between the 3rd and 4th cm from the tip of the blade. The sections were aged for 24 hours by floating them on distilled water at 25°C in darkness. After this period they were blotted and transferred to screwcap vials containing 1 ml of test solution and 250 units of penicillin G. Four sections were floated in each vial, and each fraction was tested in duplicate or triplicate samples. After 48 hours of incubation at 25°C in darkness the sections of each vial were extracted with 80 percent ethanol; chlorophyll retention was expressed by measuring the optical density of the extracts at 665 mμ. In this assay the kinetin response at concentrations between 0.003 and 3.0 μg/ml appears to be linear if plotted on a logarithmic scale. The advantage of this test over the one described by Osborne (*7*) lies both in the great uniformity of the tissue which is always taken from the same portions of leaves of identical physiological age and in the higher sensitivity of the assay to kinetin.

Eluates from two regions of the chromatogram retarded chlorophyll degradation in barley leaf sections (Fig. 1). The response of the first fraction exceeded the response obtained by the optimum kinetin concentration of 30 mg/liter. This experiment was repeated five times with different batches of sunflower plants, and the same results were obtained. A number of nutritional factors were tested for their possbile interference with the bioassay. Sugars, inorganic nitrogen (ammonium nitrate, potassium nitrate, and ammonium sulfate), organic nitrogen sources (amino acids, casein hydrolyzate), and a complete modified White's medium gave negative results. Therefore the activity of the two fractions of root exudate is attributed to specific factors which are translocated from the root to the shoot and which play a regulatory role in the metabolism of the leaves. Experiments have shown already that one of the two fractions (R_F 0.5 to 0.8) very actively induces cell division in tissue cultures of soybean callus. This test is considered specific for the detection of kinetin-like substances (*8*).

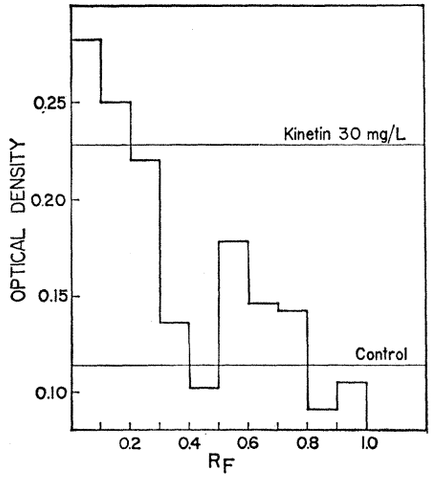

Fig. 1. Paper chromatogram of root exudate obtained from seven plants (44 ml of sap).

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2 June 1964

Self-Sterile Auxotrophs and Their Relation to Heterothallism in Sordaria fimicola

Abstract. *Eighty morphological mutants in the homothallic fungus* Sordaria fimicola *were tested on liquid minimal medium for nutritional requirements. Five had nutritional requirements, one for adenine, three for arginine, and one for lysine. All five were from among the eighty single gene mutants that were also partially or completely self-sterile. Nutritional requirements and centromere-locus intervals provide better criteria than morphological characters for selecting self-sterile mutants at complex loci governing heterothallism.*

Although extensive genetic investigations have been carried out on the homothallic ascomycete *Sordaria fimicola* in the past decade, attempts to obtain mutants with specific nutritional requirements have all met with failure. In all these past investigations tests were carried out on minimal agar medium in plates, and the failure was attributed largely to the lack of conidia (*1*). In another homothallic species *S. macrospora* studied by Esser (*2*) and Heslot (*3*), no auxotrophic mutants have been reported.

The ascospores of *S. fimicola* (Fig. 1*a*) have been shown (*4*) to be as good a tool as conidia for obtaining mutants of various morphological characters. Bearing in mind that inheritable morphological characters are the phenotypic expression of physiological processes under gene control, I set out to test a large number of morphological mutants for nutritional deficiencies. With the exception of a few ascospore color mutants, all of those tested were self-sterile as a result of the same mutation that was expressed morphologically.

The minimal medium of Beadle and Tatum (*5*) adopted in this study was modified in that sucrose was replaced by glucose as the only carbon source and the ingredients were dissolved in water distilled in glass twice. In order to eliminate any carry-over in the inoculum, the mutants were grown on water agar medium for 2 to 3 days, after which small inocula were cut out at the edge of the colony and transferred to 125-ml erlenmeyer flasks containing 15 ml of the minimal medium. The flasks were placed on a gyratory shaker for 5 to 10 days before the results were recorded. The use of liquid rather than agar minimal medium

Table 1. Auxotrophic mutants of *S. fimicola*.

