NEW CRISP GENES AND CRISP-MODIFIERS IN *NEUROSPORA CRASSA*

LAURA GARNJOBST AND E. L. TATUM

Rockefeller University

Received April 21, 1970

ONE of the first morphological mutants reported in *Neurospora crassa* is the dwarf strain known as crisp (*cr*) (LINDEGREN 1936). It has been described as follows: "short aerial hyphae with tight clusters of more brightly colored conidia than the wild type" (LINDEGREN 1936), and "early conidiation uniform over an agar surface" (PERKINS 1959).

In the present study 14 crisp strains of independent origin were examined. Crisp progeny from backcrosses of some strains × wild type retained slight characteristic differences in phenotype. These differences suggested that complementation might occur in some pairs, but none was found. Evidence is presented that two crisp-like strains, which do form wild-type heterocaryons with each of the 14 crisp strains, and with each other, represent new crisp loci in linkage group I.

Further interest in the crisp alleles arose when it became clear that spontaneous crisp-modifier mutations were often present in older crisp cultures. Although the phenotype had remained typically crisp, this appearance proved to be deceptive as shown in isolates from plated conidia and in progeny from crosses. This paper describes the effect on crisp morphology of a number of modifier mutations which appeared during the course of this study.

MATERIALS AND METHODS

Strains: The isolation numbers and origins of the crisp strains, and centromere distances of the mutant genes are given in Table 1. The wild types RL21*a* and RL3–8*A*, derived from the LINDEGREN wild types 1*A* and 25*a*, were used in backcrosses to obtain a fair degree of isogenicity and to assure the same heterocaryon genotype. The markers used were: *al-2* (albino-2), isolation No. 15300; *arg-5* (arginine-5), 27947; *inos* (inositol), 89601; *nic-2* (nicotinic-2), Y31455; and *nic-3* (nicotinic-3), Y31881.

Media: The following general media were used: (1) minimal, with or without added supplements (VOGEL 1964); (2) glycerol-sucrose complete (GSC) medium (VOGEL 1964); (3) synthetic crossing medium (WESTERGAARD and MITCHELL 1947); and (4) ST. LAWRENCE sorbose medium (LESTER and GROSS 1959). Cultures were grown at 30°C; for formation of protoperithecia and development of perithecia the slants were held at 25°C. All ascospores isolated in order from asci were treated with a 1:10 dilution of Chlorox (5% sodium hypochlorite) and retained for seven days for further ripening before they were heat-shocked for 30 min at 60°C.

Heterocaryon tests: The crisp *a* strains used were reisolates from the third to fifth backcrosses to wild type. The *A* strains used were from crosses of these *a* strains × RL3-8*A*. All were tested for unisexual heterocaryon formation with a spreading colonial mutant *spco-5* (R2450), *a* or *A*, of known heterocaryon genotype, *C,D;E* (WILSON and GARNJOBST 1966).

TABLE 1

Origin of crisp strains investigated and centromere distances of mutant genes

Locus symbol, Strain Isolation No.	Origin: strain, treatment	Centromere distance (number of asci)
cr-1		
B74	UV, 74A	7.6 (33)
B122*	UV, 74A	10.0 (8)
B123	UV, 74A	6.3 (24)
F945	?	5.0 (10)
L-crisp†	Probably spont.	11.1 (27)
S4358	Spont.; from 37401 × 37401	4.5 (22)
R2103	UV, 89601	5.0 (10)
R2104	UV, 89601	6.3 (8)
R2360	UV, 89601	4.2 (36)
R2414	UV, 89601	5.0 (10)
R2433	UV, PERKINS *a*, wild type	5.0 (20)
R2476	UV, 89601	3.3 (15)
R2482	UV, 89601	4.2 (12)
R2501	UV, 89601	6.3 (8)
		Av. 6.0 (243)
cr-2		
R2445‡	UV, 89601	15.4 (39)
cr-3		
R2509‡	UV, 89601	10.9 (28)

* B122—Aberration suspected (PERKINS 1962, p. 1186).
† LINDEGREN's crisp—obtained from Fungal Genetics Stock Center (FGSC No. 20).
‡ These two strains have paler pigmentation and delayed conidiation as compared to the *cr-1* strains.

