

Light and the Asexual Reproduction of *Pilobolus*

Responses to light play an important role in the development and reproduction of this fungus.

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Pilobolus, a fungus assigned to the order Mucorales, differs from all other members of this order of terrestrial Phycomycetes in that it discharges its sporangia explosively. Because of this spectacular method of spore dispersal *Pilobolus* has attracted the attention of biologists for almost three centuries, but the genus is of current interest not only because of the dramatic way in which it is adapted to its environment but also because it has attributes which make it of interest in the study of fundamental biological problems.

All of the species of *Pilobolus* except one occur on the dung of herbivorous animals. The mycelium grows beneath the surface of the substratum, and after a few days it produces a crop of sporangiophores. When each sporangium reaches maturity it is shot off forcibly, and if, at the end of its trajectory, it strikes a blade of grass, the sporangium adheres to it. If a grazing animal eats a blade of grass with adherent sporangia, the thousands of spores contained within each sporangium pass unharmed through its digestive tract and emerge with the dung, in which they germinate to repeat this asexual reproductive cycle. *Pilobolus* also reproduces sexually in a manner similar to that of other Mucorales.

Pilobolus is widely distributed, and although it is not often observed in the field because its sporangiophores are ephemeral, it appears frequently on samples of dung brought to the laboratory and kept under moist conditions for a few days (Fig. 1). The genus has been reported from many parts of the world, and it occurs on the dung of many kinds of herbivorous ani-

mals. For example, I have isolated strains from such exotic sources as caribou dung collected near Point Barrow, Alaska, and wallaby dung collected at Wilson's Promontory at the southernmost tip of Australia (1).

One question which arises immediately is, Why does *Pilobolus* grow on dung? To be sure, one species, *P. oedipus*, has been reported to grow on decomposing algae along river and canal banks, but all of the other species have been found only on dung. Is the genus restricted to this habitat only by the mechanics of its spore dispersal, or are nutritional factors involved as well? A partial answer to this question may be found in the results of nutritional studies.

Nutritional Requirements

Early attempts to grow *Pilobolus* on synthetic media of known chemical composition were unsuccessful. The fungus grew well only on dung or on aqueous extracts of dung. Within the past decade, however, investigations in several laboratories have provided some information on the nutritional requirements of some members of the genus. First, through fractionation of aqueous extracts of cow dung, it was found that the material in dung which was required by *Pilobolus* was heat-stable and soluble in water but not in fat solvents. It was not adsorbed by anionic or cationic exchange resins, but it was strongly absorbed by charcoal and certain other nonspecific adsorbants. The growth-promoting activity of this factor can be imitated by hemin, but relatively high concentrations (10 mg/ml) are required (2). Second, workers at the Lederle Laboratories (3) found that fer-

mentation liquors were a rich source of a material (to which they gave the somewhat inappropriate name "coprogen") which was very active in promoting the growth of *Pilobolus*. Third, Neilands (4) discovered a family of iron-containing compounds, the ferri-chromes, which were also very active in promoting the growth of *Pilobolus*. Now, hemin, coprogen, and the ferri-chromes have one property in common: they all contain iron in chelated form. It appears, therefore, that one distinctive nutritional requirement of *Pilobolus* is that it be supplied with iron in specially chelated form. Although the identity of the material in dung remains unknown, it seems probable, from the work of Lyr (5), that bacteria in the dung are a major source of this growth factor. It is possible, therefore, that this factor is similar to coprogen, or that the two are identical.

In addition to their requirement for specially chelated iron, some species of *Pilobolus* have a near-requirement for fatty acids. Growth is very poor in media containing carbohydrates as a source of carbon, but growth is vigorous in media containing appropriate concentrations of sodium acetate. All of the species of *Pilobolus* tested have grown on synthetic media containing hemin, acetate, a nitrogen source, and inorganic salts (2). By far the most active nitrogen source is ammonia (ammonium ion). Both vegetative growth and reproduction are greatly stimulated by ammonia in certain concentrations (6).

It appears from the results of nutritional studies that *Pilobolus* is bound to its habitat as much by its nutritional requirements as by its method of spore dispersal, and that its nutrition is as specialized as its morphology. Moreover, from a practical point of view the results of nutritional studies have been useful because they have made it possible to grow the fungus under much more rigidly controlled conditions than is possible on a dung medium. Also, the growth and development of the fungus can be observed more conveniently on synthetic media than on natural substrata.

Growth and Development

On transparent synthetic agar media it is possible to follow the growth of the mycelium and the development of the asexual reproductive structures of *Pilobolus* in detail (Fig. 2). The my-

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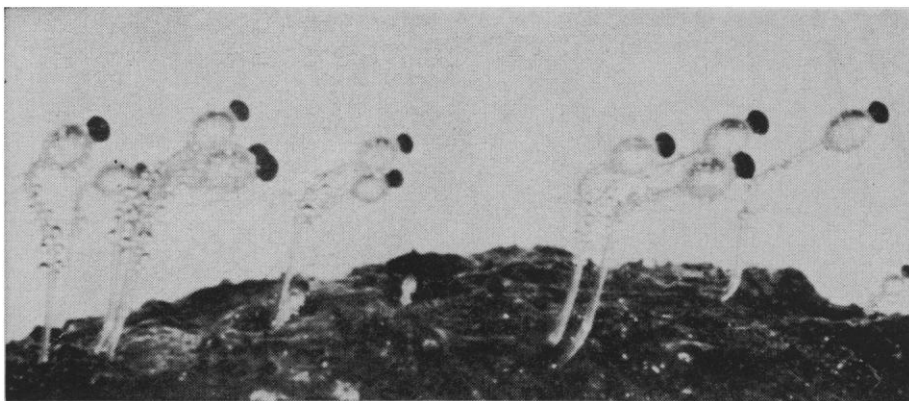


