GENETICS OF

FEMALE STERILITY IN NEUROSPORA

A GENETIC STUDY OF FEMALE STERILITY IN *NEUROSPORA CRASSA*

by

OREST MARTIN MYLYK, B.Sc., M.Sc.

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

July 1971

DOCTOR OF PHILOSOPHY
(Biology)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: A Genetic Study of Female Sterility in *Neurospora crassa*

AUTHOR: Orest Martin Mylyk, B.Sc. (University of Alberta)
M.Sc. (University of Alberta)

SUPERVISOR: Dr. S. F. H. Threlkeld

NUMBER OF PAGES: xi , 143

SCOPE AND CONTENTS:

In a general way, this study was directed towards the understanding of processes concerned with the sexual reproduction of *Neurospora crassa*. It was prompted by an observation of Threlkeld that crosses between Neurospora strains derived from different genetic backgrounds occasionally produce sterile progeny. This specific project was concerned with finding naturally occurring variants expressing some form of sterility, studying their genetics and complementation, and characterizing the mutants found for several properties.

A search for sterility uncovered patterns of perithecial and ascospore production among strains being

tested for their ability to participate in crosses and also resulted in the finding of a female sterile variant. Subsequently a rather complex pedigree of crosses involving female sterile strains followed by conidial analysis and genetic and complementation studies of selected strains revealed a total of eight mutants causing this phenotype, all appearing to have arisen spontaneously among some of the strains used. Some linkage data were obtained, and the mutants were characterized for growth characteristics and crossing ability under different cultural conditions. Possible interpretations of the findings were considered.

ACKNOWLEDGMENTS

My thanks are especially due to Dr. S. F. H. Threlkeld for his suggestion of a research project, for his advice and encouragement during the study, and for his influence on some of my attitudes towards research and other things. I am also grateful to Dr. J. J. Miller, Dr. D. Carr, and Dr. F. Ziegler for their helpful suggestions and criticisms during the study.

Technical help was provided at various times by Mr. Leslie Gyorkos, Mrs. Shanta Thomas, Mr. Simon Morris, and Mr. Bob Boardman. I am very grateful to these people. Also I would like to thank Mrs. Linda Dowd for her kindness in typing this thesis.

The financial support of the National Research Council of Canada and the Ontario Graduate Fellowship Fund is gratefully acknowledged.

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INTRODUCTION

1. Introductory Remarks

In considering a morphogenetic process as any developmental change in form either towards greater complexity or greater simplification, Turian (1966) distinguishes two main phases for the ascomycetes: (a) a simplicative morphogenesis corresponding to the germination of spores, and (b) an elaborative morphogenesis corresponding to the reproductive phenomenon of spore production. These are separated by a more or less prolonged period of mycelial growth.

The first phase involves the morphological transition from a differentiated spore, through a physiological change in which a proliferative capacity is regained, to a redifferentiated but simpler structure, the germ tube or first vegetative hypha. The second involves a change in the physiological state from the vegetative to the reproductive followed by a more direct differentiation of asexual spores or a more complicated morphogenesis of sexual spores following the differentiation of relatively elaborate sex organs.

Most studies within this realm have dealt with the description of events occurring at the morphological level,

whereas the underlying biochemical and physiological processes and their genetic control are largely unknown (Turian, 1966). Neurospora has been no exception, either for its morphogenesis in general or for the sexual development which is the specific subject of this study.

It has been said (e.g. Raper, 1966) that Neurospora is often used for experimental purposes for which it is not best suited; that other organisms may provide advantages superior to those of Neurospora for particular studies. In the present state of insufficient knowledge (as Turian, 1966, refers to it) of processes concerned with development in the Ascomycetes, such a statement cannot be thoroughly evaluated in this regard. Certainly the use of other organisms has made significant contributions to this area of study, for example the ease with which mutants for the sexual development have been obtained in Sordaria (see Esser and Kuenen, 1967) or the certainty with which sex hormones were demonstrated in Ascobolus (see especially Bistis, 1965). Yet without detracting from the usefulness of these studies, the use of Neurospora is not without its advantages. Westergaard and Hirsch (1954) and Turian (1966) have pointed out the potential of studying the sexual development by observing the morphogenetic effects of changes in cultural conditions. In addition Turian (1966) has pointed out the advantage of being able to make biochemical, physiological, and genetic studies all on the

same organism. The volume of knowledge available in these fields alone makes the use of Neurospora seem attractive. For the level of organization involved no other organism has been so intensively studied, making possible the evaluation of new information in a larger perspective.

Apart from a personal bias in choice of an experimental approach, the use of genetics seems particularly attractive in studying sexual development in Neurospora. In the first place, the use of mutants seems valuable because in present concepts of molecular genetics a mutation represents one primary block at the biochemical level and all consequences of the mutation can be attributed to this block. Secondly, in the search for mutants, little more than a basic knowledge of the life cycle is required. Mutants can be detected by observing the ability of different strains to participate in crosses without any preconceived knowledge of the underlying processes affected. Finally, a genetic approach may permit the detection of developmental blocks attributable to mutations where they may otherwise go undetected, or at least could not be detected without great difficulty using other means (see sections 54, 55, 67).

Ultimately, the finding of the effect of each gene at the biochemical level, with the identification of enzymes, structural proteins, regulatory molecules, etc., would be desirable, but even before this is done or if it cannot be done, a genetic approach should permit the

construction of models concerned with the action and interaction of genes and gene products. The handling of mutant strains alone should provide clues towards the understanding of the underlying processes. In short, with the potential for manipulating genes and chromosome aberrations in Neurospora, it is attractive to suggest that the possibilities in studying the sexual development in this way may be more limited by the imagination of the experimentation than by the disadvantages of some characteristics of the organism.

2. Morphological studies on the sexual development

Neurospora species are either (a) heterothallic, in which each strain is of one of two mating types and crosses only occur between those of opposite mating type, e.g. N. crassa and N. sitophila (Shear and Dodge, 1927), (b) homothallic, in which each homokaryotic strain can proceed through the entire sexual cycle, e.g. N. terricola (Gochenaur and Backus, 1962), or (c) secondarily homothallic, in which most ascospores produced contain nuclei of opposite mating type, and a culture arising from such a spore is capable of producing perithecia and spores, e.g. N. tetrasperma (Shear and Dodge, 1927); in those species some ascospores (Shear and Dodge, 1927) and conidia (Dodge, 1928) produced are homokaryotic and strains derived from them behave similarly to those of the heterothallic species.

A complete description of the sexual development for any Neurospora species is not available. However studies involving the different stages and using N. crassa, N. sitophila, and N. tetrasperma provide a composite picture of events that occur.

The development of the female reproductive structure, the protoperithecium, begins with the formation of a rather blunt-ended helically coiled hypha (Colson, 1934; Dodge, 1935b; Backus, 1939; see Fig. 1a). The coil becomes septate, and the basal cells as well as branches of the parental hyphae grow out to initiate in the formation of a sheath which consists of a dense intertwining mass of threads several cell layers thick and enclosing the ascogonium (Colson, 1934; Dodge, 1935b; Backus, 1939).

The ascogonium is thought to be the terminal portion of the original coiled structure, and is a short coil of 5-10 multinuclear cells somewhat greater in diameter than those of the sheath or the vegetative hyphae (Backus, 1939). In N. tetrasperma where nuclei of both mating types are usually already present in the ascogonium, it ends blindly in the sheath (Colson, 1934). However in the heterothallic species which depend on fertilization from the outside, receptive hyphae called trichogynes develop (Dodge, 1935b; Backus, 1939). They are slender and usually septate hyphae, tapering towards their distal ends, which grow out from the terminal cell of the ascogonium and in more

complicated cases from the penultimate and antipenultimate cells as well (Backus, 1939; see Fig. 1b). They zigzag between the cells of the sheath, often growing for some distance around in a circle before finding a place to emerge (Dodge, 1935b). (Other hyphae grow out from the outer layer of the sheath and may be mistaken for trichogynes, but they are not connected with the ascogonium and hence cannot act as receptive hyphae; Dodge, 1935b).

Development ceases following protoperithecial formation in cultures of individual mating types, whether in N. crassa, N. sitophila, or homokaryotic cultures of N. tetrasperma (Shear and Dodge, 1927; Colson, 1934, Dodge, 1935b; Backus, 1939). However when nuclei of opposite mating type are brought together in the ascogonium, this stimulates further growth of the fruiting body, the formation of the ostiolar papilla, and the development of ascogenous hyphae leading towards the formation of asci and the production of ascospores (Dodge, 1935b).

Male sex organs do not develop in Neurospora (Colson, 1934), but microconidia, macroconidia, and hyphae of opposite mating type can act in fertilization (Dodge, 1935b). The latter include hyphae from mycelial mats, germ tubes, trichogynes, and hyphae from perithecia (Dodge, 1935b; Backus, 1939).

During fertilization in the heterothallic species,

trichogynes branch in the proximity of cells of the opposite mating type. They often become coiled and contorted upon approaching conidia, and form several finger-like processes for enclosing them (see Fig. 1c). Following contact, a slender cytoplasmic bridge forms and much of the conidial content passes into the trichogyne. Later the portion of the trichogyne in the region of contact appears to undergo gelatinization and soon afterwards the perithecium exhibits signs of growth. If fertilization involves hyphae, the trichogyne becomes helically coiled and wraps around the hypha before fusion occurs. (See Backus, 1939, for the above).

Once nuclei of opposite mating type are present in the ascogonium, the precise sequence of events is not clear. However from genetic studies and from what is thought to occur in ascomycetes in general, it is assumed that a pair of nuclei of opposite mating type become associated and undergo conjugate division (Catcheside, 1951; Emerson, 1966). Then ascogenous hyphae grow out of the ascogonium, pairs of the resulting nuclei enter them, and the hyphae form crozier-shaped hooks (Dodge, 1927; Colson, 1934; Singleton, 1953). The nuclei undergo a conjugate division with the spindles being somewhat parallel so that a pair of nuclei of opposite mating type come to lie in the bend of the hook (see Fig. 1d). Then two crosswalls are laid down, one across the tip and one across

the base of the crozier, enclosing the pair of unlike nuclei in the penultimate cell (primary ascogenous cell) and leaving one nucleus in each of the other portions of the hook (Singleton, 1953).

The two haploid nuclei in the primary ascogenous cell fuse, and the cell takes on the clavate shape of an ascus (Dodge, 1927; Colson, 1934; McClintock, 1945; Singleton, 1953). Three nuclear divisions occur, the first two being the two divisions of meiosis and the third a mitosis (Lindegren, 1933; Colson, 1934; McClintock, 1945; Singleton, 1953). Then spores are delimited and an additional nuclear division occurs within them (Dodge, 1927; Colson, 1935; McClintock, 1945; Singleton, 1953).

(Mitchell, 1960a, 1960b, 1964, 1965; has questioned whether some events concerned with ascus development and presented in the preceding two paragraphs have been accurately described. However, if inaccuracies occur, a better working model is yet unavailable.)

The events preceding the delimitation of spores differ somewhat in N. crassa and N. sitophila from those in N. tetrasperma. In the former species the spindles lie apart from one another during the divisions so that at the time of delimitation one nucleus occurs in each spore (Singleton, 1953; see Fig. 1e). In N. tetrasperma they overlap at the second and third divisions, so that following

first division segregation of the mating type locus (this usually occurs, Lindegren, 1932) pairs of nuclei of opposite mating type are always proximal and become enclosed in each spore (Dodge, 1927; Colson, 1934; see Fig. 1f).

Although it has not been followed in Neurospora, from what has been observed in other ascomycetes, it is presumed that the basal and terminal cells of the hook can fuse, forming another ascogenous hypha which develops into a crozier, and the entire sequence can be repeated several times (Singleton, 1953).

During ascus formation, the developing perithecium grows and darkens from the accumulation of melanic pigments. At maturity the ascospores are ejected through the ostiole, the opening at the apex of the perithecium (see Esser and Kuenen, 1967; also see Fig. 1g for a perithecium with asci prior to their ejection).

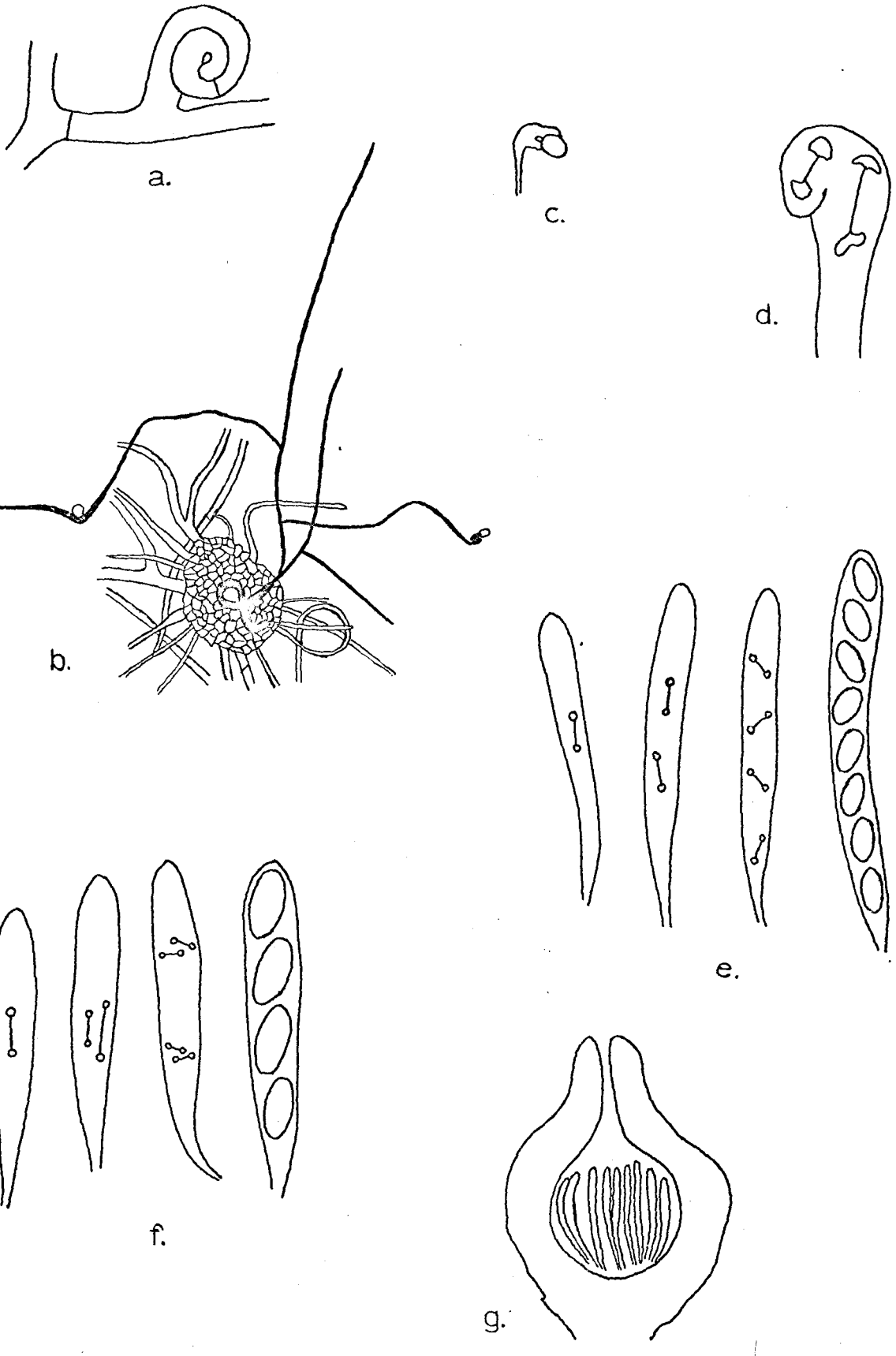
The genetic control of the sexual development in Neurospora.

3. Levels of control

The genetic control of the sexual development can be conveniently considered at two levels: (a) the control of mating type, and (b) the control of various stages of the development. A variety of studies have reflected some aspects of the genetic complexity of these phenomena, the action of the genes, their consequences in the morphology, and the cellular processes affected.

Figure 1. Stages of the sexual development in Neurospora. The different stages and sources from which diagrams were adapted are: (a) development of a coiled hypha from a vegetative one (N. tetrasperma; Colson, 1934), (b) development of trichogynes from an ascogonium (both shown in black; N. sitophila; Backus, 1939), (c) finger-like structures produced by a trichogyne for enclosing a conidium during fertilization (N. sitophila; Backus, 1939), (d) nuclear division in the crozier-shaped hook of an ascogenous hypha (note spindle orientation; N. tetrasperma; Colson, 1934), (e) nuclear division in the ascus of a heterothallic Neurospora species leading towards the formation of spores (note spindle orientation; nuclear division within the spores is not shown; N. crassa; Pincheira and Srb, 1969b), (f) nuclear division in the ascus of a secondarily homothallic Neurospora species leading towards the formation of spores (note spindle orientation; nuclear division within the spores is not shown; N. tetrasperma; Pincheira and Srb, 1969b), (g) perithecium with asci (spores not shown in asci; N. sitophila; Shear and Dodge, 1927).

Figure 1



4. Mating type

Mating type is controlled by the one gene - two allele system (Lindegren, 1932) found throughout the non-homothallic ascomycetes (Raper, 1960). The locus is thought to be highly stable since mutants for it have not been found (Raper, 1960). In addition to its role in the sexual processes, it has the property in the heterothallic species of usually preventing heterokaryon formation between strains of opposite mating type (Beadle and Coonradt, 1944; Garnjobst and Wilson, 1956). This, as well as other considerations (see Olive, 1958), suggests the possibility that it may be a complex locus (Pittenger, 1957, cited by Newmeyer, 1970). However, the heterokaryon incompatibility may be alleviated by a mutation for an unlinked gene called tolerant or tol (Newmeyer, 1968, 1970). In addition the postulated components of the complex locus have not been found separable by recombination (see especially Newmeyer, 1970). Hence thus far there is no experimental evidence establishing its complexity.

5. The genetic control of various stages of the sexual development

The genetic control of protoperithecial development has been demonstrated by the finding of a number of genetic variants for this phase of the development. These include genes affecting the density of protoperithecial production

(Aronescu, 1933; Howe, 1964) as well as mutants causing female sterility and lacking them or defective in their formation and normal functioning (Dodge, 1946; Mitchell et al., 1953; Srb, 1957; Fitzgerald, 1963; Barbesgaard and Wagner, 1959; Horowitz et al., 1960). The round spore mutant reported by Mitchell (1966) and a mutant reported by Vigfusson (1969), both female sterile, may also have blocks for this part of the development. As well as the chromosomal genes demonstrated or suggested in most of the above, Fitzgerald (1963) has also interpreted the variation in expression of some of his strains on the basis of epigenetic factors.

The female sterile mutants have implicated the functioning of some cellular processes during protoperithecial development. The mutants cyt-1 (C 115) and cyt-2 (C 117), being defective in cytochromes (Mitchell et al., 1953), suggest that respiration requires the proper functioning of cytochromes during this phase of the development (Turian, 1966). This is in addition to requiring the tricarboxylate cycle to function (see Turian, 1966). In another line of work the failure of a number of female sterile mutants to produce tyrosinase in normal amounts (Barbesgaard and Wagner, 1959) has supported previous findings of a relationship between melanin production (and hence tyrosinase) and protoperithecial formation (Hirsch, 1954). However the relationship is yet

unclear since Horowitz et al. (1960) were able to induce tyrosinase in otherwise tyrosinase repressed mutants without alleviating their female sterility.

In contrast with the various mutants found for female sterility, only one report of male sterility occurs (Vigfusson, 1969). The rarity of this may reflect that the genome from the male parent alone can influence only a short period during the sexual cycle (probably fertilization). This is in contrast with the duration of the influence of the genome from the female parent alone for the full development of the protoperithecium.

A number of mutants for ascus development have been described. The finding that the mutant peak-2 (pk-2, also called biscuit or bis, see Perkins, 1962) produces non-linear asci in homoallelic crosses (Murray and Srb, 1959, 1962) prompted Srb and his associates to screen for others affecting ascus morphology, and this was done primarily by screening for non-wild type growth as is characteristic of pk-2. In this way 12 other mutants in N. crassa were found (Srb and Basl, 1969a, 1969b; Pincheira and Srb, 1969a) as well as two in N. tetrasperma, one of which is allelic to pk-2 of N. crassa (Novak and Srb, 1969). The importance of dominance relationships for the expression of these mutants demonstrates that the genomes from both parents interact in this phase of the development (see especially

Srb and Basl, 1969a).

Some of the above mutants have been observed to affect spore delimitation by a disruption of spindle orientation in N. crassa (Pincheira and Srb, 1969a) and N. tetrasperma (Pincheira and Srb, 1969b). This is in agreement with suggestions of McClintock (1945) and Singleton (1953) that spore arrangement is dependent on an interaction between ascus wall and spindle arrangement. The most pronounced effects were observed in N. tetrasperma in which spindle overlap is considered particularly important in the development of 4-spored asci (see section 2). When pk-2 was transferred to that species from N. crassa, 5-8 spored asci in pk x pk crosses occurred at a frequency of 42%, and even + x pk crosses had abnormally high frequencies of such patterns.

