Culturing and Using the Vinegar Eel

By DONALD F. GALEN

The little-studied and seldom-used vinegar eel, *Turbatrix* (or *Auguillula*) *aceti,* is a harmless, nonparasitic, small roundworm, visible to the naked eye when held up to a bright light. Hardy and free-living, it is found in bulk cider vinegar. (Your local grocery is not a likely source for these worms, because most commercial vinegar has been pasteurized.) The eelworm is not only a good subject for a variety of studies in zoology and nematology but is also an excellent organism for use in general-biology classes in high school and college.

The techniques for culturing vinegar eels are simple and require very little preparation. In my laboratory and classroom, large populations of these eelworms have been maintained for the past eight years (Galen, 1969).

Materials and Methods

Primary Technique

The materials are half-pint glass milk bottles; white plastic foamlike plugs that are nonabsorbent and nontoxic; cider vinegar that is free of chemical preservatives; apple cider; long-nosed glass pipettes, 145 mm long, which are disposable; and a culture of vinegar eels, obtainable from a biology supply house. An adequate amount of eelworms would be a unit culture for 25 students. Substitutions for the containers and other expendables are readily made.

Clean the milk bottles and prepare about five bottles. (The number of bottles will depend on the number of eelworms desired). Add 60 parts apple cider and 40 parts cider vinegar to each bottle. Using a pipette, add 20 to 30 drops of stock vinegar-eel culture to the medium in the milk bottles and insert the plastic plugs. As the medium evaporates, replace it by adding more of the vinegar and cider combination.

Alternative Techniques

Any culture medium that contains cider vinegar (without preservatives) gives the vinegar eels the pH they need. The second ingredient is one that provides microorganisms, such as bacteria or *Mycoderma aceti,* as food.

The following techniques may also be used to culture vinegar eels:

1. Vinegar and apple medium (Carolina Biological Supply Co., 1968): Vinegar eels will flourish in a medium of 200 ml of cider vinegar and a 2.5-cm piece of apple, fresh or dried. Inoculate with eelworms and subculture as needed.

2. Bulk-vinegar medium (Silvan, 1966): Place several pipettes full of bulk vinegar containing vinegar eels (or an old *Turbatrix* culture) in 50 ml of pure cider vinegar. Silvan recommends subculturing every few weeks and keeping the container loosely covered in order to admit air.

3. Vinegar and solid-agar medium (Silvan, 1966): Mix 100 parts (by weight) of cider vinegar with 3 parts (by weight) of powdered agar in a container and heat until the agar has dissolved. Avoid prolonged heating. Place 10 drops from a thick population of vinegar eels on the bottom of a clean petri dish. Cool the vinegar–agar medium to 40 C and pour a small amount (cover the bottom) over the eelworms. Swirl the petri dish immediately and allow the medium to harden. The slowly moving vinegar eels can now be observed in the dense

Author's address: Biology Department, East High School, 515 N. 48th St., Phoenix, Ariz. 85008.

Transparent body of the vinegar eel permits students to investigate its internal organs. Blunt end is anterior. 400 ×

medium with the help of a microscope. Eelworms will live for only a few days under these conditions but can be observed with ease.

Discussion

The vinegar eel is an interesting animal to study in the classroom, and it can survive under many conditions. It will tolerate a wide range of temperatures —from 0 to 35 C—and can live for several days at 37 C, but it dies at 45 C. According to Behringer (1967), eelworm cultures can be maintained indefinitely at temperatures between 20 and 30 C if the evaporated vinegar is replaced. The eelworm tolerates a considerable range of hydrogen-ion concentrations—from pH 1.6 to pH 11—for various periods of time (Goodey, 1963).

The female eelworm is 2 mm in length, the male only 1.4 mm. The eggs are fertilized internally, and development takes place within the female's body. The thin membrane around the egg ruptures in the uterus, whence the young are born in an active condition. A female may produce as may as 45 young—an equal number of each sex. The life span of the vinegar eel is 10 months or more.

The eelworm can easily be recognized by its lack of circular muscles and by its rapid lateral lashing movement, brought about by longitudinal muscles. This rapid whipping motion can be reduced by adding a drop of methyl cellulose or polyvinyl alcohol to the slide. The worms usually congregate near the surface of the culture containers. Some investigators believe this is because of their need for oxygen, but Goodey (1963) has suggested negative geotaxis.

Eelworms create problems at vinegar factories when they contaminate the acetifier. However, the worms can be controlled by maintaining a temperature of 40 to 44 C. It is probable that vinegar flies (*Drosophila funebris*) and related flies act as distributors of vinegar eels, inasmuch as the flies are common at vinegar factories (Goodey, 1963).

Aids to Instruction

What does the instructor do with vinegar eels once he has successfully cultured them? Have any science projects used eelworms? What laboratory experiments can be done with them? Here are some suggestions.

Parasitic-roundworm substitute. The vinegar eel makes an excellent substitute for parasitic roundworms when none are available.

Chemicals (Silvan, 1966). Vinegar eels are scarcely affected by aspirin, ephedrine, thyroid extract, and even piperazine adipate, which is a drug used to rid animals of parasitic roundworms related to vinegar eels. Silvan believes the eelworms are unaffected because the chemicals do not dissolve in the acid solution in which the worms live. The chemicals are all soluble in alcohol, and an interesting project might be carried out by introducing small amounts of alcohol into the culture medium. Undenatured alcohol would enhance success.

Anatomic detail (Needham, 1959). Eelworms are ovoviviparous and transparent; therefore one can observe all stages of development. Details of the alimentary canal and other internal organs can be observed through the transparent body.

BSCS Yellow Version (1968). Locomotion is demonstrated in this laboratory exercise by the free-living vinegar eels and is closely correlated with the pattern of their body muscles.

Miscellaneous laboratory exercises. Subjects of exercises on vinegar eels described by Behringer (1967) include embryo development, digestion and excretion, pH of digestive organs, and the survival rate in high and low pH ranges. These are simple exercises.

REFERENCES

Behringer, M. 1967. Use of the vinegar eel. *American Biology Teacher* 29 (7): 515-522.

Biological Sciences Curriculum Study. 1968. *Student laboratory guide: Biological science, an inquiry into life,* 2nd ed. Harcourt, Brace, & World, Inc., New York.

Carolina Biological Supply Co. 1968. *Culture media and methods for various microorganisms.* Privately printed, Gladstone, Ore.

Galen, D. F. 1969. Culturing of organisms for the classroom teacher. *Journal of the Arizona Academy of Science* 5 (4): 251-253.

Goodey, J. B. 1963. *Soil and fresh water nematodes,* 2nd ed. John Wiley & Sons, New York.

Hegner, R. W., and K. A. Stiles. 1959. *College zoology,* 7th ed. Macmillan Co., New York.

Needham, J. G., ed. 1959. *Culture methods for invertebrate animals,* 1st ed. Dover Publishing Co., New York.

Silvan, J. 1966. *Raising laboratory animals,* 1st ed. Natural History Press, Garden City, N.Y.