DICTYOSTELIUM DISCOIDEUM, A NEW SPECIES OF SLIME MOLD FROM DECAYING FOREST LEAVES[1]

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INTRODUCTION

In the course of studies made in this laboratory on the amoeboid population of soil and decaying vegetable matter, myxamoebae in large numbers have been regularly encountered. Thom and Raper (*8*)[3] reported that the amoeboid stage of some Myxomycetes formed a normal part of the microbiological population of field soil and decomposing crop residues, and could readily be isolated and grown in artificial culture. Following this, Raper and Thom (*6*) studied the distribution of the Acrasieae in soil and reported *Dictyostelium* as a common component of the soil population from widely scattered areas and from many types of soil, while *Polysphondylium* was repeatedly isolated from vegetable remains, particularly forest litter, and occasionally from soil.

These results were in close agreement with earlier work by Krzemieniewski (*3*) and extended her observations in this field. It was reported previously (*6*) that the vast majority of cultures of *Dictyostelium* isolated apparently belonged to the single species *D. mucoroides* Bref. Continued studies and isolations since that time have shown an equally large proportion belonging to that species and support the writer's earlier findings. However, not all cultures isolated could be identified with *D. mucoroides*. A form closely agreeing with the description of *D. sphaerocephalum* (Oud.) Sacc. and March., as given by Olive (*4*) in his comprehensive study of the group, has been occasionally obtained from decaying forest litter. *D. purpureum* Olive has been isolated once, from decaying sphagnum. Still another form has been isolated that does not agree with the description of any published species and differs fundamentally in some respects from the other members of the genus. It has seemed desirable, therefore, to describe it as a new species, to review briefly its life cycle, and to discuss at some length the formation and behavior of certain structures not seen in other species.

TECHNICAL DESCRIPTION

Dictyostelium discoideum, n. sp.

Soris griseo-albis vel citrinis, rotundatis, apiculatis, plerumque 125μ–300μ diam.; sorophoris griseo-albis, ex discis expansis oriundis, basi rigidis, ad apicem tenuibus, flexuosis attenuatis, 1.5–3 mm altis; discis basilaribus cellularibus, conicis, bases sorophororum circumvallentibus et sustentantibus, 150μ–400μ diam.; sporis anguste ellipticis, hyalinis, 6μ–9μ × 2.5μ–3.5μ.

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[2] The writer is indebted to Charles Thom, Division of Soil Microbiology, for valuable counsel and criticism regarding the work, and to Edith K. Cash, Division of Mycology and Disease Survey, for preparing the Latin diagnosis.

[3] Reference is made by number (italic) to Literature Cited, p. 147.

Hab. in foliis putrescentibus in silva decidua, North Carolina.

Colonies grown on hay and dung agar; sori grayish white to pale lemon yellow, rounded, apiculate, commonly 125μ–300μ in diameter, with larger and smaller specimens frequent; sorophores grayish white, arising from expanded disklike bases, upright, rigid below, 30μ–80μ in diameter, tapering to thin flexuous above, 5μ–15μ in diameter, 1.5–3 mm high, less commonly longer or shorter; basal disks cellular, conical, surrounding and supporting bases of sorophores, 150μ–400μ in diameter; spores narrowly elliptical, hyaline, 6μ–9μ by 2.5μ–3.5μ.

Isolated from decaying leaves from deciduous forest, North Carolina.

One of the most striking characters of this species, which is only suggested in the mature sorocarp [4] by a trail of slime leading away from the base, is the peculiar behavior of the pseudoplasmodium. In other described species of *Dictyostelium*, three of which, *D. mucoroides*, *D. sphaerocephalum*, and *D. purpureum*, the writer has studied in culture, the fruiting stalk is produced from the point where the myxamoebae congregate. In this species the myxamoebae come together to form an aggregate, or pseudoplasmodium, as in the other and more common forms, but in the ordinary laboratory culture, instead of developing into a sorocarp immediately, the myxamoebae compact themselves together to form an elongated cylindrical mass which moves as a unit across the agar plate for a greater or less distance before pausing to complete its cycle of development. The formation, structure, and behavior of this "migration pseudoplasmodium" will be considered in greater detail later.

ISOLATION AND CULTURE

Dictyostelium discoideum was isolated from decaying leaves collected in a hardwood forest of the North Carolina mountains in the summer of 1933. The dominant trees were beech, birch, oak, and buckeye, and the sample consisted largely of the partially decomposed leaves of these trees, together with some weed residues. The sample had a pH of 4.65.

In isolating the organism, culture methods similar to those reported by Raper and Thom (*6*) were used. The sample was ground in a clean mortar with approximately 5 parts of sterile water, and the resulting suspension was streaked upon mannite agar plates. The plates were incubated for 3 weeks at 18° to 20° C. At the end of this time spores from sori uncontaminated by fungi were transferred to

[4] Following Zopf's use of the terms "sorus" for the spore mass and "sorophore" for the supporting structure, or stalk, Harper (*2*) introduced the term "sorocarp" to include the whole fruiting structure.

EXPLANATORY LEGEND FOR PLATE 1

A.—Mature sorocarp photographed from the side, showing the typical lemon-shaped sorus, the erect, evenly tapered sorophore, and the expanded basal disk. × 15.

B.—Spores. × 900.

C.—Vegetative myxamoebae growing in a bacterial colony; killed and stained with rose bengale and photographed in situ on the culture plate. × 250.

