Sorocarp Development by a Newly Discovered Ciliate

Abstract. *A recently discovered predatory ciliate has an unusual developmental pattern in which the swimming cells cease feeding and aggregate on a substrate near the surface of a liquid medium. The aggregate then rises aerially, producing an acellular stalk and a sorus of encysted cells. Sorogenesis requires alternate light and dark periods.*

Before the recent discovery (*1*) of a ciliate that produces aerial fruiting bodies (Fig. 1A), the cellular slime molds—a group of mycetozoans of increasing importance in studies of developmental biology—and a labyrinthulid-like protistan (*2*) were the only eukaryotes known to have independent trophic cells that later aggregate and form masses of cells that develop into aerial sorocarps (*1*, *3*). Only recently have we been able to culture the ciliate and study its life cycle. Certain similarities between its life cycle and that of cellular slime molds, as well as the unique aspects of its developmental pattern, should ensure its usefulness in future developmental studies.

Swimming cells of the sorogenic ciliate (Fig. 1B), which are characterized by an apical slitlike mouth and longitudinal ciliary rows (kineties), resemble those of the genus *Enchelys* of the order Gymnostomatida. The organism has been collected ten times in temperate to tropical areas of the world, including the coastal plain to mountains of North Carolina. It has been found only on dead plant parts (capsules, pods, dried fleshy fruits, and twigs) that have remained attached to the plants. Several days after an appropriate substrate is placed in a layer of water in a petri dish, the sorocarps arise on the moist substrate (Fig. 1A) or on floating viscous films. Repeated efforts to culture the ciliate in liquid media to which various bacteria were added were unsuccessful. Recently, some of the swimming cells were observed ingesting those of another ciliate, an unidentified member of the genus *Colpoda*. The latter was isolated and grown without difficulty in the presence of bacteria both in liquid culture and on agar media. When sorocysts were added to liquid media they germinated and produced swimming cells which, if those of *Colpoda* were also present, fed actively on them, multiplied, and produced sorocarps a few days later.

The isolate described here was obtained from old blackened figs collected about $2^1/_2$ m aboveground from a tree at Wau Ecology Institute in Papua New Guinea. Good growth and sporulation are obtained by the following procedure: (i) growth of the food ciliate in the presence of *Escherichia coli* in 100 ml of liquid medium containing 1 percent lactose and 0.5 percent yeast extract in a 250-ml Erlenmeyer flask placed on a shaker at 100 cycles per minute for 2 days; (ii) addition of sorocysts or swimming cells of the predatory ciliate to the medium and return of the flask to the shaker for 2 days; and (iii) transfer of about 10 ml of the suspension to a petri dish containing a layer of hay-infusion agar, followed by the addition of hay-infusion liquid with a suspension of *E. coli*, bringing the level of the liquid to 2 to 3 mm above the agar surface. The addition of more sorocysts or predatory swimming cells to the culture dish at this time ensures earlier and more abundant sorocarp production. Bacteria from the original substrate are invariably present in the cultures. Within 2 to 5 days, sorocarps develop around the periphery of the plate or on bits of sterilized dead plant parts inserted into the agar in such a manner that they slightly protrude above the water surface. Sorocarps also develop on floating viscous films deposited by bacteria or by introduced pollen grains. In all cases the bases of the sorocarps appear to remain in contact with the water surface throughout their development.

When the trophic cell of the sorogenic ciliate is feeding, its mouth comes into contact with a swimming *Colpoda* cell, expands, and draws the food cell into the interior, where as many as four or five ingested cells, giving the predatory cell a stout appearance, may be observed. Multiplication is by binary fission. Encystment of individual swimming cells in liquid medium is common. No essential difference between these and sorocysts has been detected. No evidence of sexuality has been observed, but more detailed studies are needed to determine its presence or absence.

