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A genetic study of female sterility in *Neurospora crassa*

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SUMMARY

A genetic analysis following the initial detection of a female sterile variant resulted in the finding of seven or eight different female sterile mutants, most or all of which are on linkage groups I and II. They were present in heterokaryotic condition in already existing strains, except one which originated spontaneously during the study. All mutants fail to produce functional protoperithecia. Most of them, however, are able to function as female parents in heterokaryons. All mutants differ morphologically from the wild type, most being subtly different, but two being appreciably different. The apparently high frequency of occurrence of female sterile mutants suggests that protoperithecial development is under an elaborate genetic control. Differences in vegetative morphology appear to be a common property of mutants affecting the early stages of the sexual development.

1. INTRODUCTION

Of different possible approaches to studying the sexual part of the life cycle in the fungi, the use of mutants seems particularly attractive. Mutants can be isolated on the basis of their effects on differentiation without a preconceived knowledge of the underlying processes they may represent. Once they are available they can be manipulated experimentally towards an eventual understanding of events affected at the molecular level.

A number of female sterile mutants have been reported in *Neurospora* (Dodge, 1946; Mitchell, Mitchell & Tissières, 1953; Westergaard & Hirsch, 1954; Srb, 1957; Fitzgerald, 1963; Barbesgaard & Wagner, 1959; Horowitz *et al.* 1960; Mitchell, 1966; Terenzi & Reissig, 1967; Vigfusson, 1969; Tan & Ho, 1970; Bhattacharya & Feldman, 1971; Ho, 1972; Weijer & Vigfusson, 1972). This paper describes a genetic study in which seven or eight female sterile mutants were also found. The mutants fail to produce functional protoperithecia, and may be useful along with the others in studying the early sexual stages in this organism. The study was prompted by the finding of one of us (SFHT) of instances of 'sterility' among progeny from some crosses and was directed towards a better understanding of the nature of these problems. A technical note based on some observations has been published (Mylyk & Threlkeld, 1972).

2. MATERIALS AND METHODS

(i) *Strains*

The wild types used were Abbott *a*, 74-OR23-1 *A* (abbreviated OR *A*), and 74-OR8-1 *a* (abbreviated OR *a*). Mutant strains were *ad-4 A* (allele No. F2, linkage group IIIR, FGSC No. 442), *pan-2 A* and *a* (B3, VIR), *pan-2 A* (B5, VIR), *nic-3 A* and *a* (Y31881, VIIL), *al-2 a* (15300, IR), and *ad-3A A* (2-17-186, IR). The linkage testers were 'alcoy' *A* and *a* described by Perkins *et al.* (1969). The strains Abbott *a*, 74-OR23-1 *A*, *ad-4 A*, and probably 74-OR8-1 *a*, were obtained from the Fungal Genetics Stock Center (FGSC), and *ad-3A A* was obtained from A. J. F. Griffiths. The *pan-2*, *nic-3*, and *al-2* strains were synthesized by crossing the mutants into a St Lawrence genetic background.

(ii) *Media*

Sucrose medium: the Westergaard & Mitchell (1947) medium having 2% sucrose and 1·5% agar. Glucose medium: the same but having glucose instead of sucrose. Malt-peptone medium: described previously (Threlkeld, 1962). Sorbose medium: prepared as described previously (Threlkeld, 1962), but having 1% sorbose, 0·05% glucose, and 0·05% fructose (see Brockman & de Serres, 1963). Where necessary, adenine was added in a concentration of 40 mg/l, pantothenic acid (Ca salt) was added at 80 mg/l, and nicotinamide was added at 80 mg/l.

(iii) *Technical procedures*

Most procedures were carried out using established methods as can be found in Davis & de Serres (1970). Crosses were made in 15 × 150 mm test tubes containing 5 ml sucrose medium, by inoculating a tube with the female parent of a cross, and after seven days' growth, by fertilizing the culture with a 1–2 ml conidial suspension of the male parent. Ascospores were isolated to 10 × 75 mm tubes containing 1 ml glucose medium. Tests for biochemical mutants were carried out on plates of sorbose medium. All vegetative cultures, tests, and crosses were at 25 °C unless otherwise indicated.

