A SURVEY OF NEW MORPHOLOGICAL MUTANTS IN *NEUROSPORA CRASSA*

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THIS investigation of morphological mutants in *Neurospora crassa* was undertaken to examine a wide spectrum of such strains and to obtain information regarding their number and distribution in each of the seven linkage groups. This information, as well as a comparative study of their morphological characteristics, is important both theoretically and practically in facilitating further investigations of the biochemical defects involved.

The present report describes the linkage to known markers of 90 morphological strains of independent origin. Of these, 58 have designated as new loci; the remainder appear to represent alleles either of the new or previously described loci. The order of the genes has been determined only in group V (MORGAN, GARNJOBST and TATUM 1967).

The differences between strains in type of mycelial growth, both from the ascospore and upon transfer, and the distinct differences between some strains in hyphal morphology have suggested a new and practical classification of the morphological mutants. Six classes are defined, illustrated, and discussed.

MATERIALS AND METHODS

Strains: The conidia used in the ultraviolet irradiation experiments were from the inositol requiring strains 89601–3–3*A*, 89601–4–10*a*, or 89601–4–12*A*, all of the heterocaryon genotype *C, D; E* (WILSON and GARNJOBST 1966). These strains were derived from *inos* (89601*a*, from DR. NORMAN GILES) through crosses with wild type SR17–3*A*, as indicated below. SR17–3*A* was obtained from a cross of SY7*A* with LINDEGREN 25*a*.

89601*a* × SR17–3*A* ⟶
1–2*a* (*inos*)

Backcross 1: 89601–1–2*a* × SR17–3*A* ⟶
2–36*a* (*inos, C, D; E*)

Backcross 2: 89601–2–36*a* × SR17–3*A* ⟶
3–24*a*, 3–3*A* (*inos, C, D; E*)

Sib-cross: 89601–3–24*a* × 89601–3–3*A* ⟶
4–12*A*, 4–10*a* (*inos, C, D; E*)

Methods used to obtain mutants: The procedures beginning with the production of conidia to isolation of colonies from irradiated conidia grown in plates containing St. Lawrence sorbose medium are described by LESTER and GROSS (1959). DRS. E. REICH, CAROLYN SLAYMAN, and S. R. GROSS, who isolated all the strains prefixed R (Rockefeller) except the incidental spontaneous mutants, used the inositol-death technique as described by LESTER and GROSS, with only minor differences. The colonies from the sorbose-agar plates were transferred to slants of complete medium (GSC) (VOGEL 1964), and incubated at 30°C.

Selection of mutants for further study: Each mutant was crossed with the wild type, either RL3–8*A* or RL21*a*. These wild-type strains were chosen from among the progeny of LINDEGREN's 25*a* × 1*A*. The strain previously used (SR17–3*A*) proved to produce a rather large number of four-spored asci and for this reason was discarded. This character was not present in the *C, D; E inos* strains selected for the radiation experiments.

Ascospores (50 to 100) were isolated at random from the first cross of each mutant with the wild type and new mutant reisolates selected. When an approximately 1:1 segregation was not obtained, additional crosses with wild type were made in succession, each with a new reisolate. By means of these backcrosses, minor modifiers were sometimes eliminated, and double mutants recognized and separated. Only mutants with morphological characteristics clearly distinguishable from wild type and which showed approximately equal numbers of mutants and wild type in progeny crosses were retained for further study. A few mutants not previously assigned to any linkage group were added to the list: some with the prefix B were originally isolated by DR. VAL WOODWARD at Brookhaven National Laboratory, and others with the prefix S or Y were obtained earlier at Stanford or Yale Universities.

Marker strains: The marker strains selected for determination of linkage were the well known nutritional mutant strains listed in Table 1. Additional markers sometimes were needed and are given in Tables 2 through 8, together with the recombination percentages obtained, in cases of linkage. Morphological mutants were not used except in a few crosses because double mutants often are not readily recognized with certainty, and some morphological mutants fail to cross with one another.

All marker strains used had been backcrossed two or three generations to the wild types RL3–8*A* or RL21*a*, the number depending upon the wild-type origin of the marker strains, and sometimes upon the presence of a spontaneous mutation (aconidial, peach, osmotic, and a few other types). At intervals, new cultures were grown from the lyophilized culture collection of these strains, or were again crossed with the wild type and new reisolates selected. All reisolates of the marker strains were tested in plates containing synthetic crossing medium (WESTERGAARD and MITCHELL 1947) to be sure protoperithecial formation was adequate. The wild-type strains were replaced every three months with new cultures regrown from lyophilized conidia.

Crosses with marker strains: When mutant isolates were obtained giving a clear 1:1 segregation of wild type to mutant, the first cross analyzed was with *al-2; pan-1*. The ascospores of at least 12 asci (sometimes 20) were isolated in order and retained for seven days at 25° before heat shocking. When the number of unripe ascospores shot on the sides of the cross tubes and/or the presence of large ascospores was excessive in either the final cross with wild type or with *al-2; pan-1*, the strain was dropped as unsuitable for this survey, unless an ascospore lethal gene was discovered.

Usually each mutant was crossed with six right arm markers, starred in Table 1, at the same time, using the marker strains as the protoperithecial parents. Except for the first marker cross mentioned, ascospores (at least 100) were isolated at random approximately 14 to 20 days after formation of perithecia. For most of the mutants listed in Tables 2 through 8, all crosses which were fertile were analyzed. Thus, usually 500 random ascospores were tested for each mutant. The presence of the *inos* gene in many of these crosses probably prevented overlooking linkage to this marker.

Heterocaryon tests: Allelism between morphological mutants within each linkage group was tested by heterocaryon tests, especially between strains with similar linkage relations and/or morphological characteristics. Clear-cut results were obtained within 24 to 30 hours at 30° by using plates containing a minimal medium (VOGEL 1964). The pairs were inoculated centrally. Negative complementation results were supplemented by tests with appropriate *C,D;E* tester strains.

Criteria for locus designation: Ideally, the identification of new loci would be based on complete genetic evidence on map location and nonallelism, heterocaryon complementation to give clear-cut wild-type growth, and morphological dissimilarity of strains. It has not always been feasible to meet all these criteria for practical reasons such as infertility of mutant intercrosses and failure of complementation between known nonallelic strains. In some cases, assignment has

been based on two criteria or rarely, only on morphology. No complementation between alleles has yet been found with morphological mutants, including the nine recurrences of crisp, four of ragged, and five of biscuit here reported. Also, in this survey, the use of morphological and genetic criteria has led to the same conclusions on allelism.

It should also be pointed out that for practical reasons, recombination and complementation tests with morphological mutants other than those under study have not been made routinely. The new strains seem to differ from those previously described either in their reported linkage relations or morphological characteristics. In most instances, the morphology of each mutant has been compared directly with that of known mutants with similar descriptions and linkage relations.

RESULTS

Genetic data: The recombination data indicating linkage to known markers, and the centromere distances, are given for each mutant listed in Tables 2 to 5 and 7 to 9. The tables also include the results of mutant intercrosses, and of heterocaryon tests. Table 6 lists group V mutants, for which genetic data are given by MORGAN, GARNJOBST and TATUM (1967).

