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Genetic recombination in *Neurospora crassa* and *N. sitophila*

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1. INTRODUCTION

Information on the genetical nature of specific differences is somewhat scarce because of the fact that individuals of different species seldom hybridize. Fincham (1951) was the first author to undertake a comparative genetic study of the mating-type chromosomes of *Neurospora crassa* and *N. sitophila*. The segregation of mating-type in the two species had been studied by various authors (Lindegren, 1932; Aronescu, 1933; Whitehouse, 1942, 1948).

It was established from the data of these authors that the mating-type locus segregates at the second division at a much higher frequency in *N. sitophila* than in *N. crassa*. Fincham (1951) calculated from these data the mean centromere distance of mating-type in *N. sitophila* to be 24 as against 6·4 map units in *N. crassa*. However, these distances are subject to variation from cross to cross and with the particular strains used. Fincham in his study showed that this peculiar difference in the positions of mating-type which lies on the left arm of linkage group I is also shown by the mutant gene crisp, *cr*, on the right arm. He found the map distance of *cr* to be 35 units in *N. sitophila* and 4 units in *N. crassa*. The other genetic markers studied by Fincham were pink, *pk*, albino, *al-2*, and ginger, *g*; *pk* is distal to mating-type, *mt*, while *al-2* and *g* are distal to *cr*.

The fact which emerged from Fincham's work was that the parts of the mating-type chromosome distal to *mt* and *cr* seemed to be similar in the two species in respect of the genes present, their order and the amount of recombination between them. He thus concluded that the most likely difference between the two species lay in the extent to which the centromere interfered with crossing-over in its vicinity.

The foregoing conclusion needs confirmation in view of the heterogeneity in some of Fincham's data. Since the comparative studies involved the transfer of marker genes from one species to the other and the fact that most of Fincham's data came from the third, fourth and fifth backcrosses, the reported heterogeneity in recombination frequencies could be due to the effects of structural heterozygosity (Pontecorvo, 1959; Perkins, 1959).

In view of the evidence which indicates that the centromere regions of the chromosomes of many organisms are most susceptible to alteration in recombination frequencies under different environmental variables such as temperature (Plough,

1917, 1921; Mather, 1938, 1939; Rifaat, 1956), a confirmation and extension of Fincham's work would be of interest. Of particular interest would be the centromere distance of marker genes situated in *N. crassa* between mating-type and its centromere and other markers close to the centromere in other linkage groups after transfer to *N. sitophila*. Information of this kind would also help to show whether the high crossing-over frequency in the centric regions of the mating-type chromosome of *N. sitophila* is localized or not and whether it is a property shared by other chromosomes in the complement. The present investigation has, therefore, been undertaken in order to provide answers to some of the problems which obviously have a bearing on the possible causes of evolutionary divergence of the two species.

The results that will be discussed are from five-point crosses involving the mating-type chromosome and three-point crosses involving linkage groups VI and VII. The choice of linkage groups VI and VII was conditioned by the fact that at the beginning of this investigation they, together with linkage groups I and II, were the only chromosomes in the *N. crassa* complement of seven with well-mapped genetic markers on both arms. The present paper gives the first comprehensive study of its kind involving a comparison of genetic recombination in the two species.

2. MATERIALS AND METHODS

(i) *Strains*

With the diverse wild-type stocks of *Neurospora crassa* in use and the evidence (Frost, 1961) that they give variable centromere distances for a number of loci, the following strains were chosen as standard parents for backcrossing and testing. Except for the designation of mating-type as *mt* instead of *sex*, the nomenclature used throughout this paper is that of Barret *et al.* (1954).

N. crassa: Lindegren 1A, Lindegren 25a.

N. sitophila: NS1a, NS2A; these are re-isolates of some English strains isolated from a Chichester timber yard by Ramsbottom & Stevens (1935).

The following mutant strains with their original isolation numbers were used for this investigation out of the eighteen initially chosen.

Linkage group I—arg-3 (30300) arginine; *cr*, crisp; *al-2* (15300) albino; *nic-1* (3416) nicotinamide.

Linkage group VI—ad-8 (74A-Y152-M7) adenine; *ylo* (Y 30539y) yellow conidia; *pan-2* (74A-YU370-1A) pantothenic acid.

