GENETICS OF A COLONIAL MICROCONIDIATING MUTANT STRAIN OF NEUROSPORA CRASSA*[1]

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WILD type strains of *Neurospora crassa* produce asexual spores in the form of multinucleate macroconidia and have a spreading growth habit. The value of this organism for biochemical and genetical research would be greatly increased if strains were available having restricted colony size and producing exclusively uninucleate microconidia. These characteristics would permit manipulation of Neurospora by bacteriological techniques and allow rapid establishment of homocaryotic lines. This paper reports the genetical analysis of a mutant strain (Y-8743) having these characteristics, the interaction of the genes concerned, and the development of derived strains.

Specifically, Y-8743 differs from the wild type in two clearly defined ways. First, it grows at a rate of less than 0.05 mm/hr, at 25°C, forming a button-like thallus, or colony, on semi-solid medium, and forming pellets in liquid shake cultures. Second, the only asexual spores produced are microconidia. In contrast, the wild type grows at about 4 mm/hr, produces a web-like mycelium on semi-solid medium, and forms a tangled mycelial mass in liquid shake culture. The asexual spores produced are predominantly multinucleate macroconidia.

EXPERIMENTAL

Materials and Methods

Strain Y-8743 was derived from an ascospore isolated from a cross involving material treated with sodium 20-methylcholanthrene-11,14-endo-α,β-succinate. Conidia of strain 1A were treated with this carcinogenic agent, added to protoperithecia of strain 25a, ascospores subsequently isolated, and the resulting cultures tested by the technique of BEADLE and TATUM (1941, 1945) for the detection of mutants. Originally strains 1A and 25a were isolated from a cross of LINDEGREN'S stocks La and LA (BEADLE and TATUM 1945). Other genes concerned with colonial growth, to be discussed later, occur in strains Y-5331 and Y-5296. The former originated from material treated with methyl-

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TABLE 1

Genes used in the linkage studies

GENE CHARACTER	MUTANT NUMBER*	SYMBOL	LINKAGE GROUP		CENTROMERE DISTANCE**	NO. ASCI ISOLATED†
			NUMBER	REF.‡		
sex	—	*A* or *a*	I	(1)	7.8	755
albino-2	15300	*al-2*	I	(2)	27.4	919
peach	—	*pe*	II	(3)	11.2	381
fluffy	—	*fl*	II	(3)	29.0	632
leucineless	33757	*leu*	III	(4)	8.5	236
tryptophanless	10575	*tpt-1*	III	(4)	21.5	242
pyridoxinless	44602 (37803)	*pdx* (*pdx*)	IV	(5)	10.1	360
pantothenicless	5531	*pan*	IV	(5)	26.3	332
cholineless	34486 (34542)	*chol-1* (*chol-1*)	IV	(5)	24.4	166
lysineless	33933	*lys*	V	(5)	3.5	99
isoleucine-valineless	16117	*i-val*	V	(5)	9.4	160
inositolless	37401	*inos*	V	(5)	26.3	249
riboflavinless	51602	*rib*	VI	(5)	2.0	123
adenineless	3254	*ad*	VI	(5)	1.8	248
sulfonamide-requiring	E-15172	*sfo*	VII	(5)	31.6	117

* Numbers with no letter preceding them identify the strain as originating at STANFORD UNIVERSITY, while the letter Y preceding a number identifies it as originating at YALE UNIVERSITY. The letter E refers to a strain obtained by DR. S. EMERSON at the CALIFORNIA INSTITUTE OF TECHNOLOGY.

** Uncorrected for double crossovers.

† See table 1A for compilation of data.

‡ (1) C. C. LINDEGREN (1936), (2) M. V. G. HUNGATE (1945), (3) C. C. LINDEGREN and G. LINDEGREN (1939), (4) F. P. HUNGATE (1946). (5) Four additional linkage groups have been demonstrated making a total of seven which agrees with the cytological data (MCCLINTOCK 1945). These groups have been arbitrarily assigned numbers IV, V, VI, VII pending further standardization. Groups IV and V correspond to the 4th and 5th group from the left as figured by BEADLE (1946).

cholanthrene, the latter from material treated with nitrogen mustard. Table 1 lists the genes used in the genetical investigation, their symbols, their linkage groups, their centromere distances and the numbers of asci on which these values are based. Table 1A gives the compilation of data including the sources used to calculate centromere distances in table 1.

Crosses between strains were made on corn meal agar and on synthetic crossing medium (WESTERGAARD and MITCHELL 1947). The presence of a mutant gene for a biosynthetic reaction was determined by failure of a culture to grow on liquid minimal medium. In crosses where several genes controlling biochemical reactions were segregating, the requirements of a culture were determined by an omission series. The morphological characters were determined whenever possible on cultures grown directly from the ascospores on the standard Neurospora complete medium in 7.5×1.0 cm tubes. The presence of

TABLE 1A

Compilation of data used to calculate centromere distances in Table 1

NUMBER OF ASCI		CENTROMERE DISTANCE	DATA SOURCE*
TOTAL	2ND DIV. SEGREG.		
	sex		
449	58	6	11
178	36	10	12
16	2	6	1
50	7	7	4
62	14	11	authors
—	—	—	
755	117	7.8	
	albino-2 (15300)		
140	64	23	10
100	57	28	7
98	57	29	16
14	12	43	17
39	24	32	18
20	4	10	14
121	69	28	4
387	216	28	authors
—	—	—	
919	503	27.4	
	peach		
103	23	11	12
278	63	11	13
—	—	—	
381	86	11.2	
	fluffy		
110	68	31	11
278	157	27	13
27	13	24	9
217	128	29	authors
—	—	—	
632	366	29.0	
	isoleucine-valineless (16117)		
31	8	13	1
21	5	12	3
60	7	6	5
20	1	2	8
28	9	16	authors
—	—	—	
160	30	9.4	

NUMBER OF ASCI		CENTROMERE DISTANCE	DATA SOURCE*
TOTAL	2ND DIV. SEGREG.		
	leucineless (33757)		
79	12	8	9
129	22	9	15
28	6	11	authors
—	—	—	
236	40	8.5	
	tryptophanless (10575)		
229	94	21	9
13	10	38	authors
—	—	—	
242	104	21.5	
	pyridoxineless (44602) (37803)		
332	70	11	2
28	3	5	authors
—	—	—	
360	73	10.1	
	pantothenicless (5531)		
257	135	26	2
75	40	27	authors
—	—	—	
332	175	26.3	
	cholineless (34486) (34542)		
139	70	25	2
27	11	20	authors
—	—	—	
166	81	24.4	
	lysineless (33933)		
71	4	3	6
28	3	5	authors
—	—	—	
99	7	3.5	
	adenineless (3254)		
10	1	5	9
99	4	2	8
37	3	4	5
20	0	<2	15
82	1	1	authors
—	—	—	
248	9	1.8	

