HETEROCARYONS BETWEEN STRAINS OF NEUROSPORA CRASSA WITH DIFFERENT CYTOPLASMS

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MITCHELL and MITCHELL (1952) and MITCHELL, MITCHELL and TISSIERES (1953) have shown that two strains of *Neurospora crassa*, *poky* and *mi 3*, of independent origin, differ from wild type and from each other in growth rate and cytochrome content. The differences are inherited maternally and thus appear to be associated with the cytoplasm. The object of this work has been to show what type of cytoplasm results when heterocaryons are made between strains having *poky* and *mi 3* cytoplasm or between either and wild type. It was hoped to show in particular (1) whether *poky* and *mi 3* cytoplasm interact to give wild type, (2) whether intermediate states of cytoplasm are to be found, and (3) whether either mutant, *poky* or *mi 3*, is "dominant" over wild type in a way which would suggest infection.

MITCHELL *et al.* (1953) report growing mixed cultures of *poky* and *mi 3* strains and obtained from the mixture no strain with a growth rate faster than either; but there is no certainty that heterocaryon formation occurred in these cultures. In the present work, the components of all heterocaryons were marked with biochemical mutant nuclear genes, usually pantothenicless (5531) in one component and p-aminobenzoicless (1633) in the other, so that when the two strains were grown together on minimal medium the heterocaryon might be at an advantage. It will be seen that heterocaryons were in fact formed, that is mixture of nuclei occurred and there was opportunity for mixture of cytoplasm.

MATERIALS AND METHODS

The strains used in the heterocaryons to be described were derived from two strains, 5531A (*pan-in-wild cytoplasm*) and 1633A (*pab-in-wild*) (BEADLE and TATUM 1945). Sometimes the two original strains themselves were used. (Throughout, "wild" will be used to denote wild type cytoplasm, and "+" wild type in respect of nuclear genes.) 5531A and 1633A were selected for use because they were found to form a heterocaryon together very readily. Also, to the author's knowledge, no case of a suppressor of either gene had been found²: in some circumstances a single strain carrying such a suppressor might be mistaken for a heterocaryon. The marker genes were introduced into *poky* and *mi 3* strains, and reisolated in wild cytoplasm, by the crosses shown in table 1, which involved the following strains: (1) a *poky* strain, 2303-2a (+-in-*po*), obtained by Mrs. M. B. MITCHELL from a cross of *po*-1720-1A (MITCHELL and MITCHELL 1952); (2) an *mi 3* strain, 2543-3a (+-in-*mi 3*) (MITCHELL *et al.* 1953); (3) a standard wild type, 2293-2a (+-in-wild).

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² The 5531A and 1633A cultures used in the present work and the *pan* and *pab* strains derived from them were all tested on minimal medium: there was no indication that any of them carried a suppressor.

TABLE 1
Derivation of strains used in heterocaryons

Cross: Maternal (protoperithecial) parent first	Progeny: Ascospore isolates used in heterocaryons
2303-2a (+-in-po) × 1633A (<i>pab-in-wild</i>)	<i>pab-in-po</i> -1 to -7
2543-3a (+-in-mi 3) × 1633A (<i>pab-in-wild</i>)	<i>pab-in-mi</i> 3-1 and -2
2293-2a (+-in-wild) × 1633A (<i>pab-in-wild</i>)	<i>pab-in-wild</i> -2
2303-2a (+-in-po) × 5531A (<i>pan-in-wild</i>)	<i>pan-in-po</i> -1
2543-3a (+-in-mi 3) × 5531A (<i>pan-in-wild</i>)	<i>pan-in-mi</i> 3-1
<i>pan-in-mi</i> 3-1 × 5531A (<i>pan-in-wild</i>)	<i>pan-in-mi</i> 3-2 to -6
2543-3a (+-in-mi 3) × 5531A (<i>pan-in-wild</i>)	<i>pan-in-mi</i> 3-7 to -9
5531A (<i>pan-in-wild</i>) × <i>pan-in-mi</i> 3-1	<i>pan-in-wild</i> -2 and -3

Ascospore isolates from these crosses were tested as to their ability to form heterocaryons with a tester strain, in the case of the first crosses examined with one of the two original strains 5531A or 1633A, and the few isolates enumerated in table 1 were selected for further use. The majority, often more than 90%, were discarded because they were not found to form heterocaryons readily with the tester strain, that is, when the growth of a strain and the tester together on minimal medium was less than that of either on supplemented medium. For later crosses, tester strains which themselves formed a heterocaryon readily with one of the original strains were used. It was usually found that where one of two strains formed a heterocaryon readily with 5531A and the other with 1633A they also did so together. It was possible therefore to use isolates in heterocaryons with several strains. It was presumably a result of using 5531A and 1633A as the original tester strains, that most heterocaryons examined were between strains of mating type A.

