THE MUTATION *SK(ad-3A)* CANCELS THE DOMINANCE OF *ad-3A*+ OVER *ad-3A* IN THE ASCUS OF NEUROSPORA

A. M. DELANGE[1]

Department of Botany, The University of British Columbia, Vancouver V6T 1W5, Canada

Manuscript received January 15, 1980
Revised copy received December 16, 1980

ABSTRACT

A newly induced mutant of Neurospora, when crossed with an *ad-3A* mutant, produces asci with four viable black and four inviable white ascospores. The survivors always contain the new mutant allele, never *ad-3A*. The new allele, which is called *SK(ad-3A)* (for spore killer of *ad-3A*), is located at or very near the *ad-3A* locus.——In crosses homozygous for *ad-3A*, each ascus contains only inviable white ascospores. This defect in ascospore maturation is complemented by the wild-type allele, *ad-3A*+ (crosses heterozygous for *ad-3A* and *ad-3A*+ produce mainly viable ascospores), but it is not complemented by the new *SK(ad-3A)* allele (all *ad-3A* ascospores from crosses heterozygous for *SK(ad-3A)* and *ad-3A* are white and inviable). In crosses homozygous for *SK(ad-3A)* or heterozygous for *SK(ad-3A)* and *ad-3A*+, each ascus contains only viable black ascospores. *SK(ad-3A)* does not require adenine for growth, and forced heterokaryons between *SK(ad-3A)* and *ad-3A* grow at wild-type rates and produce conidia of both genotypes with approximately equal frequency. Thus, the action of *SK(ad-3A)* is apparently restricted to ascospore formation. Possible mechanisms of the action of this new allele are discussed.

INVIABILITY of meiotic products is most easily detected in fungi, especially ascomycetes, since all products of a meiotic division are present in a single ascus, and viable and inviable products can usually be distinguished by their different color. [Some exceptions are the ascospore lethals *le-1* and *le-2* in Neurospora, which produce normal black ascospores (MURRAY and SRB 1961; GARNJOBST and TATUM 1967)]. Three types of mutants are known to produce inviable meiotic products: (1) meiotic mutants that produce inviable aneuploid progeny as a result of nondisjunction (*e.g.*, SMITH 1975; DELANGE and GRIFFITHS 1980b), (2) chromosome rearrangements that produce inviable deficiency progeny (see PERKINS 1974), and (3) genes that affect maturation and pigmentation of ascospores carrying a specific allele; this class comprises some cases of meiotic drive, autonomous ascospore lethals and recessive ascospore lethals (see PERKINS and BARRY 1977).

Cases of meiotic drive have been encountered in several species (see ZIMMERING, SANDLER and NICOLETTI 1970). Examples are Segregation Distorter (*SD*) factors in Drosophila (see HARTL and HIRAIZUMI 1976), *Sk* of Neurospora

[1] Present address: Department of Biology, University of South Carolina, Columbia, South Carolina 29208.

(TURNER and PERKINS 1979; RAJU 1979), "sex ratio" in *D. pseudoobscura* (see POLICANSKY and ELLISON 1970), Gamete-eliminator (*Ge*) in tomato (RICK 1966, 1970), and the Pollen-killer gene (*Ki*) of wheat (LOEGERING and SEARS 1963). In all cases, homoallelic meioses involving a sensitive allele produce viable meiotic products, but heterozygous combinations of a killer and a sensitive allele produce many nonfunctional products carrying the sensitive allele. Thus, the killer allele is preferentially recovered from these meioses.

Ascospore lethal mutants characteristically result in the abortion of the mutant-bearing ascospores. The mutant may be expressed in an autonomous fashion (*i.e.*, in crosses of *mutant* × *mutant* or *mutant* × *wild type*, all mutant ascospores are aborted), or it may act in a recessive manner (*i.e.*, in the cross *mutant* × *mutant* all ascospores are aborted, but the cross *mutant* × *wild type* produces only viable ascospores). Examples of autonomous lethals are *asco* (STADLER 1956), *tan* (NAKAMURA 1961), *cys-3* (MURRAY 1965) and *ws* (PHILLIPS and SRB 1967) in *Neurospora*. Examples of recessive ascospores lethal mutants are *ad-3A* and *ad-3B* in *Neurospora* (see GRIFFITHS 1970).

