OOSPORE GERMINATION IN TWO SPECIES OF *CHARA*[1]

by

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ABSTRACT

Germination of the oospores of *Chara contraria* Kützing and *C. zeylanica* Willd. was investigated with respect to the effect of a variety of environmental factors, among them, photoperiod, presence or absence of oxygen, culture medium, and temperature. The results indicated that the oospores of the species investigated require a period of dormancy before germination. This period may be shortened by treatment at low temperature (5° to 7°C), but if this is omitted, germination follows ultimately, but is delayed. Germination occurs under both aerobic and anaerobic conditions, contrary to reports in the literature. The ontogeny of the protonema and of the primary axis in early germination has been described for *Chara contraria* and is essentially similar to the general pattern described by earlier investigators for other species of the genus. Studies of the organism in culture indicated clearly the important role of the rhizoids in generating secondary protonemata and, thus, in the colonization of a given substrate.

The species of *Chara* are submerged aquatics attached to the substrate by rhizoids. They are colonial plants and form dense patches on the bottom of fresh and brackish bodies of water. Some grow in deep water, some in shallow, some in still water and some in rapidly running streams; perennial species grow intermingled with annual ones. They prefer oligotrophic waters and never grow in water which is polluted.

In nature, as streams and ditches containing *Chara* become dry, the oospores remain as agents of survival during dormancy. When the rain again fills dry stream beds, the oospores germinate and start a new generation. Oospores in nature, however, also germinate in habitats where they are not subjected to desiccation.

The writer has been interested in studying the early morphogenesis of *Chara*, and undertook experiments to germinate the oospores of *Chara contraria* and *C. zeylanica*.

Ross (1959), Proctor (1960), Carr and Ross (1963) and Forsberg (1965) have reported some of their experiments on germination of the oospores of different species of *Chara*. Carr and Ross, and Forsberg all emphasize the requirement of anaerobic condition for the germination of the oospore of *Chara*.

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The writer devised an agar-plate method as a convenient way of germinating *Chara* oospores. With this method, the development and morphogenesis of the sporelings of *Chara contraria* were studied.

The materials used were oospores of *Chara contraria* collected from the dried plants and from mud, and oospores collected from the living plants of *C. zeylanica*. The method of selection and sterilization of the oospores for germination is described as follows: Oospores were separated from the soil particles and debris layer by means of a series of soil-sieves, and were retained in the finest U. S. standard sieve number 70 with nominal openings of 0.21 mm. The oospores were then picked out with a small forceps under the stereoscopic binocular microscope, and were washed and sterilized as follows. The oospores were immersed in detergent solution,(2) sonicated mechanically(3) for 10 sec., washed, and then rinsed with sterilized water ten times. The oospores were then sterilized by adding 3 drops of Clorox(4) to the tube containing 6 ml of water, and oospores were left in Clorox for 1 min. and then washed with sonication, five times.

The clean, sterilized oospores were introduced into Petri dishes with sterilized 2% plain agar. The oospores were then picked up from the agar surface with flamed needles or small forceps and arranged separately on Petri dishes containing proteose yeast-extract agar.(5) These plates were then stored in an incubator of 37°C three days for testing the presence of bacteria. If any bacteria were present, the bacterial colonies were obvious around the *Chara* oospores. Only sterile oospores were picked out and planted.

EXPERIMENTS ON GERMINATION

A series of experiments were carried out in investigation of oospore germination. Both Petri dishes and test-tubes were used. The media for the germination experiments included: distilled water, BBM, Chu's No. 10 medium, Forsberg's medium,(6) soil water, and 0.5% water agar. A layer of mineral oil was added in some of the tubes with liquid media to maintain an anaerobic condition for comparison. *Chara* sediments(7) were added in a set of distilled water tubes. The experimental tubes and Petri dishes were incubated in a culture room under fluorescent illumination of 250 ft-c maintained on 12-hr light and 12-hr dark cycle at a temperature of 22°C.