RESULTS

Complementation tests: Originally there were 16 strains that appeared to be crisp: 14 typical crisp and two crisp-like (Table 1). All showed linkage to mating type. Both *a* and *A* reisolates of each strain were tested for complementation in all possible pairwise combinations (120 for each mating type). All tests were negative except pairs containing the crisp-like strains R2445 or R2509. These formed wild-type heterocaryons with each of the true crisp strains and with each other. To be certain that anastomosis (both fusion and flow) occurred, all negative tests were repeated after introducing nutritional markers *nic-3* or *inos* into each of the crisp strains. In all 91 pairs of the 14 crisp alleles, growth took place on sucrose minimal agar (slants and/or growth tubes), but the growth was definitely crisp, not wild-type. Thus it appears that heterocaryon formation did occur, and that no complementation between the crisp alleles took place.

Genetic data on the crisp mutants: Crosses of the true crisps with each of the crisp-like strains, and intercrosses of the crisp-like strains produced both parental and tetratype asci. From the occurrence of wild-type recombinants and from complementation tests, it appears that the three crisp mutants represent three nonallelic gene loci. The new loci are designated crisp-2 (R2445) and crisp-3

(R2509), and the locus previously called crisp will henceforth be designated crisp-1.

The mutant progeny from either (1) *cr-1* × *cr-2*, or (2) *cr-1* × *cr-3* were easily scored for crisp, crisp-like and double mutants, the latter because of their more restricted growth. The results obtained were as follows: (1) *cr-1* × *cr-2*, 20 asci; centromere distances (C.D.) *cr-1*, 5.0; *cr-2*, 20.0; recombination 15% (14 parental ditype, 6 tetratype asci); (2) *cr-1* × *cr-3*, 19 asci; C.D. *cr-1*, 5.6; *cr-3*, 13.2; recombination 13.2% (14 parental ditype, 5 tetratype asci).

Crosses between *cr-2* and *cr-3* have produced few perithecia and the mutant progeny were practically indistinguishable, except for the more compact double mutants in asci containing a pair of wild types. In spite of this problem, however, analysis of an intercross of *cr-2* × *cr-3* was carried out. The 25 asci (79.5% germination) included four in which only two pairs grew. *cr-2* and *cr-3* showed 10.9% recombination (23 asci) based on the wild-type pairs (five tetratype and 18 parental asci). Centromere distances could only be estimated because of the difficulty in distinguishing *cr-2* and *cr-3* progeny.

Additional information regarding the order and distance between *cr-1* and *cr-2* was obtained from 22 asci isolated from a 4-point cross, *cr-2*, *nic-2* × *cr-1* (R2433), *al-2*. The numbers of asci showing parental ditype, nonparental ditype and tetratype segregation for each pair of genes were as follows: *nic-2*, *cr-1*, 17:0:5; *cr-1*, *cr-2*, 18:0:4; *cr-2*, *al-2*, 14:0:8; *nic-2*, *cr-2*, 14:0:0; *cr-1*, *al-2*, 13:0:9. Numbers of second division segregation asci were: *nic-2*, 4; *cr-1*, 7; *cr-2*, 9; *al-2*, 12. This is consistent with the following map relations:

centromere–9.1–*nic-2*–11.4–*cr-1*–9.1–*cr-2*–18.2–*al-2*.

A second 4-point cross *cr-3*, *nic-2* × *cr-1* (R2433), *al-2* produced no perithecia in several attempts. Asci from the cross *cr-3* × *al-2* then were analyzed. For 18 asci, the centromere distances were 8.8 for *cr-3* and 22.2 for *al-2*, while *cr-3* and *al-2* showed 19.4% recombination.