D.—Vegetative myxamoebae stained as in *C* and photographed in higher magnification, showing bacterial cells in the surrounding medium. × 900.

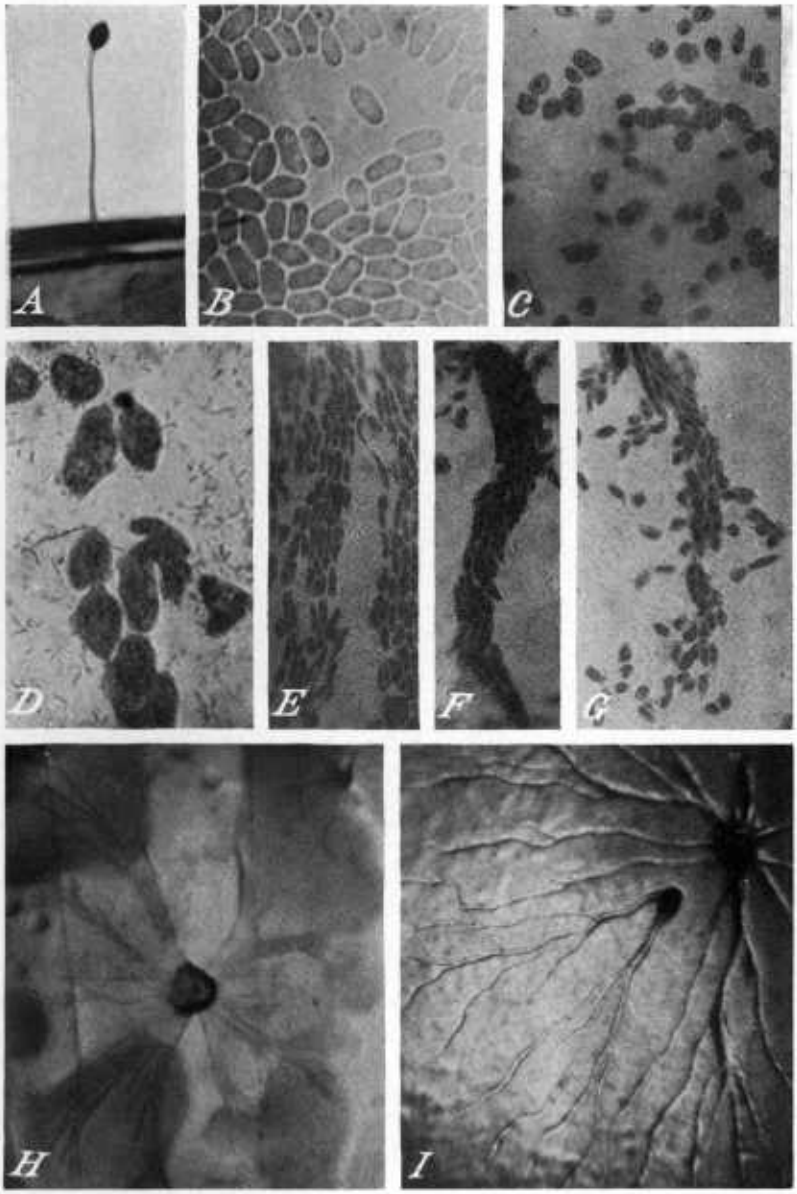
E.—Early stage in the orientation and aggregation of myxamoebae to form a pseudoplasmodial stream. In the lower part of the picture but little orientation of the myxamoebae is evident and aggregation is just beginning, while above a definite stream is already formed. × 250.

F.—A slightly later aggregation stage, but one in which the myxamoebae remain in a single layer. Their elongate Limax form and rather uniform orientation are shown. Movement is toward the top of the photograph. × 250.

G.—A somewhat later stage in aggregation of the myxamoebae, which here have become compacted together to form a ropelike structure. × 250.

H.—Entire pseudoplasmodium with streams of myxamoebae radiating in all directions from the aggregation center shown as an irregular darkened area. Note that the streams can be traced directly to regions of heavy bacterial growth, which appear as darker areas in the photograph. × 15.

I.—Somewhat later stage of aggregation, showing a large pseudoplasmodium surrounding a smaller one. In an earlier stage the entire colony was flowing toward a common center, upper right; then for some reason a main stream was severed, and as a result two fruiting masses will develop. × 15.



