*	355	30	7·8	95·4
3	*cr*(r6Abb 12)9*A*	569	75	11·6	96·5

Heterogeneity between crosses in recombination frequency

	χ^2	D.F.	*P*
Crosses 1–3	4·0	2	> 0·10

* In crosses 1 and 2, crisp strains were crossed with *me-6*(r7L)9*A*. In cross 3, the crisp strain was crossed with *me-6*(r7L)8*a*.

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Highly fertile crosses were obtained between all *cr*(r6Abb 12) strains and *aur*, *nic-1*(r6L) strains. The latter were made the protoperithecial parents because although *cr*(r6Abb 12) strains formed many protoperithecia they remained sterile after applying conidia of opposite mating type by various methods. Table 4 gives the data obtained from three crosses involving *cr*(r6Abb 12) strains of *a* mating type with *aur*, *nic-1*(r6L)7*A* and from ten crosses between *cr*(r6Abb 12) strains of *A* mating type with *aur*, *nic-1*(r6L)2*a*. The two crosses (3 and 7) involving *cr*(r6Abb 12)7*a* and 9*A* (Table 4) were made at a different time from the other crosses and the ascospores were fully analysed but only the numbers of those genotypes which would have been recovered by the selective plating method were used to calculate the recombination frequencies. It will be noticed that the viabilities of normal-sized, black ascospores from these two crosses were greater than those from the other crosses. The ascospores from the two oustanding crosses had been dispersed from the perithecia for about six weeks whereas those from the other crosses had been dispersed for only one week.

Table 4. *Recombination frequencies between* cr *and* nic-1 *in crosses of Abbott 12 × Lindegren ancestry*

Cross*	Crisp parent of cross	No. of progeny: Parental *cr*,+	No. of progeny: Recombinant +,+	% Recombination	% Germination of ascospores
1	*cr*(r6Abb 12)5*a*	86	28	24·6	92·1
2	*cr*(r6Abb 12)6*a*	486	177	26·7	87·6
3	*cr*(r6Abb 12)7*a*	414	137	24·9	96·0
4	*cr*(r6Abb 12)1*A*	102	41	28·7	87·4
5	*cr*(r6Abb 12)3*A*	157	41	20·7	85·1
6	*cr*(r6Abb 12)4*A*	99	28	22·0	84·7
7	*cr*(r6Abb 12)9*A*	150	39	20·6	94·8
8	*cr*(r6Abb 12)13*A*	262	106	28·8	84·4
9	*cr*(r6Abb 12)15*A*	223	58	20·6	78·1
10	*cr*(r6Abb 12)19*A*	149	57	27·7	83·0
11	*cr*(r6Abb 12)21*A*	148	56	27·4	80·1
12	*cr*(r6Abb 12)25*A*	163	47	22·4	79·7
13	*cr*(r6Abb 12)28*A*	300	110	26·8	85·0

Heterogeneity between crosses in recombination frequency

	χ^2	D.F.	*P*
Crosses 1–13	14·9	12	> 0·10

* In crosses 1–3, crisp strains were paternal and crossed to *aur*, *nic-1*(r6L)7*A*. In crosses 4–13, crisp strains were paternal and crossed to *aur*, *nic-1*(r6L)2*a*.

The viability of normal-sized, black ascospores from cross 12, *cr*(r6Abb 12)25*A* × *aur*, *nic-1*(r6L)2*a*, increased from 79·7% after one week from the time of dispersal to 92·0% after four weeks. Therefore some of the ascospores which had been

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dispersed for one week were probably not fully ripened and appeared to be non-viable. The lower viability may have biased the recombination frequency if the non-viable ascospores were predominantly of both parental genotypes or both recombinant genotypes. Since the numbers of crisp to non-crisp progeny were in good agreement with the expected 1:1 ratio, the non-viable ascospores could not have been of a single genotype. The recombination frequencies in the eleven crosses with lower ascospore viabilities were homogeneous (Het. $\chi^2 = 12{\cdot}4$; D.F. = 10; $P = > 0{\cdot}10$) and this homogeneity was retained when data from the two crosses with higher ascospore viabilities were included (Het. $\chi^2 = 14{\cdot}9$; D.F. = 12; $P = > 0{\cdot}10$).