This paper reports a unique case where a mutant allele (*ad-3A*) of *Neurospora crassa* can be expressed as either a recessive or an autonomous ascospore lethal, depending on whether it is crossed with wild type (*ad-3A* × *ad-3A*$^+$ produces mainly viable ascospores) or with the newly induced mutant *SK(ad-3A)* (all *ad-3A* progeny from *ad-3A* × *SK(ad-3A)* are aborted).

MATERIALS AND METHODS

Strains: The following mutant alleles were used during this study: *leu-3* (R156), *un-3* (55701t), arg-1 (36703), *nic-2* (43002) *al-2* (74-Y-112-M38), *tol* (N38), *ad-3A* (2-17-19, 2-17-124, 2-17-186, 2-17-232, 2-17-233, 2-17-814, 2-17-825, 2-31-2, 2-32-10, 2-33-3, 2-33-4, 2-33-22, 2-33-30, 2-33-34, 5-5-4, 5-5-23, 5-5-47, 5-5-52, 5-5-74), *ad-3B* (2-17-114, 2-17-76, 2-17-82, 2-17-85, 2-17-99 and 2-17-128). The last five alleles of *ad-3B* complement allele 2-17-114, and none of the *ad-3A* alleles complement each other. The approximate map distances (RADFORD 1972) of mutations on LG I are shown in FIGURE 1. Discrepancies between these values and those obtained in this study may be partly due to variability caused by genetic background.

Heterokaryon

				centromere				
Component 1	*leu-3*	+	*a*	*arg-1*	+	*ad-3B*	+	+
Component 2	+	*un-3*	*A*	+	*ad-3A*	+	*nic-2*	*al-2*
Approximate map distance (RADFORD 1972)	10		0.1	10	9	0.3	4	28

FIGURE 1.—Linkage group I (LG I) markers of the two nuclear components of strain DL917. The map distances between the markers are not drawn to scale. These components contain identical copies of all other linkage groups (see text).

The procedure used in the isolation and detection of mutant strain DL917 has been described elsewhere (DELANGE and GRIFFITHS 1980a; see first section of RESULTS). This strain is composed of two nuclear components of genotypes *leu-3 a arg-1 ad-3B* and *un-3 A ad-3A nic-2 al-2*; each component carries the *tol* mutation that allows normal growth of (*A* + *a*) heterokaryons (NEW-

MEYER 1970; DELANGE and GRIFFITHS 1975), and identical (but unknown) *het* genotype necessary for vigorous growth of the heterokaryon (GARNJOST and WILSON 1956). DL917 was detected by its production of about 60% white aborted ascospores.

The two ascospore isolates (917–36 and 917–38) were obtained from crosses between DL917 and the wild-type strains ORA and ORa, respectively. Their genotypes are *leu-3 a arg-1 ad-3B*; *tol*; *Cde* (917–36) and *un-3 A ad-3A nic-2 al-2*; *tol*; *Cde* (917–38), where *C*, *d* and *e* are alleles of three heterokaryon-compatibility loci. The presence of the *tol* mutation and *Cde* genotype in both strains allows the formation of a heterokaryon between these strains. The identity of the two adenine-requiring mutants *ad-3A* and *ad-3B* was determined, where necessary, by heterokaryon tests with *ad-3A* and *ad-3B* tester strains (see DELANGE and GRIFFITHS 1975).

Procedures: Routine manipulations and media have been described previously (DAVIS and DESERRES 1970; DELANGE and GRIFFITHS 1980a).

Crosses were performed by the simultaneous inoculation of two strains of opposite mating type (*A* and *a*) into 18 × 150 mm test tubes containing 5 ml liquid crossing medium and a strip of filter paper (NEWCOMBE and GRIFFITHS 1972). All crosses were incubated at 25°.