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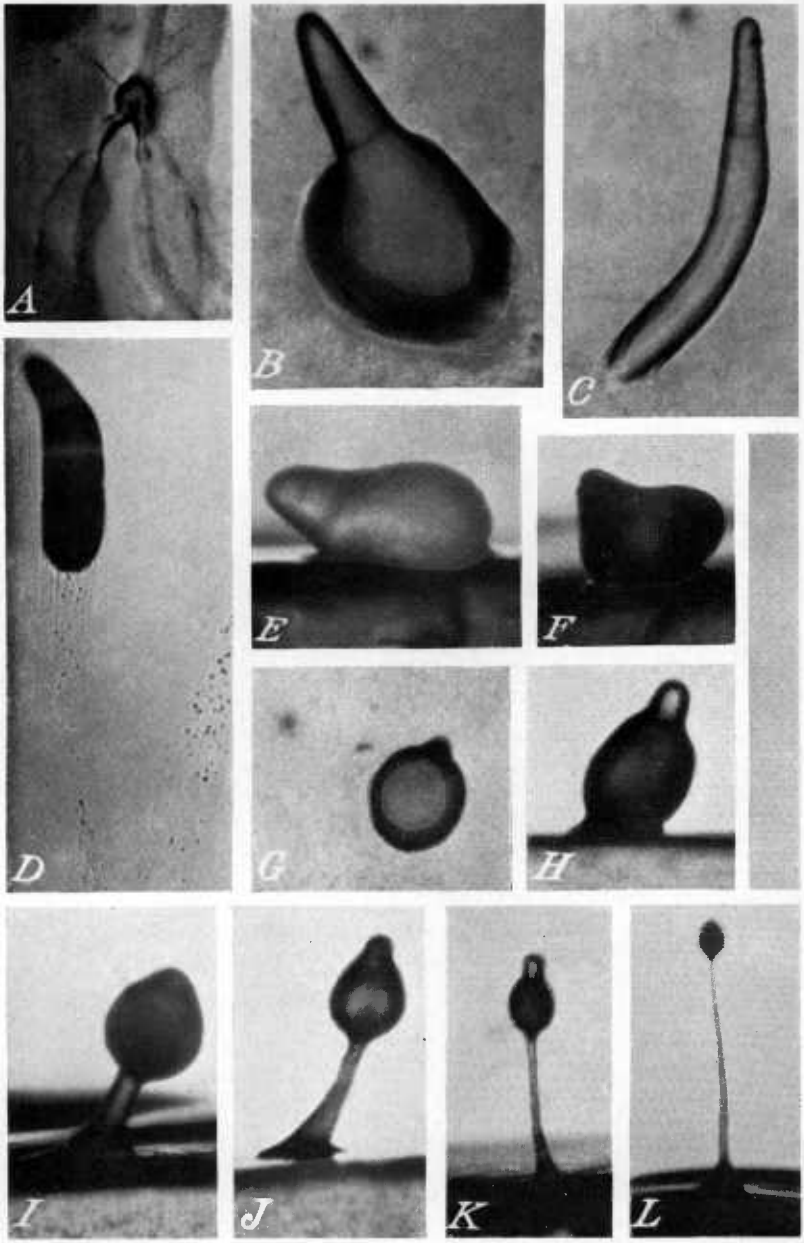

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fresh plates of hay- and of dung-infusion agar adjusted to a pH of 6.0. The organism has been grown continuously upon these media and in Petri-dish cultures since that time. Transfers have been made at intervals of 2 to 3 weeks, although it is not necessary to transfer so frequently to maintain the stock culture. Cultures have been grown for the most part in a dark incubator at a temperature of 18° to 20°.

In photographing and in measuring the dimensions of myxamoebae and in studying their relation to one another in the pseudoplasmodium it is essential to disturb the organisms as little as possible. It is particularly difficult to obtain and to maintain a true picture of them as they naturally occur in culture. If they are transferred to slides they change shape and position during the process, and if they are observed on the agar plate the concentration of light and heat when high powers are used induces the same changes. To obviate these difficulties as far as possible, photographs and measurements of myxamoebae have been made of specimens killed and stained in situ with rose bengale[5]. This preparation kills the organisms immediately and stains them a darker red than the underlying medium, so that they stand out clearly and their actual form and position, as they occur in culture, are preserved. However, the stain has a decided disadvantage in that it stains the whole organism indiscriminately and does not permit cytological study. Only the figures shown in plate 1, *C* to *G*, are photographs of myxamoebae thus killed and stained in situ; other photographs are of unstained, living material. All the photographs were made with a Leitz Makam camera.

VEGETATIVE STAGE

When large numbers of spores (pl. 1, *B*) are planted upon fresh dung- or hay-infusion agar plates, germination begins within the first day and continues for 2 or 3 days thereafter. It is characterized by a swelling of the spore contents and a longitudinal splitting of the spore case, from which the protoplast emerges as a myxamoeba. The process is similar to that in *Dictyostelium mucoroides* and agrees closely with Olive's figures (*4, pl. 6, figs. 42, 44*).

In the vegetative stage following germination, the myxamoebae are quite hyaline and finely granular, and show little differentiation into endoplasmic and ectoplasmic regions. They are uninucleate and

[5] One-percent rose bengale, or erythrosin, in 5-percent solution, to which is added a trace of calcium chloride.

EXPLANATORY LEGEND FOR PLATE 2

A.—A pseudoplasmodium in approximately the same stage of development as that shown in plate 1, *I*, with streams of myxamoebae leading in from the bacterial streaks on either side of the aggregation center. × 15.

B.—A migrating pseudoplasmodium leaving a bacterial colony. × 50.

C.—Migrating pseudoplasmodium. The transverse line in the anterior end marks the point of contact with the agar surface. × 50.

D.—Migrating pseudoplasmodium with a slime streak marking its former path. Another slime streak cuts diagonally across the lower right-hand corner of the photograph. × 50.

E.—Side view of a pseudoplasmodium just before it ceases migration. × 50.

F.—Side view of a pseudoplasmodium in which movement has ceased and sorocarp formation is beginning. × 50.

G.—Similar stage of development as that shown in *F*, but viewed from above. × 50.

H.—Early stage in formation of the sorocarp. Mass of myxamoebae becoming divided into spore- and sorophore-forming regions. × 50.

I, J.—Successive stages, showing the sorogenic mass ascending the sorophore as it develops. × 50.

K.—Still later stage, showing a well-formed apical papilla in which active sorophore formation is taking place. × 30.