TABLE 1A—*continued*

NUMBER OF ASCI		CENTROMERE DISTANCE	DATA SOURCE*	NUMBER OF ASCI		CENTROMERE DISTANCE	DATA SOURCE*
TOTAL	2ND DIV. SEGREG.			TOTAL	2ND DIV. SEGREG.		
	inositolless (37401)				riboflavinless (51602)		
80	47	29	7	111	4	2	2
79	43	27	4	12	1	4	authors
90	41	23	authors	—	—	—	
—	—	—		123	5	2.0	
249	131	26.3					

* (1) E. A. ADELBERG (unpublished), (2) HOULAHAN, M. B., G. W. BEADLE, and H. G, CALHOUN (1949), (3) BONNER, TATUM and BEADLE (1943), (4) D. M. BONNER (unpublished). (5) H. R. BUSS (1944), (6) A. H. DOERMAN (1946), (7) N. H. GILES (unpublished), (8) M. B. HOULAHAN (1944), (9) F. P. HUNGATE (1946), (10) M. V. G. HUNGATE (1945), (11) C. C. LINDEGREN (1933), (12) C. C. LINDEGREN (1936), (13) C. C. LINDEGREN and G. LINDEGREN (1939), (14) D. E. NEWMEYER (unpublished), (15) D. C. REGNERY (1947), (16) A. M. SRB (1946), (17) H. J. TEAS (1947), (18) E. ZIMMER (1946).

the gene controlling microconidiation was determined by microscopic examination for microconidia or by segregation from outcrosses to suitable strains.

Centromere distances in map units were calculated by dividing by two the percentage of asci showing second division segregation (LINDEGREN 1933, 1936). The term serially (that is, orderly) isolated asci refers to those asci from which the ascospores were dissected and their order maintained. Unless stated otherwise, all isolations reported in this paper were serial.

Genes Controlling Mutant Characteristics in Strain Y-8743

In a preliminary experiment Y-8743a was outcrossed to the wild type (cross 3) and 100 ascospores were isolated at random. The results (table 2) give the following four classes in about equal numbers: (1) wild type (spreading-macroconidial), (2) pink clumpy, (3) colonial-sterile, and (4) colonial-microconidial as shown in figure 1. These frequencies show good agreement with the hypothesis that strain Y-8743 differs from wild type by two non-linked genes. Table 2 gives the results of the Chi Square test on these data and lists the symbols adopted for each class; P equals 0.78, which is satisfactory agreement between observed and expected. Since in cross 3 only 77 spores germinated out of 100 isolated, the existence of a fifth and lethal class could not be precluded. To explore this possibility, Y-8743a was crossed with 1A (cross 1) and spores of nine asci isolated serially. In six asci the same four classes were recovered while in three asci only two types segregated. An additional intercross between *col-1, M* and *Col-1, m* (cross 8) also gave rise to no new types in 13 asci. Since only four classes were obtained in the progeny from all the crosses, it was concluded that strain Y-8743 differs from the wild type by two genes.

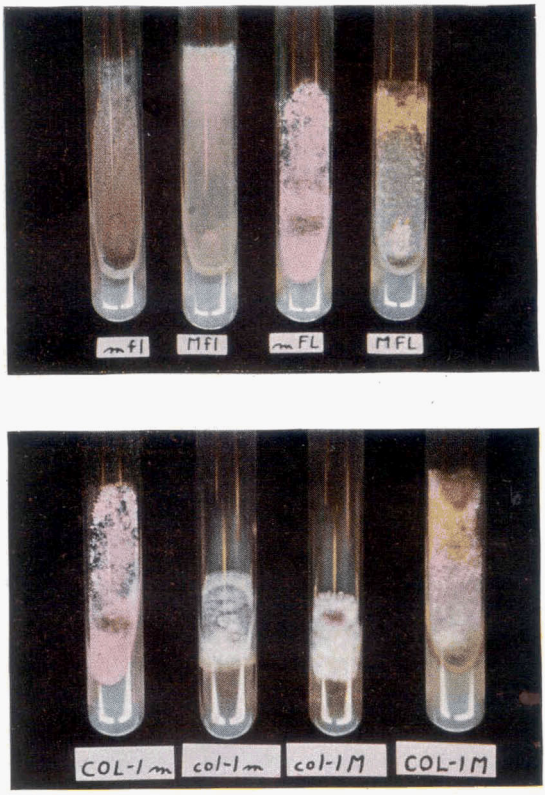

FIGURE 1.—Ascus segregations of *Neurospora crassa*. Upper: segregation types from cross of fluffy with pink-clumpy showing first division segregation of fluffy and second division segregation for microconidial. Left to right, fluffy microconidial (*m*, *fl*), fluffy (*M*, *fl*), pink-clumpy (*m*, *Fl*) and wild type (*M*, *Fl*). Lower: segregation of Y-8743 showing first division segregation of microconidial and second division segregation of colonial-1. Left to right, pink-clumpy (*Col-1*, *m*), colonial microconidial (*col-1*, *m*), colonial sterile (*col-1*, *M*), and wild type (*Col-1*, *M*).

Some interaction of genes was apparent because one of the classes, colonial-sterile (*col-1, M*) although it contains the allele *M*, produces no asexual spores, and another class, pink clumpy (*Col-1, m*) although it contains the allele *m*, produces both macro- and microconidia. In addition to producing both macro- and microconidia, a strain of the genotype *Col-1, m* shows a somewhat different growth habit and coloration from the wild type (see fig. 1). The spores, mostly macroconidia, are borne all over the surface of an agar medium rather than predominantly at the top of a slant or at the outer margin of a Petri plate as in wild type cultures. This distribution of conidia gives the culture a clumpy appearance. Several genes modifying this clumpy character have been noted but have not been investigated. Cultures of the genotype *Col-1, m* are pink

TABLE 2

Chi Square test on random isolates from outcross of Y-8743 (cross 8743-3)

MORPHOLOGICAL CHARACTERIZATION		SYMBOL	OBSERVED FREQUENCY
TYPE OF GROWTH	CONIDIATION		
spreading	macroconidia	*Col-1, M*	20
spreading	macroconidia+microconidia	*Col-1, m*	23
colonial	none	*col-1, M*	15
colonial	microconidia	*col-1, m*	19
		total	77

$\chi^2=1.698$; P=0.78 (on hypothesis that the mutant differs from the wild type by two independently segregating genes).

or peach rather than orange as in wild type. The pink character is always expressed in cultures differing from the wild type by the gene *m*. For these reasons the genotype *Col-1, m* has been called pink clumpy. The genetics of genes colonial-1 (*col-1*) and microconidial (*m*) will be discussed separately.

