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Sexuality and Genetic Behavior in the Fungus *Hypomyces* (*Fusarium*) *solani* f. sp. *cucurbitae*

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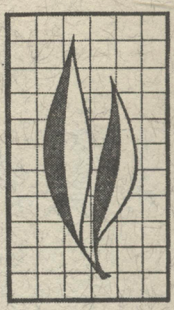

The host-specialized pathogen, *Fusarium* (*Hypomyces*) *solani* f. sp. *cucurbitae* causes rots of the stem, root, and fruit of cucurbits in many places in the world. The fungus is seed-borne, soil-borne, and sometimes air-borne. The individual clone is usually hermaphroditic but has been found in nature in a uni-sexual form. Although the Fusarium is heterothallic, rarely are the two mating types (+ and –) found together. When they are mated, the perithecia may be either red, or white, depending upon the genetic constitution. Races, as distinguished by host response, are not interfertile. Mutations of the normal hermaphrodite may give rise to male, female or neuter forms.

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Sexuality and Genetic Behavior in the Fungus *Hypomyces* (*Fusarium*) *solani* f. sp. *cucurbitae*[1]

INTRODUCTION

AS NOTED BY FINCHAM AND DAY (1965), the genetics of fungi is understood more because fungi are excellent experimental material for basic genetic research—than because these organisms are interesting as such. "Methodology in Basic Genetics" (Burdette, 1963) illustrates how fungus work has furthered understanding of the processes of meiotic and mitotic recombination, genic conversion, and extrachromosomal inheritance. The species studied by geneticists were chosen mainly on the basis of their adaptations for genetic experimentation, not of their importance to man.

Nevertheless, the genetics of plant-pathogenic fungi has received considerable study in the past two or three decades. Such work was carried out mainly by plant pathologists interested in the organisms as well as in the biological phenomena involved. Some genetic studies have helped to explain the mechanisms governing both pathogenicity and resistance in the host. The selectivity of fungicides may also become better understood through studies of the genetics of resistance. Some of the successful genetic studies by plant pathologists were indicated in a symposium of a decade ago (Holton, 1959).

Many plant-pathogenic fungi not only possess the interesting (and often specific) characteristic of pathogenicity but also are suitable for genetic experimentation. For example, valuable attributes for such study are the high degree of natural variability (example in fig. 1) and short generation time of the cucurbit root-, stem-, and fruit-rot pathogen, *Hypomyces solani* f. sp. *cucurbitae*. Further, the products of meiotic recombination are rather well preserved in the ascus. The unsuitability of the organism for obtaining ordered tetrads (Georgopoulos, 1963*a*) is not a serious difficulty, since methods have been devised (Whitehouse, 1957) by which centromeres can be mapped from unordered tetrad data.

During the last 30 years a number of staff members and students at this laboratory have worked on the variability, sexuality, and genetics of *Hypomyces solani* f. sp. *cucurbitae*. Two races of this fungus are known: race 1 causes cortical rot of roots and stems, as well as a fruit rot; and race 2 causes only fruit rot (Toussoun and Snyder, 1961). This paper considers only race 1, bringing together important knowledge to date on inheritance and clarifying some of the concepts on sexuality in light of present knowledge of sexual behavior in *Hypomyces*. The genetic control of sexual reproduction is discussed, therefore, in more detail than has so far been possible, and is compared with the sexuality of certain related fungi.

[1] Submitted for publication February 20, 1975.

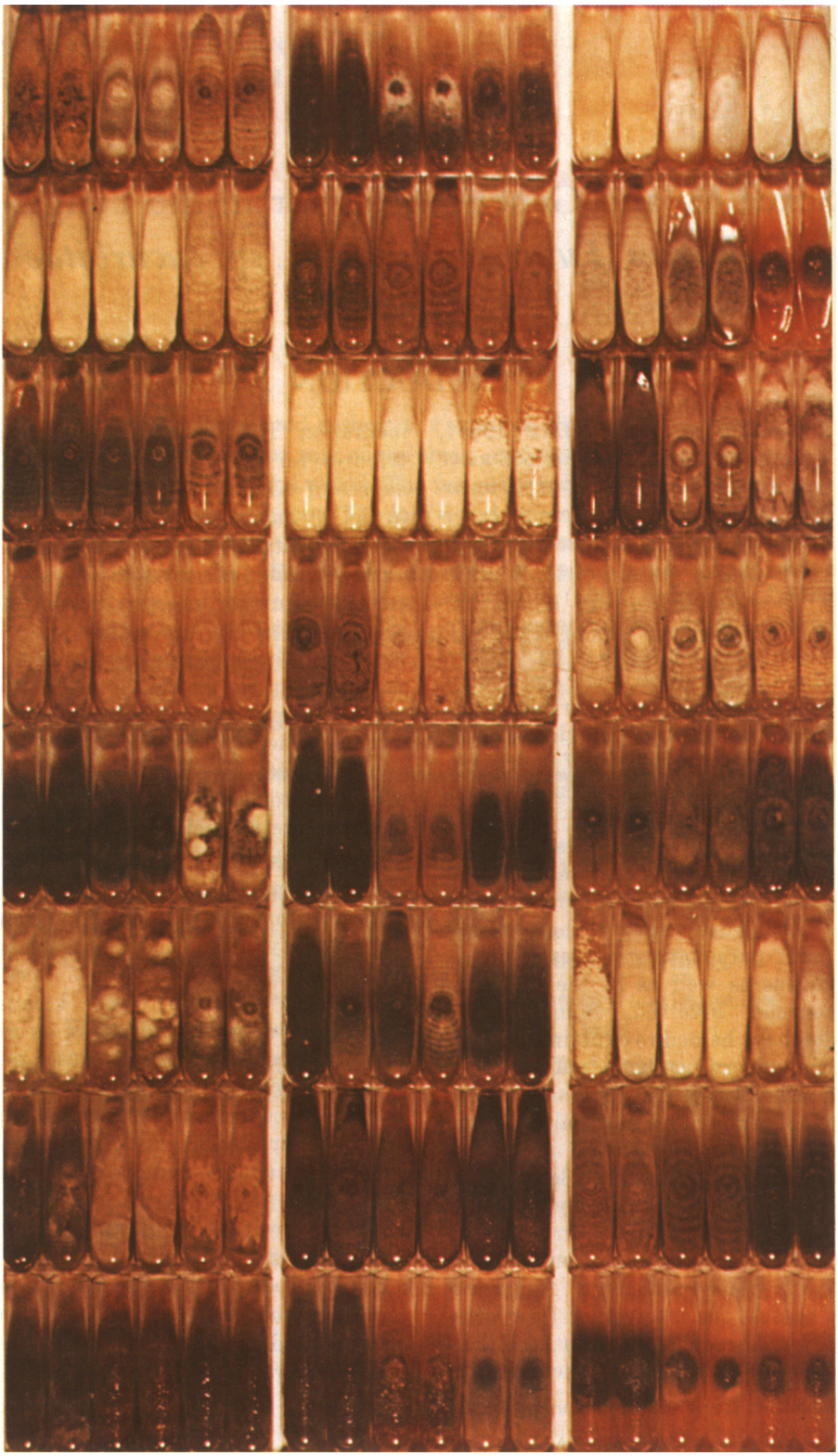

Fig. 1. Variation in cultural appearance in *Hypomyces solani* f. sp. *cucurbitae* race 1.

BIOLOGICAL AND TAXONOMIC CONSIDERATIONS

Hypomyces solani f. sp. *cucurbitae* has a perfect and an imperfect stage. It is haploid practically throughout its life cycle. Meiotic division takes place immediately (El-Ani, 1956) after formation of the diploid nucleus in the ascus mother cell. Therefore, barring mutation, each parent produces only one kind of gamete, which permits determination of the genotypes from the phenotypic ratios of the F_1. Diploids have not been produced in this organism, and no information is available on the dominance of particular alleles.

