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Sexual Hormones of Achlya and Other Fungi

Molecules controlling genesis of sex organs in two fungi and attraction of sperm in a third are characterized.

A. W. Barksdale

Until last year, a structure could not be assigned to any of several substances known to function as regulators of sexual reproduction in fungi. In 1968, structural formulas were proposed for sirenin, a sperm-attractor in *Allomyces*, and for antheridiol, the initiator of sexual reproduction in *Achlya*, and the agents controlling the formation of gametangia in *Blakeslea trispora* were shown to be trisporic acids B and C, carotenogenic compounds isolated from that fungus in 1964. Sirenin is an oxygenated sesquiterpene, the trisporic acids are terpenoid C_{18} carboxylic acids, and antheridiol is a sterol having the carbon skeleton of stigmasterol.

There is evidence (*1*) for hormonal regulation of sexual reproduction in five other genera: the ascomycetes *Ascobolus* (*2*), *Bombardia* (*3*), and *Saccharomyces* (*4*), and the aquatic oomycetes *Sapromyces* (*5*) and *Dictyuchus* (*6*). In addition, compounds similar or identical to those of *Blakeslea trispora* are involved in sexual reproduction of fungi belonging to five other mucoraceous genera: *Mucor, Phycomyces, Rhizopus, Zygorhynchus*, and *Philobolus* (*7*). These genera are but a small minority among the more than 3500 genera of fungi.

The regulatory substances have been termed hormones because the cells that respond to the regulatory molecules are separated in space from the cells that secrete them. The distance between the two kinds of cells is usually not more than a millimeter or two. For the fungi, their environment—either water or air—serves as a medium through which the hormones diffuse. Like other hormones, those from fungi are highly active in minute quantities. Sirenin, which has a molecular weight of 236, is effective in a concentration of $10^{-10}M$ (23.6 picograms per milliliter). Antheridiol exhibits the same order of activity. Though much more hormone is secreted than is needed to elicit a response, the amount secreted is, nevertheless, very small. This is one reason why so few fungal hormones have been isolated.

The Water Molds

The genus *Achlya* belongs to a family of aquatic fungi known commonly as water molds. These molds form a major part of the fungous flora of ponds and lakes, where they grow on twigs, fruits, dead insects, and other organic debris fortuitously provided. Members of the genera *Achlya* and *Saprolegnia* are frequently found in association with diseased and moribund fish and fish eggs. Whether they are the primary cause of disease or are only opportunists has not been established. Among species of *Achlya* isolated from infected fish, *A. bisexualis* and *A. flagellata* are the only ones shown able to grow and develop on experimentally wounded fish, a demonstration which seems to implicate these species as agents of disease (*8*).

Water molds are most abundant in shallow, sheltered water where oxygen and food are plentiful. Here vegetative growth is best. The motile, biflagellate asexual spores of water molds may be

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found in large numbers in the oxygen-rich layers of open water. As many as 5000 of these zoospores per liter have been counted in a sample of surface water taken near the margin of Lake Windermere in England (*9*). The nonmotile sexual spores are thick-walled, and much larger and heavier than the zoospores. They sink to the bottom, where they may lie dormant for some time before germinating.

Water molds have colorless filamentous cells that are large enough to be seen with the unaided eye. The cells—or hyphae, as they are called—are multinucleate and lack crosswalls, except for those delimiting the reproductive organs. A hypha elongates only at its tip. Occasionally, a hyphal tip may branch, but normally branches arise laterally. The branched hyphae form a cottony mesh known as the mycelium.

The asexual and sexual reproductive organs are formed at the tips of hyphae and hyphal branches; rarely, the sex organs are intercalary. Several hours before an organ is delimited, the growth in length is checked and growth proceeds according to a pattern set by the particular organ to be formed. The zoosporangium is usually cylindrical and often little wider than the hypha bearing it. The nuclei enclosed in the zoosporangium become the nuclei of the zoospores without undergoing further division.

The antheridium is cylindrical or lobed, whereas the oogonium is more or less spherical. The nuclei within the sex organs undergo one or two divisions. A single division—a mitotic one—has been reported in all but three of the cytological studies made of gametogenesis; in these three, two meiotic divisions have been reported (*10*). Confirmatory evidence that meiosis occurs during gametogenesis and that the nuclei in the hyphae are diploid has been obtained by Bryant and Howard (*11*), who have made a microspectrophotometric analysis of nuclear DNA in *Saprolegnia terrestris*.

