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A survey of cellular slime molds at selected sites on the James River, Virginia

Nathaniel Lemaster Armistead III

ABSTRACT

Forty-three sample sites representing seven plant associations were surveyed for cellular slime molds in the James River basin from the head-waters to the mouth of the James River. *Dictyostelium mucoroides*, *D. minutum*, *D. purpureum*, *D. discoideum*, *Polysphondylium violaceum* and *P. pallidum* were found in all associations. *Dictyostelium lacteum* was found in all but the Maple-Basswood association. The remaining species were unique to the Alluvial Hardwood association and the following respective forest types: *D. polycephalum* to Oak-Hickory, *D. giganteum* to Oak-Hickory and Mixed Mesophytic, and *D. rosarium* to Mixed Mesophytic. A percentage similarity test indicated that, with regards to the observed dictyostelid flora, sample sites were most similar to those within the same plant association.

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by

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INTRODUCTION

Cavender and Raper (1965 b,c) observed that the abundance and diversity of species of Acrasieae (cellular slime molds or dictyostelids) in a given area varies with the plant cover and the amount of decomposing organic matter. Moreover, these investigators found that the basic requirements for optimal development of dictyostelids are moderate temperature, high oxygen tension, a near saturated atmosphere, sufficient soil moisture and an adequate bacterial food supply.

Plant associations are used as indicators for the comparison of acrasiean populations because they reflect the environmental differences in the soil that affect dictyostelid development. Cavender and Raper (1965a) used the six associations described by E.L. Braun (1950) to classify the deciduous forests of Eastern North America in their study of acrasiean populations in that area. Nine out of the twenty-two members of the Acrasieae found in temperate North America were frequently isolated (Cavender and Raper 1965 b,c) confirming previous work by Raper (1951).

The flood plain of the James River in Virginia supports a variety of deciduous forest associations and has not been examined previously for dictyostelids. This thesis is primarily concerned with the results of a survey of dictyostelids at selected sites in the James River basin.

Some incidental observations concerning possible ecological relationships between dictyostelids are included.

Seven of the plant communities of Virginia described by Harvill, Stevens and Ware (1984) are present in the areas surveyed and collection sites were established accordingly: (1) Mountain Pine-Oak Heath, (2) Pine-Oak Heath, (3) Oak-Hickory Forest, (4) Alluvial Hardwood Forest, (5) Beech-Maple-Tuliptree Forest, (6) Mixed Mesophytic Forest, and (7) Maple-Basswood Forest. These communities were found in the Coastal Plain, Piedmont, Blue Ridge and Appalachian Mountain provinces of the state. Average annual rainfall and temperature varied across the survey area from 44.0 inches and 59.5^{o} F in the Coastal Plain to 39.2 inches and 53.8^{o} F in the Appalachian Mountains (National Climatic Data Center, 1985).

MATERIALS AND METHODS

Collection of Samples:

Samples were taken from the soil surface and from the humus layer of forests at selected sites along the banks of the James River in the Fall season. Sample locations are listed in Table 1 and are numbered to correspond to the map in Figure 1. Ten samples of approximately 50 grams each were taken along a 30 meter transect at each site to ensure representative sampling of the area population. Soils were scraped from the forest floor and placed in "zip-lock" plastic bags. Samples were maintained at 4^{o} C until plated out within 48 hours of collecting.

Preparation of Medium:

A broth was prepared by boiling 5 g Timothy Hay (*Phleum pratense*) per liter of distilled water for 15 minutes; the infusion was then filtered through cheesecloth, and the pH of the filtrate adjusted to 6.2 with a buffer solution consisting of 7.5 g KH_2PO_4 and 3.1 g Na_2HPO_4 * 7 H_2O in 100 ml of distilled water. Fifteen grams of agar were added per liter of filtrate and the medium was sterilized at 121^{o} C for 15 minutes.

A slightly modified "Cavender Method" (Cavender and Raper, 1965a) was used to isolate the clones of dictyostelids and to provide a qualitative comparison of the cellular slime mold populations. Hay infusion agar plates were poured a day before inoculation. Ten grams of a sample were measured into a 500 ml flask containing 90 ml of sterile distilled water, giving an initial dilution of 1:10. The mixture was agitated on a rotary shaker at 200 rpm for 10 minutes to break up soil particles and to distribute spores and myxamoebae. The 1:10 dilution was used to prepare a 1:25 dilution and 0.5 ml of this suspension was added to each plate so that each plate represented 1/50 gram of soil. To this was added 0.4 ml of a 24 hour culture of *Escherichia coli* grown in nutrient broth at 25^{o} C and diluted by suspending 1 ml of the culture in 15 ml sterile distilled water. The mixture of bacteria and soil suspension was distributed evenly over the agar surface by tilting the plates back and forth and rotating them counterclockwise and then clockwise five times each. The lids of the plates were tilted up slightly (to allow free water to evaporate) before being incubated at 20^{o} C.

Population Counts:

Plates were examined beginning three days after inoculation and then each day for a week after plating. The positions of the clones were marked and identification completed after fructifications developed. Individual specimens were isolated for further study by transferring them to plates containing medium and *E. coli*.

Dictyostelids were identified by the form of their fruiting bodies (Fig. 2) following the taxonomy used by Raper (1984).

Calculations:

The total number of clones from each sample site and the average number of clones per plate were recorded. These data were used to calculate the absolute density, frequency and relative density according to the methods published by Cavender and Raper (1965a). Since the material plated out was diluted at 1:50, the absolute density of each species per gram of soil was determined by multiplying the number of clones per plate for that species by 50. The total of the average absolute densities for all of the species at a site represents the absolute density of all Acrasieae per gram of soil. Frequency was determined by dividing the number of transect points at which a species occurred by 10 (the total number of transect points) and multiplying by 100. Relative densities were calculated by dividing the number of clones of each species by the total number of clones of all species in the population at that site and multiplying by 100.

A percentage similarity test (Wolda, 1981) was performed to test the uniqueness of dictyostelid floras as defined by plant associations. This algorithm generates a percentage of similarity between two entities by making pair-wise comparisons of their various components. As adapted for this study, relative densities of the dictyostelid species were used in pair-wise comparisons of all sites in the study. Specifically, for any two sites the lowest values for relative density for each species present in both sites are added together to yield the percent similarity of the two sites.

In order to present an overall picture of dictyostelid populations in the James River basin, data from all sample sites were pooled together in Figure 3a. Since each of the forest types included different numbers of sample sites, a normalization procedure was necessary. Average relative densities and frequencies for dictyostelid species in each forest type were prorated according to the number of sites sampled for each given forest type; the prorated values were then combined to give the overall relative densities and frequencies for each species throughout the James River basin.

RESULTS

A total of forty-three sites were sampled in the Coastal Plain, Piedmont, Blue Ridge, and Appalachian provinces of the state. Plant associations were unevenly distributed in the survey area. The dominant plant associations sampled in the Coastal Plain and Piedmont were Beech-Maple-Tuliptree and Alluvial Hardwoods respectively. There was no dominant plant association found among the sites sampled in the Blue Ridge province and Alluvial Hardwoods and Mixed Mesophytic associations were co-dominants in the Appalachian Mountains. The corresponding plant associations for sample sites are indicated in Table 1. Values for the percentage similarity between sample sites are listed in Table 2. Frequencies and relative densities of the dictyostelids observed in this study are summarized by forest type in Table 3.

Oak-Hickory Forest:

The Oak-Hickory association is common on the Piedmont and to a certain extent on the Coastal Plain. *Quercus alba*, *Q. prinus*, *Q. velutina*, *Q. stellata*, *Q. coccinea*, *Carya tomentosa*, *C. glabra*, *Nyssa sylvatica*, *Castanea* pumila, Pinus virginiana and P. echinata are the trees typically found in this association. Quercus alba, Carya tomentosa and C. glabra were the most numerous trees found at the four sites that were sampled. Platanus occidentalis and Liriodendron tulipifera were present at one site each. Nine species of Acrasieae were found of which the five species, Dictyostelium lacteum, D. minutum, D. mucoroides, Polysphondylium violaceum and P. pallidum occurred at consistently high frequencies. Relative densities were highest for D. minutum, D. mucoroides and P. pallidum. Despite their high frequencies, D. lacteum and P. violaceum had lower densities than other frequent species. Populations ranged from 735-1135 clones per gram of soil.

