LINKAGE DATA FOR GROUP V MARKERS IN NEUROSPORA[1]

WALTER N. STRICKLAND, DAVID D. PERKINS AND CATHERINE C. VEATCH

Department of Biological Sciences, Stanford University, Stanford, California

Received April 24, 1959

THIS paper presents linkage data from crosses with markers in linkage group V of *Neurospora crassa* (Group E of HOULAHAN, BEADLE and CALHOUN 1949). Several new markers are reported, and the map relations of a number of other genes are clarified. Many of these data were obtained in the course of an investigation of interference (STRICKLAND unpublished). Certain of the new markers are in positions where they should be useful for bracketing regions of special interest (e.g., spray, which was first mapped in the present study, has already been employed in investigations of recombination at the complex *am* locus (PATEMAN 1958)). A total of at least 17 gene loci are now known to belong to group V. These are listed, with references, in Table 1.

TABLE 1

Loci of linkage group V

Symbol and name*	Standard mutant	References†
ad-7 : adenine-7	44411	B, MITCHELL 1958
am : amination deficient	47305	BARRATT unpublished; FINCHAM 1951, 1959; FINCHAM *et al.*, 1957a,b
asp : asparagine	S1007	TANENBAUM *et al.*, 1954; FREESE 1957a; DUBES 1953
bis : biscuit	B6	PERKINS 1959
G : Gulliver	....	REISSIG 1958
hist-1 : histidine-1	C84	B, GILES 1956; FREESE 1957a,b
i : enhancer of *am*	....	FINCHAM *et al.*, 1957b
inos : inositol	37401	B, GILES 1956; GILES *et al.*, 1953; FREESE 1957a; PITTENGER 1954; LESTER *et al.*, 1959
iv-1 : isoleucine-valine-1	16117	B, GILES 1956; PITTENGER 1954
iv-2 : isoleucine-valine-2	39709	B, PITTENGER 1954; FREESE 1957b
lys-1 : lysine-1	33933	B
lys-2 : lysine-2	37101	B, GOOD 1952
me-3 : methionine-3	36104	B, FREESE 1957a
pab-1 : para-aminobenzoic-1	1633	B, GILES 1956; FREESE 1957a; DRAKE 1956
pab-2 : para-aminobenzoic-2	H193	B, DRAKE 1956
pab-3 : para-aminobenzoic-3	71301	(Not a separate locus from *pab-2*. DRAKE 1956)
pl : plug	B118	PERKINS 1959
sp : spray	B132	PATEMAN 1958; PERKINS 1959
val : valine	45201	PERKINS 1959
wl : woolly	B66	PERKINS 1959

* Mutants of doubtful value as markers, and mutants that are probable alleles at already established loci are indented.
† B: Documented in BARRATT *et al.*, 1954 compilation. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.

[1] Supported by research grants from the National Science Foundation (3840), and from the National Institute of Allergy and Infectious Diseases, Public Health Service (E1462).

MATERIALS AND METHODS

Experimental materials and methods were as described by PERKINS (1959). The morphological mutants *bis*: biscuit (B6), *pl*: plug (B118), *sp*: spray (B132), and *wl*: woolly (B66) originated in experiments of DR. V. W. WOODWARD (see PERKINS 1959,Table 1). Scoring of *am* is cleaner at 25°C than at higher temperatures. Patterns of defective ascospores suggest that *wl* (B66) may be associated with a chromosomal aberration. An unlinked aberration may also have been present in some early crosses involving *asp*(S1007).

EXPERIMENTAL RESULTS

Data from 2- and 3-point crosses are presented in Table 2, and from 4- and 5-point crosses in Table 3. The results are summarized in the form of a map in Figure 1.

Gene order: These data provide new information on a number of gene sequences: *sp* is located between *iv-1* and *am*, *bis* between *me-3* and *pab-2*, *me-3* between *pab-1* and *bis*, *val* at or near *iv-1*, *lys-2* rather far left of *inos*, and *ad-7* some distance right of *bis*. *asp*(S1007) and *pl* are close together far out in the right arm.

