

Exploring the Terrestrial Habitats Of an Aquatic Protozoan

JO ANNE MUELLER

TEACHING STUDENTS to ask stimulating, thought-provoking questions is the key to developing probing scientific minds. Questions demand answers which, in turn, lead to more specific questions and answers. An especially interesting animal that can be used to develop this question-answer process is the ciliated protozoan *Colpoda cucullus*. The following activities take students into the field as well as the laboratory as they seek to understand this organism.

C. cucullus, for decades considered a "soil" protozoan, is predominantly a vegetation-associated species that exploits the fluctuating moisture content of the terrestrial environment (Mueller and Mueller 1970). This holotrichous ciliate can be obtained from virtually any piece of vegetation, eliminating the necessity of ordering animals from a biological supply house. Most biology students are surprised to find *Colpoda* on a blade of grass, a dead clover head, corn silks, flowers, or even a small piece of tree bark. Although they cannot be detected on dry vegetation, if the material is placed in water, *Colpoda* will excyst within one to two hours and are then clearly visible as they swim about. From this simple observation, what questions can you help your students formulate and how might these questions be answered? Perhaps the most obvious questions are these: (i) What are the habitats of *Colpoda cucullus*? (ii) How are the protozoans dispersed? (iii) What adaptations make possible their surviving in the habitats they occupy?

Habitats

Investigating the habitats of *Colpoda* involves relatively simple procedures. Make collections of soil, natural waters, and a variety of plants. Place the dry collections in water and observe for activity. The only equipment needed are petri dishes or other culture vessels, a notebook for recording notations about the samples, and a low-powered microscope. Students quickly discover that these organisms are associated with terrestrial vegetation and the soil beneath vegetation but are rarely found in pond or lake water. The data in table 1 identify several representative *Colpoda* habitats. These data (or similar data collected by a class) can stimulate many questions. Encourage students not only to ask questions but to seek answers as well. For example, why aren't these aquatic cells present in lakes and ponds? How might you find out? With a

micropipette place *Colpoda* in 1 cm³ of water samples taken from a lake or pond. After 24 hours no *Colpoda* will be detectable, but the other organisms in the samples will appear the same as the control to which no *Colpoda* were added. To determine if the *Colpoda* have died or encysted, allow the fluid to evaporate and then rewet the samples with distilled water. I have observed that only *Colpoda* excyst, grow, and reproduce in the rewetted samples even though the original heterogeneous collections contain many types of protozoans that encyst and excyst under proper conditions. This observation indicates that conditions in the natural collections were not suitable for growth and reproduction of *Colpoda*, perhaps because of antibiotic products from the other animals or because of competition for food. *Colpoda* exposed to these incompatible conditions encyst. Rewetting the samples not only allows the *Colpoda* to excyst, but with the demise and absence of their competition, *Colpoda* can extend their range into a habitat previously occupied by other species. This single experiment vividly demonstrates the survival potential of *Colpoda*.

Your students might be interested in learning if these protozoans are found only on the soil surface or if they are also found below the surface. Take a core of soil to determine the number of *Colpoda* present at various levels.

Although most plants harbor *Colpoda*, the organism is never found in association with certain plant species. Students can test these species to determine if they produce a substance toxic to *Colpoda*. Submerge plant parts (flowers, stems, leaves and fruits) in dis-

The author is research biologist at the University of Evansville, Evansville, Ind., 47702. She has at present two research programs in progress: an ecological study of the fate of killer and sensitive paramecia after they are introduced into a paramecium-free lake; and a gel diffusion study of the antigenic properties of the kappa particles found in killer paramecia. Mueller obtained an M.A. from Indiana University in 1962 while she was working as a research assistant for T. M. Sonneborn. She has over 40 scientific publications dealing mainly with killer paramecia, other protozoans, and mouse physiology. Her special interests include playing the harp and cultivating mushrooms in her basement, and she has a book in progress on the cultivation and use of mushrooms.



pollen are distributed by the same methods—wind and animal activity (fig. 1).

How else are *Colpoda* distributed? Herbivores eat grasses and other small plants on which *Colpoda* reside. Do the protozoan cysts escape the digestive process of these animals, and are they discharged from the body in the feces? This can be learned by collecting newly deposited feces from herbivores at a zoo, horse stable, or a farm, or it might be simpler to have a student bring in a pet rabbit. If a rabbit is used, feed the animal clover several days prior to collecting the feces because *Colpoda* may not be present in commercially prepared rabbit pellets. Make collections of fresh feces because after deposition feces may become infected with *Colpoda* present in the soil.