Mountain Pine-Oak Heath:

This type of community is common in the Blue Ridge and westward, most often on sandstone substrata. Pinus pungens, P. rigida, Quercus ilicifolia and Q. prinus are the characteristic trees of this association; Q. prinus, P. strobus and P. rigida were the most abundant trees at the three sites sampled. Transects at these sites extended down rocky hillsides to stream bottoms where Fagus grandifolia, Liriodendron tulipifera and Acer saccharum were sparsely present. A thick humus layer composed mostly of pine needles covered the soil surface. Seven species of Acrasieae were found with D. mucoroides, D. lacteum and

P. pallidum having the highest frequencies. Dictyostelium mucoroides had the highest relative density, followed by P. pallidum. Population sizes ranged from 235-485 clones per gram of soil.

Mixed Mesophytic Forest:

Nine sites were sampled in which mixtures of Liriodendron tulipifera, Quercus rubra, Q. alba, Acer saccharum, A. rubrum, A. pensylvanicum, Carya ovata, Tilia, and Pinus strobus occurred. Sites were moist but well drained with deeply melanized soils and a mull humus layer. Nine species of Acrasieae were found; however, Dictyostelium giganteum and D. rosarium occurred in only one of the locations. Dictyostelium minutum, D. mucoroides, Polysphondylium violaceum and P. pallidum dominated the dictyostelid flora with consistantly high frequencies and relative densities. Population sizes ranged from 345-875 clones per gram of soil.

Maple-Basswood Forest:

The Maple-Basswood association occurs on moist soils in the mountains of Virginia. Acer saccharum and Tilia americana are typically the climax dominants of these communities. Two locations were sampled in which T. americana was abundant and either A. saccharum or A. saccharinum were the dominant species of maple. Areas were similar to the Mixed Mesophytic sites. Rich, moist soils were covered by thick layers of humus. Six species of Acrasieae were present at these sites. Polysphondylium violaceum and P. pallidum had similarly high frequencies; however, P. violaceum had greater relative densities. In this association D. minutum was found less frequently than in the Mixed Mesophytic Forest and D. lacteum was absent. Population sizes were 215 and 375 clones per gram of soil.

Alluvial Hardwood Forest:

This association is found on flood plains and their larger tributaries where clay, silt, sand or gravel have been deposited by running water. The characteristic trees of this community are Acer negundo, A. saccharinum, Betula nigra, Fraxinus americana, Ulmus americana, U. rubra and Carya cordiformis. Seventeen sites were sampled at which U. americana, A. negundo, A. saccharinum and B. nigra were the most commonly seen trees. Ten species of Acrasieae were found at these sites. Polysphondylium violaceum, P. pallidum, D. mucoroides and D. lacteum were the most frequently observed species. Polysphondylium violaceum had the highest relative densities followed by P. pallidum and D. mucoroides. Although it was found frequently in samples, D. lacteum had low relative densities. Population sizes in the Alluvial Hardwood communities ranged from 205-865 clones per gram of soil.

Pine-Oak Forest:

Pinus taeda is the dominant tree of this association.

Pinus palustris, Quercus laevis and Q. cinerea are found to a lesser extent in this community. Two sites were sampled in which P. taeda was the dominant and Q. cinerea and Q. alba were found. The soils were dry and covered by a humus layer composed mostly of pine needles. Seven species of Acrasieae were found of which D. lacteum, D. mucoroides and P. pallidum had the highest frequencies. Dictyostelium mucoroides had the highest relative density followed by P. pallidum. Population sizes were 785 and 815 clones per gram of soil.

Beech-Maple-Tuliptree forest:

Fagus, Acer saccharum, A. barbatum, Liriodendron and Quercus rubra are characteristic trees of the Beech-Maple-Tuliptree association. Samples were taken at five sites where the most common trees were Liriodendron tulipifera and Fagus grandifolia. Small numbers of A. saccharum, Ilex opaca, Liquidambar styraciflua and Pinus taeda were also found at these sites. Soils were dry and sandy with a thin layer of humus. Seven species of Acrasieae were observed at these sites. Dictyostelium minutum, D. mucoroides, P. violaceum and P. pallidum occurred at the highest frequencies. Dictyostelium minutum was the only species to have high relative densities. Population sizes were 435-590 clones per gram of soil.

DISCUSSION

Plant associations were found to characterize populations of dictyostelids in this survey. These associations were distributed unevenly throughout the state, typically being best represented in a single province. The Coastal Plain, Piedmont, Blue Ridge and Appalachian provinces differ in soil and climatic conditions which in turn influence the distribution of plant communities in the survey area.

Four of the plant associations distinguished in this survey correspond to those employed in the study of dictyostelids in eastern North America by Cavender and Raper (1965c). Alluvial Hardwoods of the present study match their Bottomland Hardwoods and the Beech-Maple-Tuliptree association matches their Beech-Maple. The present study separated Mountain Pine-Oak from Pine-Oak whereas these associations were combined by Cavender and Raper. The remaining three plant associations; Oak-Hickory, Maple-Basswood and Mixed Mesophytic, have the same characteristic flora in the present study as in the study of Cavender and Raper (1965c).

Although Alluvial Hardwoods and Bottomland Hardwoods associations have the same dominant trees, soils of the former are composed of clay, silt, sand or gravel and are drained better than the "earthworm mull humus" soils described by Cavender and Raper (1965c). In general, dictyostelid populations in the Alluvial Hardwoods association were similar to those of Cavender and Raper's Bottomland Hardwoods, with the exception of Dictyostelium minutum and D. discoideum which were present at higher frequencies and relative densities in the Alluvial Hardwoods association. Additionally, D. giganteum and D. rosarium were observed in the present study but were not reported in the survey by Cavender and Raper (1965c).

The Beech-Maple-Tuliptree of this survey is similar to the Beech-Maple association of Cavender and Raper with the exception of Tuliptree being present as a dominant in this survey. The average number of dictyostelids per gram of soil was higher in the Beech-Maple-Tuliptree association than the Beech-Maple. In addition, D. discoideum was found at low frequencies and relative densities in this survey but was absent from the Beech-Maple sites sampled by Cavender and Raper (1965c).

The Mountain Pine-Oak Heath and Pine-Oak associations in the present study contained different species of Pine and Oak and were in different parts of the state. The first association was found in the Appalachian Mountains and the second in the Coastal Plain and Piedmont. The types of species of acrasieae found and their frequencies and relative densities in the two Pine-Oak associations were very similar to each other and to those reported by Cavender and Raper (1965c) in their Pine-Oak association.

In terms of these measures of dictyostelid communities, there is no reason to split up the Pine-Oak association; however, absolute densities were found to differ. In the present study, the Mountain Pine-Oak Heath ranged from 235 to 485 clones per gram of soil and the Pine-Oak from 785 to 815. Cavender and Raper (1965c) reported 250 to 900 clones per gram of soil in their Pine-Oak association. Although the same species of acrasieae were present, the difference in absolute densities indicates some subtle difference in these two dictyostelid communities.

The higher plants that characterize the Oak-Hickory, Maple-Basswood and Mixed Mesophytic associations in the present study are identical to those used in the study by Cavender and Raper (1965c). Dictyostelid populations in these associations were similar in both surveys with a few exceptions.