Classification of morphological mutants: A really useful classification would of necessity take into consideration, at least, the type of growth (1) during development of the mycelium from the ascospore and (2) in a test tube upon transfer. Patterns of growth were also examined (3) in Petri plates at two different temperatures (25° and 34°C) and on two different media (minimal and complete) as well as on a minimal agar medium at the optimum temperature (usually 30°C). These three different methods of distinguishing strains morphologically,

TABLE 1

Description of chief marker strains used in linkage tests

Linkage group and arm	Locus symbol†	Isolation No.	Centromere distance‡
IL	*sex*	.....	6–10
IR*	*al-2*	15300	29–33
IL	*leu-3*	R156	about 28
IIR*	*arg-5*	27947	3–5
IIR	*arom-1*	Y7655	18–28
IIIR	*leu-1*	33757	8–13
IIIR*	*prol-1*	21863	3–7
IVR*	*pan-1*	5531	28–35
IVR	*pdx-1*	37803	8–11
VR	*inos*	89601	27–32
V	*lys-1*	33933	2–7
VIR*	*tryp-2*	S4266	9–18
VIL	*chol-2*	47904	about 21
VIIR*	*nt*	39401	21–32
VIIL	*nic-3*	Y31881	about 20

* Mutants were crossed with these markers usually on the same day. The following double mutants were used: *al-2; pan-1* and *al-2; nic-3*.

‡ Taken from BARRATT *et al.* (1954 compilation) except *leu-3*, *arg-5* and *nic-3* from PERKINS *et al.* (1962).

† Symbols are abbreviations for albino, leucine, arginine, aromatic, proline, pantothenic acid, pyridoxine, inositol, lysine, tryptophan, choline, nicotinic acid or tryptophan, nicotinic acid.

TABLE 2

Description of morphological mutants in linkage group I

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin: treatment and strain	Comments and tests for allelism†	Isolated by‡
colonial-7, *col-7* (1)	S4357	5.7%, *sex* (86 asco.); 27.5%; *al-2* (10 asci); CD 2.6 (38 asci).	Spontaneous, from 37401 × 37401	Phenotypically het⁻ with all strains tested (Y2330, R2386, R2423, R2431).	..
col-11 (1)	R2439	26.1%, *al-2* (10 asci); 50.0%, *sex* (11 asci); CD 35.0 (10 asci).	UV, 89601	Distal from *al-2*.	ER
col-12 (1)	R2440	21.6%, *sex* (76 asco.); 13.6%, *sex* (20 asci); 31.8%, *al-2* (11 asci); CD 27.4 (32 asci).	UV, 89601	Slow growth from ascospores.	ER
crisp-1, *cr-1* (5)	S4358	10.5%, *sex* (95 asco.); CD 7.1 (24 asci), R. arm.	Spontaneous, from 37401 × 37401	.....§	..
cr-1 (5)	R2103	20%, *sex* (88 asci); CD 5.0 (10 asci), R.	UV, 89601	.....	SG
cr-1 (5)	R2104	17.4%, *sex* (80 asco.); CD 6.3 (8 asci), R.	UV, 89601	.....	SG
cr-1 (5)	R2360	4.3%, *sex* (12 asci); CD 4.2 (36 asci), R.	UV, 89601	Located also by PERKINS *et al.* (1962)	SG

cr-1 (5)	R2412	13.3%, *sex* (44 asco.), R.	UV, 89601	"Fine-grained" crisp.	CWS
cr-1 (5)	R2433	0%, *sex* (10 asci); CD 5.0 (20 asci), R.	UV Perkins *a*, wild type	.....	CWS
cr-1 (5)	R2476	8.0%, *sex* (50 asco.); CD 5.0 (10 asci), R.	UV, 89601	.....	ER
cr-1 (5)	R2482	25.0%, *sex* (12 asci); CD 4.1 (12 asci), R.	UV, 89601	Cresent of conidia at top of slant and conidia over surface of slant.	ER
cr-1 (5)	R2501	12.6%, *sex* (48 asco.); CD 6.3 (10 asci), R.	UV, 89601	.....	ER
crisp-2, *cr-2* (5)	R2445	14.0%, *sex* (47 asco.); CD 10.0 (25 asci).	UV, 89601	Pale pigment, delayed condiation, fine conidia in clumps over agar surface. See GARNJOBST and TATUM (1968).	ER
crisp-3, *cr-3* (5)	R2509	26.8%, *sex* (41 asco.); CD 12.5 (12 asci).	UV, 89601	Pale pigment, delayed conidiation (later than *cr-2*), fine conidia uniform over agar surface. See GARNJOBST and TATUM, (1968).	ER
frost, *fr* (5)	R2499	28.5%, *sex* (63 asco.); 25.7%, *leu-3* (77 asco.); CD 20.4 (27 asci).	UV, 89601	Original (Class 1) is a double mutant: *fr* and a modifier of *fr*. Probably an allele of B110. R2499 × B110 not fertile. + B110, not w.t.	ER

TABLE 2—Continued

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin; treatment and strain	Comments and tests for allelism†	Isolated by‡
morphological-1, *mo-1* (4)	R2436	9.0%, *sex* (65 asco.); 50.0%, *al-2* (12 asci); CD 8.3 (12 asci).	UV, 89601	Slow growth from ascospores.	ER
mo-5 (4)	R2487	20.0%, *sex* (50 asco.); 35.0%, *al-2* (10 asci); CD 14.7 (17 asci).	UV, 89601	Produces few conidia; sometimes an exudate at top of slant.	ER
ragged, *rg* (1)	R2357	6.8%, *sex* (29 asci); 30.9%, *al-2* (84 asco.); CD 1.1 (30 asci).	UV, 89601	Allele of B53 (0 w.t./187 asco.). Located also by PERKINS *et al.* (1962) and named erupt, *er.*	SG
rg (1)	R2506	15.0%, *sex;* 13.5%, *sex* (37 asco.); 30.0%, *al-2* (10 asci); 10 asci all 1st div.	UV, 89601	Allele of B53 (× B53, 0 w.t./68 asco.; 10 asci, all parental).	ER
rg (1)	R2513	27.5%, *sex* 25.0%, *al-2* (10 asci); CD 10.0 (10 asci).	UV, 89601	Allele of B53 (× B53, 0 w.t./85 asco.).	ER
rg (1)	R2530rg	23.2%, *sex* (43 asco.); 30.0%, *sex* (10 asci); 18 asci all 1st div.	UV, 89601	R2530 original is a double mutant: a colonial (*rg*) and a flat morph. (*spco-13*) linked to *tryp-2* in VI. Allele of B53 (× B53, not fertile). Results of heterocaryon tests between all *rg* strains, not w.t.	ER
ropy-6, *ro-6* (5)	R2431	12.1%, *sex* (99 asco.); CD 3.8 (13 asci).	UV, 89601	Allele of snowflake, *sn* (?) + Y2330, + R2423, w.t.	CWS

semi-colonial-1, *smco-1* (3)	Y2330	4.3%, *sex* (12 asci); 0% *sex* (85 asco.); CD 10.0 (20 asci), R.	Nitrogen mustard; LINDEGREN 1*A*	× S4357, 0 w.t./72 asco. + R2436, w.t.	RWB
smco-2 (3)	R2386	20.0%, *rg* (10 asci); CD 11.1 (34 asci).	UV, 89601	× S4357, 14 w.t./63 asco. × Y2330, 15 w.t./37 asco. + S4357, not w.t. + Y2330, + R2523, both w.t.	SG
smco-3 (3)	R2423	9.5%, *sex*; 28.9%, *al-2* (20 asci).	UV, 89601	× S4357, 2 w.t./12 asco. × Y2330, 10 w.t./74 asco. × R2386, 1 w.t./19 asco. + S4357, not w.t. + Y2330, + R2436, w.t.	CWS
smco-5 (3)	R2442	2.5%, *sex* (40 asco.); 18.1%, *al-2* (11 asci); CD 13.6 (11 asci).	UV, 89601	Morphological growth only for 4–7 days after ascospore germination. × S4357, 25 w.t./71 asco. × Y2330, 14 w.t./43 asco. × R2386, 16 w.t./54 asco. × R2423, not fertile.	ER
spco-11 (2)	R2502	16.6%, *sex* (9 asci); CD 21.0 (19 asci).	UV, 89601	Occasionally produces conidia at top of slant. Original is a double mutant: *spco-11* and an *al-2* allele. × R2487, 14 w.t./76 asco. + R2487, w.t.	ER
spco-12 (2)	R2510	25.0%, *sex* and 35.0%, *al-2* (10 asci); 20.0%, *sex* (80 asco.); 22.0%, *al-2* (80 asco.).	UV, 89601	Downy center and "lacy" growing border. X R2487, 1 w.t./41 asco.; × R2502, 15 w.t./69 asco. + R2487, not w.t.; + R2502, w.t.	ER

