In a comparable series, the order of mating was reversed. The progenies produced by the 257 females that received marked sperm from both wild and SR males showed that their eggs were fertilized by both types of sperm and in the same ratio as the eggs produced by females that received either kind of sperm alone. The sex ratio of the 19,010 offspring from females inseminated by both kinds of males was, for normal sperm, 89.1 percent females, and for SR sperm, 6.3 percent females. The male-producing factor was not therefore transmitted by the egg alone. From the records of adult emergence, the sperm taking part in fertilization appears to have been random, and not selective as has been suggested for this as well as other arrhenotokous species (*2*).

Although the mode of action of the male-producing factor is unknown, its presence, as in the case of nonreciprocal cross incompatibility in the pteromalid wasp *Nasonia (Mormoniella) vitripennis* (Walker) reported by Saul (*4*), appears to hinder successful fertilization, either by preventing the sperm from successfully entering the egg, or by preventing the sperm nucleus from uniting with the female pronucleus after it has entered the egg. In *D. fuliginosus* the action of the factor is clearly independent of the agents considered to be responsible for altering the sex ratio of arrhenotokous animals. The factor can be increased and presumably decreased by selection and is transmitted by females to their sons. Its effects are not due to selective mortality but may be associated with male sterility. Although the sex ratio of certain selected crosses are constant, the factor is, no doubt, widely scattered through natural populations and might account for the variability of the sex ratio of this species and quite possibly the remarkable variability of the sex ratio that characterizes so many species of Hymenoptera both in the laboratory and in the field.

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References

1. P. W. Whiting, *Advan. Genet.* **10**, 295 (1961).
2. S. E. Flanders, *Quart. Rev. Biol.* **21**, 135 (1946).
3. A. Wilkes, *Can. Entomologist* **95**, 183 (1963).
4. G. B. Saul, *Z. Vererbungslehre* **92**, 28 (1961).

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Dispensable and Indispensable Genes in Neurospora

Abstract. *Twenty-four recessive lethal mutations were detected in a heterokaryon by the method of Atwood and Mukai. All controlled indispensable functions. Genetic analysis revealed that six of these failed to transmit the defect to their progeny. These mutants are believed to be semilethal genetic changes which express a lethal phenotype only in certain genetic backgrounds.*

The hereditary determinants of a microorganism can be divided into two classes: dispensable genes, whose loss or inactivation can be overcome by complete medium (that is, auxotrophs), and indispensable genes, whose loss results in lethality even in the presence of a complete medium. In *Neurospora* the relative proportions of the two classes have been estimated by two methods which gave quite different results. Horowitz (*1*) found that 14 out of 26 *Neurospora* mutants whose phenotype was temperature dependent grew on complete medium at the temperature at which they were unable to grow on minimal medium; these mutants represent dispensable genes. On the other hand, Atwood and Mukai (*2*) employed a heterokaryon method by which they demonstrated that only two out of 26 mutants examined were dispensable.

In the present study, a genetic analysis of mutants isolated by the Atwood-Mukai heterokaryon method suggests a partial explanation for this discrepancy.

The heterokaryon used in these experiments consists of two components, both of which are identifiable by their morphological and biochemical characteristics. One nucleus contains the genes *al-2* (colorless conida), *me-2* (methionine requirement) and *igloo* (a new morphological marker, linked to *al-2*). The other nucleus contains the genes *tr-1* (tryptophan requirement), and *flat* (another morphological marker). Both nuclei contain the temperature colonial gene, *cot*, but the heterokaryon is nutritionally like the wild type.

Conidia from the heterokaryon were filtered through glass wool and plated on minimal medium (*3*) at 33°C. Colonies were picked up and grown on minimal slants until conidiation, at which time they were tested for recessive lethals by plating on methionine-containing medium, also at 33°C. If no *igloo* colony types arose, this was presumed to be evidence of a recessive lethal in the *igloo* nucleus and the strain was set aside for further testing. With this method six spontaneous and 26 ultraviolet-induced mutants were isolated. The frequency was 0.95 percent for the spontaneous mutation and 8.6 and 12.5 percent for the ultraviolet induced mutations at 70 percent and 20 percent survival, respectively. All the mutants isolated were found to be indispensable, that is, none gave *igloo* colonies on Horowitz (*4*) complete medium.