Sorocarp development has been studied primarily on dead plant fragments inserted into the culture plates. When the predator cells have increased greatly in number and the food cells are much less abundant, the former begin to aggregate on the substrates close to the surface of the water. Under normal day and night conditions, aggregation begins in early morning hours. The aggregates first appear as single layers of cells on the substrate surface. They soon become thicker as additional cells swim in singly from the liquid medium and the peripheral cells of the original layer swim toward a common center. Aggregated cells are constantly in irregular motion. Some of them briefly move just outside the mass singly or in small adherent groups and

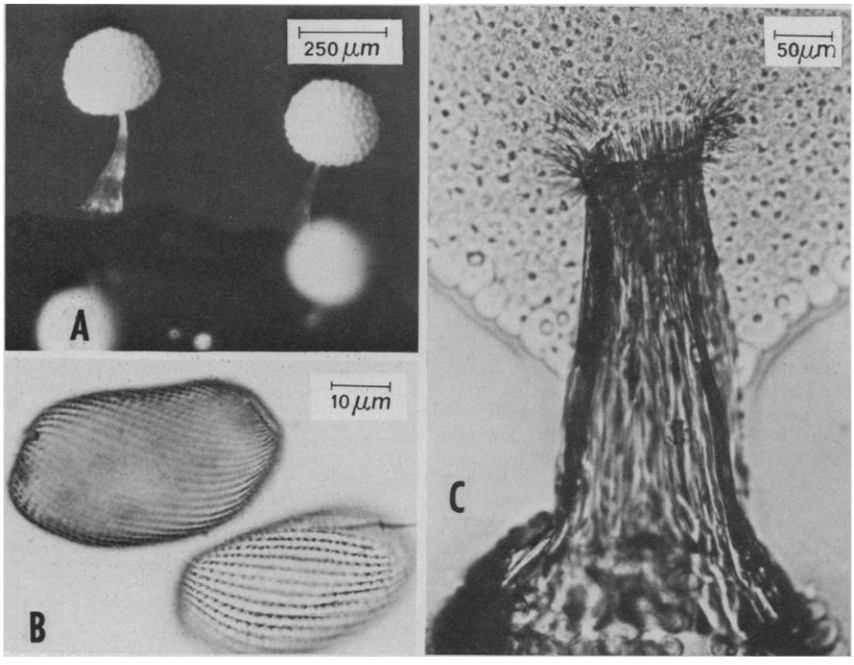

Fig. 1. (A) Sorocarps of ciliate on pokeberry pedicel removed from culture dish. (B) Silver-impregnated cells, showing longitudinal ciliary rows and apical mouthpart (upper cell). (C) Immature sorocarp lying on agar surface, showing broad base with embedded cells, longitudinally furrowed stalk with fibrous cupule at apex, and mass of sorogenic cells.

0036-8075/78/1103-0530$00.50/0

then back into it. Cells swimming into an aggregate have ceased feeding and move more slowly than the trophic cells. Rapidly swimming ones that approach an aggregate pass by without showing any attraction toward it.

About 2 hours after the flat aggregation layer is first detected, a compact mass of cells is produced which then develops into a hemispherical shape and protrudes above the water surface (Fig. 2A). During this stage of development the cells begin to secrete a mucous matrix in which they become embedded. In addition, a sheath develops around the exterior of the hemispherical mass. From this stage until the end of stalk formation, the cells retain a somewhat rounded shape and show a turbulent movement caused by ciliary activity. They constantly rotate and change position within the mucous matrix. All developmental stages from ensheathed aggregate to mature sorocarp float on water if they become detached from the substrate.

The mucous mass of cells that comprise the sorogen emerges from the top of the hemispherical ensheathed aggregate. As the sorogen rises aerially it produces a longitudinally furrowed, apically tapered stalk (Fig. 2, B to D). The sorocarp is supported by a broad base consisting of the sheath that formerly surrounded the mature aggregate, an internal gelatinous matrix firmer than that of the sorogen, and, frequently, relatively immobile cells embedded in the matrix (Fig. 1C and Fig. 2, B to D). The latter become encysted during development. Normal sorogenesis appears to occur only under very humid conditions. If the lid of the culture dish is removed for a few minutes and replaced during sorogenesis, abnormal and incomplete sorocarps generally develop.