Fusions between vegetative cells are unknown in the Oomycetes, that taxonomic group to which the water molds belong, and plasmogamy occurs only during sexual reproduction. Shortly after the oogonial initial is formed, the antheridial branch begins to grow toward it. Upon reaching the female initial, the branch curves around it (Fig. 1e), and the antheridium is delimited by a crosswall which forms near the tip of the branch. A crosswall at the base of the oogonial initial delimits the oogonium, and within this organ from one to 20 uninucleate oospheres (eggs) are produced. Fertilization is accomplished by the passage of nuclei through fertilization tubes that extend from the antheridium to each egg (Fig. 2a). As the oospore (fertilized egg) matures, the wall thickens and a large eccentric oil globule forms from the coalescence of smaller globules (Fig. 2b).

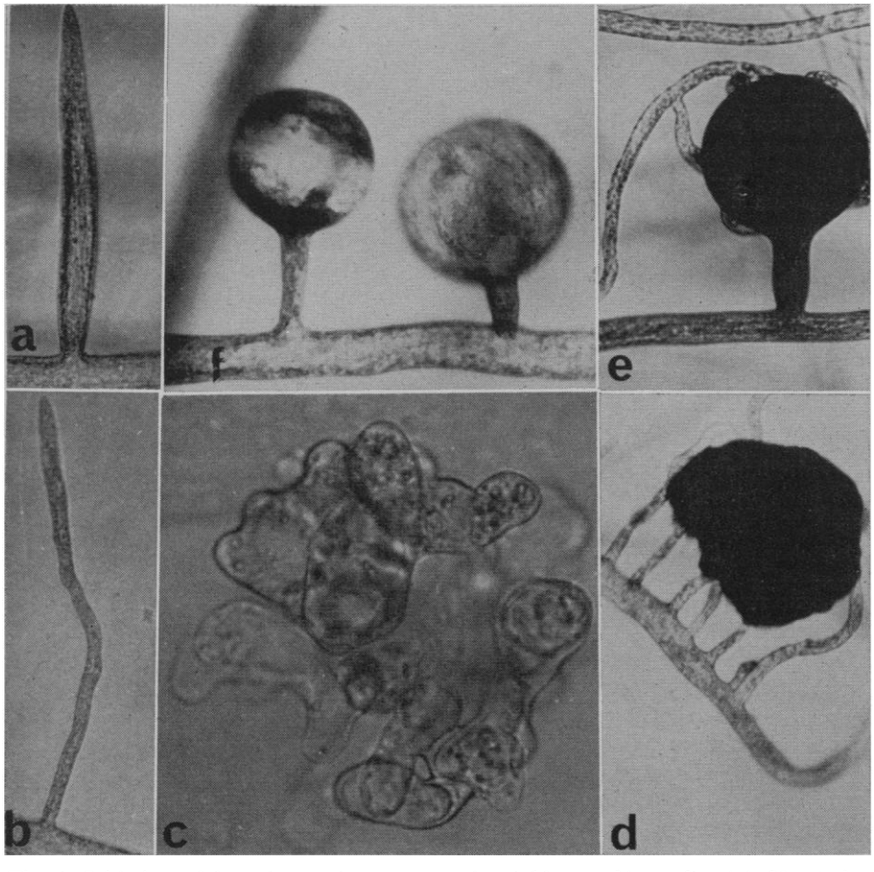

Fig. 1. Initiation of branches and sex organs in *Achlya ambisexualis*. (a) Vegetative branch and (b) antheridial branch, both initiated by antheridiol (× 140); (c) cluster of antheridia induced to form by synthetic antheridiol (× 800); attraction of antheridial branches to (d) antheridiol-treated plastic particle, and to (e) oogonial initial (× 175); (f) aborting oogonial initials initiated by hormone B (× 220).

Discovery of Sexual Hormones in Achlya

In most species of *Achlya* the oogonia and antheridia are borne on the same thallus. There are two species, however, in which the cooperation of two individuals is needed for the production of sex organs. If kept apart, each is usually sterile, but when mycelia of opposite sex are allowed to grow near one another, antheridial branches will develop on one and grow to the oogonial initials produced by the other. The riddle posed by the existence of sterile strains of *Achlya* (strains which failed to produce sex organs in culture) was made somewhat less puzzling by the discovery (*12*) in the closely related water mold *Dictyuchus* that sex organs are formed when two individuals are brought together in the right combination. When a number of sterile *Achlya* strains were paired in various combinations, sex organs were produced by one of the pairs, and this pair was described as a new species, *Achlya bisexualis* Coker (*13*).

