Exploring the Terrestrial Habitats Of an Aquatic Protozoan

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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 cm^3 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-

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Table 1. Habitats of *C. cucullus*.*

Collection sites	*Number of collections*	*Percentage of collections containing* C. cucullus
Small herbaceous plants	540	88
Bark from trees over two inches in diameter (2 x 2 cm samples)	210	98
Soil beneath nonarboreal vegetation (6 cm^3/sample)	428	83
Barren soil (6 cm^3/sample)	176	28
Soil supporting vegetation from depressions alternately wet and dry (6 cm^3/sample)	222	96
Ponds and lakes—littoral zone (20 cm^3/sample)	28	7

*Modified from Mueller and Mueller (1970)

tilled water at 4° C for 48 hours. Place the fluid, containing any soluble substances that might have been extracted, with *Colpoda* and observe for ill effects. Some extracts cause *Colpoda* to vacuolate and die. The *Aminita* mushrooms cause rapid lysis and death. Few *Colpoda* are recovered from mushrooms although many kinds of mushrooms are not detectably toxic; in fact, most mushrooms crushed in distilled water support a lush growth of *Colpoda*. Perhaps the dearth of *Colpoda* on mushrooms is due to the brief life span of the fruiting body which is so short as to be insufficient for the introduction and subsequent reproduction of the organism.

This process of collecting and observing can also be used to familiarize students with the life cycle of *C. cucullus* (Barker and Taylor 1931; Penn 1936) (see fig. 1). Beginning as a trophozoite 40 microns long, the animal swims by means of cilia, feeds, and grows to a maximum length of 110 microns. At this point in its development the protozoan forms into a reproductive cyst inside of which the cell divides twice (rarely only once) yielding four small trophozoites each about 40 microns long; these, upon excystment, begin the cycle again. Excystment is delayed if the environment becomes dry. When the food supply is deficient or the environmental moisture falls below the minimum necessary for activity, the animal forms a resting cyst in which cell division does not occur; thus, a single trophozoite will emerge when conditions are conducive for excystment. The trophozoite usually consumes bacteria (Goodey 1916), but it can also grow saprozoically in a sterile medium of dissolved nutrients (Taylor and van Wagtendonk 1941).

Dispersal

Your students might notice that encysted *Colpoda* are similar in size and shape to many kinds of pollen. Are *Colpoda* cysts distributed in the same fashion as pollen grains? It is well known that pollen is blown by wind and carried by insects and other animals. To determine if *Colpoda* are carrried by wind, place shallow water-filled vessels covered with window screen near ground level, on a ladder or other structure, and on tops of buildings. After a week or two, bring the collections into the laboratory and add culture fluid so that the animals can reproduce and thus become detectable. A solution of 0.15% Cerophyl (available from Cerophyl Laboratories, Inc., 4722 Broadway, Suite 259, Kansas City, Mo. 64112) containing *Aerobacter aerogenes* is an adequate culture fluid. The relative number of animals in the cultures can be determined by comparing the length of times it takes for a certain population density to develop within each of the collections after the addition of culture fluid.

The successful collection of *Colpoda* in this manner indicates that wind plays a role in their dispersal. At

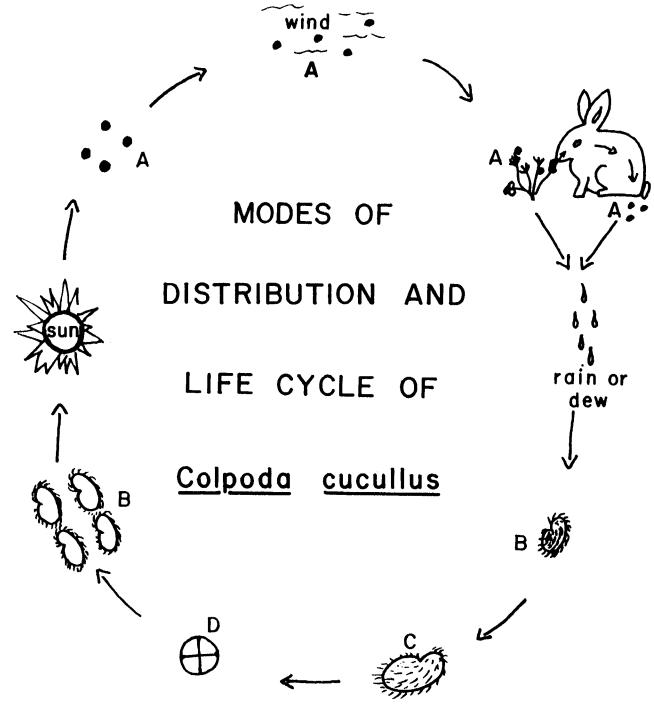

Fig. 1. Modes of distribution and life cycle of *Colpoda cucullus*. A, resting or protective cyst. B, trophozoite, 40 microns long. C, trophozoite, 110 microns long. D, reproductive cyst.

what height is the concentration of airborne *Colpoda* greatest? As you might suspect, the protozoans are more dense near the ground.

How else is pollen carried? Honeybees play a vital role in cross pollination by redistributing pollen that is collected in the baskets on their hind legs as they go from one plant to another. Students can collect bees foraging on clover with a small aerial net. It is preferable to collect bees while they are working clover since a single clover head may contain over 300 *Colpoda*! Isolate each bee in a test tube and place the tube in a refrigerator for 15–30 minutes. After the bee is inactivated by the cold, remove the pollen from the legs and place it into one cm^3 of water. *Colpoda* will probably not be immediately detectable, but after several hours they can be seen actively swimming about (Mueller and Mueller 1969). Clearly, *C. cucullus* and

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 cm^3 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 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.

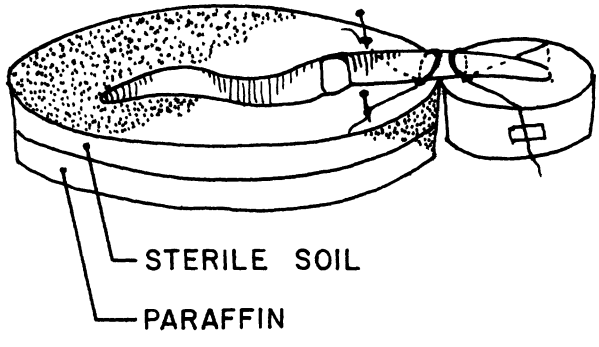

Fig. 2. Procedure for collecting earthworm castings in culture fluid. A heavy thread is tied around the posterior one-third to one-fourth of the animal and secured so that the anus is immersed in culture fluid. To keep the anterior part of the animal in position, tie another thread slightly in front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

[a]Modified from Mueller and Mueller (1970)

[b]Averages are based on 24 isolations

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 cm^3 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.

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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.

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