Abbreviations in this and the following tables: asco: ascospores (isolated at random); CD: centromere distance; 1st div.: first division segregation; het-: heterocaryon incompatible; R. (or L.) (following centromere distance): right (or left) arm of linkage group; UV: ultraviolet light; w.t.: wild type.

* Classes are: (1) true colonials (*col*); (2) spreading colonials (*spco*); (3) semi-colonials (*smco*); (4) miscellaneous spreading morphologicals not previously named (*mo*); (5) miscellaneous morphologicals previously given descriptive names; (6) environmentally influenced morphologicals (*moe*), including temperature-sensitive colonials (*cot*).

† In this and succeeding tables + indicates heterocaryon tests, × indicates crosses.

‡ The initials refer to persons who isolated the strains: R. W. BARRATT, R. COLBURN, E. DIACUMAKOS, S. GROSS, J. NADING, E. REICH, C. W. SLAYMAN, and V. WOODWARD.

§ In all combinations of *cr-1* strains, heterocaryons not wild-type.

TABLE 3

Description of morphological mutants in linkage group II

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin: treatment and strain	Comments and tests for allelism†	Isolated by‡
balloon, *bal* (1)	R2409	0%, *arg-5* (11 asci); 37.2, *arom-1* (172 asco.); 11 asci all 1st div.	Spontaneous, from abn-1. + S1441 × RL3-8*A* wild type.	Conidia rarely produced at top of slant. Probably an allele of *bal* (B56) (× B56, 0 w.t./85 asco.; + B56, not w.t.).	. .
col-10 (1)	R2438	27.7%, *arg-5* (180 asco.); CD 25.0 (10 asci).	UV, 89601	Slow growing, compact; easily scored in liquid media. × B56, 5 tetratype and 5 parental asci. × R2409, 4 tetratype and 6 parental asci.	ER
temperature-sensitive colonial-5, *cot-5* (6, 1 at 30°)	R2446	23.8%, *arg-5* (88 asco.); CD 22.7 (11 asci).	UV, 89601	Older colony forms short aerial hyphae at 25° and 30°; very little if any growth at 34°. Reverts readily. + R2438, w.t. (48 hr).	ER
cot-5 (6, 1 at 30°)	R2454	20.7%, *arg-5* (82 asco.); CD 20 (10 asci).	UV, 89601	Phenotype similar to R2446. × R2446, not fertile. + R2446, not w.t.	ER
cot-5 (6, 1 at 30°)	R2479	16.7%, *arg-5* (79 asco.); CD 25.0 (10 asci).	UV, 89601	Phenotype similar to R2446 and R2479. × R2446, × R2454, not fertile. + R2446, + R2454, not wild type.	ER

dapple, *da* (3)	R2375	Near centromere (PERKINS *et al.* 1962).	UV, 89601	.....	SG
ro-3 (5)	R2354	CD 18.0, L. arm (located by PERKINS *et al.* 1962).	UV, 89601	Curled hyphae and clumps of conidia at top of slant.	SG
ro-7 (5)	R2470	24.3%, *arg-5* (189 asco.); CD 25.0 (12 asci).	UV, 89601	+ R2354, w.t.; + R2438, w.t.	ER
ro-9 (5)	R2526	8.4%, *arg-5* (95 asco.); 13 asci all 1st div.	Spontaneous, from R2360 × RL21*a*	.....	..
spco-14 (2)	R2536	6.7%, *arg-5* (74 asco.); 10 asci all 1st div.	UV, 89601	Few scattered conidia. + R2375, + R2409, w.t.	LN

* Classes are: (1) true colonials (*col*); (2) spreading colonials (*spco*); (3) semi-colonials (*smco*); (4) miscellaneous spreading morphologicals not previously named (*mo*); (5) miscellaneous morphologicals previously given descriptive names; (6) environmentally influenced morphologicals (*moe*), including temperature-sensitive colonials (*col*).
See Table 2 for other footnotes and abbreviations.

TABLE 4

Description of morphological mutants in linkage group III

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin: treatment and strain	Comments and tests for allelism†	Isolated by‡
col-13 (1)	R2471	4.0%, *tyr-1* (48 asco.); CD 30.0 (10 asci).	UV, 89601	Occasionally forms short flare of hyphae and conidia at top of slant.	ER
col-14‖ (1)	R2503	8.3%, *prol-1* (12 asci); 12 asci all 1st div.	UV, 89601	Flat colony, yellow pigment, no conidia. × B54, 1 tetratype and 9 parental asci.	ER
col-15 (1)	R2531	5.0%, *tyr-1* (57 asco.); CD 35.0 (10 asci).	Spontaneous, from poky (*mi-1*) × RL21*a* w.t.	× R2471, 0 w.t./181 asco. + R2471, w.t. Close to but probably not an allele of R2471.	ER
col-16‖ (1)	R2539	9.5%, *prol-1* (63 asco.); CD 5.0 (10 asci).	UV, 89601	× R2503, 6 w.t./93 asco. + R2503, w.t. (48 hr).	ER
mo-4‖	R2467	10.0%, *prol-1* (90 asco.); 14 asci all 1st div.	UV, 89601	× *spg*, 6 w.t./150 asco. + R2539, w.t. + *spg*, w.t. + R2503, w.t. (slow)	ER
spco-15‖ (2)	R2537	10.3%, *spg* (97 asco.); 18.1%, *prol-1* (66 asco.); 10 asci all 1st div.	Spontaneous, from 37401 × 37401	× B54, not fertile. × R2503, 5 w.t./73 asco. × R2539, 9 w.t./90 asco. + R2467, + R2503, + R2539, w.t.	..

‖ These strains not allelic by heterocaryon tests.
See Table 2 for footnotes and abbreviations.