Gene controlling colonial growth (colonial-1)

To determine whether or not a single gene differentiates the colonial character from the wild type, the usual crosses were made. A colonial-sterile (*col-1, M*) isolate was outcrossed to the wild type (*Col-1, M*) (cross 10) and spores of 21 asci were isolated. Cultures grown from ascospores showed four *col-1, M* and four *Col-1, M* genotypes in each ascus. An intercross (cross 12) between *col-1, m* and *Col-1, m* was made and the spores of 18 asci dissected. Again only the two parental types were recovered. An additional cross was made between two colonials of the genotypes *col-1, m* and *col-1, M*. This colonial by colonial cross produced poor perithecia and all the spores of only two asci germinated. All spores, including those from the complete and incomplete asci, gave rise to cultures of colonial growth type. The above data show that the single gene, colonial-1 (*col-1*), differentiates between a culture derived from Y-8743 having colonial growth and one having spreading growth.

TABLE 3

Centromere data on Col-1 locus

CROSS NUMBER	NUMBER OF ASCI		CENTROMERE DISTANCE	*col-1* PARENT	*Col-1* PARENT
	2ND DIVISION SEGREGATION	TOTAL			
8743-1	5	9	27.8	Y-8743a	1A
8743-8	8	15	26.8	Y-8743-3-2	Y-8743-3-69
8743-10	14	21	33.3	Y-8743-3-17	Y-8743-3-69
8743-12	11	18	30.5	Y-8743-3-3a	Y-8743-3-55A
8743-22	10	17	29.4	Y-8743-2-6A	multiple
8743-25	13	29	22.4	Y-8743-2-6A	44602-1-2a
8743-26	9	17	26.5	Y-8743a	SY-7A
8743-28	4	10	20.0	Y-8743-2-6A	Y-8743-21(1-1)a
8743-29	13	27	24.1	Y-8743-2-6A	34486a
8743-45	5	9	27.8	Y-8743-2-6A	Y-8743-1(8-3)a
SY-8743-1	1	4	12.5	Y-8743-2-6A	SY-4a-f8
Total and average	93	176	26.4		

$\chi^2 = 5.28$; n = 10; P = 0.88.

Table 3 shows the segregation of the *col-1* allele in 176 asci from 11 crosses; *col-1* is located 26.4 map units from the centromere as calculated from data uncorrected for double crossing over. A Chi Square test for homogeneity of the data in the 11 crosses gives a value of 5.28. P calculated from this equals 0.88, which substantiates the data as homogeneous.

MCCLINTOCK (1945) has shown *Neurospora crassa* to have seven chromosomes. GILES (unpublished) has combined characters from five linkage groups (I *al-2* and *sex*, III *tpt-1*, IV *pan*, V *inos*, VI *rib*) into one genotype. In order to determine whether or not gene colonial-1 is in any of these groups, Y-8743 was crossed with this multiple stock (cross 22) and spores of 14 asci isolated. The recombinations of the *col-1* gene with the markers are given in table 4.

TABLE 4

Recombination of genes in "multiple stock" (see text) with col-1

LINKAGE GROUP	GENE	NO. ASCOSPORE PAIRS		% RECOMBINATION
		PARENTAL	RECOMBINATION	
I	*al-2*	23	23	50.0
III	*tpt-1*	26	20	43.5
IV	*pan*	46	0	00.0
V	*inos*	26	20	43.5
VI	*rib*	30	16	35.0

Since the gene *col-1* shows no recombination in 46 ascospore pairs with *pan* and yet shows essentially random recombination with the other four markers, it was placed tentatively in linkage group IV. Crosses were made with cultures having genes known to be in the same arm of linkage group IV as follows: *pdx*, located 10.1 map units from centromere (cross 25); *chol-1*, located 24.4 map units from centromere; and additional data were obtained on linkage with *pan* which is 26.3 units from the centromere (cross 49). No recombinations were observed between *col-1* and *pan* in 194 ascospore pairs (table 5). This obvi-

TABLE 5

Linkage of Col-1 with genes in linkage group IV
(centromere distance Col-1=26.4 units)

GENE CHARACTER MUTANT NUMBER	CENTROMERE DISTANCE*	NO. ASCOSPORE PAIRS			% CROSSINGOVER		CHI SQUARE‡	P
		ISOLATED	OBSERVED RECOMBINED	EXPECTED RECOMBINED**	OBSERVED	EXPECTED**		
Pyridoxineless 44602	10.1	112	24	18.2	21.4	16.3	0.92	0.35
Cholineless 34486	24.4	108	8	2.2	7.4	2.0	7.6	<0.01
Pantothenicless† 5531	26.3	46	0	0.05	0.0	0.1	0.01	
		148	0	0.15	0.0	0.1	0.03	
		194	0	0.20	0.0	0.1	0.04	0.97

* Not corrected for double crossovers.
** Calculated from centromere values.
† Data from two crosses (n=2).
‡ Calculated from chromatid pairs (number ascospore pairs divided by 2).

ously shows good agreement with the expected crossing over based on their centromere distances. The linkage data between *col-1* and *chol-1*, and between *col-1* and *pdx* show poor agreement between the observed and expected crossing over based on centromere data. The *col-1*×*pdx* cross gave 21.4 percent of crossing over while only 16.3 percent was expected based on centromere distances. The *col-1*×*chol-1* cross gave 7.4 percent of crossing over while only 2.0 percent was expected based on the centromere distances. Since the centromere distances are uncorrected for double crossovers, the linkage data no doubt give a more valid estimate of the true gene relationships than that obtained by comparing centromere distances.

It was concluded from these results that the *col-1* locus is in linkage IV, very closely linked (within one unit) with *pan* and about 7.4 units from *chol-1* and 21.4 units from *pdx*.

Gene controlling microconidiation (m)

To determine whether or not a single gene controls microconidiation in strain Y-8743, crosses were made between *Col-1, m* and each of the following biochemical mutants having wild type morphology: *ad* (cross 3254-1), *i-val* (cross 39), and *leu* (cross 36). Altogether, the spores from 76 asci were analyzed. Four pink clumpy (*Col-1, m*) and four wild type cultures (*Col-1, M*) were obtained from each ascus and no other morphological type appeared. In two asci dissected from the cross *col-1, m* by *col-1, M* (previously mentioned) only parental types were recovered. Accordingly, microconidiation is controlled by the single gene *m* in strain Y-8743.

