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Echinostelium minutum (Myxomycetes)
Plasmodial Phase (Protoplasmodium)

3 Illustrations

Echinostelium minutum (Myxomycetes) Plasmodial Phase (Protoplasmodium)

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General Remarks[1]

Systematic Position

The myxomycetes or true plasmodial slime molds have long fascinated biologists because they possess taxonomic characteristics of both plants and animals. They are particularly interesting in their capacity for forming amoeboid, multinucleate masses of protoplasm termed plasmodia which can differentiate into organized fruiting bodies. ALEXO-POULOS [4] and OLIVE [15] have suggested recently that *Echinostelium minutum* de BARY (Order Echinosteliales, Class Myxomycetes, Division Mycota), the subject of the present study, is one of the most primitive myxomycetes. This film records the developmental sequences of its plasmodial phase in an attempt to clarify the phylogenetic position of this species within the Class Myxomycetes.

Life Cycle

Due to the diversity of stages in their life history, the myxomycetes are especially favorable organisms for morphological studies on development. In *E. minutum* the spore gives rise to one myxamoeba. This can transform into a flagellate cell (swarm cell) when placed in a liquid environment or encyst under unfavorable conditions. Populations of myxa-moebae or swarm cells subsequently produce microscopic, multinucleate masses of protoplasm termed protoplasmodia by ALEXOPOULOS [2]. In-

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[1] Film data and summary of the Film (English, German, French) see p. 13 and 14.

dividual protoplasmodia can divide by binary fission (plasmotomy), can form a cyst (sclerotium) under unfavorable environmental conditions, or can differentiate into a single sporophore which typically contains less than 75 spores (Fig. 1).
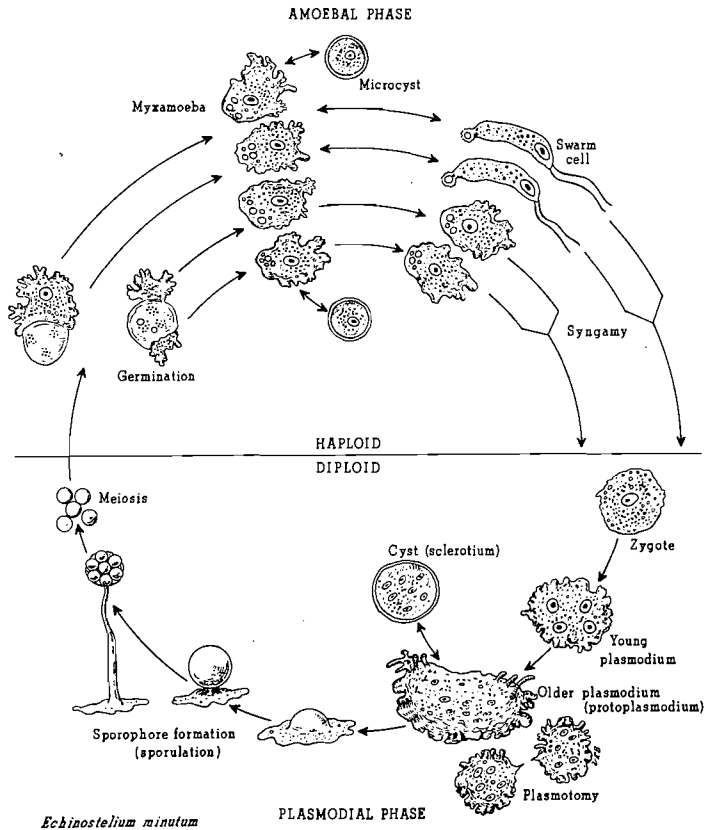

Fig. 1. Life history diagram of *E. minutum*

Plasmodial Formation

The origin of the plasmodium is one of the most vexing questions in myxomycete biology. The controversies that have arisen in the literature are due to the difficulty inherent in observing the behavior of nuclei which are 3—10 μm in diameter, to the unavailability to earlier workers of interference-contrast or phase-contrast optics which are necessary