The imperfect stage

The asexual stage of the fungus belongs to the genus *Fusarium* of the *Tuberculariaceae,* which is characterized by multicellular, hyaline, fusiform, and curved macroconidia, normally produced in sporodochia. Fungi of this genus are very common on a variety of substrates the world over, being active in the decay of organic matter everywhere. Characteristic of their broad saprophytic potentialities are two recent reports, one of an *F. moniliforme* living at the expense of hydrocarbons of diesel fuel (Flippin, Smith, and Mickleson, 1964), and one of an *F. oxysporum* being a very common contaminant of even saturated solutions of colchicine (Smith, 1964). Those studying the microflora of soils and of plant residues are particularly familiar with the high frequency in which fusaria are isolated. Because of their abundance and saprophytic aggressiveness, they have many times been considered responsible for diseases that likely are caused primarily by other organisms. Nevertheless, many fusaria are virulent pathogens, causing extensive damage to agricultural crops. The genus *Fusarium* is one of the most difficult in which to distinguish species, undoubtedly because of the great variability of its members. A classification system devised by Wollenweber (Wollenweber and Reinking, 1935) divides the genus into sixteen sections and a number of subsections. This system was later modified by Snyder and Hansen (1940, 1941, 1945), who developed a new species concept for the fusaria. Their studies on variability made it clear that the number of valid species in the genus should be reduced. The newer classification is based on the relative stability of some morphological characters as determined by the single-spore culture method. Spore shape has proved to be one of the more stable characteristics, far more reliable in taxonomy than sclerotium formation or size, spore septation, or spore dimensions. Unsuited for the separation of species are physiological characters such as colony pigmentation, ecologic preference, rate of growth, homothallism or heterothallism, and pathogenesis. Nine species are now recognized as valid (Snyder and Hansen, 1954*a;* Snyder and Toussoun, 1965). Figure 2 is a diagram of the classification of the *Fusaria.*

In addition to the macroconidia, *Hypomyces solani* f. sp. *cucurbitae* asexually produces microconidia and chlamydospores. Many strains also produce small sclerotia in culture. On the basis of the shape of the macro- and microconidia and the position of the chlamydospores, the imperfect stage belongs to the species *Fusarium solani,* which combines three species, seven varieties, and three forms of the *Martiella* section of Wollenweber (Snyder and Hansen, 1941). *Fusarium solani* is a very large species, of which the pathogenic clones represent only a small fragment. The ability of this organism to attack members of the family Cucurbitaceae distinguishes it from all other clones of the species and justifies the trinomial *F. solani* f. sp. *cucurbitae.*

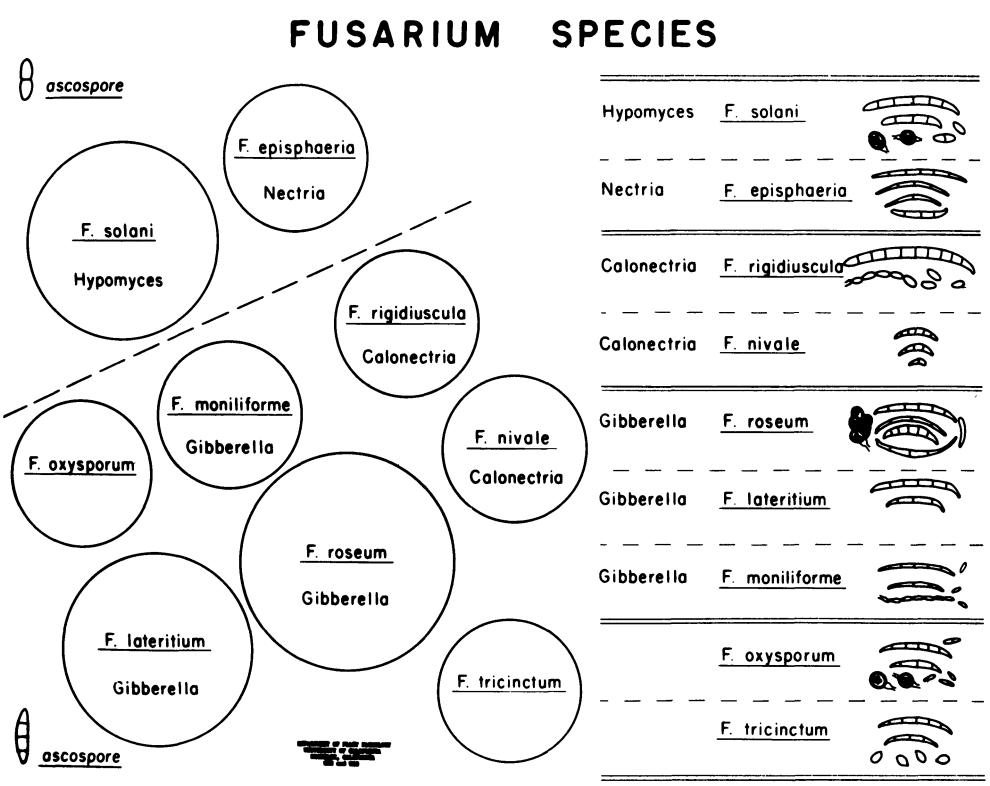

Fig. 2. Diagrammatic representation of the species in Fusarium. Right: the nine species are designated together with their perfect stages, where known. The drawings accompanying the names depict the general morphologic bases for the separation of the species: the shape of the macroconidia; the presence or absence of microconidia, their shape, and whether they are borne in chains or not; and the presence or absence of chlamydospores. It is not possible in such a chart to show all the variation within a species; for example, in some isolates of *F. episphaeria* chlamydospores may occur, whereas in some clones of *F. roseum* they may be absent. Left: the broken diagonal line separates the species into two large groups on the basis of ascospore morphology. Species producing a one-septate ascospore at maturity, usually constricted at the septum, are *Nectria* and *Hypomyces* as indicated by the names in the circles, whereas those having usually two- to four-septate ascospores at maturity are *Calonectria* and *Gibberella*. The size of the circles indicates an approximation of the relative extent of variability in each of the species. *Fusarium oxysporum* and *F. tricinctum* are placed in the group with the *Gibberella* species because of certain similarities.

The perfect stage

When sexual reproduction occurs, the perfect stage of the fungus is produced. The perfect stage is described here, and the genetic requirements for sexual reproduction to take place are discussed in the section on sexuality.

The female sexual organ is the ascogonium, but no specialized male organs, such as the antheridia of *Venturia inaequalis* (Keitt and Langford, 1941), are produced. Any nucleated part of the living thallus can act as the fertilizing agent. The ascogonium is located within a protoperithecium, which develops into a perithecium when the ascogonium is properly fertilized. How fertilization takes place has not been studied in detail. It is assumed that the

process is similar to that observed in *Neurospora sitophila,* in which also all living cells of the proper strain are capable of inducing transformation of protoperithecia into perithecia. In *N. sitophila* a trichogyne system originates from the ascogonium. When branches of the trichogyne come in contact with cells of a complementary strain, a union is effected and the greater part of the protoplasm of the donor cell enters the trichogyne through a narrow cytoplasmic bridge (Backus, 1939).

The transfer of nuclei from the donor to the recipient organ has not been followed microscopically in *Hypomyces solani* f. sp. *cucurbitae,* and no trichogynes can be recognized by color or thickness among the mycelial threads that exist around or on the protoperithecia in unspermatized cultures. Indirect evidence, however, points to the existence of specialized receptive hyphae. First, no fertilization results when compatible cells are placed on the hyphae at a distance from protoperithecia (Hirsch, 1948). Second, when a culture bearing protoperithecia is flooded with a conidial suspension from a compatible strain, as described later, the erect hyphae observed on the protoperithecia fall on the surface of the culture and do not reappear in the following days. In contrast, when the spores are taken from an incompatible strain, the threads reappear and can be seen under the dissecting microscope after the first day, as if sterile water had been used. These threads remain in place until conidia of a proper strain are added. When mixtures of spores of two compatible strains are plated thickly in the same plate, of the many protoperithecia that form, few produce perithecia unless the plate is flooded with sterile water. This also indicates that the transfer of nuclei takes place through hyphae normally not in contact with the surface of the rest of the thallus. Even in a homothallic clone of the same species, flooding increases the formation of perithecia (Hwang, Hansen, and Snyder, 1947).

Perithecia bearing mature ascospores are usually produced with 8 to 10 days of spermatization. They are bright-colored and fleshy, characteristic of the family Hypocreaceae of the Sphaeriales. Atypical perithecia, including nonostiolate ones, are occasionally produced. Such atypical perithecia of mutant strains of race 2 of the cucurbit pathogen have been described in some detail by Toussoun (1957). One particular mutant in this race produced only spherical, nonostiolate perithecia—always smaller than usual, but fertile, though incapable of liberating their ascospores. Only a very few of these perithecia were observed to develop a beak with an ostiole after several months. A similar cleistothecial strain has recently been described in *Gelasinospora calospora,* and the significance of the observation to the pyrenomycete systematics has been discussed (Maniotis, 1965). The production of sterile perithecia in our race 1 is mentioned in the following section.

Typically the perithecia are borne on the surface of the medium, have a beak, an ostiole (occasionally two), and a rough outer wall, and contain a large number of asci. The ascospores—for the most part, eight per ascus—are hyaline, always two-celled, often slightly constricted at the septum, and minutely striated. The color of the ascospore ooze is mentioned later. Two genera with one-septate ascospores are recognized (Dingley, 1951) in the Hypocreaceae: *Hypomyces,* with apiculate, and *Nectria,* with non-apiculate, ascospores. If this criterion were accepted, the cucurbit-rot fungus would be a *Nectria.* It was named *Hypomyces* by Snyder and Hansen, following Wollenweber (1913), who was of the opinion that the apiculate character is a stage in development of the ascospores, and classified all nectrioid chlamydospore producers in *Hy-*

pomyces. Following Booth (1960), Dingley (1961) created a new variety of the species, *Nectria haematococca,* for this pathogen, though she apparently has not examined its perfect stage. Whatever the proper name of the organism is, all papers on its genetics and ecology have referred to it by the trinomial *H. solani* f. sp. *cucurbitae,* which this publication follows for the sake of continuity.

