CAVE CRICKETS (ORTHOPTERA: RHAPHIDOPHORIDAE) AS VECTORS OF DICTYOSTELIDS (PROTISTA: DICTYOSTELIIDA)[1]

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ABSTRACT Our study was done to determine if cave crickets (*Ceuthophilus gracilipes gracilipes*) are able to transport dictyostelid cellular slime molds into and within caves. Large cave crickets were captured from Pigeon Roost Cave in northwestern Arkansas. Crickets were individually washed to remove dictyostelid spores, and fecal pellets collected aseptically from the washed crickets. Five species of dictyostelids, assigned to two genera (*Dictyostelium* and *Polysphondylium*), were recovered from the surface of six crickets, and a single species (*D. sphaerocephalum*) from one sample of fecal pellets. Since cave crickets forage outside the cave, they can introduce dictylostelids to caves from outside sources, and can serve as vectors for transporting dictyotelids within caves. The present study is the first to demonstrate that cave-dwelling invertebrates are capable of transporting these organisms.

KEY WORDS: Rhapidophoridae, *Ceuthophilus,* dictyostelids, cave, Arkansas, U.S.A.

Dictyostelid cellular slime molds (Dictyosteliida: Dictyosteliidae) are single-celled, eukaryotic, phagotrophic bacterivores usually present and often abundant in terrestrial ecosystems. These organisms represent a normal component of the microflora in soils and apparently play a role in maintaining the natural balance that exists between bacteria and other microorganisms in the soil environment. Dictyostelids are most abundant in the surface humus layer of forest soils, but they also occur in the soil-like material found in caves. In a recent study, 17 dictyostelid species were recovered from over 100 caves in eastern North America (Landolt et al., 2006). Five species of dictyostelids were recovered in more than 25 different caves, and three additional species were present in more than 20 different caves. In general, based on available data, the distribution of dictyostelids in caves appears to be rather patchy, but in the microhabitats where they do occur, these organisms can exhibit surprisingly high levels of abundance and diversity.

Unlike many microorganisms, dictyostelids produce spores that appear to have a rather limited potential for dispersal. In the dictyostelid life cycle, the unicellular amoeboid cells that represent the vegetative stage aggregate to form a structure called a pseudo-plasmodium, which then gives rise to one or more fruiting bodies (sorocarps), each bearing one to several masses of spores (sori). Because the spores are embedded in a mucilaginous matrix that dries and hardens, they stand little chance of being dispersed by wind (Cavender, 1973; Olive, 1975). Various animals, ranging from invertebrates to amphibians, small mammals, and birds are capable of dispersing the spores of dictyostelids by means of ingestion-defecation (Suthers, 1985; Huss, 1989; Stephenson and Landolt, 1992). Dictyostelids may be introduced to caves by bats (Stephenson and Landolt (1992). However, are there other organisms that could serve as vectors for

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dictyostelid spores, either for short range dispersal within a cave or introduction to the cave from outside?

Cave and camel crickets (Orthoptera: Rhaphidophoridae) represent an important component of the animal communities associated with caves in North America, and several species are considered critical members of these communities (Benoit et al., 2004; Taylor et al., 2005). Cave and camel crickets forage on the surface at night and then return to caves to roost during the day. In doing so, organic material in the form of cricket fecal material, cricket eggs, and cadavers of crickets are introduced to the cave, which supplies nutrients to a number of invertebrate species. In addition to transferring organic material, 14 genera of fungi were recovered from external body surfaces and internal contents of the raphidophorid *Hadenoecus cumberlandicus* inhabiting two Kentucky caves (Benoit et al., 2004). Most of these fungi were common soil saprophytes typically found in caves, but two plant pathogen species, which were isolated only internally, were believed to have been acquired during foraging outside the cave. The presence of fungi externally and internally on cave crickets plus their surface foraging and subsurface roosting behavior suggests that these organisms also might be capable of vectoring slime molds. Our primary objective of the study reported herein was to investigate the potential role that these crickets may play in the dispersal of dictyostelids into and within caves.

