NEW DATA ON LINKAGE GROUP III MARKERS IN *NEUROSPORA CRASSA*

By JANET E. CRAWFORD*

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Summary

Linkage data on eight loci in linkage group III of *Neurospora crassa* have been obtained by analysing random segregants from two- and three-point crosses. The order of the loci is *me-8*, *ad-4*, *leu-1*, *his-7*, *thi-2*, *ad-2*, *try-1*, *ro-2*. The order of the closely linked loci, *his-7*, *thi-2*, *ad-2*, and *try-1* was previously unknown.

I. INTRODUCTION

This paper presents linkage data obtained from two- and three-point crosses with eight markers in linkage group III of *Neurospora crassa*. Many of the data were obtained as a preliminary to the fine structure mapping of the *tryptophan-1* locus. Proximal and distal flanking markers were required for the mapping and since the order of the loci in the region of *try-1* was not firmly established (Barratt *et al.* 1954; Perkins and Ishitani 1959; Perkins, Glassey, and Bloom 1962), experiments were carried out to determine the correct order. The group III loci mapped are listed in Table 1.

II. MATERIALS AND METHODS

Crosses were made at 25°C on slopes of crossing medium (Westergaard and Mitchell 1947) containing folded strips of filter paper and supplemented with the requirements of the female parent. The female parent was grown for 5 days to allow the production of protoperithecia and then fertilized with a dense conidial suspension of the second parent. The perithecia began shedding ascospores after 7–14 days but ascospores were not analysed until 3 weeks after fertilization to allow for any differential ripening of perithecia.

The ascospores were collected from the walls of the tubes with a platinum loop of sterile distilled water. They were germinated by heat treatment (50 min at 56°C) on plates of SGF medium supplemented with the growth requirements of both parents. SGF medium is Vogel's medium (Vogel 1955) in which the 2% sucrose is replaced by 0·5% sorbose, 0·0125% glucose, and 0·025% fructose. Sorbose is used to produce compact colonies (Tatum, Barratt, and Cutter 1949). The combination of the three sugars overcomes the reduced germination produced by sorbose plus either glucose or sucrose (de Serres, Kølmark, and Brockman 1962).

After 12–15 hr growth, a random sample of germinated ascospores was isolated and grown for 24 hr on SS medium supplemented with the growth requirements of both parents. SS medium is Vogel's medium (Vogel 1955) in which the 2% sucrose is replaced by 0·5% sorbose and 0·1% sucrose. The sorbose–sucrose combination gives less restricted growth than does the sorbose–glucose–fructose combination.

* Department of Genetics, John Curtin School of Medical Research, Australian National University, Canberra.

The colonies were classified by testing for growth on doubly or singly supplemented SS plates, depending on the number of nutritional markers in the cross. In three-point crosses involving *ropy-2*, the germinated ascospores were grown on doubly supplemented slopes of Vogel's medium (Vogel 1955), scored for *ro-2*, then tested on singly supplemented SS plates.

Thiamine-2 could not be scored on plates because conidia and mycelium contain sufficient thiamine to support growth of *thi-2* mutants in the absence of exogenous thiamine. This problem was overcome by growing the germinated ascospores on slopes lacking thiamine and testing the conidia formed on a further set of slopes without added thiamine.

TABLE 1

LOCI OF LINKAGE GROUP III

Locus Symbol and Name	Mutant Isolation Number	References*
ad-2 (adenine-2)	Y83–M32	Beadle and Tatum (1945), Barratt *et al.* (1954), Perkins and Ishitani (1959)
ad-4 (adenine-4)	Y112–M16	Beadle and Tatum (1945), Barratt *et al.* (1954), Giles, Partridge, and Nelson (1957), Perkins and Ishitani (1959), Perkins and Murray (1963)
his-7 (histidine-7)	K520	Catcheside (1960), Webber and Case (1960)
leu-1 (leucine-1)	33757	Beadle and Tatum (1945), Barratt *et al.* (1954), Giles, Partridge, and Nelson (1957), Perkins and Ishitani (1959), Perkins, Glassey, and Bloom (1962), Perkins and Murray (1963), Ahmad *et al.* (1964)
me-8 (methionine-8)	K66	Murray (1960), Perkins and Murray (1963)
ro-2 (ropy-2)	B20	Perkins (1959), Perkins and Ishitani (1959), Perkins, Glassey, and Bloom (1962)
thi-2 (thiamine-2)	9185	Beadle and Tatum (1945), Barratt *et al.* (1954), Perkins and Ishitani (1959), Perkins, Glassey, and Bloom (1962)
try-1 (tryptophan-1)	A9–10575	Beadle and Tatum (1945), Barratt *et al.* (1954), Perkins and Ishitani (1959), Ahmad and Catcheside (1960), Catcheside (1960), Perkins, Glassey, and Bloom (1962), Perkins and Murray (1963), Ahmad *et al.* (1964).

* The references cited include only those describing the original isolation of the mutants and those relevant to the subject of linkage in group III.

Growth factors were added at the following concentrations: thiamine, 1 mg/100 ml; L-histidine hydrochloride, 50 mg/100 ml; L-leucine, 20 mg/100 ml; L-methionine, 50 mg/100 ml; L-tryptophan, 40 mg/100 ml; adenine, 40 mg/100 ml.