TABLE 6

Centromere data on M locus

CROSS NUMBER	NUMBER OF ASCI		CENTROMERE DISTANCE	*m* PARENT	*M* PARENT
	2ND DIVISION SEGREGATION	TOTAL			
8743-1	2	8	12.5	Y-8743a	1A
8743-11	1	22	25.0	Y-8743-3-53A	Y-8743-3-59a
8743-13	3	7	21.5	Y-8743-3-39A	37401a
8743-14	3	13	11.5	Y-8743-3-3a	Y-5331A
8743-15	4	22	9.1	Y-12504-1-13A	37401a
8743-21	9	18	25.0	Y-8743-14(11-1)a	Fluffy A
8743-22	3	14	10.7	Y-8743-2-6A	multiple a
8743-24	2	14	7.2	Y-8743-13(19-5)a	16117A
8743-25	5	28	8.9	Y-8743-2-6A	44602-1-2a
8743-26	5	13	19.2	Y-8743a	SY-7A
8743-27	1	12	4.2	Y-8743-21(5-3)A	SY-4a
8743-28	3	10	15.0	Y-8743-2-6A	Y-8743-21(1-1)a
8743-29	7	26	13.5	Y-8743-2-6A	34486a
8743-30	4	10	20.0	Y-8743-21(2-1)	Y-8743-24(13-5)
8743-31	2	6	16.7	Y-8743-21(13-7)a	4540A
8743-32	3	9	16.7	Y-8743-21(5-3)A	3416a
8743-34	5	11	22.7	Y-8743-21(13-7)a	4545A
8743-35	11	35	15.7	Y-8743-3-3a	E-15172(2-35-I-9)
8743-36	3	29	5.2	Y-8743-3-39A	33757a
8743-37	5	10	25.0	Y-8743-21(13-7)a	5531A
8743-39	7	22	15.9	Y-8743-3-39A	16117a
8743-40	7	21	16.6	Y-8743-21(5-3)A	33933a
8743-41	2	8	12.5	Y-8743-1(9-1)a	SY-7A
8743-42	1	5	10.0	Y-8743-1(9-2)a	SY-7A
8743-43	1	6	8.3	Y-8743-1(8-3)A	SY-4a
3254-1	14	33	21.2	Y-8743-3-3a	3254-6381-5A
Total and average	113	392	14.4		

$\chi^2 = 26.3$; n = 25; P = 0.40.

Table 6 shows the segregation of *m* in 392 asci from 26 crosses. The gene *m* is located 14.4 units from the centromere as calculated from data uncorrected for double crossing over. A Chi Square test for homogeneity of the data from 26 crosses gives a value of 26.3. P calculated from this equals 0.40.

Gene *m* recombines at random with the five markers in the multiple stock previously described (cross 22) (see table 7), hence does not appear to be located in any of these groups. Wherever possible, these linkage groups were further checked by making additional crosses with strains marked in both

TABLE 7

Recombination of genes in multiple stock (see text) with gene m

LINKAGE GROUP	GENE	NO. ASCOSPORE PAIRS		% RECOMBINATION
		PARENTAL	RECOMBINATION	
I	*al-2*	18	30	62.5
III	*tpt-1*	26	22	45.8
IV	*pan*	32	16	33.3
IV	*col-1*	32	20	39.3
V	*inos*	24	24	50.0
VI	*rib*	34	18	34.6

arms at centromere distances corresponding to that of *m*. The data in table 8 show that the gene *m* is not in linkage groups I, III, IV, V, or VI. Other crosses were made with peach (*pe*) and fluffy (*fl*) in linkage group II; and with *sfo*, the sulfonamide-requiring strain E-15172 (Emerson 1947) which is not in groups I, III, IV, V, VI (Emerson, personal communication) nor in II according to our data, and therefore is assumed to be the first marker in group VII. Gene *m* showed no recombination in 200 ascospore pairs with *pe* and 31.5 percent recombination with *fl*, while it showed random assortment with *sfo* (table 8). Therefore *m* is located in linkage group II,[3] and is closely linked to or identical with peach. Cultures of strains containing the gene *pe* appear the same as cultures containing the gene *m*, thus substantiating their identity. Furthermore, a heterocaryon between *pe*, *ad* and *pan*, *m* on minimal agar results in a culture having the typical morphology of *pe* or *m*. Recalculation of the LINDEGRENS' (1939) recombination data on *pe*, *tu*, and *fl* gives 24.9 percent crossing over between *m* and *fl* (140 asci) (table 8). The centromere values of *m* and *pe*—14.4 and 11.3—show about the same percentage difference (27 percent) as the crossover measurements between *m* and *fl*, and *pe* and *fl*—31.5 to 24.6—or 28 percent. The constancy of the percentage differences between our data and the Lindegrens' suggests that the two sets were collected under different environments such that chiasma frequency was affected. From the above data and reasoning it is concluded that the gene designated *m* throughout this paper is the same locus as *pe*. Since microconidiating stocks designated as *m* were in

[3] Recently in this laboratory a biochemically deficient strain (Y-2492) has been found to be closely linked to fluffy.

TABLE 8

Recombination data of gene m with marker genes in each linkage group (centromere distance gene m = 14.4)

GENE CHARACTER		CENTROMERE DISTANCE MAP UNITS	NO. ASCOSPORE PAIRS		PERCENT RECOMBINATION		
LINKAGE GROUP AND ARM	MUTANT NUMBER		ISO-LATED	RECOM-BINED	OB-SERVED	EXPECTED* RIGHT ARM	EXPECTED* LEFT ARM
I Right	Albino-2 15300	27.4	542	294	54.4	13.0	—
Left	Sex	7.8	129	69	53.5	—	6.6
II Right	Peach (LINDEGREN)	11.2	200	0	0.0	3.2	—
	Fluffy (LINDEGREN)	29.0	559	176	31.5	14.6	—
III Right	Leucineless 33757	8.5	118	70	59	5.9	22.9†
	Tryptophaneless 10575	21.5	48	22	45.9	7.1	—
IV Right	Pyridoxineless 44602	10.1	112	58	51.8	4.3	24.5†
	Cholineless 34486	24.4	108	50	46.3	10.0	—
	Colonial-1 Y-8743	26.4	404	197	48.7	12.0	—
	Pantothenicless 5531	26.3	168	78	46.5	11.9	—
V Right	Isol.-valineless 16117	9.4	132	56	42.4	5.0	—
	Inositolless 37401	26.3	180	80	44.4	11.9	—
Left	Lysineless 33933	3.5	114	64	56.2	17.9†	10.9
VI Right	Adenineless 3254	1.8	66	31	46.9	12.6	16.2†
	Riboflavinless 51602	2.0	52	18	34.6	12.4	16.4†
VII Right	Sulfonamide-requiring E-15172	31.6	136	64	47.1	17.2	—

* On basis of linkage assuming gene *m* on right or left arm as indicated.

† Checked across centromere. These data are much less reliable than indicated because the errors of double crossovers are additive when genes are on opposite sides of centromere.

use in several laboratories prior to this conclusion and the genetics of strain Y-8743 was presented at the Eighth International Congress of Genetics, the authors suggest that this allele be called *pe*^{m} to alleviate future confusion.