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for critical observation of living cells, and to the lack of convincing time-lapse documentation of plasmodial formation. Three pathways can be visualized for plasmodial development: heterothallism, involving the fusion of nuclei derived from two clones of uninucleate cells differing in mating type; homothallism, involving the fusion of nuclei derived from a single clone of uninucleate cells; apogamy, not involving the fusion of nuclei. The multinucleate condition of the plasmodium may develop from the repeated mitosis of the initial, diploid fusion nucleus in the two former cases or from division of a monoploid (haploid?) nucleus in apogamic development. An increase in the nuclear population of a plasmodium may also be obtained by coalescence of plasmodia or coalescence of zygotes and plasmodia. The morphological study by Ross [16] and the genetical investigation by COLLINS [5] on *Didymium iridis* confirm that this taxon is heterothallic. The phase-contrast studies by Ross [17], [18] on *Perichaena vermicularis* have shown that this species is homothallic. If the occurrence of synaptonemal complexes can be used to indicate the presence of nuclear fusion and meiosis in a life history, the reports by HASKINS, HINCHEE and CLONEY [8] on *E. minutum* and by ALDRICH and MIMS [1] on *Physarum pusillum* indicate homothallic development for these species. The investigations by VON STOSCH [19] and KERR [10], [11] support strongly the contention that some strains of *Didymium nigripes* develop by apogamy.

Plasmodial Types

The phaneroplasmodium, the aphanoplasmodium, and the protoplasmodium are the three general types of myxomycete plasmodia currently recognized (GRAY and ALEXOPOULOS [6]). A potential fourth type, which shares attributes of the foregoing, has been observed in species of the Order Trichiales by MCMANUS [13]. The phaneroplasmodium, characteristically developed by members of the Order Physarales, has granular cytoplasm, displays a fleshy fan at its advancing front and has thick, tubular veins (up to 1 mm in diameter) which possess a gellified ectoplasm and a rhythmically, reversibly streaming endoplasm. An aphanoplasmodium, characteristic of members of the Order Stemonitales, consists of a system of slender (20—40 µm in diameter), flattened, non-granular, veins which, with the exception of the pre-sporulation phase, lack a gelled ectoplasm. The protoplasm of the veins displays a rhythmically reversible flow which may vary from rapid to slow or even imperceptible. The protoplasmodium, exhibited by species of the Order Echinosteliales, is granular, lacks a system of veins or channels, displays irregular protoplasmic streaming, and always remains diminutive (20—300 µm in width). The plasmodia of the species of Trichiales studied to date have advancing fans with granular veins which typically

lack a gelled ectoplasm, and have lateral vein extensions which display irregular streaming. Hence, this plasmodial type exhibits characteristics of the phaneroplasmodium, the aphanoplasmodium, and the protoplasmodium respectively.

The Protoplasmodium

Although the protoplasmodial type was observed, apparently first in *Licea parasitica* by ZUKAL [21], it was not documented carefully and designated as such until the study by ALEXOPOULOS [3] on *E. minutum*. The initial conception of the protoplasmodium based on these latter
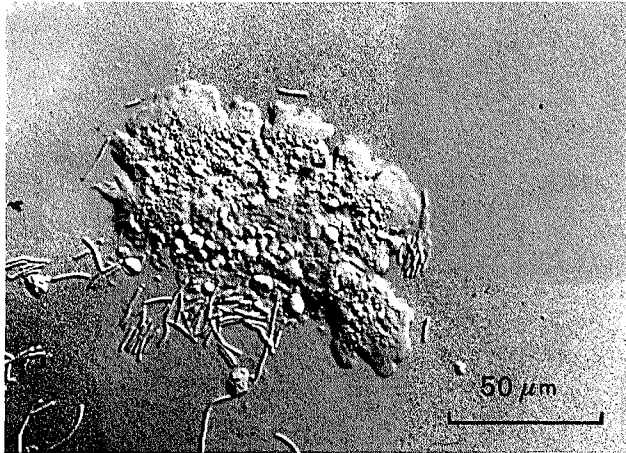
Fig. 2. Migrating protoplasmodium

studies has been broadened due to the investigations by McMANUS [14] on *L. biforis* and *Cribraria violacea*. She reports that in *L. biforis* the individual protoplasmodium gives rise to several sporangia. In *C. violacea* the feeding plasmodium is round in appearance whereas the migrating form stretches out into a network of small veins. Here each protoplasmodium gives rise to a single sporophore.

The isolate (American Type Culture Collection 22345) used for this film develops a protoplasmodium 20—250 μm in width. The young form is round to oval while the mature form is reniform. A migrating, feeding protoplasmodium usually displays a hyaloplasmic anterior margin which may be deeply lobed in contrast to the posterior (Fig. 2). Protoplasmodial mitosis is different from that of myxamoebal mitosis in that it is intranuclear and

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centrioles are absent (HASKINS, unpublished). Subsequent to mitosis a protoplasmodium undergoes plasmotomy. This results in the formation of two daughter plasmodia which often differ in size and nuclear number. The observations of the current study indicate that protoplasmodial coalescence does not occur in this isolate. This is in agreement with the study by ALEXOPOULOS [3] made on another isolate of *E. minutum*. The observation by MCMANUS [12], [14] of the absence of protoplasmodial coalescence in *Clastoderma debaryanum* and *L. biforis* which suggests that this might be a general attribute of the protoplasmodium is thrown into doubt by the report of WOLLMAN and ALEXOPOULOS [20] which demonstates plasmodial coalescence in *L. biforis*.