(2) Detergent solution was prepared by adding 5 drops of detergent stock solution to 5 ml of sterile water. Detergent stock solution was freshly prepared by adding 5 drops of Tween 80 (a non-ionic surfactant; Atlas Powder Co., Wilmington, Delaware) into 100 ml of sterile distilled water and warmed gently.

(3) Disontegrator, system eighty, Ultrasonic Industies, Inc., Plainview, L. I., New York.

(4) Clorox—A commercial bleaching solution produced by the Clorox Company, Oakland, California, with active ingredient, sodium hypochlorite 5.25% by wt., inert ingredients 94.75% by wt.

(5) 0.1% proteose-peptone and 0.3% yeast-extract with BBM agar.

(6) BBM=Bold's Basal Medium (Bischoff and Bold, 1963). Chu's No. 10 medium (Chu, 1942). Fosberg's medium (Fosberg, 1665a).

(7) Debris consisting of dried *Chara* and silt from the collection site.

Some of the oospores were pretreated in the refrigerator at 5–7°C and at the freezing point, while some were stored dry at room temperature.

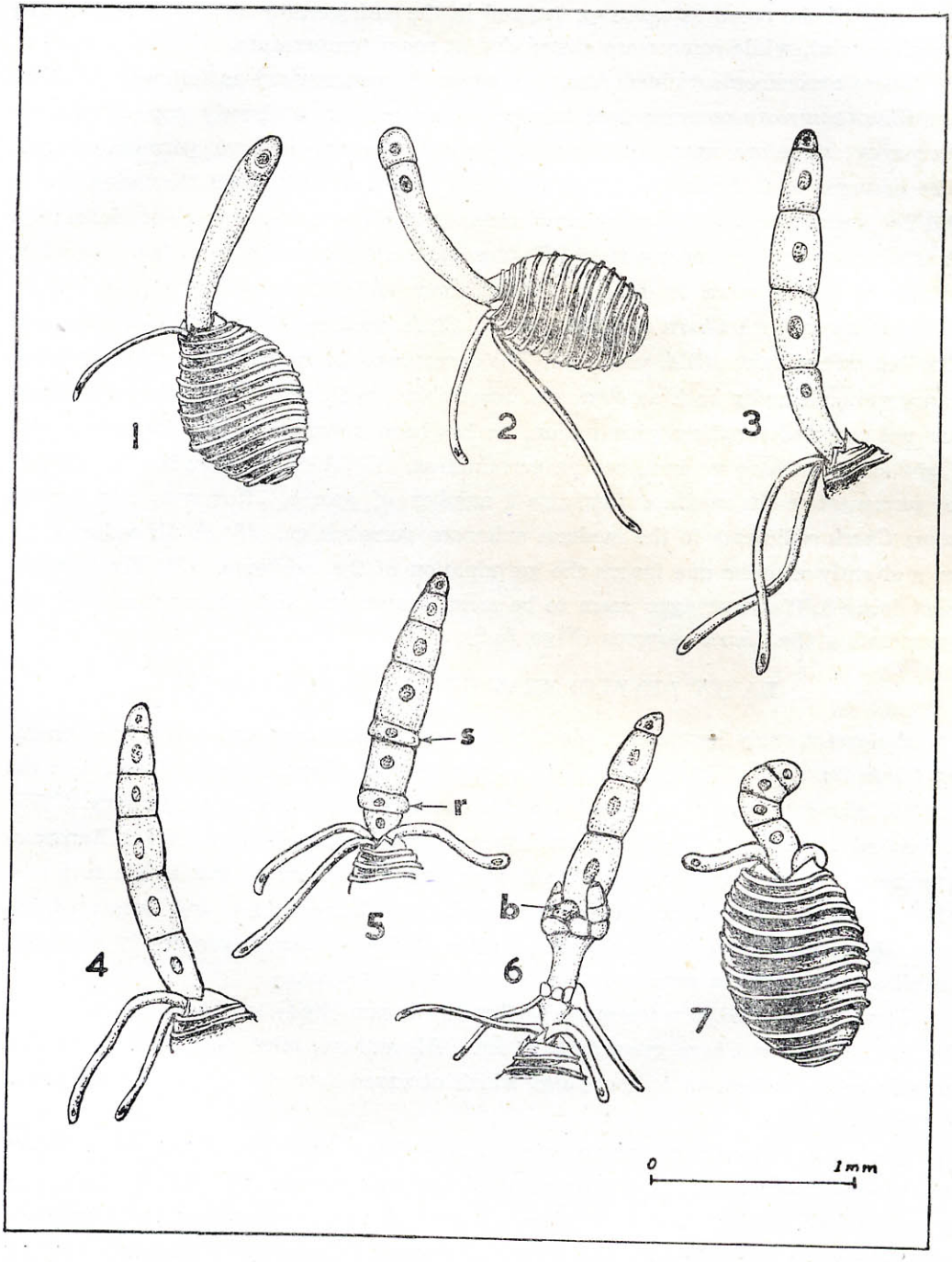
These experiments yielded data which may be summarised as follows: (1) The dry *Chara contraria* oospores used in these experiments had already completed their dormancy; therefore, even without pretreatment, they would have germinated well. The oospores of *C. zeylanica*, which had been picked directly from the living plants did not germinate unless they received some pretreatment to break their dormancy. A week to ten days cooling at 5–7°C was sufficient to accomplish this. (2) The effects of media were as follows: water agar and Texas soil water, excellent; distilled water with *Chara* sediment, good; Chu's medium, Forsberg's solution and distilled water, fair; BBM, poor, (3) The oospores of *Chara contraria* and *C. zeylanica* can germinate under both aerobic and anaerobic conditions. Anaerobic conditions are not required for their germination, as has been reported in the literature. (4) Light seems to have no influence on germination. (5) A single spore is more difficult to germinate in the medium than are a number of spores. However, addition of some *Chara* sediments to the medium enhances germination. (6) A pH value of 7.0 or a slightly alkaline one favors the germination of the oospores. (7) Petri dishes containing 0.5% water-agar seem to be most convenient and effective method for germinating the *Chara* oospores (Figs, 8, 9).