Dominance and reversion

The term dominance was first used to describe the condition in a diploid organism heterozygous for a character in which one allele is expressed to the exclusion of the other. Dominance is a measure of allele activity at an arbitrary 1:1 ratio. It gives no quantitative evaluation of the activity such as would be given by the minimum allele ratio at which one allele is expressed to the exclusion of the other. PONTECORVO (1946) has recently discussed in detail the similarities and differences between the diploid condition and that existing in a heterocaryon (dicaryon) in a haploid organism. BEADLE and COONRADT (1944) have shown that in Neurospora the use of heterocaryons provide a general method for studying dominance relations between mutant genes and their normal alleles; and that in a heterocaryon between two mutant strains the nuclear ratio can vary over wide ranges depending upon the component nuclei. They found certain alleles—especially pantothenicless—which led to wild type growth in a heterocaryon when in allele ratios as one-sided as 15:1 and 17:1. In an extension of this approach SANSOME (1947) has pointed out that heterocaryons offer an important new field for the study of gene expression as related to allele dosage.

In the present investigation an experiment using heterocaryons was conducted to determine the dominance relations between *col-1* and *Col-1*, that is, the effect at a 1:1 allele ratio. Mixtures of macro- and microconidia were made in varied ratios (table 9) and inoculated into flasks of liquid complete medium. The microconidia were from a strain of genotype *col-1*, *m*, pregerminated for 24 hours in a complete medium at 25°C. The macroconidia were taken directly from a 48 hour agar slant culture of a strain of genotype *Col-1*, *m*. The flasks were shaken for 72 hours at 25°C in order to provide the opportunity for heterocaryon formation. "Growth tubes" containing a complete agar medium

TABLE 9

Growth rates of heterocaryons between col-1, M and Col-1, m in various nuclear ratios

NO. CONIDIA PER FLASK		RATIO $\frac{\text{MICRO}}{\text{MACRO}}$	PROBABLE NUCLEAR RATIO	GROWTH RATE MM/HR
MICRO (*col-1*)	MACRO (*Col-1*)			
5×10^5	0	∞ :0	∞ :0	0.01
5×10^5	5×10^2	1000:1	100:1	0.55
5×10^5	5×10^3	100:1	10:1	1.12
5×10^5	5×10^4	10:1	1:1	2.95
2.5×10^5	2.5×10^5	1:1	1:10	3.00
5×10^4	5×10^5	1:10	1:100	3.00
0	5×10^5	0:∞	0:∞	3.00

were inoculated with mycelium from the flasks and incubated at 25°C. Inoculation of the growth tubes from the flasks was found to be a necessary step since direct inoculation of the growth tubes by mixtures of macro- and microconidia resulted in the wild type overgrowing the colonial strain regardless of the ratios. The tubes were marked twice daily and the growth rates of the heterocaryons calculated by plotting time in hours against distance in millimeters. Heterocaryons made at each ratio showed straight line plots indicating no selection of either nuclear type during growth along the growth tube. Although no check was made for the actual formation of heterocaryons and no biochemical markers were used to assure heterocaryosis, nor was the nuclear ratio determined at the end of the growth tube; nevertheless, the quantitative response shown in table 9 and the straight line growth curves are considered reasonable evidence for the formation of heterocaryons. The best available estimate of the nuclear ratio is given in column four of table 9 which is calculated on the assumption that a macroconidium contains about ten nuclei (1–20 or more have been observed). It seems reasonable to conclude that at a nuclear ratio of approximately 1:1 with respect to alleles *col-1* and *Col-1*, the wild type allele is dominant.

The dominance relations between gene *m* and its wild type allele *M* have not been investigated.

The *col-1* gene reverts spontaneously to an allele mediating much more rapid growth rate. LINDEGREN and LINDEGREN (1941b) also reported the spontaneous reversion of their colonial strain "button" to the wild type. Results obtained from plating microconidia from a 10 day old culture of genotype *col-1*, *m* show a reversion frequency of the order of magnitude of between 1 in 10^6 and 1 in 10^7 nuclei. Whether or not the reversions are to alleles other than the wild type has not yet been determined. The possibility that the reversion is due to a mutation at another locus giving rise to a suppressor of colonial growth has not been eliminated.

The gene *m* has never been observed to revert to the allele *M* but exhaustive tests have not been made to examine this point.

Other genes affecting colonial growth

Strains having a colonial growth habit have been recorded in the literature several times. LINDEGREN and LINDEGREN (1941b) describe a mutant obtained from ultraviolet treated material called "button" which, they state, produces colonies six mm in diameter. The Lindegrens' data indicate that button differs from the wild type by a single gene. BEADLE and COONRADT (1944) used two colonial mutants (2608 and 3100) and two semi-colonials (221 and 5801) in their heterocaryon studies. They defined a culture as colonial when the growth rate was less than 1.0 percent of normal, and as semi-colonial, when the rate was about 1.0 percent of normal. BONNER (1946), HOROWITZ, HOULAHAN, HUNGATE, and WRIGHT (1946), MCELROY, CUSHING and MILLER (1947), and MILLER and MCELROY (1948) all reported colonial strains derived from material treated with mustard gas and nitrogen mustard.

In this investigation experiments have been made with two other colonial

growing strains (Y-5331 and Y-5296). A heterocaryon between these two strains grows at a wild type rate (Dr. KATHERINE WILSON, personal communication), which shows that the two strains differ genetically. Dr. WILSON has made hyphal tip isolates from the heterocaryon and has demonstrated the presence of each type of nucleus. Colonial-1 and Y-5331 also form a heterocaryon which has wild type growth rate. To date it has been impossible to form a heterocaryon between *col-1* and Y-5331 showing a wild type growth rate. These tests plus data to be presented in the following section prove the three strains to be genetically different. Y-5331 has been termed colonial-2 (*col-2*) and Y-5296, colonial-3 (*col-3*).

Ascospores from 13 asci were isolated from the cross of Y-5331 by a strain having the genotype *Col-2*, *m* (cross 14-2). All asci segregated with four colonial and four spreading growth cultures showing that Y-5331 differs from the wild type by the single gene *col-2*. A similar cross between Y-5296 and *Col-3*, *m* (cross 14-1) was made and the ascospores from six asci were isolated. These asci also showed a typical Mendelian segregation for a single gene difference. Thus Y-5331 and Y-5296 each represent a single gene difference from the wild type. Spontaneous reversion to strains having a more rapid growth rate has been observed in *col-2* cultures. Ascus segregation data suggest that genes *col-2* and *col-3* are both within ten map units of the centromere.

Interaction of genes

It has been shown that the gene *m* in a wild type genotype is expressed as a pink clumpy culture forming both macro- and microconidia but forming predominantly macroconidia. In the presence of *col-1*, the gene *m* results in the production of microconidia only. In the presence of the *M* allele, a culture with the genotype *col-1* is conidialess. Therefore the gene *col-1* affects the phenotypic expression of the alleles *m* and *M*.