Fig. 1. Sporangiophores of *Pilobolus* sp. on cow dung. (About $\times 5$)

celium, which grows beneath the surface of the medium, may be somewhat restricted, as in *Pilobolus crystallinus* Fries and its relatives, or it may spread rapidly to fill the medium in a petri dish in a few days, as is the case with *P. kleinii* van Tiegh or *P. sphaerosporus* Palla. The mycelium is coenocytic; the hyphae are devoid of crosswalls except for those which delimit reproductive structures or wall off small branch hyphae from which the protoplasm has been withdrawn. Sooner or later, depending on the species and

conditions, the mycelium begins to form trophocysts, which are first recognizable as swollen regions along certain hyphae. These swellings enlarge as protoplasm flows into them, and finally each trophocyst is completed by the formation of crosswalls which isolate it from the parent hypha. Later, a stout hypha, the sporangiophore, grows from each trophocyst, increases in length, emerges from the medium, and continues to elongate in the air. After a time the sporangiophore stops elongating and its tip swells to form the

sporangium, which is later separated from the rest of the sporangiophore by a crosswall. Sooner or later the upper part of the sporangiophore swells to form a conspicuous subsporangial swelling or vesicle, and the wall of the sporangium becomes jet black. In the meantime the contents of the sporangium cleave to form a large number of multinucleate spores. As the sporangium reaches maturity its wall separates at the base, so that the gelatinous material surrounding the spores is exposed. Finally, the wall of the subsporangial vesicle ruptures suddenly just below the sporangium. The sporangium is shot off, propelled by the liquid in the sporangiophore and the empty sporangiophore is thrown flat on the medium by the recoil.

The force with which the sporangia are discharged is impressive. Sporangia which strike the lid of a petri dish make a clearly audible "plink," and those whose progress is not interrupted are thrown for remarkable distances. All records for altitude and distance are held by sporangia reported by Buller (7); he found that large sporangia of *Pilobolus kleinii* and *P. longipes* are shot to a height of 6 feet 0.5 inch.

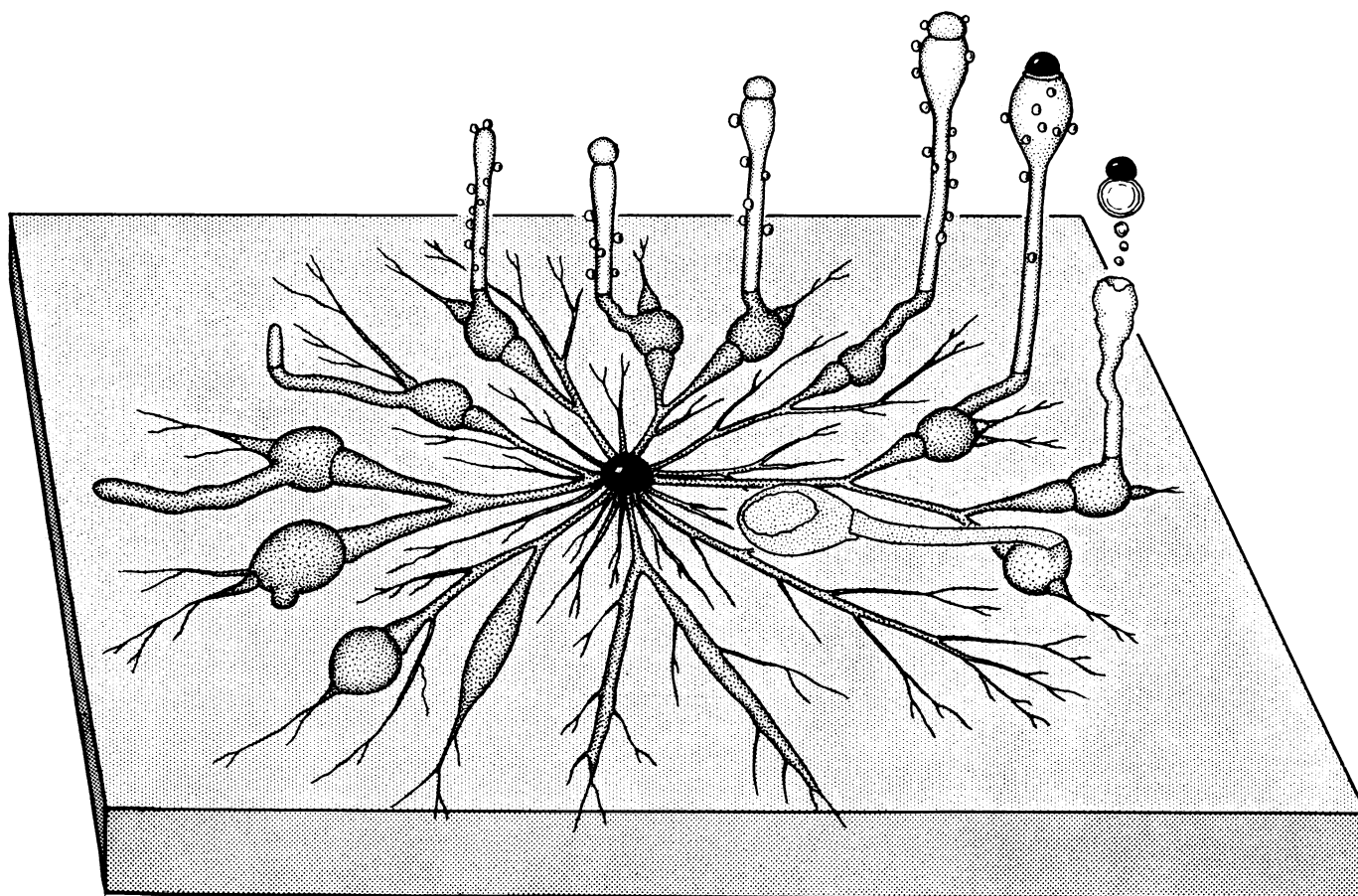


Fig. 2. Schematic drawing (based on *P. crystallinus*) showing the development of asexual reproductive structures of *Pilobolus*. Trophocysts and sporangia are represented as developing in clockwise sequence on hyphae emanating from a sporangium.

