A NEUROSPORA MUTATION THAT ARRESTS PERITHECIAL DEVELOPMENT AS EITHER MALE OR FEMALE PARENT

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ABSTRACT

A mutant of *Neurospora crassa* fails to produce perithecia when crossed as either the male (fertilizing) parent or the female (protoperithecial) parent. This mutant is unique in that it appears to be due to a single mutation that blocks sexual development when crossed as either parent. As either a male or female parent, the mutant, *fmf-1*, produces perithecia blocked at a diameter of 120 microns and containing no meiotic figures; normal perithecia are over 400 microns in diameter. The mutant maps to linkage group IL near *arg-1*. Forced heterokaryons have been made between *fmf-1* and *fmf-1*+ nuclei. These heterokaryons are fertile when crossed, and *fmf-1* can participate as either the male or female component; the mutation is thus heterokaryon recessive and nuclear nonautonomous. Homokaryotic *fmf-1* conidia were purified from a mixed conidial population derived from such a heterokaryon; these conidia failed to function as the male parent, suggesting that the *fmf-1*+ gene product is not contained in the conidium. In mixed mating-type heterokaryons, formed using *tol*, *fmf-1* participates in ascospore formation and triggers perithecial development. Moreover, *tol* suppresses the action of *fmf-1* if present in both components of a cross.———These data suggest that (1) *fmf-1* acts in the perithecium at some time between fusion of the conidium with the trichogyne and the onset of meiosis; (2) the *fmf-1* gene product is not contained in conidia; and (3) both mating types may enter the protoperithecium when a mixed mating-type heterokaryon is used as the male parent.

THIS paper deals with a mutant of *Neurospora crassa*, *fmf-1*, that has nearly normal vegetative growth, but is unable to complete successfully the sexual cycle as either the male or female parent. In wild-type crosses, the female (protoperithecial) parent initiates the cycle by producing small (40 micron) protoperithecia as a response to environmental conditions. These bodies remain undeveloped until triggered by cells of the opposite mating type (male parent). Within 12 hours after contact with the male parent, the protoperithecia can be observed to develop into perithecia by enlarging, developing a dark layer of melanin and undergoing a number of other morphological changes leading to meiosis and eventually culminating in the production of several hundred ripe

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ascospores per perithecium (see JOHNSON 1978 for a more detailed description of some of these events).

A number of mutants blocking the normal developmental process have been isolated (for review, see MYLYK and THRELKELD 1974; JOHNSON 1978). Most of these mutants occurred spontaneously and blocked development only as the female parent. Both WEIJER and VIGFUSSON (1972) and RAJU and PERKINS (1978) have reported on strains that were both male and female sterile. WEIJER and VIGUSSON (1972) screened for male-sterile mutants after UV mutagenesis and discovered that most of the male-sterile mutants recovered were also female sterile; in the two mutants tested, independent segregation of the two phenotypes indicated that each resulted from a separate mutational event. RAJU and PERKINS (1978) reported on several types of genetic events that give rise to the "barren" phenotype; the largest class are chromosomal aberrations that block further perithecial development, apparently by causing meiotic or premeiotic abnormalities. These aberrent strains act as zygotic dominants in that they are effective when crossed as either the male or female parent.

I will present data that suggest that *fmf-1*$^+$ (female and male fertile) is a single locus necessary for successful crossing, both as the male and as the female component. The dominance relationships of *fmf-1* are examined both in the diploid zygotic state and in a heterokaryon. Mixed mating-type heterokaryons were formed containing *fmf-1* and *fmf-1*$^+$ nuclei; these heterokaryons were used to cross *fmf-1* and to examine the time of action of the *fmf-1*$^+$ gene product in development. Finally, it will be shown that *tol* (NEWMEYER 1970) suppresses *fmf-1* in some as yet unknown manner. Some of these data have appeared in preliminary form (JOHNSON 1975, 1976b, 1978).

MATERIALS AND METHODS

Strains and media: Auxotrophic mutations used in these analyses include: *ad-2 (STL2)*, *pyr-3 (KS43)*, *trp-4 (Y2198)*, *pan-1 (5531)*, *leu-3 (R156)*, *leu-3 (47313)*, and *arg-1 (B369)*. Other mutations include *tol (N83)* (NEWMEYER 1970), which suppresses the vegetative incompatibility associated with different mating types; *per-1 (AB11)* and *per-1 (PBJ1)* (HOWE and JOHNSON 1976), which produce light perithecia and white ascospores instead of the normal black structures; *cr-1 (B122)*, a morphological colonial; and *al-2(15300)*, an albino mutant. Several strains containing multiple mutations were obtained from the Fungal Genetics Stock Center; other strains were constructed as required (Table 1).

