Genet. Res., Camb. (1972), **19**, *pp.* 115–119
Printed in Great Britain

Interspecific crosses and crossing-over in *Neurospora*

By K. D. NEWCOMBE and S. F. H. THRELKELD
Department of Biology, McMaster University, Hamilton, Ontario, Canada

(*Received* 27 *October* 1971)

SUMMARY

Through a series of backcrosses the centromere region of linkage group I of *Neurospora crassa* was transferred to the *N. sitophila* genome, and through another series of backcrosses the centromere region of linkage group I of *N. sitophila* was transferred to *N. crassa*. Strains thus synthesized showed, in further crosses, that the *N. sitophila* centromere region acts as a dominant enhancer of cross-over frequencies across linkage group I of the two species.

1. INTRODUCTION

Of the various methods of tackling the question of the mechanism of recombination in eukaryotes, one of the more attractive is through the study of genetic variation of recombination frequencies. Some measure of the success of this approach has been demonstrated with fungi. Thus, for example, genetic variation of recombination frequencies has been related to specific genes acting as dominant suppressors, or recessive enhancers of crossing-over, with the effect often restricted to short regions of the genome (e.g. Catcheside & Austin, 1969).

This paper reports on a further inherited variation in recombination frequencies in *Neurospora*, in which an increase in recombination appears as a dominant effect. The work reported is developed from an earlier report by Fincham (1951), which showed that cross-over frequencies on linkage group I of *N. sitophila* were much higher than those over the similar region in *N. crassa*.

It will be shown that the centromere region, or a part of that region, from linkage group I of *N. sitophila* has the effect of increasing recombination on linkage group I of *N. crassa*, when inserted into that linkage group, and that when replaced in *N. sitophila* by the similar region from *N. crassa*, recombination on linkage group I in *N. sitophila* is decreased. It will be further shown that the increase in recombination frequency appears as a dominant effect.

2. MATERIALS AND METHODS

Methods not described below have been described elsewhere (Threlkeld, 1962).

Two sets of strains were constructed from progeny of initial crosses of *N. crassa* × *N. sitophila*. One set was developed from repeated backcrosses of *N. crassa* to *N. sitophila*, and the other set from repeated backcrosses of *N. sitophila* to *N. crassa*. In both sets of backcrosses it was possible to follow the source of the centromere region of linkage group I. The region from *N. crassa* was marked with

https://doi.org/10.1017/S0016672300014336 Published online by Cambridge University Press

Table 1. *Details of synthesized strains*

Isolate	Markers	Ancestry and backcrosses	Presumed centromere	Presumed genetic background (> 95%)
E 509		*N. crassa* × *N. sitophila*	*N. crassa*	*N. crassa**
E 423	*rg cr ylo a*	*N. crassa*	*N. crassa*	*N. crassa*
M 15	*rg cr a*	*N. crassa* × *N. sitophila*[5]	*N. crassa*	*N. sitophila*
M 194	*ylo A*	*N. crassa* × *N. sitophila*[6]	*N. sitophila*	*N. sitophila*
169-7	*rg cr A*	*N. crassa* × *N. sitophila*[6]	*N. crassa*	*N. sitophila*
157-8	*rg a*	*N. crassa* × *N. sitophila*[6]	*N. crassa*	*N. sitophila*
202-1	*ad-5 hist-2 cr a*	*N. crassa*	*N. crassa*	*N. crassa*
P 367	*hist-2 cr a*	*N. crassa*	*N. crassa*	*N. crassa*
P 109	*ad-5*	*N. crassa*	*N. crassa*	*N. crassa*
K 4	*ad-5 cr a*	*N. sitophila* × *N. crassa*[7]	*N. sitophila*	*N. crassa*
B 6	*A*	*N. sitophila* × *N. crassa*[6]	*N. sitophila*	*N. crassa*
B 68	*ad-5 hist-2 cr a*	*N. sitophila* × *N. crassa*[6]	*N. crassa*	*N. crassa*
B 40	*ad-5 hist-2 cr a*	*N. sitophila* × *N. crassa*[6]	*N. crassa*	*N. crassa*
B 38	*A*	*N. sitophila* × *N. crassa*[6]	*N. sitophila*	*N. crassa*
30 JA-5	*A*	*N. sitophila* × *N. crassa*[5]	*N. sitophila*	*N. crassa*
K 110	*A*	*N. sitophila* × *N. crassa*[7]	*N. sitophila*	*N. crassa*
B 56	*cr A*	*N. sitophila* × *N. crassa*[6]	*N. sitophila*	*N. crassa*
B 27	*ad-5 a*	*N. sitophila* × *N. crassa*[6]	*N. sitophila*	*N. crassa*
B 44	*ad-5 hist-2 cr a*	*N. sitophila* × *N. crassa*[6]	*N. crassa*	*N. crassa*
20 JR-16	*A*	*N. sitophila* × *N. crassa*[5]	*N. sitophila*	*N. crassa*

* Based on differences of fertility in *N. crassa* and *N. sitophila* crosses.