L.—Mature sorocarp. × 16.

regularly possess a single contractile vacuole, which occupies a posterior position in the cell. Food vacuoles and vacuoles surrounding foreign bodies are often clearly evident.

In shape the myxamoebae vary widely. They are not infrequently elongate or of irregular shape, owing to the presence of extended pseudopods, but are more commonly rounded or broadly triangular. Plate 1, *C* and *D*, illustrates clearly this condition. The photographs are of myxamoebae killed and stained with rose bengale and photographed in situ on the agar plate, and thus faithfully show them as they appear when actively vegetating. In hanging-drop cultures, the myxamoebae are more irregular in outline and frequently show few to many thin, finely pointed pseudopods, particularly in the anterior region. In size the vegetative myxamoebae vary from 12μ to 20μ by 8μ to 12μ, with the majority about 14μ to 16μ by 9μ to 11μ. Measurements in such amoeboids are subject to rapid change with variations in shape, however, and cannot be relied upon with the same confidence as measurements of spores and other definite walled structures. It is obvious that an amoeba, while remaining constant in volume, would show, when flattened and actively moving, entirely different dimensions from those it would show when rounded and relatively quiescent.

The present studies add little to the controversial subject of the nutrition of myxamoebae. The vegetative myxamoebae of *Dictyostelium discoideum* reach their maximum development in bacterial colonies, outside the limits of which they occur as scattered individuals only. The writer has not yet been able to determine satisfactorily whether the greater amount of moisture in the bacterial colonies induces the concentration of the myxamoebae there, or whether the myxamoebae feed upon the products of bacterial metabolism or utilize substances in the substratum set free by bacterial activity, or whether they feed directly upon the bacterial cells. It is not uncommon to see myxamoebae with vacuoles enclosing bacteria, but to determine whether the bacteria are undergoing digestion or are merely present as so much inert material, as Olive (*4*) has indicated, is quite another matter. Pinoy (*5*), working with *D. mucoroides* and *D. purpureum*, and later Skupienski (*7*), working with the former species only, have reported the presence of bacteria as essential to the growth of these species. Pinoy considered the myxamoebae as parasitizing the bacterial colonies, while Skupienski believed that a symbiotic relation exists. Just what the relation is between the two in *D. discoideum* the writer is not prepared to say; certainly the myxamoebae of this species are not hindered in any way by the presence of a fair growth of "contaminating" bacteria.

Under favorable culture conditions the vegetative stage, that is, the period from spore germination to the beginning of pseudoplasmodium formation, lasts several days, and during that time the myxamoebae increase tremendously in number. The first pseudoplasmodia appear in 3 to 4 days, and others continue to develop for a similar period. The two stages, vegetative and fructifying, are thus seen to proceed simultaneously for a time in the same culture. Cell division of the vegetating myxamoebae of this species has not been studied.

AGGREGATION STAGE

Following a vegetative period, the myxamoebae congregate toward definite centers and form aggregations preparatory to fruiting. The process here agrees so closely with that in other *Dictyostelia* to which Harper (*2*) and Olive (*4*) have given such detailed study that it is only necessary to discuss it briefly. The first evidence of aggregation is the appearance of certain areas of closely packed myxamoebae. The stimuli involved in this aggregation have never been determined, but these strategically placed individuals apparently exert some stimulus which reaches myxamoebae in the surrounding area and causes them to become elongated and oriented in the direction of these centers and to flow toward them. Gradually the orientation proceeds outward and the colony of aggregating myxamoebae takes the form of definite streams converging toward definite centers. Plate 1, *E* to *G*, shows different stages in this process.

The formation of a pseudoplasmodial stream is shown first by the orientation and aggregation of scattered myxamoebae (pl. 1, *E*). In the outer edges of the area involved they appear only slightly different from vegetative forms and show little orientation, whereas toward the center of the developing aggregate the orientation is much more pronounced, the myxamoebae are crowded together, and the pseudoplasmodial stream is an accomplished fact. Plate 1, *F*, shows a slightly later stage, in which the myxamoebae are definitely out of the vegetative condition and are actively moving toward the aggregation centers. However, they are not compacted together to any particular degree and still remain in a single layer on the agar surface. The degree of elongation of the aggregating myxamoebae is shown here. In higher magnifications they are clearly differentiated into endoplasmic and ectoplasmic areas, the latter occupying a broad band at the anterior end of the myxamoebae. As the stream becomes slightly older, the myxamoebae are interlocked and overlap to such a degree as to form a compact ropelike mass (pl. 1, *G*).

The three photographs (pl. 1, *E–G*), although not of the same stream nor even of the same aggregate, are fairly typical of successive stages in the orientation of the myxamoebae and the formation of the pseudoplasmodium, and at the same time fairly represent sections of the stream successively nearer the aggregation center. All three photographs were made near the tips of streams, as it was quite impossible to photograph the larger streams near the centers and show details of individual myxamoebae.

Plate 1, *H* and *I*, and plate 2, *A*, show a lower magnification of entire aggregations. A pseudoplasmodium with streams of myxamoebae radiating in all directions from the center, which appears as a darkened irregular area, is shown in plate 1, *H*. The streams can be traced directly to regions of heaviest bacterial growth, which appear as darker areas in the photograph. Plate 2, *A*, which was taken at about the same stage in development, shows this condition equally well. The myxamoebae are being drawn primarily from the two bacterial streaks shown on either side of the aggregation center. The two pseudoplasmodia shown in plate 1, *I*, represent a later stage of development, in which many small streams have merged to form larger streams which appear as definitely raised and ropelike struc-

tures. The streaming and aggregation of the myxamoebae continue until a compact, elongate cylindrical mass is formed.

