INTERACTIONS BETWEEN AN EXTRACHROMOSOMAL FACTOR, POKY, AND NUCLEAR GENES IN *NEUROSPORA CRASSA*¹

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POKY (*mi-1*), is a maternally inherited mutant of *Neurospora crassa* (MITCHELL and MITCHELL 1952). Its phenotype is characterized by a long lag period, slow growth and defects in the cytochrome system during the early growth period. Older poky cultures recover to a considerable extent, approaching, but not attaining, either the wild-type growth rate or normal concentrations of the cytochromes (HASKINS, TISSIERES, MITCHELL and MITCHELL 1953; MITCHELL, MITCHELL and TISSIERES 1953). This asexual cycle of growth and cytochrome abnormalities is repeated on conidial transfer.

This study was undertaken to attempt to modify the life history of poky. Since poky is a transient defect, and is reestablished from a "cured" culture on conidial transfer, experiments were undertaken to perpetuate the "cured" state by preventing conidiation in two ways: by continuous hyphal growth, and by introducing the gene, fluffy, which blocks conidiation. Since poky mycelia, unlike wild-type, accumulate large quantities of free fatty acids (HARDESTY and MITCHELL 1963), attempts were made to determine whether the combination of a fatty acid-requiring mutant with poky cytoplasm would result in complementation, so that normal growth would prevail with suitable concentrations of oleic acid in the medium. Other chromosomal genes were introduced into the poky strain to attempt to modify its phenotype with the hope of gaining further insight into nuclear-cytoplasmic interactions. The gene *f* ("fast"), a modifier of the slow growth but not of the defective cytochromes of the poky phenotype (MITCHELL and MITCHELL 1956), was mapped in order to distinguish it from other modifiers which might be found.

These experiments show that the slow-growth aspects of poky can be modified to approach wild type by continuous hyphal growth over a long period, but that certain mutant genes introduced into the poky strain greatly decrease the rate of growth.

MATERIALS AND METHODS

Neurospora crassa strains 3627-1A (slow-growing poky) and 3627-3A (*f* carrying poky) from the Fungal Genetics Stock Center were crossed to inositol-requirer (*inos*, 89601), with the poky strain as the protoperithecial (maternal) parent. *inos* isolates from each cross were selected for measurement of growth rate in growth tubes (RYAN, BEADLE and TATUM 1943). The mold was grown at 25°C on minimal medium (VOGEL 1956) containing 1% sucrose, 1.5% agar

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and supplemented with 50 μ g inositol/ml. At the beginning of the experiment, each tube was inoculated at one end with conidia. The front line of growth of the mycelium was recorded on the tube every 24 hours. When the growth approached the distal end of the tube, before conidiation in that region could occur, a piece of agar with nonconidiating mycelium attached was cut out under sterile conditions, and transferred to the end of a fresh growth tube, so that the mycelium could continue growth without delay.

For linkage determination two and three-point crosses were made between *f* poky and *inos*; between *f* poky, *inos*, and RL 21 (Rockefeller-Lindegren wild type); and between *f* poky *inos* and Y112M23 (*ad-7*) obtained from Dr. N. H. GILES, JR.

To test the interaction of the extrachromosomally determined trait poky with different chromosomal genes, crosses were made between *f* poky and microconidial, fluffy (*pe^m*, *fl* strain Y8743) and an oleic acid mutant (R23660, *ol* [PERKINS, GLASSEY and BLOOM 1962]). Thus, poky strains were synthesized that were fluffy, microconidial and fatty acid requirers in all possible combinations, with or without *f*.

RESULTS

A comparison of the length of the lag period and growth rates of wild type, two slow poky isolates and one *f* poky can be seen in Figure 1 and Table 1. All conform closely to the original descriptions by MITCHELL and MITCHELL (1952, 1956). The effect of the *f* gene is to increase the growth rate of the poky strain almost to wild type, and to decrease the lag in germination of both conidia and ascospores. The cytochromes in young *f* poky isolates are the same as in the slow poky cultures, as previously shown by MITCHELL and MITCHELL (1956) and confirmed by D. J. L. LUCK (personal communication).

The location of *f* in linkage group V is shown in Figure 2.