Dictyostelium mucoroides, *D. discoideum* and *D. polycephalum* were reported more frequently in the Maple-Basswood association of Cavender and Raper than in the Maple-Basswood association of the present study. Further, *D. minutum* and *D. giganteum* were observed in sample sites of the Maple-Basswood association in this study but were absent in Cavender and Raper's (1965c). In sample sites of the Mixed Mesophytic association of this study, *P. violaceum* was the dominant dictyostelid species. *Dictyostelium giganteum* and *D. rosarium* were observed but *D.*

polycephalum was not found. *Dictyostelium minutum* was the dominant dictyostelid in Cavender and Raper's (1965c) Mixed Mesophytic association and *D. polycephalum* was observed infrequently.

Dictyostelid populations of the Oak-Hickory associations in both studies were very similar. The only difference was the report of *Acytostelium leptosomum* by Cavender and Raper in the Oak-Hickory association. This species was not observed in the present survey but was reported by Cavender and Raper (1965c) in the Oak-Hickory, Mixed Mesophytic and Pine-Oak associations at low frequencies and relative densities. Two species of acrasieae, *D. giganteum* and *D. rosarium*, were observed in this study but were not reported by Cavender and Raper (1965c).

Similarities between plant associations:

The percentage similarity (Table 2) test justified the grouping of sample sites by plant associations. Similarities of dictyostelid populations averaged ninety percent or more in the Beech-Maple-Tuliptree, Pine-Oak, Oak-Hickory, Mixed Mesophytic, and Maple-Basswood associations. Sample sites of the Alluvial Hardwoods and Mountain Pine-Oak Heath associations had average similarities of 85 percent. With only a few exceptions, similarity values between sites within a given plant association are greater than values for sites in different associations. Least similar sites in different plant associations had similarity values as low as 24 percent.

The strongest similarities in dictyostelid populations of different plant associations occurred between Mountain Pine-Oak Heath and Pine Oak, and between Mixed Mesophytic and Alluvial Hardwoods associations. Analysis of the average frequencies and relative densities for the dictyostelids in these associations (Fig.3) confirmed these results of the percent similarity test.

Three sites in the Alluvial Hardwood association had percent similarities to sites of the Mixed Mesophytic association that were comparable to their percent similarities to other Alluvial Hardwoods sites. However, these similarity values were not as high as those found within the Mixed Mesophytic association. Similarities in dictyostelid populations of the Alluvial Hardwood association to those of the Mixed Mesophytic may be related to the similarity of flora in the two associations. The sample site at Howardsville (Rt.602) had the lowest percent similarities to other sites of the Alluvial Hardwoods association but did not share any greater similarity to sample sites in other associations.

Percent similarity values for dictyostelid populations among Mountain Pine-Oak Heath sample sites were relatively low compared to values found within other associations; these values were greater, however, than those to all other sites with the exception of the Pine-Oak. Indeed, mountain Pine-Oak Heath sample sites held greater percent similarities to the Pine-Oak sites than to each other. The predominant trees in both associations were species of pine; *Pinus taeda* in the Pine-Oak, and *P. strobus* and *P. rigida* in the Mountain Pine-Oak Heath association. These trees reflected a difference in soils in the areas in which they were found. Sites of the Pine-Oak association in the Coastal Plain and Piedmont provinces were on soils that were sandy and supported a more dense population of slime molds than the rocky hillsides of the Mountain Pine-Oak Heath sites in the Appalachian province. Content of dictyostelid species was similar between sites of the two associations, however the total absolute densities were much higher in the Pine-Oak sample sites than in the Mountain Pine-Oak Heath sites.

Frequencies and relative densities of dictyostelid populations of the different plant associations are summarized in Figure 3.

Notes on individual species:

Dictyostelium mucoroides, *Polysphondylium violaceum* and *P. pallidum* were found at all of the sites sampled. They appeared in 65-75 percent of the soil samples and, together with *D. minutum*, had individual average relative densities of 20-25 percent, accounting for 87 percent of the population of dictyostelids observed in this survey. *Polysphondylium pallidum* was the most frequent of these four species while

P. violaceum was the most abundant.

Polysphondylium violaceum was the most important species in the Maple-Basswood Forest, the Mixed Mesophytic Forest and the Alluvial Hardwood Forest. It was particularly frequent in the Alluvial Hardwood association although relative densities averaged higher in the Maple-Basswood Forests. The lowest frequencies of *P. violaceum* occurred in communities that were predominantly pine. The average frequency for this species was 35 percent in the Mountain Pine-Oak Heath and Pine-Oak Forest sites, and the average relative density was six percent. Frequencies were slightly higher in the Beech-Maple-Tuliptree communities, but average relative densities there were the lowest for this species. *Polysphondylium violaceum* was consistently present in at least 30 percent and often 50 percent of the samples from sites in this study; however, relative densities were either very high as in the Maple-Basswood communities or very low as in the pine associations. When conditions were favorable for *P. violaceum*, the positive response to the conditions was more apparent than with other species of Acrasieae such as *D. mucoroides* or *P. pallidum*.

The average frequency of occurrence of *D. mucoroides* overall was 65 percent. With the exception of the Maple-Basswood forest type, *D. mucoroides* was observed at frequencies of 50 percent or more. Greater variation was seen in the relative densities independent of the frequencies. For example, relative densities averaged 13 percent in the Beech-Maple-Tuliptree sites at a frequency of 72 percent compared with an average relative density of 39 percent in the Mountain Pine-Oak Heath locations at an average frequency of 50 percent. This variability in frequency of D. mucoroides populations may be explained on the basis of competition with other dictyostelids. Under conditions that are favorable to two or more species of Acrasieae a pattern of dominance develops. All dictyostelids do not utilize food sources equally (Kuserk, 1980; Raper, 1937; Singh, 1947 a,b) and one species may dominate the others by changing its own growth and germination rates in response to environmental conditions or by inhibiting the growth of other species (E. Horn, 1971; D. Mcqueen, 1971 a,b). Dictyostelium minutum is more prevalent in the Beech-Maple-Tuliptree Forest than D. mucoroides and although D. mucoroides is found at high frequencies, its relative densities are low possibly due to inhibition by D. minutum. Where D. minutum decreases in frequency and abundance, as in the Mountain Pine-Oak Heath Forest, D. mucoroides becomes more abundant.

Dictyostelium mucoroides was the most prevalent and abundant species in the Mountain Pine-Oak Heath and Pine-Oak Forest sites. Frequencies of D. mucoroides and P. pallidum were the most nearly constant of those observed in this study. The only deviation for D. mucoroides was in the Maple-Basswood Forest where P. pallidum and P. violaceum were the only species of Acrasieae present in more than 50 percent of the samples.

In addition to having constant frequencies, P. pallidum had the most nearly constant relative densities in the different forest types of any of the observed dictyostelids. Polysphondylium pallidum reached its highest relative densities in the Mountain Pine-Oak Heath and Pine Oak Forest types, and was least abundant in the Beech-Maple-Tuliptree sites. Like D. mucoroides, P. pallidum had higher densities when P. violaceum was less abundant. The presence of American Elm and Striped Maple was consistently associated with the higher frequencies and relative densities of P. pallidum as noted earlier by Cavender and Raper (1965c).

In terms of prevalence and abundance, D. minutum was the most variable species of the four most commonly observed dictyostelids in this survey. Cavender and Raper (1965c) reported it to be the dominant member of the Acrasieae in the deciduous forest of eastern North America. However, D. minutum was the third most abundant species found in all of the sites sampled in this study and the fifth most frequently observed. When the sites were divided into groups by forest type, D. minutum was the most prevalent species in the Oak-Hickory, Beech-

Maple-Tuliptree and Mixed Mesophytic Forest associations and the most abundant species in the first two of these forest types. Numbers of _D. minutum_ generally increased where layers of undecomposed leaves were prevalent. The highest relative densities and frequencies for this species were reached in the Beech-Maple-Tuliptree Forest where _D. minutum_ accounted for 67 percent of the population. Although other species were present at frequencies greater than 50 percent in this association, _D. minutum_ was the only abundant species in any of these samples. _Dictyostelium minutum_ did not appear to inhibit the abundance of dictyostelids in other forest types, so it may be hypothesized that Beech-Maple-Tuliptree locations contain a factor such as a particular food source which particularly favors _D. minutum_.

