LINKAGE RELATIONS OF NEW MORPHOLOGICAL MUTANTS IN LINKAGE GROUP V OF *NEUROSPORA CRASSA*

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THIS paper presents detailed genetic information on 18 morphological mutant strains of *Neurospora crassa*, linked to markers in group V, and listed by GARNJOBST and TATUM (1967). The selection of this group for further analysis is based primarily on the presence of well spaced biochemical markers in the right arm of the chromosome, and on the fact that five of the six morphological classes described by GARNJOBST and TATUM (1967) are represented. The data indicate the sequence of 11 new loci and their approximate map locations.

A total of nine morphological mutants have been previously placed in this group (STRICKLAND, PERKINS, and VEATCH 1959; PERKINS, GLASSEY, and BLOOM 1962; DURKEE, SUSSMAN, and LOWRY 1966). Of these nine, four, spray, *sp* (B132), ropy-4, *ro-4* (B38), biscuit *bis* (B6), and washed, *wa* (R2359), are included in the present study.

MATERIALS AND METHODS

Strains: With few exceptions, the following nutritional marker strains were used as the protoperithecial parents in crosses: *iv-1* (requires isoleucine and valine), isolation number 16117; *inos* (inositol), 89601; *me-3* (methionine), 36104; and *asp* (asparagine), S1007.

To insure some degree of isogenicity, the marker strains were backcrossed to the wild type (RL3-8*A* or RL21*a*) at least three times. The morphological mutant strains were also crossed to these wild types, and reisolates were selected which showed a 1:1 segregation of mutant to wild type (GARNJOBST and TATUM 1967).

Crossing and maintenance: The general methods of crossing and maintenance of cultures used were as described by GARNJOBST and TATUM (1967).

Ascospore isolations: Ordered ascospore isolations were used in most crosses so that centromere distances could be calculated. The usual procedure was followed except for the method of removing intact asci from a perithecium. It was found that a short period of drying of perithecia would induce expulsion of intact asci thus eliminating the necessity of their separation from the jelly. Eleven to 13 days after their formation, several perithecia were placed on a dry 4.5% agar block and left uncovered for a period of 30 to 60 minutes. As they began to dry, each perithecium was squeezed gently with jewelers' forceps whereby groups of asci were expelled onto the surface of the agar block. In most cases, 20 to 24 asci were isolated from 8 to 10 perithecia.

Random isolations from some crosses provided additional recombination data, particularly on areas which were found to be more than 40 map units from the centromere.

Linkage: Assignment of the morphological mutants to linkage group V was based on their recombination with the markers *inos* or *me-3*. In additional crosses, the intervals between mutant and marker loci ranged from 10 to 20 map units to place the mutant genes in a particular region of the linkage group. The mutant—marker intervals in the final crosses ranged from 2 to 10 map

units (Table 1). This eliminated as nearly as possible the occurrence of undetected multiple exchanges, thus making corrections unnecessary.

Tests for allelism: Both crosses and tests for complementation between mutants were used whenever possible to establish the separate identity of closely linked loci. Although the results from crosses between mutants are considered most critical in settling the question of allelism, this criterion could not always be applied because of low germination or infertility of crosses.

All heterocaryon tests were carried out using Petri plates as described by GARNJOBST and TATUM (1967). The results were judged to be positive for complementation when wild-type growth was obtained. All strains were tested against stocks of *inos* (37401) and *al-2*; *nic-3* (15300; Y31881), both of the heterocaryon genotype *C,D;E*, before being tested with each other. While positive tests might indicate interallelic complementation, this was considered unlikely when the phenotypes of the strains were clearly different.

RESULTS

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

In all crosses represented in Table 1, the percent germination was calculated from the total number of spores isolated. In most instances, all four pairs of ascospores from each ascus were represented. Asci with both members of a pair missing were not used in calculations unless the identity of the missing pair of spores could be deduced without question from the remainder of the ascus. Although reversion was not a major problem, it was found that some mutant strains