Normally, cells are not deposited in the elongating stalk, which consists of a longitudinally furrowed sheath surrounding a mucous-gelatinous matrix. Each ridge of the sheath is lined with a long fiberlike strand, a structure that is lacking in the furrow. Although the periphery of the sorogen's matrix appears to be more glutinous than its interior, a distinct sheath is apparent only at the base of the sorogen, where sheath and fibers coordinate to form a cupule that soon adds to the elongating end of the stalk. Cells extend from the cupule into the narrowing stalk apex for a short distance (Fig. 2C). As the sorogen rises the cupule becomes narrowed and wrinkled basally as it converts into the elongating end of the stalk, while new cupule material is continuously deposited above the stalk apex. The fibers of the cupule extend above the sheath portion, where they appear more narrow (Fig. 1C and Fig. 2, B to D).

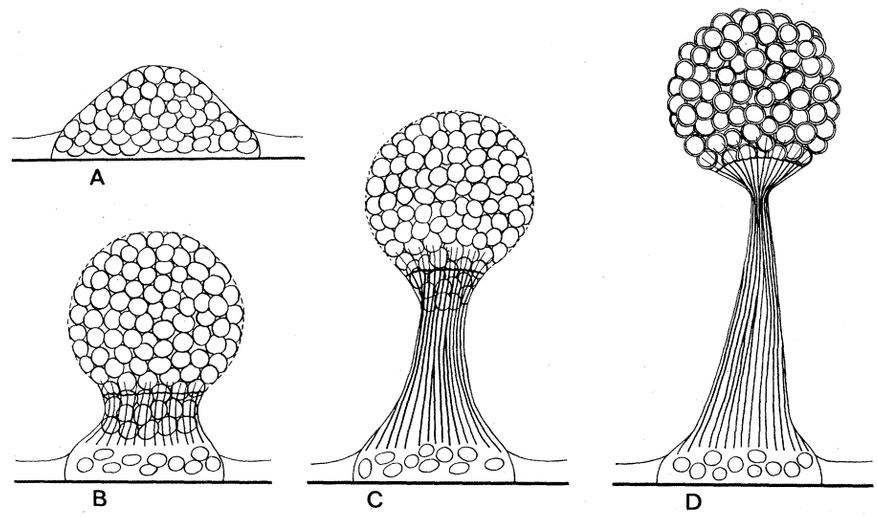

Fig. 2. Diagrammatic illustrations of stages in sorogenesis. (A) Hemispherical ensheathed aggregate protruding above surface of water. (B) Sorocarp in early stage of stalk development. (C) Intermediate stage of stalk development. (D) Mature sorocarp with tapered, longitudinally furrowed stalk and fibrous cupule at base of sorus. Note cells in stalk base and extension of stalk fibers above sheath in (B), (C), and (D). Dotted lines around sorus in (B) and (C) denote boundary of mucous matrix.

Stalk development, starting with the ensheathed aggregate, is complete within 30 to 45 minutes. Apical tapering of the stalk is correlated with a decreasing number of sorogenic cells protruding into its tip (Fig. 2, B and C). When stalk elongation ceases, the cells of the developing sorus rotate in a fixed position as walls are deposited around them. Rotation slows down and ends during sorocyst maturation. There is a slight contraction in height of the sorocarp as it matures. A fibrous cupule persists at the base of the sorus (Fig. 2D).

The force that motivates the rise of the sorogen as it deposits the stalk remains a major question. From the limited information available, it seems most likely that as the sorogenic cells—especially those that extend into the stalk apex—continue to secrete mucous material, the latter absorbs water that diffuses in from the stalk base, thus increasing the volume of the matrix and forcing the sorogen upward. Stalk furrowing would also tend to force the matrix and sorogen upward. Whether the stalk fibers are contractile and contribute to the rise of the sorogen remains to be determined.