Other mutants influencing spore development have been observed to affect the relative numbers produced (Novak and Srb, 1969a) or to affect their delimitation (Dodge, 1934, 1935a; Mitchell, 1966; Leary and Srb, 1969; Novak and Srb, 1969) or maturation (Lindegren, 1934a; Stadler, 1956; Threlkeld, 1965; Phillips and Srb, 1967). Of particular interest are the round spore mutants in N. crassa (Mitchell, 1966) and N. tetrasperma (Novak and Srb, 1969) because all spores in crosses to the wild type are of this shape (even though only half of those in

N. crassa have the round spore genotype). This demonstrates a dominance relationship and hence the determination of the final shape of the spore seems to represent the last stage in the development where both parental genomes interact since the expression of mutants for spore maturation depends on the genotype of each individual spore.

The production of perithecia without spores has been observed at various times (e.g. Lindegren et al., 1939). This is consistent with the suggestion of Lindegren et al. (1939) that perithecial development is under a different genetic control from that of the ascogenous system.

As well as discrete mutants, quantitative (polygenic) inheritance also affects some aspects of the sexual development. Selective breeding has been found to affect perithecial production (Lindegren et al., 1939) and ascospore development (Pateman, 1955, 1959).

6. Intent of this study

In a rather general way, this study was directed towards the understanding of processes involved in the sexual development of Neurospora. It was prompted by an observation of Threlkeld that crosses between strains having different genetic backgrounds occasionally produce sterile progeny.

With this potential source of genetic variation and the unavailability of efficient methods for detecting

mutants after inducing them, a search was made for these naturally occurring variants. Female sterile strains were found, and this study consists largely of the genetics and complementation of the female sterile mutants. An overall description is presented in section 20.

The species Neurospora crassa was used exclusively in this work.

MATERIALS AND METHODS

7. Abbreviations and conventions used

w.t. - wild type

Ab - an Abbott (wild type) strain

Li - a Lindegren (wild type) strain

OR - an Oak Ridge (wild type) strain

fs - a phenotypically female sterile strain

fs^+ or + - a phenotypically female fertile strain

fs (19-2) - a strain expressing this particular female sterile mutant

* ad - an adenine requiring strain

* ad^+ or + - a strain not requiring adenine for growth

* ad-4 - a strain having the ad-4 genotype

m.t. - mating type

A - A mating type

a - a mating type

complex asci - asci having patterns for female sterility that are not characteristic of one gene segregation, i.e. 1+:3fs and 0+:4fs

Unless otherwise noted, the first parent, say m, of a cross, say m x n, represents the female parent.

* ad can be substituted by any other mutant designation.

TABLE 1

Strains obtained or developed for this study

Designation		Genotype	Allele or isolation no(s)	Description	m.t.	Linkage groups	Genetic background	Source
1228	*	w.t.		Abbott w.t.	A			M. B. Mitchell
351	*	w.t.		Abbott w.t.	a			M. B. Mitchell
354	*	w.t.		Lindegren w.t.	A			M. B. Mitchell
353	*	w.t.		Lindegren w.t.	a			M. B. Mitchell
953	*	ylo-1, tryp-2	Y30539y, S4266	yellow, tryptophaneless	a	VI L, VI R	mixed	D. D. Perkins
442	*	ad-4	F2	adenineless	A	III R	St. Lawrence	M. E. Case
402	*	hist-1	C91	histidineless	a	V R	mixed	M. B. Mitchell
72	*	pyr-1	H263	pyrimidineless	A	IV R	mixed	D. D. Perkins
ORA		w.t.	74-OR23-1A	Oak Ridge w.t. (FGSC # 987)	A		St. Lawrence	F. J. deSerres
ORa		w.t.	74-OR8-1a	Oak Ridge w.t.	a		St. Lawrence	F. J. deSerres
ad-3		ad-3A	2-17-186	adenineless	A	I R	St. Lawrence	F. J. deSerres
ad-3		ad-3A	2-17-186	adenineless	a	I R	St. Lawrence	F. J. deSerres
129	*	nic-3	Y31881	nicotinateless	a	VII L	mixed	D. D. Perkins
467	*	ad-1, pan-2	3254, B5	adenineless, pantothenateless	a	VI L, VI R	mixed	M. E. Case
469	*	ylo-1, pan-2, tryp-2	Y30539y, B3, 75001	yellow, pantothenateless tryptophaneless	A	VI L, VI R, VI R	mixed	M. E. Case
272	*	arg-6, al-2	29997, 15300	arginineless, albino	a	I R, I R		D. D. Perkins
997	*	T(I, II) 4647, al-1; T(IV, V) R2355, cot-1; T (III, VI) 1, ylo-1	_____; C102(t); Y30539y	"alcoy" linkage tester	A	I R, II R; IV R, V R; III R, VI L		D. D. Perkins

Designation	Genotype	Allele or isolation no(s)	Description	m.t.	Linkage groups	Genetic background	Source
998 *	T(I, II) 4647, al-1; T(IV, V) R2355, cot-1; T(III, VI) 1, ylo-1	_____; C102(t); Y30539y	"alcoy" linkage tester	a	I R, II R; IV R, V R; III R, VI L		D. D. Perkins
1189	nic-3	Y31881	nicotinateless	A	VII L	St. Lawrence	developed
1681	nic-3	Y31881	nicotinateless	A	VII L	St. Lawrence	developed
1680	nic-3	Y31881	nicotinateless	a	VII L	St. Lawrence	developed
1604	pan-2	B5	pantothenateless	A	VI R	St. Lawrence	developed
1273	pan-2	B3	pantothenateless	A	VI R	St. Lawrence	developed
1635	pan-2	B3	pantothenateless	A	VI R	St. Lawrence	developed
1643	pan-2	B3	pantothenateless	A	VI R	St. Lawrence	developed
1633	pan-2	B3	pantothenateless	a	VI R	St. Lawrence	developed
2333	al-2	15300	albino	a	I R	St. Lawrence	developed

* designation corresponds to Fungal Genetics Stock Center (FGSC) number.

8. Strains used

The strains obtained or developed for this study are listed in Table 1. The nic-3, pan-2 (B5), pan-2 (B3), and al-2 strains in a St. Lawrence background were synthesized by backcrossing the mutants in 129 nic-3 a, 467 ad-1 pan-2 (B5) a, 469 ylo-1 pan-2 (B3) tryp-2 A, and 272 arg-6 al-2 a to the highly isogenic OR w.t.s. The backcrosses were carried out for at least seven generations in each case.

Other strains, selected or developed during the study, will be presented at appropriate times.

Strains developed in other laboratories were acquired through the Fungal Genetics Stock Center (F.G.S.C.) now located at Humboldt State College, Arcata, California 95521, with the exception of the OR a w.t. which was obtained from S. F. H. Threlkeld and the ad-3A strains which were obtained from A. J. F. Griffiths.

9. Culture media

The culture media were made up from the following constituents:

Sucrose medium

4X salt solution	250 ml
sucrose	20.0 g
Difco Bacto agar	15.0 g
distilled H_2O up to	1000 ml

4X salt solution

KNO_3	4.0 g
KH_2PO_4	4.0 g
$MgSO_4.7H_2O$	2.0 g
$CaCl_2.6H_2O$	0.4 g
NaCl	0.4 g
biotin	16.0 µg
trace element solution	1.0 ml
distilled H_2O up to	1000 ml

Trace element solution

$Na_2B_4O_7.10H_2O$	0.01 g
$CuSO_4.5H_2O$	0.1 g
$FePO_4.2H_2O$	0.2 g
$MnSO_4.4H_2O$	0.02 g
$ZnSO_4.7H_2O$	2.0 g
$NaMoO_4.2H_2O$	0.02 g
distilled H_2O up to	250 ml

Malt-peptone medium

4X salt solution	250 ml
sucrose	5.0 g
glucose	10.0 g
malt extract	2.0 g
BDH peptone	2.0 g
Difco Bacto agar	15.0 g
distilled H_2O up to	1000 ml

Glucose medium

Same as sucrose medium with glucose substituted for sucrose.

Sorbose medium

4X salt solution	250 ml
sorbose	10.0 g
glucose	0.5 g
fructose	0.5 g
Difco Bacto agar	20.0 g
distilled H_2O up to	1000 ml

Wherever necessary, biochemicals were supplemented in the following quantities:

adenine	40 mg/l	
anthranilic acid	80 mg/l	(for tryp-2)
L-arginine	200 mg/l	
cytidine	80 mg/l	(for pyr-1)
L-histidine monohydrochloride	80 mg/l	
nicotinamide	80 mg/l	
pantothenic acid (Ca salt)	80 mg/l	

Inorganic salts (Westergaard and Mitchell, 1947) and trace elements (Beadle and Tatum, 1945) are in concentrations used by Threlkeld (1961, also personal communication) for standard laboratory purposes.

Aliquots of medium were in the quantities of 5 ml per large test tube (15 x 150 mm), 1 ml per small test tube

(10 x 75 mm), and approximately 30 ml per plate (9 cm diameter disposable petri dish).

Sterile techniques

Standard laboratory procedures included the autoclaving of media, solutions, pipettes, etc. for 15 min. at 15 lb. pressure. Needles, twists, and loops were dipped in 95% alcohol and flamed prior to use and the mouth of each tube was flamed upon opening. Conidial isolates and some biochemical tests and crosses were carried out in a room decontaminated by ultraviolet light. Such procedures were recommended by Threlkeld (personal communication).

10. Crossing procedures

Crosses from which asci or random spores were analyzed were usually made in large tubes of sucrose medium and less often in small tubes.

As a general rule a small inoculum of a strain used as a female parent was placed on the medium with a wire twist. After 7 days it was fertilized by adding a conidial suspension of the male parent using a Pasteur pipette and the tube was shaken on a vortex mixer to spread the suspension over the surface of the culture.

Some crosses during the early part of the study were made by inoculating the medium simultaneously with both strains used in the crosses but those attempts were

not as successful as the ones using the former procedure.

In the screening for genetic variants for sterility where large numbers of crosses were made, the crosses were made almost exclusively in small tubes of sucrose medium with some repeats being carried out in large tubes. Since each strain was crossed to a number of others, a conidial suspension of each female parent was made up and added to the required number of tubes with a Pasteur pipette. After 7 days conidial suspensions of male parents were added and the tubes were shaken on a vortex mixer.

All crosses were maintained at 25°C as recommended by Westergaard and Mitchell (1947).

11. Tests for female sterility

Tests for female sterility were varied somewhat until enough experience was gained in handling female sterile strains to establish simple, efficient, and reliable procedures. The following routine was established. (a) (i) Small tubes of sucrose medium were inoculated in duplicate with the strains being tested for female sterility. After 7 days several drops of a conidial suspension of one mating type were added to one tube with a Pasteur pipette and a suspension of the opposite mating type was added to the other tube. The tubes were shaken on a vortex mixer to spread the suspension over the surface of each culture. The cultures were maintained at 25°C.

(ii) The above procedure was modified by growing up only one culture of each strain and adding a conidial suspension containing both mating types to the tube after 7 days.

Both methods are equally reliable (see section 57). The first was used in all cases where linkage data between mating type and female sterility were required. It provided mating types of all fs$^+$ progeny. The second method, requiring half as many tests as the first, was used where linkage data were not required.

(b) The strains used in the conidial suspensions above were intially the OR w.t.s. but they were replaced by the female sterile strains 18-1 fs A and 1-3 fs a derived from the cross OR A x 126 ad-4 fs a (see Fig. 2) as soon as these strains became available. In some tests specified in the text the strain 2333 al-2 was used as well as 1-3 fs a. The female sterile strains were generally used as males to avoid the possibility that wild types intended as males could grow up in the tubes and become fertilized by the culture growing in the tube. This could have happened in cases where the medium dried out and separated from the wall of the tube exposing a surface on which new growth could occur. Conidia do not germinate on a surface having a grown culture (Backus, 1939).

(c) Single ascospore isolates being tested were scored for

female sterility one week after the conidial suspensions of strains used as males were added to the cultures. Heterokaryons involving female sterile strains were maintained and checked periodically for at least an additional two weeks.

(d) Strains being tested for female sterility using the above procedures were usually scored for their superficial growth habit since it was realized early in the study that female sterile strains differ from the wild type in this way. (They can be scored visually with 99% accuracy, see section 58). Where the differences were subtle and difficult to detect, this was most effectively done 3-5 days after subculturing the strains for female sterility tests.

(e) (i) Wherever asci were analyzed, one spore from each identical pair was tested at least once for female sterility using method (i) in (a) and at least 10 asci from each cross were retested using both methods. Wherever an ascus pattern for female sterility was characteristic of more than one gene, that ascus was included in the sample to be retested and all spores of that ascus were included in the tests. (All spores from each ascus were isolated during dissection.)

(ii) Each random spore was tested at least once for female sterility using either method described in (a). When

the ratios of +:fs in crosses conformed to those that could be expected or easily explained, the tests were not necessarily repeated (but the reliability of such tests was supported by the classification based on growth habit). Wherever the data did not conform to such ratios, particularly where the proportion of female sterility was small, the female sterile progeny and in some instances 10% of the fs^+ progeny were retested from those crosses. (iii) Conidial isolates were tested at least once using a male parent of appropriate mating type (18-1 fs A or 1-3 fs a) with all female sterile isolates and at least 10% of the fs^+'s being tested similarly a second time. Prior to using this routine, the interval between inoculation of female and male parents was varied somewhat but the one week interval was used at least once for each culture tested. Cultures were observed for a longer period of time after the male parent was added.

12. Ascus isolation and dissection

Asci were usually dissected two to three weeks after the male parent was added to a cross. This was at least one week after spores were first shed and observed on the walls of the tubes. It was found to be a convenient time for finding numerous mature asci in developing perithecia as well as yielding high germination of ascospores.

Perithecia were removed from the tubes with a wire

loop and placed in a drop of 10% sucrose on a glass slide. The perithecia were cut open with needles and rosettes of asci were cut into smaller clusters having in the order of 5-10 asci per cluster. These were transferred to drops of 10% sucrose on blocks of 4% agar and left to dry. At the appropriate stage of dryness the asci were removed individually from the clusters, placed elsewhere on the agar and stretched to some extent. After further drying until the walls of the asci had either broken or could easily be broken, individual spores were removed with a needle and transferred to small tubes of glucose medium.

In the above 10% sucrose was used because asci were observed to burst due to osmotic shock when placed in distilled water or lower sucrose concentrations. A similar finding has been described (Lamb, 1967).

Tubes containing the ascospores were sealed with parafilm or entire racks of tubes were placed in plastic bags to prevent drying. They were left for two weeks at 25°C for further maturation of spores. Then the spores were heat shocked at 60°C for 40 min in a water bath and the tubes were transferred to a 25°C incubator.

13. Random spore isolation

Spores were removed from the walls of crossing tubes 4-5 weeks after addition of the male parent whenever good germination was required and any time after spores

were shed when this was not critical. This was done with a wire loop or needle and the spores were placed in drops of distilled water or 10% sucrose on blocks of 4% agar. They were left to dry and at the appropriate stage of dryness when spores would easily stick to a needle, they were removed and placed in small tubes of glucose medium. They were then heat shocked in a water bath at 60°C for 40 min and transferred to a 25°C incubator.

To avoid a bias in selecting healthy looking spores during their isolation, the blocks of agar were marked off into squares and all spores from a particular square were removed before proceeding to another such square.

14. Biochemical testing

Tests for biochemical requirements were carried out on plates of sorbose medium. Each strain was tested on one plate lacking the appropriate supplement and one containing it. Wherever a second mutant segregated, the appropriate supplement for that mutant was added to both plates in which the first was being tested.

The plates were incubated at 25°C and scored after 72 hr.

15. Mating type testing

Female sterile strains were added with a wire twist to 1-2 week old cultures of OR w.t.s. Several drops of

distilled water were added to each tube and the tubes were shaken on a vortex mixer to spread the inoculum over the surface of each wild type culture. The cultures were then incubated at 25°C for at least 3 days before scoring.

Mating types of *fs*$^{+}$ cultures were often obtained from tests for female sterility using the method described in (a) (i) of section 11. Otherwise duplicate cultures were inoculated in tubes of sucrose medium and after 7 days incubation at 25°C conidial suspensions of OR *A* and OR *a* strains were added to the different tubes. The tubes were shaken on a vortex mixer and the tests were scored 3 days later for perithecial production.

These tests are admittedly less efficient than a method described by Perkins *et al*. (1962) in which *A* and *a* tester strains are grown on plates and then a number of cultures being tested are inoculated in different positions on each plate. However, of several methods attempted in making crosses, the above was found most reliable and hence was expected to be most effective in revealing peculiarities of strains in crosses. This was thought to be particularly important for the type of study being carried out.

16. Special modifications of standard procedures

In some crosses segregating for ad-4 where only ad^+ progeny were analyzed, the medium on which random spores were isolated was not supplemented with adenine. One week after the spores were isolated the grown cultures (all ad^+) were retained and others (ad mutants and non-germinated spores) were discarded.

In crosses involving the "alcoy" linkage testers, where the cot mutant segregating grows normally at 25°C but forms small colonies at 34°C, the spores were placed in a 34°C incubator after heat shocking. After 3 days the cultures were separated into two lots, one containing the grown up cot^+ progeny and the other lot containing cot strains and non-germinated spores. Then the cultures were transferred to a 25°C incubator so that the germinated cot cultures could grow (see Perkins et al., 1969, for his procedure).

In some cases the cultures above which grew up at 25°C but had not grown at 34°C were retested to distinguish between cot mutants and late germinating cot^+'s. In those cases subcultures were made and incubated at 34°C for 3 days at which time they were scored.

17. Complementation tests

Strains of the same mating type form heterokaryons if they have the same allelic combinations for heterokaryon

compatibility loci (Garnjobst, 1953, 1955). For complementation tests, female sterile strains having the same heterokaryon compatibility alleles as the OR w.t.s. were used (C d e; Wilson and Garnjobst, 1966). They were obtained by crossing the female sterile strains to pan-2 and nic-3 mutants in a St. Lawrence background and selecting double mutants, i.e. pan-2 fs and nic-3 fs, which could form heterokaryons with the ad-3A strains which also have a St. Lawrence background.

Strains being tested for the above were inoculated in duplicate in small tubes of glucose or sucrose minimal medium. Several drops of a conidial suspension of ad-3A A were added to one tube and a suspension of ad-3A a was added to the other. If the cultures grew up on the minimal medium, the double mutants were considered to have formed heterokaryons with the ad-3A strains and to have the appropriate combination of heterokaryon compatibility factors. The mixtures of double mutants and ad-3A strains of opposite mating type acted as controls since they could not form heterokaryons (Beadle and Coonradt, 1944) and could not grow.

Heterokaryons between the nic-3 fs and pan-2 fs strains were synthesized by inoculating each pair of strains on a minimal medium. When the heterokaryons grew up they were subcultured once to check for vigorous growth

and then used in complementation tests.

The heterokaryons were tested for female sterility in triplicate on a sucrose minimal medium. Only those of A mating type were used although presumably the a mating type would have produced comparable results. As male parents, the strain 1-3 fs a (see section 11) was used twice and the strain 2333 al-2 a was used once.

In crosses involving heterokaryons where progeny were analyzed, some strains used in heterokaryons as female parents differed from the above but methods used were the same.

18. Conidial isolates

Conidial suspensions were prepared by pouring sterile distilled water into tubes containing four day old Neurospora cultures, shaking the cultures on a vortex mixer, and pouring the suspensions through glass wool filters to remove mycelial fragments. Following haemocytometer counts to estimate the concentrations of conidia, the suspensions were diluted serially and 0.1-0.5 ml quantities of the dilutions were spread onto plates of sorbose medium at concentrations of 50-250 conidia per plate. After 18-24 hours, blocks of medium with individual germinated conidia were removed with a needle and transferred to tubes of glucose medium. This was done with the use of a 40X or 80X stereoscopic microscope.

To ensure that biased samples of conidial isolates were not used, the medium in the plates was marked off into squares with a dissecting needle prior to removal of the isolates. Then all of the germinated conidia in one square were removed prior to the removal of isolates from another.

19. A technical problem involving the ad-4 mutant

In crosses where ad-4 segregated and progeny were tested for female sterility, the progeny expressing ad-4 could not be scored reliably for female sterility. Repeated tests did not give consistent results and genetically identical sister spores from asci did not always provide identical results either. Since segregation patterns for female sterility in asci were critical in interpreting the genetic complexity of the system, use of ad-4 was discontinued as soon as possible. (No solution to the problem has become apparent.)

Quantitative results from crosses segregating for ad-4 were based on ad^+ progeny and only generalizations could be made about ad-4 progeny.

RESULTS

20. A description of the study and a note on the presentation of the results

In a search for Neurospora strains expressing some form of sterility, a variant for female sterility was found. The genetics was pursued for three generations of crosses to OR w.t.s. From these studies a genetically complex system was apparent, but the precise number of genes, the crosses in which each gene segregated, and the source of each mutation was not clear. Some strains appeared to be heterokaryotic for female sterility.

In resolving the genetic complexity and isolating the different mutants for female sterility, conidial isolates, further crosses, and complementation tests were carried out. Finally some characterizations of the mutants were made and the validity of some testing procedures was established.

The results are presented in a number of phases approximating those in which the study was carried out:

Phase 1. The search for genetic variants for sterility.