III. Results and Discussion

Data from two- and three-point crosses are presented in Table 2 and the results are summarized in the form of a linkage map in Figure 1. Figure 1 contains only the values for percentage recombination between adjacent loci.

TABLE 2

TWO- AND THREE-POINT CROSSES WITH MARKERS IN GROUP III

The numbers of ascopores analysed and the classes to which they belong are given for each cross. The left-hand number of each pair of complementary classes represents the genotype containing the wild-type allele of the leftmost marker. Region 1 is between the left-hand and middle markers and region 2 is between the middle and right-hand markers. The mutant isolation numbers correspond to the loci in a cross, read from left to right. The order in which the crosses are listed corresponds to the order of the left-hand markers on the linkage map

Zygote Genotype and Recombination Percentage	Recombination								Total	Mutant Isolation Numbers
	Parental Combinations		Singles Region 1		Singles Region 2		Doubles Regions 1 and 2			
+ *ad-4* *leu-1* *me-8* + + 3·9 2·9	111	151	9	2	4	4	0	0	281	K66 Y112–M16 33757
+ *leu-1* + *ad-4* + *try-1* 4·9 28·0	63	34	3	4	22	17	0	0	143	Y112–M16 33757 10575
+ *his-7* *try-1* *ad-4* + + 19·6 5·1	54	50	17	10	4	3	0	0	138	Y112–M16 K520 10575
+ *ad-2* *leu-1* + 17·3	52	38	13	6	—	—	—	—	109	33757 Y83–M32
+ + *try-1* *leu-1* *ad-2* + 6·3 3·7	53	76	2	7	2	3	0	0	143	33757 Y83–M32 A9
+ *thi-2* + *his-7* + *ad-2* 1·5 1·5	60	70	1	1	0	2	0	0	134	K520 9185 Y83–M32
+ *thi-2* + *his-7* + *try-1* 2·1 3·2	140	130	2	4	6	3	0	0	285	K520 9185 10575
+ *ad-2* + *his-7* + *try-1* 3·1 3·8	81	41	3	1	4	1	0	0	131	K520 Y83–M32 10575
+ *try-1* *his-7* + 4·3	64	71	1	5	—	—	—	—	141	K520 A9
+ + *ro-2* *his-7* *try-1* + 4·4 7·2	63	59	4	2	3	7	0	0	138	K520 10575 B20
+ *ad-2* *try-1* *thi-2* + + 4·2 4·2	67	65	5	1	4	2	0	0	144	9185 Y83–M32 10575

Errors in establishing gene orders due to variations in crossing over frequency were avoided by using three-point rather than two-point crosses. By analysing a series of overlapping three-point crosses, genes can be ordered unambiguously.

The order *me-8 ad-4 leu-1* agreed with that obtained by Perkins and Murray (1963). The data verified the sequence *ad-4 leu-1 try-1 ro-2* obtained by Perkins and Ishitani (1959). The latter suggested the order *try-1 thi-2 ad-2* but expressed doubts since the order of *thi-2* and *ad-2* with respect to *try-1* was based on a single isolate in each case. The data of Perkins, Glassey, and Bloom (1962) supported the order *ad-2* (*thi-2*) *try-1*, no recombinants being obtained between *ad-2* and *thi-2* and a total of five in three crosses, between *ad-2* and *try-1*. The present data are more extensive and make it highly probable that the order is *thi-2 ad-2 try-1*.

His-7 was found to belong to linkage group III by Catcheside (1960) and by Webber and Case (1960) and to be closely linked to *try-1*. They were unable to place it proximally or distally because two-point crosses were used. The present work places it proximal to *thi-2*.

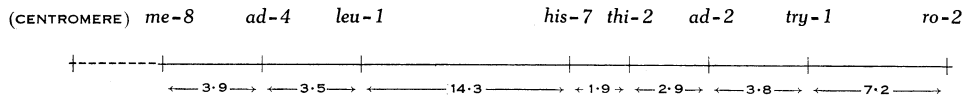

Fig. 1.—Partial map of linkage group III. The values below the line are the weighted mean recombination percentages between adjacent loci. The map is based on data from the present paper only. Only direct estimates of the recombination percentages between adjacent loci were used in calculating these values except for the *leu-1 his-7* interval. This value was obtained from four indirect estimates using values for longer intervals.

The data agree with that of Perkins (1959) in that there was no negative interference. This is shown by the absence of double crossovers. The numbers of ascospores classified in each cross are too small for any decision to be made on the presence or absence of positive interference.

The present data show the same variability of crossing over between pairs of genes as was found in earlier work (Perkins 1959; Perkins and Ishitani 1959; Perkins, Glassey, and Bloom 1962). This is almost certainly due to the diverse genetic backgrounds of the mutants rather than to sampling errors. Even in stocks derived from the same wild type, genes are known which affect the frequency of intergenic recombination, e.g. *rec-2*, which, when homozygous, doubles the frequency of recombination between *pyrimidine-3* and *leucine-2* in linkage group IV (Smith 1966).

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V. References

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