Under unfavorable conditions such as lack of food or accumulation of metabolic wastes, a protoplasmodium of *E. minutum* rounds up and a refractile membrane develops around the cell. That this refractile boundary actually represents a wall, is indicated by an electron microscopical investigation made on protoplasmodial cysts (HASKINS, unpublished).

Sporulation

Within 24 to 36 hours of the time it is deprived of a bacterial food supply, a protoplasmodium of *E. minutum* differentiates into a single sporophore consisting of a globose spore-mass mounted atop a tapering, acellular stalk (HASKINS [7]). This conversion, which typically produces a mature sporophore within 2 to 3 hours, commences with the appearance of a hemispherical nodule in the center of the protoplasmodium. Fifteen minutes after fruiting body inception, a stalk, which continues to elongate for approximately the next 2 hours, develops beneath the initial. At the cessation of stalk elongation synchronous nuclear division occurs in the sporangium. This is concomitant with the progressive, centripetal cleavage which divides the sporogenous protoplasm into uninucleate, amoeboid protospores (Fig. 3a—d). These are subsequently invested with spore walls. Because it is possible to monitor accurately the number of nuclei present in the immature sporangium, and because the number of nuclei present in the protospore stage is twice that found in the immature sporangium, it can be concluded that there is evidence for only one wave of nuclear division before spore formation. The light and electron microscopical study by HASKINS, HINCHEE and CLONEY [8] suggests that two meiotic divisions occur within the spore case of this species. The contention of endosporic meiosis is supported by the observation of normal and synaptonemal complexes in the early stages of the first nuclear division.

Methods

Protoplasmodia were grown on GPY/10 agar[1] spread with a bacterial food source, *Enterobacter aerogenes*. Sporophore formation occurred 24—36 hours following subculture to 1.5% (w/v) Ionagar No. 2 (Oxoid). Protoplasmodia were photographed on a thin layer of agar pressed against a coverslip (HEUNERT [9]), on agar in a Ross microchamber (ROSS [16])
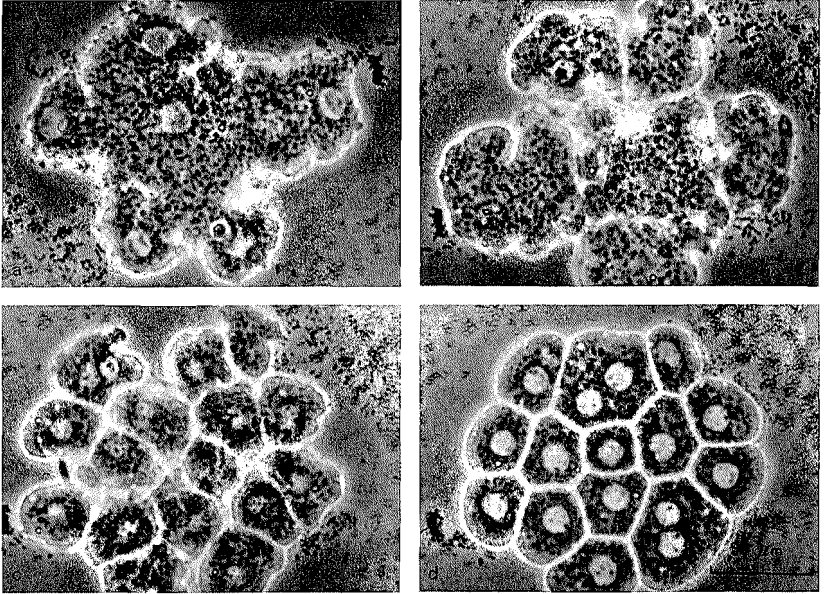

Fig. 3a—d. Synchronous nuclear division concomitant with progressive, centripetal sporangial cleavage
a: Centripetal sporangial cleavage occurs concurrently with nuclear division
b: The sporangial cleavage produces multinucleate packets — c: Progressive cleavage produces 16 amoeboid protospores — d: Two pairs of the amoeboid protospores fuse due to excessive coverslip pressure. Two other protospores are binucleate because of incorporation of degenerating nuclei

or at an agar/air interface in a coverslip microchamber. Photographs were taken on 35 mm film (Eastman Double X, London) using an Askania-Z-camera. A Zeiss WL microscope equipped with bright field, Zernike phase-contrast, or Nomarski interference-contrast optics was used.