Phases 2, 3 and 4. The analysis of the three generations of crosses to OR w.t.s.

Phase 5. The detection of mutants for female sterility in heterokaryotic strains and their segregation in crosses.

Phase 6. The genetics and complementation of selected female sterile strains.

Phase 7. The characterization of mutants.

Phase 8. The validation of some testing procedures.

21. The choice of strains in these studies

In the screening for genetic variants for sterility, strains having a variety of genetic backgrounds were used (section 22). However once a variant for female sterility was found the genetics was pursued with the extensive use of strains having a common background (St. Lawrence). The reasons for this follow.

The detailed genotypes of any Neurospora strains, for genes affecting the sexual reproduction, are not known. It is only known that the genes are present in allelic combinations that interact to result in perithecial and spore production in various crosses. In theory, these combinations could differ between strains but the results could be the same. Hence to circumvent problems in the interpretation of results that could be attributed to such differences, the strains in a St. Lawrence background were used. Their isogenicity provided the assurance that all crosses were being made to a similar combination of these alleles.

In later stages of the study (Phase 5 onwards), the pan-2 and nic-3 mutants were used in crosses and

complementation tests. They were chosen for a number of reasons: (a) they are indistinguishable from the wild type in growth rate and growth habit when strains are cultured on an appropriately supplemented medium, (b) they are non-leaky, i.e. they do not grow, even slowly, on a minimal medium, (c) they do not seem to affect perithecial and spore production, (d) pan-2 is a spore colour marker (Threlkeld, 1965).

22. Phase 1. The search for genetic variants for sterility problems.

Subsequent to the finding of Threlkeld (personal communication) that crosses between strains having different genetic backgrounds occasionally produce sterile progeny, a search for such naturally occurring variants was made.

A number of strains having different genetic backgrounds were crossed and their progeny were tested for sterility patterns in crosses. These included the Abbott and Lindegren w.t.s. because strains derived from them were noted by Threlkeld to express sterility.

The crosses made and strains selected were:

1228 Ab A x 953 ylo-1 tryp-2 a

↓

15 ylo-1 tryp-2 A strains

351 Ab a x 442 ad-4 A

↓

15 ad-4 a strains

354 Li A x 402 hist-1 a

↓

15 hist-1 A strains

353 Li a x 72 pyr-1 A

↓

15 pyr-1 a strains

(The male and female parents in these crosses were not noted; variants for chromosomal genes were sought.)

To facilitate the synthesis of heterokaryons that were being considered for further studies, and to have genetic markers to follow in crosses, the strains chosen expressed the markers segregating from the mutant strains. This tended towards a bias in the selection of the genome from these strains. To offset this the mating types from the w.t.s. were chosen. It was thought particularly important to retain genetic variation from the Abbott and Lindegren strains.

All 30 A strains were crossed to all 30 a strains, once using the A and a strains as female and male parents respectively; then the reciprocal crosses were carried out, making a total of 1800 crosses.

From these tests two observations were made: (a) that perithecial and spore production seem to be largely dependent on the female parent, and (b) that the cross 351 Ab a x 442 ad-4 A segregates for female

sterility. The first observation has not been studied quantitatively but the validity of the observation and possible implications are considered in the discussion. The study of female sterility is presented in the following sections.

For the next phase of the study, strain 126 ad-4 a was chosen from 351 x 442 and the genetics of female sterility was pursued in a cross to OR A.

23. Phase 2. The cross OR A x 126 ad-4 a

The crosses analyzed in this and the next two phases of the study are presented in the pedigree of Fig. 2. The data from these crosses and patterns of selected asci are presented in Tables 2-6.

Strain 126 ad-4 a was chosen from 351 x 442 on the basis of a low percentage of spore abortion observed in a cross to OR A. This was in contrast with the 20% spore abortion observed in the previous cross.

A preliminary analysis of the cross OR A x 126 (Fig. 2) provided the following random spore data: 117 + +, 157 + fs, 94 ad +, 120 ad fs (Table 6; % germination was not recorded). These data do not conform to a 1 +:1 fs ratio (X^2 = 8.9, 1 d.f., Table 6), nor does the 57% female sterility suggest any other simple genetic ratio. The genetic complexity inferred from these results prompted the use of ascus analysis in further studies.

From this same cross (Fig. 2) 182 asci were analyzed. It was in the repeated testing of these asci that the problem in scoring ad-4 strains for female sterility was realized (section 19), preventing the use of these strains in quantitative considerations. However the ad^+'s produced reproducible results and occurred in the asci in the following patterns: 40 2 +:0 fs, 96 1 +:1 fs, 42 0 +:2 fs (4 asci incomplete; Table 2). These data fit the ratio 1 +:1 fs (X^2 = 0.0, 1 d.f.) which is consistent with one gene segregation for female sterility in which the gene is not linked to ad-4. No linkage between female sterility and m.t. was noted in the cross (R > P, Table 5).

The difference between these results and those from random spores could not be attributed to problems in testing for ad-4 since ad^+ progeny among the random spores deviated from a 1 +: 1 fs ratio (X^2 = 5.8, 1 d.f., Table 6). Furthermore the failure to score ad-4 progeny accurately for female sterility prevented the drawing of conclusions about the number of genes required to explain the results. Hence one ascus was selected from this cross (ascus 19, Table 3) and used in further studies.

24. Phase 3. Crosses involving strains from ascus 19 of OR A x 126 ad-4 a.

(a) The use of ascus 19

Ascus 19 (Table 3) was chosen for further study

for two reasons. First of all, the four meiotic products in the ascus were easily distinguishable. Secondly the ad-4 strains produced perithecia and spores in one test for female sterility, suggesting that they are fs^+ and that all female sterility occurs in the ad^+ strains of the ascus. This would have permitted further analysis with a minimal use of the ad-4 strains. However these strains have not functioned as female parents in repeated tests and hence a more detailed analysis has been extended to them.

One strain from each spore pair of ascus 19 was crossed to an OR w.t. strain (Fig. 2) and asci were isolated from the crosses.

25. (b) OR A x 19-2 fs a

In this cross 102 of 105 complete asci (i.e. those in which at least one spore from each identical pair germinated) had patterns of 2+:2 fs (Table 2). However 3 asci segregated for "complex" patterns of 1+: 3 fs, suggesting that at least two genes were segregating in the cross (Table 2; also see definition of complex asci in section 7).

The following considerations were made in explaining the data. If a heterokaryotic parent or a mixture of two strains, say the heterokaryon (m + n), is crossed to a homokaryotic strain, say p, most perithecia contain progeny

from one type of cross, i.e. m x p or n x p (Catcheside, 1951; although mixed perithecia, i.e. those in which both crosses are found, seem to occur regularly: Sansome, 1947; Nakamura and Egashira, 1961; Weijer and Dowding, 1960; Radford and Threlkeld, 1970). The 3 complex asci above were consecutive ones dissected, suggesting that they came from one perithecium. Furthermore two female sterile spore pairs in each ascus expressed a colonial morphology (designated col) which was not observed elsewhere among the asci. This suggested that one parent in the cross was heterokaryotic and carried this mutant. Since OR A had been in use longer than 19-2 and was more likely to have accumulated mutations, it was thought to be the heterokaryotic parent.

On the basis of the asci which did not segregate for col, strain 19-2 was considered to have one mutant site for female sterility 38.7 m.u. from the centromere and not linked to m.t. (Table 4).

26. (c) OR a x 19-4 fs A

In this cross 103 of 113 complete asci had patterns of 2+:2 fs, 10 asci had patterns of 1+:3 fs, and 2 asci had patterns of 0+:4 fs (Table 2). At least two genes were required to explain female sterility in this cross.

The complex asci did not appear randomly among the asci dissected. They appeared in the following sequences: 49-51, 55-58, 78, 80, 81, 83, and 84 (in order

Figure 2. Some crosses analyzed in studying the genetics of female sterility. Abbreviations: ad = ad-4, fs = female sterile, fs? or ? = not certain if fs or fs^+, r.s. = random spores, incl. = including; for other conventions and designations, see sections 7 and 8. Asterisks (*) refer to crosses repeated using pan-2 strains in a St. Lawrence background instead of the OR w.t.s. for random spore analysis.
For the cross 351 x 442, the female parent was not noted. For all others, the OR w.t. (or pan-2) strains were used as female parents.

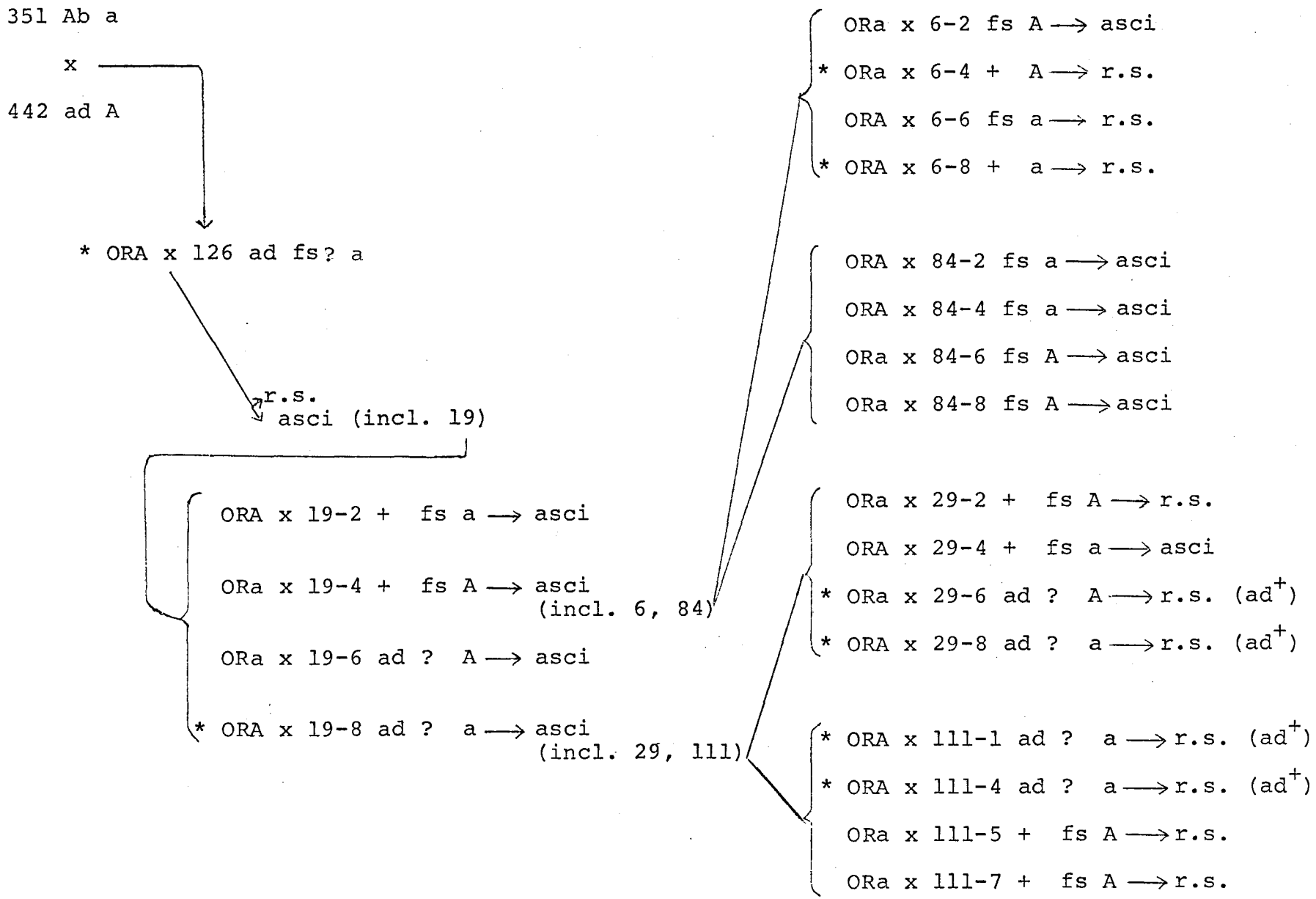

351 Ab a
x
442 ad A
* ORA x 126 ad fs? a
r.s.
asci (incl. 19)
ORA x 19-2 + fs a → asci
ORa x 19-4 + fs A → asci (incl. 6, 84)
ORa x 19-6 ad ? A → asci
* ORA x 19-8 ad ? a → asci (incl. 29, 111)
ORa x 6-2 fs A → asci
* ORa x 6-4 + A → r.s.
ORA x 6-6 fs a → r.s.
* ORA x 6-8 + a → r.s.
ORA x 84-2 fs a → asci
ORA x 84-4 fs a → asci
ORa x 84-6 fs A → asci
ORa x 84-8 fs A → asci
ORa x 29-2 + fs A → r.s.
ORA x 29-4 + fs a → asci
* ORa x 29-6 ad ? A → r.s. (ad+)
* ORA x 29-8 ad ? a → r.s. (ad+)
* ORA x 111-1 ad ? a → r.s. (ad+)
* ORA x 111-4 ad ? a → r.s. (ad+)
ORa x 111-5 + fs A → r.s.
ORa x 111-7 + fs A → r.s.

Figure 2

TABLE 2

Spore patterns of female sterility in asci studied

Cross	ad-4$^+$ spores from asci segregating for ad-4					Asci where all germinated spores could be tested				
	2+:0fs	1+:1fs	0+:2fs	inc.	Total	2+:2fs	1+:3fs	0+:4fs	inc.	Total
ORA x 126 ad-4 fs a	40	96	42	4	182					
ORA x 19-2 fs a						102	3		9	114
ORa x 19-4 A						101	10	2	3	116
ORa x 19-6 ad-4 A	113		1	1	115					
ORA x 19-8 ad-4 a	63	39	12		114					
ORa x 6-2 fs A						46	1		8	55
ORA x 84-2 fs a						54			1	55
ORA x 84-4 fs a						50	1		4	55
ORa x 84-6 fs A						51	2		2	55
ORa x 84-8 fs A						59			1	60
ORA x 29-4 fs a						48	3		4	55

inc. = incomplete asci, i.e. those in which spores from only three or fewer indentical pairs germinated.

TABLE 3

Spore patterns of female sterility in selected asci

Cross	Ascus No.	Spore No. 1	2	3	4	5	6	7	8
ORA x 126 ad-4 a	19	fs a	fs a	fs A	fs A	ad-4 a	ad-4 a	ad-4 A	ad-4 A
ORA x 19-2 fs a	10	col fs a	col fs a	col fs a	col fs a	A	A	fs A	fs A
ORa x 19-4 fs A	6	fs A	fs A	A	A	fs a	fs a	a	a
	84	fs a	fs a	fs a	fs a	fs A	fs A	fs A	fs A
ORa x 19-6 ad-4 A	110	fs a	fs a	fs A	fs A	ad-4 A	ad-4 A	ad-4 a	ad-4 a
ORA x 19-8 ad-4 a	29	fs A	fs A	fs a	fs a	ad-4 A	ad-4 A	ad-4 a	ad-4 a
	111	ad-4 a	ad-4 a	ad-4 a	ad-4 a	fs A	fs A	fs A	fs A
ORa x 6-2 fs A	2	A	A	fs A	fs A	a	a	fs a	fs a
	5	sl fs a	-[1]	a	a	sl fs A	sl fs A	fs A	fs A
ORA x 84-2 fs a	52	fs a	fs a	a	a	fs A	fs A	A	A
ORA x 84-4 fs a	45	fs a	fs a	fs a	fs a	A	A	fs A	fs A
ORa x 84-6 fs A	32	sl fs A	-	sl fs A	sl fs A	fs a	fs a	a	a
ORA x 29-4	2	fs a	fs a	a	a	fs A	fs A	A	A
	44	fs a	-	fs a	fs a	A	A	fs A	fs A
	48	fs a	fs a	fs a	fs a	fs A	fs A	A	A

1. Spore did not germinate.

TABLE 4

Linkage tests for gene-centromere and gene-mating type distances using ordered tetrads

Cross	Linkage between fs and centromere				Linkage between fs and mating type				
	Ascus pattern 1st div'n	Ascus pattern 2nd div'n	Total	Gene centromere distance (m.u.)	PD	TT	NPD	X^2 for 1:1 of PD:NPD	Distance (m.u.)
ORA x 19-2 fs a	35	67	102	38.7	17	62	23	-[1.]	
ORa x 19-4 fs A	38	63	101	36.3	21	64	16	0.7	
ORa x 6-2 fs A	22	24	46	29.0	11	28	7	0.9	
ORA x 84-2 fs a	31	23	54	23.0	39	15		78.0**	14.6
ORA x 84-4 fs a	24	26	50	29.0	8	29	13	-	
ORa x 84-6 fs A	15	36	51	43.2	7	39	5	0.3	
ORa x 84-8 fs A	28	31	59	29.3	39	20		78.0**	18.0
ORA x 29-4 fs a	12	36	48	48.0	1	39	8	-	

1. No linkage because NPD > PD (non-parental ditypes exceed parental ditypes).

** Significant at the p = 0.01 level.

Distances with correction for multiple crossovers and interference were determined from graphs provided by Barratt et al. (1954).
Testing for linkage by comparing PDs with NPDs was suggested by Perkins (1953).

TABLE 5

Linkage tests between female sterility and mating type based on progeny from tetrads

Cross	Data considered	Parental types	+ A (p)	+ a (q)	fs A (r)	fs a (s)	Total	X^2 for 1:1 of P:R
ORA x 126 ad-4 a	all ad^+s	p, s	74	102	102	79	357	-[1.]
ORa x 19-4 fs A	all germ'd spores	q, r	101	115	128	114	458	2.9
	asci with 1+:3 fs and 0+:4 fs	q, r	2	8	22	16	48	
ORA x 19-8 ad-4 a	all ad^+s	p, s	84	81	32	31	228	0.0

P - Parental types
R - Recombinant types

1. - no linkage because R > P

LEGEND FOR TABLE 6

From all crosses segregating for *ad-4* except OR*A* x 126 *ad-4* *a*, only *ad*$^+$s were retained.

* significant at the p = 0.05 level
** significant at the p = 0.01 level
1. aborted spores segregated in a proportion of 33%
2. estimated from *ad*$^+$ progeny

TABLE 6

Segregation for female sterility from random spore analyses

Cross	+	fs	Total	% germ'n	X^2 (1 d.f.) for 1+:1 fs	% fs	Cross	+	fs	Total	% germ'n	X^2 (1 d.f.) for 1+:1 fs
							1635x351	116	0	116	97	
							1633x442 ad-4[1.]	91	12	103	87[2.]	
ORAx126 ad-4	117 ad[+]	157 ad[+]	274		5.8*	57	1635x126 ad-4	47	54	101	84[2.]	0.5
	94(?)ad	120(?)ad	214		3.2	56						
	211	277	488		8.9**	57						
ORax6-4	21	2	23	92		8.7	1633x6-4	50	0	50	83	
ORAx6-6 fs	46	65	111	92	3.3							
ORAx6-8	109	4	113	94		3.5	1635x6-8	48	0	48	80	
ORax29-2 fs	15	8	23	92	3.5							
ORax29-6 ad-4	99	9	108	90[2.]		8.3	1633x29-6 ad-4	55	0	55	84[2.]	
ORAx29-8 ad-4	124	8	132	~100[2.]		6.1	1635x29-8 ad-4	62	0	62	~100[2.]	
ORAx111-1 ad-4	104	16	120	~100[2.]		13.3	1635x111-1 ad-4	61	0	61	~100[2.]	
ORAx111-4 ad-4	130	21	151	~100[2.]		13.9	1635x111-4 ad-4	61	0	61	~100[2.]	
ORax111-5 fs	12	11	23	92	0.0							
ORax111-7 fs	11	10	21	84	0.0							

of dissection). The first 48 asci had patterns of 2+:2 fs; this sequence is expected with a probability of $(101/113)^{48} = 0.0045$ if the asci occur at random.

These results suggested that complex asci occurred more frequently in some perithecia than in others, and hence that one parent was heterokaryotic for a female sterile mutant. Furthermore, since OR a had been in use longer than 19-4, it was postulated to be the heterokaryotic parent.

The number of genes segregating in this cross, other than the one thought to have come from a heterokaryotic parent, was not clear. The large proportion of asci with 2+:2 fs patterns could be explained by one gene or any number of closely linked genes. These patterns suggested that the gene(s) was (were) in the order of 36.3 m.u. from the centromere (Table 4). No linkage to m.t. was apparent, either on the basis of the complex asci with 2+:2 fs patterns (Table 4) or on the basis of germinated spores in all asci (Table 5).

27. (d) OR a x 19-6 ad-4 A

In this cross all ad^+ progeny in 113 of 114 complex asci were fs^+ (Table 2) whereas both ad^+ spore pairs in one ascus (no. 110, Table 3) were female sterile. In addition most ad-4 strains produced perithecia and spores when tested for female sterility, suggesting that they

too were *fs*$^+$. It was attractive to think that the *ad-4* mutant could interfere with tests in otherwise *fs*$^+$ strains, but that it could not restore fertility to those with other female sterile mutants.

A number of considerations were made in explaining female sterility in ascus 110:

(i) that the female sterility may reflect a problem in testing.

(ii) that quantitative inheritance may play a role in which a rare allelic combination may result in female sterility.

(iii) that crossing over between *ad-4* and a closely linked female sterile mutant may have resulted in the one unusual ascus pattern.