In plate 1, *I*, is further shown a very large pseudoplasmodium surrounding, as it were, a much smaller one, and in this case it is obvious that in an earlier stage the whole colony of myxamoebae was flowing toward a common aggregation center. Then, from some cause either foreign to or within the pseudoplasmodium or its component cells, a new center appeared, and as a result two fruiting masses will develop, each producing a sorocarp. This may well raise the question of just what determines the size of an aggregation. As Van Tieghem (*9*), Olive (*4*), Harper (*2*), and others have shown, the myxamoebae that make up a pseudoplasmodium retain their individuality, are mechanically separable, and react more or less independently of one another; none the less it is equally apparent that there is some stimulus or force which influences and determines what myxamoebae shall go into one aggregation and what into another, and consequently how large the fruiting structures shall be. In an even culture of myxamoebae, what determines whether there shall be more and smaller or fewer and larger sorocarps? For the present the whole subject must remain a matter of conjecture.

The developing pseudoplasmodium may be of almost any conceivable shape. In a fresh culture with an even distribution of myxamoebae, the form is typically radially symmetrical. But in an older culture where previous aggregations have already withdrawn the myxamoebae from certain areas, or in fresh cultures where there is an uneven growth of amoeboids, the centers once formed draw the myxamoebae from wherever they can. A resulting pseudoplasmodium may consist of a single extended stream, or of two streams extending out in opposite directions, or may be triangular as myxamoebae are drawn from a sector uninfluenced by previously developing masses, or even crescent-shaped as it forms at the periphery of a bacterial colony.

MIGRATION STAGE

Thus far the development of *Dictyostelium discoideum* follows closely that of other species of the genus. But at this point a stage occurs which does not appear in any of the Acrasieae described. The myxamoebae come together to form pseudoplasmodia which, instead of initiating a sorophore formation at once, gradually become transformed into compact, elongated cylindrical masses by the continued streaming and crowding together of their component myxamoebae. Sorophore formation is delayed, and between the appearance of the compact cylindrical pseudoplasmodium, as described above, and the beginning of sorophore formation, a period ensues during which the pseudoplasmodium moves as a unit over the surface of the agar until it reaches a suitable location for sorocarp formation. As shown earlier in this paper, the myxamoebae grow most luxuriantly in bacterial colonies, and the pseudoplasmodia are regularly formed within the limits of these colonies; thus the migratory stage may well be a demonstration of negative hydrotaxis, that is, a concerted movement on the part of the pseudoplasmodium to reach a drier situation in which to develop the fruiting structure. Plate 2, *B*, shows such a pseudoplasmodium leaving a bacterial colony.

In shape the typical migrating pseudoplasmodium is elongate cylindrical, with the anterior end tapering to a greater or less degree and the posterior end rather blunt and somewhat flattened horizontally (pl. 2, *C*). However, variations from this form are not uncommon; the moving mass may be longer and narrower, with a less pointed tip, or shorter and relatively thick, with the anterior end rounded rather than pointed. It may be either straight or slightly curved (pl. 2, *C*). As it moves, the greater portion of the mass is regularly in intimate contact with the agar surface, but with the pointed frontal portion slightly raised above it; the transverse line in the upper half of the pseudoplasmodium (pl. 2, *C*) marks the point where contact with the agar surface is interrupted. In long narrow specimens the mass may be raised in the central portion as well.

In size the migrating pseudoplasmodia vary widely, both as to relative dimensions of length and breadth, as pointed out above, and as to total volume. But in well-developed cultures on hay-infusion agar, which have been incubated for 5 or 6 days at 18° C., typical specimens show dimensions of about 0.8 to 1.2 mm by 0.15 to 0.25 mm, with extremes measuring as much as 2.0 mm in length and as little as 0.4 to 0.5 mm by 0.07 to 0.1 mm. With some exceptions, volume is clearly dependent upon the extent of the aggregating mass from which the migratory structure developed, but the factors which determine the relative proportion of length to breadth are not so obvious.

If a migrating pseudoplasmodium is crushed in a drop of water under a cover slip, the entire mass is seen to be composed of apparently similar and undifferentiated cells. There is not the slightest indication of a stalklike structure, and the myxamoebae readily and quickly separate from one another. They are at first rounded, almost spherical, with diameters averaging 8.5μ to 9μ, but after the colony of dissociated myxamoebae has been allowed to stand for 15 to 30 minutes the organisms tend to become more elongated, show a differentiation into the endoplasmic and ectoplasmic areas, and begin a slow but perceptible amoeboid movement which marks the beginning of a new organization, or reorganization, of the myxamoebae into new fruiting masses. On the other hand, if a similar pseudoplasmodium is killed and stained in situ and then removed from the agar surface and broken apart, the myxamoebae are seen to be not round but elongated and somewhat angular in outline. As would be expected, the long axes are oriented in the direction of movement. The average dimensions of the myxamoebae at this stage are about 12μ to 13μ by 5μ to 6μ. Movement of the migrating pseudoplasmodium as a unit is obviously accomplished by the concerted and, at the same time, independent action of the countless individual myxamoebae of which it is composed.