Stocks were maintained on Vogel's minimal medium (VOGEL 1964), supplemented as needed (Min or Min + supplement). Minimal medium containing sorbose (MinS) to induce colonial growth was used as described previously (JOHNSON 1976a). All crosses were performed on crossing medium (WESTERGAARD and MITCHELL 1947) containing 2% sucrose and supplements for auxotrophs as needed (Wx or Wx + supplements).

Tests for colonial growth and auxotrophic requirements were performed as previously described (JOHNSON 1978).

Filtration of conidial suspensions: Conidia obtained from two heterokaryotic strains (Table 1, strains 19, 22) were grown in liquid Min + adenine (ad). At various times after inoculation the cultures were filtered through two layers of sterile cheesecloth. The filtrate was diluted and one aliquot was added to plates of *per-1 (PBJ1) col-4 a* (1 ml of conidial suspension containing 10^3 conidia per ml) and another plated on MinS, MinS + ad and MinS + uridine, as previously described. From the crossed plates, the number of perithecia that eject black spores

TABLE 1

Strains used in these experiments

Strain number	Genotype	FGSC number	Strain number	Genotype	FGSC number
1	*pyr-3 (KS43) A*	——	15	*tol pan-1 fmf-1 a*	3110
2	*ad-2 (STL2) A*	——	16	*tol trp-4 fmf-1 A*	——
3	*per-1 (PBJ-1) pyr-3 A*	2548	17	*tol trp-4 fmf-1 a*	——
4	*per-1 (ABI-1) ad-2 A*	2547	18	*tol pan-1 fmf-1 A* and	
5	*per-1 (PBJ-1) col-4 a*	2549		*tol trp-4 fmf-1+ a*	——
6	*al-2 ad-2 a*	——	19	*per-1 (ABI-1) ad-2 A* and	
7	*leu-3 cr-1 a*	247		*fmf-1 pyr-3 A*	——
8	*leu-3 arg-1 a*	1216	20	*per-1 (PBJ-1) pyr-3 A* and	
9	*pyr-3 fmf-1 (PBJ6) A*	3108		*fmf-1 ad-2 A*	——
10	*tol pan-1 A*	——	21	*tol pan-1 fmf-1 A* and	
11	*tol pan-1 a*	1949		*tol trp-4 fmf-1 a*	——
12	*tol trp-4 A*	2336	22	*per-1 (ABI-1) ad-2 A* and	
13	*tol trp-4 a*	2337		*pyr-3 A*	——
14	*tol pan-1 fmf-1 A*	3109			

was determined; these black spores are *per-1*+ and therefore must have resulted from crosses of the *per-1*+ male nucleus and the *per-1* female nucleus. The total fraction of conidia that were *ad-2* or *pyr-3* homokaryons or heterokaryons was determined from the MinS plates, as described by JOHNSON (1976a).

Crossing, fertility determinations, formation of heterokaryons, tests for auxotrophy and mating type: These techniques have been described previously (JOHNSON 1976a, 1978). Data on diameters of perithecia are presented as mean perithecial diameter ± standard deviation. For convenience, the term "perithecium" will be used to describe fruiting bodies larger than protoperithecia (*i.e.*, 50 μ and larger) found on a strain that has been crossed whether or not they are fecund.

RESULTS

Block point of fmf-1: One of the mutants isolated in an earlier screen for female-sterile mutants turned out also to be male sterile (JOHNSON 1976b, 1978). As will be shown, the mutant is recessive in heterokaryons and can be analyzed and manipulated genetically by this means. This mutant (lab isolate and allele designation, PBJ6) has been termed female and male fertility number one, *fmf-1*, in accordance with nomenclature suggested by Ho (1972). The diameter of perithecia in crosses of *fmf-1* as either the male or female parent averages 120–130 microns (Figure 1a, b and Table 2, crosses 2 and 3) as compared with a 300-micron average diameter in wild-type crosses (Figure 1c and Table 2, cross 1). The mean diameter of *fmf-1* as the female in seven repeats was 120μ ± 10μ; as the male in three repeats it was 125μ ± 7μ. It is likely, therefore, that the *fmf-1*+ gene product is necessary at the same time and functions in a similar role as either the male or female.

Wild-type perithecia attain a diameter of 120μ within 15 hours after crossing (JOHNSON 1975). The mutant perithecia attain their full diameter by 26 hours after crossing (Figure 2) and show no further increase.