Linkage group VII—nic-3 (Y31881) nicotinamide or 3-hydroxyanthranilic acid; *arg-10* (B317) arginine.

The wild-type strains of *N. crassa* and *N. sitophila* together with the mutants *arg-3*, *cr*, *al-2* and *nic-1* were kindly supplied by Dr H. L. K. Whitehouse. Fertile strains of wild-type *N. crassa* and *N. sitophila* were also supplied by Dr L. C. Frost. All other mutant strains of *N. crassa* used for this investigation were obtained from the Fungal Genetics Stock Centre, Hanover (U.S.A.).

(ii) *Methods*

The methods used for making crosses and dissecting asci were essentially those described by Whitehouse (1942) and Beadle & Tatum (1945). For initial interspecific crosses, malt–bacto–peptone agar medium devised by Frost (1955) was found satisfactory. Intraspecific crosses were successfully made on either malt–bacto–peptone agar or glucose minimal agar medium. Marker genes were transferred from *Neurospora crassa* to *N. sitophila* by hybridization and repeated backcrossing. In most of the interspecific crosses, *N. sitophila* was the protoperithecial parent. Biochemical mutants were easily scored by the use of a selective medium, sorbose minimal agar. Since all the mating-type tests involved tetrads, the use of protoperithecial tester parents in petri dishes was found quite satisfactory. All cultures and crosses were incubated at 25°C.

3. RESULTS

Initial interspecific crosses were found always to be highly sterile. Crosses were, however, stimulated by the addition of asparagine to culture media (Beadle & Tatum, 1945). A few perithecia were generally produced after about 6–8 weeks (interspecific crosses take 1½–2 weeks) and the production of asci was very poor. A high degree of abortion of spores was observed in initial interspecific crosses and most of the asci never had more than one or two ripe ascospores. Some ripe ascospores, however, germinated quite well but were invariably sterile except one or two mutant hybrids on backcrossing. In view of the smallness of the ascospore samples from initial crosses, triplicate crosses were generally made to ensure a fairly large sample. The number of mature ascospores sampled varied from about ten to eighty depending on the cross. Percentage germination in early interspecific crosses was as low as ten. The first backcross in a few cases showed higher fertility and percentage germination. The second backcross was more fertile with an increase in the production of perithecia.

As every progeny ascospore would vary in the degree of hybridity and hence the degree of fertility when further backcrossed, a large number of mutants had to be sampled. For example, out of the thirty-two *pan-2* mutant progeny from a fourth backcross to *N. sitophila*, only one gave a few ripe asci on backcrossing. In this connexion, correlation between size and abundance of perithecia and fertility was very slight, large perithecia being often completely sterile and small ones often highly fertile.

After the third backcross fully ripe asci were obtained for the mutants *nic-1*, *bal* (balloon), *me-7* and *arg-5*. For *arg-3*, *cr*, *arg-10*, *nic-3*, *tryp-3* and *ylo* fully ripe asci were obtained after the fourth backcross. Since it is essential for the purpose of this investigation that marker genes are linked to *N. sitophila* centromeres, markers segregating at the second division were chosen for further backcrossing. This was done repeatedly in subsequent crosses. On backcrossing some of the second division segregant mutants, fully ripe asci were not always obtained; in fact, many turned out to be completely sterile without the production of perithecia.

Thus, as an insurance, mutant hybrids from random spores as well as spores from tetrads were backcrossed. In some cases backcrosses with random spore mutants gave fully ripe asci which were used for subsequent backcrossing. Five of the original number of mutants used in the transfer experiments were not successfully incorporated into *N. sitophila* genome. These are *hist-2* (histidine), *me-6*, *leu-3* (leucine), *tryp-2* and *tryp-3* (tryptophan).

As soon as fertility was ensured after the sixth backcross, the double mutants *arg-3*, *cr* and *al-2*, *nic-1* were synthesized in large numbers and further backcrossed.