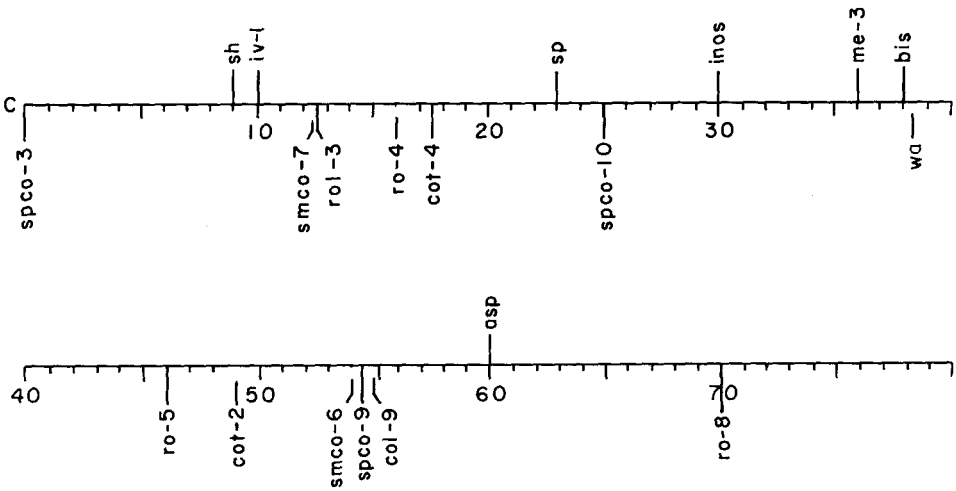

FIGURE 1.—Partial map of linkage group V, summarizing the data from the present paper. The centromere is indicated by the letter C. The symbols written above the line represent gene loci which were positioned by other workers. With two exceptions (*ro-4* and *wa*) the symbols written below the line represent previously unmapped loci. In every instance, all available data from 2- and 3-point crosses were taken into consideration in assigning locus order. Map positions were based when possible on 3-point crosses in which all distances between the three loci were consistent, or alternatively, were based on recombination data with the nearest marker. When locus order is uncertain, the symbol of the best located locus only is attached to the map line.

TABLE 1

Results of ordered ascus isolations from two-, three- and four-point crosses of morphological mutants with group V biochemical markers

Zygote genotype and percent recombination	Parental strands	Recombinations: crossover strands recovered in Regions I-IV									Total asci; percent germination	Marker isolation numbers
		I	II	III	IV	I, II	I, III	I, IV	II, III	I, II, III		
C — *spco-3* — + — + / 0 — + — 42.1 — *inos* — 2.6 — *me*	42	0	32	2	.	.	.	.	.	.	19 83.0	R2365 89601–36104
C — + — *smco-7* / 4.5 — *iv* — 2.3 — +	82	4	2	.	.	.	.	.	.	.	22 94.0	R2497 16117
C — + — *rol-3* — *cot-4* — *inos* / 10.0 — iv — 2.5 — + — 5.0 — + — 10.0 — +	60	6	2	4	6	.	.	2	.	.	20 83.5	R2498–R2101 16117
C — *sp* — + / 35.7 — + — 11.9 — *me*	48	26	6	.	.	4	.	.	.	.	21 93.0	B132 36104
C — *spco-10* — + / 23.8 — + — 11.9 — *me*	58	16	6	.	.	4	.	.	.	.	21 87.5	R2488 89601–36104
C — + — *spco-10* — *inos* / 7.2 — *iv* — 23.8 — + — 4.8 — +	56	4	18	4	.	2	.	.	.	.	21 84.9	R2488 16117
C — *ro-4* — + / 25.0 — + — 22.2 — *me*	46	10	8	.	.	8	.	.	.	.	18 93.7	B38 36104
C — + — + — *bis* / 37.6 — *inos* — 7.5 — *me* — 0 — +	46	28	4	.	.	2	.	.	.	.	20 93.5	R2413 89601–36104

TABLE 1 (Continued)

Zygote genotype and percent recombination	Parental strands	Recombinations: crossover strands recovered in Regions I-IV									Total asci; percent germination	Marker isolation numbers
		I	II	III	IV	I, II	I, III	I, IV	II, III	I, II, III		
C — + — *inos* — 27.2 — + — *me* — 4.6 — + — *bis* — 0 — +	64	20	.	.	.	4	.	.	.	.	22 96.5	R2452 89601–36104
C — + — *me* — 22.9 — + — *bis* — 6.3 — +	74	16	.	.	.	6	.	.	.	.	24 97.5	R2460 89601–36104
C — *inos* — 29.2 — + — *bis* — 14.6 — + — *asp* — 37.4 — +	52	6	.	10	.	.	14	.	4	8	24 90.0	R2460 S1007
C — + — *inos* — 20.4 — + — *me* — 6.8 — + — *bis* — 0 — +	68	14	2	.	.	4	.	.	.	.	22 88.5	R2465 89601–36104
C — + — *inos* — 25.0 — + — *me* — 6.3 — + — *bis* — 2.1 — +	68	20	4	.	.	2	2	.	.	.	24 96.0	R2475 89601–36104
C — + — *inos* — 15.9 — + — *me* — 6.8 — + — *wa* — 2.3 — +	68	12	4	2	.	2	.	.	.	.	22 96.5	R2359 89601–36104
C — + — *inos* — 41.0 — + — *me* — 4.6 — + — *smco-6* — 13.7 — +	48	24	.	4	.	4	8	.	.	.	22 96.5	R2477 89601–36104
C — + — *iv* — 7.5 — *inos* — 30.0 — + — *col-9* — 27.5 — +	44	.	12	10	.	2	2	.	8	2	20 92.5	R2417 16117