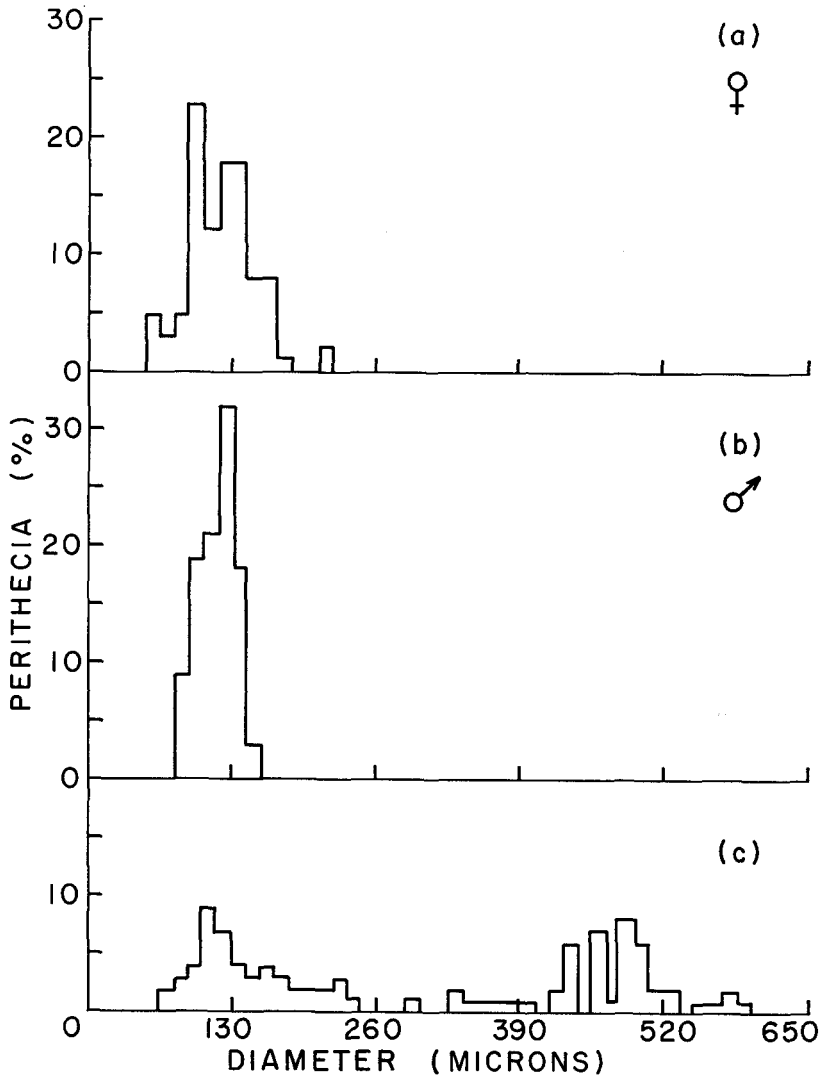

FIGURE 1.—Histograms show the diameter of perithecia at two weeks after crossing; (a) *fmf-1 pyr-3 A* × *col-4 a*; (b) *col-4 a* × *fmf-1 pyr-3 A*; (c) *col-4 a* × *pyr-3 A*.

Two other developmental events normally parallel increase in perithecial diameter. Melanin accumulates within the walls of the maturing perithecium and a number of hyphae are formed by the outer perithecial wall (JOHNSON 1978). The levels of pigmentation and the number of hyphae found in perithecia of *fmf-1* as either the male or female are qualitatively similar to wild-type perithecia of similar diameters. No internal cavity is formed within these perithecia and no ascogenous hyphae form: again wild-type perithecia of similar sizes are similar in their degree of development.

Mode of action of the fmf-1$^+$ *gene product; action in heterokaryons-single*

TABLE 2

Results of crosses of fmf-1*

Cross number	Strains: Female	Strains: Male	Average perithecial diameter ± S.D. (μ)	Ascospores produced
1	*fmf-1*+ *A*	*fmf-1*+ *a*	291 ± 106	+
2	*fmf-1 A*	*fmf-1*+ *a*	127 ± 31	—
3	*fmf-1*+ *A*	*fmf-1 a*	130 ± 16	—
4	*fmf-1 per-1*+ *A* + *fmf-1*+ *per-1 A*	*fmf-1*+ *per-1 a*	285 ± 139	+ (*fmf-1*)
5	*fmf-1*+ *per-1 a*	*fmf-1 per-1*+ *A* + *fmf-1*+ *per-1 A*	313 ± 67	+ (*fmf-1*)
6	*fmf-1 tol A* + *fmf-1*+ *tol a*		212 ± 47	+ (*fmf-1*)
7	*fmf-1 tol A* + *fmf-1 tol a*		117 ± 37	—
8	*fmf-1*+ *A*	*fmf-1 tol a* + *fmf-1*+ *tol A*	321 ± 106	+ (*fmf-1*)
9	*fmf-1 tol A*	*fmf-1*+ *tol a*	†	+ (*fmf-1*)
10	*fmf-1*+ *tol A*	*fmf-1 tol a*	†	+ (*fmf-1*)
11	*fmf-1 A*	*fmf-1 a*	small‡	—
12	*fmf-1 tol A*	*fmf-1*+ *a*	small‡	—
13	*fmf-1*+ *tol A*	*fmf-1 A*	small‡	—

* Auxotrophic markers are not listed. Additional tests were performed, in all cases, by reversing mating types to the opposite of those given. Representative data from individual experiments are presented.