TABLE 5

Description of morphological mutants in linkage group IV

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin: treatment and strain	Comments and tests for allelism†	Isolated by‡
col-5 (1)	B28	25.0%, *pan-1* (10 asci); CD 26.4 (36 asci).	UV, ST74*A*	Reverts readily; not a good marker	VW
col-6 (1)	S1302	21.0%, *pan-1* (76 asco.); 28 asci all 1st div.	UV, Y8743–17 (13–7)*a*	Slow germination of ascospores in all crosses.	RWB ELT
col-8 (1)	R2356	13.2%, *pan-1* (83 asco.); 4.1%, *pan-1* (12 asci); CD 30.9 (34 asci), R.	UV, 89601	Forms fluff of hyphae at top of slant. × B28, not fertile.	SG
col-8 (1)	R2523	13.4%, *pan-1* (52 asci); CD 29.8 (82 asci), R.	UV, 89601	Flat, slow-growing colony, yellow pigment. Probably an allele of R2356 (× R2356, not fertile in four attempts); + R2356, not w.t.	ER
cot-3 (6,1 at 34°)	R2006	25.0%, *pan-1* (88 asco.); 10.6%, *cot-1* (66 asco.); CD 25.0 (10 asci).	UV, 89601	Colonial growth at 34°; wild-type growth at 25°. × C102 (*cot-1*), 7 w.t./66 asco. + *cot-1*, w.t.	SG
ascospore-lethal-1 *le-1* (5,1)	B55	1.4%, *pan-1* (200 asco., 35.2% germ.); CD 24.6 (44 asci), R.	UV, ST74*A*	Normal appearing ascospores; no germination except after special treatment (see text).	VW

TABLE 5—Continued

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin: treatment and strain	Comments and tests for allelism†	Isolated by‡
le-1 (5,1)	S4355le	2.5%, *pan-1* (20 asci); CD 16.6 (× ST74*A*, 18 asci); CD 26.2 (× *pan-1*, 21 asci).	UV, Y8743–17 (13–7)*a*	Allele of B55. Distinctive flat mycelium, no conidia; ascospores normal in appearance; strain has three mutant genes *m, fl, le-1*).	RC
medusa, *med* (5)	R2401	8.7%, *pan-1* (21 asci); 7.7%, *pan-1* (90 asco.); 5.0%, *me-5* (88 asco.); CD 20.3 (32 asci).	Spontaneous, from cross abn-1 × RL3–8*A* wild type	Slow growing, spreading, with distinctive grooves on agar surface. (Figure 18).	..
ropy-like-1, *rol-1* (5)	B31	0%, *pdx-1* (88 asco.); CD 14.7 (44 asci), R.	UV, ST74*A*	.....	VW
smco-4 (3)	R2435	7.5%, *pan-1* (20 asci); CD 30.0 (20 asci), R.	UV, 89601	Original strain is a double mutant: ascospore-lethal; morph. not linked to lethal character.	ER
smco-8 (3)	R2505	7.1%, *pan-1* (17 asci); 1.3%, *pan-1* (73 asco.); CD 33.1 (28 asci), R.	UV, 89601	Sometimes flares out at top of slant. × R2435, 12 w.t./84 asco. + R2435, w.t. + Y8743c (col-1), w.t. + R2356, + R2523, w.t.	ER
smco-9 (3)	R2508	2.5%, *pan-1* (10 asci); 23.8%, *spco-1* (10 asci); CD 29.0 (31 asci), R.	UV, 89601	× B148 (*spco-1*), 5 pr. w.t./10 asci; × R2435, 1 w.t./59 asco.; × R2505, 11 w.t./85 asco. + Y8743c, + R2356, + R2435, + R2505, + R2523, w.t.	ER
spco-8 (2)	R2462	23.4%, *pan-1* (24 asci); CD 31.3 (24 asci).	UV, 89601	.....	ER

See Table 2 for footnotes and abbreviations.

TABLE 6

*Classification of morphological mutants in linkage group V**

Name and locus symbol; (class)†	Strain isolation No.	Origin: treatment and strain	Comments	Isolated by‡
biscuit, *bis* (5,1)	R2413	UV, 89601	Allele of B6, *bis*.	CWS
bis (5,1)	R2452	UV, 89601	Allele of B6, *bis*.	ER
bis (5,1)	R2460	UV, 89601	Allele of B6, *bis*.	ER
bis (5,1)	R2465	UV, 89601	Allele of B6, *bis*.	ER
bis (5,1)	R2475	UV, 89601	Allele of B6, *bis*.	ER
col-9 (1)	R2417	UV, 89601	Small, slow-growing colony. Reverts readily.	CWS
cot-2 (6,1 at 34°)	R1006	UV, 89601	Not quite wild-type morphology at 25°.	SG
cot-4 (6,1 at 34°)	R2101	UV, 89601	Mycelium intermediate between colonial and wild type at 25°.	SG
ro-5 (5)	R2428	UV, 89601	.....	CWS
ro-8 (5)	R2520	UV, 89601	.....	ER
rol-3 (5)	R2498	UV, 89601	.....	ER
shallow *sh* (5)	R2371	UV, 89601	Located and named by PERKINS *et al.* (1962).	SG
smco-6 (3)	R2477	UV, 89601	.....	ER
smco-7 (4)	R2497	UV, 89601	Crescent of conidia at top of slant.	ER
spco-3 (2)	R2365	UV, 89601	.....	SG
spco-9 (2)	R2480	UV, 89601	.....	ER
spco-10 (2)	R2488	UV, 89601	.....	ER
washed, *wa* (2)	R2359	UV, 89601	Located and named by PERKINS *et al.* (1962). Our first designation as *spco-2* now omitted from *spco* series.	SG

* For linkage and order of genes, see MORGAN, GARNJOBST and TATUM (1967).
† Classes are: (1) true colonials (*col*); (2) spreading colonials (*spco*); (3) semi-colonials (*smco*); (4) miscellaneous spreading morphologicals not previously named (*mo*); (5) miscellaneous morphologicals previously given descriptive names; (6) environmentally influenced morphologicals (*moe*), including temperature-sensitive colonials (*cot*).
‡ See Table 2.

together with the availability and examination of a large number of mutants have suggested that a new classification might now be feasible and useful.

At a time when few morphological mutants were known, it seemed adequate to divide them into colonial and non-colonial types. A precedent was set by C. C. LINDEGREN in Neurospora in naming mutant strains according to an outstanding,

TABLE 7

Description of morphological mutants in linkage group VI

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin: treatment and strain	Comments and tests for allelism†	Isolated by‡
mɔe-2 (6)	R2532	13.7%, *tryp-2* (87 asco.); CD 5.2 (20 asci).	UV, 89601	. . . . .	ER
spco-7 (2)	R2457	21.4%, *tryp-2* (98 asco.); 10 asci all 1st div.	UV, 89601	Acon dial. × R2532, not fertile. + R2532, w.t.	ER
spco-13 (2)	R2530spco	15.7%, *tryp-2* (95 asco.); CD 5.0 (10 asci).	UV, 89601	R2530 original strain is a double mutant (*rg* in I and *spco-13*). × R2457, × R2532, not fertile. + R2457, + R2532, not w.t. (R2530spco phenotypically het⁻).	ER

These three strains are assigned to different loci chiefly because of distinct differences in morphology.
See Table 2 for footnotes and abbreviations.

readily distinguishable character. However, now that the list of morphological strains has grown, it has become increasingly difficult to assign meaningful names to them as well as to select short locus symbols that avoid letter combinations not already in use.

Although the following more or less empirical classification based on visible characteristics may not be completely adequate, it seems both feasible and practical to follow it until the biochemical differences are known. Even so, the visible differences probably will continue to be useful.

Class 1 includes the true colonials (Figures 1–3), those that retain the restricted growth at the optimum temperature. Although some of these are readily distinguishable from one another as, for example, balloon (*bal*) and colonial-2 (*col-2*) (smooth versus a pebbled surface), some others are not. Therefore, the series begun but later abandoned, i.e., *col-1*, *col-2*, *col-3*, etc., has been readopted. The first new colonial mutant listed in the tables is *col-5*.

Class 2 comprises the so-called spreading colonials (*spco*) (Figures 4–6). These strains begin as a colony (from the ascospore or upon transfer), but growth does not remain restricted; it slowly spreads over the surface of a slant, retaining essentially the same type of morphological growth. For this reason, the strain previously known as *col-4* (B148), a spreading colonial type, has been included in Class 2, and the locus redesignated in this paper as *spco-1*.