Y-5331, differing from the wild type by the single gene *col-2*, is a strain producing neither macro- nor microconidia. Cross 14-2 between Y-5331 (*col-2*) and a pink clumpy stock (*Col-2*, *m*) was made and the spores of 13 asci dissected. The ascus isolations show two genes segregating—*col-2* and *m*—demonstrating that Y-5331 contains the wild type allele *M*. The genotype *col-2*, *m* produces microconidia abundantly. Thus the situation is essentially the same as with *col-1;* in the presence of *m* both colonial genotypes produce microconidia whereas in the presence of *M* both are conidialess.

Y-5296, differing from wild type by the single gene *col-3*, is also a colonial strain producing neither macro- nor microconidia. Cross 14-1 between Y-5296 and a strain known to differ from wild type by the gene *m* was made and the spores from 11 asci isolated. The germination of the ascospores from this cross was poor with all the segregation types represented in only five asci. In these asci no conidiating colonial types were obtained although in several cases the ascus segregation was such that the gene *m* was present with *col-3*. Thus the situation is different with *col-3* than with either *col-1* or *col-2;* since in the presence of *col-3* and either allele *m* or *M*, no conidia are produced.

In strains differing from wild type by the gene albino-2, a small amount of

pinkish pigment is produced in the conidia, especially in cultures exposed to continuous illumination. Miss PATRICIA ST. LAWRENCE (personal communication) has observed that this pigmentation is increased by using glycerol instead of sucrose as a carbon source. She has also observed that no pigment whatever is developed in albino cultures having the gene *m* regardless of the type of media used to date. Thus the gene *m* changes the pigment from orange to pink or peach in otherwise wild type cultures and completely prevents any pigment formation in albino-2.

Microconidia in Neurospora

Microconidiation was first described in *N. sitophila* by DODGE (1930). In a later paper (1932) he reported microconidiation in *N. tetrasperma* and *N. crassa* and found the character to be inherited. He believed that microconidia (microspores or spermatia), although produced by both sexes, represent ancestral male elements which can revert to perform a vegetative function.

DODGE (1930) described microconidia as "minute hyaline spherical to pear-shaped bodies about 2.5 to 3.5 microns in diameter" and observed that they can germinate directly into a hypha, but more slowly than macroconidia. Our measurements of 45 microconidia gave an average size of 2.43×3.98 microns (2.0×3.0 to 3.0×5.0). Our data substantiate the observation that microconidia germinate more slowly than macroconidia. Colonies appear in 36 hours at 35°C from microconidia of spreading strains as contrasted with less than 18 hours at 35°C from macroconidia. Ninety-five percent of the colonies appear within 62 hours at 25°C on a complete medium from microconidia of colonial microconidiating strains.

It is well known that ordinary monilioid conidia, or macroconidia, contain several nuclei (see fig. 2). The reported evidence concerning the nuclear condition of microconidia is somewhat incomplete. DODGE (1932) concluded that "because of their very small size, they (microconidia) are probably, in general, uninucleate." ZICKLER (1937) examined fixed and stained microconidia of *Bombardia lunata*, a closely related genus, and found them to be uninucleate. LINDEGREN and LINDEGREN (1941a, 1941b) and SANSOME, DEMEREC and HOLLAENDER (1945) stated that *N. crassa* produces uninucleate microconidia but offered no evidence.

Inadequate cytological techniques in the past have prevented use of the *direct* method in determining the number of nuclei in Neurospora microconidia but recently well fixed and clearly differentiated preparations have been obtained (fig. 2) with ROBINOW'S techniques (1942, 1944, and personal communication) using acid Giemsa stain.[4] Examination of several thousand microconidia at various ages showed them to be more than 99 percent uninucleate. The number of nuclei in macroconidia, using the same technique, varies from one to 20 or more.

Also an *indirect* method was devised which gives supporting evidence of the

[4] Dr. V. M. CUTTER has also obtained clearly differentiated preparations with the technique of DELAMATER (1948).

uninucleate nature of microconidia. A heterocaryon was established by inoculating the two deficient strains—*col-1*, *m*, *pdx* and *col-1*, *m*, *n-t*[5] onto a minimal agar slant. Microconidia produced from this heterocaryon were plated on a complete medium and 151 colonies isolated. Transfers from these colonies were tested on (1) minimal agar, (2) minimal agar plus nicotinamide (2 micrograms/cc), and (3) minimal agar plus pyridoxine (1 microgram/cc). No colo-

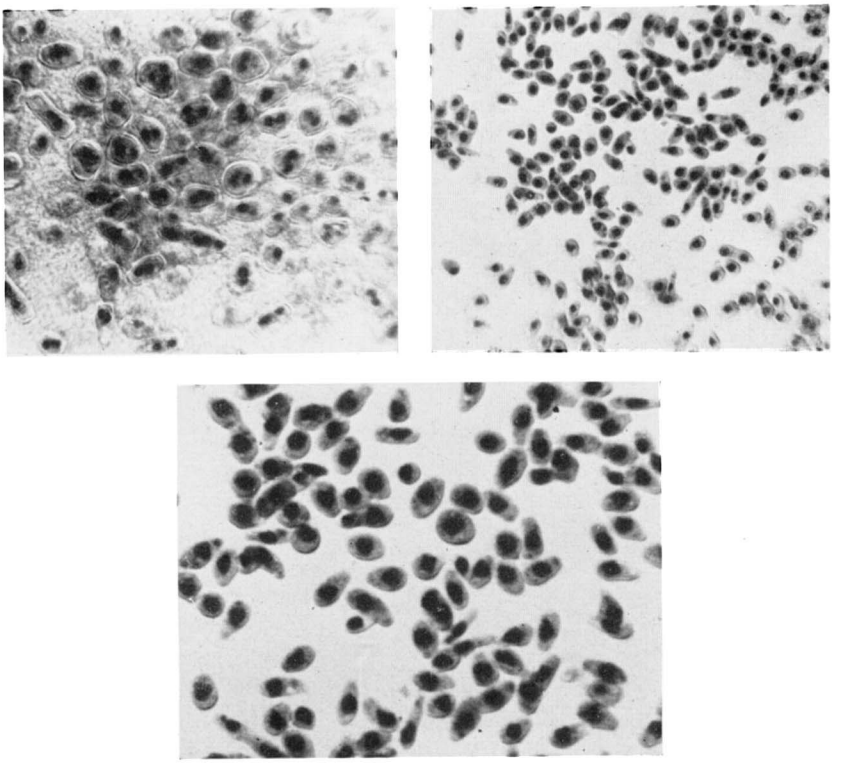

FIGURE 2.—Conidia of *Neurospora crassa*. ROBINOW'S acid Giemsa stain. Upper right: microconidia of strain Y-8743-21(13-7)a showing one nucleus in each conidium. ×1375. Lower: the same, ×2850. Upper left: macroconidia of wild type strain SY 7A showing several nuclei in each conidium. ×1375.