When sporangiophores were aimed at a 45-degree angle above the horizontal, the maximum range was 8 feet 0.5 inch for *P. kleinii* and 8 feet 7.5 inches for *P. longipes*.

From the maximum height to which sporangia are shot, Buller calculated that the muzzle velocity would be 19.6 feet per second if air resistance is neglected. Pringsheim and Czurda (8) attempted to measure the muzzle velocity directly by allowing sporangia to pass through holes in a rotating disk and strike a second disk rotating on the same shaft. From the results, they calculated that the muzzle velocity was approximately 14 meters per second. In order to avoid some of the errors inherent in this technique, Donald Kennedy and I arranged sporangiophores of *P. kleinii* in such a way that, in their flight, they partially occluded, in succession, two beams of light 3 millimeters apart. The partial interruption of the beams was detected by a photocell connected to an oscilloscope in such a way that the interval between the interruptions could be measured. Preliminary results indicated that the muzzle velocity of relatively small sporangia of *P. kleinii* ranged from 5 to 10 meters per second, but further trials will be required to establish the range of velocities for this and other species more precisely.

Response to Light

All of the stages in asexual reproduction which precede the dramatic and climactic discharge of the sporangium are influenced by light. Indeed, it would be difficult to overemphasize the importance of light in the life of *Pilobolus*, because almost all of its activities are affected by this environmental factor. For purposes of discussion, the responses of the fungus to light may be divided into two categories: morphogenic responses, in which the response is not oriented, and tropisms, in which the direction of the response is related to the source of the stimulus.

Various species of *Pilobolus* differ in their morphogenic responses to light. *Pilobolus kleinii*, for example, requires light for the first step in the development of the asexual reproductive apparatus, the development of trophocysts. The mycelium of this species grows vigorously in the dark, but no trophocysts are formed unless the specimen is exposed to light. Only a brief exposure is required, and when mycelia were

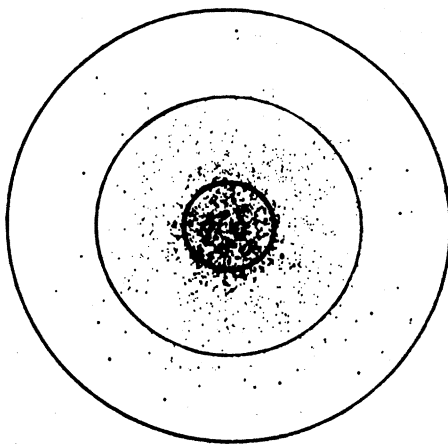


Fig. 3. Sporangia of *Pilobolus kleinii* adhering to a paper target of which the center was illuminated (see text).

exposed to visible light of various wavelengths from a monochromator, only light of wavelengths shorter than about 510 millimicrons was effective in inducing the formation of trophocysts (9). These spectral limits, together with the fact that trophocyst formation is suppressed by L-lyxoflavin, which is a competitive inhibitor of riboflavin, but not by diphenylamine, which interferes with the synthesis of some carotinoids, suggest that the photosensitive pigment may be a flavin. Of course, other interpretations are possible, but in any case it is interesting that this step, which requires light in *P. kleinii*, can be carried out in complete darkness in other species.

Pilobolus crystallinus is capable of forming trophocysts and sporangiophores in continual darkness, but the sporangiophores become abnormally long—up to several centimeters, as compared with the usual length of about 0.5 centimeter—and sporangia do not form unless the culture is exposed to light. Brefeld reported similar behavior in *P. microsporus* (10). *Pilobolus sphaerosporus*, on the other hand, not only produces normal sporangiophores but forms and discharges sporangia in continual darkness. However, even those species which are able to form part or all of the asexual reproductive apparatus in darkness produce more structures after exposure to light.

All species of *Pilobolus* form sporangia periodically when exposed to a suitable daily alternation of light and darkness. *Pilobolus kleinii*, for example, begins to discharge sporangia about 3 hours after the beginning of the light period when grown on a schedule of 12 hours of light and 12 hours of darkness per day. Because of its ability to

form sporangia in the dark, *P. sphaerosporus* is more suitable for studies on periodicity, and it has been shown to have an endogenous rhythm which is susceptible of entrainment (11).

The phototropic responses of *Pilobolus* have been studied far more intensively than its morphogenic responses to light (12). It is well known that the fungus discharges its sporangia toward a source of light, and the accuracy with which the sporangiophores direct the sporangia at an illuminated target has been demonstrated by many workers. To illustrate this accuracy, a target of translucent paper was attached to the underside of the lid of a container that was opaque except for a hole 1 centimeter in diameter behind the bull's-eye of the target, and a culture of *Pilobolus kleinii* growing on synthetic medium in a petri dish 10 centimeters in diameter was placed in the container, together with moist blotting paper to maintain the relative humidity at a high level. As shown in Fig. 3, many sporangia struck the bull's-eye of the target, and most of them landed within 1 centimeter of that circle. In this instance, the target was placed 10.7 centimeters above the culture, so that a circle 1.0 centimeter in diameter subtended an arc of approximately 5 degrees. Thus, most of the sporangia struck the paper within about 5 degrees of the illuminated center of the target, despite the fact that sporangia near the margin of the petri dish were aimed at a considerable angle to the vertical. Of course, the fungus has no means of compensating for the effect of gravity on the trajectory of the sporangium, so its accuracy decreases as the distance to the target is increased.