Two procedures were used routinely in testing for female sterility: (*a*) strains being tested were grown for 7 days in 10 × 75 mm test tubes containing 1 ml sucrose medium, then conidial suspensions of the female sterile strains 18-1 *A* and 1-3 *a* (derived from a cross between the wild type OR *A* and a strain *ad-4 fs-1 a*) were added to separate cultures of each strain, and the tubes were shaken on a Vortex mixer to spread the suspension over the surface of each culture; (*b*) the procedure was that in (*a*) except that a conidial suspension having a mixture of *both* strains 18-1 and 1-3 was used, and this mixture was added to each culture being tested. Both methods were found equally reliable. The first had the advantage of providing the mating types of *fs*$^+$ strains, whereas the second required half as much testing as the first. The female sterile strains 18-1 and 1-3 were used to avoid the possibility that the strains intended as male parents could grow up in the tubes and

function as female parents, thereby providing false results. Tan & Ho (1970) similarly used female sterile testers as males in their study.

Examinations for developing perithecia were continued for at least one week following attempted fertilization. From extensive observations over longer periods of time it was found that all cultures from the crosses studied could be correctly scored as *fs* or *fs*$^+$ after one week except for some heterokaryons used in complementation tests, where developing perithecia were occasionally first noticed as late as 10–12 days after fertilization.

(iv) *A problem in using* ad-4

The mutant *ad-4* interferes with the reliable testing for female sterility under the conditions used, so that repeated tests on individual isolates or tests of sister spores from asci did not always yield identical results. Hence only data from *ad-4*$^+$ strains could be used unless further testing confirmed the presence or absence of female sterile mutants in *ad-4* strains.

(v) *Definitions and conventions*

fs: female sterile. *fs*$^+$ or + : female fertile. Complex ascus: an ascus having the pattern 1+ : 3*fs* or 0+ : 4*fs*, indicating segregation for more than one female sterile mutant (ascus patterns were usually treated as tetrads, i.e. in terms of four pairs of identical spores, even though all eight spores were isolated and those in complex asci were all tested for female sterility). Designation of crosses: the first strain referred to in a cross is the female parent.

3. RESULTS

(i) *A description of the study leading to the finding of seven or eight female sterile mutants*

Female sterility was initially detected in the cross Abbott *a* × *ad-4 A* (FGSC 442). The genetics of this trait was pursued for three generations of crosses to the wild types OR *A* and OR *a*. It became apparent that a number of genes were segregating. It was also evident that certain strains were heterokaryotic for female sterility, i.e. mutations had occurred, so that the strains contained both female fertile and female sterile nuclei, and the female sterile mutants segregated among the crosses.

The complexities became apparent from several types of observations. All of the above crosses produced female sterile progeny, even though both parents of some crosses were female fertile. A number of crosses produced a small proportion of asci with complex patterns, indicating more than one female sterile mutant segregating in each such cross. In some instances several asci segregated for a morphological characteristic that was not found in the majority of asci from the cross, and the morphologically different strains were female sterile.

Following the realization that female sterile mutants were segregating from heterokaryotic parents, the wild types Abbott *a*, OR *A*, and OR *a*, and the mutant

strain *ad-4 A* (FGSC 442) were tested to see if they were heterokaryotic. It was thought that since these strains had been in use for some time, they were more likely to have accumulated mutants, and hence become heterokaryotic, than isolates selected during this study and used in crosses to the OR wild types. Conidial isolations of the wild types yielded female sterile cultures, confirming that Abbott *a*, OR *A*, and OR *a* were indeed heterokaryotic. Some crosses, described later, confirmed that *ad-4 A* (FGSC 442) was also heterokaryotic.