Achlya ambisexualis Raper is the other species in which sex organs are produced by the cooperation of two individuals. This species and *A. bisexualis* were used by John R. Raper in his classic experiments showing that sexual reproduction in certain species of *Achlya* is initiated and controlled by diffusible substances reciprocally secreted by the sexually reacting pair. These same substances, or hormones, appear to function also in some of the hermaphroditic species of *Achlya* (*14*).

In Raper's experiments, the molds to be studied were grown on the surface of an agar medium or on halved seeds of the hemp plant, *Cannabis sativa* (Fig.

3). Of the many kinds of insects, larvae, and seeds tried as substrates for cultivation of water molds in the laboratory, the seed of hemp has proved to be the most satisfactory, and use of these seeds by students of water molds has become standard. The use of hempseed, however, has one drawback. If the seed is viable, the investigator must be licensed to possess a narcotic, since the female hemp plant yields marihuana. Hence, the hempseed used in mycological laboratories has been steam-heated and is nonviable. Since the heat-treated seed is an ingredient in feed for pet canaries, it can be bought from a supplier of bird feed.

For convenience in the discussion that follows, I call the thalli that produce antheridia "males" and those producing oogonia, "females." Working with 2- to 4-day-old hempseed cultures, each prepared by placing an inoculated half-seed in a dish containing 10 milliliters of water, Raper found that lateral branches were produced within 4 hours on the hyphae of the male when Seitz-filtered water in which a female had grown was poured over the male. When the water in which a male had grown was poured over the female, there was no response. However, when the water in which the male hyphae had reacted by branching was filtered and poured over a female, oogonial initials were produced. Clearly, a secretion of the vegetative hyphae of the female initiated the sexual reaction by inducing the formation of antheridial branches, and the sexually activated male then secreted a substance that induced the formation of oogonial initials in the female. The female secretion was called "hormone A" and the male secretion was called "hormone B." The growth of the antheridial branches to the oogonial initials and the formation of antheridia, Raper concluded, are controlled by a specific substance secreted only by the oogonial initials. It was postulated that this substance, called "hormone C," had a dual function: attraction of the antheridial branches and delimitation of the antheridium. The cleavage that gives rise to oospheres was attributed to the action of a fourth substance, called "hormone D," secreted by the antheridium. Thus it appeared that each stage in the formation and conjugation of the sex organs was controlled by a different hormone (*15*).

Raper found the number of antheridial branches produced to be directly proportional to the concentration of hormone A. This relationship was demonstrated in the following manner. A filtrate from hempseed cultures of the female was concentrated to one-tenth its original volume by evaporation of water, and from this concentrate a series of dilutions was made, such that they contained concentrations varying from 1/1000 to 10 times the concentration of the original filtrate. A hempseed culture of the male was placed in a sample of every dilution, and for every concentration of hormone the average number of branches produced per 3-millimeter apical segment of hypha was determined (*16*).

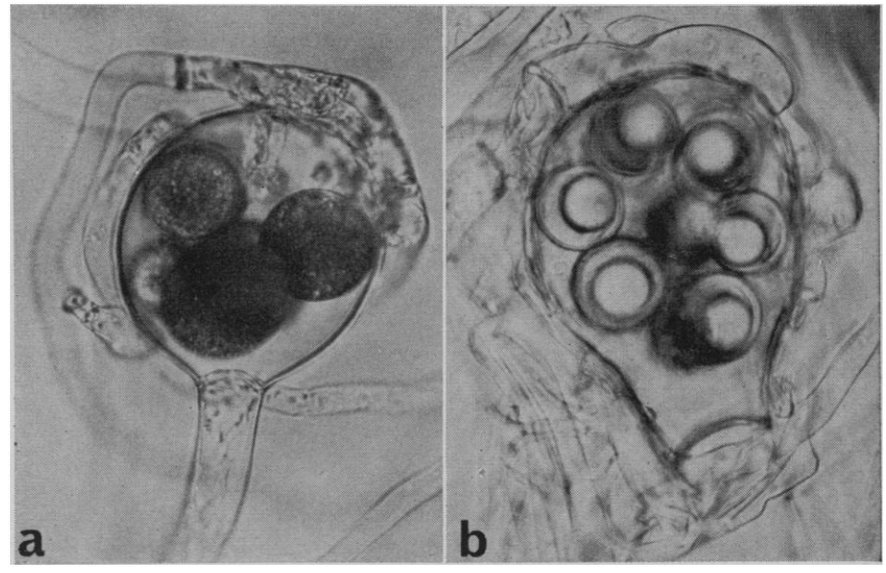

Fig. 2. Sex organs of (a) *Achlya ambisexualis*, showing fertilization tubes extending from antheridium to eggs inside the ooagonium, and of (b) *A. bisexualis*, showing mature eggs (× 560).