Table 1. Summary of results of the tetrad analysis of spontaneous and induced mutants. Unless more than four spores germinated on the methionine-containing dissection agar, the spores were not transferred to small slants of complete agar. The last column indicates whether or not the mutants behaved according to the assumptions made by Atwood and Mukai.

Origin of mutants	No. examined	No. giving aberrant pattern
Spontaneous	6	1
Ultraviolet	18	5

All of the spontaneous mutants and a sample of the "ultraviolet" mutants were tested for allelism by the method of Atwood and Mukai (*2*). None gave identical complementation patterns and therefore all were different by this criterion.

Eighteen of the "ultraviolet" mutants and all six of the spontaneous mutants were crossed to *cot a* and asci were dissected on methionine medium and germinated at 33°C. Control crosses of *Igloo* A × +a were also dissected, and no lethal genetic factors were found to be segregating.

Crosses were judged as either normal or aberrant. A heterokaryon mutant was said to be normal if no tetrads were dissected in which more than four spores out of eight grew. If more than four spores grew, and if the ascus in question was segregating for the *igloo* gene, then the spores were transferred to slants and allowed to grow. Strains with this behavior were aberrant. Table 1 gives the data for the dissections. Since crosses were always dissected on methionine medium, the other nucleus

(which contains *tr-1*) is automatically excluded.

Since a total of 6 out of 24 of the isolates examined do not appear to be carrying recessive lethals as judged by genetic analysis, it is felt that this offers a partial explanation for the discrepancy between the Horowitz and Atwood-Mukai methods of ascertaining the proportion of indispensable genes. The aberrant heterokaryon mutants are thought to be semilethal genetic changes which will act as fully lethal in the debilitating genetic background employed by the nature of the heterokaryon method. However, when crossed to wild type the progeny spores will possess a new genetic background, and at least some of the asci will carry more than four viable spores. Although the Atwood-Mukai heterokaryon employed different genetic markers, many of their isolates were probably semilethal genes of this sort.

It should also be pointed out that 6 out of 24 is a minimum estimate for the fraction of semilethal genes present. The possibility exists that others were not separated from their debilitating background in the asci dissected and were classed as fully lethal.

If the hypothesis of a large class of semilethal genes among those mutants isolated by the heterokaryon method is correct, then one would expect that in unselected material slow-growing mutants that do not respond to complete medium would greatly outnumber biochemical mutants. This is indeed the case, as Mitchell, Mitchell and Tissières (*5*) have shown. Another prediction is that the effects of the semilethal gene could be observed among the spores from the aberrant mutant crosses. Only three strains were examined in this fashion and in one (S13A) segregation for a growth-retarding factor was observed. However, in the other two strains neither growth tests nor visual observation unambiguously demonstrated a semilethal factor.

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References

1. N. H. Horowitz, *Advan. Genet.* **3**, 33 (1950).
2. K. C. Atwood and F. Mukai, *Proc. Natl. Acad. Sci. U.S.* **39**, 1027 (1953).
3. H. J. Vogel, *Microbial Genet. Bull.* **13**, 42 (1956).
4. N. H. Horowitz, *J. Biol. Chem.* **171**, 255 (1947).
5. M. B. Mitchell, H. K. Mitchell, A. Tissières, *Proc. Natl. Acad. Sci. U.S.* **39**, 606 (1953).