Of the six least common cellular slime molds observed in this survey, _D. lacteum_, _D. purpureum_ and _D. discoideum_ were the most often found; _D. lacteum_ was more prevalent overall than _D. minutum_ but never in great numbers. An average of 50 percent of the samples contained _D. lacteum_ but its average relative density was only six percent. The Alluvial Hardwood locations had the highest frequencies of _D. lacteum_ followed by the Oak Hickory, Pine-Oak and Mountain Pine-Oak Heath associations where frequencies of 50 percent were observed. _Dictyostelium lacteum_ was not found in the samples from the Maple-Basswood Forests and was least frequently observed in the Beech-Maple-Tuliptree and Mixed Mesophytic communties.

In a study by Cavender and Raper (1965c) _D. purpureum_ ranged from nearly absent in the northern forests to being one of the most important species in the southern United States. It was observed in 33 percent of the samples in the present study and represented three percent of the total population. The highest frequencies for this species were reached in the Alluvial Hardwood Forest and the highest relative densities were in the Beech-Maple-Tuliptree communities. A reverse correlation was noted between _D. purpureum_ and _D. discoideum_ in which _D. purpureum_ was more frequently seen when _D. discoideum_ was absent from the site. Sites where _D. discoideum_ was not found had an average frequency of 48 percent for _D. purpureum_ compared to an overall frequency of 33 percent for this species. Although _D. purpureum_ did not appear in large numbers, it was an important member of the population, occuring in 39 of the 43 sites in this survey.

Dictyostelium discoideum was observed in 26 sites at an overall frequency of 15 percent. This species usually prefers forests with a heavy layer of undecomposed leaf litter, conditions which are also known to favor _D. minutum_. The prevalence and abundance of the two species varied in a comparable manner in three of the forest types. The Oak-Hickory and Mixed Mesophytic associations were favorable for both species while the Alluvial Hardwood Forest was not. Dictyostelium discoideum attained its highest frequencies in the Oak-Hickory Forest and its highest relative densities in the Maple-Basswood Forest. It did not comprise a significant part of any of the populations in this survey and made up only two percent of the total number of dictyostelids that were found.

Dictyostelium polycephalum was observed in the Oak-Hickory and the Alluvial Hardwood Forests. It has been referred to as an "indicator" of Silver Maple-American Elm lowland hardwood forests where it was sometimes present in 60-70 percent of the samples (Cavender and Raper, 1965c). However, only eight of the twelve locations where D. polycephalum was observed in this study contained Silver Maple, American Elm or both, and D. polycephalum was not found in six other sites where these trees were present. There was no increase in frequency or abundance of D. polycephalum when found in associations containing Silver Maple and American Elm. Frequencies of this species never exceeded 20 percent in this study and the average relative densities were less than one percent.

Dictyostelium giganteum and D. rosarium were the least frequently observed members of the Acrasieae in this survey. Both were observed in the Mixed Mesophytic and the Alluvial Hardwood Forest associations; D. giganteum was also found at one site in the Oak-Hickory Forest. Of the two species, *D. giganteum* is considered to be the more common in the eastern United States. It is similar in appearance to *D. mucoroides* but with longer sorophores and was not described as a separate species until 1947. The most favorable site for this species was an Oak-Hickory association where it was observed in 30 percent of the samples at a relative density of eight percent. *Dictyostelium rosarium* also reached frequencies of 30 percent but favored the Alluvial Hardwood forest type where it was found at three sample sites. Although the two species were observed at the same frequencies, *D. giganteum* was twice as abundant as *D. rosarium*. Both species occurred in four sites each. They had two sites in common (Maidens-Rt.522 and Indian Rock-Rt.614) which may indicate a preference for the same conditions.

This survey is the first known study of dictyostelid populations in soils along the James River. Soils of seven plant associations were studied and compared on the basis of percent similarity of relative densities. Stronger similarities were observed among dictyostelid populations within the same plant associations than among populations of differing plant associations, with one exception; dictyostelid populations of the Mountain Pine-Oak Heath association had higher similarities to populations of the Pine-Oak association than to each other. The only difference between populations of these two associations was a higher total absolute density in the Pine-Oak than the Mountain Pine-Oak Heath associations.

All samples were collected in the Fall, which together with Spring, is when conditions for growth are best for cellular slime molds. An extended survey in different seasons would more clearly define the variation in compositions of dictyostelid communities due to seasonal changes. Also, the effect of factors such as the presence or absence of particular plants on the numbers and types of cellular slime molds found in a particular environment needs further investigation. The "indicators" and criteria currently used to correlate distributions of cellular slime molds are broad and do not clarify the differences in microenvironments that pertain to the growth and development of dictyostelids.

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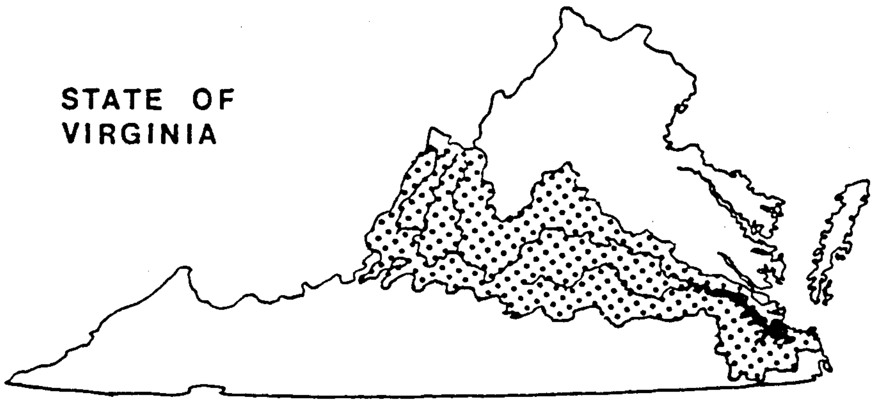
Fig. 1. Distribution of sample sites on the James River. Numbers correspond to those for sites listed in Table 1.