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[1] GPY = 0.1% Difco yeast extract, 0.1% $MgSO_4 \cdot 7 H_2O$, 1% Difco peptone, 1% glucose. GPY/10 is a 1 : 9 dilution of GPY. GPY/10 agar was solidified with 1.5% Difco Bacto agar.

Film Contents[1]

Plasmodial Development
Mitosis
1 f/s to 4 f/min

1. Mitosis in a uninucleate protoplasmodium. A uninucleate protoplasmodium is distinguished from a myxamoeba by the occurrence of intranuclear mitosis and the absence of cytokinesis following nuclear division. Whether the uninucleate protoplasmodium in the center of the field is a product of nuclear fusion, or has developed by apogamy remains unclear. At the beginning of the scene the large, central nucleolus begins to fade and the chromosomes become arranged on a prominent metaphase plate. Anaphase and telophase occur and as the nucleolar fragments reappear the daughter nuclei become more distinct. At the termination of mitosis the binucleate protoplasmodium begins to ingest bacteria.
Frame width 155 μm, exposure frequency 15 f/min, phase-contrast, elapsed time 2 h.

2. Mitosis in a uninucleate protoplasmodium. The uninucleate plasmodium in the center of the field is more mature than the one observed in scene 1, above. It displays numerous phase-bright cytoplasmic granules, flame-shaped pseudopodia, contractile vacuoles, and a slime sheath. The movement of cytoplasmic granules slows as metaphase, anaphase, and telophase occurs. Phagocytosis and cyclosis resume at the completion of nuclear division.
Frame width 98 μm, exposure frequency 4 f/min, phase-contrast, elapsed time 3 h. 48 min.

3. Mitosis in a binucleate protoplasmodium. This binucleate plasmodium is the same one recorded in scene 2, above. The two nuclei proceed through mitosis in synchrony and following division the movement of granules and phagocytosis are evident in the 4-nucleate plasmodium.
Frame width 155 μm, exposure frequency 30 f/min, phase-contrast, elapsed time 35 min.

4. Mitosis in a 4-nucleate protoplasmodium. Mitosis of the nuclei is synchronous and results in an 8-nucleate plasmodium. This plasmodium is the same one recorded in scene 2 and 3, above.
Frame width 155 μm, exposure frequency 1 f/sec, phase-contrast, elapsed time 11 min.

5. Migrating protoplasmodium. A migrating protoplasmodium is polarized. The hyaloplasmic anterior is deeply lobed and a cluster of bacteria enmeshed in the slime sheath trail behind the plasmodium.

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[1] The headlines in *italics* correspond with the subtitles in the film.

Frame width 195 μm, exposure frequency 1 f/sec, phase-contrast, elapsed time 10 min.

6. Migrating protoplasmodium. As the plasmodium migrates over the agar substratum, bacteria are rolled before the deeply lobed anterior margin. Numerous cytoplasmic blebs and bacteria enmeshed in the slime sheath drag from the posterior (Fig. 2).

Frame width 195 μm, exposure frequency 1 f/sec, interference-contrast, elapsed time 8 min. 21 sec.

Phagocytosis
Plasmotomy
15 f/min and 8 f/min

7. Phagocytosis. Anterior pseudopodia actively ingest bacteria and debris is expelled from the posterior of the protoplasmodium. Note that a hyaline slime sheath surrounds the cell.

Frame width 120 μm, exposure frequency 15 f/min, phase-contrast, elapsed time 42 min. 24 sec.

8. Plasmotomy. At the beginning of the scene a solitary protoplasmodium is observed. It consists of two distinct lobes connected by a narrow stem. As these lobes pull apart and the stem breaks, daughter plasmodia are formed.

Frame width 300 μm, exposure frequency 15 f/min, phase-contrast, elapsed time 53 min.

9. Plasmotomy. A cleft which forms on one side of the protoplasmodium leads to the development of two lobes. Division of the cell in two is accompanied by reversing, rhythmic streaming.

Frame width 400 μm, exposure frequency 8 f/min, interference-contrast, elapsed time 1 h. 29 min.