C — 34.8 — + — *me* — 13.5 — *ro-5* — +	54	26	6	.	.	6	.	.	.	.	23 93.0	R2428 89601–36104
C — 31.0 — + — *inos* — 4.8 — + — *me* — 26.2 — *ro-8* — +	50	10	.	8	.	2	12	.	.	2	21 97.0	R2520 89601–36104
C — 11.4 — + — *iv* — 31.8 — *spco-9* — +	58	2	20	.	.	8	.	.	.	.	22 96.0	R2480 16117
C — 32.0 — + — *inos* — 3.6 — + — *me* — 35.7 — *spco-9* — +	32	4	.	6	.	.	12	.	.	2	14 96.5	R2480 89601–36104

Numbers of progeny are given in the body of the table. Since the use of ordered asci makes possible the recovery of all progeny, reciprocal classes are not listed. Regions are numbered from left to right with the centromere (C) as the first marker. In the last column, the isolation numbers of the morphological mutants are listed first.

TABLE 2

Results of random isolations from two- and three-point crosses of morphological mutants with group V biochemical markers

Zygote genotype and percent recombination	Parental combinations		Recombinations: Singles region I		Singles region II		Doubles regions I,II		Total; percent germination	Marker isolation numbers
ro-4 + + / + *inos* *me* (14.1, 2.8)	66	85	13	8	1	0	1	3	177/189 94.0	B38 89601–36104
inos + *cot-2* / + *me* + (6.3, 13.6)	66	89	6	4	9	15	1	1	191/200 95.5	R1006 36104
inos *ro-5* + / + + *asp* (18.1, 14.7)	59	65	16	11	13	8	1	4	177/200 88.5	R2428 S1007
smco-6 + / + *asp* (6.1)	95	76	6	5	.	.	.	.	182/200 91.0	R2477 S1007
inos *col-9* + / + + *asp* (16.4, 4.9)	67	62	15	12	1	7	.	.	164/200 82.0	R2417 S1007
asp + / + *ro-8* (11.1)	77	92	11	10	.	.	.	.	190/200 95.0	R2520 S1007
inos *me* + / + + *spco-9* (6.4, 18.1)	34	39	2	2	8	7	1	1	94/100 94.0	R2480 89601–36104
spco-9 + / + *asp* (5.8)	84	79	4	6	.	.	.	.	173/200 86.5	R2480 S1007

Conventions as in Table 1 with the following exceptions: The left-hand number of each pair of complementary classes represents the genotype that contains the mutant allele of the leftmost marker. Regions are numbered from left to right. In the total and percent germination column, the first figure refers to the number of germinants and the second, to the total number of ascospores isolated.

occasionally revert to normal morphology while others (*bis*, and colonial-9. *col-9*, R2417) do so frequently.

Some minor variations were found in recombination frequencies for identical intervals in different crosses. It is believed, however, that these differences are due primarily to sample size. In most instances, these variations in recombination values did not affect the order of gene loci. The questionable cases will be discussed separately.

With the possible exception of spreading colonial-3 (*spco-3*), which is very close to the centromere (Table 1), all of the morphological mutants thus far mapped in linkage group V are in the right arm, at locations ranging from the centromere to a distance of 70 map units. There are two major groupings of gene loci. The first extends 25 units from the centromere; the second covers a range of 34 map units in the interval between *me-3* and *ro-8* (Figure 1).

In the first major grouping, there is close linkage between four loci. Although the most probable sequence is semi-colonial-7 (*smco-7*), ropy-like-3 (*rol-3*), *ro-4* and colonial temperature sensitive-4 (*cot-4*) (Figure 1), the order of *rol-3* and *smco-7* is questionable since they appear to be very closely linked. No wild types were observed among 154 progeny from a cross between the two mutants. Positive complementation tests obtained with these two mutants indicate that they are not alleles. The placement of *rol-3* is firmly established on the basis of both centromere distance (CD 13.0, 69 asci representing three crosses) and recombination with *iv-1* (Table 1). The centromere data from the cross of *smco-7* with *iv-1* (*smco-7*, CD 6.8, 22 asci; *iv-1*, CD 4.5, 22 asci) support the conclusion that *smco-7* is distal to *iv-1* by 2.3 map units. On the basis of 134 asci (combined data from all crosses involving *iv-1*) the centromere distance of *iv-1* was 9.7. This distance is in close agreement with that found by BARRATT, NEWMEYER, PERKINS, and GARNJOBST (1954), and STRICKLAND *et al.* (1959). It seems probable that the low value obtained for the centromere distance of *iv-1* in the cross with *smco-7* is due to sampling error.