In distinguishing and isolating the different mutants, further genetic tests and some complementation tests were carried out using strains believed to represent the entire spectrum of female sterile mutants. These included conidial isolates from the wild types and ascospore isolates from various crosses, especially from complex asci. Female fertile strains used in further genetic analyses were *pan-2(B3) A* and *a* and *nic-3 A* and *a* (the biochemical mutants do not interfere with female fertility). They were presumed homokaryotic, as they were recent ascospore isolates. From the crosses female sterile strains having the heterokaryon forcing markers *pan-2* and *nic-3*, and being heterokaryon compatible with strain *ad-3A A* (having a St Lawrence genetic background), were selected and used in complementation tests. Furthermore, some crosses between female sterile strains and the alcoy linkage testers were studied.

The genetic and complementation data, along with morphological characteristics, distinguished a total of seven or eight female sterile mutants. Most of them could be attributed to spontaneous mutations occurring in the strains Abbott *a*, *ad-4 A*, OR *A*, and OR *a*, with one mutation apparently originating in an ascospore isolate from one of the crosses.

(ii) *The distinction of the different female sterile mutants*

Genetic and complementation data representative of the different female sterile mutants are presented in Tables 1 and 2. On the basis of complementation tests alone (Table 2), it is possible to distinguish between at least five mutants, with data for complementation between *fs-3* and *fs-6* being unavailable, and *fs-5* being excluded from the tests. The mutants *fs-3* and *fs-6* were distinguished on the basis of linkage considerations, as *fs-3* maps to the left of the mating type locus on linkage group I (see below), whereas *fs-6* is unlinked to mating type (Table 1). The mutant *fs-5* was excluded from the complementation tests because it is slow growing and is difficult to handle. It was considered a mutant different from the others on the basis of its slow growth and morphology. The above considerations suggest seven different mutants. However, closer examination of the data, described in the following section, suggests that *fs-n* may be either one mutant or two closely linked ones. Hence as many as eight different mutants were found.

(iii) *The genetics of the female sterile mutants*

The genetic data are considered for the different mutants individually below.

(*a*) *fs-1*. The mutant *fs-1* (isolates 19-2 and 2494, Tables 1 and 2) was a heterokaryotic component of strain *ad-4 A* (FGSC 442), as became evident from

the following considerations. From the cross *pan-2(B3) a* × *ad-4 A* (FGSC 442), 12 of 103 *ad*$^+$ progeny were female sterile, including the isolate 2494. This small proportion of female steriles could have occurred if the *ad-4* strain were heterokaryotic, or if it were homokaryotic for a female sterile mutant linked to *ad-4*. In crosses between *fs-1* isolates and the alcoy linkage testers (Table 1), *fs-1* was found to be linked to *al-1*, showing that it must be on linkage group I or II, whereas *ad-4* is on III. Hence strain 442 must be heterokaryotic, having *fs-1* as a component.

Table 1. *Genetic data from crosses involving select female sterile strains*

		fs$^+$ × select strains					Linkage to alcoy markers†		
				Random spore segregation§					
Mutants	Select strains	Ascus patterns‡	1st:2nd division segregation	+	fs	Recombination with mating type	*al-1* (I or II)	*cot-1* (IV or V)	*ylo-1* (III or VI)
fs-1	19-2	102 2+:2fs‖	35:67				**		NS
	2494						*	NS	NS
fs-2	10-4			56	61				
fs-3	84-2, 84-8	113 2+:2fs	60:53			See text			
fs-4	2326			21	33	22·2%, **			
fs-5	5-5, 32-1			50	47				
fs-6	2351			109	96	NS	**		NS
fs-n¶	2324			27	32	52·6%, NS	**	NS	NS
	29-4	48 2+:2fs, 3 1+:3fs	12:36 (2+:2fs asci)						
	2-2			98	98	40·8%, **	**	NS	NS
	44-1			92	101	44·6%, NS	**	NS	NS
	44-3			39	41	35·0%, **			
	44-4			108	92	39·0%, **	**	NS	NS
	44-8			91	105	44·8%, NS	**	*	*

* χ^2 significant at the $P = 0{\cdot}05$ level. ** χ^2 significant at the $P = 0{\cdot}01$ level. NS χ^2 not significant.