Heterocaryons were made on minimal slants. Unless otherwise stated, the two components were inoculated on to the same point in the middle of a slant, one on top of the other, using approximately equal dry inocula (mycelium and conidia) from young cultures. These first slant cultures will be referred to throughout as *heterocaryon cultures*. In many cases they were tested as to their cytoplasmic properties by growth in flasks in minimal medium, as described by MITCHELL *et al.* (1953). Pads of mycelium resulting from four days' growth in flasks were examined for cytochrome content by observation with a hand spectroscope, and their dry weight was determined as a measure of the growth rate of the cultures. However it was not known to what extent any particular heterocaryon culture, or the mycelium grown from it in flasks, was in fact heterocaryotic. It was possible that it might consist of a mixture of the heterocaryon and its separate components, or even of the components alone supporting each other through the medium. In many cases therefore a transfer was made from the heterocaryon culture to a minimal plate from which a few single hyphal tips were isolated. Each one was transferred to a slant of minimal medium. The resulting cultures will be referred to as *hyphal tip cultures*. They were tested by growth in flasks in the same way as for the heterocaryon cultures. It could be assumed that the hyphal tips which grew had been heterocaryotic, and this was always confirmed by the recovery of both marker genes in those cases where single hyphal

TABLE 2

Control results

Dry weights from 4 days' growth in flasks of components of heterocaryons (marked with pab or pan) on supplemented medium

	<i>wild</i> (22 observations)	<i>poky</i> (35 observations)	<i>mi 3</i> (32 observations)
lowest	79 mg	2 mg	7 mg
highest	114 mg	22 mg	46 mg
average	87 mg	11 mg	30 mg

tip cultures were crossed to wild type (+-*in-wild*). Most attention will be given, therefore, to the cytoplasmic properties of the hyphal tip cultures.

An indication should be given of how much information was obtained from the tests of cultures by growth in flasks. Pads of *poky* mycelium have about 15 times, and of *mi 3* about 5 times, the concentration of cytochrome *c* in wild type pads (MITCHELL *et al.* 1953). With the hand spectroscope used in these experiments, it was possible to distinguish readily, on the basis of accumulation of cytochrome *c*, between *poky* and *wild* mycelium on the one hand and between *mi 3* and *wild* on the other. But it was not possible to distinguish with certainty between *poky* and *mi 3*, for which the difference in concentration of cytochrome *c* was offset by a difference in size of the pads. Single pads were examined. Also, with this hand spectroscope, it was not possible to detect the cytochrome *b* absorption band which distinguishes *wild* and *mi 3* mycelium from *poky*.

When heterocaryon or hyphal tip cultures were tested in flasks they were compared with their component strains grown in flasks of supplemented medium at the same time. The growth rates of these controls are summarized in table 2. It is clear that on the basis of growth rate, as well as cytochrome content, it is easier to distinguish between *poky* and *wild* and between *mi 3* and *wild* than between *poky* and *mi 3* for which the range of dry weights overlaps. An intermediate state of cytoplasm might most readily be detected in heterocaryons between *poky* and *wild* strains. It is satisfactory that the control growth rates agree with results previously obtained for different isolates of *poky* and *mi 3* strains not carrying biochemical marker nuclear genes, e.g. the samples quoted by MITCHELL and MITCHELL (1956). Except that perhaps *pab-in-mi 3* isolates grew rather slowly, there was no indication of interaction between the *pab* and *pan* genes and the *poky* and *mi 3* cytoplasms.

RESULTS

Heterocaryons between strains having wild and poky cytoplasms

Table 3 shows the results obtained for heterocaryons between three *pan-in-wild* strains (5531A and two reisolates of it) and five different isolates of *pab-in-poky*.