In the Acrasieae the production of slime is characteristic, but in *Dictyostelium discoideum* it manifests itself in an earlier stage and in a somewhat different manner. As the migrating pseudoplasmodium moves over the agar surface it produces and leaves behind a trail of slime which persists and can be seen leading away from any migratory mass or mature sorocarp. The material is quite tenacious, for when a sorocarp or pseudoplasmodium is removed, invariably a portion of the slime streak, often 2 to 3 cm in length, remains attached to it.

Plate 2, *D*, shows a pseudoplasmodium with a trail of slime clearly marking the path it has traveled; cutting diagonally across the lower right-hand corner of the same photograph is the trail of another pseudoplasmodium. Embedded in the slime streaks are scattered myxamoebae that have been left behind; microscopically they appear as vacuolated cells of irregular shape. As will be seen later, the number of myxamoebae thus left behind varies with the rate of progress made by the migrating structure.

Regularly the migration pseudoplasmodium produced by an aggregating colony of myxamoebae remains as a unit as it creeps over the plate, and forms a single sorocarp. However, the mass not uncommonly divides, either leaving behind a mass of myxamoebae which produces an independent fructification or splitting into two masses each of which continues to move as a typical migratory structure. Secondary divisions of these pseudoplasmodial masses have occasionally been observed. In order to study their habits to best advantage, the migrating structures were removed from the cultures in which they grew and transplanted to fresh plates where they were less crowded and where their movements could be followed more easily.

In contrast to this splitting of a pseudoplasmodium into more or less equal masses, in older cultures there frequently occurs a gradual fragmentation of the entire structure. In such cases comparatively small masses of myxamoebae are cast aside, and these proceed to develop into small atypical sorocarps at the point where they are cut loose from the larger, parent structure. A large pseudoplasmodium may in this way give rise to a whole series of miniature fruiting bodies lining its path; and intermixed with the sorocarps countless myxamoebae remain stranded, either singly or in groups of varying size. Just what brings this condition about is not entirely clear, but it is no doubt influenced by the drying out of the agar and perhaps through the accumulation of waste products as well.

In cultures grown at 18° to 25° C. the migration pseudoplasmodia are formed and behave in the manner described above. When cultures are grown at 30° to 32° the pseudoplasmodia are formed in the same way but behave very differently. In such cultures the majority never leave the bacterial colony in which they are formed, but proceed to form sorocarps at the points where the aggregates develop. Sorocarps formed under such conditions, however, are not unlike those which develop from migrating pseudoplasmodia. Further studies are in progress concerning the growth and behavior of the species in response to changes in its environment.

SOROCARP FORMATION

Following the period of migration described above, the pseudoplasmodium ceases forward movement and builds an erect fruiting structure which, in accordance with Harper's usage, may be called the sorocarp. This development is shown in a series of photographs (pl. 2, *E–L*), all of which, with the exception of plate 2, *G*, are side views of successive stages.

The first evidence of this development is a relative shortening and thickening of the pseudoplasmodium. Forward movement of the mass becomes progressively slower until it ceases altogether. The myxamoebae, however, continue to crowd together, and the whole

structure takes on a more rounded appearance (pl. 2, *E*). In plate 2, *F*, this tendency has progressed further, and what has been the pointed anterior end now appears as a raised knob at one side; the whole colony is here becoming definitely transformed into an upright structure. Plate 2, *G*, shows a slightly more advanced stage as viewed from above. The structure presents a rounded, almost symmetrical form, with the apex of the developing sorocarp lifted above the mass.

In plate 2, *H*, the structure is more erect and is becoming definitely differentiated into sorophore and spore-forming parts, and it is in structures presenting this appearance that the early stages of sorophore formation are to be found. As development continues and the sorophore becomes longer and sufficiently strong to support the weight, the mass of myxamoebae slowly begins its ascent. In plate 2, *I*, this is clearly shown; the basal portion of the sorophore is complete and is securely anchored to the substratum, and the spore-forming mass is quite removed from it. This particular figure, however, is somewhat atypical in that it shows no pronounced apical region where sorophore formation is taking place. In the developing sorocarp of *Dictyostelium mucoroides*, as pointed out by Harper (*2*) and as seen in culture, the pseudoplasmodium extends down the sorophore as a band either applied to one side or coiled spirally around it, and may form a continuous stream between the substratum and the sorogenic mass. Such a condition has not been observed in *D. discoideum;* the pseudoplasmodium, or spore-forming mass, climbs the developing sorophore as a compact unit.

Plate 2, *J*, is a somewhat later stage and more nearly presents the usual picture. The apical region is composed of undifferentiated myxamoebae, which later become extremely vacuolated and contribute to sorophore development. The myxamoebae making up the bulbous mass below are destined to form spores; in fact, those nearest the periphery have already become differentiated at this stage. The fact that the stalk and base in this figure are heavier in proportion to the size of the sorocarp as a whole than usually is the case is due to exposure to the dry air of the laboratory preparatory to and during the process of photographing. A still more advanced stage is shown in plate 2, *K*, in which the relative proportions of parts in the developing structure are more nearly normal. The well-formed apical papilla indicates that active sorophore formation is in progress. Mature sorocarps are shown in plates 1, *A*, and 2, *L*, all the myxamoebae having become differentiated either into stalk cells or spores.