STATE OF VIRGINIA



JAMES RIVER BASIN

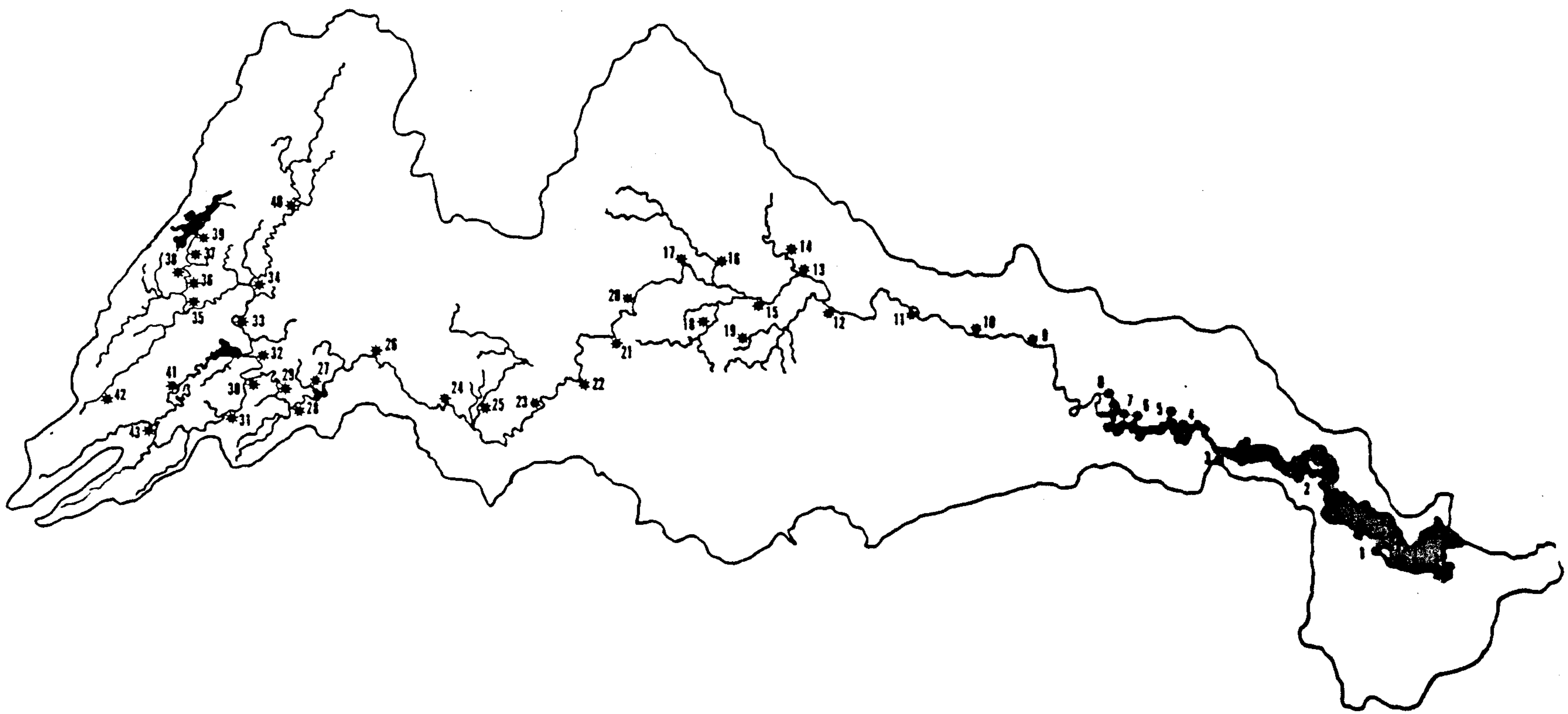


Fig. 2. Illustrations of the 10 species of Acrasieae observed in the soils of deciduous forests on the James River. A. *Dictyostelium mucoroides*, B. *D. giganteum*, C. *D. purpureum*, D. *Polysphondyllium violaceum*, E. *P. pallidum*, F. *D. rosarium*, G. *D. polycephalum*, H. *D. lacteum*, I. *D. minutum*, J. *D. discoideum*.

Dictyostelid Fruiting Bodies

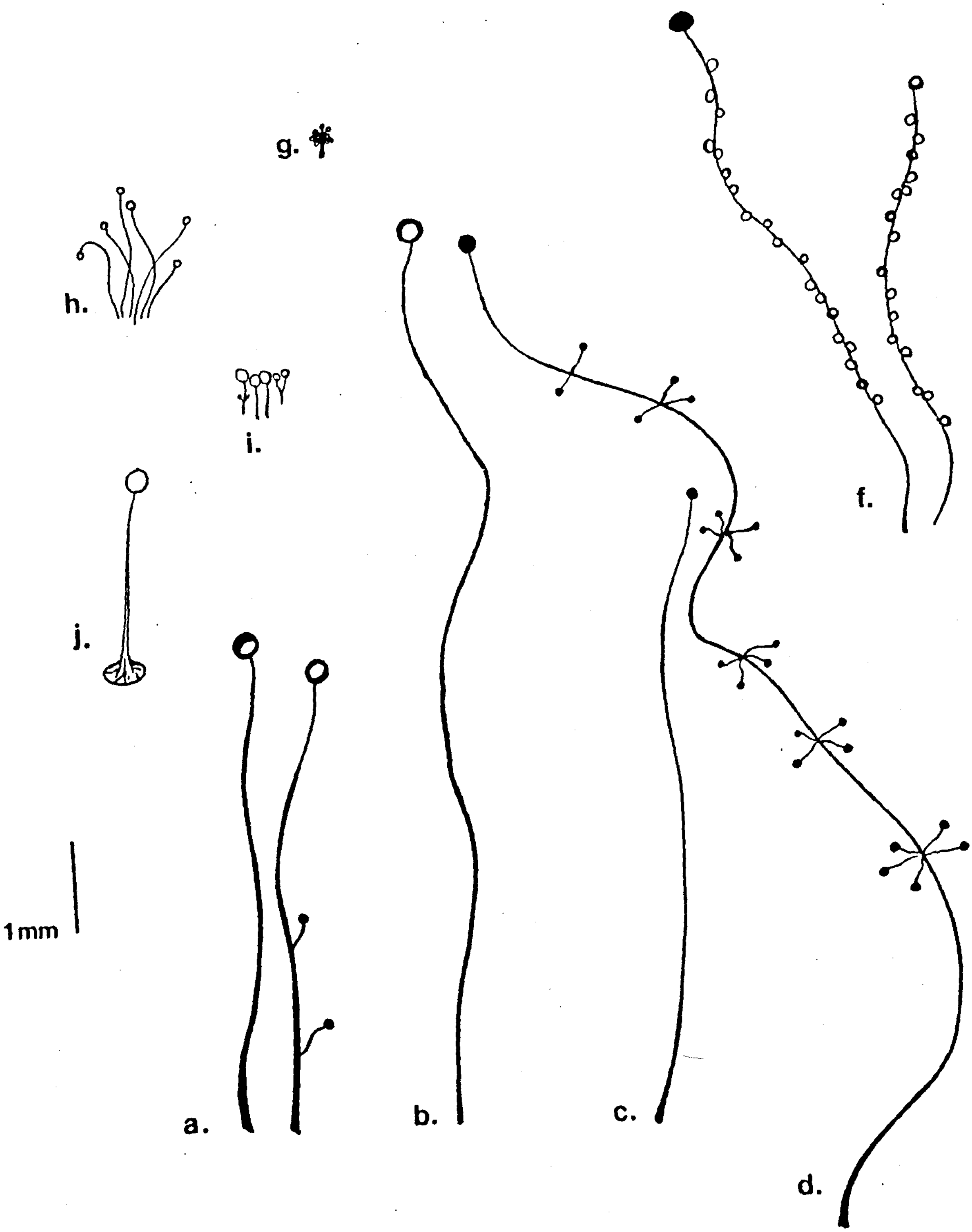
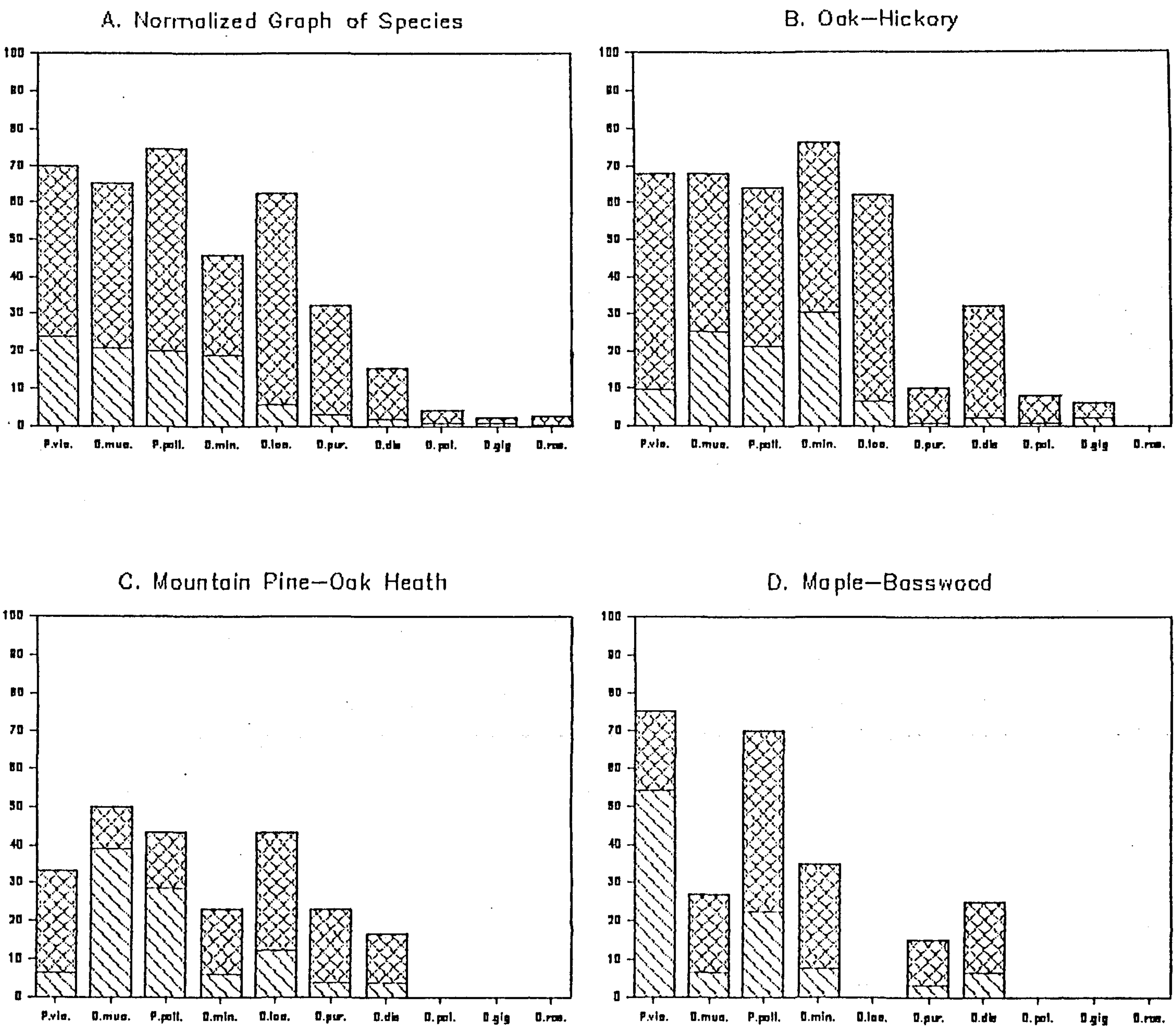
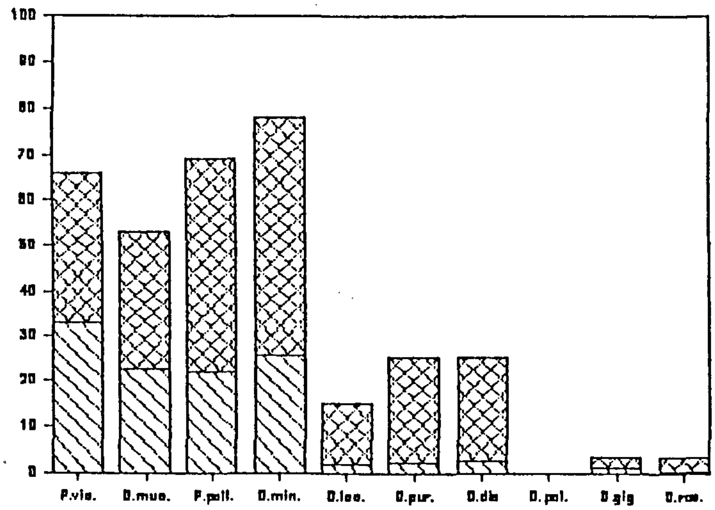