Relations to environment

In most of the work in this laboratory, the fungus has been grown on potato-dextrose agar, pH 6.2, prepared in our laboratory. For study of variation and inheritance of morphological characters, isolates were grown from single spores on the same batch of medium and under the same temperature and light conditions. The wild-type strains used have no special nutrient requirements beyond a carbon source, a nitrogen source, and a few inorganic salts. Nutritionally deficient mutants have been produced by mutagenic treatments. Czapek's Dox agar supports growth and production of asexual spores, but pigmentation and production of sporodochia and protoperithecia are greatly reduced or absent. The effect of a number of nitrogen sources and of carbon/nitrogen (C/N) ratios on the number of perithecia and on the ability to produce ascospores was studied recently (Hix and Baker, 1964). Not only the kind of amino acid but also the isomer is probably important for production of the perfect stage, as has been found for race 2 (Toussoun, 1962). Perithecia have not been found in nature. This cannot reflect unsuitability of substrate, since the perfect stage is produced readily on artificially inoculated fruits when the proper genetic factors are present. These factors, however, have never been found in the same field. It is also likely that environmental conditions are not conducive to formation of the perfect stage at the time susceptible tissues are available, at least in California.

The disease symptoms caused by *Hypomyces solani* f. sp. *cucurbitae* were described recently (Toussoun and Snyder, 1961). This pathogen is the only forma of *Fusarium solani* known to infect the seed internally. Up to 100 per cent of the seed may be invaded. Other formae may be disseminated with seed but be carried on the outside (Nash and Snyder, 1964). The disease occurs only occasionally in plantings of susceptible varieties, but when it does it may cause up to 75 per cent loss. Its sporadic appearance seems to be due to the limited longevity of chlamydospores in the soil (Nash and Alexander, 1965). Improved ability to clean up infected seed lots with aging may be similarly explained.

METHODS OF STUDYING *Hypomyces* GENETICS

This section briefly describes how the perfect stage of *Hypomyces solani* f. sp. *cucurbitae* is obtained, and the progeny are analyzed.

When compatible strains of *Hypomyces* are grown together in the same plate, few perithecia will develop, though protoperithecia of both strains may be present. The sexual fruiting bodies are obtained in large numbers if the recipient strain is grown under favorable conditions in PDA slants until it has developed protoperithecia and is then spermatized with cells from the donor strain. This spermatization is usually accomplished by pouring a suspension of conidia from the donor strain on the recipient culture. The slant is left tilted for a few minutes, and the excess water is then poured off. This method of mating permits accurate knowledge of the direction of transfer of nuclei in

a particular cross, the importance of which is emphasized later. As shown in crosses of strains carrying different alleles for perithecial color (referred to later), all perithecia that are produced following this kind of mating derive from protoperithecia of the recipient culture. Not until a month or more later may small thalli of the donor strain inside the culture tube form protoperithecia. Some of these protoperithecia may develop from the reciprocal cross or be fertilized by recombinant ascospores (secondary fertilization). Obtaining progeny during only the first 10 to 25 days after spermatization avoids such perithecia. The cultures used are always kept on a laboratory bench in the presence of diffuse daylight.

For random analysis of progeny, the ascospore mass exuded from the ostiole of the perithecium is removed with the tip of a sterile, cool, and slightly wetted transfer needle under a dissecting microscope. The spore mass is then suspended in a small volume of sterile water, and the suspension is poured on a thin layer of solidified water agar in a Petri dish. An adhesive substance coating the ascospores makes it necessary to soak the ascospore mass in the water blank, preferably for one to two hours with periodic agitation, so that spores will be separated. Since some clumping may still remain, the spores are checked under the compound microscope before being transferred (Georgopoulos, 1963*a*).

Tetrads are obtained 8 to 10 days after spermatization. The spore ooze is first removed from the perithecium, which is then carefully picked up with a fine forceps, with as little conidial and mycelial contamination as possible, and transferred to a drop of sterile water in a sterile Petri dish. After the perithecium is washed in a number of such drops, it is crushed with a needle, and asci are thus extruded, in various stages of development, singly or in groups, as well as free ascospores. With a little experience, suitable asci (i.e., asci in which the spores are mature enough to germinate but still all inside the ascus) can be recognized under the high power of the dissecting microscope. Such asci are picked up singly with a small droplet of water in a capillary, and transferred to a layer of water agar in a Petri dish. After 6 to 9 hours, the asci, all of the ascospores of which will probably germinate, can be recognized by initiation of germination in some of their ascospores, and by considerable swelling of the others. A block of water agar with the ascus is then transferred to a flamed slide and placed on the stage of the compound microscope. The ascus is dissected with a micromanipulator and a very fine and inflexible glass needle. Ascospores, thus well separated, are allowed to germinate and establish in the agar before they are transferred. This technique of obtaining and dissecting single asci in *Hypomyces* involves minor modification of the technique described by El-Ani (1952).

THE GENETICS OF CULTURAL APPEARANCE

The most striking difference in cultural type in *Hypomyces solani* f. sp. *cucurbitae* is associated with dual phenomenon: the existence of two types, conidial (sporodochial) and mycelial. The former produce colored sporodochia with large numbers of macroconidia and relatively scant mycelium, and the latter give more abundant mycelial growth, no sporodochia, few macroconidia, and relatively numerous (as compared with the wild type) microconidia, and lack pigmentation. It has been shown (Hansen and Snyder, 1943) that mycelial types arise from conidial types by a single-gene mutation. The

same pair of alleles is related directly to sexual behavior; its effects are discussed in the sections on sexuality.

The mycelial types are always white, whereas the sporodocial strains of *Hypomyces solani* f. sp. *cucurbitae* may vary in color from light blue to dark brown when grown on PDA in diffuse daylight. This color is associated with the conidia, not the mycelium. Single-spore transfers of sporodochial strains maintain the same color if grown under the same conditions, unless some have mutated. However, this character not only is highly influenced by the environment but also seems not to be inherited in a simple Mendelian fashion. When two conidial strains, widely different in pigmentation, one brown and one blue, were crossed, and 70 of the resultant ascospores were cultured under identical conditions, only a few looked like either parent. In the majority the color was somewhere between brown and blue, preventing classification of the progeny on the basis of color (El-Ani, 1952). Apparently, each parent carries a number of genes related to pigmentation, and reassortment of the genes from both parents in the products of meiosis determines to what extent each ascospore isolate will look more brown than blue, and vice versa.

The woolly mutants have been described (El-Ani, 1952) as characterized by irregular clumps of conidia and aerial mycelium. The mutation has been observed to occur in both the sporodochial and the mycelial types. Crosses to the wild type produced a 1:1 ratio of normal to woolly. A cross of woolly × woolly produced no wild-type progeny.

Another cultural type, termed "button" (Prasad, 1949), is genetically controlled and is characterized by a leathery texture and complete lack of aerial mycelium, in addition to slow growth on PDA. No button × button crosses have been studied, and it is not known whether button-type mutants may result from mutations at different loci. One such mutant was found to grow more or less normally on PDA supplemented with biotin; with the amount of biotin normally present in PDA, the strain could grow only as button (Prasad, 1949). It is not known whether additional biotin would increase the production of mycelium by other button-type strains. Some of these mutants are not known to produce any protoperithecia; others produce a few. Genetic evidence has been obtained (Georgopoulos, 1963*b*) that the inability of one such mutant (*b*-55) to produce protoperithecia has resulted from mutation other than at what is discussed later as the locus for femaleness. The effect of the button mutations on sexuality is probably indirect and may be overcome by proper nutrition, in contrast to the main two sex mutations, to be discussed.

Completion of the sexual cycle is prevented by a mutation that also produces large amounts of dark pigments, which diffuse in the agar medium on PDA. Protoperithecia are not prevented, and size increases some in response to the addition of cells from a compatible strain, but the perithecia are atypical in appearance and never contain asci and ascospores (Georgopoulos, 1963*b*). The dark pigmentation in this case is controlled by one locus (*stp*-4), and the sterile perithecium character is always associated with the dark pigments in the progeny. It is therefore likely that the effect of this mutant gene on sexuality is also indirect, through the production of some toxic metabolites, perhaps the pigments themselves. Filtrates from a sterile culture of the homothallic *Sordaria fimicola* have been shown (Carr and Olive, 1959) to contain thermostable substances that are able to prevent sexual reproduction in normally self-fertile strains. For these reasons the *stp*-4 mutation was discussed here instead of in the sections on sexuality.