Class 3 consists of the semi-colonials (*smco*), which begin as a small colony (from the ascospore and upon transfer) and sooner or later produce a flare of wild type-appearing hyphae (with or without conidia) (Figures 7–9). The presence or absence of a flare in some isolates of a strain appears to depend upon the degree of moisture present and/or an oxygen or CO_2 gradient as in a test tube. For example, scoring often becomes difficult in cultures grown from isolated ascospores held for five to seven days for ripening.

It is necessary to point out that under changing environmental conditions such as differences in light, temperature, and especially moisture, some strains, or some reisolates thereof, assigned to Class 1 may resemble those of Class 3. When the mycelium is near the top of the slant, a short hyphal flare and clumps of conidia may be formed, though rarely. This response is not known to occur in such strains at a constant temperature of 30°, and for this reason these strains are retained in Class 1.

Class 4 is a miscellaneous group of spreading morphological (*mo*) strains readily distinguishable from the wild type (Figures 10–12). The morphological differences in these are not easily defined; they are sometimes described as scanty, or they have fine hyphae and reduced conidiation.

Class 5 also is a miscellaneous group, each strain having a distinctive type of mycelial growth (Figures 13–18). The descriptive published names, such as crisp, ropy, frost, ragged, etc., have been retained, and this method of naming could be extended in the future to other strains equally distinctive. The locus symbol of strains previously known as crisp, *cr* has been redesignated *cr-1*.

Class 6 includes the colonial temperature-sensitive strains (*cot*) and other

TABLE 8

Description of morphological mutants in linkage group VII

Name and locus symbol, (class)*	Strain isolation No.	Percent recombination with known marker; centromere distance (CD)	Origin: treatment and strain	Comments and tests for allelism†	Isolated by‡
colonial-17, *col-17* (1)	B5	13.7%, *nt* (175 asco.); 22.0%, *do* (9 asci); CD 17.4 (46 asci).	UV, ST74*A*	Very slow growing colony. × R2450, 5.5% recom. (9 asci).	VW
environment sensitive morphological-1, *moe-1* (6)	Y6821	5.0%, *nt* (10 asci); CD 27.5 (40 asci), R.	Methylcolanthrene (1*A* conidia × 25*a*)	Spreading mycelium; pale yellow pigment; aconidial; wide collar at top of slant.‖	RWB
moe-1 (6)	R2408	11.7%, *nt* (77 asco.).	Spont. from cross LINDEGREN 1*A* × 25*a* w.t.	An allele of Y6821.‖	
moe-1 (6)	R2529	18.9%, *nt* (74 asco.).	Spont., 37401 × RL21*a* w.t.	Allele of Y6821 and R2408.‖	ED
le-2 (5,1)	R2411	6.7%, *me-7* (45 asco.); 43.0%, *pan-1* (15 asci); CD 7.6 (33 asci), L.	UV, 89601	Ascospores normal in appearance; germination rare (2 or 3/100 asco. in some crosses).	CWS
mo-2 (4)	R2464	29.3%, *nt* (82 asco.); CD 17.0 (9 asci).	UV, 89601	.....	ER
mo-3 (4)	R2466	4.5%, *nt* (66 asco.); CD 30.0 (10 asci); R.	UV, 89601	Fine hyphae, scant mycelium.	ER

rol-2 (5)	R2459	24.2%, *nt* (95 asco.); CD 5.0 (20 asci).	UV, 89601	.....	ER
spco-4 (2)	R2367	Linked to *nic-3* (PERKINS, pers. commun. 1964), L.	UV, 89601	.....	SR
spco-4 (2)	R2481	25.5%, *me-7* (90 asco.); 41.3%, *nt* (87 asco.); 15.2%, *nic-3* (86 asco.); CD 30.5 (52 asci).	UV, 89601	Aconidial, fine hyphae. Allele of R2367 (0 w.t./257 asco.) (+ R2367, not w.t.).	ER
spco-5 (2)	R2450	19.7%, *nt* (91 asco.); 25.0%, *do* (10 asci); 5.5%, *col-4* (9 asci); CD 3.2 (47 asci).	UV, 89601	× B5, 1 tetratype and 8 parental asci. + Y5331 (*col-2*), + R2481, w.t. + B5, not w.t.	ER
spco-6 (2)	R2456	22.0%, *nt* (92 asco.); 10.0%, *do* (10 asci); 22.2%, *col-2* (9 asci); CD 5.2 (48 asci).	UV, 89601	× Y5331, 4 tetratype and 9 parental asci. × R2450, 7 w.t./93 asco. +B5, + Y5331, + R2450, + R2481, w.t.	ER

‖ In all combinations, crosses not fertile; results of heterocaryon tests, not wild-type.
See Table 2 for other footnotes and abbreviations.

TABLE 9

Unusual or incompletely analyzed morphological mutants

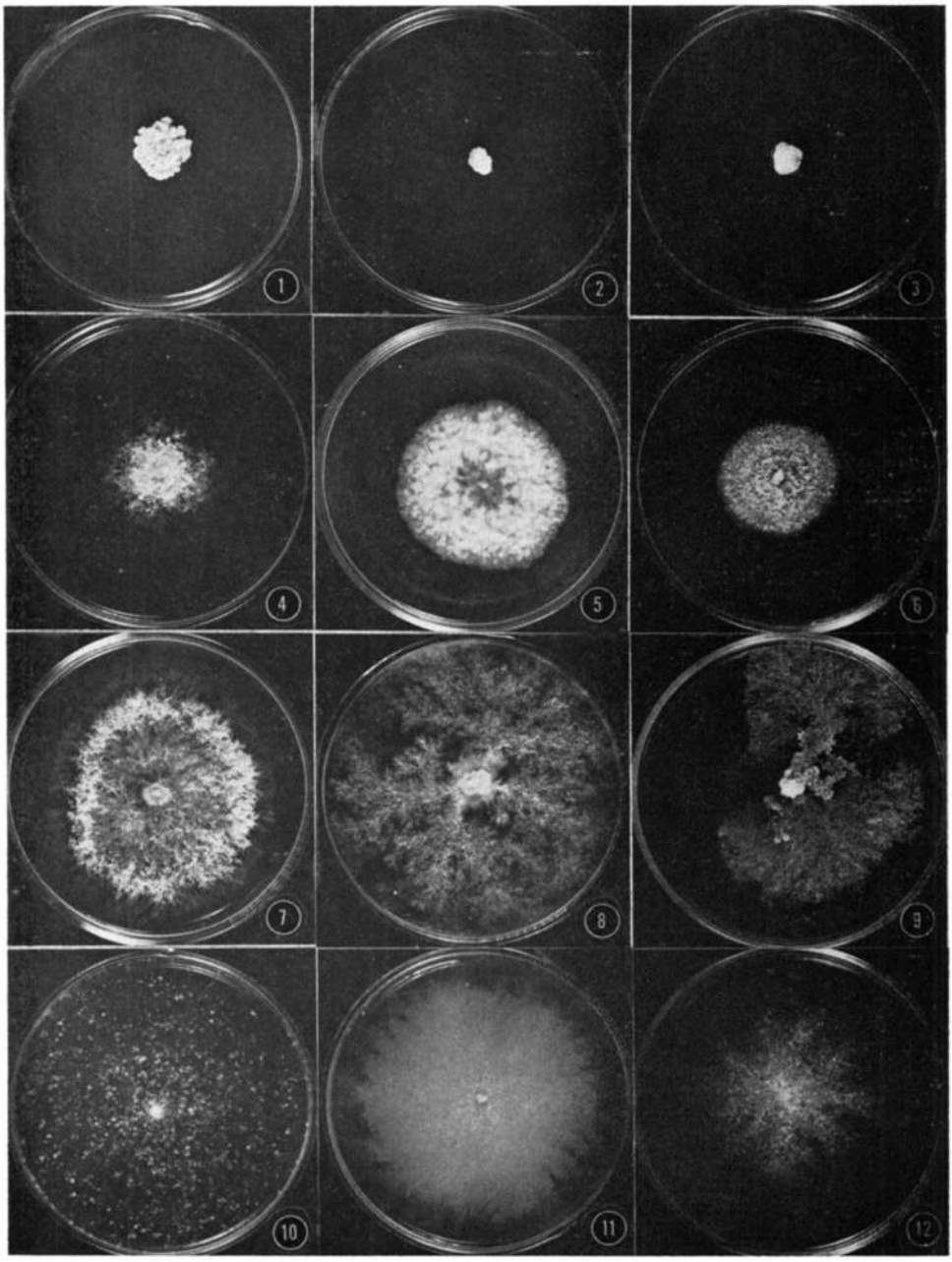
Strain isolation No., (class)*	Origin: treatment and strain	Description and comments	Isolated by‡
R2361 (5)	UV, 89601	36.7% recom., *sex* (15 asci); exudate; possibly allele of os.	SG
R2394 (2)	UV, 89601	Aberration: linked to *sex* and *inos* (on basis of wild-type segregants). Shows no linkage to *me-3*.	ER
R2403 (2)	UV, 89601	30.9% recom., *arom-1* (71 asco.). 45.0%, *arg-5*; CD 22.5 (20 asci), R. Markers in III, VI not tested; not on R. of I, II, IV, V, VI.	CWS
R2406 (5)	UV, Perkins *a* (wild type)	CD 28.6 (21 asci); exudate.	CWS
R2441 (4)	UV, 89601	Very slow growth; no cross walls present. Occasionally better growth and few conidia and some cross walls.	ER
R2472 (4)	UV, 89601	Suspected aberration: Many white spores expelled. Cross × wild type gave a 1:1 segregation of mutant to wild type (70 asco.). Darkens agar.	ER
R2473 (5)	UV, 89601	29.1% recom., *al-2* (12 asci); 34.6% recom., *al-2* (98 asco.). CD 29.1 (12 asci). R2473 × *os* (49 asco.), no wild-type progeny.	CWS
R2499fr mod (4)	UV, 89601	CD 18.2 (52 asci). More exudate at top of slant and under agar on synthetic crossing medium than on minimal or complete media. Original is a colonial double mutant (mod, *fr*).	ER