(iv) that crossing over between a female sterile mutant and a closely linked suppressor may have occurred giving rise to the unusual pattern.

(v) that one parent in the cross may be heterokaryotic for female sterility.

The tests for female sterility in ascus 110 were reproducible, suggesting that the first alternative was unlikely. However the rarity of the ascus required testing for segregation of female sterility in further crosses before the alternative could be ruled out. Of the other possibilities, that of a heterokaryotic parent seemed

most attractive because others were concerned with rare recombinants whereas female sterility was found in both ad$^+$ spore pairs of this ascus.

In view of the above considerations and the large proportion of fs$^+$ progeny among ad$^+$'s and apparently among the ad-4 strains, 19-6 was tentatively considered as fs$^+$. The heterokaryotic parent, if one existed, was postulated to the OR a because it had been in use longer than 19-6 and because the cross OR a x 19-4 also seemed to involve a heterokaryotic parent.

28. (e) OR A x 19-8 ad-4 a

The ad$^+$ progeny in asci from this cross occurred in the following patterns: 63 2+:0 fs, 39 1+:1 fs, 12 0+:2 fs (Table 2). Approximately one quarter of the ad$^+$ spore pairs were female sterile (165 +, 63 fs; X^2 = 0.8, 1 d.f. for 3:1). No linkage to m.t. was apparent (X^2 = 0.0, Table 5).

Because of the problem in scoring ad-4 strains, the genetics of female sterility was not clear. Hence several possibilities had to be considered. They were: (i) that a female sterile mutant linked to ad-4 may be present in 19-8, (ii) that a female sterile mutant and a suppressor may be present in 19-8 with the genes neither being linked to each other nor to ad-4, and (iii) that a female sterile mutant may have been inherited from a heterokaryotic parent.

In considering the alternatives, the possibility of a heterokaryotic parent was discouraged. This was because OR A was more likely to be heterokaryotic than 19-8, yet in OR A x 19-2 only the easily recognized col could be attributed to a heterokaryotic parent. In contrast a substantial proportion of ad^+'s were female sterile in this cross, and none resembled col.

The possibility of a suppressor was attractive because of the following observations: All strains from some asci produced perithecia and spores. If this meant that the ad-4 strains were fs^+, the results could not be explained by (i) above but fitted the possibility of a suppressor. Furthermore, the proportion of female sterile ad^+'s (1/4) fitted the model of (ii) particularly well.

The final explanation, regardless of its nature, would have to distinguish between the proportion of female sterile strains segregating in OR A x 126 ad-4 a (approx. 1/2 among ad^+'s) and that in this cross.

29. (f) Considerations regarding the genetic complexity of female sterility as revealed by the above crosses

All crosses involving strains from ascus 19 produced female sterile progeny. Of the alternative explanations proposed, none was irrefutable. However some statements could be made with reasonable certainty

and used in planning further studies. These were:

(i) that 19-2 has a single mutant site for female sterility.

(ii) that 19-6 is *fs*$^{+}$.

(iii) that *col* was inherited from a heterokaryotic parent (probably OR *A*).

(iv) that a female sterile mutant segregating in OR *a* x 19-4 was inherited from a heterokaryotic parent (thought to be OR *a*).

Thus far the genetics of OR *A* x 19-2 seemed relatively clear, whereas that of the other crosses did not. For the next phase of the study two asci were selected from each of OR *a* x 19-4 and OR *A* x 19-8 and strains from these asci were crossed to OR w.t.s. The objectives in this were not only in resolving the genetic complexity but in isolating the mutants for female sterility. This was thought to be particularly important for studies leading towards the understanding of processes involved in the sexual development of *Neurospora* and the proposed crosses were considered as good sources of mutants other than the one present in strain 19-2.

Phase 4. Studies on asci selected from OR a x 19-4 fs A and OR A x 19-8 ad-4 a

30. (a) *The selection of strains*

The asci chosen for studying the genetics of

OR *a* x 19-4 were asci 6 and 84 (Table 3). Ascus 84 was chosen for expressing more than one mutant and for having female sterility distributed among all spores (pattern 0+:4 *fs*). Ascus 6, on the other hand, was chosen for having the pattern 2+:2 *fs*. It was thought that if a heterokaryotic parent had contributed to the segregation of female sterility in this cross, ascus 84 should express the gene coming from a heterokaryotic parent but ascus 6 should not. This was because ascus 6 came from the sequence of 48 consecutive asci having patterns of 2+:2 *fs* (section 26).

The asci chosen for studying the genetics of OR *A* x 19-8 were asci 29 and 111 (Table 3). The *ad*$^{+}$ strains in both asci were female sterile, but the *ad-4*'s differed; in one test those in ascus 29 produced perithecia and spores whereas those in ascus 111 did not. In the event that these differences were not entirely attributable to problems in testing *ad-4* strains, both asci were used.

In crosses involving strains from these asci, the use of OR w.t.s was continued despite the possibility that the strains may be heterokaryotic. This was because no other strains with a similar background and of both mating types were readily available. Furthermore only the easily recognized *col* could be attributed to a heterokaryotic OR *A* strain thus far with reasonable

certainty and a female sterile mutant possibly occurring in OR a could be considered in the evaluation of further crosses. This seemed less serious than introducing strains with other genetic backgrounds (see section 21).

For further analysis, a culture from each spore pair in these asci was crossed to an OR w.t. and asci or random spores were isolated (see Fig. 2).

31. (b) Ascus analysis of OR A x 84-2 fs a and OR a x 84-8 fs A

In these crosses all complete asci expressed the pattern 2+:2 fs (Table 2) characteristic of one gene segregation for female sterility. Linkage to m.t. was found with map distances of 14.6 and 18.0 m.u. occurring in the respective crosses (Table 4). Gene-centromere distances were 23.0 and 29.3 m.u. (Table 4).

The same gene was thought to segregate in both crosses since linkage to m.t. was found here but not in OR A x 84-4 or OR a x 84-6 (Table 4). In addition this seemed to be the gene inherited from a heterokaryotic parent in OR a x 19-4 (section 26), the reason being that linkage to m.t. was not detected in that cross and it would likely have been masked if the gene segregated in a small proportion of asci.

One difficulty arose in interpreting the results in this way: If the interpretation were correct, then

strain 19-4 rather than OR *a* would have to be postulated as the heterokaryotic parent. This was on the basis of data from complex asci of OR *a* x 19-4. In these asci, 2 of the *fs*$^+$ spore pairs were *A* whereas 8 were *a*. Since the gene is linked to m.t., the *fs*$^+$ allele likely came from the *a* parent and the *fs* allele from the *A* parent.

Statistical considerations support the likelihood that the female sterile mutant was inherited from 19-4 if one parent were heterokaryotic. Supposing that it had come from OR *a* instead and recombined with m.t. with a frequency of 15.5% (based on the complete asci from OR *A* x 84-2 and OR *a* x 84-8, uncorrected for multiple cross overs or interference), then the expected numbers of *fs a* and *fs A* progeny in the complex asci would be 3.1 and 6.9. In comparing these with the observed results, $X^2 = 9.1$ (1 d.f., Yates correction) which is easily significant at the $p = 0.01$ level.

An alternative explanation was considered: that a heterokaryotic parent may not occur in OR *a* x 19-4, but that a gene for female sterility and a linked suppressor may be present in 19-4. This would also have led to a masking of linkage to m.t. in the cross because the mutant would only have been expressed by a small proportion of progeny. However, this cannot explain the non-random occurrence of complex asci in the cross, and on that

basis the suggestion of a heterokaryotic parent seems more attractive.

32. (c) Ascus analysis of OR a x 6-2 fs A, OR A x 84-4 fs a, OR a x 84-6 fs A, and OR A x 29-4 fs a

The majority of ascus patterns in these crosses were 2+:2 fs: 46 of 47 in OR a x 6-2, 50 of 51 in OR A x 84-4, 51 of 53 in OR a x 84-6, and 48 of 51 in OR A x 29-4 (complete asci only; Table 2). The remaining patterns were 1+:3 fs: 1 of 47, 1 of 51, 2 of 53, and 3 of 51 in the respective crosses (Table 2).

Since the asci with 1+:3 fs patterns were relatively rare, a number of alternatives were considered in explaining these patterns. They included some of those considered in explaining ascus 110 of OR a x 19-6 ad-4 A (section 27):

(i) that the patterns may reflect a problem in testing for female sterility.

(ii) that female sterility may occasionally be attributable to quantitative effects; that rare combinations of genes may cause female sterility in addition to that of single genes segregating in the crosses.

(iii) that some female sterile strains may have pairs of closely linked mutants and that rare cross overs between the genes may result in 1+:3 fs patterns.

(iv) that some genes for female sterility may have closely linked suppressors, with rare cross overs between the genes causing the female sterile mutants to be expressed in some progeny.

(v) that parental strains in the crosses may be heterokaryotic for female sterility.

In the crosses OR a x 6-2 and OR a x 84-6, the possibility of a heterokaryotic parent seemed particularly attractive for the following reasons: In the complex asci of these crosses, two of three female sterile spore pairs expressed a growth habit found in only one other ascus of each cross. The growth was particularly retarded (and is designated as slow or sl). Since it did not occur in all asci of the crosses, it seemed to have come from a heterokaryotic parent, and since OR a was the common parent and had been in use longer than the other strains, it was tentatively considered as that parent.

Assuming the above explanation to be correct, (until further crosses could test for other alternatives) only one mutant for female sterility had to be postulated to occur in 6-2 and 84-6. From complete asci with 2+:2 fs patterns, gene-centromere distances of 29.0 and 43.0 m.u. were found in the respective crosses to OR a (Table 4), and no linkage to m.t. was apparent in either case (X^2 = 0.9,

X^2 = 0.3 respectively, 1 d.f., Table 4). Furthermore the same mutant seemed to be present in both strains. The reasons follow.

Any female sterile mutant present in ascus 6 must occur in strains 6-2 and 6-6 because 6-4 and 6-8 are $\underline{fs}^+$ (Table 3). (This is assuming that no suppressed genes occur.) Since only one mutant could be attributed to 6-2 and hence 6-6, strain 19-4, which is female sterile, must be homokaryotic for this mutant and must have transmitted it to ascus 84. Since this mutant is not linked to m.t. but that in 84-2 and 84-8 is linked (section 31), then it must occur in strain 84-4 and 84-6.

The explanations for complex asci in OR $\underline{A}$ x 84-4 and OR $\underline{A}$ x 29-4 were not as apparent as those for the above crosses, although the above consideration that 84-4 may have only one mutant suggests that a parent in the cross may be heterokaryotic. However, regardless of the explanations, since the 1+:3 $\underline{fs}$ asci were relatively rare, the genes segregating in asci with 2+:2 $\underline{fs}$ patterns could be considered in the order of 26.0 and 37.5 m.u. from the centromere in the respective crosses (Table 4) with no linkage to m.t. being apparent (NPD > PD in both crosses, Table 4).

33. (d) Random spores analyzed from crosses between OR w.t.'s and strains from asci 6, 29, 111

Crosses between the female sterile strains 6-6, 29-2, 111-5, and 111-7 and OR w.t.'s (Fig. 2) produced progeny in the ratio of 1+:1 fs (X^2 < 3.86 in each case, Table 6). The data were consistent with one gene segregation in each of the crosses.

Crosses between the fs^+ strains 6-4 and 6-8 as well as the ad-4 strains 29-6, 29-8, 111-1, and 111-4 and the OR w.t.s (Fig. 2) produced a small proportion of female sterile progeny in each case (3.5-13.9%, Table 6; only ad^+ progeny were considered where ad-4 segregated).

The results from the latter crosses could be explained either by female sterile mutants inherited from heterokaryotic parents or mutants and suppressors occurring in the parental strains with recombinants expressing female sterility among the progeny. In addition where ad-4 strains were crossed, female sterile mutants linked to ad-4 could be postulated to occur in the strains and could have segregated for a small proportion of female sterile ad^+ progeny.

If female sterile mutants were segregating from OR w.t.s in some of these crosses and those in (b) where explanations of complex ascus patterns were not clear, then the mutants would not necessarily be those considered thus

far. This is because only the female sterile mutants col and sl could be attributed to these wild types with reasonable certainty, whereas the above crosses were clearly segregating for others.

34. (e) Considerations regarding the genetic complexity of female sterility revealed by the above crosses

Thus far the following mutants for female sterility were apparent:

(i) the mutant in 19-2, not linked for m.t. (section 25)

(ii) the mutant in 19-4, 6-2, 6-6, 84-4, and 84-6, not linked to m.t. (section 32), and possibly the same as in 19-2

(iii) the mutant in 84-2 and 84-8, linked to m.t. (section 31)

(iv) the col mutant (section 25)

(v) the sl mutant (section 32).

However, the precise number of genes, the crosses in which each gene segregated, and the strain in which each mutation originally occurred could not be asserted. Furthermore the explanations for female sterility in various crosses were not clear and alternatives had to be considered.

The next two phases of the study were planned primarily towards resolving these uncertainties and isolating

the mutants involved. Tests were carried out along the following lines:

(i) conidial isolates of selected strains to test if they were heterokaryotic for female sterile mutants

(ii) crosses, designated by asterisks in Fig. 2, were made using recently isolated pan-2 strains in place of OR w.t.s to detect where female sterile strains segregating could be attributed to the heterokaryotic wild types

(iii) crosses were made between strains thought to represent all genes for female sterility and recently isolated pan-2 and nic-3 strains to test for genetic segregation, obtain some linkage data, and select strains for complementation tests (see section 21 for reasons in using these mutants)

(iv) complementation tests

(v) linkage tests using the alcoy linkage testers. The information obtained from these tests permitted some final linkage considerations using ascus data.

Phase 5. Detection of female sterile mutants in heterokaryotic strains and their segregation in crosses

35. (a) Detection of mutants in heterokaryotic strains

Conidial isolates were carried out for strains 351 Ab a, 442 ad-4 A, OR A, and OR a, all of which functioned repeatedly as female parents in crosses.

Fifty of 147 isolates from 351 Set 2 were female sterile (although all 79 from set 1 were *fs*$^+$, Table 7), as were 48 of 214 isolates from OR *A* and 19 of 218 isolates from OR *a* (Table 7). Hence these three strains are heterokaryotic for female sterility.

The two sets of conidial isolates from 351 were carried out at different times. The different findings presumably indicate different nuclear ratios of +:*fs* in the two subcultures used. The possible significance of this will be considered in a later section (section 62).

The isolates from strain 442 produced ambiguous results because of the *ad-4* mutant. However the strain does have a mutant for female sterility. From the cross between 442 and 1633 *pan-2 a* (a recent single spore isolate with a St. Lawrence background), 12 of the 103 *ad*$^+$ progeny were female sterile (Table 6). Since strain 442 has functioned repeatedly as a female parent, and the perithecial and spore production has been judged to exceed the leakiness of the mutant it contains (Group 19-2, see section 53), it appears that the strain is heterokaryotic for the mutant.

36. (b) Detection of female sterile mutants segregating in crosses involving heterokaryotic parents

The crosses in Fig. 2 repeated using the recent ascospore isolates 1635 *pan-2 A* and 1633 *pan-2 a* (in a

TABLE 7

Conidial isolates from wild type strains and from synthesized heterokaryons

Culture tested	Set No.	+	fs	% fs	+ +	+ nic	pan +	Total	% survival[1]
351 Ab a	1	79	0	0				79	76
	2	97	50	34				147	98
ORA		166	48	22				214	97
ORa		199	19	13				218	99
(1604 + 2243)					4	4	32	40	100
(2243 + 2441)					10	1	24	35	88
(2243 + 2481)					10	4	23	37	92

1. % survival is not likely indicative of differential survival of different genotypes because most conidial isolates failing to grow up were found to have been lost on the sides of tubes during their transfer from plates.

St. Lawrence background) instead of the OR w.t.s were those involving ad-4 strains whose genotypes were not certain, and the fs^+ strains from ascus 6 whose progeny in crosses to OR w.t.s segregated for female sterility.

The ad^+ progeny of 1635 x 126 ad-4 a segregated for female sterile ad^+s (Table 6), confirming that this strain has a mutant for female sterility. Crosses to the ad-4 strains 19-8, 29-6, 29-8, 111-1, and 111-4, however, failed to produce female sterile progeny (only ad^+s were tested) as did those to the wild types 6-4 and 6-8 (Table 6). Hence these strains are fs^+, and the female sterile progeny produced in crosses to OR w.t.s can be attributed to mutants in these heterokaryotic strains.

The finding that strain 19-8 ad-4 a is fs^+ and the strong indication that 19-6 ad-4 A is also fs^+ (section 27) suggest that a mutant present in strain 126 ad-4 a occurs in strains 19-2 and 19-4 of ascus 19 from OR A x 126 (Fig. 2) and it is that transmitted to strains 6-2 and 6-4 of ascus 6 and strains 84-4 and 84-6 of ascus 84 in the cross OR a x 19-4 (see section 32).

These data also establish that the female sterile mutant linked to m.t. and occurring in strain 84-2 and 84-8 of ascus 84 from OR a x 19-4 (section 31) was inherited from a heterokaryotic parent in the cross. This is because the mutant is not found in ascus 6 of that cross. It cannot

occur suppressed in strains 6-4 and 6-8 (otherwise it would be expressed among progeny in crosses between these strains and those expressing pan-2) and it has not been detected in strains 6-2 or 6-6 (only one mutant needs to be postulated to occur in these strains and it is not linked to m.t.; section 32).

Phase 6. Genetics and complementation of selected female sterile strains

37. (a) Selection of female sterile strains for further study

Strains thought to express all female sterile mutants segregating were selected for these studies. They included conidial isolates from 351 Ab a, OR A, and OR a and a single spore isolate derived from 442 ad-4 A, female sterile strains cccurring in or derived from complex asci of all crosses in which they were found, and strains considered to have individual mutants on the basis of ascus patterns. The latter were chosen in some cases to distinguish between genes inherited from homokaryotic and heterokaryotic parents.

These strains are listed below (also see Table 3 for ascus patterns):

Ascospore isolates

Cross	Strains selected	Ascus pattern	Comments
1633 x 442 ad-4	2494 pan-2		fs from heterokarotic 442
OR A x 126	19-2	2+:2 fs	

Cross	Strains selected	Ascus pattern	Comments
OR A x 19-2	10-2, 10-4	1+:3 fs	both col
OR a x 19-6	110-2	2+ fs:2 ad-4 ?	
OR a x 6-2	2-4	2+:2 fs	
	5-1, 5-5, 5-8	1+:3 fs	5-1, 5-5 are sl
OR A x 84-2	52-2	2+:2 fs	
OR A x 84-4	45-1, 45-4, 45-8	1+:3 fs	
OR a x 84-6	32-1, 32-4, 32-6	1+:3 fs	32-1, 32-4 are sl
OR A x 29-4	2-2	2+:2 fs	
	44-1, 44-3, 44-4, 44-8	1+:3 fs	
	48-1, 48-4, 48-6	1+:3 fs	

Conidial isolates

Heterokaryotic strain	Isolate No.
OR A	2324
OR a	2326
351 Ab a	2351

With the exception of strain 2494, all strains were crossed to recent ascospore isolates of nic-3 and pan-2 mutants (1681 nic-3 A, 1680 nic-3 a, 1635 pan-2 A, and 1633 pan-2 a; Table 1) and their progeny were used for several purposes. For strains in which the number of mutants was not known, the progeny were observed for segregation ratios. This was partly meant to distinguish

between testing problems or quantitative inheritance and other observations. From crosses thought to segregate for one gene or a pair of closely linked genes, female sterile strains with the biochemical mutants were selected and used in complementation tests. Finally, from some crosses linkage determinations were made.

For the assignment of genes to linkage groups, some of the above strains were crossed to alcoy linkage testers.

The strain 2494 pan-2 fs A was used directly in the complementation tests and was also crossed to an alcoy linkage tester for linkage determination.

(b) Genetic segregation in crosses involving the selected strains

38. (i) Strains from asci segregating for col and sl

Crosses involving the col strains 10-2 and 10-4 (from OR A x 19-2) and the sl strains 5-1, 5-5, 32-1, and 32-4 (the first two from OR a x 6-2, and the latter two from OR a x 84-6) segregated from 1+:1 col or 1+:1 sl (X^2 < 3.86 in each case, Table 8), suggesting that these traits are caused by mutations for single genes. The growth habits are epistatic to those of another female sterile mutant occurring in the asci (the mutant present in strains 19-2, 6-2, and 84-6; see section 36), since the double mutant strains 10-2, 5-1, and 32-4 express the col or sl morphology. However their female sterility is

expressed independently of the other mutant as is evident from the strains 10-4, 5-5, and 32-1 (which do not segregate for the other mutant, Table 8) and their col or sl progeny. All col progeny from 1681 x 10-4 and 1635 x 10-4 were tested for female sterility, and samples of four sl strains from each of 1680 x 5-5 and 1680 x 32-1 were tested.

The female sterile strains 5-8 and 32-6 segregated for 1+:1 fs as was expected (X^2 = 1.0, X^2 = 0.2 for the respective crosses, 1 d.f., Table 8), with no sl progeny appearing.

Since col and sl segregated as one gene mutant, their female sterility cannot be attributed to testing problems or quantitative inheritance. However the ease with which they were scored among these progeny on the basis of growth habit supports the hypothesis that they were inherited from heterokaryotic parents in the crosses where they were first detected. Otherwise they would have been observed in all asci from those crosses.