The results of these crosses clearly showed that *cr-1*, *cr-2*, and *cr-3* are nonallelic, but did not provide definitive information on their order. However, summation of data on centromere distances from these crosses and from those given in Table 1: *cr-1*, 6.0 (243 asci); *cr-2*, 16 (106 asci); *cr-3*, 13.9 (77 asci), suggests that the order is *cr-1*, *cr-3*, *cr-2*.

This order was confirmed by the results of crosses involving all three *cr* loci. In making these crosses, the problem of infertility was partially overcome by forming a phenotypically wild-type heterocaryon of *cr-1*, *cr-2*, *al-2* + *cr-3* and crossing it with *cr-3*. The progeny were analyzed by heterocaryon tests for complementation with each of the three crisp mutants. Four asci showing recombination between the crisp loci were obtained out of a total of 49 analyzable asci from two crosses. Examination of these recombination asci, given in Table 2, definitely eliminated four of the six possible orders of the crisp loci, on the basis of the numbers of multiple crossovers required.

Of the remaining two possibilities, the order *cr-3*, *cr-1*, *cr-2* was eliminated since ascus 2 would require 2 crossovers between the crisp loci and because the earlier summarized centromere distances of these loci placed *cr-1* closer to the

TABLE 2

Segregation patterns of crisp recombinant asci from crosses involving all three crisp loci

Zygote genotype	$\frac{a}{A}$ C $\frac{cr\text{-}1 \; +}{+ \; cr\text{-}3}$	$\frac{+}{cr\text{-}2}$	$\frac{+}{+}$	$\frac{al\text{-}2}{+}$	Crossovers required
Ascus 1	*a*	+	—	—	—	
	A	+	—	+	+	3-strand double
	a	—	+	+	+	
	(*A*	—	+	—	—)	
Ascus 2	*a*	+	+	—	—	
	a	—	—	+	+	3-strand double
	A	+	—	+	+	
	(*A*	—	+	—	—)	
Ascus 3	*a*	—	+	+	+	
	a	—	+	+	+	4-strand double
	A	+	—	—	—	
	(*A*	+	—	—	—)	
Zygote genotype	$\frac{A}{a}$ C $\frac{cr\text{-}1 \; +}{+ \; cr\text{-}3}$	$\frac{+}{cr\text{-}2}$	$\frac{+}{+}$	$\frac{al\text{-}2}{+}$	Crossovers required
Ascus 4	(*A*	—	+	—	—)	
	A	—	+	+	—	2-strand double
	(*a*	+	—	—	+)	
	a	+	—	+	+	

Notes: Ascus pairs in parentheses not recovered. Marker isolation numbers: *cr-1*, R2433; *cr-2*, R2445; *cr-3*, R2509; *al-2*, 15300. C, centromere.

TABLE 3

Summary of ordered tetrad isolations from crosses involving all three crisp loci

Zygote genotype*	Number of asci	Parental strands	Recombination strands: Singles in region 1	2	3	4	5	Doubles (Regions in parentheses)
$\frac{a}{A}$ C $\frac{cr\text{-}1 \; +}{+ \; cr\text{-}3}$ $\frac{cr\text{-}2}{+}$ $\frac{al\text{-}2}{+}$	25	71**	1**	8	0	1	18	1 (**1 & 4**)
	18	37	11	6	1	4	6	1 (**1 & 3**) 4 (**1 & 5**) 2 (**2 & 5**)
$\frac{A}{a}$ C $\frac{cr\text{-}1 \; +}{+ \; cr\text{-}3}$ $\frac{cr\text{-}2}{+}$ $\frac{al\text{-}2}{+}$	6	10	4	0	0	0	8	2 (**4 & 5**)
Totals		118	22	16	2	8	40	
Percent recombination			11.2	8.2	1.0	4.1	20.4	

* C, centromere; *cr-1*, R2433; *cr-2*, R2445; *cr-3*, R2509; *al-2*, 15300.
** Mating type determined in only one ascus in this series, No. 1, Table 2.

centromere than *cr-3*. The remaining order, *cr-1*, *cr-3*, *cr-2*, thus appeared the most probable, and was used in presenting the results of these crosses (Table 3).