Although the position of *ro-4* relative to *cot-4* is uncertain, it is felt that the order presented is the most likely one (Figure 1). The placement of *ro-4* (CD 25.0; 18 asci) to the left of *cot-4* (CD 17.0; 88 asci representing four crosses) is based on the recombination of *ro-4* with *inos* and *me-3* (Tables 1, 2). Four asci showed first division segregation for *ro-4* and second division segregation for *me-3*, whereas only two asci showed first division segregation for *me-3* and second division segregation for *ro-4*; this is the basis for choosing the order shown in Table 1 and Figure 1 rather than the contradictory order favored by the first cross in Table 2. This is also the basis for designating *ro-4* and *ro-5* as separate loci.

In the second grouping of loci, there is good evidence that five mutants (R2413, R2452, R2460, R2465 and R2475) are allelic with *bis* (B6). (1) The morphology of all five mutants is very nearly identical to that of *bis*. (2) On the basis of the results from crosses of each of the strains with the *inos*, *met-3* tester stock, only one of the mutants (R2460) gives more than 2% recombination with *me-3* (Table 1, Figure 1). (3) The six *bis* strains (B6, R2413, R2452, R2460, R2465 and R2475) were crossed in all possible combinations with one another, and in no instance were any wild-type progeny obtained. All crosses matured slowly, and although a large number of bulb-shaped perithecia were formed, many remained immature. The formation of such perithecia is typical of *bis* intercrosses (PERKINS 1962). Of the 60 to 70 apparently normal perithecia examined from each cross, most were found to be empty. The ascospores which were present were normal in appearance, and all were retained in the perithecia. An attempt was made to isolate 100 ascospores at random from each cross, but this was not possible in three cases (R2413 × R2452, 64 ascospores; R2452 × R2465, 80; R2452 × R2475, 91). The ascospore germination in the 15 crosses ranged from 64% to 96%. Because the nutritional marker (*inos*), which was present in four crosses, segregated normally, sampling appeared random. (4) Heterocaryon tests of the mutants in all possible combinations with one another gave only colonial growth.

The positioning of washed, *wa* close to *bis* is based on its recombination with *inos* and *me-3*

(Table 1, Figure 1). Crosses of *wa* by each of the *bis* strains provided the following results: (a) Wild-type progeny were obtained from all crosses thus proving that *wa* and *bis* are not alleles. (b) Of the 440 germinants from the six crosses, 25 (5.7%) showed wild-type morphology. This indicates that *wa* may be distal to *bis* by a greater distance than that shown by the 3-point cross, *wa* × *inos*, *me-3*.

Five loci are placed within a ten-unit interval between *me-3* and *asp*. Although there is some variation in results, the most probable gene sequence is *ro-5*, *cot-2*, *smco-6*, *spco-9*, and *col-9* (Figure 1). With one exception, complementation in heterocaryon tests between all combinations of pairs of these five mutants indicates that they are not alleles. Complementation tests between *spco-9* and *col-9* were inconclusive, and intercrosses were infertile. These two are tentatively considered different loci on the basis of their distinctive morphological characteristics. On the basis of recombination values obtained with *inos*, *me-3*, and *asp*, the distance between *cot-2* and *ro-5* appears to be about three map units. The results from a cross between these two mutants indicate that the distance between them is greater (7 w.t./110 germinants; 55% germination). Three mutants, *smco-6*, *spco-9*, and *col-9* are mapped on the basis of their recombination with *asp* (Tables 1, 2). Although they are close together, it is felt that the order chosen is the most likely one. Results from the crosses *cot-2* × *smco-6* (10 w.t./72 germinants; 72% germination) and *cot-2* × *col-9* (13 w.t./128 germinants; 64% germination), indicate that *col-9* could be slightly to the left, rather than to the right of *smco-6*. The results from the cross *smco-6* × *col-9* (10 w.t./145 germinants; 72% germination) indicate that the distance between these two mutants may be greater than that shown in Figure 1.

On the basis of data obtained from 2- and 3-point crosses (Tables 1, 2), the three ropy mutants in linkage group V (*ro-4*, *ro-5*, and *ro-8*) are at separate gene loci. Although tests for heterocaryon complementation between pairs of these three strains were negative, all three ropy mutants formed wild-type heterocaryons with a ropy-like mutant, *rol-3*. Each of the three ropy mutants and *rol-3* were tested for complementation with ropy-7, *ro-7* (R2470) in group III. The results were positive in all instances.

It is possible that some factor associated with the phenotype of the ropy mutants in group V prevents the formation of wild-type heterocaryons between them, but allows positive results in tests with other mutants. The fact that three similar morphological mutants at different loci within a linkage group do not form wild-type heterocaryons with one another is exceptional and warrants further investigation.

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SUMMARY

Of 18 morphological mutant strains mapped in linkage group V, 11 are assigned to new loci ranging over 70 units in the right arm.

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