In later experiments, in which an acetone extract of hormone A was diluted in distilled water or in a filtrate from hempseed cultures of the male, Raper was able to show that the number of branches produced by the male test plant in response to hormone A was greater when the hormone was diluted in culture filtrate than when it was di-

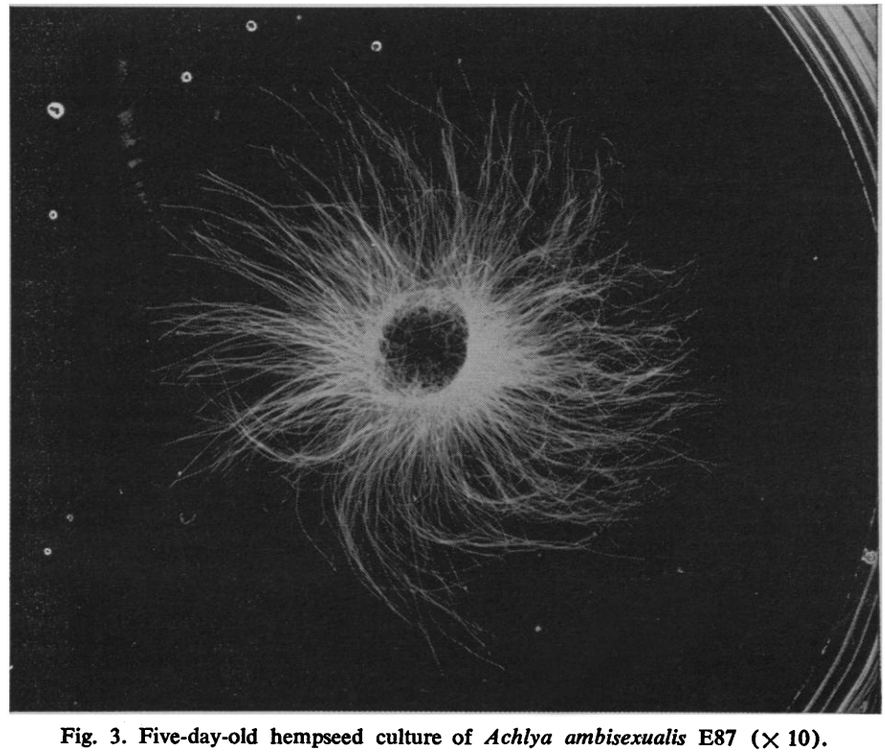

Fig. 3. Five-day-old hempseed culture of *Achlya ambisexualis* E87 (× 10).

Table 1. Effect of various concentrations of nutrient and antheridiol on the mean number of branches initiated per hypha in *Achlya ambisexualis* E87.

Dilution of E-G solution*	Mean number of branches			
	Antheridiol units per milliliter			
	1000	300	100	30
Undiluted	36.0	27.1	24.2	17.6
1:10	33.3	23.9	19.2	13.8
$1:10^2$	34.1	21.3	13.2	10.7
$1:10^3$	30.1	13.6	9.2	8.0
$1:10^4$	22.2	8.1	6.4	6.2
$1:10^5$	18.4	7.1	4.8	6.1
Salt solution†	8.1	5.3	4.6	4.5

* E-G solution contains Edamin (hydrolyzed lactalbumin), 400 mg; glucose, 2400 mg; calcium glycerophosphate, 80 mg; tris(hydroxymethyl)-aminomethane, 100 mg; $MgSO_4 \cdot 7H_2O$, 125 mg; KCl, 150 mg; trace metals; distilled water, 1 liter. † E-G solution minus Edamin and glucose.

luted in water. The augmentation of hormone A activity by the culture filtrate was attributed to the presence of another hormone secreted by the male. This substance was given the name "hormone A^1" (*17*).

Raper and Haagen-Smit were the first to attempt the isolation of hormone A. In the purification procedure that they devised, water was distilled from 1400 liters of filtrate taken from hempseed cultures of a female strain of *Achlya bisexualis*. The 393 grams of dry solids remaining was progressively fractionated until there was obtained about 2 milligrams of an amorphous, creamy white substance that was active in a concentration as low as 10^{-12} gram per milliliter (*18*).

Antheridiol

At the New York Botanical Garden, in 1965, a crystalline compound having hormone A activity was isolated by Trevor McMorris from the culture liquid of *Achlya bisexualis*, strain T5, and to this compound the name "antheridiol" was given (*19*). A structure was proposed for antheridiol by McMorris and his collaborators at the Massachusetts Institute of Technology, G. P. Arsenault and K. Biemann, in 1968 (*20*), and shortly thereafter a team of chemists at Syntex Research, headed by J. H. Fried and J. A. Edwards, succeeded in synthesizing this compound (*21*). Its activity is identical to that of the natural hormone (Fig. 1c).