EARLY DEVELOPMENT OF *CHARA CONTRARIA*

A careful study has been made of the germination stages and early development of *Chara contraria*. Pringsheim (1862), who studied *Chara fragilla* Desv., was the first to elucidate the structure of the protonema of the sporeling. DeBary (1875) published an account of oospore development and germination of several European species of *Chara*, *Tolypella*, and *Nitella*, including also *Chara contraria*. He reviewed the previous works of Pringsheim, Nordstedt, and Kamicuskies, and presented his own observations. He stated that the course of development is essentially the same in all species of these genera except for minor differences.

More recent studies were made by Sundaralingam (1954), on *Chara zeylanica*, and by Ross (1959) on *Chara gymnopitys* A. Br. All authors, other than Ross, have had to rely on the collection of sporelings which occurred sporadically in mud of aquaria or ponds.

During germination of the oospores in *C. contraria*, the tips of the spiral ridges of the oospore coat split apart forming an aperture surrounded by five valve-like projections of original tube cells (Figs. 10, 11). A primary protonema and primary rhizoid emerge from the aperture by splitting open of the valves of the outer covering of the oospore (Fig. 1). The primary protonema at first is colorless, with a single nucleus located at the tip (Fig. 1). The primary rhizoid arises much like the primary protonema but it immediately undergoes a series of divisions to form a number of subsidary rhizoids, each of which has a nucleus at the tip.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The first nucleus at the tip of the tubular primary protonema divides, and an apical cell is cut off by a transverse wall (Fig. 2). The apical cell appears yellow. This apical cell does not divide, but the tubular filament below the apical cell becomes segmented by transverse walls into 4–5 cells (Figs. 3, 4, 7, 10). This filament, designated the protonema, is of limited apical growth, future divisions occurring only in the proximal region.