Ascospore analysis was usually performed by isolating individual ascospores and testing the resulting cultures. To detect *ad*+ recombinants among the progeny from a cross between *leu-3 a arg-1 ad-3B* and *A ad-3A*, ascospores were plated on standard plating medium supplemented with leucine and arginine. The resulting colonies were transferred from the plates to slants of vegetative medium supplemented with leucine and arginine and tested for their leucine and arginine requirements. Some *leu*+ *arg*+ *ad*+ cultures were presumably the result of nondisjunction of LG I. Only the *leu arg ad*+ recombinants were used further.

The *ad-3B* mutation in strain 917–36 (*leu-3 a arg-1 ad-3B*) has been reverted in one experiment. A conidial suspension of strain 917–36 was irradiated with UV at 5×10^3 ergs/cm^2 for 30, 60 or 90 sec, and the irradiated conidia were plated on standard plating medium supplemented with leucine and arginine. The *leu arg ad*+ revertants were crossed to *ad-3A*, and the resulting *leu arg ad*+ ascospore isolates were then tested for mutant (ascospore abortion) phenotype.

RESULTS

Origin of strain DL917: The isolation of DL917 has been described elsewhere (DELANGE and GRIFFITHS 1980a). Briefly, complementing auxotrophic markers were used to select ascospores disomic ($n + 1$) for linkage group I (LG I). The instability of disomic nuclei in Neurospora (PITTENGER 1954) converts these disomics into heterokaryons containing two types of haploid nuclei that differ only with respect to their LG I (Fig. 1). These heterokaryons, which are self-fertile due to mating type (A/a) heterozygosity, allow detection of recessive meiotic mutant genes on any linkage group except LG I. DL917 was one of the heterokaryons screened for the presence of recessive meiotic mutants. When placed on appropriate crossing medium, strain DL917 produced perithecia containing about 60% aborted ascospores.

Is the ascospore abortion of DL917 caused by a recessive mutation?: If the observed ascospore abortion of DL917 were caused by a recessive mutation present in both components (*leu a arg ad-3B* and *un A ad-3A nic al*; see Figure 1) of DL917, crosses between DL917 and wild-type strains ORA and ORa should not produce ascospore abortion. Both types of crosses, *i.e.*, DL917 × ORA and DL917 × ORa, yielded primarily black viable ascospores. On this basis, it appeared plausible that the observed ascospore abortion is caused by a recessive mutation.

Analysis of ascospores from DL917 × ORA and DL917 × ORa crosses showed that ascospore abortion segregated with LG I (Table 1): (1) In cross DL917 × ORa (*i.e.*, component *un A ad-3A nic al* of DL917 × *un*$^+$ *a ad*$^+$ *nic*$^+$ *al*$^+$), all 18 *un A ad-3A nic al* progeny exhibited ascospore abortion when backcrossed to DL917; whereas, all 20 *un*$^+$ *a ad*$^+$ *nic*$^+$ *al*$^+$ progeny produced mainly black ascospores in such backcrosses. (2) Similarly, abortion segregated with the *leu a arg ad-3B* progeny from the cross DL917 × ORA; in this cross, the abortion factor was shown to be linked to markers in the centromere region of LG I. The factor appeared to be to the right of *arg-1* (one *leu a arg ad*$^+$ and three *leu a arg*$^+$ *ad*$^+$ recombinants did not carry the abortion factor), and close to or to the left of *ad-3B* (all 23 parental types *leu a arg ad-3B* and *leu*$^+$ *A arg*$^+$ *ad-3B*$^+$ were also parental for the abortion factor).

Since the components of DL917 are heterozygous for several closely linked markers in the region to which the abortion factor was mapped, it was considered unlikely that any part of this region, including the abortion factor, would have become homozygous. An apparent paradox was created by (1) the absence of ascospore abortion in the crosses DL917 × ORA and DL917 × ORa, which suggests a recessive mutation, and (2) the location of the abortion factor on LG I, which argues against the presence of the same recessive mutant allele in both components of DL917.