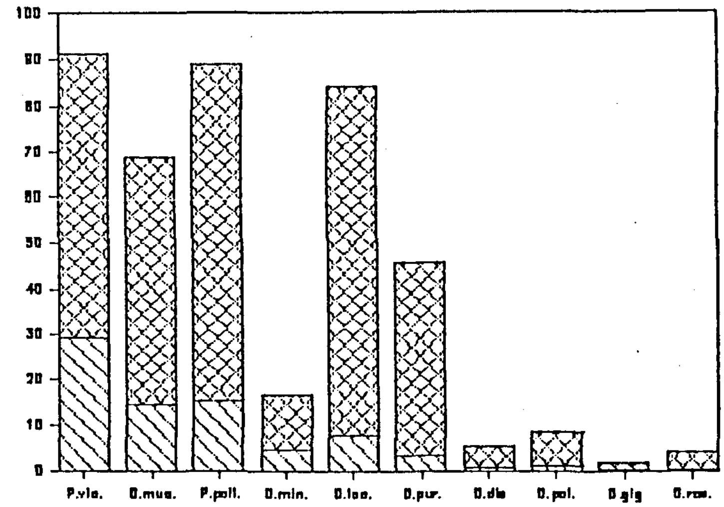
Fig. 3. Average frequencies (hatch-work bars) and relative densities (angled line bars) of Acrasieae in soils of seven forest types on the James River. The average frequencies and relative densities of dictyostelids in each forest types were normalized and combined to give a representative average occurrance throughout the survey area. A. Normalized population, B. Oak-Hickory, C. Mountain Pine-Oak Heath, D. Mixed Mesophytic, E. Maple-Basswood, F. Alluvial Hardwoods, G. Pine-Oak, H. Beech-Maple-Tuliptree. P.vio. = Polysphondyllium violaceum, D.muc. = Dictyostelium mucoroides, P.pall. = P. pallidum, D.min. = D. minutum, D.lac. = D. lacteum, D.pur. = D. purpureum, D.dis. = D. discoideum, D.pol. = D. polycephalum, D.gig. = D. giganteum, D.ros. = D. rosarium.


A. Normalized Graph of Species
B. Oak—Hickory
C. Mountain Pine—Oak Heath
D. Maple—Basswood
0
10
20
30
40
50
60
70
80
90
100
P.vio.
O.mus.
P.poll.
O.min.
O.loe.
O.pur.
O.dis
O.pol.
O.gig
O.ros.

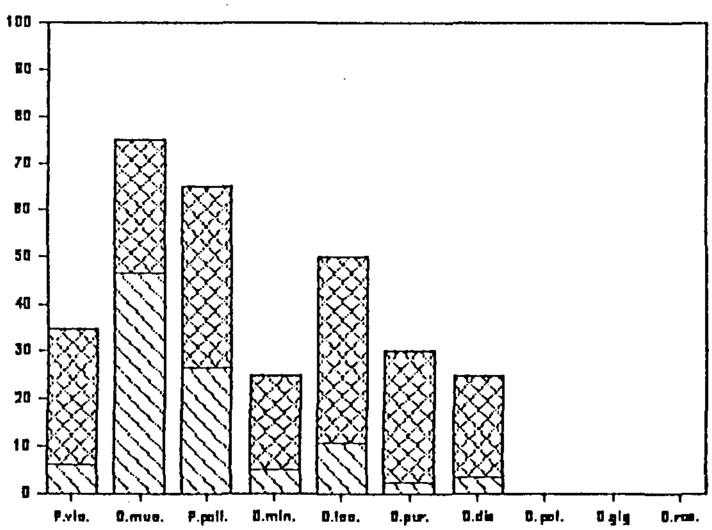
E. Mixed Mesophytic



F. Alluvial Hardwoods



G. Pine–Oak



H. Beech–Maple–Tuliptree

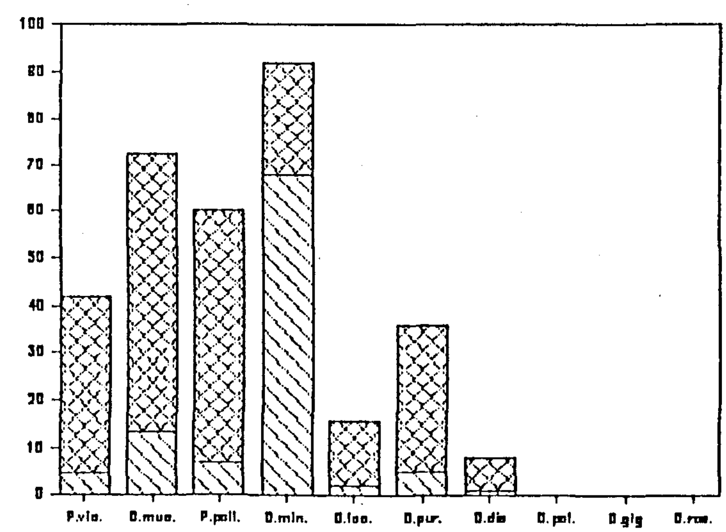


Table 1. Localities sampled for dictyostelids. Numbers correspond to those on Fig. 1. Forest types abbreviated as follows: AH=Alluvial Hardwood, BMT=Beech-Maple-Tuliptree, MB=Maple-Basswood, MM=Mixed Mesophytic, MPO=Mountain Pine-Oak Heath, OH=Oak-Hickory, PO=Pine-Oak.