† 100 random spores were isolated from each cross between the select strain and the alcoy linkage tester (germination at least 70%).

‡ Only complete asci, i.e. those in which at least one spore from each presumed identical pair germinated, are included (at least 90% of the asci from each cross).

§ Germination at least 75%.

‖ 3 complex asci segregating for *fs-2* as well as *fs-1* are excluded from these data.

¶ *fs-n* may represent one mutant or two closely linked ones (see text).

The gene *fs-1* is more likely on linkage group II than on I. In the various crosses involving this mutant, it always segregated independently of the mating type locus on linkage group I. Furthermore, it segregated independently of *fs-n*, believed to be on linkage group I (see below), in one cross + + × *fs-1 fs-n*.

(*b*) *fs-2*. The mutant *fs-2* (isolate 10-4, Tables 1 and 2) was one of two mutants initially detected on the basis of its morphology. It is somewhat colonial in its growth. It was originally found in three out of 105 asci from the cross OR *A* × 19-2. Since it did not segregate in all the asci, it was realized to have been inherited from a heterokaryotic parent. Since the strain OR *A* had been in use substantially longer

than 19-2, which was isolated shortly prior to the cross, the mutant more likely originated in OR *A*.

One cross + + × *fs-1 fs-2* showed loose linkage between *fs-1* and *fs-2*, tentatively suggesting that *fs-2* is on linkage group II.

(*c*) *fs-3*. The mutant *fs-3* (isolates 84-2 and 84-8, Table 1, and 52-2, Table 2) segregated in 12 out of 105 asci from a cross OR *a* × 19-4 *fs-1 A*. Two further crosses, using strains 84-2 and 84-8, established the order and distances: *fs-3*–(15·5)–*A*/*a*–(11·5)-centromere (from 113 asci).

Table 2. *Complementation of select female sterile strains*

		Mutants and representative strains										
							fs-n					
Mutants	Strains*	*fs-1* 2494	*fs-2* 10-4	*fs-3* 52-2	*fs-4* 2326	*fs-6* 2351	2324	2-2	44-1	44-3	44-4	44-8
fs-1	19-2	−	+	+	+	+	+	+	+	+	+	+
fs-1	2494		+	+	+	+	+	+	+	+	+	+
fs-2	10-4			+	+	+	+	+	+	+	+	+
fs-3	52-2				+	NT	+	+	+	+	+	+
fs-4	2326					+	+	+	+	+	+	+
fs-6	2351						+	+	+	+	+	+
fs-n	2324							−	−	−	−	−
fs-n	2-2								−	−	−	−
fs-n	44-1									−	−	−
fs-n	44-3										−	−
fs-n	44-4											−

* Complementation tests were carried out using female sterile derivatives from these strains having mating type *A* and having *nic-3* and *pan-2* as heterokaryon forcing markers, except strain 2494 which was of genotype *pan-2 fs-1 A* and could be used directly.

+, Complementation; −, no complementation; NT, not tested.

The male parent was the female sterile strain 1-3*a*, or, in repeated tests, *al-2 a* (both male parents produced identical results).

Since *fs-3* occurred in only some asci from cross OR *a* × 19-4 *fs-1 a*, one parent was clearly heterokaryotic for this mutant, and it appears to be strain 19-4 which was isolated shortly prior to the cross. Of the 12 complex asci in which it was found, ten were of patterns 1+ : 3*fs* and two were 0+ : 4*fs*. The ten female fertile strains from these asci segregated 2 *A* and 8 *a*. Since *fs-3* is linked to mating type, the *fs-3*$^+$ allele was likely inherited from OR *a* and the *fs-3* allele from strain 19-4. Clearly *fs-3* differs from *fs-4*, which definitely arose in OR *a* (see below), since *fs-3* and *fs-4* complement (Table 2).