nies grew on the minimal medium (1 above); all grew on either the nicotinamide or pyridoxine supplemented medium (2 or 3); and none grew on both the supplemented media (2 and 3). Therefore, microconidia produced from the genotype *col-1*, *m* are homocaryotic, and probably uninucleate. The experiment was repeated with two strains of the genotype *Col-1*, *m*, *fl*, each marked with a biochemical character. Microconidia from the heterocaryon of *tpt-1* and *lys* were plated directly on (1) a minimal medium, (2) a minimal medium plus

[5] *n-t* is the symbol for nicotinic-tryptophaneless (39401) (Beadle, Mitchell, & Nyc, 1947). The strain used was Y-17000 shown by D. M. BONNER to be the same as 39401 (personal communication).

lysine, and (3) a minimal medium plus tryptophane. Tergitol #7 was added to the media to produce discrete colonies (details to be reported elsewhere). At spore concentrations giving 636 colonies per plate on lysine and 288 colonies per plate on tryptophane, no colonies appeared on the plates containing only the minimal medium. SANSOME very recently (1947) showed somatic segregation from a heterocaryon of two component mutant types each having the gene fluffy by plating microconidia. She concluded that microconidia are "normally uninucleate." This experimental approach can be criticized on the basis that a homocaryotic microconidium may not necessarily be uninucleate. PONTECORVO (1946) stressed the fact that all the nuclei in a macroconidium are of the same genetic type in certain species of fungi known to have multinucleate conidia. Thus all that platings of microconidia from a heterocaryon can prove is that the microconidia are homocaryotic.

LINDEGREN (1933) described a macroconidialess strain, fluffy, which originated as a spontaneous mutation in selfed normal lines. He showed that it differed from wild type by a single gene located in linkage group II (LINDEGREN and LINDEGREN 1939). Fluffy normally produces no macroconidia or microconidia, but when mycelium is moistened and allowed to stand 24 hours, microconidia develop. These facts suggested that a combination of the macroconidialess character fluffy with the gene *m* might produce a new abundantly microconidiating strain with spreading growth habit. A cross of the genotype *col-2, m, Fl* (*col-1, m, Fl* can be substituted, with the same results) was made with fluffy, *Col-2, M, fl* (cross 21), and 20 asci dissected serially. In addition to the parental types, two others appeared having the genotype *col-2, M, Fl* and *Col-2, m, fl* as shown in figure 1. *col-2, M, Fl* is the same as Y-5331 as expected. *Col-2, m, fl* on the other hand, grows in a wild type spreading growth habit since it has the wild type allele *Col-2*, produces no macroconidia since it contains the *fl* gene, and produces microconidia abundantly since it contains the gene *m*.

A striking difference in time of conidiation exists between the microconidiating colonial strains and the microconidiating spreading strains. Y-8743 and Y-8743-2-6A produce microconidia in about ten days after transfer whereas Y-8743-21(13-7)a—a microconidiating spreading strain—produces microconidia on the fourth day. Spreading strains conidiate most abundantly when glycerol is substituted for sucrose as a carbon source in the complete agar medium. The reverse is true for colonial strains. Both types conidiate best at 25°C.

DISCUSSION

As described above, a strain containing one or more of the mutant genes *col-1, col-2,* and *col-3* is conidialess. Thus gene *M* controlling macroconidiation is hypostatic to all three colonial growth-controlling genes. In genotypes containing the gene *m* in addition to *col-1* or *col-2*, microconidia are produced abundantly. *m* is epistatic to or independent of *col-1* and *col-2*. In genotypes containing *col-3* and the allele *m* no microconidia are produced. Thus allele *m* as well as *M* is hypostatic to colonial-3. A plausible interpretation of these relationships is that the action of the colonial genes result in the loss of the

capacity to synthesize some unknown intermediate necessary for the production of macroconidia, the colonial character being a secondary expression of this loss. If this were the case, then in the presence of *col-1* or *col-2*, gene *m* would mediate the production of microconidia. In the presence of the wild type allele of these colonial genes, the action of the gene *m* is suppressed and a pink clumpy culture is obtained. Perhaps this suppression is the result of competition for an intermediate used in the production of macroconidia. Loss of function at the *col-3* locus leads to a block in conidiation prior to an intermediate necessary for the production of *any* conidia. Loss of function at the *fl* locus interrupts the production of macroconidiation without the secondary effect which results in colonial growth. In the presence of both gene *m* and gene *fl*, microconidia are produced abundantly.

Two origins of the alleles *m* and *col-1* should be considered. Either they are spontaneous mutations existing in the stock prior to treatment or were induced by the treatment. At the time when strain Y-8743 was isolated, phenotypically pink clumpy strains frequently (up to eight percent) appeared in isolations from control crosses of strain 1A by 25a. This suggests that the allele *m* occurred as a spontaneous mutation in the 1A or 25a stock and was present in the nucleus in which *Col-1* mutated to *col-1*. Colonial strains have never appeared in isolates from control crosses in this laboratory. Thus the mutation to colonial-1 probably occurred at the time of treatment with the carcinogen.

Strains of Neurospora containing the genes *m* and *col-1*, or *m* and *fl*, have promise as research tools. The colonial character permits direct plating whereas the uninucleate microconidial character allows the rapid establishment of homocaryotic lines. Data to be published elsewhere indicate that microconidia can be treated with mutagens or candidate mutagens and plated directly. The resulting homocaryotic cultures can be tested directly for biochemical mutants. These methods greatly facilitate the screening of chemicals as mutagens and the quantitative evaluation of known mutagens.

Genes controlling biochemical requirements have been introduced into genotypes containing *col-1* and *m*. GILES and E. LEDERBERG (1948) have used these colonial microconidiating strains in studying reversion rates of genes controlling biochemical requirements.

Strains having a wild type growth rate and producing only uninucleate microconidia have been developed by replacing the *col-1* gene with the gene *fl*. These microconidiating strains show promise in determining nuclear ratios in heterocaryons as begun by BEADLE and COONRADT (1944), in studying reversion, and in investigating mutagens.

SUMMARY

1. Strain Y-8743 differs from the wild type by two non-linked genes called colonial-1 (*col-1*) and microconidial (*m*).

2. The gene colonial-1 controls colonial growth and in the absence of *m* results in a conidialess mycelium. Colonial-1 is located in linkage group IV, 26.4 map units from the centromere as calculated from ascus segregation data uncorrected for double crossovers.

3. Gene *m* controls the production of microconidia in the presence of *col-1*. In the presence of the wild type allele (*Col-1*), the result is a pink clumpy culture producing both macro- and microconidia. Gene *m* is located in linkage group II 14.4 units from the centromere as calculated from ascus segregation data uncorrected for double crossovers. Gene *m* either is identical with or an allele of LINDEGREN's gene peach. It is suggested that the allele be designated by the symbol pe^m.