Pure antheridiol proved to be a colorless substance that is highly insoluble. Its solubility in water and in the common organic solvents, such as chloroform, ethyl acetate, ethyl ether, and acetone, is extremely low. It is slightly soluble in hot methanol but will crystallize out of solution when cooled. It was this property that afforded the means for obtaining crystalline hormone.

Though the pure substance is not soluble in methylene chloride, antheridiol is efficiently extracted from culture liquids by this solvent. Two extractions with methylene chloride remove 80 to 90 percent of the hormone activity. The concentration of hormone in the culture liquid is very low. Our strain T5, the best of a number of female strains tested for hormone A production, secretes only 20 to 40 micrograms per liter. The mycelium contains a small amount of hormone, which can be extracted with methanol or acetone, but the hormone in the mycelium represents only 10 percent of the total activity in the mold culture.

After removal of the methylene chloride, the crude extract can be purified by countercurrent distribution through use of a system of four solvents—water, methanol, ethyl acetate, and petroleum ether (boiling point, 60° to 80°C)—or by chromatography on a silica gel column with ethyl acetate as the eluting agent, followed by preparative thin-layer chromatography with a chloroform-methanol mixture as solvent (*19*). Ambiguous results were obtained in an early attempt at chromatography on silica gel, but this method has been used since with success. The highly active gum resulting from chromatography was made to yield crystalline antheridiol by adding methanol and putting this solution in the cold. Recrystallization from methanol gave colorless crystals, melting at 250° to 255°C and active at a concentration of 6×10^{-12} gram (6 picograms) per milliliter when assayed with *Achlya ambisexualis* E87.

The structure (Fig. 4) proposed for antheridiol was determined from only 20 milligrams of hormone—an exercise in frugality. The molecular weight 470 and the molecular formula $C_{29}H_{42}O_5$ were obtained by high-resolution mass spectrometry. The mass spectrum also provided evidence that antheridiol was a sterol having the carbon skeleton of stigmasterol. Additional information was obtained from the ultraviolet and infrared spectra, which revealed the presence of hydroxyl and carbonyl functions in the molecule. The presence of an α-β-unsaturated γ-lactone and an α-β-unsaturated ketone was indicated by a shift in position of the carbonyl peaks in the infrared spectrum when antheridiol was converted to its tetrahydro derivative. The nuclear magnetic resonance spectrum, obtained with difficulty because of the insolubility of the hormone, confirmed what had been deduced from the other spectra and was particularly important in helping to place the oxygen functions in the side chain.

Table 2. Effect of omitting a carbon or nitrogen source (or both) upon the number of branches produced by *Achlya ambisexualis* E87 in response to antheridiol (2.5 nanograms per milliliter).

Ingredient omitted from E-G solution	Mean number of branches per hypha	Time elapsed before branches appear (min)
None	22.2	75
Glucose	11.4	105
Edamin*	6.0	150
Glucose and Edamin	3.2	240

* Hydrolyzed lactalbumin.

Synthesis of biologically active antheridiol confirms the validity of the structure proposed for this hormone. The starting point for its synthesis was a disnor cholenic acid, readily prepared from stigmasterol (*21*). The synthesis yielded two of the four stereoisomers possible in respect to the asymmetric carbons 22 and 23. One of the isomers has hormonal activity equaling that of the natural substance; the other has 1/1000 the activity of the first. The absolute stereochemistry of the biologically active isomer is not known at present, but in due time it may be determined by x-ray analysis.

Antheridiol appears to function in several ways in the reproductive process of *Achlya* (*22*). It initiates the formation of antheridial branches, and from them it elicits a chemotropic response. It is involved, in a way not yet understood, in the formation of antheridia and is evidently essential for their formation, since antheridia are delimited only on antheridial branches and these can be initiated only by antheridiol. Finally, it stimulates the male to secrete the hormone that induces the formation of oogonial initials.

The concentration of antheridiol determines both the number of branches initiated and the time that elapses between the addition of hormone and the appearance of branches. The number of branches increases with increased concentration of hormone until an upper limit is attained. The length of time before branches appear decreases with increasing concentration until a minimum of 40 to 45 minutes is reached.