Nature of the defect: In addition to the parental *leu a arg ad-3B* and *un A ad-A3 nic al* components, DL917 may contain a small proportion of nonparental nuclei, *e.g.*, produced by somatic crossing over (PITTENGER and COYLE 1963). Therefore, to ensure that only two nuclear components of known genotype were crossed, a cross between the ascospore isolates 917–36 (*leu a arg ad-3B*) and 917–38 (*un A ad-3A nic al*) was used to analyze further the ascospore abortion observed in DL917. Examination of ascus patterns from this cross showed that most asci contained 4 black and 4 white (aborted) ascospores (4B:4W asci). A few asci had fewer than 4 black spores; none had more.

TABLE 1

Analysis of isolates from crosses of DL917 to ORA and ORa wild-type strains*

Cross	Genotype LG I	Number of progeny	% Ascospore abortion when crossed with DL917
DL917 × ORa	*un A ad-3A nic al*	18	60
	+ *a* + + +	20	0–10
	un A ad-3A + *al*	1	60
	+ *a ad-3A nic al*	1	—
DL917 × ORA	*leu a arg ad-3B*	12	60
	+ *A* + + + †	11	0–10
	leu a + +	4	0–10
	leu a arg +	1	0–10
	+ *A arg ad-3B*	3	—

* Recombinants between *ad-3A* and *al* were not tested.

† Another 7 isolates of this genotype could not be tested because of a second mutation (see DELANGE and GRIFFITHS 1980a).

A background of 10% random ascospore abortion, also observed in some crosses between mutant and wild-type strains, accounts for the extra abortion observed in addition to the 50% accounted for by 4B:4W asci. This 4:4 pattern of abortion is uncharacteristic of meiotic mutants or chromosome rearrangements, but resembles that of spore-killer and autonomous ascospore lethal mutants.

Ascospore analysis of the cross 917–36 × 917–38 revealed normal recombination and nondisjunction frequencies (Table 2). However, only one of the two LG I parental chromosomes (*leu a arg ad-3B*) was recovered from this cross (during this initial analysis no distinction was made between *ad-3A* and *ad-3B*). In addition, reciprocal crossover classes were detected only in the *arg-nic* region. Evidently, a small region or gene in the *arg-nic* region on the *un A ad-3A nic al* chromosome cannot be recovered when crossed with strain 917–36 (the *leu a arg ad-3B* chromosome). This same region or gene is normally recovered when crossed with wild-type ORa. These data suggested that the 4B:4W asci are produced by the killing action of a mutant gene located on the *leu a arg ad-3B* chromosome of 917–36 on a killer-sensitive gene located on the *un A ad-3A nic al* chromosome of 917–38. The corresponding wild-type chromosome (LG I) of ORa has neither the killer nor the killer-sensitive gene. To determine whether

TABLE 2

Genotype of 79 isolates from the cross 917–36 × 917–38 that produced 4B:4W asci

LG I markers	Genotype*	Number ascospore isolates
Parental:	*leu + arg ad + +*	33
	+ un + ad nic al	0
Crossover:		
leu-un	*+ + arg ad + +*	6
	leu un + ad nic al	0
arg-nic	*+ un + ad + +*	4
	leu + arg ad nic al	4
nic-al	*leu + arg ad + al*	21
	+ un + ad nic +	0
Double Crossover:		
leu-un/nic-al	*+ + arg ad + al*	7
	leu un + ad nic +	0
arg-nic/nic-al	*+ un + ad + al*	3
	leu + arg ad nic +	1

Recombination frequency	
This cross	Control crosses
(*leu-un*) = 13/79 (16%)	11–17%
(*un-arg*) = 0/79 (0%)	
(*un-nic*)	15–20%
(*arg-nic*) = 12/79 (15%)	
(*nic-al*) = 32/79 (40%)	30–35%
Nondisjunction frequency = 0/79 (0%)	

* During initial analyses, no distinction was made between *ad-3A* and *ad-3B*. All crossover products were later found to be *ad-3B*, as explained in text.

the killer and the killer-sensitive mutant genes are alleles of the same gene or represent different genes, precise mapping of both genes was required.