INHERITANCE OF COLOR OF PERITHECIA AND ASCOSPORES

Snyder (1940) showed that strains of *Hypomyces solani* f. sp. *cucurbitae* produce protoperithecia which are either red or white. No strain known to produce protoperithecia of one color has been observed to give rise to mutants with protoperithecia of the other color or any intermediate. Strains of both kinds are found in nature. It should be noted that of all clones of *Fusarium solani,* homothallic or heterothallic, that are so far known to reproduce sexually, the only ones known to produce perithecia of a color other than red are the white-protoperithecial strains of *H. solani* f. sp. *cucurbitae.* Probably the latter genotype has arisen from that of the red protoperithecium by mutation for the particular pigment biosynthesis. Other pigments, located in other parts of the thallus or diffusing into the medium, are not affected. The fact that the color of the protoperithecium is controlled by one pair of alleles suggests that probably one pigment is involved, and its identification would undoubtedly make an interesting study of gene action. The color of the perithecia that develop after fertilization is determined only by the genotype of the recipient strain. This is to be expected if the perithecial wall is produced by proliferation of cells derived from only the recipient thallus. If the donor strain carries the gene for the other color, half of the progeny produces red protoperithecia, and half white, for the two genes are allelic (Snyder, 1940; Georgopoulos,

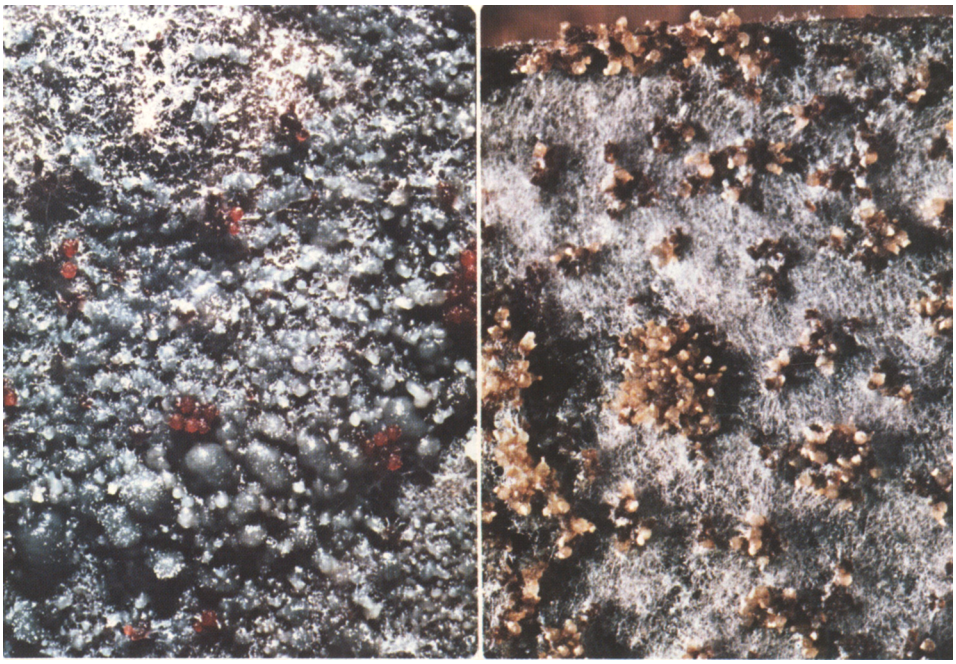

Fig. 3. Red (left and white (right) perithecia of *Hypomyces solani* f. sp. *cucurbitae* race).

1963*b*). The difference between the two colors remains quite distinct during perithecial development and aging (fig. 3). When strains differing at the perithecial color locus are mated, no doubt is left as to the direction of the cross. This helps to determine the sexual behavior displayed by each parent (discussed later in this paper). Of course, other differences in perithecium morphology, e.g., short-neck versus long-neck in *Ceratostomella* (Olson, 1949*b*), can be used similarly.

We have made no attempt to exhaust the literature on perithecial color mutations in the Sphaeriales, though they do not seem to be common. In *Neurospora crassa,* the pigment in protoperithecia is melanious in nature. Hirsch (1954) observed that environmental conditions affecting tyrosinase activity and melanogenesis similarly affect the production and normal functioning of protoperithecia. In *Hypomyces* no correlation exists between red protoperithecial pigment and sexual fruit-body formation. White-protoperithecial strains are as fertile as red-protoperithecial strains, and white perithecia contain just as many normal asci. On the other hand, the known genic determinant of the production of sterile perithecia, *stp*-4, has no effect on the color of these structures. In fact, through linkage, the large majority of the sterile-perithecial progeny will produce the red pigment (Georgopoulos, 1963*b*). A similar genetically determined difference in perithecial color (brown versus black) exists in *Ceratostomella radicicola* (El-Ani, Klotz, and Wilbur, 1957). Here again, both types have been isolated from nature; it is not known whether the one can mutate to the other, and the difference in pigmentation does not seem to affect the perithecium otherwise. The pigment of *C. radicicola,* however, is not confined to the perithecial wall but also exists in the macroconidia, the pigmentation of which is determined by the same pair of alleles.

In contrast to the color of the perithecium, the color of the ascospores of *Hypomyces solani* f. sp. *cucurbitae* can be used only with difficulty as a genetic marker. The color of the ascospore ooze is a genetically determined character and must be due to differences in spore pigmentation which cannot be recognized when individual ascospores are compared under the microscope. Otherwise, the genotype of the donor strain should not affect the color of the ooze. As described by Georgopoulos (1963*b*), only spore masses from homozygous hyaline × hyaline perithecia are colorless. Otherwise, the difference in ascospore mass color is not difficult to recognize and is not influenced by the pigmentation of the perithecium or the culture bearing it.

INHERITANCE OF PATHOGENICITY AND TOLERANCE TO SOME FUNGISTATIC COMPOUNDS

Hypomyces solani f. sp. *cucurbitae,* like certain other fungi, can be used to develop sectors tolerant to chlorinated nitrobenzenes, such as PCNB and TCNB (Georgopoulos, 1962). Furthermore, it has helped in proving the mutational origin of the sectors by demonstrating a Mendelian segregation in the progeny of crosses to the wild type (fig. 4). Three loci are now known to control tolerance to these fungistatic compounds. Each tolerant gene is epistatic, its presence rendering the phenotype insensitive to the presence of another tolerant or sensitive gene at any one locus and gives the maximum tolerance possible (Georgopoulos, 1963*a*). The same mutations produce tolerance to 2,6-dichloro-4-nitroaniline (DCNA), the active ingredient of the fungicide

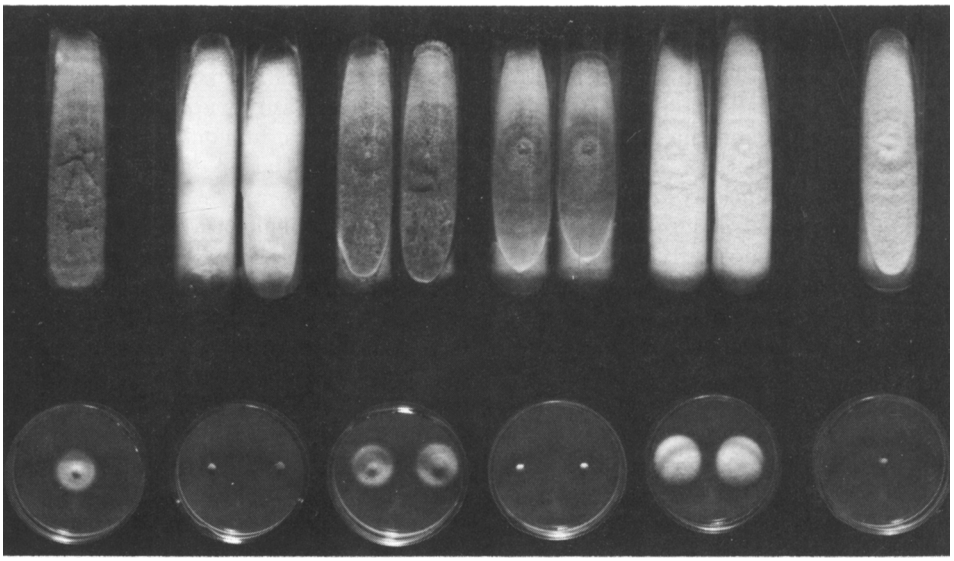

Fig. 4. Segregation for tolerance to 2,3,5,6-TCNB in *Hypomyces solani* f. sp. *cucurbitae*. Between the two parents, one sensitive (right) and one tolerant (left), and the isolates obtained from one ascus; grown on PDA slants (upper) and TCNB-agar plates (lower).

Botran. The biochemical basis of the tolerance is not yet known. Recent studies (Georgopoulos and Vomvoyianni, 1965) have shown that the development of tolerant sectors of *H. solani* f. sp. *cucurbitae* in the presence of diphenyl is the result of mutation at one of the three loci for chloro-nitrobenzene tolerance. Thus, diphenyl and some of its derivatives are similar to the chlorinated nitrobenzenes in their ability to select for the same mutants.

Because strains of *Hypomyces solani* f. sp. *cucurbitae* race 1 are unable to cross with other strains of the same species, even of race 2 (Toussoun and Snyder, 1961), little is known about the inheritance of pathogenicity in this organism. That strains vary in virulence has been shown repeatedly, but complete lack of pathogenicity has not been demonstrated. A study of the pathogenicity of PCNB-tolerant strains showed that at least one gene affecting virulence, directly or indirectly, is closely linked to one of the loci for tolerance (Georgopoulos, 1963*c*). The same locus has shown linkage to mating type (Georgopoulos, 1963*b*). Mention was made earlier of another linkage relationship, that of perithecial color to *stp*-4. A third relationship, that between the sex loci, is referred to later. From El-Ani's (1956) studies the chromosome number is believed to be n = 4, but a report (Howson, McGinnis and Gordon, 1963) does not seem to be in complete agreement.