In order to observe the morphological aspects of the phototropic responses which precede the discharge of sporangia toward light one must study sporangiophores at two stages in their development, because positive phototropism is shown both by young sporangiophores which have not begun to form a sporangium and by mature sporangiophores in which the subsporangial vesicle has attained its full size. The technique used to study the phototropism of sporangiophores at both stages was, briefly, as follows. The fungus was grown on synthetic media in petri dishes, and after the mycelium was established, the cultures were subjected to 8 hours of light followed by 16 hours of darkness daily to induce the formation of sporangiophores. Blocks of agar

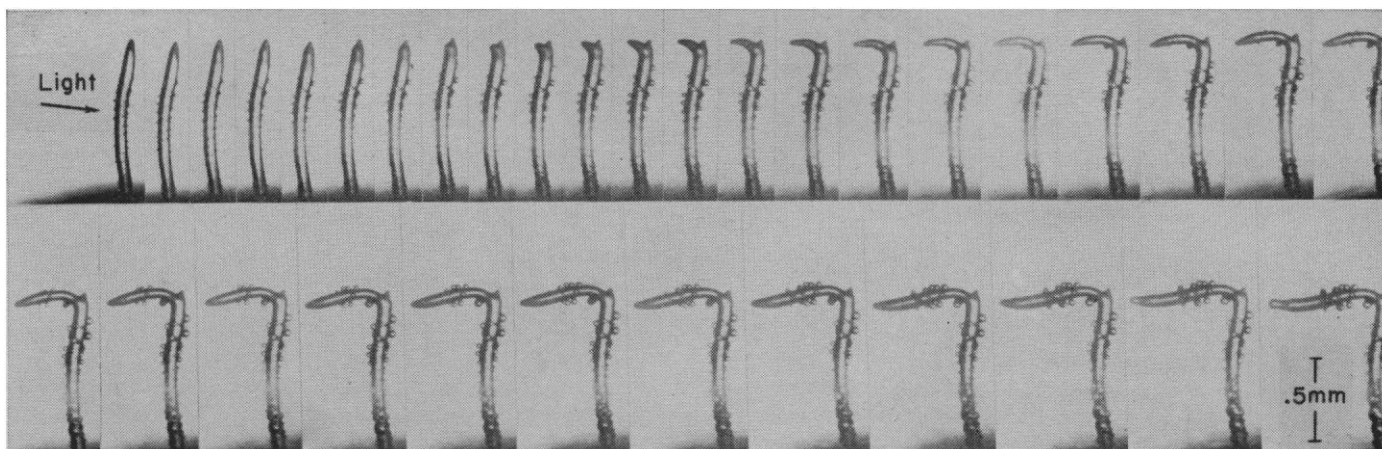


Fig. 4. Response of a young sporangiophore of *Pilobolus kleinii* to unilateral illumination. Photomicrographs were taken at 10-minute intervals.

with sporangiophores at an appropriate stage of development were cut from these cultures. Each block was laid on its side on a cover glass, which was then inverted to form the top of a small chamber mounted on a microscope slide, so that the sporangiophores could be viewed from the side with a microscope fitted with a $3\times$ objective. Light from a small microscope lamp was passed through heat-absorbing glass and directed on the sporangiophores through a window in the side of the chamber. Red light from a photographic safelight mounted in a second microscope lamp was focused on the sporangiophores by the condensing lens of the microscope to provide illumination for observation and photomicrography. Photomicrographs were taken at intervals of 5 or 10 minutes.

These observations showed that young and mature sporangiophores differ considerably in the way in which they respond to unilateral illumination, and that the response of young sporangiophores of *Pilobolus kleinii* differs in some respects from that of young sporangiophores of *P. crystallinus*. The nature of the phototropic response of a young sporangiophore of *P. kleinii* is revealed by the series of photomicrographs shown in Fig. 4. When the sporangiophore was exposed to unilateral illumination it stopped growing. After about an hour a protuberance appeared on the illuminated flank of the dome-shaped tip of the sporangiophore, and this protuberance soon assumed the shape and character of a sporangiophore apex. Growth from this new tip continued in the general direction of the light until the sporangium began to form. Usually the new tip is formed close to the original one, so that after a sporangiophore has elongated for some

time it appears to be sharply but smoothly bent toward the light. Occasionally, as in the sporangiophore illustrated, the new growth is initiated farther from the tip, so that the original tip remains visible. In such a case, the response may be said to consist of the formation of a branch on the illuminated side of the sporangiophore.

The response of young sporangiophores of *Pilobolus crystallinus* to unilateral illumination appears to be basically similar to that of *P. kleinii*, but there is a puzzling difference. As shown in Fig. 5, the sporangiophore of *P. crystallinus* also stopped growing when the light was directed on it from one side, and after a time growth recommenced, after the initiation of a new tip on the flank of the old one. In all cases observed the new tip was formed close to the original one, so branching was not evident. The sporangiophores of *P. crystallinus* differ from those of *P. kleinii* in that they do not grow more or less directly toward the light but grow toward a point above the light. Thus, after growing for some time, young sporangiophores of *P.*

crystallinus are bent to an angle which is consistently less than the angle between the light and the former axis of the sporangiophore.

Mature sporangiophores resemble young ones in that they exhibit positive phototropism, but they respond by bending toward the light rather than by initiating growth in a new direction (Fig. 6). Here again the response is preceded by a lag phase, but this appears to be much shorter in the mature sporangiophores. It is difficult to determine the minimum length of this period precisely, because of difficulties in recognizing fully matured and responsive sporangiophores and in detecting the first traces of bending, but curvature of sporangiophores of *Pilobolus kleinii* has been observed 10 minutes after the beginning of unilateral illumination. This figure is in agreement with that of Buller (7), who reported the period of latency in *P. longipes* to be 10 minutes.