*, ‡ See Table 2 for footnotes and abbreviations.

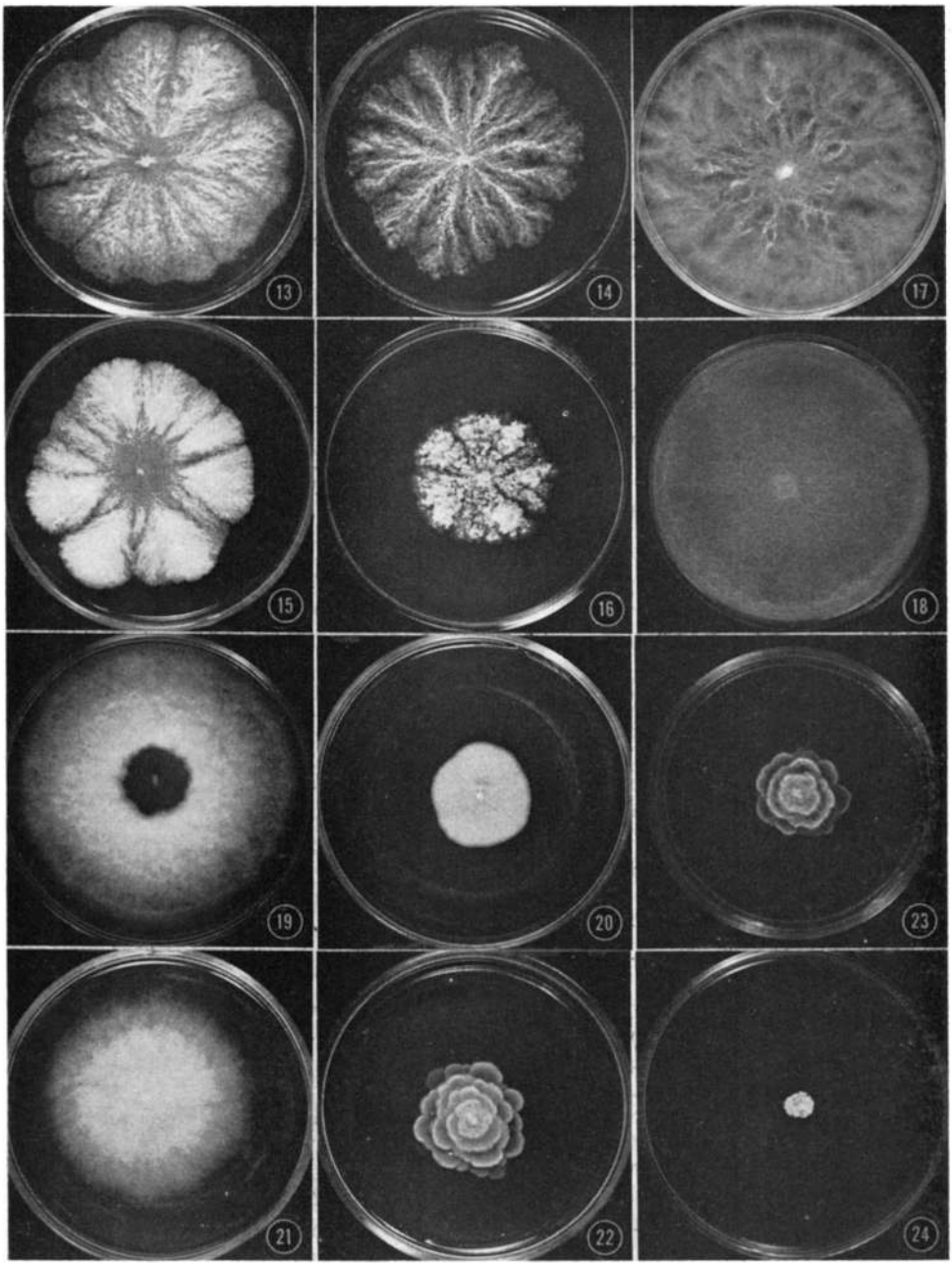
morphologicals environmentally (*moe*) readily influenced in their manner of growth by factors including moisture, pH and temperature. These strains are also classified in the tables according to type of growth on a minimal medium at various temperatures (Figures 19–24).

Although it is realized that instances of allelism between temperature-sensitive and temperature-insensitive strains may eventually be found, no such allelism has yet been found among morphological mutants. Therefore it seems practical at this time to retain Class 6. According to this classification, the locus symbol of *cot* (C102t) is redesignated *cot-1*. The heterocaryon *cot-1* + *col-1* (Y8743c) is wild type (30°).

Supplementary distinguishing characteristics: The hyphal morphology, chiefly of the spreading types, has been observed microscopically as an aid in distinguish-



FIGURES 1–12:—Photographs of colonies grown 48 hours on minimal medium in 10 cm Petri plates at 30°C. Figures 1–3: colonial (*col*) mutants R2471, R2503, R2452 (*bis*). Figures 4–6: spreading colonial (*spco*) mutants R2502, R2457, R2510. Figures 7–9: semi-colonial (*smco*) mutants Y2330, R2477, R2386. Figures 10–12: sparse, spreading morphologicals (*mo*) R2487, R2466, R2464.