39. (ii) Strain 110-2 from ascus 110 of OR a x 19-6 ad-4 A

The progeny from crosses involving this strain segregate 1+:1 fs (X^2 = 0.0, 1 d.f., Table 9). Hence testing problems and quantitative inheritance can be ruled out in explaining female sterility in this ascus and the possibility of a heterokaryotic parent is favoured (see

LEGEND FOR TABLE 8

1. Numbers in parenthesis refer to designations of strains crossed to OR w.t.s from which the preceding strains were derived, e.g. strain 10-2 was derived from strain 19-2 in the cross ORA x 19-2.

* Significant at the p = 0.05 level.

TABLE 8

Crosses involving strains from asci segregating for col or sl

Cross	col+		col	Total	% germ'n	X^2 (1 d.f.) for 1+:1 col	X^2 (1 d.f.) for 1+:1 fs among col+
	+	fs					
1681 x 10-2 (19-2)[1.]	21	10	26	57	95		
1635 x 10-2	21	15	22	58	97		
	42	25	48	115	96	3.8	4.3*
1681 x 10-4 (19-2)	29		29	58	97		
	27		32	59	98		
	56		61	117	97	0.2	
	sl+		sl	Total	% germ'n	X^2 (1 d.f.) for 1+:1 sl	X^2 (1 d.f.) for 1+:1 fs among sl+
	+	fs					
1681 x 5-1 (6-2)	21	8	21	50	83	0.6	5.8*
1680 x 5-5 (6-2)	25		27	52	87	0.1	
1680 x 5-8 (6-2)	20	27		47	94		1.0
1680 x 32-1 (84-6)	25		20	45	75	0.6	
1680 x 32-4 (84-6)	17	11	22	50	83	0.7	1.3
1681 x 32-6 (84-6)	24	21		45	90		0.2
Pooled data from 1681 x 5-1 and 1680 x 32-4	38	19	43	100	83	2.0	6.3* X^2_{het} = 5.8+1.3 -6.3 = 0.8

TABLE 9

Genetic ratios in crosses between selected female sterile strains and biochemical markers

Cross		+	fs	Total	% germ'n	Ratio tested (+:fs)	X^2 (1 d.f.)
1681 x 110-2	(19-6)[1.]	23	14	37	74		
1635 x 110-2		14	25	39	78		
		37	39	76	76	1:1	0.0
1681 x 45-1	(84-4)	16	20	36	72		
1635 x 45-1		14	25	39	78		
		30	45	75	75	1:1	3.0
1681 x 45-4	(84-4)	14	22	41	82		
1635 x 45-4		10	32	42	84		
		24	54	83	83	1:3	1.5
						1:1	14.8**
1680 x 45-8	(84-4)	25	20	45	90		
1633 x 45-8		20	19	39	78		
		45	39	84	84	1:1	0.4
1681 x 2-2	(29-4)	19	26	45	90		
1635 x 2-2		20	27	47	94		
		39	53	92	92	1:1	2.1
1681 x 44-1	(29-4)	109	130	239	93		
1635 x 44-1		102	114	216	84		
		211	244	455	88	1:1	2.4
1681 x 44-3	(29-4)	18	21	39	78		
1635 x 44-3		21	21	42	84		
		39	42	81	81	1:1	0.1
1681 x 44-4	(29-4)	131	104	235	91		
1635 x 44-4		113	108	221	86		
		244	212	456	88	1:1	2.2
1680 x 44-8	(29-4)	118	107	225	87		
1635 x 44-8		122	121	243	94		
		240	228	468	91	1:1	0.3

Cross	+	fs	Total	% germ'n	Ratio tested (+:fs)	X^2 (1 d.f.)
1681 x 48-1 (29-4)	28	17	45	90		
1635 x 48-1	17	19	36	72		
	45	36	81	81	1:1	0.5
1681 x 48-4 (29-4)	19	29	48	96		
1635 x 48-4	28	21	49	98		
	47	50	97	97	1:1	0.1
1680 x 48-6 (29-4)	26	16	42	84		
1633 x 48-6	22	19	41	82		
	48	35	83	83	1:1	2.0
1681 x 2351 (c.i. of 351)	105	93	198	95	1:1	0.7
1680 x 2324 (c.i. of OR A)	14	15	29	91		
1633 x 2324	12	19	31	97		
	26	34	60	94	1:1	1.0
1635 x 2326 (c.i. of OR a)	7	17	24	75		
1681 x 2326	14	16	30	94		
	21	33	54	84	1:1	2.7

1. Numbers in parenthesis refer to designations of strains crossed to OR w.t.s from which the preceding strains were derived, e.g. strain 110-2 was derived from strain 19-6 in the cross ORa x 19-6.

** Significant at the p = 0.01 level

c.i. = conidial isolate.

section 27). This supports the suggestion that strain 19-6 ad-4 A is fs^+ (section 27).

40. (iii) Strains from ascus 45 of OR A x 84-4

Strains 45-1 and 45-8 segregated for 1+:1 fs in these crosses (X^2 = 3.0, X^2 = 0.4 respectively, 1 d.f., Table 9), whereas 45-4 segregated for 1+:3 fs (X^2 = 14.8, 1 d.f., for 1+:1 fs; X^2 = 1.5 for 1+:3 fs, d.f.; for 1+:3 fs; Table 9). Hence two independently assorting genes must segregate in the ascus with the individual mutants occurring in 45-1 and 45-8 and the double mutant occurring in 45-4. This establishes that one mutant came from a heterokaryotic parent, presumably OR A, in the cross OR A x 84-4 since no other asci with complex patterns were found in that cross. Hence strain 84-4 seems to have a single mutation for female sterility.

41. (iv) Strains from asci of OR A x 29-4

All of the strains 2-2, 44-1, 44-3, 44-4, 44-8, 48-1, 48-4, and 48-6 segregated for 1+:1 fs (X^2 < 3.86 for each case, 1 d.f., Table 9). Since two mutant sites have to be postulated in explaining the 1+:3 fs patterns in asci 44, 48, and one other ascus in OR a x 29-4, it appears that these mutants are closely linked. This is because some of the above strains should be double mutants, yet they segregate for the ratio 1+:1 fs.

Such a combination of mutants was likely to have

been inherited from OR A in the cross OR A x 19-8 rather than one being inherited by strain 29-4 in that cross and an additional one segregating in complex asci from OR A x 29-4. This is because a second and closely linked mutant inherited in the latter cross should have been detected in non-parental ditypes (0+:4 fs) rather than tetratypes (1+:3 fs). However a pair of closely linked mutants inherited in the first cross would easily explain the 1+:3 fs patterns in OR A x 29-4.

It is unlikely that these results can be attributed to errors in handling the strains. At least two spore pairs must be represented among the strains 44-1, 44-3, and 44-4, (all fs a) and 44-8 (fs A) must represent a third, regardless of dissecting errors or spore slippage in the ascus. In addition all strains tested from two asci (44 and 48) rather than just one segregated for 1+:3 fs making the possibility of errors seem even less likely.

42. (v) Conidial isolates from wild type strains

The female sterile conidial isolates 2324 (from OR A), 2326 (from OR a), and 2351 (from 351 Ab a) segregated for 1+:1 fs (X^2 < 3.86 for each strain, 1 d.f., Table 9). These data are consistent with one gene segregation, although, as mentioned in (iv) above, a pair of closely linked mutants likely occur in some nuclei of OR A and hence they both may occur in strain 2324.

(c) Complementation tests

43. (i) Strains selected for complementation tests

Female sterile strains with nic-3 and pan-2 mutants (Table 10) were selected from crosses segregating for single genes or pairs of closely linked mutants. This was done according to the procedure in section 17 and the strains are thought to represent all mutants for female sterility except sl. This mutant was not included in various tests because its poor growth makes sl strains difficult to handle.

44. (ii) Complementation patterns

The strains from which nic-3 fs and pan-2 fs strains were derived can be placed into at least five complementation groups (Fig. 3). The groupings are:

(1) 19-2, 2-4, 45-1, 2494

(2) 52-2 and 2351

(3) 2326

(4) 10-4 (col)

(5) 2324, 45-8, 2-2, 44-1, 44-3, 44-4, 44-8, 48-1, 48-4, 48-6

Complementation tests were not carried out between mutants in 52-2 and 2351 because strains having these mutants and different biochemical markers were not available. However in Fig. 3 they are considered separately because different mutants have been established in these strains on the

TABLE 10

Strains selected for complementation tests

Cross	Strains selected	
	nic-3 fs A	pan-2 fs A
1681 x 19-2 (126)[1].	2496	
1635 x 19-2		2503
1681 x 10-4 col (19-2)	2486	
1635 x 10-4 col		2490
1680 x 2-4 (6-2)	2435	
1633 x 2-4		2441
1189 x 52-2 (84-2)	2243	
1681 x 45-1 (84-4)	2446	
1635 x 45-1		2451
1680 x 45-8 (84-4)	2458	
1633 x 45-8		2462
1681 x 2-2 (29-4)	2477	
1635 x 2-2		2481
1681 x 44-1 (29-4)	2398	
1635 x 44-1		2417
1681 x 44-3 (29-4)	2510	
1635 x 44-3		2514
1681 x 44-4 (29-4)	2404	
1635 x 44-4		2421
1680 x 44-8 (29-4)	2409	
1633 x 44-8		2424
1635 x 48-1 (29-4)		2519
1681 x 48-4 (29-4)	2521	
1635 x 48-4		2527
1680 x 48-6 (29-4)	2534	
1633 x 48-6		2536

Cross	Strains selected	
	nic-3 fs A	pan-2 fs A
1681 x 2351 (c.i. of 351)	2507	
1633 x 442		2494
1680 x 2324 (c.i. of ORA)	2467	
1633 x 2324 (c.i. of ORA)		2473
1681 x 2326 (c.i. of ORa)	2414	
1635 x 2326 (c.i. of ORa)		2430

1. Strain 19-2 was derived from strain 126 (enclosed in parenthesis) in a cross to an OR w.t.:ORA x 126. This convention is used throughout the table.

c.i. = conidial isolate.

Figure 3. Complementation patterns of selected female sterile strains.

For designation of groups, see section 49.

Designations for double mutant strains, i.e. pan-2 fs and nic-3 fs are followed, in parentheses, by female sterile strains from which they were derived.

+ = perithecia and spores produced

- = no perithecia or spores produced

Numbers accompanying complementation data in bottom right hand corners of squares indicate the number of sets of three cultures involving heterokaryons of the pairs of strains were tested for female sterility. Asterisks indicate exceptions to the heterokaryons that could be scored for female sterility on the basis of growth habit.

Figure 3

GROUP	panic A	19-2				52-2	2326	2351	10-4	2-2									
		2503 (19-2)	2441 (2-4)	2451 (45-1)	2494 (442)	- (52-2)	2430 (2326)	- (2351)	2490 (10-4)	2473 (2324)	2462 (45-8)	2481 (2-2)	2417 (44-1)	2514 (44-3)	2421 (44-4)	2424 (44-8)	2519 (48-1)	2527 (48-4)	2536 (48-6)
19-2	2496 (19-2)	- *	-	-	-		+		+	+	+	+	+	+	+	+	+	+	+
	2435 (2-4)	-	-	-	-		+		+	+	+	+	+		+	+			
	2446 (45-1)	-	-	-	-		+		+	+	+	+	+		+	+			
	- (442)																		
52-2	2243 (52-2)	+	+	+	+ *		+		+	+ *	+	+	+	+	+	+	+	+	+
2326	2414 (2326)	+	+	+	+		-		+	+	+	+	+	+	+	+ 3	+	+	+
2351	2507 (2351)	+	+	+	+		+		+	+	+	+	+	+	+	+	+	+	+
10-4	2486 (10-4)	+	+	+	+		+		-	+	+	+	+	+	+	+	+	+	+
2-2	2467 (2324)	+	+	+	+		+		+	-	-	-	-	-	-	-	-	-	-
	2458 (45-8)	+	+	+	+		+		+	-	-	-	-	-	-	-			
	2477 (2-2)	+	+	+	+		+		+	-	-	- 2	- 2	-	- 2	- 2			
	2398 (44-1)	+	+	+	+		+		+	-	-	- 2	- 2	-	- 2	- 2			
	2510 (44-3)	+	+	+	+		+		+	-	-	-	-	-	-	-			
	2404 (44-4)	+	+	+	+		+		+	-	-	- 2	- 2	-	- 2	- 2			
	2409 (44-8)	+	+	+	+		+		+	-	-	- 2	- 2	-	- 2	- 2			
	- (48-1)																		
	2521 (48-4)																-	-	-
	2534 (48-6)																-	-	-

basis of linkage tests (section 47).

The formation of heterokaryons was established in a few cultures: 10 of 35 conidial isolates from (2243 + 2441) and 10 of 37 conidial isolates from (2243 + 2441) did not have nutritional requirements (Table 7). For other cultures the relatively rapid growth on a minimal medium, compared with the poor growth of cultures judged not to complement when strains were being selected for these tests, was considered to infer that heterokaryons were formed.

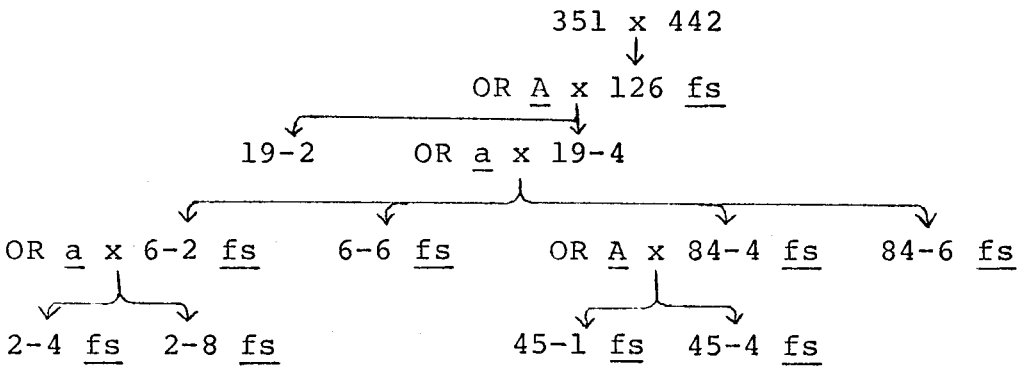
It can be noted that the mutants in strains 2494, 2326, 2351, and 2324, derived from the heterokaryotic strains 442 ad-4 A, OR a, 351 Ab a, and OR A, respectively, are in different complementation groups.

45. (iii) Inheritance patterns revealed from complementation tests

The female sterile mutant occurring in strain 19-2 of ascus 19 from OR A x 126 (Fig. 2) seems to be that occurring in the heterokaryotic strain 442 ad-4 A since the mutants in 19-2 and 2494, a derivative of 442, do not complement (Fig. 3). Hence strain 126 isolated from 351 x 442 must have inherited this mutant from 442 and transmitted it to ascus 19 in the above cross (see also Fig. 2).

The occurrence of this mutant in strains 2-4 and

45-1, since they do not complement with 19-2 or 2494 (Fig. 3), supports the suggestion that the same mutant must be present in 19-2 and 19-4 (section 36) because it must have been transmitted to these strains through 19-4 in the following sequence of crosses:



Strains 2-4 and 45-1 could not otherwise have inherited the mutant since strain 442 was not used elsewhere in the pedigree (Fig. 2) than in the original cross.

The presence of the mutant has already been established in strains 6-2, 6-6, 84-4, and 84-6 (section 36). It must occur in strains 2-4 and 2-8 from OR a x 6-2 because strains 2-6 and 2-8 are fs^+ (Table 3). In addition it must occur in strain 45-4 as well as 45-1 because strain 45-4 is a double mutant having this and the mutant present in 45-8 (see section 40, also next paragraph).

The complementation patterns of mutants in strains 45-1 and 45-8 from ascus 45 of OR A x 84-4 support the suggestion that one parent in the cross is heterokaryotic (section 40). Furthermore the heterokaryotic parent must

be OR A because the mutant(s) in strain 45-8 does (do) not complement with that (or those) in 2324, the conidial isolated from OR A (Fig. 3).

The strains from asci 2, 44 and 48 of OR A x 29-4 do not complement with 2324, a conidial isolated from OR A (Fig. 3), supporting the consideration that the mutants were inherited by strain 29-4 from a heterokaryotic OR A strain in OR A x 19-8 (sections 36, 41, Fig. 2). However the complementation tests do not permit a distinction between the two mutants postulated to segregate among those strains (section 41). The strains from ascus 44 do not complement with one another nor do those from ascus 48 (Fig. 3). Hence not only must the mutants be closely linked (section 41) but non-complementing as well.

Although two female sterile mutants must be postulated to occur in strain 29-4 (section 41), it is not clear whether both mutants are present in strain 2324. Presumably the mutations occurred at different times so that some nuclei in OR A must have one mutant and others must have both.

The mutant linked to m.t., present in strain 52-2 (derived from 84-2 in the cross OR A x 84-2, section 37), and postulated to have come from a heterokaryotic parent in the cross OR a x 19-4 (section 31),

is not the same as that occurring in strain 2326, a conidial isolate of OR a (52-2 and 2326 complement, Fig. 3). This is consistent with the suggestion that strain 19-4 is heterokaryotic for this mutant (section 31) as well as being homokaryotic for and expressing another (see above in this section).

(d) Linkage tests

(i) The assignment of genes to linkage groups using the alcoy linkage testers

The alcoy linkage testers (Perkins, 1964; Perkins et al., 1969) have three reciprocal translocations, one involving linkage groups I and II, a second involving the linkage groups III and VI, and a third involving linkage groups IV and V. These strains (997 A and 998 a, Table 1) also have the markers al-1, ylo-1, and cot-1, with al-1 occurring at the breakpoint of the first translocation, ylo-1 occurring close to the second, and cot-1 being close to the third.

If a mutant on linkage group I or II in a strain with a normal chromosome arrangement is crossed to an alcoy linkage tester, it will usually show linkage with al-1. Similarly if it is on linkage group III or VI it will usually show linkage with ylo-1, and if it is on linkage group IV or V it will usually show linkage with cot-1. If the mutant is on linkage group VII or is distant from the breakpoint of one

of the reciprocal translocations, it will not show linkage with any marker. On the basis of these considerations, from one cross it is often possible to assign a mutant to one of two linkage groups (see Perkins, 1964; Perkins et al., 1969).

The mutant in 19-2, 45-1, and 2494 (strains from one complementation group, section 44, Fig. 3) showed linkage with al-1 ($X^2 > 3.86$, 1 d.f., in each case, Table 11) but not with ylo-1 or cot-1 ($X^2 < 3.86$, 1 d.f., in each case, Table 11). Hence this mutant is on linkage group I or II.

The mutant in 2351 showed linkage with al-1 ($X^2 = 64.5$, 1 d.f., Table 11) but not with ylo-1 ($X^2 = 0.0$, 1 d.f., Table 11) or cot-1 ($X^2 = 0.0$, 1 d.f., Table 11). Hence it is also on linkage group I or II.

The pair of closely linked mutants postulated to occur among 2324, 45-8, 44-1, 44-4, 44-8 (sections 41, 45; strains from one complementation group, sections 44, 45, Fig. 3) showed linkage with al-1 ($X^2 > 3.86$, in each case, Table 11) but, with the exception of 998 x 44-8, did not show linkage with ylo-1 or cot-1 ($X^2 < 3.86$, 1 d.f., in these crosses, Table 11). Hence these mutants seem to be on linkage group I or II.

Although linkage was found between female sterility and all mutants in 998 x 44-8, the value of X^2 was higher

TABLE 11

Linkage determinations using the alcoy linkage testers

Group	Cross	al+ (a)	al fs (b)	+ + (c)	+ fs (d)	Total (n)	% germ'n	X^2_L
19-2	997 x 19-2 (126)	32	2	12	25	71	71	26.0**
	998 x 45-1 (84-4)	30	17	12	29	88	88	10.2**
	998 x 249.4 (442)	26	15	12	20	73	73	4.9*
		88	34	36	74	232	77	36.5**
2351	997 x 2351 (c.i. of 351)	44	3	0	29	76	76	64.5**
2-2	998 x 45-8 (84-4)	35	13	8	32	88	88	24.0**
	997 x 2-2 (29-4)	25	11	14	25	75	75	8.3**
	997 x 44-1 (29-4)	33	7	8	37	85	85	35.6**
	997 x 44-4 (29-4)	38	10	7	23	78	78	24.8**
	998 x 44-8 (29-4)	36	17	7	31	91	91	20.3**
	998 x 2324 (c.i. of ORA)	29	8	8	45	90	90	37.4**
		ylo + (a)	ylo fs (b)	+ + (c)	+ fs (d)			
19-2	997 x 19-2 (126)	7	13	5	12	37	71	0.0
	998 x 45-1 (84-4)	5	15	7	14	41	88	0.2
	998 x 249.4 (442)	9	15	3	5	32	73	0.5
		21	43	15	31	110	77	0.3
2351	997 x 2351 (c.i. of 351)	0	15	0	14	29	76	0.0

Group	Cross	ylo + (a)	ylo fs (b)	+ + (c)	+ fs (d)	Total (n)	% germ'n	X_L^2
2-2	998 x 45-8 (84-4)	4	14	4	18	40	88	0.4
	997 x 2-2 (29-4)	7	13	8	11	39	75	0.2
	997 x 44-1 (29-4)	3	17	5	20	45	85	0.0
	997 x 44-4 (29-4)	2	7	5	16	30	78	1.2
	998 x 44-8 (29-4)	5	7	2	24	38	91	10.5**
	998 x 2324 (c.i. of ORA)	4	20	4	25	53	90	0.5
		cot + (a)	cot fs (b)	+ + (c)	+ fs (d)			
19-2	998 x 2494	17	15	21	20	73	73	0.0
2-2	998 x 45-8 (84-4)	18	17	25	28	88	88	0.2
	997 x 2-2 (29-4)	17	16	22	20	75	75	0.0
	997 x 44-1 (29-4)	19	22	22	22	85	85	0.1
	997 x 44-4 (29-4)	18	16	27	17	78	78	0.8
	998 x 44-8 (29-4)	31	23	12	25	91	91	4.8*
	998 x 2324 (c.i. of ORA)	20	28	17	25	90	90	0.0

For designations of groups, see section 49.

$$X_L^2 = \frac{(a - b - c + d)^2}{n}$$

* significant at the p = 0.05 level

** significant at the p = 0.01 level

c.i. = conidial isolate.