hist-2 (C 94) in backcrosses of *N. sitophila* to *N. crassa*, and with *rg-1* (B 53) in backcrosses of *N. crassa* to *N. sitophila*, while in both sets the *N. sitophila* centromere region was unmarked. Our data indicate that both *rg-1* and *hist-2* are very close to the centromere of linkage group I in *N. crassa*; we have found no second-division segregation for *rg-1* in approximately 300 asci, and the frequency of second-division segregation for *hist-2* has been found to be less than 1%. For the purpose of transferring the centromere region from *N. sitophila* to *N. crassa*, following the initial cross of *N. crassa hist-2* × *N. sitophila* (wild type), *hist-2*$^+$ progeny were always backcrossed to *hist-2* (*N. crassa*) strains. To transfer the centromere region from *N. crassa* to *N. sitophila* an initial cross between wild types of the two species was made in the hope of enhancing the productivity of the early crosses of the backcross programme. The backcross programme was then initiated with a cross of *rg-1* (*N. crassa*) strain to an isolate (E 509) of the original cross between the wild types; subsequent crosses were made between *rg-1* progeny and the *N. sitophila* wild-type strain. In order to further minimize the probability that the marker concerned had become separated from its parental chromosome by a cross-over, where possible, progeny were selected from asci in which the centromere marker was seen to segregate at first division.

The wild-type *Neurospora* strains used to initiate the programmes were: *N. sitophila A* (Cambridge strain), *N. sitophila A* (HSSf32APC), *N. crassa* (Lindegran 25*a*), and *N. crassa A* (75-OR23-1A), and the marked strains, all of which were in

https://doi.org/10.1017/S0016672300014336 Published online by Cambridge University Press

N. crassa backgrounds were *ad-5, hist-2, cr, a* (Y152M40, Y152M14, B123); *hist-2A* (C 94); *rg cr* (B 53, B 123); and *ylo–1A* (Y30539y). These strains have been described in more detail by Barratt & Ogata (1970); all the markers are located on linkage group I, except *ylo* which is on linkage group VI. Details of the synthesized strains are listed in Table 1. The last column in the table refers to the presumed genetic background of these strains. Strain E509 obtained from a cross of *N. sitophila* × *N. crassa* is thought to be largely *N. crassa* on the basis of good fertility of E509 × *N. crassa* crosses, and poor fertility of E509 × *N. sitophila* crosses. Except for the pure *N. crassa* strains E423, 202–1, P367 and P109, the presumed genetic background (95 %) for the remainder of the strains is based on the number of backcrosses to the species identified as the background, with selection in each generation for the relevant centromere marker (see the column headed 'Presumed centromere').

The synthesized strains permitted the following types of crosses: with a predominantly *N. sitophila* background, it was possible to make crosses with the linkage group I centromere regions homozygous for *N. crassa*, i.e. CC (*N. sitophila*) crosses, and crosses with the linkage group I centromere regions heterozygous for the two species, i.e. CS (*N. sitophila*). Unfortunately the data from the former of these two types of crosses is sparse because of the poor fertility of *rg* × *rg* crosses. With a predominantly *N. crassa* background, crosses of the following types were studied: CC (*N. crassa*), CS (*N. crassa*), and SS (*N. crassa*); these are respectively, homozygous for *N. crassa* centromere regions (CC), centromere regions heterozygous for the two species (CS), and centromere regions homozygous for *N. sitophila* (SS), all in *N. crassa* genetic backgrounds.

3. RESULTS

Crosses showing comparisons of centromere arrangements in the *N. sitophila* background are summarized in Table 2. Recombination frequencies of the two CS (*N. sitophila*) crosses are not significantly different from each other, and they are clearly much greater than the frequencies for the CC (*N. crassa*) cross. The CC cross with the *N. sitophila* [CC (*N. sitophila*)] background has the *N. crassa*-like recombination frequency, and is not significantly different from the CC (*N. crassa*) cross which is essentially a *N. crassa* cross. The levels of significance for homogeneity (χ^2) are: for (i) CC (*N. crassa*) and CC (*N. sitophila*) $P > 0{\cdot}3$, and for (ii) CC (*N. sitophila* and CS (*N. sitophila*) $P < 0{\cdot}01$. This indicates that the presence of the homozygous (CC) combination of *N. crassa* centromere regions for linkage group I in *N. sitophila* has the effect of reducing crossing-over in the region to the level of that found in *N. crassa*. The presence of one centromere of an *N. sitophila* source nullifies this effect and restores the cross-over frequency to that expected in *N. sitophila* strains (e.g. Fincham, 1951). It is possible that this enhancement of cross-over frequencies may be extended to other linkage groups, as suggested by the increased frequency on linkage group VI, between *ylo* and the centromere; however, this increased frequency is not statistically significant.

https://doi.org/10.1017/S0016672300014336 Published online by Cambridge University Press

Table 2. *Relationships between cross-over frequencies and centromere regions in* N. sitophila *and* N. crassa