Identity of the killer-sensitive mutant gene: A more precise mapping of the sensitive gene within the *arg-nic* region utilized the identification of *ad-3A* and *ad-3B* alleles in crossover products of the cross 917–36 × 917–38. All products were *ad-3B* rather than *ad-3A*; therefore, the sensitive gene was located near or at the *ad-3* loci.

To test the possibility that the *ad-3A* mutation itself could not be recovered in these crosses, 19 independently derived *ad-3A* alleles were used in crosses with strain 917–36. As a control, five *ad-3B* mutants and seven wild-type strains were also crossed with 917–36. Crosses with each of the 19 *ad-3A* alleles resulted in 4B:4W asci; in contrast, no abortion was detected in the remaining crosses. Since the *ad-3A* and *ad-3B* mutants were all derived from the same wild-type strain, the identity of *ad-3A* as the killer-sensitive mutant gene is firm.

Chromosomal location of the spore killer gene: The mutation on the *leu a arg ad-3B* chromosome that causes *ad-3A*-containing ascospores to abort will be referred to as spore killer of *ad-3A*, or *SK(ad-3A)*. In order to determine the location of *SK(ad-3A)* on LG I, 253 isolates from the cross between 917–36[*leu a arg ad-3B SK(ad-3A)*] and a wild-type strain [FGSC 1228, which is *ad-3A*$^+$ and therefore resistant to *SK(ad-3A)* action] were tested for the killer character. No recombinants between *SK(ad-3A)* and *ad-3B* were recovered. Thus, *SK(ad-3A)* is closely linked to *ad-3B*.

To show that *ad-3B* is not required for the killing of *ad-3A*-containing ascospores, the *ad-3B* mutation in strain 917–36 was reverted. The killing action of *leu a arg ad*$^+$ revertants clearly demonstrated that *SK(ad-3A)* was still present and acted independently of the *ad-3B* mutation.

A more precise mapping of *SK(ad-3A)* with respect to the *ad-3A* and *ad-3B* loci was obtained by isolating *leu a arg ad*$^+$ recombinant progeny from crosses between 917–36 *(leu a arg ad-3B SK(ad-3A))* and two strains containing alleles 2–17–814 or 2–17–825 of *ad-3A* (Figure 2). These wild-type recombinants were obtained at a frequency of 0.1%, not significantly different from the expected frequency (approximately 0.15%). All four recombinants contained the *SK(ad-3A)* mutation. Therefore, since *SK(ad-3A)* is very closely linked to *ad-3B* (0/253 recombinants), but nearer *ad-3A* than *ad-3B*, it was concluded that *SK(ad-3A)* and *ad-3A* are very tightly linked and may be alleles of the same gene.

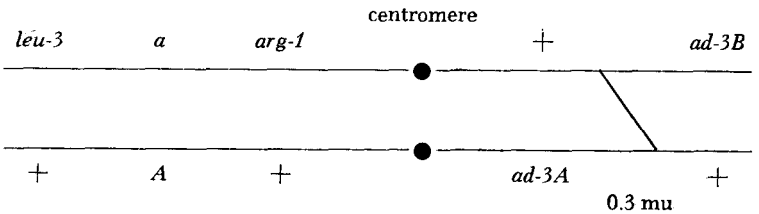

FIGURE 2.—Selection of *ad*$^+$ recombinants to locate *SK(ad-3A)* with respect to *ad-3A* and *ad-3B* loci.

Other characteristics of ad-3A *and* SK(ad-3A) *mutants*: The only known effect of *SK(ad-3A)* is its killing action on *ad-3A*-containing ascospores. Thus, crosses homozygous for *SK(ad-3A)* produce only black ascospores. In addition, *SK(ad-3A)* cultures grow at wild-type rates and do not require adenine for growth.