Ten replicates of one of these heterocaryons, *pan-in-wild-3* with *pab-in-po-4*, were made, using as far as possible the same technique. The growth of the ten was uniform as heterocaryon cultures and much alike when these were transferred to minimal plates. Two replicates were selected as showing most difference from each other in

their growth on plates: Replicate 4, thought to be growing more slowly, and Replicate 5, thought to be growing faster. Single hyphal tips of these were isolated as recorded in the table.

The results in table 3 indicate that all these heterocaryons had wild type cytoplasm. All heterocaryon cultures and hyphal tip cultures examined had *wild* characteristics, and those heterocaryon cultures which were not tested gave every appearance of growing at wild type rate.

Two results not appearing in table 3 were thought at first to be exceptional. Heterocaryon cultures of 5531A (*pan-in-wild*) with *pab-in-po-6* and 5531A with *pab-in-po-7* appeared to be intermediate between *poky* and *wild*, in accumulation of cytochrome *c* and growth rate (32mg dry weight in both cases). One of these, 5531A with *pab-in-po-6*, was transferred to a minimal plate where its growth was slow and irregular so that hyphal tips were isolated with difficulty. Two out of five of these grew on minimal and were shown to resemble wild type in accumulation of cytochrome *c*, but to resemble *poky* in growth rate (10 mg and 18 mg dry weights). The type of growth on the plate suggested at once that this heterocaryon was not stable, and that this and not a *poky* cytoplasm might account for the low growth rate of the hyphal tip cultures. The cytoplasm may in fact have been *wild* as indicated by the cytochrome observations. It is considered likely that neither *pab-in-po-6* nor *pab-in-po-7* formed a stable heterocaryon with 5531A. It is of interest that both strains were found later to be mating type a.

TABLE 3

Heterocaryons between strains having wild and poky cytoplasms (pan-in-wild with pab-in-poky)

<i>pan-in-wild</i> component	<i>pab-in-po</i> component	Heterocaryon culture properties†	Hyphal tip cultures	
			Survival*	Properties†
5531A (<i>pan-in-wild</i>)	<i>pab-in-po-1</i>	wild	11/13	all wild
5531A (<i>pan-in-wild</i>)	<i>pab-in-po-2</i>	wild	6/9	all wild
5531A (<i>pan-in-wild</i>)	<i>pab-in-po-3</i>	wild	5/9	all wild
5531A (<i>pan-in-wild</i>)	<i>pab-in-po-4</i>	wild	— (Hyphal tips not isolated)	—
5531A (<i>pan-in-wild</i>)	<i>pab-in-po-5</i>	wild	—	—
<i>pan-in-wild-2</i>	<i>pab-in-po-1</i>	— (Culture not tested)	4/7	all wild
<i>pan-in-wild-2</i>	<i>pab-in-po-2</i>	—	4/7	all wild
<i>pan-in-wild-2</i>	<i>pab-in-po-4</i>	—	2/7	both wild
<i>pan-in-wild-3</i>	<i>pab-in-po-1</i>	—	5/7	all wild
<i>pan-in-wild-3</i>	<i>pab-in-po-2</i>	—	6/7	all wild
<i>pan-in-wild-3</i>	<i>pab-in-po-4</i>	—	1/7	wild
Replicates of <i>pan-in-wild-3</i> with <i>pab-in-po-4</i>				
Replicate 4		—	5/7	all wild
Replicate 5		—	5/7	all wild

* Number surviving on minimal medium/number isolated.

† Cytoplasmic properties as indicated by observations of both cytochrome content and growth rate.

One heterocaryon was made between strains having *wild* and *poky* cytoplasm with the genes reversed, that is between the isolates *pab-in-wild-2* and *pan-in-po-1*. The heterocaryon culture and six surviving hyphal tip cultures of seven isolated were tested in flasks, and all appeared *wild*.