Table 1 gives data for combinations of other nuclear genes with poky cyto-

FIGURE 1.—Comparison of growth rates of wild type and three poky isolates, one of which carried the *f* (fast) gene.

FIGURE 2.—Map location of the *f* gene on linkage Group V. Centromere distance, 61 asci: 36.9.

Results of two- and three-point crosses:

	Total*	Percent recombination
<i>f-inos</i> (all crosses)	517	8
<i>f-inos</i> (3-point)	209	10
<i>inos-ad-7</i> (3-point)	209	8
<i>f-ad-7</i> (3-point)	209	18

* Number of ascospores if isolated at random, or ascospore pairs, if isolated in order.

plasm. It was of interest to establish whether a poky that formed no conidia would still have the initial lag period and slow growth rate of poky, and whether it could maintain the wild-type rate of growth indefinitely. As can be seen from Table 1, aconidial poky, fluffy isolates carrying the *f* gene had a shorter lag and more rapid growth rate than the fluffy strain with wild-type cytoplasm; slow poky, fluffy isolates were not only slower in growth than non-poky, fluffy but were also slower than ordinary poky isolates. The same was true of a slow poky, microconidial strain.

HARDESTY and MITCHELL (1963) showed that young cultures of poky accumulate large quantities of free fatty acid. Two isolates of a double mutant (*ol*, poky) that required fatty acid and carried the poky trait were studied. Each had a

TABLE 1

Growth rate of normal and mutant strains in growth tubes (25°)

	Lag (days)*	Rate during lag (average) mm/hr	Rate after lag (average) mm/hr
Wild type†	2	1.0	3.3
Fast poky (<i>f</i>)†	3–4	1.3	3.2
Slow poky No. 43	7	0.9	3.0
Very slow poky No. 80	8	0.7	2.6
Non-poky fluffy	1	1.9	3.2
Fast poky, fluffy (<i>f</i>)	1	1.7	3.2
Slow poky, fluffy‡	4–21	1.0	2.1 (1.6–2.2)
Slow poky, microconidial§	16+	1.8	1.9
Non-poky <i>ol</i>	2–4	..	2.5
Poky <i>ol</i>	24	..	1.7

* Lag is defined as the number of days between inoculation of a culture and its achievement of a stable rate of growth.

† Three isolates tested.

‡ Seven isolates tested (one grown continuously for 57 days—maximal growth fluctuated from 1.6 to 2.2 mm/hr).

§ Four isolates tested (one grown continuously for 46 days. After 16 days rate fairly constant at 1.9 mm/hr).

|| Two isolates tested.

longer lag and slower growth rate in growth tubes at every concentration of oleic acid supplement tested (from 0 to 0.1%) than did either single mutant (*ol* or *poky*). This was confirmed in liquid medium. Not only was there no complementation between the *poky* trait and the fatty acid requirement, but the combination of the two defects was more detrimental than either alone (Table 1).

The other attempt to modify the life history of the *poky* strain proved more successful. Slow *poky* cultures were maintained in a state of continuous hyphal growth for periods up to 180 days. Conidia were removed at various intervals and tested on slants or growth tubes for measurement of germination time and growth rate. After the initial lag, the vegetative growth rate remained comparable to wild type throughout the experiment (Figure 3). When conidia were sampled before Day 40, the pregermination lag period and the rate of growth were similar to those of the parent cultures, indicating a retention of the *poky* condition. Sometime between Days 40 and 80 a change took place in the cultures, so that the lag in germination of conidia and growth rate approached that of wild type. This effect was obtained with several different *poky* isolates (Table 2).

The cultures were then crossed with wild type to determine whether mutation to a new "*f*" type of gene had occurred. Ascospores were isolated in order from each of 14 asci. All the progeny demonstrated the long lag period and slow growth rate characteristic of the *poky* phenotype (Table 3). When these progeny were maintained in a state of continuous vegetative growth, they too became

FIGURE 3.—Growth rates of four *poky* isolates maintained in a state of continuous hyphal growth up to 180 days.