Table 1. *Recombination in linkage group I (mating-type chromosome) of* Neurospora crassa *and* N. sitophila. *Tetrad data from five-point cross*

$$\frac{mt^+ \quad arg\text{-}3 \quad cr \quad al\text{-}2 \quad nic\text{-}1 \quad g}{mt^- \quad + \quad + \quad + \quad + \quad +}$$

	N. crassa			*N. sitophila*			Percent recombination		
Interval	PD	T	NPD	PD	T	NPD	*N. crassa*	*N. sitophila*	χ^2 (2 × 2)
mt–arg-3	238	84	17	106	204	26	17·4 ± 1·4	38·1 ± 1·7	144·4*
mt–cr	205	114	19	81	208	45	22·5 ± 1·4	44·6 ± 1·6	147·8*
mt–al-2	93	212	34	54	225	57	41·3 ± 1·7	50·5 ± 1·5	22·5*
mt–nic-1	72	227	38	54	217	65	45·0 ± 1·4	51·6 ± 1·6	12·2*
arg-3–cr	237	99	1	129	203	8	15·0 ± 1·3	32·2 ± 1·4	111·2*
arg-3–al-2	106	212	17	52	243	45	36·7 ± 1·4	49·0 ± 1·4	41·1*
arg-3–nic-1	91	218	27	52	240	48	40·5 ± 1·4	49·4 ± 1·5	21·9*
cr–al-2	137	188	13	84	234	17	31·7 ± 1·4	40·0 ± 1·4	20·2*
cr–nic-1	104	215	17	69	245	25	37·1 ± 1·4	43·5 ± 1·4	11·4*
al-2–nic-1	260	73	1	253	91	0	11·2 ± 1·1	13·2 ± 1·2	2·4
al-2–g†	23	26	0	34	33	1	26·5 ± 3·6	25·7 ± 3·2	0·04

* $P < 0{\cdot}001$.

† Data from Fincham (1951).

After the eighth backcross multiple mutant strains of *N. sitophila* with the genotype *arg-3*, *cr*, *al-2*, *nic-1* were synthesized. The same procedure was used for the synthesis of the triple mutants *ad-8*, *ylo*, *pan-2* and *nic-3*, *me-7*, *arg-10* after the ninth backcross. The counterpart multiple mutant strains were also synthesized in *N. crassa* after individual backcrossing of marker genes to the standard wild-type about three to four times to ensure a fairly homogeneous genetic background. The mutants *bal*, *arg-5*, and *pe* (peach) in linkage group II were also transferred, but *pe* was abandoned because of the difficulty of scoring it against the wild-type and *bal* was left out because of its poor conidiation and growth properties. From a sample of sixteen tetrads it appeared that the segregation of *arg-5* might be identical in the two species.

The tetrad data and recombination frequency tests on the mating-type chromosome are shown in Table 1. Because fertility was sometimes impaired in crosses with mutliple mutant strains, perithecia from two identical crosses had to be

sampled for the first experiment. The recombination frequencies in the two crosses did not differ significantly and so the data have been lumped together. Sufficient asci were, however, obtained from a single cross in experiments involving linkage groups VI and VII. The data are summarized in Tables 2 and 3 respectively. The

Table 2. *Recombination in linkage group VI of* Neurospora crassa *and* N. sitophila. *Tetrad data from three-point cross*

ad-8 ylo pan-2
\+ + +

	N. crassa			*N. sitophila*			Percent recombination		
Interval	PD	T	NPD	PD	T	NPD	*N. crassa*	*N. sitophila*	χ^2 (2 × 2)
ad-8–ylo	85	180	8	138	131	2	34·4 ± 1·5	25·0 ± 1·5	31·0*
ad-8–pan-2	69	190	14	128	139	4	39·9 ± 1·5	27·1 ± 1·5	35·7*
ylo–pan-2	227	44	1	248	21	2	8·4 ± 1·2	4·6 ± 1·0	13·1*

* $P < 0{\cdot}001$.

asci were classified according to whether they show, respectively, 0, 4 or 8 ascospores which are recombinant for the loci considered in pairs. These classes are: parental ditype (PD), tetratype (T), and non-parental ditype (NPD). The standard errors of recombination frequencies quoted were calculated from the formula given by Mather & Beale (1942). Percentage germination in all crosses was over 85%.