After a lag period the sporangiophore begins to bend in a region just below the subsporangial vesicle. The rate of bending increases and within a few minutes reaches a maximum, which

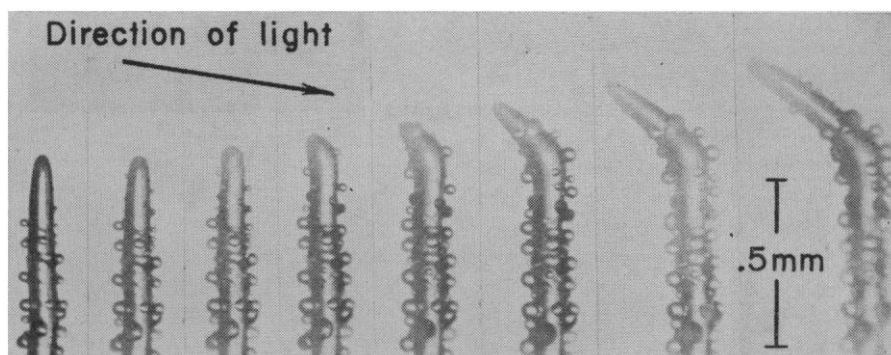


Fig. 5. Response of a young sporangiophore of *Pilobolus crystallinus* to unilateral illumination. Photomicrographs were taken at 20-minute intervals.

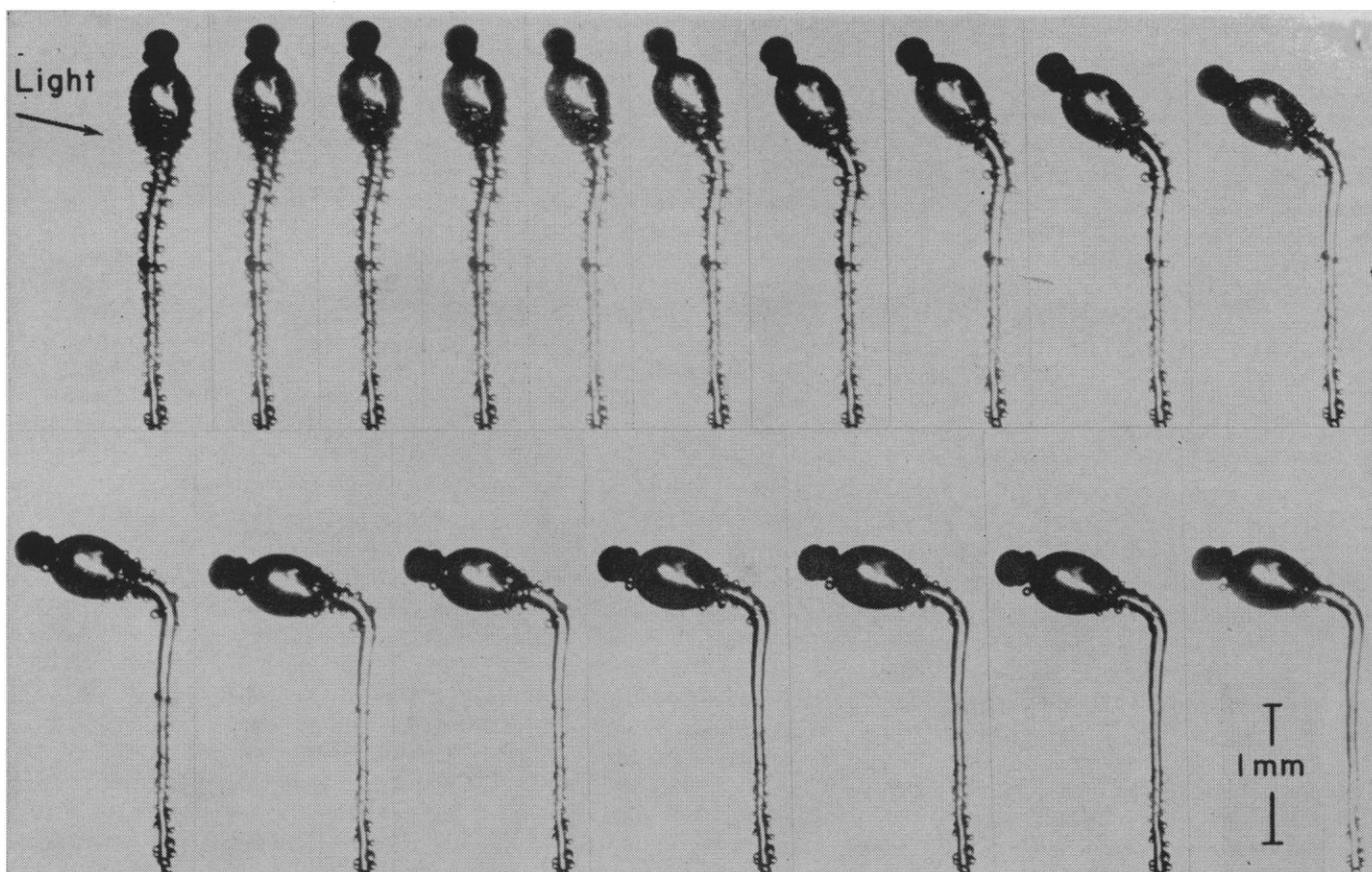


Fig. 6. Phototropic response of a mature sporangiophore of *Pilobolus kleinii*. The first photomicrograph was taken approximately 1 hour after the beginning of unilateral illumination and the others, at 5-minute intervals thereafter.

varies from 1.1 to more than 4 degrees per minute. Bending continues at almost the maximum rate until the sporangiophore bends beyond the beam of incident light; then the bending slows rapidly and reverses direction. A fully mature sporangiophore of *Pilobolus kleinii* reaches its maximum curvature about 1 hour after the beginning of unilateral illumination.

Proposed Mechanisms

That the sporangiophore overshoots is shown in Fig. 6, and if the curvature of a unilaterally illuminated maturing sporangiophore is plotted against time, the resulting curve (Fig. 7) shows that the sporangiophore may bend past the beam of light several times. Although the measurements were made too infrequently to give a detailed picture, the curve gives the impression of being the record of a damped oscillation. It may be that the lag which precedes the onset of bending is partially responsible for the damping action. It is also possible that the pigment which is present in the cytoplasm at the shoulder of the subsporangial vesicle

adjacent to the sporangium acts as an optical filter which reduces the intensity of light striking the pigmented zone at the base of the vesicle as the sporangiophore approaches the beam of light.