Place fecal samples in water and observe immediately, after several hours, and each day thereafter for several days. *Colpoda* will be present in a large percentage of the samples. If the protozoans are not immediately detectable but later become active and visible, it can be assumed that the cells were encysted when defecated but they excysted after being placed in the water. If at all possible, have the students make these collections at a zoo. A great deal can be learned while awaiting freshly deposited dung from a variety of animals.

Since *Colpoda* are found in soil as well as on plants, it might be interesting to investigate the role earthworms play in their dispersal. The following experiment defines the relationship between *Colpoda* and earthworms and can be performed by beginning biology students. Place earthworms into soil to which a high concentration of encysted *Colpoda* (around 300 per cm³ of soil) have been added. After 30 minutes, wash the earthworms in running distilled water and place them into sterile soil. Now, gently tether each worm by tying two threads around the body and securing the ends in such a way that the anterior end remains in moist sterile soil and the posterior tip is immersed in dilute culture fluid (fig. 2). As the animals ingest sterile soil, they will defecate and the castings will be collected in the culture fluid. Place the posterior end into fresh culture fluid every five minutes for 45 minutes. The castings should be observed immediately

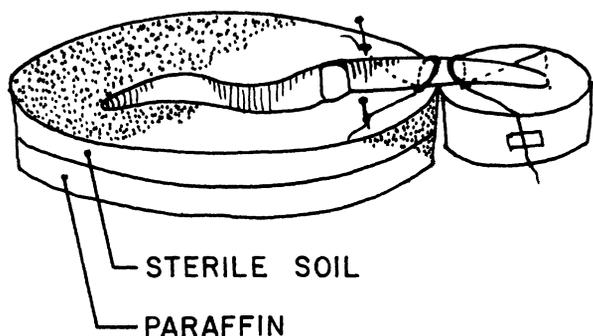


Fig. 2. Procedure for collecting earthworm castings in culture fluid. A heavy thread is tied around the posterior one-third to one-fourth of the animal and secured so that the anus is immersed in culture fluid. To keep the anterior part of the animal in position, tie another thread slightly in front of the first and secure with pins.

Table 2. Incidence of *C. cucullus* in naturally occurring intermittent waters.^a

Source of water	Average number of <i>Colpoda</i> that developed in 0.1 cm ³ fluid ^b
Dew from grass	157.3
Dew from glass plates	103.8
Rainwater	23.0
Distilled water (control)	10.2

^aModified from Mueller and Mueller (1970)

^bAverages are based on 24 isolations

and again in 24 hours. *Colpoda* cysts travel through the digestive tract of earthworms and are expelled during the first five minutes after their removal from cyst-laden soil (Mueller and Mueller 1970). Thus, the protozoans hitch a ride, although the earthworm certainly does not move *Colpoda* great distances. The same type of experiment can be performed with land snails feeding on vegetation.

From the above samplings and experiments, two conclusions emerge: (i) *C. cucullus* is abundantly present on vegetation and in soil associated with vegetation, and (ii) the protozoans are constantly being redistributed by animal activity and by passive transport in the air.

Adaptations

A single *Colpoda* transported to a suitable habitat is capable of producing a new population. One might ask why these animals survive and thrive where moisture conditions are precarious. Many microorganisms need a continuously moist environment in order to survive, but *Colpoda* encyst and excyst so quickly that they can utilize habitats unacceptable to other protozoans. In this manner they escape the control brought on through competition. Since *Colpoda* are stimulated to excyst when surrounded by water, it is instructive to investigate in the laboratory the nutritional value of naturally occurring intermittent waters in order to understand how the cells might respond when carried to these waters in nature.

Pipette feeding *Colpoda* (maintained in culture fluid with bacteria) into distilled water and leave for one hour. With a micropipette isolate cells into 0.1 cm³ aliquots of water from various sources, including dew from grass tips (collected with a micropipette), dew collected on glass plates, rainwater, and distilled water. Be sure to remove any *Colpoda* that might be in the water when it is collected. Make at least six isolations into each type of water and record the number of animals that develop from each isolate every 24 hours. Table 2 contains data from a similar experiment indicating naturally occurring intermittent waters contain the nutrition necessary for *Colpoda* to grow and

(Concluded on p. 435)

a discipline being described without interacting with the materials. When the teacher only spends 3% of his time lecturing, the remainder can be spent moderating class or team discussions, interacting with individuals or entire teams, and, in general, circulating among the various teams. We feel this situation is the epitome of the individualization of instruction. The teacher can spend time with each team or individual student discussing problems and questions unique to that group or person. This procedure combines the best of two pedagogical worlds—individual attention from the teacher and the social interaction of group discussion.