† Only a few wild-type size perithecia were produced.

‡ Not measured, but visually similar to the small perithecia usually produced by *fmf-1*.

mating type: Heterokaryons were forced using auxotrophic forcing markers (JOHNSON 1976a) and contained one nucleus that was *fmf-1 per-1*+ and another that was *fmf-1*+ *per-1* (Table 1: strains 19 and 20). In crosses to *per-1* strains, the presence of *per-1*+ (and therefore the participation of *fmf-1* in the cross) is signalled by the production of black ascospores (JOHNSON 1976a, 1977, 1978). These heterokaryons are fertile as both the male and the female parent (Table 2: crosses 4 and 5), and in both cases black spores were produced. Testing the genotype of these black spores confirmed the fact that the *fmf-1* nucleus had participated in the cross.

Action in heterokaryons; mixed mating type: Heterokaryons can be formed in which the components are of opposite mating types if both components carry *tol* (NEWMEYER 1970), which suppresses the normal vegetative heterokaryon incompatibility usually associated with opposite mating types. Strains were constructed using *pan-1* and *trp-4* as forcing markers. Since *trp-4* is tightly linked to *tol*, appropriate stocks were first constructed carrying *trp-4* (Table 1: strains 16 and 17). These were used to test strains known to be *pan-1* and of opposite mating type and that were both *fmf-1* and *fmf-1*+ (Table 1: strains 10, 11, 14 and 15) for the presence of *tol* by the ability of the two strains jointly to fuse and grow on MinS. Mixed mating-type heterokaryons were isolated from these plates (*e.g.*, Table 1: strain 18) and crossed as the male parent to appropriate females. In all cases, the cross was fertile and *fmf-1*-bearing spores were found in all perithecia examined (Table 2: cross 8).

Suppression of fmf-1 *by* tol: Mixed mating-type heterokaryons in which one

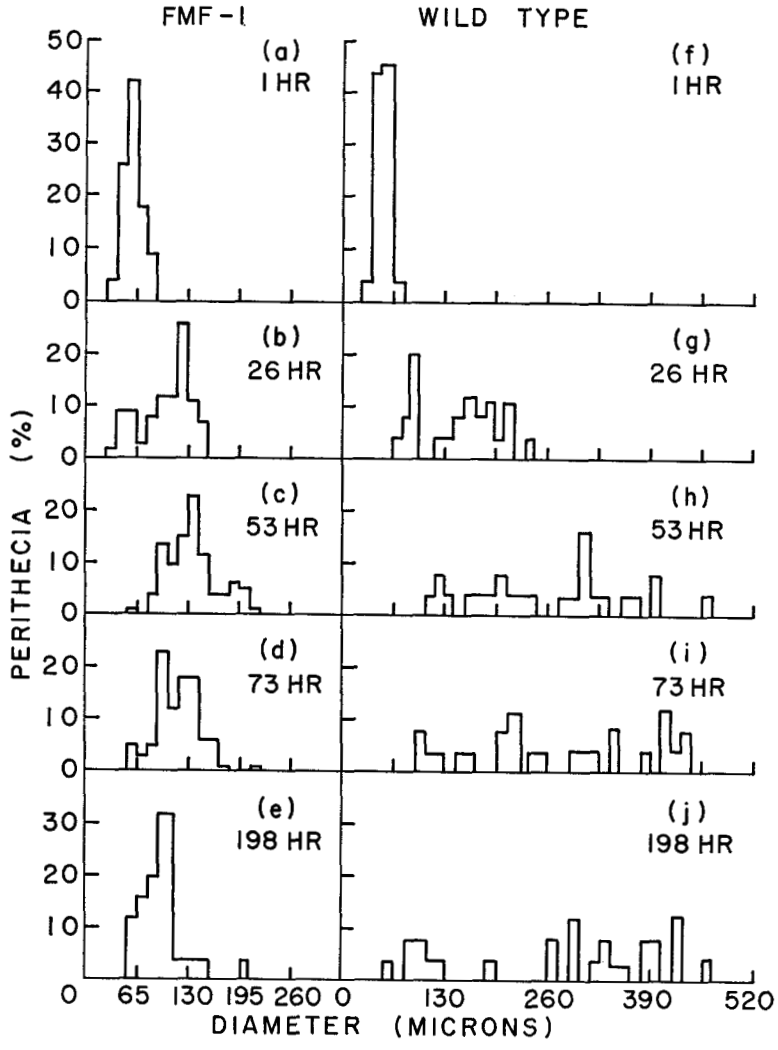

FIGURE 2.—Histograms show the diameter of perithecia produced by crosses of *fmf-1 pyr-3 A* × *per-1 col-4 a* at five times after crossing (a–e) or *fmf-1*+ *pyr-3 A* × *per-1 col-4* at five times after crossing (f–j).