The distal four cells of the protonema stop dividing and suggest in appearance the cotyledon of a monocotyledonous embryo (Figs. 6, 16). The most proximal cell forms a transverse wall, and thus two discoid, nodal cells develop (Figs. 5, 17), at both ends a cylindrical internodal cell. Of the two nodes, the upper is shoot node and the lower the rhizoidal node. Each of those two nodal cells continues division, developing into a single layer of cells comprising several central cells surrounded by a single ring of peripheral cells. The shoot node is thicker on one side than on the other, and thus a shoot bud develops laterally (Figs. 6, 10, 14, 16). The apical cell inside the shoot bud is the initial of the adult plant; it is hidden by a whorl of branchlets or "leaves," as they are usually designated (Fig. 14, 16). Rhizoids develop from the peripheral cells of rhizoidal node (Figs. 6, 14).

Except for relatively minor differences, the germination and development of the protonema of *Chara contraria* are similar to those of: *C. foetida* A. Br., *C. fragilis*, *C. crinita* Wallr., *C. scoparis* Baur. (described by DeBary, 1865), *C. zeylanica* (described by Sundaralingham, 1954), and *C. gymnopitys* A. Br. (described by Ross, 1959).

During the development of the protonema, the sequence of wall formation differs from that described by DeBary but is in accord with that described by Sundaralingam and Ross. The first wall is formed close behind the tip of the protonematal filament, and successive walls are formed basipetally. DeBary stated that the first-formed wall is the most proximal one and that it segregates the distal, densely cytoplasmic portion of the filament, which is later divided into several cells.

Adventitious protonemata may be developed from the node of a rhizoid (Figs. 12, 15, 17, 18). Their mode of development is similar to that of the primary protonema. Here again the first nucleus at the tip of the protonema divides, and an apical cell is cut off by a transverse wall. The filament below the apical cell is cut off basipetally

Figures 1–7. *Chara contraria.*

Fig. 1. Primary protonema and primary rhizoid emerged from the aperture of the oospore, with a single nucleus located at the tip.