Ten *cr*(r6L) strains of *a* mating type and three of *A* mating type were crossed with *me-6*(r7L)9*A* and *me-6*(r7L)8*a* respectively. The crisp strains were made the protoperithecial parents and all crosses were highly fertile. The recombination frequencies between *cr* and *me-6* are recorded in Table 5 and were homogeneous.

Table 5. *Recombination frequencies between* cr *and me-*6 *in crosses of Lindegren* × *Lindegren ancestry*

Cross*	Crisp parent of cross	No. of progeny: Parent *cr*,+	No. of progeny: Recombinant +,+	% Recombination	% Germination of ascospores
1	*cr*(r6L)1*a*	180	35	16·3	92·7
2	*cr*(r6L)5*a*	185	40	17·8	93·7
3	*cr*(r6L)10*a*	572	94	14·1	95·0
4	*cr*(r6L)11*a*	157	40	20·3	90·5
5	*cr*(r6L)15*a*	174	25	12·6	95·3
6	*cr*(r6L)18*a*	213	32	13·1	91·6
7	*cr*(r6L)19*a*	141	23	14·0	95·1
8	*cr*(r6L)22*a*	192	32	14·3	95·4
9	*cr*(r6L)23*a*	534	95	15·1	96·7
10	*cr*(r6L)25*a*	222	46	17·2	92·4
11	*cr*(r6L)8*A*	251	49	16·3	94·1
12	*cr*(r6L)12*A*	288	47	14·0	94·6
13	*cr*(r6L)24*A*	161	26	13·9	94·0

Heterogeneity between crosses in recombination frequency

	χ^2	D.F.	P
Crosses 1–13	9·9	12	> 0·50

* In crosses 1–10, crisp strains were maternal and crossed to *me-6*(r7L)9*A*. In crosses 11–13, crisp strains were maternal and crossed to *me-6*(r7L)8*a*.

(ii) *Recombination frequency in crosses of Lindegren wild-type ancestry*

Since the *cr*(r6L) strains showed homogeneous recombination frequencies in one interval, mutant strains backcrossed to L 1*A* were tested for homogeneity of

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recombination frequencies in other intervals. It may be seen in Table 6, however, that recombination frequencies in the intervals *cr-aur*, and *aur-nic-1* in crosses between strains which had a history of at least five backcrosses to L 1*A* were heterogeneous.

Table 6. *Recombination frequencies in the intervals* cr-aur *and* aur-nic-1 *in crosses of Lindegren wild-type ancestry*

Cross No.	Crosses*	% Recombination in intervals between *cr* and *aur*	% Recombination in intervals between *aur* and *nic-1*	% Germination of ascospores
1	*cr*(r6L)12*A* × *aur*(r6L)3*a*(♀)	34·4		91·4
2	*cr*(r8L)4*a* × *aur*(r10L)2*A*	26·3		91·0
3	(r6s2L)12*a*(♀) × *cr*(r6L), *aur*(r6L)1*A*	26·1		90·0
4	*cr*(r6L)19*a*(♀) × *aur*, *nic-1*(r6L)7*A*	41·4	8·6	89·0
5	*cr*(r6L)19*a* × *aur*, *nic-1*(r6L)7*A*(♀)	33·9	8·9	92·8
6	*cr*(r6L)19*a* × *aur*(r6L), *nic-1*(r7L)67*A*(♀)	29·1	7·5	95·0
7	*lys-3*(r6L)37*A*(♀) × *cr*(r6L), *aur*, *nic-1*(r6L)6*a*	42·2	14·1	92·0
	Heterogeneity χ^2	62·8	17·1	
	D.F.	6	3	
	P	< 0·001	< 0·001	

* For explanation of strain symbols see text, section iv.

(iii) *The effect of wild-type ancestry on recombination frequency*

A comparison was made of the recombination frequencies in two crosses with different wild-type ancestries having one parental strain in common (Tables 7*a* and 7*b*). Over 500 ascospores from each cross were analysed by the selective method. The fertilities of the crosses varied but there were no significant deviations from the expected 1:1 ratios of crisp to non-crisp progeny among parental or recombinant types, and no indication that ascospore abortion affected a single genotype.