4. Two other independently occurring colonial mutant strains of *Neurospora crassa* were investigated. Colonial growth in both of these strains (designated colonial-2 and colonial-3) is the result of a single gene difference from the wild type, and both differ genetically from colonial-1.

5. Examination of fixed and stained microconidia at various ages showed them to be more than 99 percent uninucleate. Microconidial analysis of heterocaryons demonstrated microconidia to be greater than 99 per cent homocaryotic.

6. When the gene fluffy, whose action prevents macroconidiation, is genetically combined with the microconidial gene, a strain results which produces microconidia exclusively and has a wild type growth rate. Genotypes of Neurospora containing the genes *m*, *col-1*, and *fl* in various combinations are useful as research material.

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LITERATURE CITED

BEADLE, G. W., 1946 Genes and the chemistry of the organism. Am. Scientist **34:** 31–53.

BEADLE, G. W., and V. L. COONRADT, 1944 Heterocaryosis in *Neurospora crassa*. Genetics **29:** 291–308.

BEADLE, G. W., H. K. MITCHELL, and J. F. NYC, 1947 Kynurenine as an intermediate in the formation of nicotinic acid from tryptophane by Neurospora. Proc. nat. Acad. Sci. **33:** 155–158.

BEADLE, G. W., and E. L. TATUM, 1941 Genetic control of biochemical reactions in Neurospora. Proc. nat. Acad. Sci. **27:** 499–506.

1945 Neurospora II. Methods of producing and detecting mutations concerned with nutritional requirements. Amer. Jour. Bot. **32:** 678–686.

BONNER, D., 1946 Biochemical mutations in Neurospora. Cold Spring Harbor Symp. Quant. Biol. **11:** 14–24.

BONNER, D., E. L. TATUM, and G. W. BEADLE, 1943 The genetic control of biochemical reactions in Neurospora: A mutant strain requiring isoleucine and valine. Arch. Biochem. **3:** 71–91.

BUSS, H. R., 1944 The genetics of the methionineless mutants of *Neurospora crassa*. Stanford Univ. Thesis.

DELAMATER, E. D., 1948 Basic fuchsin as a nuclear stain for fungi. Mycologia **40:** 423–429.

DODGE, B. O., 1930 Breeding albinistic strains of the monilia bread mold. Mycologia **22:** 9–38.

1932 The non-sexual and sexual functions of microconidia in Neurospora. Bull. Torrey Bot. Club **59:** 347–360.

DOERMAN, A. H., 1946 Investigations of the lysine-requiring mutants of *Neurospora crassa.* Stanford Univ. Thesis.

EMERSON, S., 1947 Growth responses of a sulfonamide-requiring mutant strain of Neurospora. J. Bact. **54:** 195–207.

GILES, N. H., JR., and E. Z. LEDERBERG, 1948 Induced reversions of biochemical mutants in *Neurospora crassa.* Amer. J. Bot. **35:** 150–157.

HOROWITZ, N. H., N. B. HOULAHAN, M. G. HUNGATE, and B. WRIGHT, 1946 Mustard gas mutations in Neurospora. Science **104:** 233–234.

HOULAHAN, M. B., 1944 The genetics of a group of biochemical mutants of *Neurospora crassa.* Stanford Univ. Thesis.

HOULAHAN, M. B., G. W. BEADLE, and H. G. CALHOUN, 1949 Linkage studies with biochemical mutants of *Neurospora crassa.* Genetics **34** (in press).

HUNGATE, F. P., 1946 The biochemical genetics of a mutant of *Neurospora crassa* requiring serine and glycine. Stanford Univ. Thesis.

HUNGATE, M. V. G., 1945 A genetic study of albino mutants of *Neurospora crassa.* Stanford Univ. Thesis.

LINDEGREN, C. C., 1933 The genetics of Neurospora III. Pure bred stocks and crossing over in *N. crassa.* Bull. Torrey Bot. Club **60:** 133–154.

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

LINDEGREN, C. C., and G. LINDEGREN, 1939 Non-random crossing over in the second chromosome of *Neurospora crassa.* Genetics **24:** 1–7.

1941a X-ray and ultra-violet induced mutations in Neurospora. I. X-ray mutations. J. Heredity **32:** 405–412.

1941b X-ray and ultra-violet induced mutations in Neurospora. II. Ultra-violet mutations. J. Heredity **32:** 435–440.

MCCLINTOCK, B., 1945 Neurospora I. Preliminary observations of the chromosomes of *Neurospora crassa.* Amer. J. Bot. **32:** 671–678.

MCELROY, W. D., J. CUSHING, and H. MILLER, 1947 The induction of biochemical mutations in *Neurospora crassa* by nitrogen mustard. J. C. C. P. **30:** 331–346.

MILLER, H., and W. D. MCELROY, 1948 Factors influencing the mutation rate in Neurospora. Science **107:** 193–194.

PONTECORVO, G., 1946 Genetic systems based on heterocaryosis. Cold Spring Harbor Symp. Quant. Biol. **11:** 193–201.

REGNERY, D., 1947 A study of the leucinelsss mutants of *Neurospora crassa.* Calif. Inst. of Technology Thesis.

ROBINOW, C. F., 1942 A study of the nuclear apparatus of bacteria. Proc. Royal Soc. London B **130:** 299–324.

1944 Cytological observations on *Bact. coli*, *Proteus vulgaris*, and various aerobic spore-forming bacteria with special reference to the nuclear structures. J. Hygiene **43:** 413–423.

SANSOME, E. R., 1947 Somatic segregation by microconidial isolation in systhesized heterokaryons of *Neurospora crassa.* Proc. Royal Soc. Edin. B **62:** 299–303.

SANSOME, E. R., DEMEREC, N., and A. HOLLAENDER, 1945 Quantitative irradiation experiments with *N. crassa.* I. Experiments with X-rays. Amer. J. Bot. **32:** 218–226.

SRB, A. M., 1946 Ornithine-arginine metabolism in Neurospora and its genetical control. Stanford Univ. Thesis.

TEAS, H. J., 1947 The biochemistry and genetics of threonine requiring mutants of *Neurospora crassa.* Cal. Inst. of Technology Thesis.

WESTERGAARD, N., and H. K. MITCHELL, 1947 Neurospora V. A synthetic medium favoring sexual reproduction. Amer. J. Bot. **34:** 573–577.

ZICKLER, H., 1937 Die Spermatienbefruchtung bei *Bombardia lunata.* Ber. d. Deutsch. Bot. Ges. **55:** 114–119.

ZIMMER, E. M., 1946 Mutant strains of *Neurospora* deficient in para-aminobenzoic acid. Stanford Univ. Thesis.