1. James River Bridge (BMT)
2. Hog Island (PO)
3. Claremont Rt.646 (BMT)
4. Sturgeon Point Rt.614 (BMT)
5. Willcox Wharf Rt.618 (BMT)
6. Benjamin Harrison Bridge (OH)
7. Shirley Plantantion (OH)
8. Malvern Hill (AH)
9. Huguenot Bridge (AH)
10. Gaskins Road (PO)
11. Maidens Rt.522 (AH)
12. Cartersville Rt.45 (AH)
13. Columbia (BMT)
14. Rivanna River Rt.6 (AH)
15. Rt.15 (AH)
16. Hardware River Rt.6 (AH)
17. Scottsville Rt.20 (AH)
18. Slate River Rt.676 (AH)
19. Willis River Rt.622*650 (AH)
20. Howardsville Rt.602 (AH)
21. Rt.56 (AH)
22. Bent Creek Rt.60 (OH)
23. Stapleton Rt.624 (OH)
24. Monacan Park Rt.130*652 (MM)
25. Monroe Rt.1202*1210 (MB)
26. Rt.130*501 (MM)
27. Indian Rock Rt.614 (MM)
28. Buchanon Rt.630 (AH)
29. Saltpete Cave Rt.688 (AH)
30. Salisbury Rt.688 (AH)
31. Fincastle Rt.606 T630 (AH)
32. Eagle Rock Rt.220*43 (AH)
33. Woods Island Rt.633 (AH)
34. Iron Gate Rt.220 (MM)
35. Jackson River Rt.60*220 (MM)
36. Jackson River Rt.1104 (MM)
37. Jackson River Rt.687 (MM)
38. Camp Appalachia Rt.666 (MM)
39. Gathright Dam Rt.605 (MB)
40. Wilton Green Rt.39 (MM)
41. Va. Mineral Springs Rt.606 (MPO)
42. Paint Banks Hatchery (MPO)
43. New Castle Hatchery Rt.42 (MPO)

Table 2. Percentage similarity test. Sample sites are listed according to plant associations. Numbers in parenthesis relate sample sites to their respective positions on the map in Figure 2.

Table 2. Percent similarity values.