The mature sorocarp is typically a symmetrical structure, consisting of a disklike cellular base which envelops and supports the base of the stalk, an unbranched sorophore of similar nature which is relatively thick and rigid below but tapers rather evenly to a thin terminal region above, and a rounded apiculate or lemon-shaped sorus. Such an expanded disklike base has not hitherto been described in the Acrasieae. When seen from above, this basal disk is roughly circular in outline, and the upright sorophore arises perpendicularly from the central region of it (pl. 3, *D* and *F*). When viewed from the side, the structure presents a somewhat conical appearance, with a more or less centrally placed cuplike depression into which the base of the sorophore fits (pl. 3, *E*).

What actually happens is that the basal portion of the upright sorophore is first formed and then the basal disk is built outward from it

at the same time that it continues to be built upward. When a young fruiting mass such as that shown in plate 2, *H*, is crushed, it is found that the basal part of the sorophore is in process of formation, and extending outward from it on all sides a band of myxamoebae are cut off by what is apparently a slime sheath not unlike that which extends upward through the mass during sorophore formation. Inside this basal sheath, the cells nearest the upright sorophore are differentiated first and later those further removed are differentiated.

At the periphery of the completed disk the highly vacuolated and horizontally flattened cells form a single layer (pl. 3, *F*). The disk becomes thicker in cross section nearer the sorophore, with the cells piled one above the other. Bordering the sorophore a sleevelike layer of disk cells extends upward for some distance (pl. 3, *E*). The whole structure is enclosed in an envelop of slime which is continuous with that of the sorophore, and extending out from one side of the disk is the trail of slime laid down by the migrating pseudoplasmodium, shown in plate 3, *D*, as a thin line curved about the base.

The sorophore is composed of a slightly bulbous base (pl. 3, *D*), which is enclosed in the basal disk described above, and a cylindrical, elongated tapering shaft which supports the sorus. Its structure is like that of *Dictyostelium mucoroides* (pl. 3, *B*, *C*, *D*, and *F*), although it is more rigid and is almost invariably formed perpendicular to the substratum to which it is attached. Its greater rigidity is due to two factors: (1) In relation to height it has a greater basal diameter and the degree of tapering is greater than in *D. mucoroides*, thus adding to its strength; and (2) the expanded cellular base rests squarely upon the substratum, thus giving the sorophore a much firmer support than in the more common species, where it is anchored by slime only.

As noted above, the bulbous base of the sorophore is formed first, and extending upward from it in the central region of the young sorocarp is a tubular slime sheath which is formed in advance of the vacuolated cells and in which these cells become packed as growth of the sorophore progresses. This sheath is continuous with the sorophore below and the greater part of it is of the same diameter; the terminal region, however, is regularly expanded into a funnellike structure. A similar structure is present in other *Dictyostelia*, and figures of it in *Dictyostelium mucoroides* have been given by Brefeld (*1, pl. III, fig. 21*) and Harper (*2, pl. 6, fig. 14*). Brefeld's figure shows the slime sheath formed for some distance beyond the enclosed, vacuolated cells; and, except in the absence of an expanded terminus, it agrees with structures commonly seen in young, rapidly developing sorocarps of *D. discoideum*. As to the origin of this sheath, it is not clear whether it is secreted by cells that are destined to become stalk

EXPLANATORY LEGEND FOR PLATE 3

A.—Terminal region of developing sorocarp, showing the funnel-shaped sheath formed in advance of sorophore formation. The apical papilla and the region surrounding the top of the sorophore are composed of amoeboid cells. Below, spores are already differentiated. × 320.

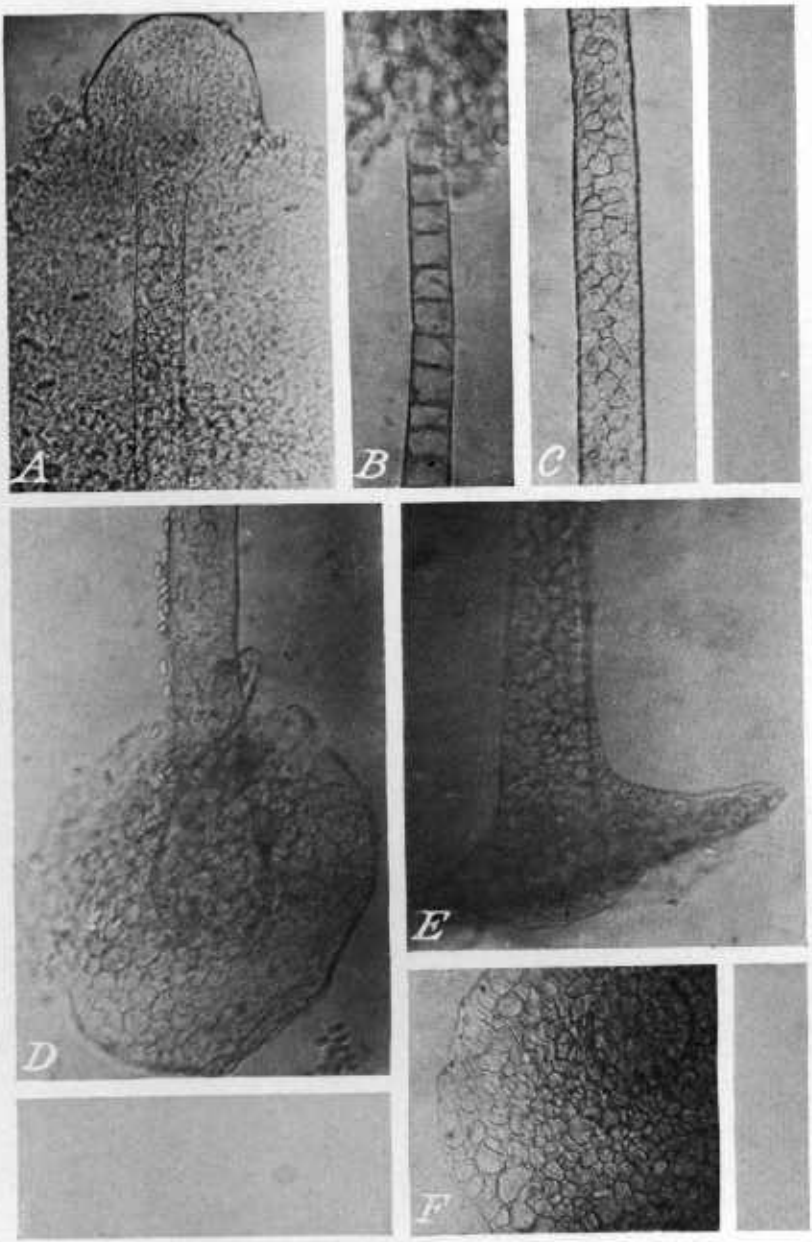
B.—Terminal region of a sorophore, consisting of cells in a single row or superimposed on other cells. × 900.