component is *fmf-1* and one *fmf-1*+ are self fertile when used as the female (Table 2: cross 6). Moreover, if either component of such a heterokaryon is used as the female component in a cross to the other component (Table 2: crosses 9 and 10), the cross is fertile. Only a few perithecia are produced in these crosses; the rest remain small and lightly pigmented as is typical of *fmf-1*. Heterokaryons in which both components are *fmf-1* are sterile and perithecial development is blocked at 117μ (Table 2: cross 7).

In all of the heterokaryon experiments tried above, forcing markers and mating

types were interchanged in all appropriate combinations with *fmf-1*, with no effect on the results of the experiments.

Absence of fmf-1$^+$ *gene product in conidia*: The conidia of *N. crassa* may contain one or more nuclei. If these conidia are derived from heterokaryotic mycelia, an individual conidium can contain both nuclear types. I have demonstrated that *fmf-1* is recessive in heterokaryons as the male. It is possible then that the *fmf-1*$^+$ gene product is cytoplasmic and might function in conidia that are themselves homokaryons and genotypically *fmf-1*. It is possible to test this hypothesis directly by isolating homokaryotic *fmf-1* conidia derived from a heterokaryotic mycelium and using these conidia to effect crosses. The procedures are described in detail in MATERIALS AND METHODS; briefly the procedures involve germinating the mixture of conidia under nutritional conditions such that only the *fmf-1* homokaryotic conidia fail to germinate. The germinating conidia can be filtered clear of the nongerminated material, some of which can then be used to effect fertilization and others plated to determine the proportion of conidia of the various genotypes.

Conidia were isolated from strains 19 and 22 and germinated in Min + ad for varying lengths of time. The proportion of conidia that were *pyr-3* homokaryons are shown in Figure 3. The proportion of *pyr-3* homokaryons rises to nearly 100% in both the experimental and control crosses by 35 hours after inoculation. The proportion of perithecia that produce the black spores indicative of *fmf-1* participation in the cross decreased to zero, while in the control nearly 100% of the perithecia produced black spores. This finding suggests that the *fmf-1*$^+$ gene product is not stored in the cytoplasm and that an *fmf-1*$^+$ nucleus must be present in the cytoplasm to allow crossing.

Experiments were also performed where conidia of strains 4 and 9 were mixed in Min + ad, ur and allowed to fuse. The results suggested that the *fmf-1* conidia gain the ability to cross with strain 5 at seven hours after mixing. Individual perithecia were opened and the rosettes of ascospores examined for the presence of asci segregating only *per-1* spores (eight light: zero black) and those segregating both *per-1* and *per-1*$^+$ spores (four light: four black). Of 19 rosettes that segregated *per-1*$^+$ in some asci (and therefore resulted from a cross of the *fmf-1* nucleus), only one was mosaic in that it also segregated *per-1 fmf-1*$^+$ in the same rosette. This finding can be compared to other systems where male heterokaryons between two stocks marked by different spore color loci gave 23% mosaic rosettes (WEIJER and DOWDING 1960) and where conidial mixtures of two strains carrying different spore markers gave 2% mosaic rosettes (NAKAMURA and EGASHIRA 1961).

Mapping of fmf-1*:* Preliminary findings suggested that *fmf-1* was linked to mating type on linkage group IL and loosely centromere-linked. Several strains containing multiple linkage group I markers (Table 1, strains 6, 7, and 8) were obtained and were used to assign map position by means of three- or four-point crosses (Table 3). The male parent in all cases wa sthe heterokaryon, *tol pan-1 fmf-1 A + tol trp-4 fmf-1*$^+$ *a* (Table 1, strain 18). This heterokaryon had previously been shown to be fertile in crosses to both mating types. The data from