With few exceptions recombination frequencies in the intervals tested were significantly higher in crosses between strains of the same wild-type ancestry than in crosses of L × Abb 12 ancestry. Crosses 19 and 17 (Table 7*b*) of Abb 4 × 12 ancestry gave significantly higher recombination values in the interval *al*(G2)-*nic-1*, homogeneous values in the interval *cr-al*(G2) but significantly lower values for the interval *cr-aur* than crosses 7 and 18 of Abb 12 × Abb 12 ancestry. The Abb 4 × Abb 12 crosses 19 and 20 gave higher recombination frequencies in the *cr-al*(G2) and *al*(G2)-*nic-1* intervals than L × Abb 12 crosses 9 and 8 respectively.

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Table 7*a*. *Details of crosses used for a comparison of recombination frequencies different with wild-type ancestries*

Cross No.	Parents of crosses*	% Germination of ascospores
1	*cr*(r6Abb 4)2*a* × *aur*(r7Abb 4)3*A*(♀)	82·0
2	*cr*(r6L)19*a* × *aur*(r7Abb 4)3*A*(♀)	92·0
3	*cr*(r6Abb 4)11*A* × *me*-6, *aur*(r6Abb 4)3*a*	88·0
4	*cr*(r6Abb 4)11*A*(♀) × *me*-6(r7L), *aur*(r6L)30*a*	97·0
5	*cr*(r6Abb 4)11*A*(♀) × *aur*, *nic-1*(r6Abb 4)13*a*	85·0
6	*cr*(r6L)12*A*(♀) × *aur*, *nic-1*(r6Abb 4)13*a*	97·0
7	*cr*(r6Abb 12)7*a* × *al*(G2), *nic-1*(r6Abb 12)4*A*	94·0
8	*cr*(r6L)19*a*(♀) × *al*(G2), *nic-1*(r6Abb 12)4*A*	80·0
9	*cr*(r6L)19*a* × *al*(G2), *nic-1*(r6Abb 12)4*A*(♀)	84·0
10	*cr*(r6L)12*A*(♀) × *me*-6(r7L), *aur*(r6L)30*a*	89·0
11	*lys*-3(r6Abb 4)17*A* × *cr*(r6L), *aur*, *nic-1*(r6L)6*a*(♀)	99·0
12	*lys*-3(r6L)37*A*(♀) × *cr*(r6L), *aur*, *nic-1*(r6L)6*a*	92·0
13	*cr*(r6Abb 12)9*A* × *aur*(r6L)3*a*(♀)	95·6
14	*cr*(r6L)12*A* × *aur*(r6L)3*a*(♀)	91·4
15	*cr*(r6Abb 12)7*a* × *aur*, *nic-1*(r6L)7*A*(♀)	96·0
16	*cr*(r6L)19*a* × *aur*, *nic-1*(r6L)7*A*(♀)	92·8
17	*cr*(r6Abb 4)2*a* × *aur*(r6Abb 12)1*A*	98·0
18	*cr*(r6Abb 12)7*a* × *aur*(r6Abb 12)1*A*	96·0
19	*cr*(r6Abb 4)2*a* × *al*(G2), *nic-1*(r6Abb 12)4*A*(♀)	97·0
20	*cr*(r6Abb 4)2*a*(♀) × *al*(G2), *nic-1*(r6Abb 12)4*A*	92·0

* For explanation of the strain symbols see text, section iv.

4. DISCUSSION

The results obtained here based on recombination frequencies from the analysis of random ascospores are in general agreement with those based on centromere distances derived from tetrad analyses reported by Frost (1961). One difference, however, was that Frost indicated that Abb 12 × L crosses gave consistently lower, but not significantly lower, centromere distances than L × L crosses but sample sizes were small. In this work where larger sample sizes were possible, Abb 12 × L crosses have given significantly lower recombination frequencies in the two intervals *cr-aur* and *aur-nic-1* (Table 7*b*) and in the *cr-me-6* interval for 43·5 % of the L × Abb 12 crosses (Table 3) compared with L × L crosses (Table 5). Recombination frequencies in the *cr-me-6* interval in the remaining 56·5 % of the crosses tested were not significantly different although they were always lower in the Abb 12 × L crosses.