In the presence of a high concentration of hormone, as many as 35 to 40 branches may be initiated on a single hypha about 3 millimeters long. The branches initiated by a single dose of hormone make their appearance simultaneously, and for 3 to 4 hours thereafter they elongate rapidly, after which time they grow more slowly. If conditions are right for antheridium formation, the tip of the branch will begin to enlarge, forming a club-shaped or lobed structure (Fig. 1c). Then, by the formation of a crosswall, the antheridium is delimited, and the nuclei within undergo meiosis.

Sources of nitrogen and energy are required for the response to antheridiol (*23*), and it can be shown that the number of branches produced is proportional to the amount of nitrogen and glucose supplied, as well as to the concentration of hormone (Table 1). The experimental results compiled in Tables 1 and 2 were obtained in the following way. The water in which a 42-hour-old hempseed culture of the male strain had grown was poured off and replaced with 10 milliliters of a nutrient solution of specified composition. To this, antheridiol was added, and later the branches initiated on the male hyphae were counted. Omission of the nitrogen source from the nutrient solution resulted in a greater reduction in the number of branches initiated than did omission of the carbon source (Table 2). Omission of both the nitrogen and the carbon sources further reduced the number of branches and greatly prolonged the time before the branches appeared.

Since antheridial and vegetative branches differ in morphology (Fig. 1, a and b), the two kinds of branches can be readily distinguished. Whether the branch initiated by antheridiol will become antheridial or vegetative in form and function is determined by the amount of nutrient available. In the experiment recorded in Table 1, all branches initiated in the undiluted solution became vegetative. In the 1 : 10 dilution, some branches were antheridial and some were vegetative. In all higher dilutions of the solution, all branches initiated developed into antheridial branches.

The "augmentation" of antheridiol activity by exogenously suppled nitrogen and carbon sources is suggestive of the augmentation of hormone A activity reported for hormone A^1 (*17*). Whether any or all of the hormone A^1 activity exhibited by filtrates of hempseed cultures of the male is attributable to hydrolytic products released from hempseed by mold enzymes remains a question (*23*). Nevertheless, the similarity between the activity of hormone A^1 and that of a mixture of amino acids and sugar is striking.

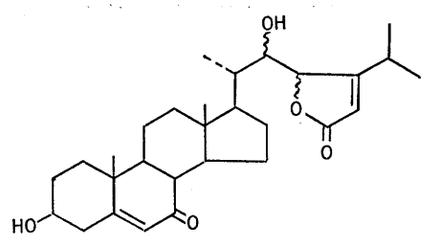

Fig. 4. Structure of antheridiol.

To demonstrate the chemotropic response of antheridial branches to antheridiol, an experiment was devised in which "plastic oogonia" were prepared by allowing antheridiol to be adsorbed on particles of polyvinyl plastic (*24*). When a few of the treated particles were sprinkled over a mat of male hyphae, antheridial branches were initiated in the vicinity of each particle and the branches were attracted to the particle (Fig. 1d). Antheridia were delimited on some of the antheridial branches, but the sexual reaction ended there, since the plastic particles were incapable of forming eggs.

Hormone B

The hormone that initiates the formation of oogonia has not been isolated, and very little is known about its secretion by the male and its action on the female. Scarcity of antheridiol has impeded work with hormone B. Though our supply of antheridiol is still inadequate, we have been able to devise an assay for hormone B and to carry out some experiments yielding information on the conditions of culture which favor its secretion (*25*).

Whereas the growing female mycelium secretes antheridiol continuously, the male secretes hormone B only at the end of the growth phase and only after it has responded to antheridiol. Unfortunately, the male may react vigorously to antheridiol without secreting any hormone B.

Following the addition of antheridiol to a culture of the male strain, the concentration of antheridiol in the culture liquid decreases until very little remains by the end of 2 to 3 hours (*26*). The fate of this added antheridiol is not known. Hormone B can be detected in the culture liquid as early as 2 hours after the addition of antheridiol, and secretion of this hormone may be continued for from 4 to 24 hours.

Hormone B, like antheridiol, can be extracted from the culture liquid with methylene chloride. In fact, residual antheridiol is removed along with hormone B, and the two hormones move at nearly the same rate on a silica gel column or thin-layer chromatography plate.

Since the male strain reacts to antheridiol and not to hormone B and the female reacts to hormone B and not to antheridiol, it is possible to assay for antheridiol or hormone B in the presence of both hormones. The female strain, *Achlya ambisexualis* 734, responds to hormone B more slowly than the male strain, E87, responds to antheridiol. Twelve to 24 hours may elapse before the oogonial initials make their appearance. If conditions are unfavorable for the development of oogonia, the oogonial initials abort rapidly (Fig. 1f).