Cross	Crossing-over by region (%) I (*mt*–*rg*)	II (*rg*–*cr*)	III (*cr*–*ylo*)	No. of spores or asci
E 423 × E 509 CC (*N. crassa*)	7·9	6·6	2·6	38 asci
M 15 × *N. sitophila* CS (*N. sitophila*)	28·9	39·5	—	19 asci
M 15 × M 194 CS (*N. sitophila*)	29·5	29·5	9·2	49 asci
169-7 × 157-8 CC (*N. sitophila*)	21·6		—	74 spores

Table 3. *Relationship between cross-over frequencies and centromeres in* Neurospora crassa

Cross	Crossing-over by region (%) I (*mt*–*ad-5*)	II (*ad-5*–*hist-2*)	III (*hist-2*–*cr*)	No. of spores
987 × 202-1 CC (*N. crassa*)	11·9 ± 1·5	2·3 ± 0·7	3·8 ± 0·9	469
P 367 × P 109 CC (*N. crassa*)	—	3·2 ± 0·8	4·0 ± 0·9	471
987 × B 44 CC (*N. crassa*)	—	2·0 ± 0·7	7·7 ± 0·5	351
987 × K 4 CS (*N. crassa*)	—	38·7 ± 2·3		444
B 68 × B 6 CS (*N. crassa*)	—	5·2 ± 1·0	33·1 ± 1·5	535
B 40 × B 38 CS (*N. crassa*)	—	8·3 ± 1·4	32·0 ± 2·3	411
30 JA-5 × 202-1 CS (*N. crassa*)	—	9·9 ± 1·5	32·0 ± 2·3	383
20 JR-16 × 202-1 CS (*N. crassa*)	—	9·6 ± 0·5	24·3 ± 2·4	333
K 4 × K 110 SS (*N. crassa*)	—	37·0 ± 2·5		357
B 56 × B 27 SS (*N. crassa*)	32·1 ± 2·5	37·1 ± 2·6		340

Data from the crosses with the *N. crassa* background are more extensive; they are summarized in Table 3. Recombination frequencies are low for the CC crosses and significantly higher for the CS and SS crosses ($P < 0{\cdot}001$). No significant differences are present between the CS and SS crosses, and, where it is possible to make comparisons, the crosses do not differ significantly from the CS crosses in Table 2, i.e. those with the *N. sitophila* background.

The two CC crosses in Table 3 do not differ significantly from each other, and neither are they significantly different from the CC crosses described in Table 2.

In both sets of crosses the frequencies of double cross-overs were insufficient to provide evidence of any strong patterns of chromosome or chromatid interference.

4. DISCUSSION

It should be noted that isolates B 68, B 40 and B 44 used in crosses (B 68 × B 6), (B 40 × B 38) and (987 × B 44) respectively, although presumed to possess the *N. crassa* centromere region as well as the *N. crassa* background, were all derived from an initial cross involving *N. sitophila*. The fact that they behave as predicted reflects the success of the backcross programmes. In no case were strains isolated

https://doi.org/10.1017/S0016672300014336 Published online by Cambridge University Press

that did not behave as predicted; thus the data presented clearly demonstrate that the *N. sitophila* centromere region of linkage group I is largely responsible for the relatively high crossing-over within linkage group I of that species, and that this region is capable of enhancing crossing-over to the same degree in *N. crassa*.

In considering some mechanism that would explain the effects described, thought must be given to (i) chromosomal aberrations, (ii) a recombination gene, or gene complex, and (iii) chromosome pairing. Chromosomal aberrations are unlikely to afford the basis of an explanation on two counts. The number of spores isolated that failed to germinate and the number of hyaline spores were low; chromosomal aberrations such as inversions should give lowered recombination frequencies in the heterozygous crosses (CS), with higher frequencies appearing in both the homozygous crosses.

Further work seeking the existence of a specific locus associated with the phenomena is under way, both from the standpoint of mapping and of examining the possible effect of a gene product in crosses with heterokaryons. An interesting speculation is the possible existence of a protein similar to that described by Alberts & Frey (1970). The possibility of chromosome pairing, perhaps through the activity of centric heterochromatin, is also open to investigation.

This study was made possible through a grant from the National Research Council of Canada.

REFERENCES

Alberts, B. M. & Frey, L. (1970). T 4 bacteriophage gene 32: a structural protein in the replication and recombination of DNA. *Nature* **227**, 1313–1318.

Barratt, R. W. & Ogata, W. N. (1970). Neurospora stock list. Fifth revision. *Neurospora Newsletter* **16**, 23–123.

Catcheside, D. G. & Austin, B. (1969). The control of allelic recombination at histidine loci in *Neurospora crassa*. *American Journal of Botany* **56**, 685–690.

Fincham, J. R. S. (1951). A comparative study of the mating type chromosome of two species of Neurospora. *Journal of Genetics* **50**, 221–229.

Threlkeld, S. F. H. (1962). Some asci with non-identical sister spores from a cross in *Neurospora crassa*. *Genetics* **47**, 1187–1198.

https://doi.org/10.1017/S0016672300014336 Published online by Cambridge University Press