Heterocaryons between strains having mi 3 and poky cytoplasm

Results for heterocaryons between four different isolates of *pan-in-mi 3* and five *pab-in-po* isolates are given in table 4. The *pab-in-po* isolates are those already mentioned as used in heterocaryons between *wild* and *poky* strains. As before, ten replicates were set up of one heterocaryon, *pan-in-mi 3-7* with *pab-in-po-3*. One, replicate 6, was observed to grow less profusely than the others, both as a heterocaryon culture and when transferred to a minimal plate. Three replicates, including this one, but not necessarily including the fastest, were selected, and single hyphal tip cultures from them examined, as recorded in table 4. We have seen that *poky* and *mi 3* cultures could not be distinguished with complete certainty with the hand spectroscope,

TABLE 4

Heterocaryons between strains having mi 3 and poky cytoplasm (pan-in-mi 3 with pab-in-poky)

<i>pan-in-mi 3</i> component	<i>pab-in-po</i> component	Heterocaryon culture properties†	Hyphal tip cultures	
			Survival*	Properties‡
<i>pan-in-mi 3-2</i>	<i>pab-in-po-1</i>	mi 3	3/10	2 tested: both mi 3
<i>pan-in-mi 3-2</i>	<i>pab-in-po-2</i>	mi 3	8/18	4 tested: all mi 3
<i>pan-in-mi 3-2</i>	<i>pab-in-po-3</i>	mi 3	— (Hyphal tips not isolated)	—
<i>pan-in-mi 3-2</i>	<i>pab-in-po-4</i>	mi 3	—	—
<i>pan-in-mi 3-2</i>	<i>pab-in-po-5</i>	mi 3	—	—
<i>pan-in-mi 3-7</i>	<i>pab-in-po-1</i>	mi 3	7/10	all mi 3†
<i>pan-in-mi 3-7</i>	<i>pab-in-po-2</i>	mi 3	—	—
<i>pan-in-mi 3-7</i>	<i>pab-in-po-3</i>	Intermediate between mi 3 and wild	6/10	all mi 3†
<i>pan-in-mi 3-8</i>	<i>pab-in-po-1</i>	mi 3	—	—
<i>pan-in-mi 3-8</i>	<i>pab-in-po-3</i>	mi 3	—	—
<i>pan-in-mi 3-9</i>	<i>pab-in-po-1</i>	po	5/7	all po
<i>pan-in-mi 3-9</i>	<i>pab-in-po-3</i>	po	3/8	all po
Replicates of <i>pan-in-mi 3-7</i> with <i>pab-in-po-3</i>				
Replicate 2		— (Culture not tested)	6/7	all mi 3
Replicate 6		—	6/7	all po
Replicate 10		—	3/7	all mi 3

* Number surviving on minimal medium/number isolated.

† Some of these hyphal tip cultures were used as protoperithecial parents in crosses with 2293-2a (+-in-wild). Both *pan* and *pab* mutants were recovered among ascospores from each cross examined.

‡ Cytoplasmic properties as indicated by observations of both cytochrome content and growth rate.

and that the ranges of their growth rates overlap. Thus the classification of cultures in this table is less satisfactory than that in tables 3 or 5.

The results in table 4 provide no evidence that *poky* and *mi 3* can interact to give wild type cytoplasm in a heterocaryon. No heterocaryon culture or hyphal tip culture was found to grow faster than its faster (*mi 3*) component. A single exception was the original heterocaryon culture of *pan-in-mi 3-7* with *pab-in-po-3*, which gave a dry weight of 62mg, and its accumulation of cytochrome *c* agreed with this in suggesting a condition intermediate between *mi 3* and *wild*. But hyphal tip cultures derived from this heterocaryon culture and those from the replicates of it which were examined did not behave similarly. One possible explanation of the result is that a suppressor of *poky* (such as that described by MITCHELL and MITCHELL 1956), or of *mi 3*, was present in the heterocaryon culture, perhaps only during its growth in the flask.

While most of the cultures in table 4 were classified as *mi 3*, the heterocaryon and hyphal tip cultures from the heterocaryons involving *pan-in-mi 3-9* consistently gave growth rates typical of *poky* cultures, though only limited support for this classification could be obtained spectroscopically. These two heterocaryons, like two between *poky* and *wild* strains discussed earlier, proved to be unusual in being between strains of opposite mating type—*pan-in-mi 3-9* is a; perithecia and ascospores formed in both heterocaryon and hyphal tip cultures. However there was evidence, for example from the vigour of their growth on plates, that these were stable heterocaryons, unlike the earlier examples. Even so the slow growth rates obtained may possibly have been associated with the difference in mating type. GROSS (1952) and GARNJOBST (1953) have noted slow growth rates of heterocaryons between opposite mating types, though GARNJOBST recorded exceptions. Difference of mating type cannot be the explanation of the *poky* result for replicate 6 of *pan-in-mi 3-7* with *pab-in-po-3*. That heterocaryons between *poky* and *mi 3* strains occasionally have *poky* cytoplasm, not *mi 3* cytoplasm, seems more likely in the light of the results to be discussed for heterocaryons between *mi 3* and *wild* strains.