Numbers in parenthesis refer to designations of strains crossed to OR w.t.s from which the preceding strains were derived.

in the test involving al-1 (X^2 = 20.3, 1 d.f., Table 11) than that for ylo-1 or cot-1 (X^2 = 10.5, X^2 = 4.8 respectively, 1 d.f., Table 11). Furthermore the significance of the X^2 value in the test involving ylo-1 is partly attributable to distorted segregation ratios in the cross. If a 2 x 2 contingency table is used for the calculation, X^2 = 4.2, 1 d.f., which approaches the p = 0.05 significance level of 3.86. (A 2 x 2 contingency table used in testing for linkage to al-1 and cot-1 results in values of X^2 = 21.9, 1 d.f., and X^2 = 5.5, 1 d.f., for the respective genes, these values resembling those in the above calculations). Hence these results do not seriously detract from the assignment of the mutant to linkage group I or II.

Mutants on different linkage groups cannot be postulated to segregate in the cross 998 x 44-8 because the progeny segregate for the ratio 1+:1 fs (44 +, 48 fs, Table 11; X^2 = 0.3, 1 d.f.).

47. (ii) Some linkage tests from random spore data

The pairs of closely linked mutants postulated to occur among 2-2, 44-1, 44-3, 44-4, 44-8, 48-2, 48-4, 48-6 and 2324 (sections 41, 44, 45) and found to be on linkage group I or II from crosses to alcoy strains (section 46), provide variable results in tests for linkage to m.t. on linkage group I. Female sterility is linked to m.t. in

crosses involving strains 2-2, 44-3, 44-4, and 48-6 (X^2 > 3.86, 1 d.f. in these crosses, Table 12) whereas it is not detected in crosses involving 44-1, 44-8, 48-4, or 2324 (X^2 < 3.86, 1 d.f., in these crosses, Table 12). These data are best explained by suggesting that the genes are on linkage group I but distant from m.t. so that linkage cannot always be detected. The variation in the results is not considered to detract from this suggestion because linkage values are variable in Neurospora (see especially Stadler, 1956).

The mutant in 2326 (a conidial isolate from OR a) is linked to m.t. (X^2 = 16.7, 1 d.f.) and is 22.2±5.7 m.u. from it on linkage group I (Table 12).

The mutant in 2351 (a conidial isolate from 351 Ab a) and found to be on linkage group I or II from a cross to an alcoy strain (section 46), shows no linkage to m.t. Hence it is attractive, tentatively, to place it on linkage group II. The data provide a distinction between this mutant and the one found in strain 52-2 (they were not tested for complementation, section 44) because the mutant in 52-2 is linked to m.t. (see cross OR A x 84-2 from which 52-2 was derived, section 31).

The mutant in 110-2 is linked to m.t. (X^2 = 27.8, 1 d.f.) and is 19.7±4.6 m.u. from it on linkage group I (Table 12). This confirms that the mutant segregating only

in ascus 110 from OR *a* x 19-6 *ad-4 A* was inherited from a heterokaryotic parent (sections 27, 39). Otherwise, since *ad-4* is in linkage group III, the mutant would have occurred among half of the *ad*$^+$ progeny in asci from that cross. This, then, establishes, beyond any reasonable doubt, that 19-6 *ad-4 A* from OR *a* x 126 is *fs*$^+$ (sections 27, 39).

The similarity of the map distance for the mutant in 110-2 and that for the one in strain 2326 suggests that the same mutant may be present in both strains. This is likely because 2326 was a conidial isolate from OR *a* and 110-2 was derived from a cross involving OR *a*.

The *col* strain 10-2 (from OR *A* x 19-2) and the *sl* strains 5-1 and 32-4 (from OR *a* x 6-2 and OR *a* x 84-6 respectively) segregate for female sterility other than that attributable to these mutants. The same mutant is present in these strains (section 45) and has been assigned to linkage group I or II (section 46; strains 19-2, 45-1, 2494 in crosses to alcoy linkage testers).

In the crosses 1681 x 10-2 and 1635 x 10-2, the *col*$^+$ progeny deviate significantly from a ratio of 1+:1 *fs* (X^2 = 4.3, 1 d.f., Table 8), the expected ratio if the two mutants assorted independently. Hence *col* seems to be on the same linkage group (I or II) as the mutant in the above strains.

TABLE 12

Tests for linkage between female sterility and mating type from random spore analysis of selected strains

Group	Cross	P types	+ A (a)	+ a (b)	fs A (c)	fs a (d)	Total (n)	% germ'n	X^2_L (1 d.f.)	% recomb'n
2-2	1681 x 2-2 (29-4)	a, d	26	23	21	26	96	92		
	1635 x 2-2	a, d	33	16	20	31	100	96		
			59	39	41	57	196	94	6.7**	40.8±3.5
	1681 x 44-1 (29-4)	a, d	54	38	48	53	193	93	2.3	
	1681 x 44-3 (29-4)	a, d	15	3	8	13	39	78		
	1635 x 44-3	a, d	13	8	9	11	41	82		
		a, d	28	11	17	24	80	80	7.2**	35.0±5.3
	1681 x 44-4 (29-4)	a, d	65	43	35	57	200	96	9.7**	39.0±3.4
	1680 x 44-8 (29-4)	b, c	41	50	58	47	196	94	2.0	
	1681 x 48-1 (29-4)	a, d	13	15	6	11	45	90		
	1635 x 48-1	a, d	11	6	7	12	36	72		
		a, d	24	21	13	23	81	81	2.1	
	1681 x 48-4 (29-4)	a, d	10	9	14	15	48	96		
	1635 x 48-4	a, d	15	13	7	14	49	98		
		a, d	25	22	21	29	97	97	1.2	
	1680 x 48-6 (29-4)	b, c	13	13	10	6	42	84		
	1633 x 48-6	b, c	6	13	17	5	41	82		
		b, c	19	26	27	11	83	83	6.4*	36.2±5.2

Group	Cross	P types	+ A (a)	+ a (b)	fs A (c)	fs a (d)	Total (n)	% germ'n	X_L^2 (1 d.f.)	% recomb'n
2-2	1680 x 2324 (c.i. of ORA)	b, c	5	10	4	9	28	88		
	1633 x 2324	b, c	6	6	8	11	31	97		
		b, c	11	16	12	20	59	92	1.5	
?	1681 x 110-2 (19-6)	a, d	19	4	4	10	37	74		
	1635 x 110-2	a, d	11	3	4	21	39	78		
		a, d	30	7	8	31	76	76	27.8**	19.7±4.6
2326	1681 x 2326 (c.i. of ORa)	a, d	4	3	2	15	24	75		
	1635 x 2326	a, d	12	2	5	11	30	94		
		a, d	16	5	7	26	54	84	16.7**	22.2±5.7
2351	1681 x 2351 (c.i. of 351)	a	48	57			105	95		
	1635 x 2351	a	61	39			100	95		
			109	96			205	95	0.8	

For group designations, see section 49.

$$X_L^2 = \frac{(\text{parental (P) types} - \text{recombinants})^2}{n}$$

* significant at the p = 0.05 level
** significant at the p = 0.01 level

c.i. = conidial isolate.
Numbers in parenthesis refer to designations of strains crossed to OR w.t.s from which the preceding strains were derived.

In the cross 1681 x 5-1, sl$^+$ progeny deviate significantly from the ratio 1+:1 fs (X^2 = 5.8, 1 d.f., Table 8) but those in 1680 x 32-4 do not (X^2 = 1.3, 1 d.f., Table 8). Data from the crosses, however, are homogeneous (X^2 = 0.8, 1 d.f., Table 8) and on the basis of pooled data, sl$^+$ progeny deviate from the 1+:1 fs ratio (X^2 = 6.3, 1 d.f., Table 8). Hence sl can also be placed, at least tentatively, on the same linkage group (I or II) as the mutant segregating in the above strains and col.

48. (iii) Linkage determinations from tetrads

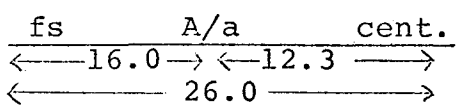
The strains 19-2, 19-4, 6-2, 84-4, and 84-6 express one mutant for female sterility (section 45) and strains 84-2 and 84-8 express another (section 31). Data from crosses between these strains and the OR w.t.s can be consolidated in making some final estimates of map distances. Furthermore some complex and incomplete asci can be used in these calculations. These include (i) complex asci where further genetic or complementation studies revealed the genotypes of spores, and (ii) incomplete asci where genotypes of missing spores can be extrapolated. The latter were used only in cases where genes segregating from heterokaryotic parents were detected in rare cases if at all and the extrapolation could be considered reliable.

For final linkage estimates from the cross OR A x 29-4, the extrapolation of genotypes in incomplete asci was not made because strain 29-4 is thought to have

a pair of closely linked mutants (sections 41, 45).

From the consolidated data, the mutant present in 19-2, 19-4, 6-2, 84-4, and 84-6 maps 35.5 m.u. from the centromere (95% confidence limits of 32.7-42.0 m.u., Table 13).

The mutant in 84-2 and 84-8 maps 16.0 m.u. from m.t. (95% confidence limits of 11.3-22.0 m.u., Table 13) and 26.0 m.u. from the centromere (95% confidence limits of 20.0-23.5 m.u., Table 13). Furthermore the m.t. locus maps 12.3 m.u. from the centromere in this cross (95% confidence limits of 8.0-17.8 m.u.) so that the genes can be positioned on the left arm of linkage group I as follows:



The closely linked genes postulated in 29-4 map 3.0 m.u. apart (95% confidence limits of 0.5-9.0 m.u.) and are distant from the centromere (48.0 m.u. with a lower limit of 34.5 m.u. with 95% confidence).

49. (iv) Some final considerations regarding the linkage relationships of female sterile mutants

From morphological characteristics and linkage and complementation tests, the strains expressing single mutants or pairs of closely linked mutants can be subdivided into seven distinct groups:

LEGEND FOR TABLE 13

Distances and confidence limits with corrections for multiple crossovers and interference were determined from graphs provided by Barratt *et al.* (1954).

PD = parental ditype
NPD = non-parental ditype
TT = tetratype

TABLE 13

Linkage determinations from ascus data

Group	Cross	Distance tested	Gene-Centromere Data				
			Ascus pattern		Total	Distance (m.u.) with 95% confidence limits	
			1st div'n	2nd div'n			
19-2	ORA x 19-2	fs	39	75	114		
	ORa x 19-4	to	38	64	102		
	ORa x 6-2	centro-	28	27	55		
	ORA x 84-4	mere	26	29	55		
	ORa x 84-6		15	39	54		
			146	234	380	35.5 (32.7-40.0)	
52-2	ORA x 84-2	fs	31	24	55		
	ORa x 84-8	to	29	31	60		
		cent.	60	55	115	26.0 (20.0-33.5)	
	ORA x 84-2	A/a	44	11	55		
	ORa x 84-8	to	44	16	60		
		cent.	88	27	115	12.3 (8.0-17.8)	
2-2	ORA x 29-4	fs to cent.	12	36	48	48.0 (34.5---)	
			PD	TT	NPD	Total	Distance (m.u.)
52-2	ORA x 84-2	fs	40	15	0	55	
	ORa x 84-8	to	40	20	0	60	
		A/a	80	35	0	115	16.0 (11.3-22.0)
2-2	ORA x 29-4	fs to fs (different mutants)	48	3	0	51	3.0 (0.5-9.0)

Group	Strains
19-2	19-2, 2-4, 45-1, 2494
52-2	52-2
2326	2326
2351	2351
2-2	45-8, 2-2, 44-1, 44-3, 44-4, 44-8, 48-1, 48-4, 48-6, 2394
10-4 (*col*)	10-4
5-5 (*sl*)	5-5, 32-1

The groups are designated on the basis of strains occurring in the groups and considered to be representative of them.

The reasoning in establishing these groups is as follows: From complementation studies, strains in the first six groups were separable into at least five groups, but complementation between strains in Groups 52-2 and 2351 was not attempted (section 44). However the mutants in these two groups were distinguished by linkage studies (section 47). Finally the distinct growth of *sl* strains suggested Group 5-5.

Two mutants need to be postulated for Group 2-2 (sections 41, 45) but only one is thought to occur in each of the remaining groups.

Linkage considerations for mutants in all groups are summarized below, making possible the tentative assignment of all mutants to specific linkage groups:

Group 19-2: linkage to al-1 of alcoy (strains 19-2, 45-1, 2494; section 46), hence on linkage group I or II; no linkage to m.t. (pooled data for 19-2, 19-4, 6-2, 84-4, 84-6; section 48), hence probably on linkage group II.

Group 52-2: 16.0 m.u. from m.t. on linkage group I (strains 84-2, 84-4; section 48).

Group 2326: 22.2 m.u. from m.t. on linkage group I (strain 2326, section 47).

Group 2351: linkage to al-1 of alcoy (strain 2351, section 46), hence on linkage group I or II; no linkage to m.t. (strain 2351, section 47), hence tentatively placed on linkage group II.

Group 2-2: linkage to al-1 of alcoy (strains 45-8, 2-2, 44-1, 44-4, 44-8, 2324; section 46); linkage to m.t. found in some crosses (strains 2-2, 44-3, 44-4, 48-6; section 47) but not in others (strains 44-1, 44-8, 48-1, 48-4, 2324; section 47), hence appears to be distant from m.t. on linkage group I.

Group 10-4: apparently linked to the mutant in Group 19-2 (strain 10-2; section 47), hence probably on linkage group II.

Group 5-5: apparently linked to the mutant in Group 19-2 (strains 5-1, 32-4; section 47), hence probably on linkage group II.

The placement of mutants in Groups 19-2 and 2-2 on different linkage groups, and hence also affecting that of Groups 10-4 and 5-5, is supported by the independent assortment of the mutants in strain 45-4 from OR A x 84-4 which is considered to have the mutants present in strains 45-1 (Group 19-2) and 45-8 (Group 2-2; section 40).

50. (v) Inheritance patterns of mutants in the cross 351 x 442 and the three generations of crosses to OR w.t.s

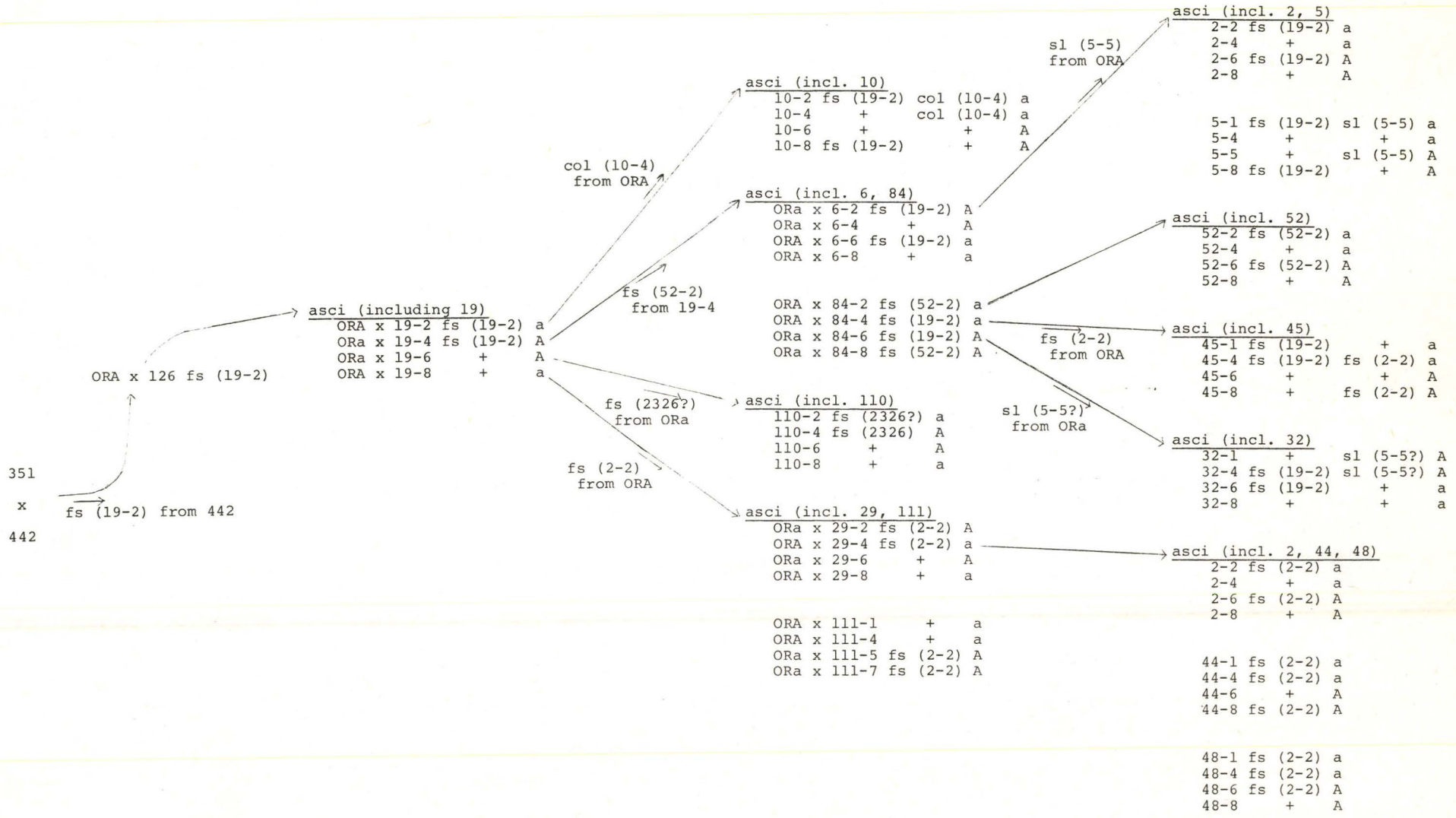
Data from the genetic and complementation studies reveal the inheritance patterns of all mutants in the cross 351 x 442 and the three generations of crosses to OR w.t.s (Fig. 2). These patterns are presented in Fig. 4 and explained below. All asci selected from the crosses for further studies are included.

Group 19-2:

Considerations in section 45 provide the following pattern of inheritance for the mutant in this group: from 351 x 442 it was transmitted to 126; from OR A x 126 to 19-2, 19-4; from OR a x 19-4 to 6-2, 6-8, 84-4, 84-6; from OR a x 6-2 to 2-4, 2-6; from OR A x 84-4 to 45-1, 45-4. In other crosses, the mutant was transmitted as follows: from OR A x 19-2 to 10-2, 10-8 (10-6 is fs^+, Table 3, and the col strain 10-4 does not segregate for this mutant, section 38, Table 8); from OR a x 6-2 to 5-1, 5-8 (5-4 is fs^+, Table 3, and the sl strain 5-5 does not segregate for the mutant, section 38, Table 8); from OR a x 84-6 to 32-4,

Figure 4. Pattern of transmission of female sterile mutants in the cross 351 Ab a x 442 ad-4 A and subsequent crosses to OR w.t.s.
Numbers in brackets following the designation fs refer to groups to which the particular mutants belong.
Small arrows accompanied by mutant designations indicate the transmission of a mutant from a heterokaryotic parent in the cross.