GENETIC CONTROL OF SEXUAL REPRODUCTION

Concepts of homothallism and heterothallism

The great diversity of sexual processes of fungi is abundantly discussed in the literature. Some reviews are those of Burnett (1956), Olive (1958, 1963), Raper (1960, 1963), and Fincham and Day (1965). In the homothallic fungi, the nuclei which undergo caryogamy and meiosis can be genetically identical. Heterothallism, on the

other hand, requires the bringing together of nuclei that are different at least at one locus. The terms homothallism and heterothallism should refer to physiological phenomena, not to morphology (Korf, 1952).

In some essentially heterothallic fungi, such as *Neurospora tetrasperma* (Dodge, 1927), genetically different nuclei are included in each ascospore at the time of its formation. Single-spore cultures can thus reproduce sexually—a condition known as secondary homothallism. In some otherwise homothallic fungi, such as *Glomerella cingulata* (Wheeler, 1954) and *Sordaria fimicola* (Carr and Olive, 1959), genetic blocks in the sexual process exist in self-sterile mutants. This situation resembles true heterothallism in the requirement for fusion of genetically different nuclei, and has been named (Olive, 1958) unbalanced heterothallism. In true heterothallism, the presence of the second thallus never induces self-fertility; and the self-sterile thalli are never complementary, e.g., never produce self-fertile forms among the progeny. In the successful matings of self-sterile mutants of homothallic fungi, the nuclei differ in usually two distinct loci, with complementation between two allelic genes being, of course, a possibility. On the basis of this latter possibility, Olive (1958) suggested that a one-locus heterothallism may have evolved from homothallism by pseudoallelic self-sterility mutations in a compound locus. This hypothesis was later supported by some experimental evidence (El-Ani and Olive, 1962).

The concepts of bi-, uni-, and asexuality

Of the mechanisms which ascomycetes employ to bring two compatible nuclei together for caryogamy, some do not permit recognition of differential male and female organs, while others do. In the first case, it is not possible to determine which component of a cross plays the role of the female and which the role of the male. In the second case, both ascogonia and antheridia are produced by some species, but only ascogonia by others. In this latter category, hybrid perithecia may arise from ascogonia that are heterokaryotic in origin, as is probably the case in *Sordaria fimicola* (Carr and Olive, 1959). This condition, again, prevents distinction between female and male. In many cases, however, production of the ascogonium is independent of the presence of both nuclei, e.g., the protoperithecia are produced by the one thallus but do not develop until the second kind of nucleus is brought to the ascogenous area (Sansome, 1946). If we consider sex as a function in these fungi, femaleness is the ability to produce protoperithecia that will give normal asci when provided with the compatible nucleus, and maleness is the ability to donate that nucleus.

It is possible for a strain to possess both femaleness and maleness, as defined above, and such a strain is termed bisexual or hermaphrodite. When only one of the two capabilities is present, the strain is unisexual female or unisexual male. Asexuality is the complete inability of a thallus to participate in sexual reproduction, either as a recipient or as a donor. Distinction may sometimes be difficult between strains of the above four types in a fungus that permits recognition of the two sex functions. In *Hypomyces solani* f. *cucurbitae* it has been greatly facilitated by the differential pigmentation of the protoperithecia and the technique by which cultures are mated in the laboratory.

Compatibility heterothallism

Of the many isolates of *Hypomyces solani* f. sp. *cucurbitae* obtained from various regions and the many laboratory mutants and ascospore isolates that have been examined, none has been

TABLE 1
RESULTS OF SPERMATIZATIONS BETWEEN UNISEXUAL STRAINS
OF *Hypomyces solani* f. sp. *cucurbitae*

Culture being conidiated		Culture providing conidia		Production of perithecia
Mating type	Unisexuality	Mating type	Unisexuality	
A	♀	a	♂	Yes
A	♀	A	♀ or ♂	No
a	♀	a	♀ or ♂	No
a	♀	A	♂	Yes
A	♀	a	♀	No
A	♀ or ♂	A	♀	No
a	♀ or ♂	a	♀	No
a	♀	A	♀	No

found capable of self-fertilization. This is due primarily to a true balanced one-locus heterothallism, which has been named (Snyder and Hansen, 1954*b*) compatibility heterothallism. Two alleles, *A* and *a* (or + and −), control this compatibility reaction. All perithecia of *H. solani* f. sp. *cucurbitae* are hybrid for the compatibility locus. Of the ascospores of any one ascus examined, half have produced *A* and half *a* mycelia (see diagram of fig. 5), independently of the reassortment of other genes known so far. Over the many years that a number of isolates have been maintained by single-spore transfers, no mutation at the compatibility locus has been encountered (Georgopoulos,

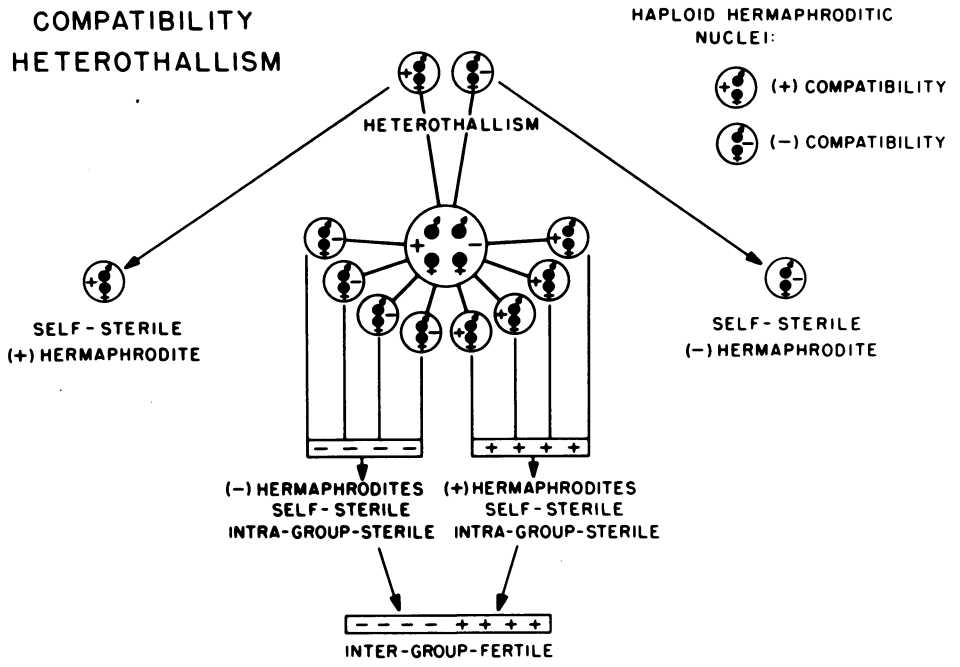

Fig. 5. Heterothallism in *Hypomyces solani* f. sp. *cucurbitae* in the absence of sex mutations.

1963*b*). In this respect, *Hypomyces* resembles *Neurospora* and is different from *Saccharomyces,* in which compatibility mutations appear to be common. Both compatibility types have never been found in the same field. In fact, as discussed in a following section, the two types still seem to be widely separated geographically.

It is assumed (Burnett, 1956; Olive, 1963) with respect to the phylogeny of homothallism and heterothallism that either may evolve from the other. Apparently, evolution of saprophytism and parasitism may also proceed in both directions. Saville (1955) suggested that saprophytes have generally arisen from parasitic forms, but he recognized that saprophytes may have returned to a parasitic habit. Whatever the direction of evolution in the fusaria, it is of interest that parasitic ability is associated with compatibility heterothallism within the species *Fusarium solani.* Though nonpathogenic heterothallic clones have been obtained, no pathogenic homothallic formae are known. The report of a homothallic strain of *Nectria haematococca* pathogenic to pea (Buxton, 1959) is apparently in error (Snyder and Alexander, 1961; Reichle, Snyder and Matuo, 1964). A one-locus heterothallism exists in the mildly pathogenic forma *Hypomyces solani* f. sp. *batatas* (McClure, 1951), as well as in race 2 of the cucurbit rot fungus (Toussoun and Snyder, 1961) and the pea pathogen (Reichle, Snyder, and Matuo, 1964). Pathogenic formae reported from Japan to reproduce sexually, namely *H. solani* f. sp. *mori* (Sakurai and Matuo, 1959), *H. solani* f. sp. *xanthoxyli* (Sakurai and Matuo, 1961), and *H. solani* f. sp. *robinae* (Matuo and Sakurai, 1965), are also heterothallic in this compatibility sense. But, as stated earlier, *H. solani* f. sp. *cucurbitae* does not cross with any of the other formae. It is also impossible to cross race 1 with race 2 (Toussoun and Snyder, 1961). There is probably no other similarity between the two races except in the ability to attack the cucurbit fruit. In other species of *Fusarium,* homothallic pathogenic formae are common e.g., in *F. roseum.*

Sex mutations

Hypomyces solani f. sp. *cucurbitae* is a monoecious fungus (Hansen and Snyder, 1943; Whitehouse, 1949), and its wild-type strains are bisexual (♀). Two mutations (*b*-55 and *stp*-4) imposing apparent unisexuality were mentioned earlier as having an indirect and probably surmountable effect on the female sex. Further, these mutations have been observed only in the laboratory and are very rare in comparison with the sex mutations to be discussed now. The sex mutations are two, one preventing femaleness and one preventing maleness. The respective loci have been named *c* and *m* (El-Ani, 1956; Georgopoulos, 1963*b*). No gene affecting both femaleness and maleness is known. This, of course, indicates that the two functions are distinct.