Some of the cultures classified as *mi 3* in table 4 should perhaps have been entered as intermediate between *poky* and *mi 3* though tending towards *mi 3*. Among all the *mi 3* cultures, growth rates for hyphal tip cultures from the same heterocaryon culture agreed well within themselves; but sets of growth rates for hyphal tip cultures from different heterocaryon cultures sometimes differed significantly. For example, the following dry weights were obtained for hyphal tip cultures of *pan-in-mi 3-7* with *pab-in-po-1*: 39mg; 33mg; 39mg; 33mg; 29mg; 30mg; 27mg, and the following for hyphal tip cultures from the original heterocaryon culture of *pan-in-mi 3-7* with *pab-in-po-3*: 26mg; 20mg; 14mg; 25mg; 24mg; 30mg. The two sets of results were obtained at the same time using cultures of the same age. The second set is more suggestive of an intermediate condition than any other obtained. However, both sets fall well within the range of control results obtained for all *mi 3* components of heterocaryons, shown in table 2.

It should be noted that good agreement of the growth rates of hyphal tip cultures derived from the same heterocaryon culture, such as that illustrated by the data in the previous paragraph, was found throughout this work.

One heterocaryon was made between strains having *poky* and *mi 3* cytoplasm with

the genes reversed, between *pab-in-mi* 3-1 and *pan-in-po*-1. The heterocaryon culture only was tested, and was classified as *mi* 3.

*Heterocaryons between strains having wild and mi 3 cytoplasm*s

Table 5 gives the results obtained for heterocaryons between 1633A (*pab-in-wild*) and four different isolates of *pan-in-mi* 3. The first results in the table, for heterocaryon cultures only, were among the earliest obtained for this combination of cytoplasm, and they suggested that the cytoplasm of the heterocaryons might be sometimes *mi* 3 and sometimes *wild*—even differing for the same heterocaryon made on different occasions (in the case of 1633A with *pan-in-mi* 3-4). This heterocaryon was therefore further repeated, and made by four methods different from the usual one to show whether they influenced the results. The methods were: (I) a larger inoculum of the *mi* 3 component than of the *wild* one was used; (II) a larger inoculum of the *wild* component was used; (III) the *pan-in-mi* 3 component was first grown alone, on a slant containing a limiting amount of calcium pantothenate such that growth just covered the slant, and then the *pab-in-wild* strain was added; (IV) the *pab-in-wild* component was first grown alone, it grew sufficiently after several days on a minimal slant, and then the *pan-in-mi* 3 strain was added. It will be seen that there was no indication, from the results for (I) and (II), that the cytoplasmic properties of the heterocaryon followed those of the component inoculated in larger amount, or, from

TABLE 5

*Heterocaryons between strains having wild and mi 3 cytoplasm*s (*pab-in-wild* with *pan-in-mi* 3)

<i>pab-in-wild</i> component	<i>pan-in-mi</i> 3 component	Heterocaryon culture Properties§	Hyphal tip cultures	
			Survival*	Properties§
1633A (<i>pab-in-wild</i>)	<i>pan-in-mi</i> 3-3	<i>mi</i> 3	— (Hyphal tips not isolated)	—
1633A (<i>pab-in-wild</i>)	<i>pan-in-mi</i> 3-5	<i>wild</i>	—	—
1633A (<i>pab-in-wild</i>)	<i>pan-in-mi</i> 3-6	<i>wild</i>	—	—
1633A (<i>pab-in-wild</i>)	<i>pan-in-mi</i> 3-4	<i>mi</i> 3	—	—
1633A (<i>pab-in-wild</i>)	<i>pan-in-mi</i> 3-4	<i>wild</i>	—	—
1633A (<i>pab-in-wild</i>)	<i>pan-in-mi</i> 3-4	Intermediate between <i>mi</i> 3 and <i>wild</i>	4/6	all <i>mi</i> 3‡
1633A (<i>pab-in-wild</i>)	<i>pan-in-mi</i> 3-4	I† <i>wild</i>	4/4	all <i>mi</i> 3‡
		II Intermediate between <i>mi</i> 3 and <i>wild</i>	4/4	all <i>mi</i> 3
		III <i>wild</i>	5/5	all <i>wild</i> ‡
		IV <i>wild</i>	5/5	all <i>wild</i> ‡

* Number surviving on minimal medium/number isolated.