Since the 1954 compilation of BARRATT, NEWMEYER, PERKINS and GARNJOBST,

TABLE 2

Two- and three-point crosses with group V markers

Zygote genotype and recombination percent	Parental combinations		Recombinations: Singles region 1		Singles region 2		Doubles regions 1 and 2		Total and percent germination	Marker isolation numbers
+ *b* *c* / *a* + +	+*bc*	*a*++	+++	*abc*	+*b*+	*a*+*c*	++*c*	*ab*+	...	(a) (b) (c)
+ + *pl* / *lys-1* *me-3* + (37.3, 37.3)	19	..	13	..	13	..	6 C=0.8	..	51 (*lys*$^+$ only) 94%	33933 36104 B118
+ + *am* / *iv-1* *sp* + (19.6, 4.3)	70	..	18	..	4	..	0	..	92^V (*iv*$^+$ only) 88%	16117 B132 47305
+ *sp* + / *iv-1* + *inos* (13.0, 17.4)	9	23	1	5	5	3	0	0	52^V 88%	16117 B132 37401
+ *val* / *iv-1* + (0)	..	..	0	..	..	..	..	..	73*^{P} 90%	16117 45201
+ *sp* *inos* / *val* + + (19.0, 9.5)	41	42	12	10	5	6	0	0	116^P 97%	45201 B132 37401

+ *am* *inos* *sp* + + 3.5 7.1	39	37	2	1	3	3	0 0	85[V] 85%	B132 47305 37401
+ *me-3* *asp* *sp* + + 14.3 30.2	24	45	7	7	19	14	2 1 C=0.6	119[V] 75%	B132 36104 S1007
+ + *bis* *hist-1* *inos* + 7.8 4.5	113	125	10	9	8	2	1 1 C=2.1 (>0.26)	269 83%	C84 37401 B6
+ *me-3* *bis* *hist-1* + + 7.6 1.4	58	74	4	7	1	1	0 0	145 48%	C84 36104 B6
+ + *pab-2* *hist-1* *bis* + 7.8 9.7	47	39	3	4	5	4	0 1 C=1.3 (>0.03)	103 31%	C84 B6 H193
+ + *pab-2* *hist-1* *bis* + 1.7 9.5	58	45	1	1	8	3	0 0	116 72%	C84 B6 H193
+ + *bis* *inos* *pab-1* + 1.1 4.0	90	77	1	1	5	2	0 0	176[P] 88%	37401 1633 B6
+ + + *inos* *pab-1* *bis* 1.8 3.7	241	239	3	5	11	7	0 1 C=3.0 (>0.08)	507 94%	37401 1633 B6
+ (+) *wl* *inos* (*pab-1*) + 9.7	55	57	5	7	..	..	.. ..	124[V] 83%	37401 (1633) B66
+ *pab-2* + *me-3* + *asp* 13.9 0	29	39	8	3	0	0	0 0	79[P] 88%	36104 H193 S1007
+ + *pl* *me-3* *asp* + 24.4 0.6	55	62	16	22	1	0	0 0	156[V] 90%	36104 S1007 B118
+ *pl* *me-3* + 23.3	12	11	4	3	..	..	.. ..	30[P] 100%	36104 B118
+ *pl* *asp* + 0	29	25	0	0	..	..	.. ..	54[P] 90%	S1007 B118
+ + *asp* *pl* 3.3	18	11	0	1	..	..	.. ..	30[P] 100%	S1007 B118

Numbers of progeny are given in the body of the table. Progeny genotypes are not designated explicitly, but the genotype of each class can be determined from the order of presentation. The left-hand number of each pair of complementary classes represents the genotype that contains the plus allele of the leftmost marker. Alleles shown in parentheses in the genotype column were not scored or recorded, nor were classes of segregants where dashes replace numbers. Regions are numbered from left to right, and isolation numbers in the last column are listed in the same order. Crosses are tabulated in a sequence corresponding to the position of their leftmost markers in the linkage group. Where gene order is uncertain, one arbitrarily chosen order has been used consistently. C=coincidence. When C exceeds one, the minimum coincidence is also given from which the observed value does not deviate significantly (2.5 percent one-sided level).

* Germinating ascospores scored on minimal plate.

[P] Data of PERKINS.

[V] Data of VEATCH, from Cox 1956.

TABLE 3

Four- and five-point crosses. Conventions as in Table 2. Coincidence values are based on pairs of crossovers both in double and in triple recombination classes