Table 3. *Recombination in linkage group VII of* Neurospora crassa *and* N. sitophila. *Tetrad data from three-point cross*

nic-3 me-7 arg-10
\+ + +

	N. crassa			*N. sitophila*			Percent recombination		
Interval	PD	T	NPD	PD	T	NPD	*N. crassa*	*N. sitophila*	χ^2 (2 × 2)
nic-3–me-7	183	132	1	169	141	7	18·8 ± 1·4	24·4 ± 1·5	3·3
nic-3–arg-10	123	179	14	81	218	18	32·8 ± 1·6	40·1 ± 1·4	1·4
me-7–arg-10	182	129	6	128	183	7	22·2 ± 1·5	31·0 ± 1·5	24·8*

* $P < 0{\cdot}001$.

The results show that in the mating-type chromosome recombination frequency between the genes is significantly higher at the 5% level in *N. sitophila* than in *N. crassa*, except in the distal intervals *al-2–nic-1* and *al-2–g*. The picture in the centromere region is, however, different because the very high recombination value obtained for the *arg-3–cr* interval in *N. sitophila* is largely due to the high crossing-over frequency in the interval bounded by the centromere and *cr*.

On the other hand, in linkage group VI recombination frequency between the genes is significantly higher in *N. crassa* than in *N. sitophila*. This difference applies also to the segregation of *pan-2* but not to *ylo*. However, in linkage group VII recombination frequencies are not significantly different in the two species except for the *me-7–arg-10* interval. In contrast to the situation in the mating-type chromosome and linkage group VI, recombination frequency between the most distally placed markers, *nic-3* and *arg-10*, is not significantly different. The genes *nic-3* and *me-7* which are closest to the centromere and lie on opposite sides of it show essentially the same frequency of segregation in the two species. The marker

Table 4. *A comparison of random spore data from crosses involving linkage group I of* Neurospora sitophila

Genotypes	Frequency: Repulsion cross (R)	Frequency: Coupling cross (C)	% Recombination: R	% Recombination: C	χ^2 (2 × 2)
arg + *al* +	89	1	*arg-3* and *cr*		
+ *cr* + *nic*	82	1	28·4	30·3	0·45
+ + + +	5	102*	*arg-3* and *al-2*		
arg cr al nic	5	144	41·6	42·6	0·11
arg + + +	10	52	*arg-3* and *nic-1*		
+ *cr al nic*	7	57	47·3	43·1	1·86
+ *cr* + +	17	21	*cr* and *al-2*		
arg + *al nic*	18	24	39·7	28·9	13·60**
+ + *al* +	34	7			
arg cr + *nic*	31	9	*cr* and *nic-1*		
+ + + *nic*	27	13	43·9	34·3	10·10**
arg cr al +	19	20			
arg cr + +	7	51	*al-2* and *nic-1*		
+ + *al nic*	5	50	15·8	11·8	3·59
+ *cr al* +	49	9			
arg + + *nic*	64	7			

* Indicates significant departure from equality for complementary genotypes, probability being less than 0·05.

** $P < 0{\cdot}001$.

genes after transfer were stable and their phenotypic effects were not altered. They were all linked to homologous chromosomes in *N. sitophila*, and there was no ambiguity as to the positions of the genes after transfer.

In view of the similarity in the segregation of *arg-3* in the mating-type chromosomes of the two species, it was thought that this might be due to some factor such as heterozygosity having a suppressing effect in the immediate vicinity of the centromere region of *N. sitophila*. Two experiments were, therefore, designed to see whether the recombination frequency in the interval *arg-3–cr* would alter radically when the two genes are in repulsion and after further backcrossing to *N. sitophila*. The double mutants *arg-3*, *al-2* and *cr*, *nic-1* were selected from tetrads analysed in the first experiment involving the mating-type chromosome of *N. sitophila*. The

cross between the double mutants would then have adjacent markers in repulsion. The multiple mutant, *arg-3*, *cr*, *al-2*, *nic-1*, also isolated from one of the tetrads was further backcrossed four times to *N. sitophila*, thus achieving a total backcrossing of twelve generations for individual markers. The random spore data from these experiments are shown in Table 4.