Occasionally, large aggregates subdivide into one large and one to several smaller ones that develop into sorocarps proportional to their size. Rarely, a small secondary sorocarp arises laterally from the lower portion of the stalk of a larger sorocarp. The sorocarps are quite variable in size, the largest exceeding 1 mm in height and possessing a sorus nearly 0.5 mm in diameter with several hundred sorocysts. The smallest have relatively short, narrow stalks that bear as few as four sorocysts apically. Sorocysts measure 18 to 32 μm in diameter.

When mature sorocarps are removed from humid culture dishes and enclosed within dry petri dishes, the sori begin to fracture as the mucous matrix dries. This results in the expulsion of sorocysts, which then land on the surface of the dish. Obviously, air currents would also contribute to their distribution during this process. After most of the sorocysts have been dispersed, a group generally remains attached to the inside of the cupule at the stalk tip.

Within 2 hours after transfer to a liquid medium the sorocysts begin to germinate, a single swimming cell emerging from a pore or slit in the cyst wall. Sorocysts stored in dried condition for as long as 20 months in an incubator at 16°C have been induced to germinate.

Sorogenesis requires alternating light and dark periods. Cultures grown in constant dark or constant light fail to produce sorocarps. Under ordinary day and night conditions in the laboratory, after aggregation occurs in the early morning, sorogenesis commonly takes place between 7 and 10 a.m. If cultures are retained in the dark on the morning when sorogenesis is due, sorocarp development continues normally. An excess of transmitted light during aggregation can cause dispersal of the cells. The time of

sorogenesis may be readily shifted by altering the light and dark periods. Soro carps that develop in culture dishes placed in a dark box with light entering from a small opening on one side show no tendency to become oriented toward the light source.

The information presented here should facilitate more detailed developmental studies of this unique ciliate.

LINDSAY S. OLIVE
Department of Botany,
University of North Carolina,
Chapel Hill 27514

References and Notes

1. L. S. Olive, *The Mycetozoans* (Academic Press, New York, 1975). Sorocarps of the ciliate were first detected by my technical assistant, C. Stoianovitch.
2. M. J. Dykstra and L. S. Olive, *Mycologia* **67**, 873 (1975).
3. J. T. Bonner, *The Cellular Slime Molds* (Princeton Univ. Press, Princeton, N.J., 1967); K. B. Raper, in *The Fungi*, G. C. Ainsworth, F. K. Sparrow, A. S. Sussman, Eds. (Academic Press, New York, 1973), vol. 4B, pp. 9-36.
4. Supported by NSF grants DEB 72-02392 and DEB 77-12652. I thank P. C. Bradbury and T. M. Sonneborn for their critical reviews of the manuscript. I also thank Dr. Bradbury for contributing Fig. 1B and information on the classification of the ciliate, and R. L. Blanton for Fig. 1A.

28 July 1978

Chimpanzee Problem-Solving: A Test for Comprehension

Abstract. *An adult chimpanzee was shown videotaped scenes of a human actor struggling with one of eight problems and was then shown two photographs, one of which depicted an action or an object (or both) that could constitute a solution to the problem. On seven of the eight problems, the animal consistently chose the correct photograph. This test of problem-solving comprehension permits the animal's knowledge about problem-solving—its ability to infer the nature of problems and to recognize potential solutions to them—to be examined.*

Köhler's pioneering experiments on tool use by chimpanzees provided early evidence for complex problem-solving capabilities in a nonhuman species. The chimpanzees, when faced with inaccessible food, fit sticks together for use as a rake, propped up poles or stacked boxes for use as ladders, pulled strings attached to distant goal objects, and moved aside physical obstructions blocking their paths (*1*). Subsequent research by other investigators has focused on behavioral mechanisms of the ape's performance. For example, many of the actions chimpanzees display in problem situations can be traced to "innate" origins in play behavior, and the behavioral progression from apparently random activity to organized, goal-directed solution behaviors may often be described in terms of trial-and-error learning (*2*). However, Köhler noted that some of his subjects arrived at solutions quite suddenly, after a period of intense activity and then quiescence. He proposed that such cases revealed "insight"—perceiving relationships between a problem and its solution—which organized successful goal-oriented behavior. Unfortunately, these intriguing observations have received little experimental attention in subsequent research.