Trisporic Acid

The system regulating sexual reproduction in *Mucor* and related genera has been investigated almost continuously since Burgeff's initial demonstration, in 1924, of diffusible substances controlling the formation and copulation of the gametangia. In the Mucorales, the gametangia are simple, multinucleate cells that fuse in pairs to form thick-walled zygotes called zygospores. Each gametangium is borne at the tip of a copulatory hypha and is delimited by a crosswall that subdivides an enlargement of the hyphal tip, known as the progametangium. Certain species are composed of two kinds of individuals differing in mating type and referred to as the plus and minus strains. Burgeff (*27*) showed that hyphae of the plus and minus strains of *Mucor mucedo* produced progametangia and were attracted to one another even when separated by a collodion membrane. He also noted that the hyphae on either side of the membrane turned yellow. This increased pigmentation associated with sexual reproduction was later found to result from increased synthesis of carotenes.

As a consequence of the colorful manner in which their sexuality is manifested, interest was aroused in the relationship between sexuality and carotene synthesis in these molds. It is true of a number of species that the plus and minus strains grown in mixed culture

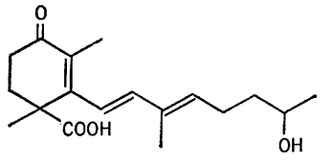

Fig. 5. Structure of trisporic acid C.

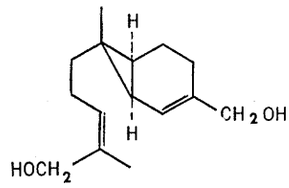

Fig. 6. Structure of sirenin.

produce more carotene than either strain cultured alone (*28*). The increase in carotene production achieved in a mixed culture of *Blakeslea trispora* is particularly striking, and it was from a mixed culture of plus and minus strains of this species that several closely related terpenoid C_{18} carboxylic acids were independently isolated in 1964 by American (*29*) and Italian (*30*) workers. When added to a culture of the minus strain, these acids markedly stimulated carotene synthesis, but they had little effect on the plus strain. Three of the terpenoid compounds isolated from *B. trispora* by Caglioti *et al.* were given the names "trisporic acids A, B and C," and structures were assigned to trisporic acids C and B (*31*). Trisporic acid C (Fig. 5) was present in the greatest amount, and trisporic acid A in the least amount, in the cultures of *B. trispora.*

It was not until 1956 that work on the isolation and identification of the progametangium-inducing substances of *Mucor mucedo* was begun. In the decade that followed, Plempel and his associates (*32*) succeeded in isolating material from the culture liquid of mated strains that behaved as a single entity during chromatography and that was highly active in inducing the formation of progametangia in both the plus and minus strains of *M. mucedo.* An empirical formula was determined and some information regarding substituents of the molecule was obtained.

Working with liquids derived from mixed cultures containing the plus and minus strains of *Blakeslea trispora*, van den Ende (*33*) isolated two compounds each of which was active in the induction of progametangia in the plus and in the minus strain of *Mucor mucedo.* He has shown these compounds to be trisporic acids B and C. At about the same time, Gooday (*34*) obtained from mycelia harvested from mixed cultures of "mating types" of *Mucor mucedo* a substance which was capable of inducing progametangia in both the plus and the minus strain. Subsequently, it has been established that this progametangium-inducing material consists mostly of trisporic acid C (*35*).

The trisporic acids are not mating-type-specific. Plempel, however, was led to postulate two mating-type-specific inducers of progametangia, which he called plus and minus "gamones." When these were not separated by chromatography he concluded that they were very similar compounds. He also postulated the secretion of two other mating-type-specific substances, called "progamones," which stimulate the secretion of gamones. From the studies of van den Ende and of Gooday it appears that synthesis of trisporic acid occurs only when the plus and minus strains are allowed to interact. That a soluble substance is involved in this interaction is indicated by the demonstration of trisporic acid synthesis when the two mating types are separated by a membrane (*33*).

Sirenin

The gametes of *Allomyces*, unlike those of *Achlya*, are motile. They differ from one another in size and color, the orange-colored male gamete being smaller than the colorless female gamete. The male and female gametangia, which are borne on the same thallus, normally discharge their gametes into water. Male gametes are attracted to the female gametangia, and they cluster about the female gametes after these are released from the gametangia. Fusion of a male with a female gamete results in a motile, biflagellate zygote. The substance secreted by the female gametes which attracts the male gametes is called "sirenin"; it has been isolated (*36*) and characterized (*37*) (Fig. 6).