Even though content retention is not the primary goal of IRA, scores on content tests of IRA students are much higher than scores on similar tests administered in years previous to its use (Seymour et al. 1973). Tangible evidence of success with IRA is shown through more class involvement than in previous years, better attendance (14% improvement in biology classes at Norman High School in 1972-73 over 1971-72), greater content mastery, and improved grades. Before the IRA program was used at Norman High School, approximately 50% of the biology grades were C's and D's. With the IRA, 50% of the grades were B's and C's. Working in teams results in students putting pressure on their peers to become involved. That peer pressure results in involvement of the learners with the content. We hypothesize that involvement is what produces the increased content mastery.

There is no doubt that one of the major advantages of any school situation is the opportunity for students to interact in a social setting. IRA provides such experience. But as science teachers we want that social experience to be provided within the context of good, sound science content. From our perspectives—teaching high school students and teaching potential teachers—the IRA program meets both content and process needs in science, and we feel it would do so in any discipline. We recommend it.

Often when a new program such as IRA is introduced, teachers have specific questions they would like answered. What follows are four questions we have been asked; the answers have been formulated from our experience with IRA at Norman High School.

1. Does the program involve team teaching? Team teaching and team planning could be used to implement IRA, but these methods aren't mandatory. We have seen success with team planning (at Norman High School in 1972-73) and without it.

2. What about students who cannot work effectively as team members? This situation should be resolved with the student's best interests in mind, which may mean letting him work alone. This solution may be at the expense of the social skills the IRA program develops. The decision will depend on the immediate circumstances.

3. How much time is spent on laboratory work in which the student interacts with laboratory materials? About 70% of class time.

4. How much time is spent on paper-and-pencil activities? Approximately 20% of class time, and the remaining 7% is devoted to class discussions.

References

- BINGMAN, R. M., ed. 1969. *Inquiry objectives in the teaching of biology*. Biological Sciences Curriculum Study—Mid-continent Regional Educational Laboratory, Kansas City, Mo.
- , P. G. KOUTNIK, L. A. SEYMOUR, L. F. PADBERG, and K. J. BINGMAN. 1974. *Inquiry role approach*. Silver Burdett Publishing Co., Morristown, N.J.
- RENNER, J. W., D. G. STAFFORD, A. E. LAWSON, J. W. MCKINNON, F. E. FRIOT, and D. H. KELLOGG. In press. *Research, teaching, and learning with the Piagetian model*. University of Oklahoma Press, Norman.
- SEYMOUR, L. A., R. M. BINGMAN, P. G. KOUTNIK, L. F. PADBERG, L. L. HAVKICEK, A. T. KOCHER, and K. A. BURTON. 1973. *Inquiry role approach field test report (1972-73)*. Mid-continent Regional Educational Laboratory, Kansas City, Mo.
- SHEEHAN, D. J. 1970. *The effectiveness of concrete and formal instructional procedures with concrete- and formal-operational students*. Unpublished doctoral dissertation. State University of New York, Albany.

Ed Marek
West High School
Norman, Okla. 73069

John W. Renner
Science Education Department
University of Oklahoma
Norman 73069

Terrestrial Habitats ...

from p. 410

reproduce. In light of this finding, plus the fact that the cells are constantly being distributed by animal activity and by passive transport in the air, it is not difficult to comprehend the abundance and universal distribution of the opportunistic *C. cucullus*.

This paper suggests but a few of the many experiments students can undertake using this easily accessible animal. Although the suggested questions have already been answered in the literature, they encourage original thought and investigation—an ideal way to turn on students to the joy of discovery.

REFERENCES

- BARKER, H. A., and C. V. TAYLOR. 1931. A study of the conditions of encystment of *Colpoda cucullus*. *Physiological Zoology* 4:620.
- GOODEY, T. 1911. A contribution to our knowledge of the Protozoa of the soil. *Proceedings of the Royal Society of London*, B, 84:165.
- MUELLER, J. A., and W. P. MUELLER. 1969. *Colpoda cucullus* and the honeybee. *Journal of Protozoology* 16:60.
- . 1970. *Colpoda cucullus*: a terrestrial aquatic. *American Midland Naturalist* 84:1.
- PENN, A. B. 1936. Reproduction in *Colpoda cucullus*. *Archiv für Protistenkunde* 88:366.
- TAYLOR, C. V., and W. J. van WAGTENDONK. 1941. Growth studies of *Colpoda duodenaria* in the absence of other living organisms. *Journal of Cell and Comparative Physiology* 17(3):349.