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Insect Fertility: Inhibition by Folic Acid Derivatives

Abstract. *Folic acid antagonists inhibited oviposition by screw-worm flies* [Cochliomyia hominivorax *(Coquerel); Diptera: Calliphoridae*]. *Fertility was unaltered when sufficient folic acid was administered simultaneously, or it was partly restored spontaneously about 2 weeks after treatment. Folic acid antagonists principally affected only maturing eggs at any age, rather than those already matured.*

Impairment of fertility of screw-worm flies [*Cochliomyia hominivorax* (Coquerel); Diptera: Calliphoridae] by chemicals is being intensively investigated as an aspect of the method of insect control wherein the males are sterilized (*1*). Among several hundred compounds screened, several 4-amino derivatives of folic acid (*2*) were highly effective, manifesting their activity by inhibition of oviposition. More precise studies of the action of these antimetabolites are summarized in this report.

Bisexual or unisexual groups of adults less than 24 hours old were fed various concentrations (0.0001 to 10 percent) in honey or sugar syrup daily for 5 or 7 days, or for 1 day, after emergence. The relevant results are shown in Table 1. In addition, single treatments of both sexes with 0.05 percent aminopterin or chloromethotrexate limited the production of viable eggs to only 5 percent. Oviposition was inhibited by less aminopterin or methotrexate when both sexes were treated simultaneously than when females only were treated. Yet, when only males were treated, eggs hatched normally, or nearly so, even at a concentration of 5 percent. Treatment of male *Drosophila melanogaster* Meigen (*4*) or *Musca domestica* Linnaeus (*5*) similarly did not affect oviposition by untreated females. The weight of evidence does not support a conclusion that fertility is affected when males are treated.

Methotrexate and chloromethotrexate were more effective than the other two compounds. Each of the first two compounds has a methyl group attached to the *p*-benzoylamino group of aminopterin, a substitution markedly enhancing activity. However, although one chlorine atom added to the benzoyl ring of methotrexate hardly reduced efficiency, two chlorine atoms (dichloromethotrexate) reduced the activity to less than that of aminopterin. Generally, folic acid antagonists powerfully inhibit folic acid enzymes by interfering with intracellular nucleic acid synthesis of rapidly proliferating cells, which in turn leads to disturbances of cell division and to chromosomal damage (*3*). Nucleic acids undergo rapid synthesis in nurse cells of young female screw-worm flies, but they are already formed in postmeiotic male germ cells, some of which have already left the testes as mature motile sperm when the adult emerges from the pupa. Therefore, effects of metabolic antagonism can only be exerted in females. Antagonism of folic acid similarly occurs and leads to inhibition of oviposition in other insects, for example, *Drosophila* (*4*), *Musca* (*5*), and *Bracon hebetor* Say (*6*).

Flies fed 0.05 percent methotrexate and 20 percent folic acid simultaneously for 24 hours after emergence were normally fecund and fertile. The number of viable eggs produced increased as the concentration of folic acid was increased from 1 to 20 percent (Fig. 1). This increase was rapid up to a concentration of 3 percent folic acid, but lessened as the concentration increased. The action of methotrexate was nullified when 400 times as much folic acid as antimetabolite was used. The reversible affinity of folic acid reductase is far greater (up to 1000 times) for these antimetabolites than for the normal substrates, folic and dihydrofolic acids

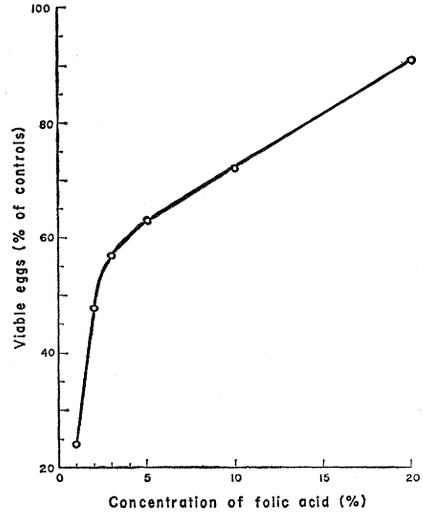

Fig. 1. Viable egg production by screw-worm flies treated orally with 0.05 percent methotrexate plus various concentrations of folic acid.