Characteristics	Requirements
st-59	
Nonautonomous spore color mutant producing hyaline inviable ascospores, slow growth	Arginine
a-3	
Abortive asci, slow growth	Arginine (partial)
st-412	
Only mycelium produced, slow growth	Arginine (partial)
st-401	
Only mycelium produced, slow growth	Adenine
st-64	
Irregular ascospore maturation, normal and abortive ascospores	Lysine

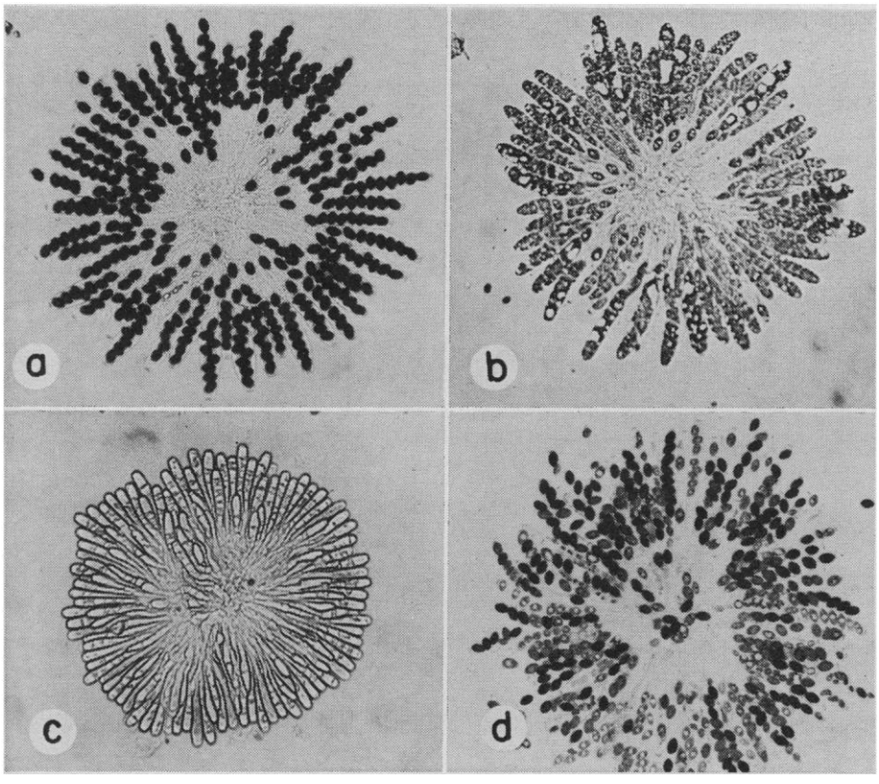

Fig. 1. Asci of *Sordaria fimicola* in (*a*) wild type with mature ascospores; (*b*) *st-59* with abortive ascospores; (*c*) *a-3*, note ascus abortion; (*d*) *st-64* with viable and abortive ascospores.

was important in detecting the auxotrophs in *S. fimicola*. Mutant *st-64* that had been isolated and tested for nutritional requirements by Gramacke (*6*) without success, was found auxotrophic by L. Simone (*7*), who used the liquid medium described.

The results show that five auxotrophs listed in Table 1 are nutritionally deficient, one being deficient for adenine, three for arginine (two partially deficient), and one for lysine. All five are partially or completely blocked in self-fertility as a result of the same gene that governs nutritional requirements. Recombination data obtained from crosses between these mutants and with other markers show that the lysine mutant (Fig. 1*d*) is situated in linkage group I, and the three arginine mutants are closely linked and situated about 2.6 crossover units from the centromere of linkage group II (Fig. 1, *b* and *c*). Recombination data from crosses between these three markers showed that three single crossover asci were found in 486 asci from *a-3* × *st-412*, but neither in 504 asci from *a-3* × *st-59* (*8*) nor in 430 asci from *st-59* × *st-412* did crossing-over occur. The data indicate that the three arginine loci represent a functional unit consisting of three sites in which *st-59* is flanked by *a-3* and *st-412*.

Complementation between these auxotrophic mutants was demonstrated by the formation of prototrophic heterokaryons and by their complete cross-fertility.

The results have significant bearing on the evolution of self-incompatibility in homothallic ascomycetes and the function of sterility loci. First, nutritional mutants with similar requirements are more likely to be controlled by pseudoalleles than are morphological or self-sterile mutants taken at random. They may thus prove to be an efficient source of material for studying complex loci of the heterothallism defined by Olive (*9*). Second, the present investigation may cast new light on the role of vital substances in the process of sexual reproduction in the ascomycetes. It is too early to predict whether a block at one point in the perithecial development is due to the same deficiency or to deficiencies of related substances. The pleiotropic effects of these loci may be interpreted as due to a failure in the synthesis of a common precursor in a single pathway for growth and reproduction or in two distinct biosynthetic pathways, one for growth and the other for reproduction. According to the first alternative, the addition of required substances at certain concentrations to the medium would be expected to induce growth and restore perithecial development and self-fertility. This however is not the case, for aside from growth, the only change observed due to exogenous supplementation is a considerable increase in the number of perithecia produced by *st-59* and *st-64* without having any effect on spore color and viability. Yet the first alternative cannot be entirely ruled out, considering that in a heterokaryon involving *st-59* and *a-3*, the selfed asci that frequently appear among crossed ones in hybrid perithecia are characteristic of their mutant type. Thus although complementation occurs in the mycelium, heterozygocity of the ascus is a prerequisite for normal ascospore development. It is quite possible that penetration of the supplement through the hyphal wall and its movement rate within the mycelium may be too low to induce normal fruiting. In mutants like *st-59*, *a-3*, and *st-64*, the requirements must therefore be synthesized within the hybrid ascus.

The second alternative simply implies that the detected requirement represents an intermediate in a pathway for growth after the block, and its relation to a distinct pathway for reproduction is traced to a common precursor blocked by a single gene. Requirements for reproduction would be unknown.

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10. I thank Dr. D. D. Perkins for criticism of the manuscript. Supported by grants from the National Institutes of Health (E-2326) and the National Science Foundation (G-14163).
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11 May 1964