(*d*) *fs-4*. This mutant arose in the wild type OR *a*, as it occurred among conidial isolates from this wild type, including isolate 2326 *fs-4 a* (Tables 1 and 2). It is located 22·2 map units from the mating type locus on linkage group I (based on 54 random spores).

(*e*) *fs-5*. Mutant *fs-5* (isolates 5-5 and 32-1, Table 1) was the second female sterile mutant initially detected on the basis of its morphology. It grows very slowly, with

most aerial growth occurring close to the surface of the medium. Cultures of this mutant eventually turn a brown colour. The mutant (or at least this morphology) segregated in several asci from two different crosses involving the wild type OR *a*. Hence it was likely a heterokaryotic component of OR *a*. In a cross + + × *fs-1 fs-5*, mutants *fs-1* and *fs-5* showed loose linkage, providing tentative evidence that *fs-5* is on linkage group II.

(*f*) *fs-6*. This mutant originated in the wild type Abbott *a*, as it was present in conidial isolates from this strain, including isolate 2351 (Tables 1 and 2). It occurs on linkage group I or II, according to the cross to alcoy (Table 1). Linkage between *fs-6* and mating type has not been detected.

(*g*) *fs-n*. In the absence of a simple and attractive explanation of the data, it is most convenient to consider *fs-n* as a pair of closely linked female sterile mutants which could not be distinguished. The designation *fs-n* may refer to either of the proposed mutants or the double-mutant. Conidial isolations of OR *A* yielded *fs-n*, demonstrating that at least one of the mutants originated in this wild type.

Strain 2324 (Tables 1 and 2) was a conidial isolate from OR *A*. The other *fs-n* strains (Tables 1 and 2) were ascospore isolates, with strains 2-2, 44-1, 44-3, 44-4, and 44-8 being progeny from a cross OR *A* × 29-4 *fs-n a*. The isolate 2-2 came from ascus 2 having the pattern 2 + : 2*fs*, and 44-1, 44-3, 44-4, and 44-8 came from ascus 44 having the pattern 1 + : 3*fs*. The four isolates from ascus 44 represent the three female sterile meiotic products, with 44-3 and 44-4 presumed to be sister spores.

Evidence that *fs-n* represents two closely linked non-complementing mutants comes from the following considerations. The cross OR *A* × 29-4 produced three complex asci out of a total of 51 (Table 1), suggesting two genes segregating in the cross. The complex asci seem to have occurred too frequently to represent gene conversion at one locus. Segregation in all crosses involving *fs-n* strains (Table 1) was consistent with the ratio 1 + : 1*fs* even though some strains (29-4, 2-2, and one meiotic product from ascus 44) should have the double-mutant genotype. This could have occurred only if the mutants are closely linked. If that is the case, however, the mutants do not complement. Complementation did not occur between derivatives from any two female sterile isolates from ascus 44 (Table 2), even though two of the meiotic products should have the different single-mutant genotypes.

The use of three rather than just two *a* isolates from ascus 44 (44-1, 44-3, and 44-4) ensures that both meiotic products of mating type *a* were included in the complementation tests. Genetic and complementation data using isolates from another ascus having the pattern 1 + : 3*fs* provided results similar to those from ascus 44, making it seem unlikely that dissecting errors could account for the unusual results.

The three asci from cross OR *A* × 29-4 having patterns 1 + : 3*fs* (Table 1) suggest that the mutants *fs-n* are approximately three map units apart. The large proportion of second division segregation patterns (Table 1) shows that they are a substantial distance from the centromere. Linkage to *al-1* of alcoy in crosses involving various *fs-n* strains (Table 1) shows that they are on linkage group I or II. Linkage to mating type in some crosses but not others (Table 1) suggests that

they are far from the mating type locus on linkage group I. (Apparent linkage of *fs-n* to *cot-1* and *ylo-1* in the cross alcoy × 44-8 *fs-n A*, is partly due to distorted segregation for the alcoy markers, and the linkage is probably too close to the borderline of significance to be important.)