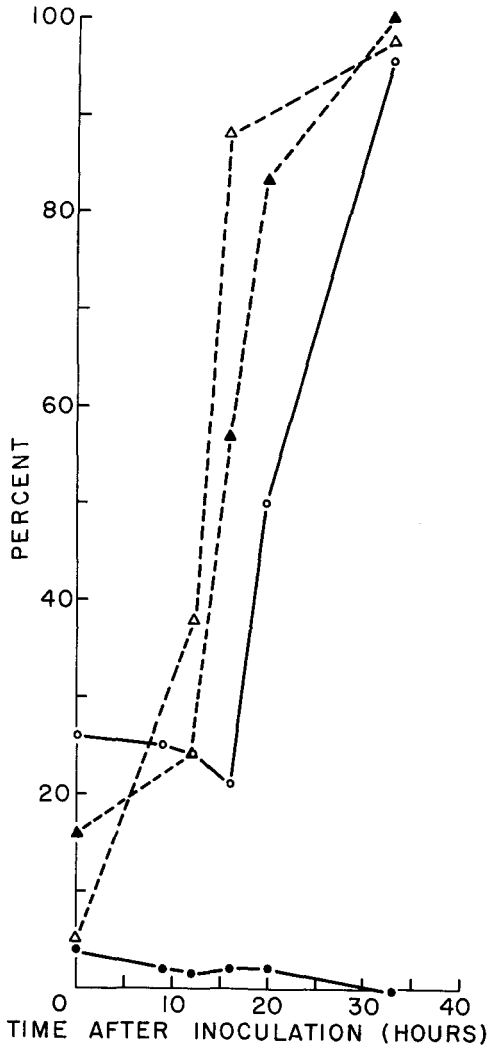

FIGURE 3.—Results of filtration experiments on conidia from either of two heterokaryons: *per-1 ad-2 A* + *per-1*+ *pyr-3 A* (Table 1 strain 22) or *fmf-1*+ *per-1 ad-2 A* + *fmf-1 per-1*+ *pyr-3 A* (Table 1, strain 19): ▲—▲—▲ indicates the percent of conidia that are uridine auxotrophs in strain 19; ●—●—● indicates the percent perithecia producing *per-1*+ spores in strain 19; △—△—△ indicates the percent of conidia that are uridine auxotrophs in strain 22; ○—○—○ indicates the percent of perithecia producing *per-1*+ spores in strain 22.

crosses 1 and 2 assign *fmf-1* to a locus between mating type and *cr-1*. However, the uncorrected data from cross 3 are not easily interpreted. The data are incompatable both with this assignment of linkages and with the known linkage relationships of *leu-3*, mating type and *arg-1*. There is a large excess of double crossover in regions I and III. This class is more than the sum of the single crossovers in I and those in III. This is true even if *fmf-1* is outside of *arg-1* and mat-

TABLE 3

Mapping of fmf-1*

Cross number	Zygote genotype (Linkage group 1 markers)	Parental Female	Parental Male	Progeny† Singles I	Progeny† Singles II	Progeny† Singles III	Doubles I & II	Doubles II & III	Doubles I & III	% Recombination I	% Recombination II	% Recombination III
Region:	I II											
1) Uncorrected	*a fmf-1*+ *al-2* *A fmf-1* *al-2*+	70	81	11,12	35,38	--	9,8	--	--	15	34	—
Region:	I II III											
2) Uncorrected	*leu-3* *a* *fmf-1*+ *cr-1* *leu-3*+ *A* *fmf-1* *cr-1*+	17	83	10,0	2,0	0,1	0,0	0,0	0,1	10	2	2
Region:	I II III											
3) Uncorrected	*leu-3* *a* *fmf-1*+ *arg-1* *leu-3*+ *A* *fmf-1* *arg-1*+	44	55	2,6	1,3	0,0	0,1	0,2	0,19	21	5	14
Corrected‡		44	0	2,6	1,3	0,0	0,0	0,0	0,0	8	4	0

* The male or conidial parent in all crosses is the heterokaryon *tol pan-1 fmf-1 A* + *tol trp-4 fmf-1*+ *a*.
† The left-hand number of each reciprocal class represents progeny that contain maternal alleles of the left most marker scored.
‡ Corrected by: 1) deleting all *arg-1*+ *leu-3*+ progeny from consideration and by 2) doubling the frequencies of *arg-1 leu-3* reciprocal progeny.

ing type. This altered linkage relationship could be explained by means of a pericentric inversion (PERKINS 1974), but there is a better explanation.

A more likely explanation is that the other (*a*) nucleus in the paternal heterokaryon can sometimes participate as the female component in a cross. If this nucleus participates, we would expect that: (1) the *trp-4* marker should be present in the progeny of the cross, (2) there would be an excess of progeny of one reciprocal type over the progeny of the other type (*e.g.*, an excess of *leu-3*$^+$ *a fmf-1*$^+$ *arg-1*$^+$ progeny in cross #3). Only in cross #3 were *trp-4* progeny detected and were there extreme differences between reciprocal classes among the progeny (the differences between reciprocal classes in cross 2 is due to uniformly poor viability of *cr-1* ascospores). One way of excluding this error is by counting any progeny that carry at least one marker of the female parent (Table 3: cross 3, corrected). In cross 3, correcting for this source of error significantly affects the outcome. It thus appears that *fmf-1* is indeed near *arg-1*. It is also apparent that in the cross to *leu-3 arg-1 a*, but perhaps not in other crosses, the *a* component of the male can participate in meiosis and produce viable ascospores.