Buller (7) suggested that the upper pigmented zone might function in triggering the discharge of the sporangium by increasing the osmotic pressure of the cell sap or weakening the wall of the subsporangial vesicle at the point where rupture occurs, but the position of this zone makes it appear equally likely that it could function as a screen which would tend to damp oscillation and act as a "fine adjustment" in aiming the sporangiophore.

The optical aspects of the phototropic response of mature sporangiophores were studied intensively by Buller, who was a pioneer in the application of the principles of optics and mechanics to the solution of biological problems. By means of diagrams constructed carefully on the basis of the refractive indices of the wall, cytoplasm, and cell sap of the sporangiophore, Buller (7) demonstrated that the subsporangial vesicle acts as a lens. When the spo-

rangium is not directed toward a source of light, the rays which enter the vesicle are concentrated on the wall, but when the sporangium is aimed directly at the light, the rays are directed to the base of the vesicle in the vicinity of a ring of protoplasm which contains an orange-red pigment. Thus, according to Buller, a sporangiophore is an ocellus which is in physiological equilibrium when the sporangium is aimed directly at a source of light. Buller's hypothesis also explains the fact that mature sporangiophores exposed to two sources of light simultaneously aim toward one light or the other when the angle between them exceeds a certain value (about 10 degrees). In fact, his explanation of the optics of phototropism was stated with such logic and clarity that it may have discouraged further investigation, because it created the impression that all problems had been solved.

Although the optical properties of young sporangiophores appear simpler than those of the mature structures, they must be reexamined in view of the discovery that young sporangiophores respond by initiation of growth on the illuminated side of the tip. Most in-

investigators have assumed that the young sporangiophore of *Pilobolus* responds in the same way as *Phycomyces*, another member of the order Mucorales. Buder (13) proposed that in *Phycomyces* the sporangiophore acts as a cylindrical lens which concentrates light on the cytoplasm inside the wall farthest from the light, and that curvature results from an increase in growth in this region. The results of most experiments have supported this hypothesis for *Phycomyces*, but for *Pilobolus* it is difficult to reconcile the hypothesis with the observation that the response of the young *Pilobolus* sporangiophore occurs on the side nearest the light. In *Pilobolus* reflection from the surface appears to be far more important than refraction by the contents of the sporangiophore.

If the cylindrical lens hypothesis is no longer tenable for *Pilobolus*, a convenient explanation for the fact that young sporangiophores of *P. crystallinus* do not grow directly toward a source of light is eliminated. Sporangiophores of *Phycomyces* also bend to an angle that is less than that between a source of visible light and the former axis of the sporangiophore. Varju, Edgar, and Delbrück (14) have explained this phenomenon as being due in part to the fact that light is refracted in a complex pattern by a tilted cylindrical lens and in part to the combined effects of light and gravity.

The effect of gravity may be discounted in the case of *Pilobolus crystallinus*, because its sporangiophores are only weakly geotropic (a recognizable geotropic curvature is detectable only after the sporangiophores have been allowed to elongate in the dark for several days); moreover, under the conditions of our study, the sporangiophores were horizontal, so that a geotropic response would have been at right angles to the plane of observation. Thus, the behavior of young sporangiophores of *Pilobolus crystallinus* remains a puzzling phenomenon, for which no explanation is at hand.

Another question not yet answered with certainty is, What is the nature of the visual pigment in *Pilobolus*? One of the first attempts to identify this photosensitive pigment was that of Bünning (15), who measured the phototropic response to light of various wavelengths obtained by means of glass and liquid filters and compared the action spectrum so obtained with the absorption spectrum of pigments extracted

from sporangiophores. He found a close resemblance between the action spectrum and the absorption spectrum, and found that the absorption spectrum, in turn, resembled the absorption spectrum of beta carotene. For over a decade the conclusion, drawn from these results, that the active pigment was a carotinoid was accepted, but after it was suggested (16) that flavins might be involved in phototropism of higher plants this interpretation was questioned, because it is difficult to distinguish between the two classes of pigments on the basis of action spectra. An additional complication was introduced by Jacob (17), who determined the limits of spectral sensitivity of young sporangiophores of *Pilobolus* both by means of interference filters and by means of cadmium- and zinc-filled gas discharge tubes with glass filters. He found that young sporangiophores of certain strains of some species were sensitive to wavelengths as long as 660 m μ .

Limits of Spectral Sensitivity

It seemed desirable, therefore, to determine the limits of spectral sensitivity by a different method. Both young and mature sporangiophores were exposed to light of various wavelengths through projection of a spectrum on cultures. After being passed through heat-absorbing glass and suitable neutral density filters, light from an incandescent lamp was passed through a slit and focused on a collimating lens before being dispersed by a prism and directed on the culture by a second lens. The apparatus was calibrated by placing a fluorescent lamp in front of the slit and noting the positions of the emission lines of mercury. Slabs of synthetic agar medium with sporangiophores at the proper stage of development were cut from petri-dish cultures which had been grown under conditions of alternating light and darkness to induce synchronous development of sporangia, and the slabs were placed in a clear