Sample Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1. James River Bridge Rt.32 (1)			Beech-Maple-Tuliptree																																							
2. Claremont Rt.646 (3)	0.98																																									
3. Sturgeon Point Rt.614 (4)	0.95	0.95																																								
4. Willcox Wharf Rt.618 (5)	0.93	0.94	0.93			Pine-Oak																																				
5. Columbia (13)	0.95	0.97	0.96	0.94																																						
6. Hog Island (2)	0.35	0.34	0.34	0.38	0.33																																					
7. Gaskins Road (10)	0.34	0.33	0.33	0.37	0.32	0.92																																				
8. Benjamin Harrison Bridge (6)	0.60	0.59	0.59	0.64	0.58	0.67	0.68			Oak-Hickory																																
9. Shirley Plantantion Rt.608 (7)	0.58	0.56	0.56	0.61	0.56	0.64	0.65	0.92																																		
10. Bent Creek Rt.60 (22)	0.62	0.61	0.61	0.66	0.60	0.67	0.68	0.95	0.90																																	
11. Stapleton Rt.624 (23)	0.57	0.55	0.55	0.60	0.55	0.68	0.72	0.91	0.89	0.89																																
12. Malvern Hill (8)	0.37	0.36	0.35	0.40	0.35	0.62	0.60	0.64	0.62	0.62	0.60																															
13. Huguenot Bridge (9)	0.42	0.41	0.39	0.45	0.40	0.56	0.56	0.65	0.62	0.61	0.62	0.92																														
14. Maidens Rt.522 (11)	0.32	0.31	0.29	0.36	0.30	0.60	0.56	0.59	0.58	0.56	0.56	0.87	0.84																													
15. Cartersville Rt.45 (12)	0.41	0.40	0.38	0.44	0.39	0.54	0.52	0.60	0.58	0.59	0.56	0.91	0.94	0.83				Alluvial Hardwoods																								
16. Rivanna River Rt.6 (14)	0.35	0.34	0.34	0.39	0.33	0.63	0.63	0.63	0.60	0.59	0.62	0.92	0.90	0.89	0.87																											
17. Rt.15 (15)	0.39	0.38	0.35	0.42	0.36	0.61	0.58	0.63	0.61	0.60	0.61	0.93	0.95	0.87	0.93	0.91																										
18. Hardware River Rt.6 (16)	0.28	0.26	0.24	0.31	0.25	0.59	0.60	0.58	0.56	0.54	0.61	0.88	0.85	0.86	0.83	0.89	0.88																									
19. Scottsville Rt.20 (17)	0.41	0.40	0.39	0.44	0.39	0.66	0.63	0.70	0.67	0.66	0.66	0.92	0.89	0.87	0.86	0.87	0.91	0.82																								
20. Slate River Rt.676 (18)	0.32	0.31	0.29	0.35	0.30	0.54	0.54	0.57	0.54	0.53	0.53	0.89	0.90	0.87	0.88	0.90	0.88	0.87	0.83																							
21. Willis River Rt.622*650 (19)	0.36	0.35	0.33	0.39	0.34	0.61	0.59	0.61	0.59	0.58	0.59	0.92	0.91	0.88	0.89	0.93	0.94	0.87	0.88	0.88																						
22. Howardsville Rt.602 (20)	0.40	0.39	0.41	0.43	0.39	0.69	0.66	0.64	0.59	0.65	0.59	0.75	0.70	0.69	0.71	0.70	0.72	0.63	0.81	0.64	0.69																					
23. Rt.56 (21)	0.41	0.40	0.39	0.43	0.39	0.61	0.58	0.65	0.63	0.62	0.62	0.93	0.94	0.89	0.91	0.91	0.95	0.85	0.94	0.88	0.93	0.76																				
24. Buchanon Rt.630 (28)	0.39	0.38	0.38	0.43	0.37	0.57	0.56	0.63	0.61	0.62	0.59	0.92	0.93	0.85	0.94	0.90	0.94	0.84	0.86	0.86	0.89	0.72	0.91																			
25. Saltpete Cave Rt.688 (29)	0.39	0.38	0.35	0.42	0.36	0.60	0.58	0.69	0.66	0.64	0.66	0.86	0.87	0.83	0.84	0.85	0.89	0.84	0.90	0.80	0.87	0.71	0.90	0.85																		
26. Salisbury Rt.688 (30)	0.40	0.39	0.39	0.44	0.38	0.64	0.61	0.68	0.65	0.65	0.65	0.91	0.92	0.89	0.90	0.93	0.95	0.85	0.93	0.86	0.93	0.76	0.96	0.93	0.92																	
27. Fincastle Rt.606 T630 (31)	0.42	0.41	0.40	0.45	0.40	0.51	0.47	0.56	0.54	0.55	0.52	0.87	0.88	0.79	0.91	0.82	0.88	0.78	0.84	0.84	0.84	0.77	0.89	0.86	0.81	0.86																
28. Eagle Rock Rt.220*43 (32)	0.38	0.37	0.37	0.42	0.36	0.70	0.68	0.72	0.70	0.69	0.68	0.89	0.84	0.85	0.82	0.87	0.85	0.80	0.92	0.80	0.86	0.80	0.88	0.84	0.85	0.89	0.77															
29. Woods Island Rt.633 (33)	0.32	0.31	0.30	0.35	0.30	0.61	0.58	0.58	0.56	0.55	0.57	0.92	0.88	0.91	0.85	0.94	0.91	0.92	0.88	0.90	0.91	0.69	0.90	0.85	0.84	0.89	0.83	0.83					Mixed Mesophytic									
30. Monacan Park Rt.130*652 (24)	0.58	0.57	0.57	0.62	0.57	0.52	0.52	0.78	0.75	0.77	0.75	0.76	0.79	0.71	0.77	0.76	0.76	0.66	0.79	0.70	0.74	0.68	0.79	0.78	0.78	0.81	0.73	0.79	0.70													
31. Rt.130*501 (26)	0.49	0.49	0.47	0.54	0.49	0.56	0.57	0.80	0.77	0.75	0.78	0.77	0.79	0.72	0.73	0.76	0.77	0.73	0.83	0.70	0.76	0.67	0.78	0.75	0.83	0.80	0.69	0.81	0.73	0.90												
32. Indian Rock Rt.614 (27)	0.54	0.53	0.51	0.58	0.53	0.46	0.47	0.73	0.78	0.71	0.71	0.68	0.72	0.67	0.72	0.66	0.70	0.61	0.71	0.62	0.67	0.62	0.71	0.73	0.72	0.72	0.65	0.71	0.62	0.90	0.85											
33. Iron Gate Rt.220 (34)	0.46	0.45	0.45	0.51	0.46	0.62	0.62	0.75	0.73	0.72	0.76	0.78	0.80	0.73	0.74	0.80	0.78	0.73	0.84	0.71	0.77	0.71	0.79	0.77	0.81	0.83	0.70	0.85	0.75	0.88	0.92	0.80										
34. Jackson River Rt.60*220 (35)	0.54	0.53	0.53	0.59	0.53	0.55	0.55	0.77	0.75	0.77	0.75	0.78	0.80	0.73	0.77	0.77	0.78	0.69	0.81	0.71	0.76	0.68	0.80	0.80	0.81	0.83	0.73	0.80	0.72	0.96	0.93	0.88	0.89									
35. Jackson River Rt.1104 (36)	0.51	0.50	0.50	0.55	0.49	0.59	0.59	0.77	0.75	0.75	0.75	0.80	0.81	0.75	0.76	0.79	0.79	0.71	0.85	0.73	0.77	0.72	0.81	0.79	0.82	0.84	0.72	0.85	0.74	0.93	0.93	0.84	0.94	0.95								
36. Jackson River Rt.687 (37)	0.51	0.50	0.50	0.56	0.50	0.59	0.59	0.79	0.76	0.76	0.77	0.78	0.81	0.74	0.75	0.79	0.79	0.73	0.83	0.72	0.77	0.69	0.80	0.78	0.83	0.84	0.71	0.83	0.74	0.93	0.95	0.85	0.93	0.95	0.97				Maple-Basswood			
37. Camp Appalachia Rt.666 (38)	0.48	0.47	0.45	0.52	0.47	0.57	0.58	0.79	0.76	0.74	0.78	0.77	0.79	0.72	0.73	0.76	0.77	0.74	0.83	0.70	0.76	0.67	0.78	0.75	0.83	0.80	0.69	0.81	0.73	0.88	0.98	0.84	0.93	0.91	0.92	0.94						
38. Wilton Green Rt.39 (40)	0.51	0.51	0.49	0.56	0.50	0.63	0.63	0.82	0.77	0.79	0.79	0.77	0.78	0.72	0.73	0.74	0.75	0.68	0.82	0.69	0.74	0.76	0.78	0.74	0.78	0.79	0.69	0.83	0.71	0.88	0.91	0.82	0.91	0.88	0.93	0.92	0.91				Mountain Pine-Oak Heath	
39. Monroe Rt.1202*1210 (25)	0.23	0.24	0.25	0.30	0.27	0.44	0.44	0.46	0.44	0.44	0.46	0.71	0.76	0.65	0.74	0.75	0.76	0.69	0.67	0.72	0.72	0.52	0.71	0.75	0.67	0.74	0.68	0.66	0.70	0.63	0.65	0.58	0.69	0.67	0.68	0.68	0.65	0.61				
40. Gathright Dam Rt.605 (39)	0.29	0.30	0.31	0.36	0.33	0.45	0.46	0.51	0.48	0.48	0.49	0.76	0.82	0.70	0.80	0.78	0.81	0.71	0.73	0.77	0.77	0.58	0.77	0.81	0.71	0.79	0.74	0.71	0.73	0.69	0.69	0.62	0.72	0.71	0.73	0.72	0.68	0.67	0.91			
41. Va. Mineral Springs Rt.606 (41)	0.34	0.33	0.33	0.38	0.32	0.84	0.83	0.69	0.65	0.69	0.73	0.65	0.60	0.66	0.58	0.69	0.64	0.66	0.70	0.60	0.65	0.73	0.64	0.60	0.62	0.67	0.54	0.73	0.67	0.55	0.59	0.48	0.65	0.57	0.62	0.61	0.59	0.66	0.47	0.53		
42. Paint Banks Hatchery (42)	0.37	0.36	0.37	0.40	0.36	0.92	0.89	0.67	0.64	0.67	0.70	0.65	0.59	0.61	0.56	0.65	0.62	0.61	0.70	0.56	0.63	0.75	0.65	0.58	0.60	0.65	0.54	0.71	0.64	0.53	0.57	0.46	0.63	0.55	0.60	0.59	0.57	0.64	0.45	0.48	0.87	
43. New Castle Hatchery Rt.42 (43)	0.38	0.37	0.34	0.39	0.35	0.92	0.88	0.69	0.66	0.67	0.73	0.58	0.57	0.56	0.55	0.57	0.61	0.60	0.67	0.50	0.58	0.66	0.61	0.55	0.65	0.62	0.52	0.64	0.57	0.52	0.60	0.49	0.62	0.53	0.58	0.58	0.61	0.64	0.39	0.42	0.80	0.88

Table 3. Frequency and relative density of Acrasieae in soils of forests. Figures are expressed in percent. F=Frequency, D=Relative Density, A=Appalachian, B=Blue Ridge, C=Coastal Plain, P=Piedmont.

Species	Oak-Hickory (C=2,P=1, B=1)		Mtn. Pine-Oak Heath (A=3)		Maple-Basswood (B=1.A=1)		Mixed Mesophytic (A=7,B=2)		Alluvial Hardwoods (P=11,A=5, B=1,C,1)		Pine-Oak (C=1,P=1)		Beech-Maple-Tuliptree (C=4,P=1)	
	F	D	F	D	F	D	F	D	F	D	F	D	F	D
Dictyostelium discoideum	32	2	16	4	25	6	25	2	5	>1	25	3	8	>1
D.giganteum	6	2	-	-	-	-	3	1	2	>1	-	-	-	-
D.lacteum	62	6	43	12	-	-	15	2	84	8	50	10	16	2
D.minutum	76	31	23	6	35	8	78	26	17	5	25	5	92	68
D.mucoroides	68	26	50	39	25	6	63	23	69	15	75	47	72	14
D.polycephalum	8	>1	-	-	-	-	-	-	8	1	-	-	-	-
D.purpureum	10	>1	23	4	15	3	24	2	46	3	30	2	36	5
D.rosarium	-	-	-	-	-	-	3	>1	4	>1	-	-	-	-
Polysphondylium violaceum	68	10	33	6	75	54	66	33	91	29	35	6	42	5
P.pallidum	64	22	43	29	70	22	69	22	89	15	65	26	60	7

VITA

Nathaniel LeMaster Armistead III was born on November 5, 1954 in Richmond, Virginia. He recieved his high school degree from St. Christophers School, Richmond, Virginia in 1973. He then recieved his Bachelor of Science in Chemistry from Hampden-Sydney College, Virginia in 1977. He later attended graduate school at the University of Richmond, Virginia where he graduated with his Master of Science in Biology in 1987. He is married to Mary Stewart Armistead.