To determine whether or not *ad-3A*-containing conidia could be obtained from vegetative heterokaryons between *ad-3A* and *SK(ad-3A)*, several heterokaryons between strain 917–36 (*leu a arg SK(ad-3A) ad-3B*) and 2–17–825a (*a ad-3A*) were allowed to grow in 50 cm race tubes. These heterokaryons grew at a rate comparable to several heterokaryons between *ad-3A* and *SK(ad-3A)*$^+$ *ad-3B*. Conidia from the beginning and the end of one race tube were isolated and their genotypes were tested. The *ad-3A* and *leu arg ad-3B* genotypes were recovered with approximately equal frequency (Table 3). Thus, *SK(ad-3A)* does not appear to affect the viability of *ad-3A*-containing conidia.

DISCUSSION

The *ad-3A* mutant in *Neurospora crassa* behaves as a recessive ascospore lethal, *i.e.*, crosses homozygous for *ad-3A* produce mainly inviable unpigmented ascospores, but most ascospores from a cross between *ad-3A* and its wild-type allele *ad-3A*$^+$ are pigmented and viable. This paper reports the isolation of a mutant that, when paired in a cross with an *ad-3A* mutant, causes the abortion of *ad-3A*-containing ascospores. The new mutant gene was called spore killer of *ad-3A*, or *SK(ad-3A)*, and was found to be located at or very close to the *ad-3A* locus. Both this close proximity of *SK(ad-3A)* to the *ad-3A* locus and its specific effect on the viability of only *ad-3A*-containing ascospores strongly suggest that this new mutant gene is an allele, control or structural, of the *ad-3A* locus. Thus, depending on which allele *ad-3A* is crossed with, *ad-3A* may act as a recessive or an autonomous ascospore lethal. While many cases of both types of lethality have been previously described, both types have never before been associated with the same gene.

The abortion of *ad-3A*-containing ascospores superficially resembles some cases of segregation distortion that involve inviability of meiotic products. For example, the inviability of *Sd*$^+$ in Drosophila (HARTL and HIRAIZUMI 1976), *Sk*^{s} in Neurospora (TURNER and PERKINS 1979), and "l" in Ascobolus (MAKARE-

TABLE 3

Analysis of conidial isolates from a heterokaryon between strain 917–36 (leu-3 a arg-1 SK(ad-3A) ad-3B) *and 2–17–825a* (a ad-3A)

Genotype	Number of isolates from Beginning of race tube	End of race tube
HK*	22	22
leu-3 arg-1 ad-3B	13	11
ad-3A	8	13

* Wild type due to complementation of the two nuclear types.

WICZ 1966), depends on the other allele of these loci and on the genetic background in the meiocytes. Since meiocytes homozygous for these alleles produce only viable products (ascospores or sperm), inviability appears to be caused by the interaction of two different alleles at a particular locus. In contrast, meiocytes homozygous for *ad-3A* produce mainly inviable ascospores. Therefore, the lethality of *ad-3A*-containing ascospores is apparently the result of a deficiency in these ascospores.

Assuming that *SK(ad-3A)* is an allele of the *ad-3A* locus, abortion of *ad-3A*-containing ascospores would be caused by a failure of the altered *SK(ad-3A)* gene or gene product to complement the deficiency in these ascospores. Since complementation is normal in vegetative heterokaryons, the reduced ability to complement the deficiency is restricted to the ascus. Several mechanisms for such reduced complementing ability of the *SK(ad-3A)* gene can be visualized.

SK(ad-3A) could be within the structural *ad-3A* gene and produce an altered polypeptide with a differentially low activity in the ascus and ascospores. Such reduced activity might affect the maturation of *ad-3A*-containing ascospores if a specific threshold level of activity is required prior to ascospore enclosure. This threshold level would not be easily reached in the case of the relatively inactive *SK(ad-3A)* gene product. A similar model has been proposed to explain some irregular features of segregation distortion in Drosophila (MIKLOS and SMITH-WHITE 1971).