Zygote genotype, recombination percent, and marker isolation numbers	Parental combinations		Recombinations: Singles Region 1		Singles Region 2		Singles Region 3		Singles Region 4		Doubles (Regions in parentheses)				Triples	Total and percent germination
+ *inos* *pab-1* *bis* / *lys-2* + + +	77	88	18	22	5	1	3	1	..	..	0 2 (1&2) C=1.3 (>0.15)	0 1 (1&3)	.. ..		0	218
19.7 3.7 2.3																73%
37101, 37401, 1633, B6																
+ *inos* *pab-1* *asp* / *hist-1* + + +	36	34	3	6	3	0	4	4	..	..	0	.. ..	.. ..		0	90
10.0 3.3 8.9																90%
C84, 37401, 1633, C123																
+ *inos* *pab-1* *bis* + / *hist-1* + + + *ad-7*	99	118	2	3	1	2	1	2	9	13	0 1 (1&4)	.. ..	.. ..		0	251
2.4 1.2 1.2 9.2																85%
C84, 37401, 1633, B6, 44411																
+ *inos* *pab-1* *bis* + / *hist-1* + + + *asp*	39	43	4	4	3	3	8	3	7	7	0 1 (1&2) C=1.2 (>0.03)	2 1 (2&3) C=2.9 (>0.72)	1 0 (2&4)	0 1 (2,3,4)		127
7.1 9.5 11.8 11.8																64%
C84, 37401, 1633, B6, C123																
+ *inos* *pab-2* + / *hist-1* + + *asp*	13	29	2	4	6	8	7	3	..	..	1 0 (1&2) C=0.6	0 1 (1&3)	.. ..		0	74
10.8 20.3 14.9																25%
C84, 37401, H193, C123																
+ + *me-3* *bis* / *inos* *pab-1* + +	114	110	0	1	2	3	1	1	..	..	0	.. ..	.. ..		0	232
0.4 2.2 0.8																77%
37401, 1633, 36104, B6																
+ + *me-3* *bis* / *inos* *pab-1* + +	..	..	..	..	..	..	..	19	..	..	.. .. (1&2)	0 .. (1&3)	0 .. (2&3)	.. 0 (1,2,3)		19 (*me*⁺ *bis*⁻ only)
37401, 1633, 36104, B6																
+ + + *asp* / *inos* *pab-1* *bis* +	40	26	3	1	1	4	1	0	..	..	0	.. ..	.. ..		0	76
5.3 6.6 1.3																76%
37401, 1633, B6, C123																
+ + + + / *inos* *pab-1* *bis* *asp*	42	27	0	1	0	2	12	11	..	..	0 1 (1&2) C=12.1 (>0.31)	0 1 (2&3) C=1.0	.. ..		0	97
2.1 4.2 24.8																97%
37401, 1633, B6, S1007																
+ + + *asp* / *inos* *pab-1* *bis* +	44	51	0	2	3	0	14	18	..	..	2 0 (1&3)	.. ..	.. ..		0	134
3.0 2.2 23.4																67%
37401, 1633, B6, S1007																

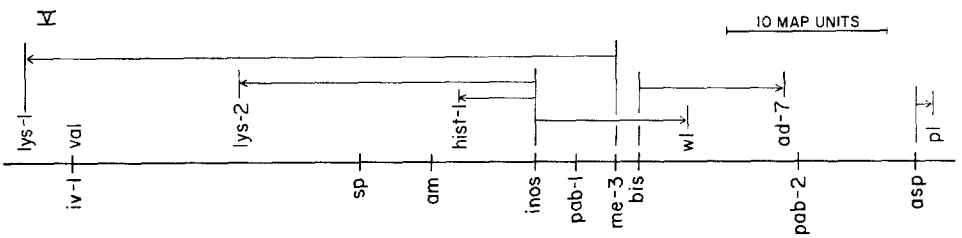

FIGURE 1.—Partial map of group V, summarizing the data from Tables 2 and 3. The sequence of loci written below the heavy line is based directly on 3-point data for all the genes involved. Above the heavy line, only the relationships indicated by arrows are based on 3-point data. Each arrow originates from the medial marker in the 3-point cross, and leads to a marker that was nonmedial. Interval lengths are imprecise owing to the variability of crossing over. The map is based solely on data from the present paper, except that the position shown for genes above the line may reflect the results of other workers in cases where our own data would require order to be decided arbitrarily or to be based only on quantitative 2-point data from different crosses. See text for sequences established by other workers. The centromere is located left of *iv-1*.

a number of sequences in group V have been reported or confirmed by other workers: centromere 10 *iv-1* 10 *hist-1* 7 *inos* 3 *pab-1* (GILES 1956); *iv-2 hist-1* 5.5 *inos* 0.9 *pab-1* 0.9 *me-3* 14.5 *asp*(1A7) (FREESE 1957a, b); centromere *sp am hist-1 inos pab-1* (BARRATT personal communication, 1956); *sp am inos* (PATEMAN 1958). Data in the present paper are consistent with these results.

The chief remaining uncertainties regarding gene order concern *lys-2* with respect to markers proximal to *inos*; *ad-7* and *wl* with respect to markers distal from *bis*; and *asp* and *pl* with respect to one another. The seriation shown for *pl* and *asp* depends on a single isolate.