Mutation at locus *c* prevents the production of protoperithecia, the mutant being morphologically as well as functionally devoid of femaleness (unisexual ♂). Mutation at the locus *m* does not affect the production of any organs, since there are no specialized male organs, the mutant being morphologically indistinguishable from the wild type but functionally a unisexual female (♀). The requirements for a c^+ and an m^+ for femaleness and maleness, respectively, are absolute and not quantitative. A *c* mutant (♂) is not known to produce even one protoperithecium under any circumstances, and no *m* mutant (♀) has ever produced a conidium capable of functioning as a fertilizing agent. A strain that is mutant at both loci is asexual or neuter (⇌). Mor-

phologically, it is indistinguishable from the unisexual male.

Lack of femaleness

In *Hypomyces solani* f. sp. *cucurbitae,* though this may not be so in other fungi, the locus *c* is also responsible for the dual phenomenon referred to earlier. A strain devoid of femaleness can thus easily be distinguished from the wild type after a few days of growth, and long before the appearance of protoperithecia in the latter. Mutation from the hermaphrodite to the unisexual male is very common in the laboratory, with the reverse mutation never observed. In nature, unisexual males are not common. This, of course, might well be due not to infrequent appearance of the mutants in the field but to reduced ability to survive. This could result from reduced pathogenicity or reduced chlamydospore formation or longevity, or from any combination of these factors. Variation is probably great in these respects also. For example, one of the mycelial strains of the collection is highly pathogenic, whereas others are not.

Lack of maleness

Mutants at the *m* locus (♀) remain conidial types, like the bisexual strains. Though they produce large numbers of conidia, none of these conidia or any other cells of the thallus are capable of fertilizing protoperithecia of the opposite mating type. All are normal in every other respect, including ability to cause disease as well as to reproduce the fungus. They also possess one of the compatibility alleles, which can be determined not only by using them as recipient strains but also by adding their conidia to cultures bearing protoperithecia. In the latter case, this can be accomplished by observing the reaction of the erect hyphae associated with the protoperithecia of the recipient culture, which are probably the trichogynes. The reaction is that of trichogyne relaxation, described in the first section, even if the conidia carry the mutant gene that prevents maleness. This is the only means of determining the compatibility gene carried by a neuter strain of unknown origin. It is not known whether this response of the trichogenes is a response to contact with the conidium, to some dissolution of cell wall, to some cytoplasmic connection, or even to nuclear transfer. All that can be said is that the mutant conidia are incapable of normal function in plasmogamy.

According to Hirsch (1948), filtration-sterilized culture media or extracts from ground mycelia of male strains are not effective in restoring the fertilizing ability of the conidia of the male-sterility mutants. No similar attempts have been made in recent years, and it is not known whether another method of sterilization might show that such preparations carry important factors. Whatever the immediate effect of the male-sterility gene might be, it is important only if present in the genome of the fertilizing parent. This association of the block with only the one sex deserves particular attention. In this case also, mutation to a unisexual (female) is very common whereas the reverse has never been observed. It is certain that mutation at the *m* locus takes place in nature. As described later, the most common North American strain of the fungus seems to be a unisexual female mutant of the *A* mating type.

Sex heterothallism

Independently of whether a fungus is homothallic or heterothallic in compatibility, the existence of unisexual strains imposes a requirement that particular thalli meet for the perfect stage to develop; it is an effective outbreeding device that reduces self-fertility in an otherwise homothallic fungus. In *Ceratostomella fimbriata,* for example, self-

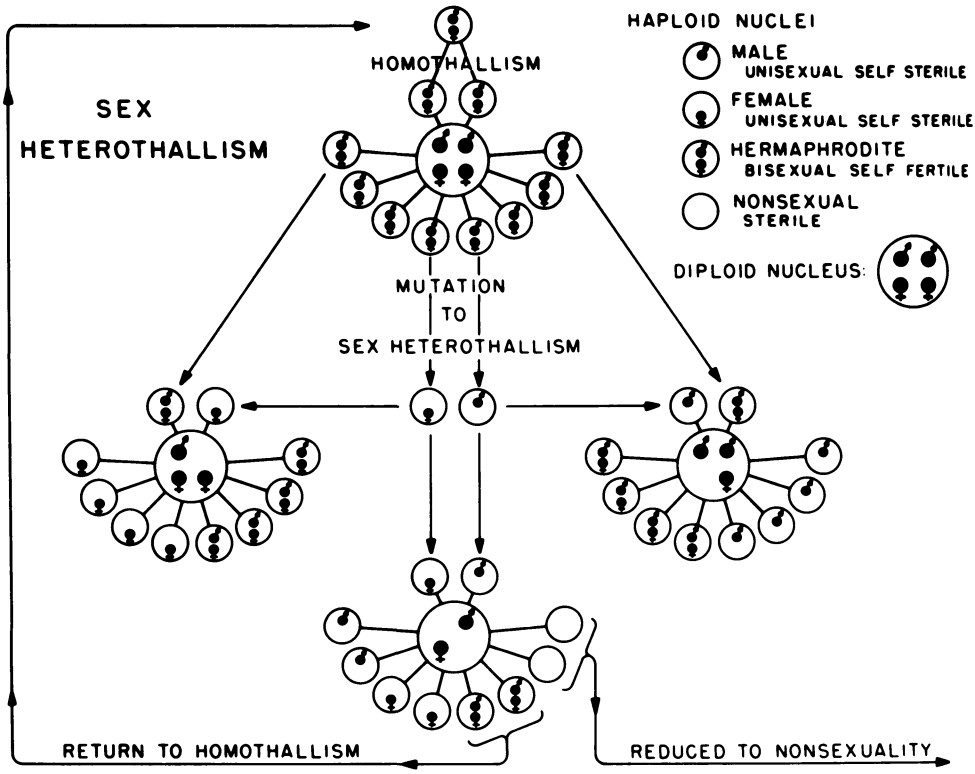

Fig. 6. Heterothallism due to sex mutations in the absence of one-locus incompatibility.

ing is probably the rule as long as the strains remain bisexual. When only unisexual mutants are available (Olson, 1949*a;* Hansen and Snyder, 1952), however, selfing does not occur. In *Hypomyces solani* f. *cucurbitae,* if the strains given are all bisexual, fertilizing *A* with *a* is neither easier nor more difficult than reciprocating. The compatibility in heterothallism is independent of the direction of the cross. The picture is different if some of the strains are not bisexual. If only unisexual cultures are given, of the eight conidiations shown in table 1, for example only two will result in the formation of perithecia, whereas if the cultures given are all bisexual each can fertilize, and be fertilized by, 50 per cent of the others.

In both homothallic (Hansen and Snyder, 1952) and heterothallic fungi (in the usual compatibility sense) that show the sex heterothallism described here, the crosses bisexual × unisexual male and unisexual female × bisexual give rise only to parental types in a 4:4 ratio in every ascus. These results might seem to indicate that sex heterothallism is controlled by one locus with three allelomorphs, one wild-type for the bisexual condition and two mutant, one for female-sterility and one for male-sterility. It is only when two unisexuals are crossed that it becomes evident that the factors for female and male behavior are not alleles, and that when both wild-type genes exist in the same thallus, the thallus becomes bisexual (♂). Random ascospore analyses by Hansen and Snyder (1946) showed that all four "sex types" are obtained when protoperithecia of a unisexual female are fertilized with conidia from a unisexual male. Thus, bisexual strains