METHODS

Ceuthophilus gracilipes gracilipes (Haldeman) is a species of rhaphidophorid cricket commonly found inhabiting caves and forest floors across central and eastern United States (Hubbell, 1936). It has been reported from a number of caves in Arkansas (Graening et al., 2004). Pigeon Roost Cave in Benton County, Arkansas, was selected for this study because the site contained *C. gracilipes gracilipes* and because human access to the cave is restricted due to the seasonal presence of federally endangered gray bats (*Myotis grisescens*). Pigeon Roost Cave is developed in Mississippian aged limestones, specifically the Boone and underlying St. Joe formations. The cave is located in an oak-hickory forest typical of the type found throughout much of northern Arkansas (Spetich, 2004).

Twelve crickets were captured alive inside the cave. While in the cave, each cricket was placed into separate 50 mL centrifuge tubes containing 7.5 mL of sterile deionized water and 0.25 mL of a wetting agent (Tween 20). The surfactant was used as a wetting agent to elute any substances carried by the crickets. The tube was shaken gently for a few seconds, after which the cricket was removed and placed in a single holding container lined with sterile paper. Crickets were removed from the cave and held in the holding container without food for 5 days at 20°C to collect fecal pellets.

Fecal pellets (frass) deposited by the crickets on the bottom of the holding container were combined into two samples. Each sample was transferred to a sterile 50 mL centrifuge tube containing 7.5 mL of sterile distilled water. The pellets in each tube were broken up with the use of a sterile glass rod and vigorous shaking of the sealed tubes.

Isolation procedures used for dictyostelids were those described by Cavender and Raper (1965). Aliquots (each 0.5 mL) of the “cricket wash” from each of the

12 tubes were added to each of two 100 x 15 mm culture plates prepared with hay infusion agar (Raper, 1984). The same procedure was followed for the two tubes containing the suspended material from the fecal pellets except that three plates were prepared from each tube. Approximately 0.4 ml of a heavy suspension of E. coli was added to each culture plate, and plates were incubated under diffuse light at 20–25°C. Each plate was examined at least once a day for several days following appearance of initial aggregations, and the location of each aggregate clone marked. When necessary, isolates were subcultured to facilitate identification. Nomenclature used herein follows Raper (1984).

RESULTS AND DISCUSSION

Five different species of dictyostelids appeared in one or both of the plates prepared with "cricket wash" from six of the twelve crickets we examined (Table 1), and three of these were recovered from a single cricket. Because Pigeon Roost Cave was one of the caves investigated for the presence of dictyostelids in a larger study of the distribution and occurrence of these organisms (Landolt et al., 2006), data are available on the species present. *Dictyostelium purpureum,* recovered from crickets, was not isolated from samples of cave soil. Two other species (*D. rosarium* Raper and Cavender and *Polysphondylium pallidum* Olive), recorded from cave soil, were not recovered from the crickets. One of the six plates prepared with suspended material from the fecal pellets yielded a single colony of *D. sphaerocephalum.*

Table 1. Occurrence of dictyostelids in the two sets of samples. Plus (+) indicates presence, minus (-) indicates absence.

Dictyostelid Species	Cricket Wash	Cricket Frass
Dictyostelium giganteum Singh	+	-
Dictyostelilum mucoroides Brefeld	+	-
Dictyostelium purpureum Olive	+	-
Dictyostelium sphaerocephalum (Oudem) Sacc and Marchal	+	+
Polysphondylium violaceum Brefeld	+	-

Because several surface-dwelling invertebrates have been reported as vectors of dictyostelid spores (Huss, 1989), the results obtained in the present study are not unexpected. However, the fact that we were able to recover dictyostelids from half of the crickets examined suggests that these organisms potentially play a role in spore dispersal. A still unanswered question is whether cave crickets can introduce dictyostelids to caves. As noted above, cave crickets forage in the litter layer on the forest floor outside the cave. Although the primary microhabitat for dictyostelids in forests is represented by the soil/humus layer that occurs at the interface of soil and the decomposing organic material (mostly leaf litter) located above the soil, dictyostelids also occur in the leaf litter itself (Stephenson and Landolt, 1996). As such, crickets would have the opportunity to come into contact with dictyostelid spores during their foraging activities in leaf litter. It seems likely that some of these spores could be introduced to caves.

In summary, our data indicate that cave crickets can serve as vectors for dictyostelid spores within caves. Since the crickets forage outside the cave, it is possible that they also introduce spores to caves from outside sources. The present study is the first to demonstrate that cave-dwelling invertebrates are capable of transporting dictyostelid spores.

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