In Table 3 the calculated lengths of interval **1** (mating type, centromere), of interval **2** (centromere, *cr-1*) and of interval **5** (*cr-2*, *al-2*) are in reasonable agreement with figures from the literature and from crosses reported earlier in this paper. However, the frequency of recombination between the crisp loci appears considerably less than in crosses reported earlier in this paper. If this is significant, it would appear that recombination may be less in crosses involving all 3 crisp mutants than in those involving only 2.

In any case, from all the data available, it seems clear that there are 3 non-allelic crisp loci, located in a fairly short region in the right arm of linkage group I, between *nic-2* and *al-2*. The results show that, from the centromere, the order is *cr-1*, *cr-3*, *cr-2*, and suggest that *cr-3* is somewhat closer to *cr-1* than to *cr-2*.

Modifiers of crisp morphology: The five modifiers described below are referred to as m-1 through m-5. These designations are used as a convenience in this paper and are not intended to serve as permanent locus symbols.

(m-1) A gene modifying crisp morphology was first detected in a *cr-1* (S4358), *inos* strain. The conidia of this apparently typical crisp culture, upon being plated on sorbose medium supplemented with 50 μg inositol/ml produced two types of colonies, one more restricted than the other. The more restricted colonies when transferred to slants of complete medium (GSC), without sorbose, consistently showed delayed conidiation as compared to *cr-1* cultures from the more numerous, slightly spreading colonies on sorbose. The restricted colonies proved to be triple mutants (*cr-1*; *inos*, m-1).

m-1 has also appeared among progeny from crosses of older *cr-1* cultures × wild type. This finding made it necessary to use, in definitive crosses, *cr-1* reisolates derived immediately from ascospores. The m-1 mutation appears not to be present in wild-type strains RL21*a* and RL3-8*A*; no culture resembling this modifier has been found among the progeny from many other crosses with these wild types.

The phenotype of the modifier alone is quite close to that of wild type, but is readily distinguished from wild type because of (1) its tendency to brown the agar, (2) its finer hyphae and conidia, and (3) the germination of almost all ascospores without heat shock. The m-1 gene, suggested symbol *mod (cr)-1*, is closely linked to *inos*, and few recombinants have been obtained.

Modifier m-1 increases the rate of growth of crisp hyphae on solid medium without sorbose, and delays conidiation (Table 4). The effect of m-1 on all 14 alleles of *cr-1* is similar, as shown by crosses of each strain × m-1, but the effect is not locus specific since in crosses of *cr-2* and of *cr-3* with this modifier, similarly modified crisp-like germinants were present in some asci.

The heterocaryon *cr-1* + *cr-1*; m-1 formed by spontaneous mutation, differs from the double mutant (*cr-1*; m-1). In the double mutant the effect of the modifier is apparent upon growth from the ascospore, whereas in the spontaneous heterocaryon no effect is observable. The phenotype of *cr-1*; m-1 + *cr-1* is crisp, at least for a considerable time. When heterocaryons *cr-1*; *nic-3* + *cr-1*; m-1, *inos*

TABLE 4

Growth of Petri plate cultures of crisp, modified crisp, and modifier (m-1) on minimal and on complete medium

Strain (inos⁻)	Colony diameter in mm at 48 hours			
	25°C		34°C	
	Minimal†	Complete	Minimal†	Complete
crisp (*cr-1*) (S4358)*	7.0	6.0	13.0	11.0
modified crisp‡ (*cr-1*; m-1)	70.0	65.0	40.0	45.0
modifier (m-1)	85.0	70.0	43.0	27.0

* The response of this crisp is typical of that of the other *cr-1* strains.
† Minimal supplemented with 50 μg inositol per ml.
‡ Only the modified crisp did not conidiate in 48 hours.