FIGURES 13–24:—Photographs of colonies grown 48 hours on minimal medium in 10 cm Petri plates at 30°C. Figures 13–15: ropy (*ro*) mutants R2470, R2431, R2520. Figure 16: ropy-like (*rol*) mutant R2498. Figure 17: R2401, medusa (*med*); Figure 18: medusa, bottom of plate, showing grooves. Figures 19–24: environment sensitive morphological (*moe*) mutants R2408 and R2532. Figures 19, 20: R2408 on minimal medium at 25° and 34°. Figures 21, 22: R2408 on complete medium at 25° and 34°. Figure 23: R2532 on minimal medium at 34°. Figure 24: R2532 on complete medium at 34°.

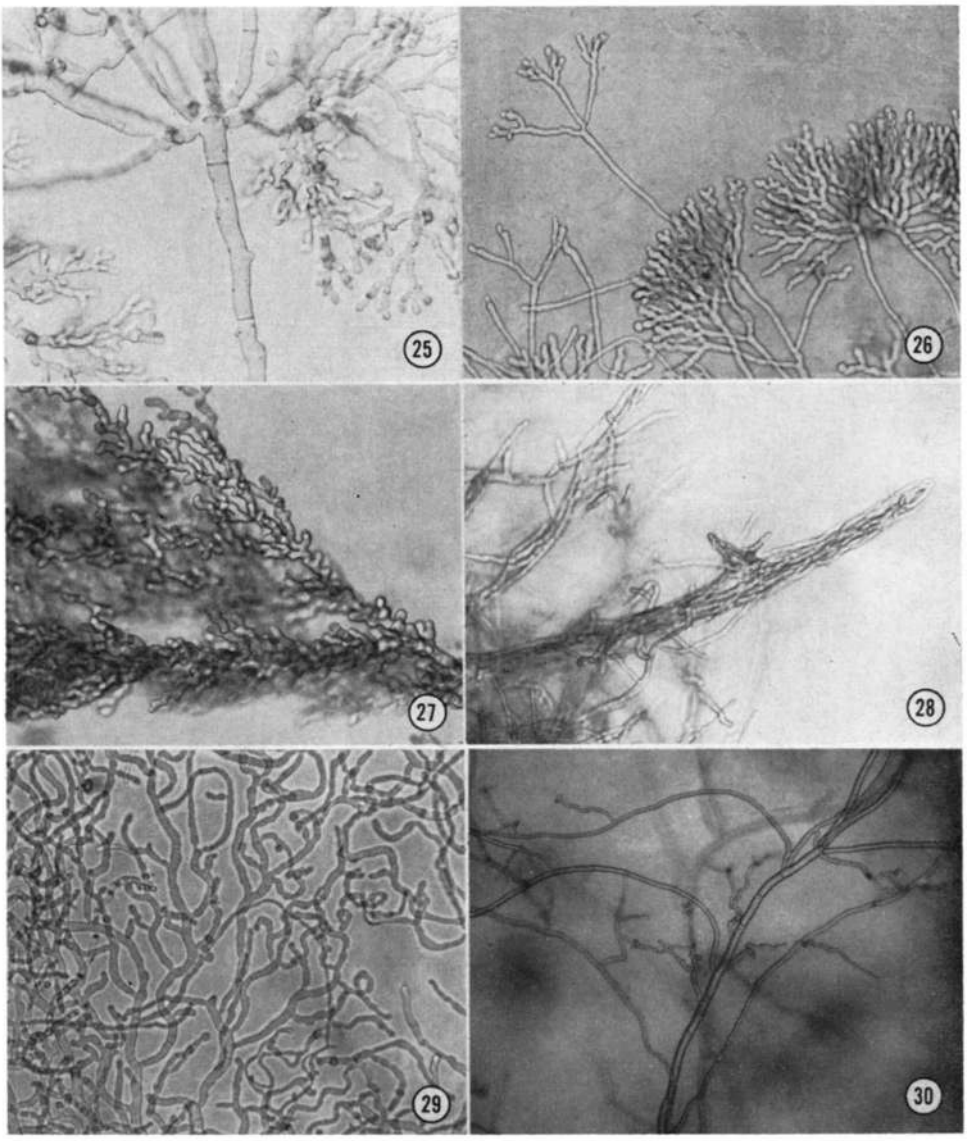
ing mutants. For this study, the wild type grown under similar conditions was the standard of comparison. Environmental factors, including also those produced during their own growth, appear important, and even in the wild type some distinctive variations in hyphal morphology were present in different areas of the same plate, though they appeared very rarely in comparison to the mutants, where distinctive differences are numerous and often of one characteristic type; for example, the multiple branching of hyphae in frost, *fr* (B110) (Figure 25), the fan-shaped hyphae in shallow, *sh* (R2371) (Figure 26) and the incomplete separation of conidia in granular, *gran* (B42) (Figure 30).

Mutants of particular interest: ropy strains: The morphological characteristics of ropy cultures are of some interest because the *ro* genes occur in at least four linkage groups (II, III, IV, V). In common with the ropy strains previously described, the new ropy strains have curled hyphae (as observed microscopically) as well as the ropy appearance on slants, topped by clumps of conidia. The strains called ropy-like in this report do not have curly hyphae but their mycelia form patterns on agar surfaces closely resembling those of the true ropy strains (cf. Figures 13, 14, 15, and 16) and for this reason the symbol *rol* (ropy-like) seems appropriate.

Ascospore-lethal strains: The three strains—B55*A* (IV), S4355*a* (IV), and R2411*A* (VII) produce normal appearing ascospores which do not germinate. The first two probably contain alleles of a previously described gene in group IV (MURRAY and SRB 1961) whereas R2411 (*le-2*) is closely linked to *me-7*. Among the over 1,000 ascospores isolated from various crosses and heat-shocked in the usual way, no germination of either B55 or S4355 was observed. The same result was obtained when about 200 ascospores from crosses with the wild types were treated with furfural (1.2×10^{-4}M). In contrast, three *le-2* germinants were obtained from two crosses— R2411*A* × 16117*a* (iv) and × 4894*a* (*me-7*)— following retention of ascospores at 25° for seven days before heat shock. These three reisolates (all *A*) were crossed with RL21*a* and asci isolated in order. In each ascus, two ascospore pairs failed to germinate.

Two other techniques produced some *le-1* (B55) ascospore cultures. The first was suggested by the work of LOWRY and SUSSMAN (1958), who used Chlorox to remove the outer walls of ascospores prior to nuclear staining. Accordingly, ascospores (from crosses with wild type), were suspended in a 1:3 dilution of Chlorox (5.25% sodium hypochlorite) and incubated for one hour at room temperature. They were then rinsed with sterile distilled water and plated on sorbose minimal medium plus furfural (1.2×10^{-4}M). The germination was very low for both wild type and mutants (B55: 1 wild type and 3 mutants; S4355: 3 wild types and no mutants). Of the three B55 reisolates, 1 was *A* and 2 were *a*, confirming that they had indeed come through the cross and had not originated as conidial contaminants.