The lost ability of *SK(ad-3A)* to complement *ad-3A* in the ascus could also be caused by changes in the control of enzyme synthesis, its modification, stabilization or an altered property of transfer through the cytoplasm in the ascus. Mutant *cys-3* (MURRAY 1965) in Neurospora may be an example of a defect in transfer in asci. It lacks a permease and is the only cysteine-requiring mutant that produces mainly light inviable ascospores.

Sk(ad-3A) represents a unique mutation that may permit the study of different processes in the development of ascospore enclosure and maturation. Analysis in this system is facilitated by the knowledge of several components. First, ascospore abortion cannot be due to a lack of adenine in the ascospores, because abortion has not been associated with other adenine-requiring mutants (*e.g.*, ISHIKAWA 1962). Abortion may be indirectly caused by the *ad-3A* mutant gene. For example, the accumulation of an intermediate (AIR) in the purine synthetic pathway in *ad-3A* mutants may be the direct cause of abortion (FISHER 1969a). Mutants in the same enzyme in Schizosaccharomyces show a correlation between the accumulation of the polymer of AIR, the accumulation of red pigment and a slight decrease in growth rate. Both the red pigmentation and the reduced growth rate can be eliminated by a secondary mutation located in the purine pathway before the formation of the intermediate AIR (GUTZ *et al.* 1974). These findings suggest that similar secondary mutations should be able to suppress the ascospore inviability, if the accumulation of AIR polymers is the direct cause of such inviability. Second, the enzyme involved in the primary defect has been partially purified and characterized (FISHER 1969b). Therefore, any altered properties of the enzyme in the *SK(ad-3A)* mutant could be deter-

mined. Finally, genetic means of analysis are available. For example, more detailed mapping of *SK(ad-3A)* may locate it within the control or structural part of the *ad-3A* locus. In addition, mutations that interact with *SK(ad-3A)* to modify the abortion phenotype should be readily obtainable.

I wish to thank A. J. F. GRIFFITHS for critical review of the manuscript. Research was supported by Grant 6599 from the National Research Council of Canada.

LITERATURE CITED

DAVIS, R. H. and F. J. DESERRES, 1970 Genetic and microbiological research techniques for *Neurospora crassa*. pp. 79–143. In: *Methods in Enzymology*, 17A. Edited by H. TABOR and C. TABOR. Academic Press, New York.

DELANGE, A. M. and A. J. F. GRIFFITHS, 1975 Escape from mating-type incompatibility in bisexual (*A*+*a*) heterokaryons. Can. J. Genet. Cytol. **17**: 441–449. ——, 1980a Meiosis in *Neurospora crassa*. I. The isolation of recessive mutants defective in the production of viable ascospores. Genetics. **96**: 367–378. ——, 1980b Meiosis in *Neurospora crassa*. II. Genetic and cytological characterization of three meiotic mutants. Genetics **96**: 379–398.

FISHER, C. R., 1969a Enzymology of the pigmented adenine-requiring mutants of *Saccharomyces* and *Schizosaccharomyces*. Biochem. Biophys. Res. Commun. **34**: 306–310. ——, 1969b Phosphoribosyl-aminoimidazole-succinocarboxamide synthetase from *Neurospora crassa*. I. Partial purification and properties. Biochem. Biophys. Acta **178**: 380–388.

GARNJOBST, L. and E. L. TATUM, 1967 A survey of new morphological mutants in *Neurospora crassa*. Genetics **57**: 579–604.

GARNJOBST, L. and F. J. WILSON, 1956 Heterokaryosis and protoplasmic incompatibility in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **42**: 613–618.

GRIFFITHS, A. J. F., 1970 Topography of the *ad-3* region of *Neurospora crassa* Can. J. Genet. Cytol. **12**: 420–424.