To what extent does the chimpanzee comprehend the elements of a problem situation and potential solutions? Our understanding of this aspect of problem-solving in chimpanzee and other species is limited by methods that rely solely on observations of subjects producing solutions to problems. It is essential to study not only the animal's problem-solving performance but also its knowledge about problem-solving. Accordingly, we designed a procedure which provided a chimpanzee with the opportunity to observe, rather than participate in, a problem situation. We simply showed the subject videotaped scenes of a human actor encountering one of several problems. The chimpanzee was then required to identify, rather than produce, a means for solving the actor's problem by choosing a photograph depicting a potential solution. By this technique, we examined the chimpanzee's capacity to recognize representations of problems and solutions, as well as its ability to perceive the relationship between each type of problem and its appropriate solution.

The subject was Sarah, an African-born female chimpanzee (*Pan troglodytes*) approximately 14 years old. She was obtained by the laboratory when less than 1 year old and was trained and tested on numerous cognitive tasks, including a simplified language (*3*). Although she had no formal experience with the problems investigated here, she did have extensive prior exposure to photographs and television programs broadcast over commercial networks, a factor which undoubtedly contributed to her performance with the visual test material.

The test consisted of two tests with four problems each. For each test, we staged one 30-second scene of a trainer struggling with each of four problems and videotaped each scene. In addition, we made photographs of either the trainer performing an action with an object or an object alone, which could constitute a solution to each problem. The two tests differed in the nature of the televised problems and in the content of the photographic solutions. In test 1, problems were of the standard variety used in animal testing and were based on those Köhler arranged for his chimpanzee subjects (Fig. 1) (*1*). Videotaped scenes showed the actor struggling to reach bananas made inaccessible in one of four ways. The photographic solutions depicted the actor performing an action with an object in the situation. In test 2, a new set of problems was drawn from events in the daily laboratory routine, and the photographic solutions merely showed objects which could constitute a solution to each problem. In this second test, "problem" was no longer defined simply as inaccessible food but ranged from a human actor locked inside a cage to a gas heater that had gone out (Fig. 2).

Each test consisted of several daily sessions of four trials each, with intertrial intervals of approximately 2 minutes. During each trial, Sarah was shown one black-and-white videotaped scene on a television monitor (Sony CVM-115 with an 11-inch screen). In the last 5 seconds of the scene, the videotape was put on "hold," thereby leaving an image of the problem situation on the screen like one of those shown in the left-hand columns of Figs. 1 and 2. The trainer then handed Sarah a covered box containing two of the set of four 8- by 10-inch color photographs, each mounted on a 10- by 12-inch piece of plywood. Afterward, the trainer left the room and closed the door. Sarah was required to open the box, select one photograph, and place it on a paper towel in front of the television screen. This aspect of the procedure was derived from a previous match-to-sample paradigm, in which Sarah was trained to place correct comparison stimuli on a towel and incorrect ones elsewhere. Sarah then summoned the trainer from an adjacent room by ringing a bell. Thus, the subject responded in the absence of the trainer, a procedure we use routinely for the control of social cues (*4*). When the trainer heard the bell, he returned to the test room and graded Sarah's answer, telling her either "Good Sarah, that's right," or "No Sarah, that's wrong," in a tone of voice one would use with a young child. At the end of every session she was given yogurt, fruit, or candy.

Before each test, Sarah was given a preliminary session in which she was

0036-8075/78/1103-0532$00.50/0