† I-IV: Heterocaryons made by methods differing from the usual one (see text).

‡ Some of these hyphal tip cultures were used as protoperithecial parents in crosses with 2293-2a (+*-in-wild*). Both *pan* and *pab* mutants were recovered among ascospores from each cross examined.

§ Cytoplasmic properties as indicated by observations of both cytochrome content and growth rate.

the results for (III) and (IV), that they followed those of the component grown first. Results obtained for a second heterocaryon (1633A (*pab-in-wild*) with *pan-in-mi* 3-2), which was examined in the same way, presented a similar picture.

All these results suggested that a heterocaryon between strains having *mi* 3 and *wild* cytoplasms might have the cytoplasmic properties of one or other component. Heterocaryon cultures tested appeared to be on different occasions *mi* 3, *wild* or intermediate. The sets of hyphal tip cultures derived from them were sometimes *mi* 3 and sometimes *wild*. In spite of the results for some heterocaryon cultures, it is not clear that any case of a heterocaryon having an intermediate type of cytoplasm was obtained, since of the sets of hyphal tip cultures, which agreed well within themselves, none was evidently intermediate. The set of hyphal tip cultures from 1633A with *pan-in-mi* 3-4 made by method (IV) approached most nearly an intermediate condition. They gave growth rates which were somewhat low for wild strains (dry weights: 74 mg; 71 mg; 71 mg; 74 mg; 71 mg) and which were significantly different from growth rates obtained at the same time and under comparable conditions for hyphal tip cultures from method (III). Observations of cytochrome agreed with the classification of both sets of hyphal tip cultures as *wild*. The heterocaryon cultures classified as intermediate may be explained by supposing them to have been mixtures perhaps of an *mi* 3 heterocaryon and its wild component (1633A (*pab-in-wild*) grows a very little on minimal medium). The case of a heterocaryon culture appearing *wild* and the hyphal tip cultures derived from it *mi* 3 may require a similar explanation.

For the heterocaryon 1633A (*pab-in-wild*) with *pan-in-mi* 3-4 (as shown in the table), and for 1633A (*pab-in-wild*) with *pan-in-mi* 3-2, marker genes were recovered from crosses of both *mi* 3 and *wild* hyphal tip cultures to wild type. Preliminary counts of *pab* and *pan* mutants at ascospore germination gave no indication that the cytoplasmic properties of the cultures were correlated with their nuclear ratios.

Six heterocaryons were made between strains having *mi* 3 and *wild* cytoplasms with the *pab* and *pan* genes reversed, using the strains 5531A (*pan-in-wild*), *pan-in-wild*-2 and *pan-in-wild*-3 with *pab-in-mi* 3-1 and *pab-in-mi* 3-2. Only heterocaryon cultures were tested and all were classified as *wild*.

Heterocaryons were also made between the following strains obtained by MRS. M. B. MITCHELL, in which the nuclear markers were C141 (histidineless) and 34556 (*pan-tothenicless*): *hist-in-wild* with *pan-in-mi* 3, and *pan-in-wild* with *hist-in-mi* 3. (The mutant *pan* (34556) has not been shown to be different from *pan* (5531)). Ten replicates were made of both of these heterocaryons in the way already described for a heterocaryon between *poky* and *wild* strains. For both, the replicates thought to be the fastest and slowest from their growth on minimal plates were selected, and hyphal tip cultures from them were examined. The hyphal tip cultures from both replicates of *hist-in-wild* with *pan-in-mi* 3 were classified as *mi* 3, and those from both replicates of *pan-in-wild* with *hist-in-mi* 3 as *wild*.

DISCUSSION

The results obtained for heterocaryons between strains having *poky* and *mi* 3 cytoplasms indicate that such strains do not interact to give wild type mould or a strain faster than *mi* 3.