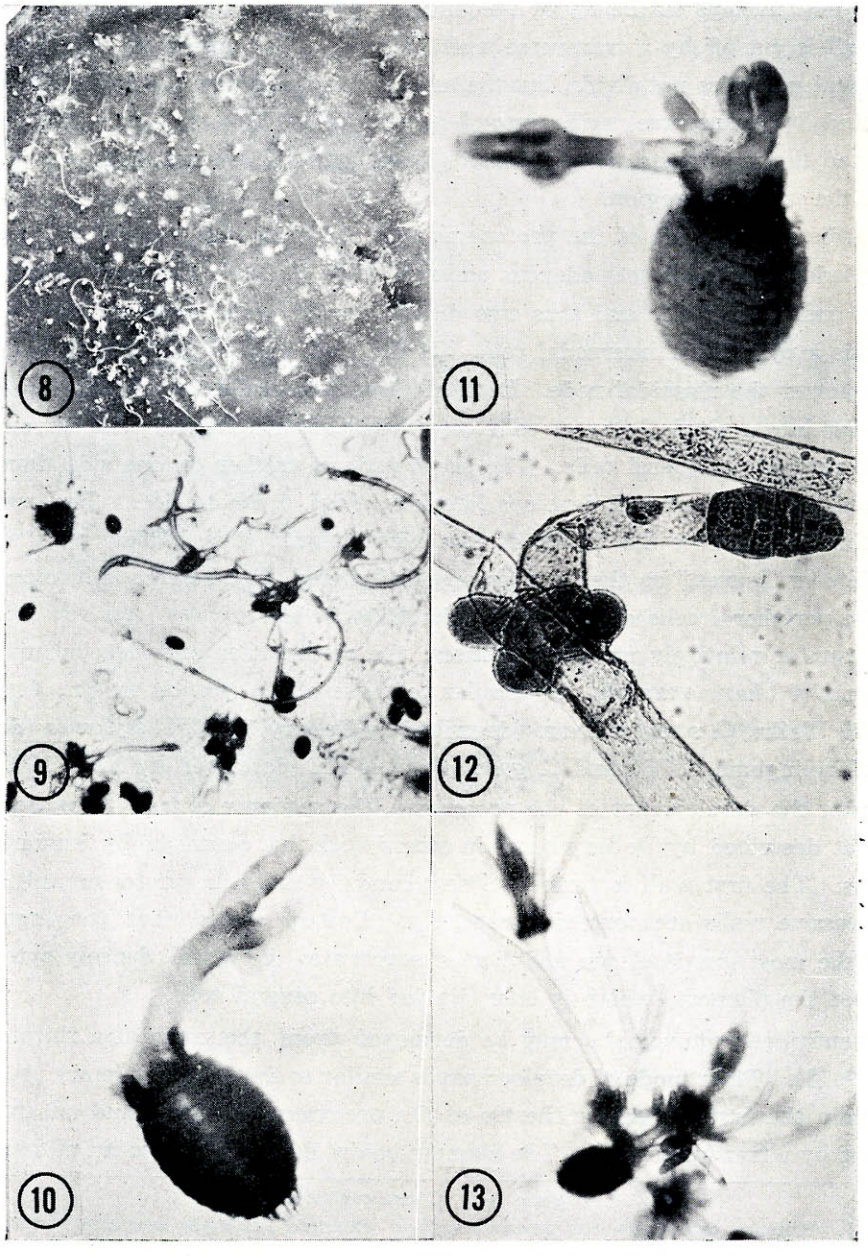
Fig. 2. An apical cell of the primary protonema is cut off by a tranaverse wall.

Figs. 3, 4. The tubular filament of the primary protonema becomes segmented by transverse walls.

Fig. 5. Two discoid, nodal cell initials developed on the primary protonema; s., shoot node initial; r., rhizoidal node initial.

Fig. 6. A shoot bud (b) is initiated from the shoot node of the primary protonema and rhizoids developed from the peripheral cells of rhizoidal node.

Fig. 7. A germinating spore with segmented primary protonema and a primary rhizoid emerge from the aperture by the splitting open of the valves of the outer covering of the oospore.