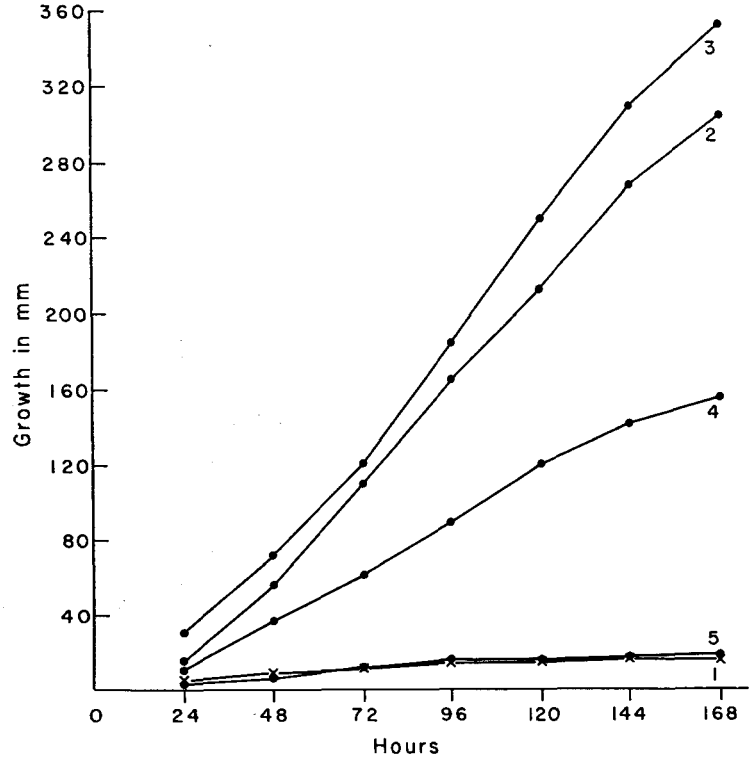

FIGURE 1.—Effect of modifier *m-1* on growth of crisp (R2476) in heterocaryons:
(1) *cr-1; nic-3* + *cr-1;* m-1, *inos;*
(2) *cr-1; nic-3* + m-1, *inos*. Controls:
(3) m-1, *inos;*
(4) *cr-1;* m-1, *inos;*
(5) *cr-1; nic-3.*
Media: (1) and (2) on minimal sucrose agar; (3) and (4) on minimal + inositol; (5) on minimal + niacin.

are formed on minimal sucrose agar, the phenotype likewise is crisp (Figure 1).

(m-2) In contrast to m-1, m-2 retards growth in the double mutant *cr-1*; m-2. This modifier has appeared only once, in the original cross of *cr-1* (R2412) × wild type; possibly it was induced simultaneously with the *cr-1* (R2412) mutation. The m-2 homocaryon phenotypically is a slow-growing wild-type strain.

(m-3) This modifier (R2526), which increases conidiation at the top of the slant in the double mutant (*cr-1*; m-3), was found among the progeny of *cr-1* (R2360) b_2 × wild type. Its phenotype when homocaryotic, is ropy. The gene, designated ropy-9 (*ro-9*), is linked to *arg-5* (GARNJOBST and TATUM 1967). It is not of special interest in this context; many morphological double mutants are slightly modified in growth habit as compared to either single mutant.

(m-4 and m-5) These two modifiers which completely suppress the crisp phenotype were obtained from an experiment described below. m-4 is a frail, pale yellow aconidial strain which upon transfer resembles m-5. m-5, a semi-aconidial strain, is bright yellow and on slants usually produces clumps of orange conidia slightly below the hyphal tips.

Spontaneous occurrence of modifiers: cr-1 cultures, immediately derived from ascospores, were transferred seven times at weekly intervals on slants of complete medium and then conidia from each culture were plated. From each, 50–100 colonies were isolated at random from 10 plates, each containing approximately 50–100 colonies.