GUTZ, H., H. HESLOT, U. LEUPOLD and N. LOPRIENO, 1974 *Schizosaccharomyces pombe*. pp. 395–446. In: *Handbook of Genetics*, Vol. 1. Edited by R. C. KING, Plenum Press. New York and London.

HARTL, D. L. and Y. HIRAIZUMI, 1976 Segregation distortion. pp. 616–666. In: *The Genetics and Biology of Drosophila*, Vol. 1b. Edited by M. ASHBURNER and E. NOVITSKY. Academic Press, New York.

ISHIKAWA, T., 1962 Genetic studies of *ad-8* mutants in *Neurospora crassa*. Genetic fine structure of the *ad-8* locus. Genetics **47**: 1147.

LOEGERING, W. Q. and E. R. SEARS, 1963 Distorted inheritance of stem-rust resistance of Timstein wheat caused by a pollen-killing gene. Can. J. Genet. Cytol. **5**: 65–72.

MAKAREWICZ, A., 1966 Colourless mutants in *Ascobolus immersus* with alternative phenotypes. Acta Soc. Bot. Pol. **35**: 175–179.

MIKLOS, G. L. G. and S. SMITH-WHITE, 1971 An analysis of the instability of segregation-distorter in *Drosophila melanogaster*. Genetics **67**: 305–317.

MURRAY, N. E., 1965 Cysteine mutant strains of *Neurospora*. Genetics **52**: 801–808.

MURRAY, J. C. and A. M. SRB, 1961 A mutant locus determining abnormal morphology and ascospore lethality in *Neurospora*. J. Heredity **52**: 149–153.

NAKAMURA, K., 1961 An ascospore color mutant in *Neurospora crassa*. Bot. Mag. Tokyo **74**: 104–109.

NEWCOMBE, K. D. and A. J. F. GRIFFITHS, 1972 Adjustable platforms for collecting shot asci. Neurospora Newsletter **20**: 32.

NEWMEYER, D., 1970 A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. Can. J. Genet. Cytol. **12**: 914–926.

PERKINS, D. D., 1974 The manifestation of chromosome rearrangements in unordered asci of Neurospora. Genetics **77**: 459–489.

PERKINS, D. D. and E. G. BARRY, 1977 The cytogenetics of *Neurospora*. Adv. Genet. **19**: 133–285.

PHILLIPS, R. L. and A. M. SRB, 1967 A new white ascospore mutant of *Neurospora crassa*. Can. J. Genet. Cytol. **9**: 766–775.

PITTENGER, T. H. and M. B. COYLE, 1963 Somatic recombination in pseudo-wild type cultures of *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **49**: 445–451.

PITTENGER, T. H., 1954 The general incidence of pseudo-wild types in *Neurospora crassa*. Genetics **39**: 326–342.

POLICANSKY, E. and J. ELLISON, 1970 "Sex ratio" in *Drosophila pseudoobscura*: Spermiogenic failure. Science **169**: 888–889.

RADFORD, A., 1972 Revised linkage maps in *Neurospora crassa*. Neurospora Newsletter **19**: 25–26.

RAJU, N. B., 1979 Cytogenetic behavior of Spore killer genes in Neurospora. Genetics **93**: 607–623.

RICK, C. M., 1966 Abortion of male and female gametes in the tomato determined by allelic interaction. Genetics **53**: 85–96. ——, 1970 The tomato *Ge* locus: linkage relations and geographic distribution of alleles. Genetics **67**: 75–85.

SMITH, D. A., 1975 A mutant affecting meiosis in *Neurospora*. Genetics **80**: 125–133.

STADLER, D. R., 1956 A map of linkage group VI of *Neurospora crassa*. Genetics **41**: 528–543.

TURNER, B. C. and D. D. PERKINS, 1979 Spore killer, a chromosomal factor in Neurospora that kills meiotic products not containing it. Genetics **93**: 587–606.

ZIMMERING, S., L. SANDLER and B. NICOLETTI, 1970 Mechanisms of meiotic drive. Ann. Rev. Genet. **4**: 409–436.

Corresponding editor: C. W. SLAYMAN