Figures 8-13. *Chara contraria.*

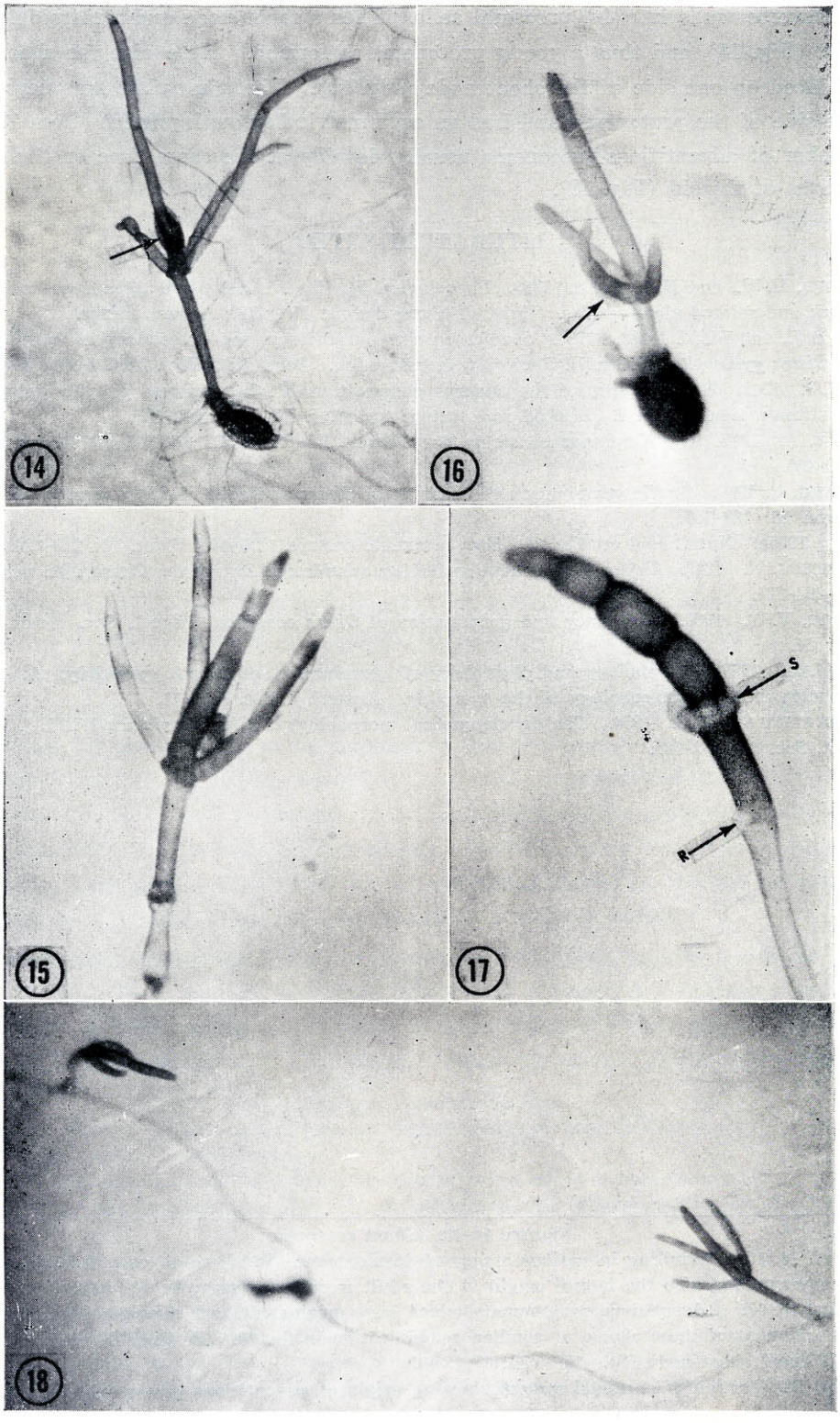
Fig. 8. Aerobic germination of oospores on surface of agar.

Fig. 9. Enlarged view of germiling from preceding. ×4.

Figs. 10, 11. Early stages in oospore germination; note the protuberant protonema, ruptured oospore wall, and rhizoid. ×30.

Fig. 12. Adventitious protonematal initial arising from nodal structure of a rhizoid. ×150.

Fig. 13. Primary and secondary protonema developed from a single oospore. ×10.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by transverse walls into 4–5 proximal cells. Then a shoot node and a rhizoidal node develop (Fig. 17), and thus a young protonema is formed. Here also the shoot bud is produced on one side of the shoot node (Fig. 15). The adult plant was developed on one side of the protonema and had an asymmetrical growth pattern (Figs. 13, 14). A number of adventitious protonema usually develop at the same time on the nodal structures of rhizoid (Fig. 18).

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Figures 14-18. *Chara contraria.*

Figs. 14, 16. Germiling in various stages of development. In all cases note the segments of the protonema and also the lateral origin of the adult main axis (arrow). 14, ×10; 16, ×15.

Figs. 15, 17. Adventitious protonemata which have originated from rhizoids; Figs. 15 and 17 are enlargement of those shown at smaller magnification in fig. 18. In Fig. 17. note rhizoidal node (R) and shoot node (S). 15, ×30; 17, ×40.

Fig. 18. Portion of rhizoidal system showing origin of adventitious protonemata. ×5.