Of the 12 *cr-1* strains tested (Table 5), five gave no evidence that morphological mutant genes other than *cr-1* were present. The non-crisp cultures obtained from the other seven *cr-1* strains were of the following classes: (1) phenotypically wild type; (2) non-crisp morphological types, and (3) intergrades in appearance between crisp and class (1) and/or class (2). One of each type of non-crisp culture (classes 1 and 2) from each *cr-1* strain was crossed with a standard wild type. All proved to be heterocaryons. At least one ascus from each cross of class (1) cultures (three independent occurrences) contained four wild-type spore pairs (Table 5). Although this result could be due to suppressor mutation, the reversion of *cr-1* to wild type seems more likely since no ascus with only one pair of *cr-1* was found.

From crosses of class (2) reisolates × wild type, pure cultures of each non-crisp morphological type were obtained. These were: strains like m-1 in appearance (three independent occurrences), m-4 (two occurrences), and m-5 (single occurrence). Crosses of each of these pure non-crisp cultures with *cr-1* and subsequent additional crosses identified the mutant genes concerned as m-1, m-4, and m-5.

DISCUSSION

The crisp phenotype can result from mutation at any one of three loci, fairly close to each other in the right arm of linkage group I. The significance of these linkage relations is not known; possibly the three loci may be involved in a sequence of reactions biochemically related to the crisp phenotype. A few such clusters of genes are known for particular biosynthetic sequences in Neurospora,

TABLE 5

Spontaneous mutations found in crisp cultures after seven weekly transfers

crisp-1 strains tested	Class of culture recovered	Mutations found in recovered culture
S4358-1-1A, *inos*‡	(2)	m-1 (linked to *inos*)
S4358-4-3 *a*, *inos*	(2)	m-5
B74-4(1-7)*A*, *nic-3*	(2)	m-4
F945-5-3*A*, *inos*	*cr* only	....
L-*cr*-3-15*A*, *inos*	(1)	Probable reversion* of *cr-1* to wild type (one ascus of 12, 4 pr. wild type/ascus)
	(2)	m-1 (linked to *inos*)
R2103-3(1-5)*A*, *inos*	*cr* only	....
R2360-3-5*A*, *nic-3*	(1)	Probable reversion* of *cr-1* to wild type (one ascus of 12, 4 pr. wild type/ascus)
	(2)	m-4
R2360-3-20*A*, *inos*	(1)	Probable reversion* of *cr-1* to wild type (10 asci of 16, 4 pr. wild type/ascus)
R2412-4-4*A*, *inos*	*cr* only	....
R2433-3(1-4)*A*, *inos*	*cr* only	....
R2476-3(3-2)*A*, *inos*	*cr* only	....
R2482-3(3-1)*A*, *inos*	(2)	m-1 (linked to *inos*)

* In the cross to wild type, no tetratype asci were found, but the possibility of a suppressor closely linked to *cr-1* has not been excluded. Results in parentheses are from the cross to wild type (see text).

‡ The first number refers to strain isolation number of the *cr-1* allele; second, to number of backcross to wild type; third, to spore number; or third and fourth, to ascus and spore number.

e.g., the aromatic (GROSS and FEIN 1960; GILES *et al.* 1967) and isoleucine-valine (WAGNER *et al.* 1964) pathways. Clusters of morphological genes in Neurospora have been suggested by recent work (GARNJOBST and TATUM 1967). However, genes in these apparent clusters were morphologically unrelated, and most morphological genes having similar and possibly related phenotypes, such as colonial or ropy, are widely and seemingly randomly distributed in the genome.

The recurrence of mutant genes which modify the phenotype of certain Neurospora strains has also been reported by MURRAY and SRB (1962). However, the spontaneous occurrence in crisp strains of reversions and of morphological mutant genes which modify the crisp phenotype seems to be unusually frequent. Even when the parent strain had been grown recently from ascospores, modifiers occasionally appeared among the progeny of crosses. They have also appeared repeatedly during continued vegetative growth.

This is in contrast to the behavior of many other morphological mutants (GARNJOBST and TATUM 1967). A particularly striking example is a fluffy reisolate (from LINDEGREN's fluffy × *inos* (37401) which has been transferred every 3 months for 10 years in this laboratory without showing any indication of mutation or the occurrence of any other modification.