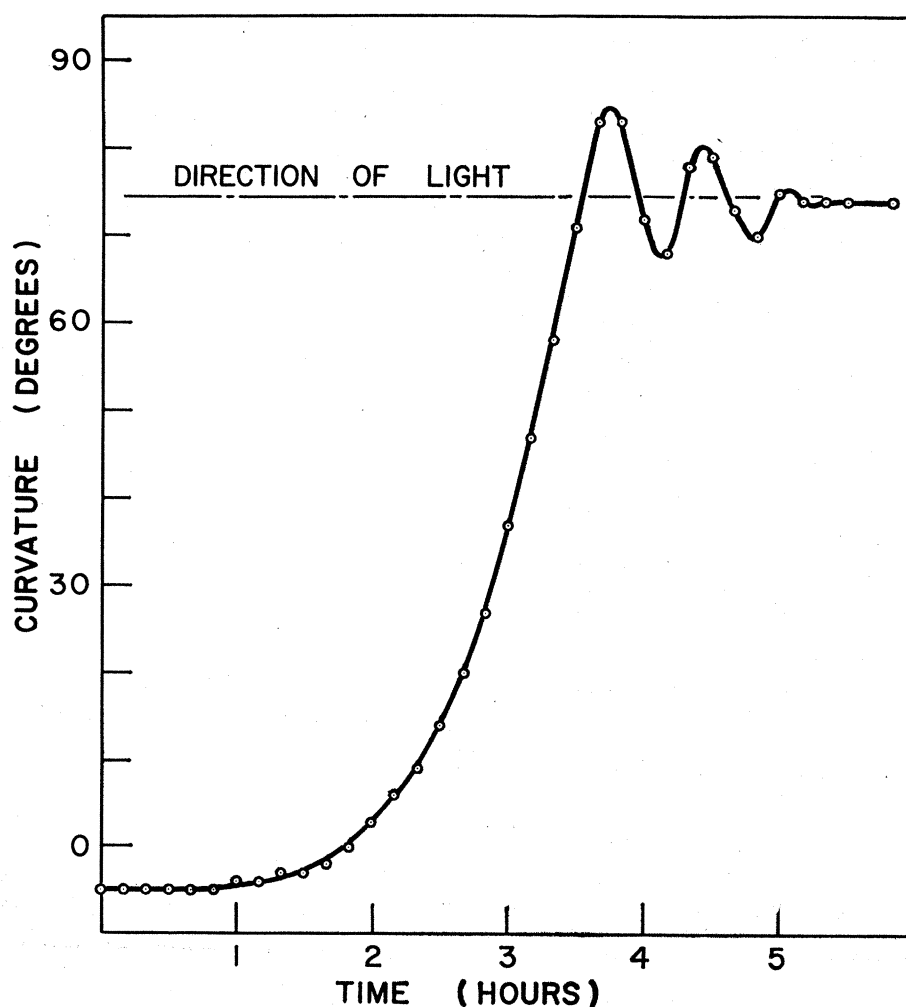


Fig. 7. Graph showing the response of maturing sporangiophore of *Pilobolus kleinii* to unilateral illumination.

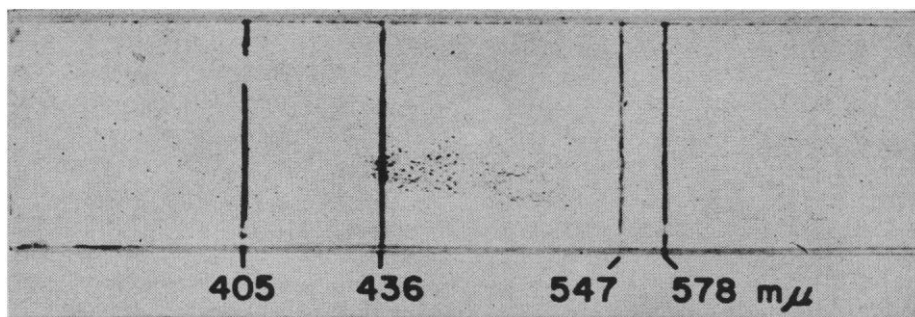


Fig. 8. Sporangia of *Pilobolus sphaerosporus* adhering to a glass plate interposed between a culture and a light dispersed by a prism. The distribution of the sporangia gives a crude action spectrum for phototropism of mature sporangiophores.

plastic box with a saturated sponge to maintain the relative humidity.

The response of mature sporangiophores was recorded by placing a plate of glass in the box between the fungus and the light. If a sporangiophore responded to light of a given wavelength it shot its sporangium toward the plate; if it did not respond, it discharged its sporangium in a random direction. Thus, the pattern of sporangia adhering to the glass gave not only an indication of the limits of spectral sensitivity but also a very crude action spectrum.

The response of mature sporangiophores was determined by direct observation. A plate of glass with the paths of the emission lines of mercury marked on it was laid in the bottom of the plastic dish, and slabs of agar medium with young sporangiophores that had just emerged from the medium were laid on the plate. After the sporangiophores had been exposed for several hours to the light dispersed by the prism, those on portions of the plate illuminated by light of wavelengths to which the sporangiophores respond appeared to lie in windrows, because they were all growing toward the light, whereas those on other portions of the plate stood erect or were bent at random.

The results indicated that mature sporangiophores of a strain of *Pilobolus kleinii* respond to wavelengths between 400 and 550 $m\mu$, but the lower limit of sensitivity could not be established with certainty, because of scattering of light by the optical system. Mature sporangiophores of *P. crystallinus* responded to a similar range of wavelengths, but those of *P. sphaerosporus* responded strongly to wavelengths between 430 and 540 $m\mu$, with a maximum response at about 480 $m\mu$ (Fig. 8).

The results obtained with young sporangiophores of *Pilobolus crystallinus*

were clear-cut. Most of the sporangiophores exposed to wavelengths shorter than about 546 $m\mu$ were bent in the direction of the light, and the response appeared to be particularly strong between about 410 and 520 $m\mu$. The pattern of response of one strain of *P. kleinii* was similar, but the demarcation between response and no response was less clear. In two out of three trials no response was observed at wavelengths above 546 $m\mu$, but in one trial the upper limit was between 546 and 578 $m\mu$.

Although the technique used for these trials was far too crude to permit us to draw conclusions with regard to the nature of the photoreceptor, the results are compatible with the suggestion that it is either a carotenoid or a flavin. Since there was no clear response of young sporangiophores to wavelength greater than about 580 $m\mu$, the results failed to confirm those of Jacob. It seems probable that this difference should be ascribed to differences in the species and strains of the fungus, but it is also possible that differences in light intensity are involved. The fact that *Entomophthora coronata* (= *Conidiobolus villosus*), another terrestrial phycomycete, responds to wavelengths as great as 630 $m\mu$ indicates that red light can be absorbed by some fungus photoreceptors (18).