Figure 4


351
x
442
fs (19-2) from 442
ORA x 126 fs (19-2)
asci (including 19)
ORA x 19-2 fs (19-2) a
ORa x 19-4 fs (19-2) A
ORa x 19-6 + A
ORA x 19-8 + a
col (10-4) from ORA
fs (52-2) from 19-4
fs (2326?) from ORa
fs (2-2) from ORA
asci (incl. 10)
10-2 fs (19-2) col (10-4) a
10-4 + col (10-4) a
10-6 + + A
10-8 fs (19-2) + A
asci (incl. 6, 84)
ORa x 6-2 fs (19-2) A
ORa x 6-4 + A
ORA x 6-6 fs (19-2) a
ORA x 6-8 + a
ORA x 84-2 fs (52-2) a
ORA x 84-4 fs (19-2) a
ORa x 84-6 fs (19-2) A
ORA x 84-8 fs (52-2) A
asci (incl. 110)
110-2 fs (2326?) a
110-4 fs (2326) A
110-6 + A
110-8 + a
asci (incl. 29, 111)
ORa x 29-2 fs (2-2) A
ORA x 29-4 fs (2-2) a
ORa x 29-6 + A
ORA x 29-8 + a
ORA x 111-1 + a
ORA x 111-4 + a
ORa x 111-5 fs (2-2) A
ORA x 111-7 fs (2-2) A
sl (5-5) from ORA
fs (2-2) from ORA
sl (5-5?) from ORa
asci (incl. 2, 5)
2-2 fs (19-2) a
2-4 + a
2-6 fs (19-2) A
2-8 + A
5-1 fs (19-2) sl (5-5) a
5-4 + + a
5-5 + sl (5-5) A
5-8 fs (19-2) + A
asci (incl. 52)
52-2 fs (52-2) a
52-4 + a
52-6 fs (52-2) A
52-8 + A
asci (incl. 45)
45-1 fs (19-2) + a
45-4 fs (19-2) fs (2-2) a
45-6 + + A
45-8 + fs (2-2) A
asci (incl. 32)
32-1 + sl (5-5?) A
32-4 fs (19-2) sl (5-5?) A
32-6 fs (19-2) + a
32-8 + + a
asci (incl. 2, 44, 48)
2-2 fs (2-2) a
2-4 + a
2-6 fs (2-2) A
2-8 + A
44-1 fs (2-2) a
44-4 fs (2-2) a
44-6 + A
44-8 fs (2-2) A
48-1 fs (2-2) a
48-4 fs (2-2) a
48-6 fs (2-2) A
48-8 + A

32-6 (32-8 is *fs*$^+$, Table 3, and the *sl* strain 32-1 does not segregate for this mutant, section 38, Table 8).

The mutant originally occurred in the heterokaryotic strain 442 (section 45).

Group 52-2:

The mutant in this group was found in strains 84-2 and 84-8 derived from the cross OR *a* x 19-4 (section 31), and in OR *A* x 84-2 it was transmitted to strains 52-2 and 52-6 (52-4 and 52-8 are *fs*$^+$, Table 3). It was first detected as one of the mutants segregating in the cross OR *a* x 19-4 and is thought to occur in a heterokaryon in strain 19-4 (sections 31, 45). The mutant probably occurs in all complex asci from OR *a* x 19-4.

Group 2326:

Strains 110-2 and 110-4 from OR *a* x 10-6 are thought to express this mutant occurring in a heterokaryotic strain OR *a*. This is based on the similarity of linkage relationships found in crosses involving strain 110-2 and conidial isolate 2326 from OR *a* (section 47).

Group 2-2:

The pair of mutants thought to occur in this group (sections 41, 45, 49) and inherited from a heterokaryotic OR *A* strain in OR *A* x 19-8 (section 41) were transmitted to strains 29-2, 29-4, 111-5, and 111-7 from OR *A* x 19-8 (29-6, 29-8, 111-1, 111-4 are *fs*$^+$, section 36). In the cross OR *A* x 29-4, they were transmitted to 2-2, 2-4 (2-4 and 2-8 are *fs*$^+$, Table 3), 44-1, 44-4, 44-8 (44-6 is

$\underline{fs}^+$, Table 3), 48-1, 48-4 and 48-6 (48-8 is $\underline{fs}^+$, Table 3). It is not possible to distinguish the single and double mutants in the strains from asci 44 and 48 (sections 41, 45).

All female sterile progeny in the cross OR $\underline{A}$ x 19-8 can be explained by the segregation of this pair of mutants.

At least one mutant for this group appeared in strains 45-4 and 45-8 of ascus 45 from OR $\underline{A}$ x 84-6 (45-8 does not complement with other strains in this group, section 45, and 45-8 has this and another mutant segregating in the ascus, sections 40, 45) and presumably was inherited from the heterokaryotic strain OR $\underline{A}$ in that cross.

Group 10-4:

The $\underline{col}$ mutant appeared in strains 10-2 and 10-4 derived from OR $\underline{A}$ x 19-2 (as well as two other asci in that cross, section 24). It is thought to have come from a heterokaryotic OR $\underline{A}$ strain in that cross (sections 24, 38).

Group 5-5:

The $\underline{sl}$ mutant appeared in strains 5-1 and 5-5 derived from OR $\underline{a}$ x 6-2 (as well as in one other ascus in that cross, section 32) and strains 32-1 and 32-4 from OR $\underline{a}$ x 84-6 (and in two other asci in that cross, section 32).

Phase 7. The characterization of female sterile mutants

51. (a) Growth characteristics

Female sterile mutants in all groups differ from the wild type in their aerial growth. With the exception of those in Groups 10-4 (col) and 5-5 (sl), the differences are rather subtle but some experience in handling the mutants permits the classification on this basis with high accuracy (99-100%, section 58).

The growth characteristics have not been carefully studied thus far, but pending this the following superficial comparisons can be made (from growth observed in small tubes of sucrose medium).

Wild type (OR)

Growth is relatively rapid with the conidia forming a band well above the surface of the medium and leaving a gap of sparse growth between the medium and the band of conidia. Pigmentation is relatively intense.

Groups 52-2, 2326, 2351, 2-2:

Growth is slower than that of w.t. and is more continuous in the culture so that there is no conspicuous gap of sparse growth. The mutants do not become as highly pigmented as the w.t.

Differences have not been noted thus far between mutants in these groups.

Group 19-2:

Growth is similar to that in the above groups in being slower, more continuous in the culture, and less highly pigmented that the w.t. However it has a rather stringy appearance as well.

Group 10-4:

Growth is probably slower than that of the w.t., but this is not as conspicuous as the colonial morphology. The mutant (col) tends to grow in clumps or clusters.

Group 5-5:

Growth of this mutant (sl) is conspicuously retarded and is restricted to the surface of the medium.

52. (b) Growth of mutants at 34°C

Dr. A. Radford and Dr. D. Newmeyer (personal communications) suggested testing female sterile mutants for growth at 34°C in the event that they may be temperature sensitive nutritional mutants. This was found to be a necessity when progeny from crosses between some strains in Groups 19-2 and 2351, and the alcoy linkage testers could not be scored for cot-1 (at 35°, section 16) because of another temperature sensitive mutant appearing to segregate in the crosses. It was thought that this temperature sensitivity may be attributable to the female sterile mutants.

Strains selected from all groups except Group 5-5 (sl) and derivatives from them were tested for growth at 34°C. The results are presented in Table 14 and considered below.

Groups 19-2 and 2351:

Strains 19-2 and 45-1 from Group 19-2 and strain 2351 from Group 2351 did not grow at 34°C, but some female sterile strains derived from them grew. Hence the female sterile mutants cannot be temperature sensitive nutritional mutants.

Groups 52-2, 2326, and 2-2:

All strains tested from these groups grew at 34°C. Hence they cannot be considered as temperature sensitive nutritional mutants.

Group 10-4:

The col strain 10-4 did not grow at 34°C and neither did the 25 strains derived from it. Hence the mutant in this group seems to be a temperature sensitive growth mutant as well as a female sterile.

The growth problems at 34°C could not be alleviated by using a malt peptone medium instead of a glucose or sucrose medium as may have been possible if col were a temperature sensitive nutritional mutant. Hence it is not yet clear why the mutant does not grow at this temperature.

LEGEND FOR TABLE 14

\+ growth at 34°
\- no growth at 34°

Tests were done in duplicate on a sucrose medium, with controls at 25°C. Those strains in column B which did not grow at 34° were also tested on glucose and malt-peptone medium with results being identical to those made on a sucrose medium.

TABLE 14

Tests of selected female sterile strains and their progeny for growth at 34°

A	B		C		D		E	F
Group	Nutritionally w.t. strains		nic strains derived from those in B		pan strains derived from those in B		Crosses between biochemical mutants and fs strains	Segregation of growth in crosses
	Strain	Growth	Strain	Growth	Strain	Growth		
19-2	19-2	-	2496	+	2503	+	1681 x 19-2	14+, 10-
	2-4	+	2435	+	2441	+		
	45-1	-	2446	-	2451	+	1681 x 45-1	4+, 8-
			2494	+			1635 x 45-1	4+, 8-
52-2	52-2	+	2243	+				
2326	2326	+	2414	+	2430	+		
2351	2351	-	2507	-			1681 x 2351	16+, 8-
10-4	10-4	-	2486	-	2490	-	1681 x 10-4	0+, 11-
							1635 x 10-4	0+, 12-
2-2	2324	+	2467	+	2473	+		
	45-8	+	2458	+	2462	+		
	2-2	+	2477	+	2481	+		
	44-1	+	2398	+	2417	+		
	44-3	not tested	2510	+	2514	+		
	44-4	+	2404	+	2421	+		
	44-8	+	2409	+	2424	+		
	48-1	not tested			2519	+		
	48-4	not tested	2521	+	2527	+		
	48-6	not tested	2534	+	2536	+		

53. (c) The leakiness of mutants

The mutant in Group 19-2 is somewhat leaky. Some strains produce perithecia and spores, but the perithecia are generally few in number, small in size, and produce small numbers of spores, if any. Leakiness is not rare, although the frequency of occurrence has not been noted, but even at its extreme the production of perithecia and spores is conspicuously below that of *fs*$^{+}$ strains.

Some instances of leakiness have been observed among *sl* strains (Group 5-5) but at best few small perithecia and spores have been produced. Although quantitative data are not available, perithecial and spore production at their extreme are even more pronouncedly different from the w.t. than the leakiness of the mutant in Group 19-2.

(d) A test for the detection of developmental blocks of female sterile mutants following fertilization

54. (i) The description of the test

Although careful observations have not yet been made, it is expected that the female sterile mutants in all groups have developmental blocks prior to or at fertilization. This is because, with the exception of the leakiness of mutants in some groups (section 53), attempted crosses do not produce pigmented structures resembling developing perithecia; they do not proceed far enough in the development

to permit mating type determinations in these tests. Furthermore, blocks in the development following fertilization would be expected to be alleviated, at least in some cases, by the complementation between nuclei of opposite mating type (see section 5).

A test has been devised which, with appropriate modifications (section 67), is expected to permit the detection of developmental blocks following fertilization. This is in addition to the blocks postulated to occur in the earlier stages of development, and is proposed in the event that the genes may have pleiotropic effects. The mating type locus in Ascobolus stercorarius has been shown to affect several stages of the sexual development in that organism (see especially Bistis, 1965), and hence a gene for development in Neurospora may affect various stages as well (even if it is not the mating type locus). This has, in fact, been observed; the round spore mutant in N. crassa is female sterile as well as affecting spore delimitation when used as a male parent (Mitchell, 1966).

For any particular mutant, the proposed test consists of the following crosses using heterokaryons:

(pan-2 (B5) + nic-3 fs) ♀ x pan-2 (B3) ♂

and (pan-2 (B5) + nic-3 fs) ♀ x pan-2 (B3) fs ♂

Both crosses have a pan x pan component, which can only result in pan progeny (this is with the exception of rare

wild types produced by recombination between the two heteroalleles of pan-2, Threlkeld et al., 1969, and pseudo-wild types produced by nondisjunction of linkage group VI, Threlkeld and Stoltz, 1970) and a nic x pan component which can result in + +, + nic-3, pan +, and pan nic progeny.

The test is based on the detection of the nic x pan component in each cross, and hence is dependent on finding + +, + nic, and pan nic progeny. This is facilitated by the use of the pan-2 mutants in the following way. Spores having the pan-2 mutant and produced on limiting concentrations of pantothenic acid are pale, whereas pan^+ spores are dark (Threlkeld, 1965). Hence the observation of a substantial proportion of dark spores in a cross indicates the production of + + and + nic progeny from the nic x pan component and provides preliminary information prior to the isolation and testing of the spores.

The recovery of the nic x pan component from the first cross should establish that the female sterile mutant can function as a female parent in a heterokaryon and that the block postulated to occur prior to or at fertilization has been alleviated. Given that this occurs, the failure to recover this component from the second cross should indicate an additional block following fertilization. This would be expected only if nuclei of opposite m.t. failed

to complement, and this would be attributable to the presence of the same female sterile mutant in each.

55. (ii) The application of the test to the mutant in Group 52-2

This test has been carried out by attempting the following crosses using the mutant in Group 52-2:

(1604 + 2243) ♀ x 1643 ♂

pan-2(B5) A nic-3 fs A pan-2(B3) a

and (1604 + 2243) x 1881

pan-2(B5) A nic-3 fs A pan-2(B3) fs a

Strain 1881 was derived from the cross 1273 pan-2(B3) A x 52-2 fs a.

The formation of a heterokaryon between strains 1604 and 2243 is supported by the finding that 4 of 40 conidial isolates from the culture did not have nutritional requirements (Table 7).

In two separate attempts of each cross on a minimal medium dark spores were observed indicating the production of + + and + nic progeny. Upon isolating and testing spores, substantial numbers of + +, + nic and pan nic progeny were found in each case: 56 of 67 and 36 of 45 germinated spores in separate attempts of the first, and 55 of 70 and 37 of 61 germinated spores in separate attempts of the second (Table 15). Hence the nic x pan component was found in each cross.

TABLE 15

Crosses involving synthesized heterokaryons used in testing for a developmental block following fertilization in the mutant in Group 52-2

Cross	+ +	+ nic	pan +	pan nic	Total	% germ'n
(1604 + 2243) x 1643	24	25	11	7	67	56
pan-2(B5) A nic-3 fs A pan-2(B3) a	21	12	9	3	45	38
(1604 + 2243) x 1881	25	15	25	5	70	58
pan-2(B5) A nic-3 fs A pan-2(B3) fs a	14	12	24	11	61	51

With the reservations discussed in section 67, these data suggest that the gene in Group 52-2 does not have a role in the sexual development following fertilization.

56. A summary of characteristics of the female sterile mutants found

See Table 16 for a summary of characteristics of the female sterile mutants.

Phase 8. Some considerations about testing procedures for female sterility

57. (a) The use of different methods for scoring female sterility in crosses

In the earlier stages of the study (up to the end of Phase 4), each strain of unknown m.t. was tested for female sterility using the method in (i) of section 11 where one culture of the strain was inoculated with a male parent of m.t. A and another with m.t. a. To facilitate some later tests, the method in (ii) of section 11 was used where a single culture was inoculated with a mixture of A and a strains.

To rule out unforeseen problems using the latter procedure, at least ten asci were selected from each cross analyzed using asci, and the strains were tested using both methods. These included all asci with complex patterns (Fig. 4) and hence mutants from all groups except

TABLE 16

A summary of characteristics of the female sterile mutants found

Group	No. of mutant sites	Strain in which mutation(s) originally occurred	Linkage group	Differ from w.t. in growth habit	Growth at 35°C as well as at 25°C	Comments
19-2	1	442	I or II (II)	Yes	Yes	Somewhat leaky
52-2	1	19-4	I	Yes	Yes	(No developmental block following fertilization)
2326	1	ORa	I	Yes	Yes	
2351	1	351	I or II (II)	Yes	Yes	
2-2	2	ORA	I or II (I)	Yes	Yes	Mutants do not complement with one another
10-4	1	ORA	(II)	Yes	No	Colonial growth
5-5	1	ORa	(II)	Yes		Conspicuously slow growth; slightly leaky

Tentative information is enclosed in parentheses; highly probable information is not.

Group 2351 (section 50). This served not only as a test for the second method but the use of the first also served as a repeat for some tests made only once up to that point.

Only one spore from each identical pair was tested from asci with 2+:2 fs patterns, but all spores were tested from those with complex patterns. Strains expressing ad-4 were omitted.

In these tests, the results were invariably the same using both methods. Hence the use of either seems equally reliable.

Although each strain was tested only once using the method in (ii) of section 11, the tests included sister spores from asci for all mutants tested, and hence the above conclusion is considered valid.

58. (b) The reliability of classifying female sterility mutants on the basis of growth characteristics

The reliability with which classification for female sterility could be made on the basis of growth characteristics of mutants (described in section 51) was noted for mutants in all groups except Group 5-5. The crosses used segregated for single mutants or, in the case of one or two strains from Group 2-2, for a pair of closely linked mutants. It is thought that the individual mutants from that group have been included since strains from all spore pairs of ascus 44 from OR A x 29-4 were

used. The ascus has the pattern 1+:3 fs (Table 3) and one female sterile spore pair should have both mutants whereas the other two should have the different individual mutants.

Strains in all groups were classified with 99-100% accuracy (Table 17), supporting the confidence of tests in crosses and demonstrating that for various purposes this method alone should be sufficiently reliable.

Because of the conspicuous growth habit of the sl mutant in Group 5-5, it is doubtful that classification of the mutant on this basis is less reliable.

59. (c) Tests for female sterility on different media and using different time intervals between inoculation of the male and female parents

As an added check to ensure that female sterility could not be attributable to testing problems, strains from all groups were tested on different culture media and using different intervals between inoculation of the female and male parents.

Selected strains were tested in triplicate on glucose and malt peptone media as well as on sucrose with the usual one week interval between inoculations of the female and male parents. In addition the strains were tested on sucrose medium using a two week interval between inoculations. All cultures were observed for five weeks following inoculation of the female parent.

TABLE 17

Accuracy with which different female sterile mutants found can be classified for female sterility on the basis of growth habit

Group	Cross	Correctly classified strains	Unclassifiable or wrongly classified strains	Total	% accuracy	Random spore or ascus data
19-2	1681 x 19-2	190	0	190		
	1635 x 19-2	199	0	199		
		389	0	389	100	r.s.
52-2	ORA x 84-2	217	2	219	99	ascus
2326	1681 x 2326	24	0	24		
	1635 x 2326	30	0	30		
		54	0	54	100	r.s.
2351	1681 x 2351	198	0	198	100	r.s.
2-2	1681 x 44-1	193	0	193		
	1635 x 44-1	168	0	168		
		361	0	361	100	r.s.
	1681 x 44-3	39	0	39		
	1635 x 44-3	42	0	42		
		81	0	81	100	r.s.
	1681 x 44-4	200	0	200		
	1635 x 44-4	176	0	176		
		376	0	376	100	r.s.

Group	Cross	Correctly classified strains	Unclassifiable or wrongly classified strains	Total	% accuracy	Random spore or ascus data
2-2	1680 x 44-8	181	2	183		
	1633 x 44-8	196	1	197		
		377	3	380	99	r.s.
10-4	1681 x 10-4	57	1	58		
	1635 x 10-4	59	0	59		
		118	1	119	99	r.s.

In all cases except for Group 5-5, strains of m.t. A were used and were fertilized with the strain 1-3. For strains from Group 5-5 (s1), where mating types were not determined, tests using male parents 18-1 and 1-3 (in separate inocula and in one; see section 11) were carried out.

For all groups except Group 5-5, the nic-3 and pan-2 derivatives (Table 10) were used in these tests. For Group 5-5, four strains were selected from the cross 1680 x 5-5. The strains are listed below:

Group	Deviation	Strains used
19-2	19-2	2496, 2503
52-2	52-2	2243
2326	2326	2414, 2430
2351	2351	2507
2-2	2-2	2477, 2481
	44-1	2398, 2417
	44-3	2510, 2514
	44-4	2404, 2421
	44-8	2409, 2424
10-4	10-4	2486, 2490
5-5	5-5	four strains from 1680 x 5-5

All cultures tested as female sterile regardless of the culture medium or the interval between inoculations.

This is in contrast with the OR w.t.s. which have been successfully used on all media and following these and other periods of growth prior to inoculation with male parents.

60. A summary of considerations supporting the validity of procedures used in testing for female sterility

The validity of testing procedures is supported by the following considerations:

(a) All genetic variants for female sterility can be attributed to discrete mutations. Even leaky mutants do not resemble w.t.s.

(b) Both testing procedures described in section 11 provide the same result.

(c) The mutants can be scored on the basis of their superficial growth characteristics in strong agreement with tests in attempted crosses.

(d) Variation in culture media and the time interval between inoculation of female and male parents is possible without altering the interpretation of results.

DISCUSSION

61. Expected vs. actual results

From Threlkeld's finding of sterile variants from crosses between strains having different genetic backgrounds, it was expected that this may be attributable to incompatibility of a general nature rather than female sterility e.g. a heterogenic incompatibility as found in Podospora anserina where crosses between different geographical races have reduced fertility or are completely sterile (see Esser, 1965). Also the female sterility initially found in this study was expected to be attributable to a genetically complex situation. This was assuming a homokaryotic condition of the parental strains (both of which functioned as female parents) following the failure of initial attempts to obtain female sterile conidial isolates from them (see section 35 for Set 1 of strain 351; also a similar number of conidial isolates from strain 442 all produced perithecia and spores in one test for female sterility, suggesting that they were all fs^+, but this was not reproducible and the conclusion could not be considered reliable). These factors prompted the concern for using a St. Lawrence background as a reference

(section 21) and tempted the postulation of suppressed genes at various times during the study.

There is no longer a reason to suggest such incompatibility factors or suppressed genes among the crosses described, although there is also no reason to rule out the possibility of their existence in Neurospora. All of the female sterile mutants found in this study can be attributed to spontaneous mutation in the strains used. Threlkeld's original observations possibly involved a few of the same mutants found in this study.