The failure of the proposed *fs-n* mutants to complement with each other poses questions about their nature. It is unlikely that the complex asci from cross OR *A* × 29-4 reflect a duplication of a segment of chromosome on which a locus for female sterility exists. The *fs-n* strains function well as male parents, whereas duplications are usually 'barren', i.e. crosses with them may produce many perithecia, but few if any spores are produced, regardless of whether the duplications are used as female or male parents (Newmeyer & Taylor, 1967; Newmeyer, 1970; Perkins, 1972). It is of interest, then, whether the mutants represent one gene or two. They could represent one gene if the actual distance between them is well below the estimate of three map units provided here. Alternatively, they may be non-complementing mutants representing completely different genes as have been found in *Aspergillus* (Pontecorvo, 1963; Apirion, 1966).

(iv) *Functioning of the mutants in crosses involving heterokaryons*

Horowitz *et al.* (1960) demonstrated that the female sterile mutant *ty-1* could proceed through crosses when used as a female parent in a heterokaryon. In the complementation tests in Table 2, where heterokaryons having pairs of female sterile mutants were tested for their effectiveness as female parents, ascospores were produced wherever perithecia were produced. Most of these mutants, then, can similarly function as female parents in heterokaryons.

An attempt was made to test if a heterokaryon could also be used in making a cross homozygous for a female sterile mutant. The cross attempted was [*pan-2(B5) A* + *nic-3 fs-3 A*] ♀ × *pan-2(B3) fs-3 a* ♂. It was made on a minimal medium. The cross has a *pan-2* × *pan-2* component heterozygous for *fs-3*, and a *nic-3* × *pan-2* component homozygous for *fs-3*. The mutant *pan-2* is a spore colour mutant, so that *pan-2* spores produced on a crossing medium lacking pantothenic acid fail to become pigmented (Threlkeld, 1965). Spores from the component heterozygous for *fs-3*, then, were expected to be *pan-2* and hence pale in colour. Half of the spores from the *fs-3* × *fs-3* component, on the other hand, were expected to be *pan-2*$^+$ and darkly pigmented. Pigmented spores occurred in appreciable numbers in two separate attempts of the cross. Furthermore *nic-3* was recovered among the progeny. Hence the *fs-3* × *fs-3* cross was demonstrated to occur.

(v) *Other characteristics of the female sterile mutants*

Although rigorous microscopic studies have not been made, it appears that all female sterile mutants found fail to produce functional protoperithecia (data are not critical enough to distinguish for any mutant whether protoperithecial development fails to begin, whether it begins but is not completed, or whether a protoperithecium is formed but is not receptive). Casual observations under a dissecting microscope (× 80 magnification), of cultures representing all mutants, failed to

reveal structures that could be interpreted as developing protoperithecia, except for a culture of *fs-5*, where several possible protoperithecia were observed. More conclusive evidence that the mutants affect protoperithecial development was obtained from the female sterility tests in which crosses were attempted using the mutants as female parents. These attempts failed to proceed far enough in the sexual development to provide an indication of the mating type of a strain, with the exception of some strains of *fs-1* and *fs-5*. These findings are in accord with those of other investigators that female sterile mutants generally produce defective protoperithecia or fail to produce them at all (for references, see Introduction). The exceptions concerning *fs-1* and *fs-5* were due to the 'leakiness' of these mutants; in the tests for female sterility, some strains of these mutants produced some perithecia and spores when they were used as females. However the numbers of perithecia and spores were distinctly below the lower limit for fs^+ strains segregating in the same crosses.