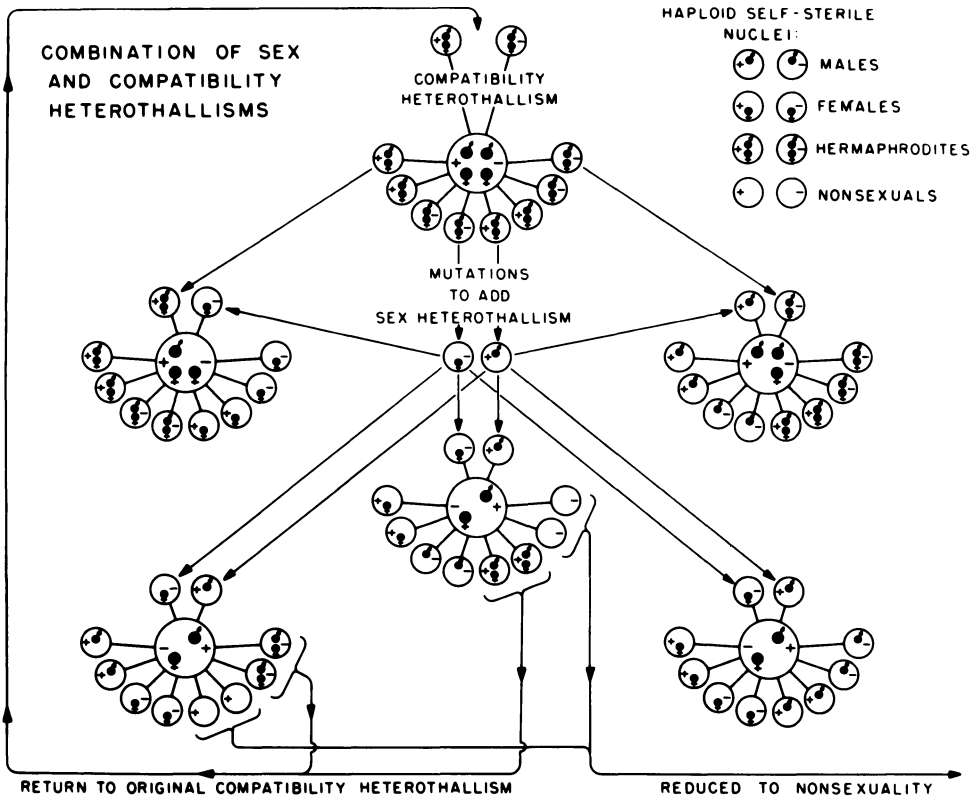

Fig. 7. Combination of compatibility heterothallism and sex-heterothallism in *Hypomyces solani* f. sp. *cucurbitae*.

which may tend to disappear in the absence of reverse mutations may be reconstituted by mating and recombination between two unisexuals. Asexual clones are also produced, not only by mutation but also from recombination. This finding was confirmed by means of tetrad analysis by El-Ani (1954), who recovered parental ditype and tetratype asci from the above cross, and by Georgopoulos (1963*b*), who recognized a nonparental ditype ascus in addition to the above two types. Figure 6 diagrams the genetics of this two-locus sex heterothallism in an otherwises homothallic fungus. Homothallic isolates of *Hypomyces solani* fit into this scheme. Figure 7 combines the two kinds of heterothallism known to exist in *H. solani* f. sp. *cucurbitae*. The symbols ♀ and ♂ are used for the respective functions, as described above, not for the morphology of the female and the male.

The loci for male-sterility and female-sterility are linked. The recombination percentages were found to be respectively 26, 23, and 16 by Hansen and Snyder (1946), El-Ani (1954), and Georgopoulos (1963*b*). Reasons have been given (Georgopoulos, 1963*b*) which might account for these differences. The two loci have been found to be centromere-linked. This was shown by the use of tetratype frequencies in the formulae of Whitehouse (1957). The only data available thus far indicate that the centromere is located between *c* and *m*, and considerably closer to the latter locus (Georgopoulos, 1963*b*).

GENETIC CHARACTERS AND GEOGRAPHIC DISTRIBUTION OF *Hypomyces solani* f. sp. *cucurbitae*

The appearance in a new area of pathogens which are not seed-borne can usually be blamed on surrounding regions. This, of course, is not true for a seed-borne organism, like *Hypomyces solani* f. sp. *cucurbitae,* which may be transferred to very remote places. In such cases, information on inherited characteristics and their stability may aid in determining the probable origin of an organism previously unknown in a region. The cucurbit-rot fungus can serve as an example.

The disease was first described in South Africa in 1932 (Doidge and Kresfelder, 1932) and ascribed to *Fusarium javanicum* v. *theobromae.* Snyder (1938) reported it from California,

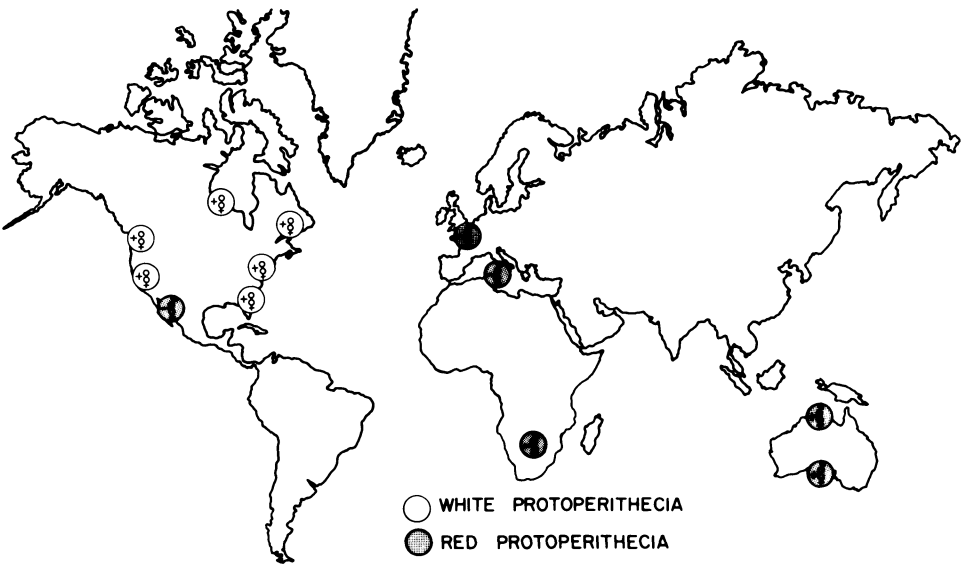

Fig. 8. World distribution of mating types, perithecial color, and sexuality of *Hypomyces solani* f. sp. *cucurbitae.*

where he had repeatedly identified it for some years. All California isolates obtained up to that time had the ability to produce protoperithecia which were white, but the perfect stage was not produced until the South African isolate was obtained and used to spermatize the white protoperithecia. In subsequent years, cultures were received from many areas of the world. Figure 8 shows the world distribution of mating types, perithecial color, and sexuality of these isolates.

Of about 20 isolates obtained from various areas of California and other parts of the United States, and at least three from Canada received between 1945 and 1961, all carry the *A* (+) gene for mating type, the gene for white perithecia, and the mutant gene at the locus for maleness, e.g. all are unisexual females. Considering that this genotype has not been found in other parts of the world, all these North American isolates probably represent a single introduction spread with infected seed. The op-

posite mating type (*a*) was obtained only once from North America (Los Angeles area). This isolate was bisexual and produced red protoperithecia. It was probably introduced from South Africa or Europe, where the same genotype is known to exist. It did not spread, because seed was not maintained from that field, and it apparently did not survive. Between 1951 and 1960 three isolates were obtained from Australia, where the fungus had probably existed since 1923 (Conroy, 1953). They belong to the same mating type as the North American isolates but are all bisexuals and produce red protoperithecia. Only two isolates have been obtained from Europe: one from Italy, in 1945, and one from Holland, in 1965. Both are of the same genotype as the culture obtained from South Africa.

From experience in the laboratory, mutations are common from bisexual to unisexual, but not vice versa. Mating type and perithecial color genes, on the other hand, appear very stable. With this in mind it should be easy in the future to trace the appearance of a new genotype in an area. It is very unlikely, for example, that any of the North American isolates would give rise to a strain that is bisexual or of the $a(-)$ mating type or has the red perithecium gene. If any such strain should be found in the United States or Canada, the chances are that it will be a recent introduction from another country. The original home of the organism is unknown. It may be in a tropical area, with both mating types present and sexual reproduction favored, giving rise to new clones.

COMPARISON WITH THE SEXUALITY OF OTHER FUNGI

Sex heterothallism versus unbalanced heterothallism

Because two complementary genes are involved, the sex heterothallism we have described should be called unbalanced heterothallism, in which two nonallelic sterility mutants are crossed—producing both self-sterile and self-fertile progeny. It is obvious that both balanced and unbalanced heterothallism may exist in the same fungus. The definition of unbalanced heterothallism refers only to the interfertility of the sterility mutants, not to the sexual behavior displayed by each. To produce interfertile mutants in *Hypomyces solani* f. sp. *cucurbitae,* however, one mutation must affect femaleness, and the other affect maleness. Since this condition is not described satisfactorily by the term unbalanced heterothallism, the term sex heterothallism is maintained.