Spontaneous mutation in a crisp culture, either back mutation or modifier mutation at other loci, is not as readily detected as in many other strains. This suggests a relative dominance of crisp morphology in heterocaryons, at least until a significant proportion of double mutant or revertant nuclei is reached.

In contrast to the situation in heterocaryons, in modified crisp cultures that are homocaryotic, the effects of the modifier genes are readily recognized. The five modifiers of crisp that were found themselves have morphological phenotypes differing from one another and distinguishable from wild type. The interactions of these modifiers with crisp also differ, ranging from a restriction of growth on sorbose and a stimulation on medium without sorbose, or a restriction of colony size, to a complete suppression of crisp phenotype.

m-1 shows no allele or locus specificity. Its effects are similar on all 14 crisp alleles of *cr-1* and on *cr-2* and *cr-3*. It is not known whether other modifiers, especially m-4 and m-5, show the same nonspecificity. Further work will be necessary to resolve this question and to elucidate the biochemical basis of the crisp phenotype and of modifiers of crisp.

The authors are greatly indebted to Mrs. ANN LAFABREGUE and Miss ANNE HAMILL for their skilled technical assistance. This work was supported in part by research grant CA-03610 from the Public Health Service.

SUMMARY

Sixteen crisp strains of independent origin have been examined. From linkage tests and results of interstrain crosses and complementation tests, it is concluded that the crisp phenotype is determined by mutation at any one of three loci in the right arm of linkage group I. The order appears to be *cr-1*, *cr-3*, *cr-2*, with *cr-1* closest to the centromere.—Genetic modifications of the crisp phenotype found included apparent reversions of *cr-1* and mutant genes which modify crisp morphology (m-1, m-2, and m-3) or completely suppress it (m-4 and m-5) in the double mutants. The phenotype of the modified crisp (*cr-1*; m-1) was not readily expressed in heterocaryons with *cr-1* (*cr-1* + *cr-1*; m-1) formed by spontaneous mutation or produced experimentally.

LITERATURE CITED

GARNJOBST, L., 1953 Genetic control of heterocaryosis in *Neurospora crassa*. Am. J. Botany **40**: 607–614.

GARNJOBST, L. and E. L. TATUM, 1967 A survey of new morphological mutants in *Neurospora crassa*. Genetics **57**: 579–604.

GILES, N. H., M. E. CASE, C. W. H. PARTRIDGE and S. I. AHMED, 1967 A gene cluster in *Neurospora crassa* coding for an aggregate of 5 aromatic synthetic enzymes. Proc. Natl. Acad. Sci. U.S. **58**: 1453–1460.

GROSS, S. R. and A. FEIN, 1960 Linkage and function in Neurospora. Genetics **45**: 885–904.

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

LINDEGREN, C. C., 1936 A six-point map of the sex chromosome of *Neurospora crassa*. J. Genet. **32**: 243–256.

MURRAY, J. C. and A. M. SRB, 1962 The morphology and genetics of wild-type and seven morphological mutant strains of *Neurospora crassa*. Canad. J. Botany **40**: 337–349.

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

PERKINS, D. D., M. GLASSEY and B. A. BLOOM, 1962 New data on markers and rearrangements in Neurospora. Canad. J. Genet. Cytol. **4**: 187–205.

VOGEL, J. H., 1964 Distribution of lysine pathways among fungi: evolutionary implications. Am. Naturalist **98**: 435–446.

WAGNER, R. P., A. BERQUIST, T. BARBER and K. KIRITANI, 1964 Genetic blocks in the isoleucine-valine pathway of *Neurospora crassa*. Genetics **49**: 865–882.

WESTERGAARD, M. and H. K. MITCHELL, 1947 Neurospora. V. A synthetic medium favouring sexual reproduction. Am. J. Botany **34**: 573–577.

WILSON, J. F. and L. GARNJOBST, 1966 A new incompatibility locus in *Neurospora crassa*. Genetics **53**: 621–631.