Conclusion

The responses of *Pilobolus* to light, as well as its specialized nutrition, are manifestations of the adaptation of the fungus to its environment, and some of the aspects of asexual reproduction are so striking as to make teleological interpretation a temptation. For example, the periodic formation of sporangia ensures that sporangiophores will be at a reactive stage when they are

exposed to light; the relatively crude phototropic response of young sporangiophores permits them to grow out of crevices and away from the substratum; the precise phototropism of mature sporangiophores guarantees that sporangia will be shot toward the sun or open sky; and the timing of sporangial discharge—in some species, at least—is such that the range is nearly maximal. But teleology is unfashionable; moreover, the series of events which culminates in the explosive discharge of the sporangium is only the first stage in a system of asexual reproduction. The second stage, in which the spores are transported passively inside the digestive tract of an animal, is less dramatic but results in transport over far greater distances. It should be emphasized that all aspects of asexual reproduction—the subtle as well as the striking—are important, because they are part of a system. Since this system depends on all of its components, *Pilobolus* could not have evolved prior to the advent of herbaceous angiosperms and grazing mammals. The present widespread distribution and frequent occurrence of the fungus are objective and eloquent testimonials to the survival value of its system of asexual reproduction.

Most of the information accumulated on asexual reproduction in *Pilobolus* has been descriptive of the more spectacular and obvious features. It is to be hoped that in the future more attention will be devoted to some of the subtler aspects. The status of our knowledge of phototropism in *Pilobolus* is very elementary as compared with the detailed analyses of the behavior of *Phycomyces* sporangiophores made by Castle, Delbrück, Shropshire, and others (19). The biochemical mechanisms involved in phototropism have not been studied. Even the nature of the photoreceptive pigment is not known—nor is it known in *Phycomyces* and higher plants. Perhaps more significant is our ignorance of the chain of events that lie between the reception of light by this as yet unknown pigment and the formation of wall material.

Indeed, information on this aspect of phototropism might contribute to an understanding of some general aspects of morphogenesis in fungi. In coenocytic organisms, such as *Pilobolus*, the body is basically a tube which swells in certain localized areas as a result of synthesis of wall material; in fact, all structures formed by *Pilobolus* could be accounted for by two mechanisms:

one which would bring about localized synthesis of wall material to produce swellings, and one which would form crosswalls to delimit the structures. Since in *Pilobolus* it is possible through suitable manipulation of light to determine the regions in which wall synthesis occurs, it may be possible to study the biochemistry and fine structure of these regions and thus explore a significant and intriguing channel in the archipelago of events which stretches between genetic information and physical shape.

References and Notes

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NEWS AND COMMENT

Nuclear Energy for India: U.S. Position on Safeguards Raises Concern of IAEA

Supporters of the International Atomic Energy Agency (IAEA) are readying mourning bands and casting accusatory looks toward the Kennedy administration, despite public and private assurances that the administration is dedicated to promoting a bright future for the IAEA.

There is substantial evidence that a bright future is, in fact, what the administration wishes for the IAEA; but the matter is not a simple one, and friends of the agency can be forgiven if they conclude that there is a disturbing discrepancy between American words and deeds.

This discrepancy has been evident throughout the 5-year history of the IAEA, an organization that slowly and painfully grew out of the Atoms-for-Peace proposal put forth by President Eisenhower in 1953. Now numbering 78 member nations, IAEA is a large and busy enterprise, devoted in large part to communicating peaceful nuclear technology to underdeveloped nations. But the original justification for its founding—to provide assurance that the major nuclear powers, in spreading nuclear technology, would not be contributing to the spread of nuclear weapons—has never been realized in actual operations.

The reasons for this failure are technical and political. Nuclear power

came along far more slowly than was expected when Eisenhower first made his proposal. Thus there proved to be no immediate grounds for fears that nations newly equipped with power reactors could surreptitiously divert nuclear materials to weapon production. In the meantime, the two major nuclear powers had entered into a large number of bilateral agreements with nations seeking the benefits of nuclear technology. The bilateral agreements, of which the United States now has 44 and the Soviet Union 14, were in most instances preferred by the recipient nations, who came to look upon a nation-to-nation relationship as more prestigious than assistance channeled through the IAEA. This feeling was fostered by the Soviets' traditional aversion to international inspection, and, politically, it put the United States in a poor competitive position when lesser-developed nations suggested they would look toward the Soviets if the United States insisted upon working through the IAEA. In all bilateral arrangements, the United States and, presumably, the Soviets have insisted that their own inspection and safeguards accompany assistance, with the result that the IAEA has not come to play a significant role in inspection.

The agency, meanwhile, has justified its \$7 million budget and 600 em-

ployees by evolving into a useful service organization for training technicians and developing international standards in such areas as health, safety, and waste disposal. It has also sponsored what are generally regarded as extremely useful conferences and symposiums on a wide variety of subjects related to the peaceful development of atomic energy.

However, the agency's failure to make any headway in a safeguards or inspection role has been a constant source of concern to its supporters, since this role has been regarded from the first as the main justification for the agency's existence. The creeping development of nuclear energy has until recently provided an explanation for this failure, but the safeguards issue has now presented itself in a concrete form, namely, India's decision to buy a 380-megawatt nuclear power plant from this country.

India's Plans

There is no doubt that there will be outside inspection of the Indian plant; the United States has made it clear that it will not permit shipment of the nuclear materials unless it has ironclad assurance that the installation operations will be open to trustworthy inspection. Rather, the question is whether the inspectors will come from this country or from the IAEA. The agency regards the decision as an extremely critical one for its future since the Indian installation, larger than any that has yet been sold abroad, would for the first time give a nuclear have-not nation the capacity for acquiring a sizable amount of weapon-grade plutonium. What disturbs the IAEA is that United States officials, apparently in deference to India's opposition to IAEA inspection, have stated that this