The region of major interest is that bounded by *arg-3* and *cr*. The amount of recombination between these genes is not significantly different for the repulsion and coupling crosses. The repulsion data also compare reasonably well with the data from tetrad analysis (see Table 1). The difference in the amount of recombina-

Table 5. *Percentage second division segregation of genes in* Neurospora crassa *and* N. sitophila

		No. of asci				Percent B		
		N. crassa		*N. sitophila*				
Genes	Class	A	B	A	B	*N. crassa*	*N. sitophila*	χ^2 (2 × 2)
L.G. I								
mt		247	94	117	220	27·6 ± 2·7	65·2 ± 2·6	96·5**
arg-3		296	42	302	39	12·4 ± 1·7	11·4 ± 1·7	0·2
cr		263	77	134	207	22·6 ± 2·3	60·7 ± 2·6	104·4**
al-2		133	204	100	242	60·5 ± 2·6	70·8 ± 2·4	7·5*
nic-1		121	221	104	238	64·6 ± 2·6	69·6 ± 2·4	1·9
g†		17	33	23	41	66·0 ± 6·7	64·1 ± 6·0	0·5
L.G. VI								
ad-8		82	195	133	138	70·4 ± 2·4	51·0 ± 3·0	59·9**
ylo		248	27	253	18	9·8 ± 1·7	6·6 ± 1·6	1·8
pan-2		250	25	266	5	9·0 ± 1·7	1·8 ± 0·8	13·7**
L.G. VII								
nic-3		186	130	171	147	41·1 ± 2·8	46·2 ± 2·8	1·7
me-7		304	14	302	16	4·4 ± 1·1	5·0 ± 1·2	0·1
arg-10		187	129	138	181	40·8 ± 2·8	56·7 ± 2·7	16·1**

Class A = 1st division segregation asci.
Class B = 2nd division segregation asci.
† Data from Fincham (1951). L.G. = linkage group.
* $P < 0{\cdot}01$. ** $P < 0{\cdot}001$.

tion in the *cr–al-2* interval between the coupling and repulsion crosses in *N. sitophila* (Table 4) might be due to differences in genetic background due to the different programmes of backcrossing to which the parent strains had been subjected. It may be pointed out that random spore data provide a more satisfactory estimate of recombination frequency between genes showing close linkage than when loose linkage is involved. However, the results indicate quite well that the recombination frequency between *arg-3* and *cr* retained its characteristic *N. sitophila* value regardless of the phase of the cross.

When the behaviour of *arg-3* was discovered, fresh transfer experiments were begun with the mutants *lys-4*, *hist-3*, *ad-3B*, and *nic-2* which lie in that order