The second method, though crude, also was successful in producing germination of *le-1* (B55) ascospores. Ascospores were washed in a 1:10 dilution of Chlorox to kill conidia, ground gently in a mortar, and plated on sorbose- furfural medium. 1402 young colonies were examined microscopically, 27 of which were recognized as mutant colonies. Of the 27, it was possible to isolate only 20



FIGURES 25–30:—Photographs of hyphae growing on minimal medium at 27°C (150×); Figure 25: frost (*fr*) B110, showing multiple hyphal branching; Figure 26: shallow (*sh*) R2371; Figure 27: ascospore lethal mutant (*le-1*) B55; Figure 28: sponge (*spg*), showing a bundle of fine hyphae formed by inter-hyphal fusions; Figure 29: curled hyphae of ropy (*ro-4*) B38; Figure 30: sparsely branched hyphae of granular (*gran*) B42.

because the other seven overlapped nearby wild-type colonies. Each sex (mating type) was represented: 15 *A* and 5 *a*. These results also indicate that at least some of the colonies had arisen from ascospores and not from conidia.

Temperature-sensitive mutants: Six mutants were isolated during this survey:

three are in linkage group II (R2446, R2454, and R2479), one in IV (R2006), and two in V (R1006 and R2101). Only the strains in group II (*cot-5*) appear to be alleles. These are essentially colonials which grow at 25° to 30° but scarcely at all at 34°. Reversions have appeared frequently, especially upon transfer from cultures retained for some time at 5°.

The other new strains, *cot-2* (R1006), *cot-3* (R2006) and *cot-4* (R2101) grow colonially at 34°. At 25°, *cot-3* becomes completely wild-type in appearance, but the other two retain, to some extent, a colonial morphology.

Cultures inhibited by GSC: Of the cultures clearly inhibited on complete medium, only two have been investigated somewhat further. These are *le-1* (B55) and *moe-1* (R2408). The GSC effect on these two strains could be produced by minimal medium plus yeast extract alone, but not by minimal plus N-Z-Case (enzymatic hydrolysate of casein) or minimal plus vitamins. In trying to find the component of yeast extract active for *le-1*, some growth inhibition was obtained with adenine (and adenosine and deoxyadenosine), somewhat less inhibition with guanine and its derivatives, and essentially no effect with pyrimidines. In one experiment, growth in liquid medium was inhibited 50% by adenine (1 mg/ml), while growth of the wild-type control was not affected significantly.

An unusual morphological type: Upon germination from the ascospore, strain *smco-5* (R2442) grows as a flat colony and retains this type of growth for about six days when a few conidia are formed and a wild-type mycelium is developed. Although, upon transfer, the mycelium does not return to the morphological stage, the ascospores formed in a subsequent cross again germinate as described. A long period of mycelial development upon germination from the ascospores also takes place in some biochemical mutants; for example, in *rib-2* (Y30539r) (GARNJOBST and TATUM 1956).

DISCUSSION

The morphological mutants studied show the following distribution of 58 new loci in the various linkage groups: Group I, 14 loci; II, 6; III, 6; IV, 9; V, 11; VI, 3; and VII, 9. The percent distribution is almost the same as the distribution of 122 biochemical loci (BARRATT and OGATA 1966), and approximately in proportion to the maximum established map lengths of the linkage groups from published maps (PERKINS 1959; PERKINS and ISHITANI 1959; MALING 1959; STRICKLAND, PERKINS and VEATCH 1959; PERKINS, GLASSEY and BLOOM 1962). From these findings, it appears that both classes of mutant loci are distributed similarly in all seven linkage groups, possibly in proportion to the chromosome lengths.

Preliminary linkage maps, constructed from the genetic data (given in the tables) and heterocaryon tests for allelism, appear to show some clusters of morphological mutants, often near the centromeres. Such a pattern, if substantiated by further work, would indicate either that the genes in certain regions are more susceptible to mutation by ultraviolet irradiation, or that genes con-

trolling morphology are concentrated in specific regions of the chromosomes, or that crossing over near the centromeres is less per unit cytological length.

Twenty-eight recurrences of mutation at the new or previously described morphological loci have been found. Of these, 18 are at previously described loci: crisp (9), ragged (4), and biscuit (5). Few double mutants and no triple mutants were encountered. The double mutants of some interest are R2436 (*al-2, mo-1,* both in I), R2502 (*al-2, spco-11,* both in group I), and R2530 (*rg* in I, *spco-13* in VI).

The method of selecting strains for linkage determinations probably eliminated those with gross aberrations. Two strains, however, gave some evidence of such alterations: R2394 and R2472 (Table 9).

A few strains did not show linkage to any of the markers used, including one marker in each of the occupied left arms. Markers near the centromere in the unoccupied left arms also were tested without conclusive results. The fact that almost all of the mutants in the fairly large sample of randomly obtained strains were found to be linked to known markers suggests that comparatively few genes are present in the remaining unmarked chromosome regions, including the unoccupied left arms.

Several general conclusions may be drawn regarding the behavior of morphological mutants. One of these is that their morphological characteristics are relatively constant, and autonomous in that in general they are only slightly modified by environmental conditions or nutrition. Higher temperatures and richer media in general tend to cause somewhat more restricted, denser growth on agar. Another finding has been the rather frequent minor modification of growth habit resulting from mutations at other loci, either of spontaneous origin or introduced from other strains by crosses. In general, too, double morphological mutant cultures seem to be more restricted in growth than either single mutant culture.

These observations are consistent with the possibility that morphology is dependent on the rates of intracellular reactions, competing and interrelated, both catabolic and biosynthetic, as suggested for *col-2* (Y5331) (BRODY and TATUM 1966). In this instance, the primary effect of the *col-2* mutation appears to be in the structure of glucose-6-phosphate dehydrogenase, resulting in changes in its reaction kinetics and temperature stability. The mutants described in the present paper should be useful in further biochemical studies attempting to define the primary lesions concerned with morphological characters. Some groups, such as the temperature-sensitive strains and those represented by recurrent alleles and modifiers, are particularly promising for such studies.

Much more work remains to be done on morphological mutants. More detailed genetic studies are needed on the mutants here reported, so that they can be located with greater precision, as has been done for linkage group V in the accompanying paper (MORGAN, GARNJOBST and TATUM 1967). The effect of other mutagens on the spectrum of morphological mutants should be studied.

Finally, it is hoped that studies on the genetics and biochemistry of morphology in Neurospora can contribute definitive understanding of the more general phe-

nomenon of morphological differentiation. In Neurospora, this understanding will depend on further genetic and biochemical and morphological studies, and their correlation. Aspects such as hyphal form and structure, cell-wall composition (MAHADEVAN and TATUM 1965, 1967), and the metabolism and enzymology of morphological mutants (BRODY and TATUM 1966, 1967) seem particularly important and promising areas for future investigations.

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SUMMARY

Ninety-eight morphological mutants, mostly obtained by ultraviolet irradiation of conidia of the inositoless strain 89601, have been studied morphologically and genetically. Although each mutant strain has its own characteristic growth habit, they have been classified into reasonably well defined and distinguishable groups. These are (1) the true colonials (*col*), (2) spreading colonials (*spco*), (3) semi-colonials (*smco*), (4) sparsely growing morphologicals (*mo*), (5) particularly distinctive mutants, given descriptive symbols (*cr*, *ro*, etc.), and (6) strains particularly sensitive to environmental modification (*cot*) and (*moe*).—Of the 98 mutant strains examined, linkage relations have been established for 90 mutant genes, 58 of which have been designated as new loci. The remainder appear to represent allelic recurrences of these and previously described mutant genes.—The distribution of the new loci in different linkage groups is: I, 14; II, 6; III, 6; IV, 9; V, 11; VI, 3; and VII, 9. This distribution is similar to that of the known biochemical loci. Also, as for biochemical loci, there is considerable variation in the frequency of mutation at different loci. Morphological loci may not be randomly distributed within each linkage group.—The principal morphological characteristics of most of these mutants are also completely autonomous, in that they are only slightly affected by environmental and nutritional conditions.

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