62. Variation in nuclear ratios in heterokaryons

From conidial isolates and some of the crosses to OR w.t.s., it appears that at least some mutants for female sterility can occur in heterokaryons in different nuclear ratios. This includes mutants in Groups 2351, 2-2, and possibly 2326; data for others are inconclusive.

The two sets of conidial isolates from strain 351, in which 0 of 79 (0%) and 50 of 147 (34%) conidial isolates respectively were female sterile, indicate differences in nuclear ratios in the two subcultures of the strain heterokaryotic for the mutation Group 2351. The mutants in Group 2-2 and occurring in OR A range from being undetected (e.g. cross OR A x 84-2 which had no complex asci) to occurring in a large proportion of nuclei (cross OR A x 19-8 in which 63 of 165 ad^+ spore pairs among complete

asci are female sterile, this being 63 of 114 alleles (55%) inherited from the OR parent). That in Group 2326 ranges from being undetected (e.g. cross OR a x 84-8 which has no complex asci) to occurring among more than 80% of the progeny from the crosses OR A x 6-4 and OR a x 29-6 (more than 16% of the alleles inherited from the OR parent; see Table 6).

From data available thus far it is not possible to attribute these differences to any selective advantage of the mutant (or wild type) alleles. It is just as likely that by using small inocula, different subcultures of strains by chance received varying proportions of the nuclear types. However, in retrospect, this explains some difficulties encountered in the interpretation of results in the three generations of crosses to OR w.t.s.

63. Dependence of perithecial and spore production on the female parent

Of the 1800 crosses between strains of various genetic backgrounds from which the dependence of perithecial and spore production on the female parent was observed (section 22), 250 were repeated once and another 200 were repeated twice. (Since the crosses constituted a screening procedure, there was no reason to repeat all of them.) Of the different crosses, 450 involved ad-4 strains as female parents and hence are of questionable reliability (see

section 19). However even their perithecial and spore production tended to be of the same order regardless of the male parents or the ability of the male parents to function as females in the reciprocal crosses. Repeated tests provided similar patterns. Hence, until more quantitative data are available, these generalizations are considered reasonably reliable and prompt the following considerations.

Since a strain of either mating type can produce protoperithecia in the absence of one of the opposite mating type, only the genome from the female parent seems to act in protoperithecial production. The above data suggest, furthermore, that the potential of the protoperithecia to develop into perithecia is also largely dependent on that parent.

Presumably the male parent is restrictive in perithecial production in the sense that with limiting numbers of conidia, only a certain number of fusions with trichogynes can occur, and this is probably not only dependent on the absolute numbers of the conidia but on the ability of a conidium of a particular genotype to participate in such a fusion. However, with conidia being produced in abundance, as is usually the case in N. crassa, this may not be a significant limiting factor. Even if a particular type of conidium has a low probability of fusing with a

trichogyne, its large numbers may ensure fertilization to the greatest extent.

The dependence of spore production on the female parent may only be reflective of the expectation that the number of spores produced is dependent on the number of perithecia produced. This itself does not seem to conflict with previous findings (see section 5) that both parental genomes interact during much of the development within the perithecium.

These observations, in the context of previous findings (section 5) suggest the following pattern for the genetic control of the sexual development in N. crassa. Mating type determines whether or not a cross can occur. Given that it can, the female parent determines the degree of protoperithecial formation and is the major factor in determining perithecial production. This in turn determines, to a great extent, the potential of spore production. On the other hand, the genome from the male alone may influence fertilization, but the usual abundance of male cells provides little restriction on perithecial and spore production. Both parental genomes interact in the development of the ascogenous system in the formation of asci and up until the time that spores are delimited and their final shape is determined. Following this the maturation of spores in largely dependent on the genotypes

of the individual spores.

64. The genetic complexity of the sexual development

Although spontaneous mutants are expected to occur in Neurospora strains, and are often detected (see Lindegren and Lindegren, 1941), it was surprising to find that 4 of the 10 strains obtained (8 used in the screening for genetic variants, section 22, and the OR w.t.s; others cannot be evaluated) have accumulated mutants for female sterility. The mutation that seems to have occurred in the recent single spore isolate 19-4 was particularly unexpected.

Thus far the only studies methodical enough to reflect the genetic complexity of any phase of the sexual development in Neurospora are those of Srb and his associates (see section 5) in which 13 mutants for ascus development were found despite the difficulty in screening for such mutants. Their findings suggest that that phase of the sexual development is under a relatively elaborate genetic control.

In this context, the abundance in which mutants for female sterility were found seems to suggest an elaborate genetic control for protoperithecial development as well (even though thus far the blocks have not been observed), and this can possibly be extrapolated to infer the genetic complexity of the sexual development in general.

65. Relationship between growth habit and female sterility

The finding that all female sterile mutants differ from the wild type in their growth habit is probably indicative of a more general property of mutants affecting protoperithecial production, and this may even be a common feature of those affecting the sexual development. Several previously described female sterile mutants in Neurospora have been noted to differ from the wild type in their growth characteristics (Mitchell et al., 1953; Srb, 1957; Horowitz et al., 1960), and from the subtlety of some differences observed in this study, there is reason to suspect that others may have similar effects but that these have not been noted.

Of other mutants concerning the sexual development, all of those found by Srb and his associates to affect ascus morphology (section 5) also have abnormal growth characteristics, but this may not be a representative group since most of them were screened on the basis of growth characteristics (see especially Srb and Basl, 1969). However, mutants concerned with the sexual development in other ascomycetes, in particular Sordaria (see Esser and Kuenen, 1967) have also expressed differences in mycelial growth, and hence support the likelihood that the relationship is a common one. This suggests that a promising line of work leading towards the understanding of underlying

processes concerned with the sexual development may be that concerning hyphal morphology (Burnett, 1968).

The relationship between female sterility and growth habit (hyphal morphology?) suggests that these mutants may have an altered cell wall composition (de Terra and Tatum, 1963; Bartnicki-Garcia, 1968), although this need not be the primary effect of the gene. Barber et al. (1969) found the mutant pk-2, which affects ascus morphology and also has a non-wild type growth, to differ from the wild type in a number of bands in protein patterns on electropherograms (although the use of highly isogenic strains was not made), suggesting that various cellular processes may be affected by that mutant. The female sterile mutants may have several effects as well.

A possible alteration in cell wall composition prompts the consideration that at least some of the mutants may have developmental blocks at the stage of fertilization, i.e. the fusion of conidia with trichogynes. If this is the case, then it is also necessary to postulate differences in the cell wall composition of conidia and trichogynes. Otherwise the same mutants should also fail to act as male parents, but this is not the case. If the male sterile mutants of Vigfusson (1969) have blocks at fertilization, then they may reflect such structural differences.

66. Genetics and complementation of the two mutants in Group 2-2

The need to postulate two mutant sites in Group 2-2, originating in the heterokaryotic strain OR A, is not accompanied by a simple or attractive model fitting the genetic and complementation data concerning that group. Until the mutants are more carefully mapped and complementation between them is attempted over a wide range of nuclear ratios in heterokaryons, several possibilities have to be considered in explaining the two closely linked non-complementing mutants.

If the distance between the mutant sites is better represented by the lower limit of 0.5 m.u. at the 95% confidence level than by the 3.0 m.u. determined on the basis of data available thus far (section 48), or if the actual distance falls below this limit, then the mutant sites can be postulated to occur in the same cistron or in different cistrons of a gene cluster, possibly similar to the aromatic (arom) cluster (Giles et al., 1967). If not, then the results may be tentatively explained on the basis of a duplicated chromosome segment or on the basis of mutations for two completely different genes.

If the mutant sites, say fs_1 and fs_2, are in different cistrons of one gene cluster, then it can be postulated that both lie in one unit of transcription, and at least one, say fs_1, is a nonsense or frame shift

mutation. If transcription proceeds in the direction from $\underline{fs}_1$ to $\underline{fs}_2$, then the genotypes $\underline{fs}_1$ + and $\underline{fs}_1$ $\underline{fs}_2$ lack both functional polypeptides and + $\underline{fs}_2$ lacks the one for the $\underline{fs}_2$ cistron. Hence with all three female sterile types failing to produce the functional polypeptide for the $\underline{fs}_2$ cistron, complementation cannot occur among the different female sterile strains occurring in asci with patterns of 1+:3 fs (genotypes + +, + $\underline{fs}_2$, $\underline{fs}_1$ +, $\underline{fs}_1$ $\underline{fs}_2$) derived from the cross OR A (+ +) x 29-4 ($\underline{fs}_1$ $\underline{fs}_2$, see section 41).

A duplication of the right tip of linkage group I can be suggested in explaining the results. The likelihood that the mutant sites are on the right arm of that linkage group is inferred from the variability of linkage to the mating type locus on the left arm of the linkage group (suggesting a loose linkage, section 47) but a highly significant linkage to al-1 in crosses to the alcoy linkage testers (section 46, Table 11) which marks the breakpoint of the translocation of the right arm of the linkage group (Perkins et al., 1969). In addition Newmeyer and Taylor (1967) have suggested that a duplication for the right tip may normally exist in wild type strains since part of it is dispensable.

In explaining the genetic and complementation data on this basis, a duplication for the right tip of

linkage group I in a tandem arrangement seems necessary. This sequence and one possible series of events leading to the occurrence of asci with patterns of 1+:3 fs from the cross OR A x 29-4 are diagrammed in Fig. 5.

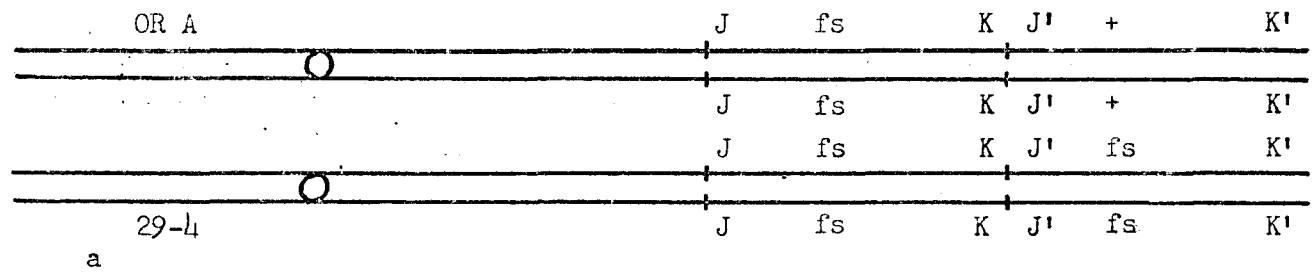
If both chromatids having the w.t. allele occurred in different meiotic products, patterns of 1+:3 fs could not occur. Hence the production of these patterns requires either the loss of one w.t. allele or the transmission of both to one meiotic product. Fig. 5 shows how a loss of one wild type allele could occur. The OR A component of the cross is postulated to be heterozygous for the female sterile mutant. (This is only necessary for the formation of zygotes in the production of these patterns. Other zygotes may involve other types of components from the heterokaryotic OR parent.) Strain 29-4, on the other hand, must only have the mutant form of the allele.

The transmission of both wild type alleles to one meiotic product could result from the pairing between the regions JK and J'K' from the different chromatids of the OR parent in Fig. 5a followed by a crossover distal to fs of JK and + of J'K' (not illustrated). This would result in genotypes of the chromatids from the OR parent being fs and + fs fs, whereas those from strain 29-4 would both be fs fs, and the resulting ascus pattern would be 1+:3 fs.

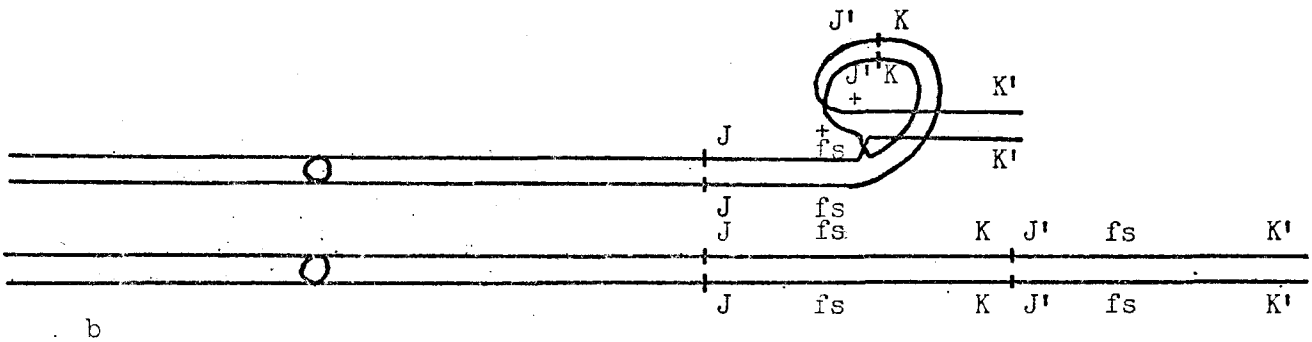
Fig. 5. Chromosome sequences in strains OR A and 29-4 and possible events leading to the production of asci with patterns of 1+:3 fs from the cross between these strains.

(a) linkage group sequences of strains OR A and 29-4, (b) pairing of the duplicated regions of one chromatid and crossing over involving one strand of the chromatid, (c) chromosome sequence following crossing over; note an acentric ring, (d) ascus pattern resulting from the preceding events.

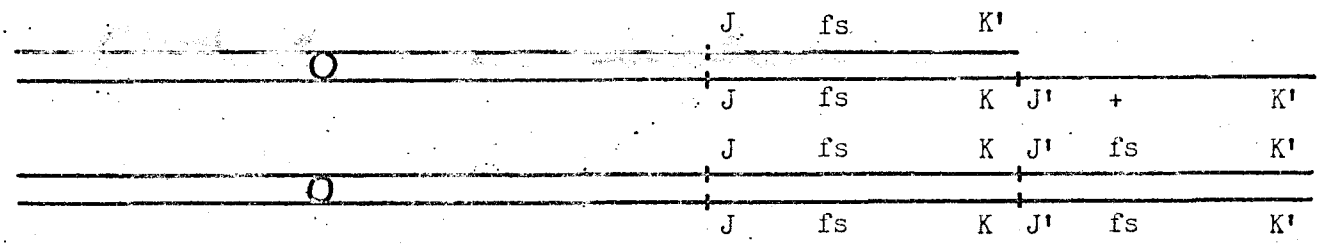
Figure 5



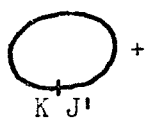
a



b



c



lost

d

Genotype	Phenotype
fs	fs
fs	fs
fs +	+
fs +	+
fs fs	fs
fs fs	fs
fs fs	fs
fs fs	fs

The possibility of a duplication of this type is unattractive for the following reasons: Mitotic crossing over in Neurospora appears to be a relatively common phenomenon (see especially Threlkeld and Stoltz, 1970). If this is the case, the above type of duplication would likely be eliminated in somatic nuclei by the pairing of duplicated segments followed by crossovers similar to that shown in Fig. 5. (Note that the chromosome from strain 29-4 did not have to participate in crossing over to result in the loss of the segment.)

As an alternative explanation, mutants for two distinct genes which do not complement can be postulated. Mutants for distinctly different genes (unlinked) which do not complement in heterokaryons have been found in Aspergillus (Apirion, 1966). The failure to complement may mean that the different genes are responsible for the production of different polypeptides of a protein, and that in a heterokaryon the protein is not produced in sufficient quantities to alleviate the defect. This seems unattractive, however, because even though perithecial and spore production varied somewhat in the complementation tests, no deliberate attempt was made to control nuclear ratios in the heterokaryons, yet only one set of three tests, for the heterokaryon (2414 + 2424) and one of three cultures, for the heterokaryon (2477 + 2430), failed to

produce perithecia and spores (see Fig. 3). This is in contrast with a failure of perithecia and spores to form in any heterokaryon involving any pair of strains in Group 2-2 despite the fact that some sets of three tests were repeated (see Fig. 3). Perhaps a more favourable explanation is one similar to that suggested by Apirion (1966) in explaining why the different mutants in Aspergillus fail to complement in heterokaryons but complement in diploids: the products of both genes may be assembled into one entity in the nucleus prior to its release into the cytoplasm. In this way only defective forms could be produced by both nuclear types and hence complementation could not occur.

67. The detection of developmental blocks following fertilization

A recovery of the nic x pan component in the first but not in the second test described in section 54, and applied in section 55 to the mutant in Group 52-2, would have suggested a developmental block following fertilization as well as before. However the recovery of the component from both crosses poses another question: does a block following fertilization exist but is alleviated in some way?

In a cross involving an uncommon self-sterile "bisexual" strain, i.e. one which can participate in

crosses to both mating types but cannot produce perithecia and spores by itself, Lindegren (1936) found that a cross attempted between this (f A/f a) and another strain (F A) resulted not only in zygotes of the type F A x f a but also those of the type f A x f a. He suggested that a hormone was produced by the strain F A permitting the cross f A x f a to occur. Whether or not a hormone was produced (sex hormones have not been irrefutably demonstrated to exist in Neurospora; see Raper, 1960; Turian, 1966), this data suggests that a gene product of some sort was produced, possibly by the cross F A x f a, permitting the other cross to occur. This possibility is supported by data from crosses between mutants for the sexual development in the homothallic Sordaria macrospora. When crosses between sterile strains were attempted, occasional selfings were found in addition to the crosses, i.e. some perithecia produced spores of only one parental type or the other as the homokaryotic wild type strains normally do. Gene products permitting selfing of otherwise self-sterile strains could not be detected in culture filtrates, suggesting that an intracellular transport had occurred.

In the event that such transport occurs between developing perithecia in the above test, it may be necessary to modify it by using individual conidia as male parents

to fertilize the heterokaryotic females so that only one perithecium can develop in each culture. Stronger evidence reflecting that a developmental block following fertilization does not occur would require the finding of the nic x pan component of the second cross in such a perithecium in the absence of a pan x pan component.

By using both the modified and unmodified tests, and by determining whether the nic x pan component can occur alone in a perithecium or if it has to be accompanied by the pan x pan one, some inferences about the extent to which gene products are transported may be possible.

Regardless of restrictions found from future experience in applying this test, the possibility of making allelic crosses for at least some female sterile mutants has been demonstrated and can be used as a tool in handling these strains.

68. The potential for studying the sexual development in Neurospora

The genetic complexity of the sexual development in Neurospora inferred from this and other studies offers attractive possibilities for a genetic approach to studying this system. It suggests a potential for precise experimental control of the different steps once a full spectrum of mutants is available and once sophisticated enough experimental procedures are devised for manipulating

the mutants to provide information about the underlying processes.

Despite these features, N. crassa in its present form is not an ideal organism for this order since no simple screening procedures for isolating such a spectrum of mutants has been proposed. Even the best conceivable methods using the organism may have limitations since some of the stages are controlled by genomes from both parents and hence only dominant mutants may be easily detected.

The use of Sordaria macrospora has proven most effective for the isolation of developmental mutants in that organism (see Esser and Kuenen, 1967). This is because the species is homothallic and any homokaryotic culture which does not produce and eject mature spores is observed as a mutant. However the use of that species has its limitations. The homothallic condition, for instance, causes difficulties in controlling crosses.

For these purposes the development of a homothallic Neurospora strain with essentially an N. crassa genome seems particularly attractive. Such a strain would permit the easy detection of mutants and also certain types of studies where a homothallic condition is necessary or convenient. Furthermore, once the mutants are found they could be transferred into a heterothallic strain for other studies.

With the availability of at least five different homothallic species of Neurospora (Gochenaur and Backus, 1962; Nelson et al., 1964; Mahoney et al., 1969; Frederick et al., 1969), it may be possible, through a series of interspecific crosses, to develop such a strain. Any information deduced using such a system could be evaluated in the large perspective of knowledge available on N. crassa.

SUMMARY

(a) From qualitative observations, perithecial and spore production in N. crassa seem to depend largely on the female parent used in a cross. It is interpreted that perithecial production is highly dependent on the genome from the female parent, whereas the apparent dependence on this parent of spore production may only reflect that the number of perithecia produced restricts the number of spores produced.

(b) Eight spontaneously occurring mutants for female sterility were found. Two of them are closely linked and thus far have not complemented with one another despite being able to complement with other mutants. Alternative explanations are that the mutant sites may occur in one cistron, two different cistrons in a gene cluster, or in entirely distinct genes.

(c) The apparent abundance of mutants for female sterility suggests that protoperithecial development is under an elaborate genetic control, and this is likely a characteristic of the sexual development in general in Neurospora and in other ascomycetes.

(d) All female sterile mutants differ from the wild type in their superficial aerial growth characteristics.

This may reflect a common property of mutants concerning the sexual development in general, and suggests that a promising line of investigation leading to the understanding of processes involved in the sexual development may be the study of processes concerned with corresponding changes in hyphal morphology.

(e) A test for detecting blocks in the sexual development following fertilization has been proposed. This is in the event that the mutants for female sterility, believed to affect protoperithecial development, also affect later stages of the development. With appropriate modifications the test may be useful in inferring transport of gene products within and between developing perithecia. For at least some mutants, the procedure should be useful as a tool in making allelic crosses involving some mutants for female sterility.

(f) The genetic complexity of the sexual development in Neurospora inferred from this and other studies suggests that a genetic approach may be particularly useful in studying this system despite some of the disadvantages in using Neurospora as a representative ascomycete.

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