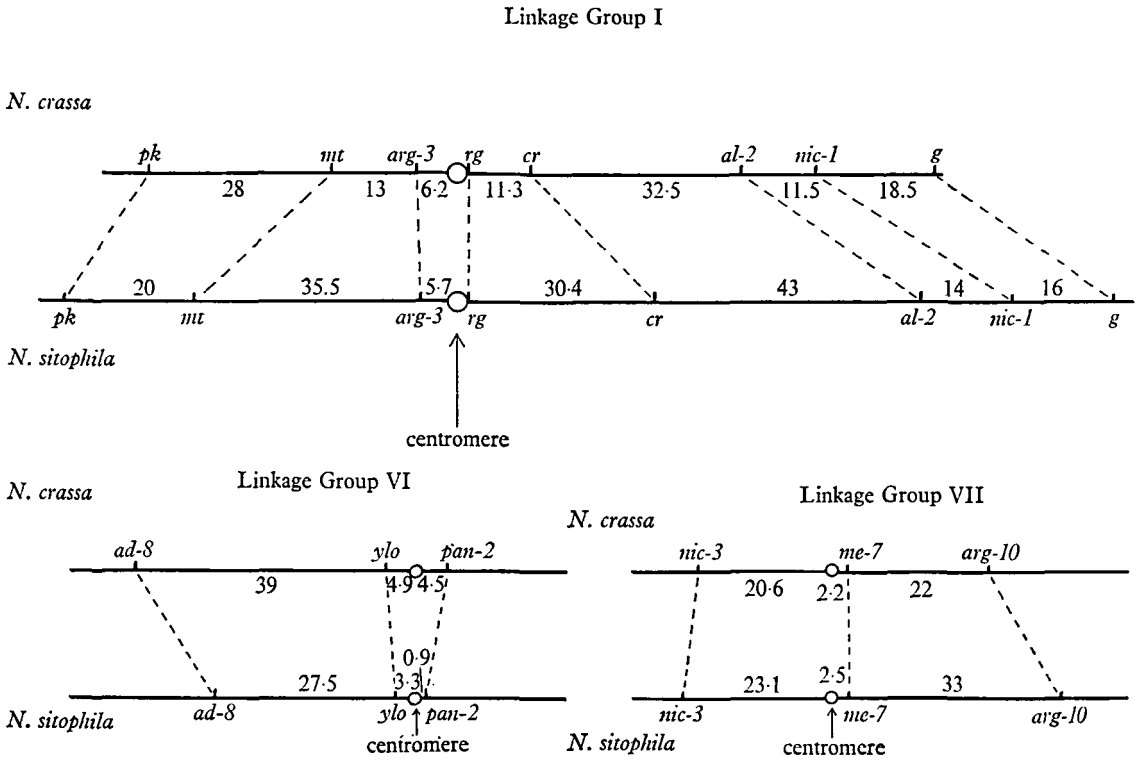

Fig. 1. Maps of linkage groups I (mating-type chromosome), VI and VII of *Neurospora crassa* and *N. sitophila*, based on the frequency of tetratype or second division segregation. From an analysis of over 300 tetrads, the marker ragged, *rg*, has been shown to segregate always at the first division in the two species (Threlkeld, personal communication).

between the centromere and *cr* in the right arm of the mating-type chromosome of *N. crassa*. The map distance between *lys-4* and *cr* varies from 5 to 9 units. This was done with a view to determining whether a region adjacent to the centromere in the right arm of the mating-type chromosome of *N. sitophila* would behave similarly to the *arg-3–centromere* region. All the mutants except *ad-3B* failed to hybridize with *N. sitophila*. The hybrid progeny ascospores from the cross between the mutant *ad-3B* and *N. sitophila* were completely sterile on further backcrossing. Different strains of the four mutants were tried but none showed any better fertility with *N. sitophila*.

The frequencies of second division segregation for marker genes in *N. crassa* and *N. sitophila* are shown in Table 5. The maps of linkage groups I, VI and VII of the two species have been constructed on the basis of second division segregation or tetratype frequency (see Fig. 1). The map distances were corrected by using the tetrad mapping function curve on page 16 of Barratt *et al.* (1954).

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4. DISCUSSION

It is often suggested, and some evidence from *Drosophila* shows, that the centromere controls crossing-over in its vicinity (Beadle, 1932; Mather, 1936, 1938), although Charles (1938) finds no support. In accordance with Mather's ideas, Fincham (1951) suggested that the *Neurospora* interspecific difference lay in the interference properties of the centromere.

However, though Fincham's actual data are confirmed, his theoretical conclusion is probably wrong, in as much as the *arg-3–centromere* interval, the closest of all to the centromere, shows no difference between the two species. The results also invalidate the deductions of Frost (1955), based largely on Mather's hypothesis, that the centric regions of other chromosomes of *N. sitophila* would probably show a similar pattern of high crossing-over frequency to that of the mating-type chromosome. There is certainly a difference in crossover distribution between the two species, which could be under genetic control and perhaps of a similar kind to that inferred from cytological observation in higher plants (Darlington, 1930, 1935).

In the other linkage groups studied there is no clear difference between the two species—the segregation of *pan-2* is variable even within *N. crassa*.

SUMMARY

Mutant genes in linkage groups I (mating-type chromosome), VI and VII have been transferred from *Neurospora crassa* to *N. sitophila* by hybridization and repeated backcrossing. Recombination between these genes has been studied from five-point crosses involving linkage group I and three-point crosses involving linkage groups VI and VII of the two species.

The results show significant differences in the amount of recombination between some of the genes in the proximal regions of the mating-type chromosomes of the two species. They indicate proximal localization of crossovers in the mating-type chromosome of *N. sitophila*. The results also show significant differences in recombination frequency between the genes in linkage group VI and a close similarity in linkage group VII. They further show that the centromere in the two species may not be interfering with crossing-over in its vicinity to such an extent as to be of any evolutionary significance.

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