This work has provided data, previously lacking, on recombination frequencies in Abb 4 × Abb 4 Abb 12 and × Abb 12 crosses. In general, recombination frequencies in the intervals tested were significantly higher in these crosses than in L × Abb 4 or L × Abb 12 crosses but not always as high as in L × L crosses (Table 7*b*). This agrees with the work of Stadler (1956*a*) and Stadler & Towe (1962) who showed

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Table 7*b*. *Heterogeneity tests of recombination frequencies in various intervals in linkage group I in pairs of crosses with different wild-type ancestries but with one parental strain common in each pair of crosses*

Cross No. (see Table 7*a*)	Ancestries of crosses	Total progeny tested	% Recombination and heterogeneity χ^2 test (D.F. = 1)					
			cr *me-6*	*me-6* *aur*	*cr* *aur*	*aur* *nic-1*	*cr* *al*(G2)	*al*(G2) *nic-1*
1	Abb 4× Abb 4	358			22·9			
2	L× Abb 4	459			15·1 } *			
3	Abb 4× Abb 4	413	6·5	27·8				
4	Abb 4× L	473	4·0 }	19·7 } **				
5	Abb 4× Abb 4	573			36·6	9·9		
6	L× Abb 4	521			29·2 } **	6·7 } *		
7	Abb 12× Abb 12	521					29·7	4·8
8	L× Abb as	887					10·6 } **	4·7 }
7	Abb 12× Abb 12	521					29·7	4·8
9	L× Abb 12	469					17·3 } **	8·1 }
4	Abb 4× L	473	4·0	19·7				
10	L× L	512	11·9 } **	17·8 }				
11	Abb 4× L	265			27·5	2·6		
12	L× L	263			42·2 } **	14·1 } **		
13	Abb 12× L	478			17·2			
14	L× L	450			34·4 } **			
15	Abb 12× L	552			24·1	1·1		
16	L× L	463			33·9 } **	8·9 } **		
17	Abb 4× Abb 12	1201			17·1			
18	Abb 12× Abb 12	968			24·2 } **			
19	Abb 4× Abb 12	556					28·1	18·9
7	Abb 12× Abb 12	521					29·7 }	4·8 } **
20	Abb 4× Abb 12	468					33·6	13·7
8	L× Abb 12	887					10·6 } **	4·7 } **
19	Abb 4× Abb 12	556					28·1	18·9
9	L× Abb 12	469					17·3 } **	8·1 } **

* Heterogeneity χ^2 significant at $P = 0{\cdot}05$

** Heterogeneity χ^2 significant at $P = 0{\cdot}01$

that crossing-over was suppressed by 'heterozygosity'. An exception to this claim was the lower recombination frequency of 26·1 % obtained from the cross (6s2L)12*a* (♀) × *cr*(r6L), *aur*(r6L)1*A* of L × L ancestry compared with 36·6 % from the cross (r6s2L)12*a* (♀) × *cr*(r6Abb 12), *aur*(r6L)11*A* of L × (Abb 12 × L) ancestry (Het. $\chi^2 = 11{\cdot}8$; D.F. = 1; $P = < 0{\cdot}001$). Stadler (1956*a*), Towe (1958) and Stadler & Towe (1962) claimed that the centromere distance of *asco* increased with inbreeding but never decreased. This feature was noticed in this work but the recombination frequency between *cr* and *aur* (Table 6) was just significantly lower in cross 2 where each strain had been backcrossed to L 1*A* a greater number of times than in cross 1 (Het. $\chi^2 = 4{\cdot}1$; D.F. = 1; $P = > 0{\cdot}02$). It is possible that the occurrence of undetectable double cross-overs gave rise to the apparent lower recombination frequencies in the highly inbred crosses.

The phenomenon reported by Stadler & Towe (1962) of non-uniform increases of crossing-over in the marked region of linkage group VI was encountered also in this work in linkage group I. In Table 7*b*, recombination frequency between *me-6* and *aur* was greater in cross 3 of Abb 4 × Abb 4 ancestry than in cross 4 of Abb 4 × L ancestry while recombination frequencies between *cr* and *me-6* were homogeneous. In crosses 4 and 10 involving the same mutant markers but with L × L and Abb 4 × L ancestries, the recombination frequencies between *me-6* and *aur* were homogeneous, while the recombination frequency between *cr* and *me-6* in the L × L cross was higher than in the Abb 4 × L cross. One cross (7) of Abb 12 × Abb 12 ancestry gave a recombination frequency between *cr* and *al*(G2) which was significantly higher than in Abb 12 × L crosses (8 and 9), but homogeneous recombination frequencies resulted between *al*(G2) and *nic-1*.