Co-segregation of male and female sterility: In these crosses and others, the segregation of male and female sterility has been followed. No recombinants were detected among 407 progeny. This suggests that the two phenotypes are linked by less than 0.3 map units, which is consistent with both male sterility and female sterility being due to the same original mutation. Attempts were made to induce reversion, both spontaneously and by using UV; no revertants were obtained even though a strong selection pressure for the reversion of the male sterile phenotype could be brought to bear.

DISCUSSION

Block point: Perithecial development is blocked at 130μ diameter in *fmf-1*, used as either the male or the female parent in a cross. This size corresponds to 15 hours into wild-type development. Male and female sterility co-segregate, and in all genetic tests the phenotypes are genetically identical. Although no revertants have been obtained, there is ample evidence to conclude that both phenotypes result from the same genetic alteration; this is the only reported case of a single mutational event giving rise to both male and female sterility in Neurospora. Many of a series of male-sterile mutants isolated by WEIJER and VIGFUSSON (1972) turned out to be female sterile also, but apparently due to a second mutational event. RAJU and PERKINS (1978) also have reported on the "barren" phenotype, which is similar to *fmf-1* in its sterility as both male and female; however, unlike *fmf-1*, at least one duplication, DP S1229, that generated barren perithecia in crosses was autonomous in its effect; *i.e.*, it would not cross even when in a heterokaryon (JOHNSON, preliminary observations). This finding is consistent with the interpretation put forward by RAJU and PERKINS (1978). It appears that all the other developmental events normally associated with enlargement of the perithecium (accumulation of melanin, production of hyphae protruding from the outer perithecial wall, spore formation, etc.) can be

blocked by *fmf-1* as either parent at a point in perithecial development that corresponds to about 15 hours after crossing. These data suggest (see JOHNSON 1978, for additional data) that these events are highly integrated and regulated in normal perithecial development.

Autonomy of mutant action: Can genotypically *fmf-1* cells be supplemented with *fm-1*$^+$ gene product from *fmf-1*$^+$ cells in the same tissue? This question was asked at two distinct points in the life cycle: in conidia and in the perithecium. Nonautonomy of mutant action has been shown before in conidia of Neurospora (STADLER and SMITH 1968; SHELBY, DE SERRES and STINE 1975; conidia were isolated from mycelia heterokaryotic for UV-resistant and sensitive nuclei; it was found that the fraction of conidia homokaryotic for UV-sensitivity genes were phenotypically resistant, thus implying that the wild-type gene product was synthesized in the conidium prior to the separation of individual conidia. In the present study, homokaryotic *fmf-1* conidia were isolated from a heterokaryon of *fmf-1* and *fmf-1*$^+$ nuclei by filtering out the nongerminating *fmf-1* conidia. These *fmf-1* conidia failed to cross as the male parent, suggesting that *fmf-1* is conidial autonomous and that the *fmf-1*$^+$ gene product is not synthesized by the conidium prior to conidial separation. However, the purification of these *fmf-1* homokaryotic conidia required 30 hours of starvation in germination medium, and it is possible that stored *fmf-1*$^+$ gene product could be turned over in this time.

The *fmf-1* phenotype seems to be due to a defect somewhere in early perithecial development prior to 15 hours where the male and the female nuclei both must play a similar developmental role. In normal perithecial development a male cell fuses with the protoperithecial trichogyne and the walls between these two structures dissolve, allowing the male nucleus to infiltrate the cytoplasm of the trichogyne. The male nucleus then migrates down the arm of the trichogyne into the ascogonium (BACKUS 1939) where it comes eventually to lie next to a female nucleus in the distal arm of specialized hyphae called croziers. Here fusion of the two nuclei to form the zygote occurs, and the diploid nucleus immediately begins meiosis, while the crozier forms a typical ascus within which the linear meiotic divisions occur. COLSON 1934; SINGLETON 1953; FINCHAM and DAY 1971). From the block point of *fmf-1*, it seems that the normal developmental process must be interrupted somewhat before meiosis since meiosis does not take place until about 48 hours (MCNELLY-INGLE, LAMB and FROST 1966; RAJU and PERKINS 1978). No evidence of asci or ascogonial material is seen in squashes of *fmf-1* perithecia.