No stable intermediate state of cytoplasm has been shown to occur. For hetero-

caryons between *poky* and *wild* strains, where this might most easily have been detected, no set of hyphal tip cultures was found which could not confidently be classed as *wild*. For both of the other combinations of cytoplasm, the set of hyphal tip cultures which came nearest to suggesting an intermediate state of cytoplasm has been described, but the properties of each set were sufficiently typical of one of the component cytoplasm to be regarded as indicating only a slight variation in the manifestation of it.

Thus all cultures which were known to have been heterocaryotic (derived from single hyphal tips) were shown to have the cytoplasmic properties of one of their components.³ In the case of heterocaryons between *mi 3* and *wild* strains different sets of hyphal tip cultures had the properties sometimes of one and sometimes of the other component, even differing for a single heterocaryon made on different occasions from the same strains. The *mi 3* with *wild* heterocaryons were unlike those for *poky* with *wild* and *poky* with *mi 3* in having, in most cases, the pantothenicless gene in the slower component; for all the combinations of cytoplasm more information about heterocaryons with the genes reversed and about heterocaryons with different marker genes is very desirable. However, it may be that heterocaryons between the other combinations of cytoplasm, as well as *mi 3* with *wild*, may take two alternative forms, which each have the cytoplasmic properties of one component, and which perhaps arise by exchange of nuclei without exchange of cytoplasm, or by segregation of the cytoplasm from a temporary mixture. One set of hyphal tip cultures, at least, suggests that there may be a *poky* form, as well as an *mi 3* form, of *poky* with *mi 3* heterocaryons. Failure to obtain *poky* hyphal tip cultures from *poky* with *wild* heterocaryons may have been a consequence of the large difference between the growth rates associated with *poky* and *wild* cytoplasm, that is, a consequence of the selective advantage of the *wild* form of heterocaryon. If so, it is not evident why, for heterocaryons between the other combinations of cytoplasm where the growth rates of the components differed less, mixtures of hyphal tip cultures of the two possible sorts were not obtained from the same heterocaryon culture, though hyphal tips of any one set were often derived from inoculations on to more than one plate. If there are two forms of heterocaryon for each combination of cytoplasm, the form assumed by a particular culture may be decided at an early stage, perhaps when the heterocaryon is first formed, by some mechanism of which there is no indication.

It is evident from the consistent finding that *poky* with *wild* heterocaryons have the characteristics of strains with *wild* cytoplasm, that the *poky* condition is not "dominant" over *wild* in the way which might be expected if *poky* was caused by, and able to infect wild cytoplasm with, a virus. As will be clear from the previous paragraph, it does not necessarily follow that *wild* is "dominant" over *poky*, or hence that *poky* cytoplasm has a deficiency which can be repaired by *wild*. Also, the results do not indicate that either component is "dominant" in *mi 3* with *wild* heterocaryons.

SUMMARY

Heterocaryons have been made between strains of *Neurospora crassa* having *poky*, *mi 3* and *wild type* cytoplasm, in the three possible combinations. The strains were

³ The method of selecting the strains used in heterocaryons means that the possibility of a heterocaryon with a growth rate slower than that of its slower component has not been examined.

marked with biochemical mutant nuclear genes and the heterocaryons made on minimal medium. Single hyphal tips were isolated and were confirmed as having been heterocaryotic, in all cases tested, by recovery of the marker genes of both components of the heterocaryon.

The cytoplasmic properties of the heterocaryons, as indicated by tests of the single hyphal tip cultures, were: for a number of heterocaryons between strains having *poky* and *wild type* cytoplasms respectively, consistently *wild type*; for heterocaryons between *poky* and *mi 3* strains, usually *mi 3* but occasionally *poky*; for heterocaryons between *mi 3* and *wild type* strains, sometimes *mi 3* and sometimes *wild type* cytoplasm. Thus:

- (1) There was no evidence that strains having *poky* and *mi 3* cytoplasms can interact in a heterocaryon to give wild type mould or a strain faster than either.
- (2) No intermediate state of cytoplasm was found.
- (3) *Poky* and *mi 3* were not shown to be "dominant" over *wild type* in the way which might be expected if either condition was caused by an infective agent.

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