TABLE 2

Conidial tests in growth tubes to show effect of continuous hyphal growth (25°C)

Isolate	Days of continuous growth	Lag (No. days)	Average rate during lag	Average rate after lag
43 (slow poky)	0	7	0.9	3.0
	28	6	1.4	2.6
	40	4	1.4	2.6
	81	4	1.3	2.7
	117	4	1.6	2.9
	137	3	1.2	2.8
	151	3	1.5	2.5
	173	4	1.2	2.5
80 (slow poky)	0	8	0.7	2.6
	28	9	1.3	2.3
	40	11	1.3	2.3
	81	3	1.9	2.9
	117	2	2.2	3.2
	137	3	1.5	3.2
	151	2	1.5	3.1
	173	2	1.4	2.9
1-3 (slow poky)	0	7	1.1	3.3
	19	6	1.4	3.2
	38	3	1.3	2.8
	52	1	1.3	2.7
6-6 (slow poky)	0	7	0.7	3.0
	19	8	1.5	2.7
	38	3	1.7	2.8
16 (<i>f</i> poky)	0	3	1.4	3.0
	44	3.5	1.6	3.3
	80	2	2.1	3.2

Isolates (1-3) and (6-6) were progeny of a cross between "cured" poky isolate 43 (Day 88 of continuous vegetative growth) and wild type.

TABLE 3

Comparison of progeny from crosses of "cured" poky × wild type with wild type and poky strains

	Day of appearance	
	Mycelia	Conidia
Wild type	1	2
<i>f</i> poky	3	4
Slow poky	5-6 or more	6-7 or more
Cross 1		
Cured (Day 88) × wild type	5	6
Cross 2		
Cured (Day 171) × wild type	5	6

"cured" with respect to the initial growth rate and lag before conidial germination (Table 2). All the progeny of crosses with wild type once again reverted to the poky condition. This was true regardless of the length of time the poky cultures had been maintained in continuous hyphal growth (Table 3).

DISCUSSION

The slow growth of the poky strain is caused by the decreased respiratory rate which in turn is due to its defective cytochrome system. It is known from studies of aerobic bacteria that respiration of spores is very low, rising abruptly with germination, and that sporulation is preceded by a burst of respiratory activity. The activation of *Neurospora* spores is known to be accompanied by a precipitous decline in ATP and a rise in ADP concentration (reviewed by WRIGHT [1964]).

The "recovery" of both growth rate and the cytochrome system in older poky cultures is evidently stable so long as no excess demands for energy are made, and so can be maintained by continuous hyphal growth. Formation and germination of conidia, however, require bursts of energy and this probably is the cause of the reversion to the poky condition up to about the 40th day of continuous growth. The aconidial condition of fluffy may explain the short lag and rapid growth of the wild-type and *f* poky cultures. There must be some other unknown reason for the long lag and slow growth of poky fluffy isolates not carrying the *f* gene.

The change that occurs after 40 days of continuous growth may well be the result of a selection in the mitochondrial population of the growing tip for those with the most normal cytochrome system. Whatever the cause of the change, it is stable for at least 180 days of vegetative propagation and is equal to the energy demands of conidiation and of conidial germination. Since the change does not survive through a cross with the "cured" poky as the protoperithecial parent, it is not a gene mutation analogous to *f*, nor is it maternally inherited like the poky trait.

That the aconidial gene, fluffy, and the genes for microconidia formation and fatty acid requirement have such extreme effects on the growth rate of the poky cultures are interesting observations, but their significance is totally obscure. Yet, like the other observations on the poky strain in this and other studies, they will have to be accounted for when the poky puzzle is finally solved.

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SUMMARY

Poky cultures can be maintained at a wild-type growth rate by continuous vegetative growth. Reversion to the poky state occurs on germination of conidia if the cultures are allowed to conidiate before 40 to 50 days. After this time conidia germinate with approximately wild-type lag and the growth of mycelia is at wild-type rate. If these cultures are crossed to wild type, with poky as the maternal parent, the progeny are all poky in rate of germination and vegetative

growth. Poky strains that carried the genes fluffy or peach grew more slowly than any of the parental strains. A strain that carried a gene for fatty acid requirement in poky cytoplasm had a longer lag period and slower growth rate on oleic acid supplement than did either single mutant (*ol* or *poky*). The gene *f* (fast) modifying the slow-growth of poky is located in linkage group V, 8–10 units proximal to *inos*.

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