Encystment
Cyst Germination
8 f/min and 4 f/min

10. Encystment of protoplasmodia. Three protoplasmodia cease migration and retract their pseudopodia. Vigorous cyclosis and contractile vacuole activity is observed as the cyst walls form.

Frame width 195 μm, exposure frequency 4 f/min, interference-contrast, elapsed time 3 h. 3 min.

11. Cyst germination. During the germination of a solitary cyst, active cyclosis occurs, pseudopodia appear, and the hatched protoplasmodium begins active movement. No cyst wall remains upon germination.

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Frame width 155 μm, exposure frequency 8f/min, phase-contrast, elapsed time 1 h. 10 min.

12. Cyst germination. Three protoplasmodial cysts germinate; no cyst walls remain. Although the protoplasmodia come into repeated contact, coalescence does not occur.

Frame width 195 μm, exposure frequency 8 f/min, interference-contrast, elapsed time 1 h. 44 min.

13. Overview of migrating, feeding protoplasmodia. Uncompressed, reniform protoplasmodia migrate over an agar substratum feeding on a layer of bacteria. Although plasmodia often collide with one another, coalescence is not observed.

Frame width 1500 μm, exposure frequency 4 f/min, oblique bright-field, elapsed time 2 h. 18 min.

Sporophore Formation Nuclear Division Sporangial Cleavage
15 f/min to 4 f/min

14. Sporophore formation; side view of one sporophore. The appearance of a mound-shaped initial in the center of the stationary protoplasmodium is the first indication of sporulation. The acellular stalk which has formed beneath the sporangial initial continues to elongate for approximately the next 1¾ hours. No surging movements, typical of sporophore development in the Physarales, are observed during this process. A protoplasmodium migrating from the rear of the field collides with the stalk of the sporophore. The protoplasmodium moves away, returns and collides once more.

Frame width 500 μm, exposure frequency 8 f/min, bright-field, elapsed time 2 h. 11 min.

15. Sporophore formation; lateral view of a fruiting body. The sporangial initial is elevated from the substratum at the tip of an acellular stalk.

Frame width 300 μm, exposure frequency 8 f/min, bright-field, elapsed time 1 h. 12 min.

16. Sporangial nuclei in division. Synchronous nuclear division is observed in a highly compressed sporangium. This is presumed to be intranuclear because sharp separation of the nuclear and cytoplasmic regions persist during division and evidence of a nuclear membrane, present as a thin dark line, can be detected. Upon the completion of division a number of tiny nucleoli appear in each nucleus. Subsequently, some of the nuclei become arranged in pairs, trios, and quartets. A few nuclei appear to be degenerating. Because of compression, no cleavage is observed.

Frame width 155 μm, exposure frequency 4 f/min, phase-contrast, elapsed time 3 h. 57 min.

17. Nuclear division concomitant with sporangial cleavage. Synchronous nuclear division occurs in 8 nuclei of this slightly compressed sporangium. Two smaller degenerating nuclei do not divide. Initially, cleavage results in the production of 16 amoeboid protospores. Subsequently, because of excessive coverslip pressure, two pairs of protospores fuse and become binucleate. Two other protospores are binucleate because of incorporation of degenerating nuclei (Fig. 3a—d). As the scene concludes, nuclear rotation within the 14 protospores is especially prominent.

Frame width 120 μm, exposure frequency 15 f/min, phase-contrast, elapsed time 1 h. 3 min.

18. Sporophore formation; lateral view of one fruiting body. The spherical sporangial initial mounted atop the tapering, acellular stalk is progressively elevated from the substratum. When the sporophore attains its maximum height, cleavage occurs. Note the close packing of spores within the mature sporangium.

Frame width 400 μm, exposure frequency 8 f/min, bright-field, elapsed time 2 h. 28 min.

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Film Data

Summary of the Film

Mitosis in uninucleate to 4-nucleate protoplasmodia is illustrated; plasmotomy is demonstrated. Phagocytosis, encystment, and excystment are included. Sporulation, including nuclear division concomitant with cleavage is presented.

Inhalt des Films

Der Film zeigt die Mitosevorgänge vom einkernigen bis zum vierkernigen Protoplasmodium sowie die Plasmotomie. Man sieht Phagozytose, Encystierung und Excystierung sowie die Sporenbildung einschließlich der Kernteilung.

Résumé du Film

La mitose d'un protoplasmodium à un noyau en un protoplasmodium à 4 noyaux est illustrée; la plasmodie est démontrée. Phagocytose, enkystement et dékystement sont inclus. La formation de spores, y compris la division du noyau avec scission simultanée, est représentée.

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