Sirenin was extracted with methylene chloride from water in which large numbers of female gametes had been suspended. The production of a single batch of fluid containing sirenin involved five stages and required almost 20 days. To prevent contamination, the procedure at all stages but the last was carried out under aseptic conditions. To obtain a suspension composed almost entirely of female gametes, the normally hermaphroditic habit of this mold had to be circumvented. This Machlis (*38*) accomplished by selecting an interspecific hybrid in which 96 to 99 percent of the gametangia produced were female. Male gametes, obtained from another hybrid, predominantly male, were used in a bioassay in which counts were made of the gametes congregating beneath a membrane forming the bottom of a miniature cell containing a solution of sirenin.

At room temperature, sirenin is a viscous liquid. A bicyclic sesquiterpene, sirenin is one of two homologs of 2-carene (*37*). The other, identical in structure to deoxysirenin, was isolated from the essential oil of the fruits of *Schisandra chinensis* (*39*).

The response of male gametes to sirenin becomes increasingly stronger as the concentration is raised from $10^{-10}M$ to $10^{-6}M$. Increasing the concentration to $10^{-5}M$ and $10^{-4}M$ results in diminished response to sirenin, a phenomenon that may be due to saturation of the chemoreceptors in the gamete. Sirenin is removed from solution by the male gametes and is inactivated (*40*). Female gametes and asexual spores of *Allomyces* do not respond to sirenin (*41*).

When tested, hormone A from *Achlya* had no effect on the male gametes of *Allomyces* and sirenin had no effect on male hyphae of *Achlya.* The action of hormone A was not antagonized by sirenin, nor was the action of sirenin antagonized by hormone A (*42*).

Summary

A structure has been assigned to each of three compounds functioning in sexual reproduction of a fungus. All three are terpenoid in character.

Antheridiol, which is a sterol, is secreted by the female strains of *Achlya bisexualis* and *A. ambisexualis.* It acts on the male strains of *A. ambisexualis* to initiate the formation of antheridial branches, and from these branches it elicits a chemotropic response. It is involved in the formation of antheridia and is essential for their development, since only antheridial branches can give rise to antheridia and only antheridiol can initiate antheridial branches. It also stimulates the male to secrete the hormone that induces formation of sex organs by the female.

Trisporic acids B and C are terpenoid C_{18} carboxylic acids secreted by *Blakeslea trispora.* Each stimulates synthesis of carotene in the hyphae of the minus strain, and each induces the formation of progametangia in the plus as well as the minus strain of *Mucor mucedo.*

Sirenin is an oxygenated sesquiterpene secreted by the female gametes of *Allomyces*, and it acts by attracting the male gametes.

The isolation and characterization of these substances opens the way to studies of the functions they govern and of the manner in which this government is accomplished.

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Feedbacks in Economic and Demographic Transition

A neo-Malthusian and an alternative model of development are compared and tested against the real world.

Harald Frederiksen

Demographic transition and economic development are not independent phenomena. If there is such a thing as a "population problem," it cannot be understood and solved in isolation from the complex process of national development, of which economic development is but one aspect.

Needs and resources for health and family-planning programs evolve in the context of the successive stages of demographic transition and economic development. We have to agree on the nature and magnitude of the interactions between population and economic phenomena at the various stages of national development (called simply "development" hereafter) before we can agree on how much of what is most appropriate and effective in the circumstances in question.

Neo-Malthusian Model

A neo-Malthusian school believes that the process of development is impeded when the rate of population growth is high, and that this high rate of growth is the result of a rapid reduction in mortality, which in turn is the result of alien technology's increasing the effectiveness and efficiency of health services quite independently of levels of production and consumption. Let me quote from some writers who belong to this school.

> The death rate in less-developed areas is dropping very rapidly . . . and without regard to economic change. . . .
>
> The less-developed areas have been able to import low-cost measures of controlling disease, measures developed for the most part in the highly industrialized societies. The use of residual insecticides to provide effective protection against malaria at a cost of no more than 25 cents per capita per annum is an outstanding example. . . .
>
> The death rate in Ceylon was cut in half in less than a decade and declines approaching this rapidity are almost commonplace. The result of a precipitous decline in mortality while birth rate remains essentially unchanged is, of course, a very rapid acceleration in population growth. . . .
>
> In the longer run, economic progress will eventually be stopped and reversed unless the birth rate declines *or the death rate increases* [*1*].

The higher the population growth, the harder becomes the task of breaking through the Malthusian trap. A vicious spiral is set into operation. Because of a high rate of population growth, industrialization is difficult to attain. Because there is no industrialization, the birth rate and the rate of population growth remain high [2].

It may seem indecent to some to suggest that medical research first be concentrated on those diseases whose control

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