To establish the distinction between sex heterothallism and unbalanced heterothallism, it would have to be shown that, even if the two mutations are nonallelic, the perfect stage is not produced when both mutants lack the same sex in our sense. With the mutants available, this could be demonstrated only in the case of female-sterile strains of *Hypomyces solani* f. sp. *cucurbitae.* No crosses were successful between any two of strains *cm* $^+$ *stp*-4 $^+$ *b*-55, *c* $^+$*m* $^+$*stp*-4 *b*-55$^+$, and *c* $^+$*m* $^+$*stp*-4 $^+$*b*-55, always heterozygous for the $A - a$ locus. This was so when conidia from the one culture were transferred to the other, and when about equal numbers of conidia of the two strains were plated thickly on PDA so that the resulting thalli were in close contact—and probably anastomosed—and flooded with sterile water fifteen days later to assure even better mixing. Of course, there may be a number of reasons for this difference in behavior between self-sterile mutants of *H. solani* f. sp. *cucurbitae* and such fungi as

Sordaria fimicola, in which the nonallelic nature of the sterility mutations seems to be the only requirement (Carr and Olive, 1959) for cross-fertility. First, *S. fimicola* is homothallic in the usual compatibility sense. Second, heterokaryon formation is established in *Sordaria* but appears questionable in *Hypomyces* (Parmeter, Snyder, and Reichle, 1963). Finally, fertilization through the trichogyne seems to be a requirement in *H. solani* f. sp. *cucurbitae,* whereas in *S. fimicola* ascogonia can be heterokaryotic in origin (Carr and Olive, 1959).

It would be interesting to know whether *Sordaria brevicolis,* which is heterothallic and produces protoperithecia and microconidia in the presence of only the one mating-type gene (Olive and Fantini, 1961), gives unisexual female and male strains by mutation at different loci. As far as we know, only femaleless mutants have been observed in this species, but they have not been discussed in detail. The very short generation time of this fungus might make it somewhat difficult to determine what fraction of the perithecia produced after a certain conidiation were derived by fertilization of protoperithecia of one or the other parent. In *Hypomyces solani* f. sp. *cucurbitae* the female-dependence of the perithecium color leaves no doubt in this respect. This characteristic has permitted the following observations, which might help explain sex heterothallism in contrast to unbalanced heterothallism.

Mixtures of conidia of two strains, each carrying different genes for mating type and perithecium color and wild-type or mutant genes at the female- and male-sterility loci, were plated together, and protoperithecia and perithecia were counted 12 days after flooding. Protoperithecia of both colors are produced (with none developing into a perithecium), if both strains are unisexual females, though both mating-type genes (*A, a*) are present. In the homothallic *Sordaria,* intersterility is also the case when perithecial formation is prevented by mutant alleles of the same locus in the strains combined (Carr and Olive, 1959). When an *A* bisexual wild type is combined with an *a* unisexual mutant (or *a* ♀ with *A* ♀ or ♂), only one kind of perithecium develops. If the bisexual strain carries the gene for red protoperithecia and the other strain is a male, only *red* protoperithecia appear, and practically all of them produce asci and ascospores. If the same wild type is combined with a female, large numbers of protoperithecia of both colors appear, but only the *white* ones, those of the mutant, give perithecia. Of 72 red protoperithecia counted in one plate, none developed; and of 56 white ones, all produced normal perithecia. Thus, the close association with the bisexual strain of the opposite mating type, even from the outset of growth, cannot restore the femaleness or the maleness of the mutants. For the same reason, the presence of a self-fertile bisexual apparently does not induce self-fertility of a unisexual female or male in the homothallic *Ceratostomella fimbriata.* Otherwise, perithecia containing only mutants of the one kind (♀ or ♂) should be produced. In *S. fimicola,* induction of self-fertility is very common (Olive, 1956), a difference in behavior that supports the distinction between sex heterothallism and unbalanced heterothallism.

Sex heterothallism in other fungi

We have so far given a detailed account of what we call sex heterothallism. We have accepted that:

1. Sex heterothallism is of mutational origin—the loci recognized as one for femaleness and one for maleness, and not as any two loci imposing sterility, directly or indirectly.
2. Each of the sex mutants is completely unisexual (not dependent to any de-

gree on environment or other genes) and consequently self-sterile, even if the organism does not display compatibility (balanced) heterothallism.

3. The unisexuality of the mutant is not affected by the association with the bisexual (and self-fertile, in the case of fungi homothallic in the compatibility sense) wild type.

Following the recognition of sex heterothallism in *Hypomyces solani* f. sp. *cucurbitae,* similar phenomena were observed in other *Hypocreaceae* with a *Fusarium* imperfect stage. Gordon (1961) gave a brief report on species of *Calonectria, Gibberella,* and *Hypomyces,* some homothallic and some heterothallic, obtained in his laboratory. In some of the cultures, unisexuality was observed, but in the only phenotypic ratio given, the sex types were apparently misprinted. The genetic nature of the unisexuality seems established from the abstract, but it is unfortunate that details are not available. Of 40 strains of the reportedly pathogenic forma *H. solani* f. sp. *mori* that were examined by Sakurai and Matuo (1959), five were found to be unisexual males, and three asexual neuters. In *H. solani* f. sp. *radicicola* race 2, which is interfertile with *H. solani* f. sp. *pisi,* hermaphroditic, male, and female clones have been found (Reichle, Snyder and Matuo, 1964). In this laboratory, sex heterothallism has been observed in a number of saprophytic fusaria over the years, though no detailed record was kept.

Unisexual strains of a number of Ascomycetes outside the family Hypocreaceae, both homothallic and heterothallic, have been recognized. Some of the earlier reports were mentioned by Whitehouse (1949). In *Neurospora sitophila,* which, like *N. crassa* (Sansome, 1946), probably does not produce heterokaryotic ascogonia, Dodge (1946) observed that one of his stock cultures lost the ability to produce protoperithecia. This deficiency reappeared in approximately 50 per cent of the F_1 when the non-protoperithecial strain was used as a donor. It was further shown that combinations of non-protoperithecial strains of opposite mating types did not result in protoperithecium formation. The situation is similar to that with our unisexual male mutants, which also segregate in a 1:1 ratio when crossed with the hermaphroditic wild type and do not complement one another when combined among themselves. Dodge was concerned mainly with the production versus nonproduction of protoperithecia, and does not seem to have looked for lack of maleness. Unfortunately, little appears to have been done since then on the sexuality of *Neurospora.* Had male sterility mutants been recognized, the situation would be identical with that in *Hypomyces solani* f. sp. *cucurbitae.*

Recent studies by Webster (1967) have shown that both self-sterile, cross fertile males and females occur in *Ceratostomella fimbriata.* With the aid of a white mutant for perithecial color, these studies clearly demonstrate the receptive nature of the female strains in this fungus and provide an example of sex-heterothallism in a normally homothallic organism. As in *Hypomyces solani* f. sp. *cucurbitae* sex-heterothallism in *C. fimbriata* is genetically controlled by non-allelic factors with the unisexual male and female strains arising from the homothallic hermaphroditic strains via mutation. In the related fungus *C. radicicola,* unisexual mutants were observed (El-Ani, Klotz, and Wilbur, 1957) though no details were published.

In many fungi showing compatibility, heterothallism, and sexual sterility mutations, including *Hypomyces solani* f. sp. *cucurbitae* (Hansen and Snyder, 1943; Georgopoulos, 1963*b*), there is no linkage between compatibility and sex loci. This is evidence that sexual differentiation and mating type are of inde-

pendent genetic determination (Whitehouse, 1949). It should be noted, however, that independent segregation of sex and mating type was not observed in *Gibberella cyanea* (Gordon, 1961). In those species of Ascomycetes where both female- and male-sterility mutations have been recognized, the two genes are usually non-allelic. Two apparent exceptions to this generalization are discussed below.

Drayton and Groves (1952) reported that *Stromatinia narcissi* produces two kinds of morphologically distinct thalli: females, producing only apothecial fundaments; and males, producing only spermatia. Of 83 ascospores from a cross, 41 yielded male and 42 female cultures. This condition might be similar to that of *Ceratostomella fimbriata,* except that the loci for maleness and femaleness would have to be linked rather closely, which would require that a larger number of progeny be examined if crossing-over is to be detected. This hypothesis is favored by the recognition of even a very few bisexual strains (Drayton and Groves, 1952).

The second case is that of *Ascosphaera apis* (Spiltoir, 1955; Spiltoir and Olive, 1955). Here, sex organs are not produced as long as the two strains are kept separately. When female and male meet, the former produces ascogonia each consisting of a trichogyne and a "nourishing cell." The trichogynes fuse with hyphae of the male strain, which apparently never produces ascogonia. Only female and male thalli (in a 1.1 ratio) are obtained from ascospores (Olive, 1958), but we do not know the size of the sample that was examined. Further, we are not certain that the trichogyne never fuses with hyphae of a strain also capable of producing ascogonia. If it does not, some of what are called females may be bisexuals.

We believe that sex heterothallism is more common among the fungi than has been suspected. Among the reasons why it has not been recognized in more instances are the peculiarities in the sexuality of many species, mating techniques which do not permit one to know which strain contributed which sexual organ in a cross, and the failure to search for infertility in strains which from their morphology should possess the ability to fertilize. The concept of sex heterothallism introduces the idea that sexual functions may be controlled by two nonallelic genes. It is likely that, in many organisms, individuals considered to be female are bisexual hermaphrodites—hence the acceptance of a one-locus control of sex.

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