The recombination frequencies between *cr* and *me-6* in crosses of Abb 4 × L ancestry (Table 2) and between *cr* and *aur* in crosses of L × L ancestry (Table 6) did not fall into obvious groups which might have suggested control by a simple gene mechanism. Instead, a range of recombination frequencies was obtained indicating control by several factors as found for *asco* by Stadler (1956*b*) and Stadler & Towe (1962), and suggested in the work of Rifaat (1958) and Frost (1961).

The limitations of repetitive backcrossing as a means of introducing a mutant marker into a given wild-type genome depend on the random segregation of chromosomes, the frequency of recombination and position of the mutant locus. Mating type is a limitation since mutant progeny of opposite mating type to the given wild strain must be used for each successive backcross. The markers used in this work were linked to the mating-type locus but in the opposite arm. Therefore the chromosome region between mating type and the mutant locus, or between two mutant loci, would be replaced by that of the wild-type only as a result of multiple crossing-over. Frost (1961) suggested that the various wild strains are genetically different and may even carry chromosome complements which are no longer strictly homologous, therefore recombination during backcrossing may be restricted by some lack of homology between the wild-type and mutant strain of hybrid wild-type ancestry. In this work, taking random ascospore progeny from each

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successive backcross as a parent for the next did not ensure that recombination had occurred between the marker chromosome and the homologous wild-type chromosome. Prakash (1963) pointed out that this limitation could be minimized by selecting from ordered tetrads a second-division segregant for the marker in each successive backcross. Another limitation applicable to both Abbott wild strains was that each carried an ascospore abortion factor or factors. Initially, the Abbotts were crossed to mutant strains with some Lindegren ancestry and in each subsequent backcross high fertility factors, presumably of Lindegren derivation, had to be selected in order to prevent complete abortion which occurred in strictly inbred crosses of either Abb 4 or Abb 12.

Hence small chromosome regions of diverse wild-type origin may have survived repetitive backcrossing particularly in the case of the Abbott strains. Even with the higher frequencies of recombination in L × L crosses, heterogeneity persisted in some cases through at least five successive backcrosses.

It may be concluded that repetitive backcrossing of present-day marker strains of hybrid ancestry to a given wild strain may not be fully effective in attempts to obtain constancy of map distance, in spite of the considerable labour involved. Rather it would seem that inducing marker mutations anew in a given wild strain would be more successful. This work indicates that Lindegren 1*A* is the most suitable strain for such projects with the use of (r6s2L)12*a* as the closely related wild-type of opposite mating type since it is strongly protoperithecial and gives fertile crosses even on further inbreeding with Lindegren 1*A*. (The strains Lindegren 1*A* and (r6s2L)12*a* are being deposited at the Fungal Genetics Stock Center).

SUMMARY

A selection of single and double mutant strains was backcrossed repetitively at least four times to each of the wild-types Abbott 4*A*, Abbott 12*a* and Lindegren 1*A*, and then re-isolated. Twelve crisp progeny isolated from the fifth backcross to Abbott 4*A* were heterogeneous for recombination frequency in the interval between *cr* and *me-6* while thirteen crisp progeny from the fifth backcross to Lindegren 1*A* were homogeneous for recombination frequency in this interval. Thirteen crisp strains from the fifth backcross to Abbott 12*a* were homogeneous for recombination frequency between *cr* and *nic-1* loci. However, mutant strains which had been backcrossed independently to Lindegren 1*A* were heterogeneous for recombination frequency in the intervals *cr-aur* and *aur-nic-1*.

In general, recombination frequencies in crosses between strains of the same wild-type ancestry including Abbott 4 and Abbott 12 were significantly higher than those in crosses of Lindegren × Abbott 4 or Lindegren × Abbott 12 ancestry but exceptions were found. Recombination frequencies between the same markers usually, but not always, increased on inbreeding and the changes in frequency were non-uniform in the marked region in some crosses.

The limitations of backcrossing as a means of transferring a mutant marker into a wild-type genome were discussed. It was concluded that inducing marker

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mutations anew in a given wild strain, preferably Lindegren 1*A*, might be more successful in obtaining constancy of map distance.

The authors are indebted to Dr R. W. Barratt, Dr B. M. Elliott and Professor D. G. Catcheside who kindly supplied the original strains.

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