In addition to *fs-2* and *fs-5* which were first detected on the basis of having morphological characteristics appreciably different from the normal, all other mutants found differ morphologically from the wild type, but those differences are *subtle* and might have gone undetected for most mutants if their segregation had not been observed in asci. The mutants grow more slowly, are less pigmented, and form a more continuous culture in a tube than wild types (the OR wild types used for comparison produce a dense band of conidia over a gap of relatively sparse growth above the surface of the medium). Mutant *fs-1* is also somewhat stringy in its appearance. Classification for female sterility on the basis of morphology was usually met with some difficulty in cultures from germinated ascospores (except for *fs-2* and *fs-5*), but five-day-old subcultures could be classified with 99% accuracy.

Complementation for female sterility was accompanied by complementation for morphology. Female fertile heterokaryons (Table 2) were wild type in their vegetative morphology, whereas female sterile ones were not.

The mutant *fs-2* does not grow at 34 °C, but none of the others are similarly affected at this temperature.

Female sterility was not alleviated for any of the mutants on the malt-peptone or the glucose media. Also when the interval between the time of inoculation of the female sterile strain and the time of attempted fertilization was extended to two weeks (three weeks for *fs-5*), the cultures still tested as female sterile.

4. DISCUSSION

The finding of seven or eight spontaneously originating female sterile mutants, occurring in heterokaryotic condition in five strains, including a culture from a recently isolated ascospore, suggests that such mutations occur commonly in *Neurospora*. This is in agreement with other observations. Westergaard & Hirsch (1954) pointed out that vegetative cultures of *Neurospora* commonly become 'sterile' when maintained for long periods of time. From such cultures they were

able to isolate a number of female sterile mutants. More recently, Bhattacharya & Feldman (1971) found that female sterile mutants could very successfully be isolated following mutagenesis.

From the apparently high frequency of occurrence of the mutants, it seems that many loci must affect female fertility, so that collectively they mutate with a substantial frequency. This in turn indicates that protoperithecial formation or function is under an elaborate genetic control. Such a genetic complexity was demonstrated for another part of the sexual development in *N. crassa*. Srb and his associates, in their methodical search for mutants affecting ascus development, were able to find mutants at 13 different loci, despite the difficulty in screening for mutants in a part of the life cycle where two parental genomes interact (Srb & Basl, 1969*a*, *b*; Pincheira & Srb, 1969).

The common finding of female sterile mutants prompts the question of whether they may possess a selective advantage in the vegetative phase, once they occur in female fertile strains. If such an advantage exists, however, it might disappear once the mutants sector from the heterokaryon, as the female sterile mutants grow more slowly than female fertile strains.

The finding that all female sterile mutants isolated in this study differ from the wild type in their vegetative morphology suggests that this is a common property of mutants affecting the early part of the sexual development in *Neurospora*. The female sterile mutants *cyt-1* and *cyt-2* (Mitchell *et al.* 1953), *ty-1* (Horowitz *et al.* 1960), *ty-2* (Ho, 1972), *leu-1* (Mylyk & Threlkeld, 1972), *R* (Barbara Turner, personal communication), and two others detected subsequent to the study reported here, also differ from the wild type in their vegetative morphology. The subtleness of this difference in most cases suggests that a careful re-examination of additional female sterile mutants may reveal that some of them also have such a characteristic.

The ability of female sterile mutants to commonly function as female parents in heterokaryons poses questions about events occurring in the developing reproductive structures that make crosses using heterokaryons possible. It would be of interest to know what tissues must be heterokaryotic in order that products from non-mutant genes alleviate the effects of the mutants, especially in permitting crosses such as *fs-3* × *fs-3* to take place. It would be of interest to know if crosses involving certain mutants can only occur in 'mixed' perithecia, i.e. those having a non-mutant as well as a mutant female component.

The results prompt a note of caution in the study of female sterility. In view of the common occurrence of the mutants, care must be taken in choosing strains that do not contain hidden mutants that might yield misleading experimental results. The use of recent ascospore isolates as female fertile strains, and the preservation of female sterile mutants in suspended animation may be especially effective in avoiding difficulties.

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