The *fmf-1* phenotype is not completely autonomous in its effect since heterokaryons with *fmf-1*$^+$ nuclei are fertile and *fmf-1* crosses well in such heterokaryons. Whenever and wherever the defect in *fmf-1*, it is at a time and place such that the *fmf-1* nucleus is in functional communication with the *fmf-1*$^+$ nucleus. Autonomy in the sexual cycle has been examined in several other contexts. All the mutants affecting spore pigmentation in *N. crassa* (STADLER 1956; NAKAMURA 1961; MURRAY 1965; PERKINS 1974; HOWE and JOHNSON 1976) are spore autonomous and all spore morphology mutants so far screened appear to

be ascus autonomous (JOHNSON 1977; SRB and BASL, personal communication). These observations are consistent with findings on ascospore development, and suggest that the shape of the spore is determined first while all the nuclei within an ascus are in functional communication (SRB and BASL, personal communication). Only later does pigmentation begin, at a time when each spore is now programmed only by its own nucleus.

It could be that *fmf-1*$^+$ nuclei are required in the ascogonium for functional complementation to occur. If this were true, we might expect that *fmf-1*$^+$ male nuclei would more often be found participating in the formation of mosaic rosettes of ascospores, some of which would segregate *fmf-1* and others would not. There were only 5% mosaic rosettes in crosses of heterokaryons containing both *fmf-1* and *fmf-1*$^+$ nuclei. This frequently is similar to that in other systems where both components of the male were *fmf-1*$^+$ (WEIJER and DOWDING 1960; NAKAMURA and EGASHIRA 1961; JOHNSON 1976a). Perhaps the *fmf-1*$^+$ nucleus need not be physically present in the ascogonium and/or the rosette for functional complementation to occur. These arguments thus place the time of *fmf-1* action as somewhere between the fusion of the conidium with the trichogyne and ascogonial formation.

Dominance relationships: When crossed as either sex, a developmental block would be expected if *fmf-1* were dominant in its effect on development. The facts that heterokaryons between *fmf-1* and *fmf-1*$^+$ nuclei are fertile and that *fmf-1* participates suggest that such a simple dominance relationship does not occur. Moreover, the sterility phenotype can be complemented either as the male or as the female parent by an *fmf-1*$^+$-bearing nucleus that is either of the same mating type or of opposite mating type. When mixed mating-type heterokaryons are grown as the female parent, the *fmf-1*$^+$ function is required for fertility. Therefore, the action of *fmf-1*$^+$ is not solely to allow fusion of the conidium with the trichogyne and functional mixing of their cytoplasmic contents. Thus mixed mating-type heterokaryons are not just bypassing the normal action of the *fmf-1* gene product.

Interaction of fmf-1 *and* tol*:* When crosses were performed between *fmf-1* and *fmf-1*$^+$, both of which were also *tol* (NEWMEYER 1970), the crosses were fertile. Qualitative differences in the efficacy of the cross were apparent in that there were many fewer large, and therefore fertile, perithecia in these crosses than in wild-type crosses. However, there are never any fertile perithecia in *fmf-1* crosses lacking *tol*. Two possible interpretations of this finding are: (1) the *tol* gene product and the *fmf-1* gene product could functionally interact to replace the normal *fmf-1* function, or (2) *tol* could also alter incompatability functions normally found associated with mating type in sexual crosses. There are no data that can be brought to bear on these interpretations, but the latter seems much more reasonable since *tol* clearly suppresses a vegetative incompatability reaction that is inseparable from mating type (NEWMEYER, HOWE and GALEAZZI 1973). In this interpretation the *tol* gene might somehow allow *fmf-1*$^+$ gene product to flow between the nuclei of the two alternate mating types in such crosses. One possible way this exchange could occur is by allowing fusion of the

two mating types by mechanisms normally utilized only in vegetative growth and now allowed in sexual development through the interaction of *tol*. However, as pointed out earlier, the *fmf-1*$^+$ gene product must also function at some later point in development since mixed mating-type heterokaryons both of which are *fmf-1* are sterile and blocked at the normal *fmf-1* block point.

Use of mixed mating-type heterokaryon in mapping: The data presented in Table 3 are all derived from crosses where the male parent was a *tol pan-1 fmf-1 A* and *tol trp-4 fmf-1*$^+$ *a* heterokaryon. When crossed with *a* strains, only the *A* component of the male participated in the cross in two cases; both components participated in cross 3 as deduced by the fact that *trp-4* ascospores were found among the progeny of this cross. It thus appears that in some cases, but not all, only the opposite mating-type nucleus of the male is able to participate. This problem can be alleviated by: (1) marking both paternal nuclei with additional markers so that illegitimate crosses can be detected, and (2) excluding from the data all those spores that bear no marker exclusively derived from the female parent. When these procedures are followed, there appears to be no systematic problem in performing routine mapping experiments with such heterokaryons. A similar system has been used by NELSON, LITTLEWOOD and METZENGER (1975) for crossing the mutant, slime.

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