All markers so far mapped in group V are in the same chromosome arm. (A possible exception is *lys-1* which in any case is close to the centromere.) Such an apparently non random distribution of genes might be due to the fact that distal markers such as *inos* have frequently been used for linkage tests.

Linkage of *lys-2* (37101) in V was first detected by GOOD (1952). *am* was first mapped by DR. R. W. BARRATT (personal communication).

Status of new mutants: 45201 is the first valine mutant to be mapped. (The first two digits are probably correct, but not completely certain.) No recombinants were observed in a cross between *val* and *iv-1* (16117), which requires both isoleucine and valine. *asp*(C123) and *asp*(S1007) show dissimilar crossover frequencies in several crosses. Direct evidence regarding allelism is not available. C123 is allelic with two other asparagine mutants, 1A7 and 67603 (DUBES 1953).

General aspects of the results reported here have been discussed by PERKINS (1959) in conjunction with comparable data from other linkage groups.

SUMMARY

Multiple-point linkage data have been obtained on 15 loci, including five that are new, in group V of *Neurospora crassa*, using random segregants obtained by nonselective methods. All loci are in the same chromosome arm, with the possible exception of one which is close to the centromere. Chiasma interference is positive.

ACKNOWLEDGMENTS

We are indebted to DR. E. L. TATUM, DR. R. W. BARRATT, MRS. MARY B. MITCHELL, and DR. V. W. WOODWARD for providing stocks.

LITERATURE CITED

BARRATT, R. W., D. NEWMEYER, D. D. PERKINS, and L. GARNJOBST, 1954 Map construction in *Neurospora crassa*. Advances in Genet. **6**: 1–93.

COX, C. A., 1956 Map locations of some group V mutants in Neurospora. M.A. Thesis, Stanford University.

DRAKE, B., 1956 Evidence for two loci governing para-aminobenzoic acid synthesis in *Neurospora crassa*. Genetics **41**: 640.

DUBES, G. R., 1953 Investigations of some "unknown" mutants of *Neurospora crassa*. Ph.D. Thesis, Calif. Inst. of Technology.

FINCHAM, J. R. S., 1951 The occurrence of glutamic dehydrogenase in Neurospora and its apparent absence in certain mutant strains. J. Gen. Microbiol. **5**: 793–806.

1959 The role of chromosomal loci in enzyme formation. Proc. 10th Intern. Congr. Genet. **1**: 355–363.

FINCHAM, J. R. S., and J. A. PATEMAN, 1957a Formation of enzyme through complementary action of mutant 'alleles' in separate nuclei in a heterocaryon. Nature **179**: 741–742.

1957b A new allele at the *am* locus in *Neurospora crassa*. J. Genet. **55**: 456–466.

FREESE, E., 1957a Über die Feinstruktur des Genoms in Bereich eines Pab Locus von *Neurospora crassa*. Z. Ind. Abst. Vererb. **88**: 388–406.

1957b The correlation effect for a histidine locus of *Neurospora crassa*. Genetics **42**: 671–684.

GILES, N. H., 1956 Forward and back mutation at specific loci in Neurospora. Brookhaven Symposia Biol. **8**: 103–125.

GILES, N. H., and C. W. H. PARTRIDGE, 1953 The effect of a suppressor on allelic inositolless mutants in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **39**: 479–488.

GOOD, N., 1952 Lysine metabolism in Neurospora. Ph.D. thesis, Calif. Inst. Technology.

HOULAHAN, M. B., G. W. BEADLE, and H. G. CALHOUN, 1949 Linkage studies with biochemical mutants of *Neurospora crassa*. Genetics **34**: 493–507.

LESTER, H. E., and S. R. GROSS, 1959 Efficient method for selection of auxotrophic mutants of Neurospora. Science **129**: 572.

MITCHELL, M. B., 1958 Genetic recombination in Neurospora. Genetics **43**: 799–813.

PATEMAN, J. A., 1958 Aberrant recombination at the *am* locus in *Neurospora crassa*. Nature **181**: 1605–1606.

PERKINS, D. D., 1959 New markers and multiple point linkage data in Neurospora. Genetics **44**: 1185–1208.

PITTENGER, T. H., 1954 The general incidence of pseudowild types in *Neurospora crassa*. Genetics **39**: 326–342.

REISSIG, J. L., 1958 A marker for chromosome V of Neurospora. Microbial Genet. Bull. **16**: 21. (Cited by permission.)

TANENBAUM, S. W., L. GARNJOBST, and E. L. TATUM, 1954 A mutant of Neurospora requiring asparagine for growth. Am. J. Botany **41**: 484–488.