C.—Median region of a sorophore. × 320.

D.—Basal part of a sorocarp, showing the swollen base of the upright sorophore and the expanded basal disk from which it arises. The slime streak remained attached and is shown as a line curved about the base of the sorophore. × 320.

E.—Basal part of a sorocarp, showing the conical shape of the basal disk and how the end of the sorophore fits into and is attached to it. × 320.

F.—Basal disk as viewed from above, showing the end of the sorophore as a circular structure in the upper right corner of the figure. × 320.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

cells or by the surrounding cells, which later form spores. In either case it serves to separate the two groups of cells before there is any evident differentiation of either.

In plate 3, *A*, the terminal region of a more advanced sorocarp than those just under consideration is shown, and in it, in contrast to the condition in younger, rapidly developing structures, the expanded portion is just above the rounded, vacuolated cells, which later become compacted together to form a rigid sorophore. Above the developing sorophore is the apical papilla of undifferentiated amoeboid cells which contribute to its further extension. Just below and surrounding the terminal region of the sorophore are other undifferentiated myxamoebae; whether they all ultimately become spores or whether some of them join the apical mass and become stalk cells has not been determined.

In the lower part of the picture the outlines of spores already formed can be seen. Once the mass of myxamoebae has become separated from the substratum (*pl. 2, I, J*) and is ascending the sorophore as it develops, the myxamoebae at the periphery of the mass in a centrally located band begin to be differentiated into spores, and as this sorogenic mass rises continually higher on the developing sorophore more and more spores are formed. If a sorocarp such as that shown in pl. 2, *K*, is studied in a drop of water, a band of spores is seen completely encircling the developing head; if as many spores as possible are washed away and the structure is crushed under a cover slip, it becomes evident that beneath the layer of spores is a mass of cells in stages of differentiation into spores and that around the sorophore is a cylinder of cells still amoeboid which carries the mass upward. Gradually the cells in the apical region are used up in sorophore formation, and those surrounding it become spores to complete the fruiting structure.

TABLE 1.—*Comparative dimensions of parts of sorocarps of Dictyostelium discoideum from 3-week-old dung-agar cultures*

Diameter of expanded base	Size of sorophore					Diameter of sorus
	Length	Portion above basal swelling		Terminal region		
		Diameter	Cells in cross diameter	Diameter	Cells in cross diameter	
μ	*μ*	*μ*	*Number*	*μ*	*Number*	*μ*
200	1,800	42	5–6	8.5	1, 1, and 2 [1]	225
200	2,350	38	5	9	do [1]	200
210	2,210	39	5	7.5	1	230
240	2,510	45	6	9.2	1 and 2	275
205	2,250	41	5	7.5	1	240
185	2,050	32	4	7	1	225
210	2,420	37	5	8.5	1, 1, and 2 [1]	300
325	2,850	70	9–10	14	2	350
270	2,500	48	7–8	10.2	2	250
225	2,440	42	6	8.5	1 and 2	275
200	2,240	38	5–6	9	do	225
300	2,625	60	8	10	2	325

[1] The sorophore was usually 1 cell but occasionally 2 cells wide.

When sorocarps in large numbers are observed, one is impressed with the relation in size that exists between their different parts, for example, the diameter of the basal disk, sorophore, and sorus. This relationship is not exact, to be sure, but within limits it does exist. In table 1 are given the measurements of a group of sorocarps picked from dung-agar cultures 3 weeks old. Dimensions of the sori were taken in situ, and the sorocarps were then removed to slides, where other measurements were made. Roughly the ratio of the diameter of the expanded disklike base to the diameter of the sorophore just above the swollen base is 5 to 1 and the ratio of the diameter of the sorophore at this point to its diameter in the terminal region is likewise approximately 5 to 1. The relationship between length and diameter is not so regular, but in general the longer sorophores have the greater diameters, arise from the larger bases, and bear the larger sori.

In *Dictyostelium discoideum*, as in other Acrasieae, there is a striking relation between the diameter of the sorophore in the terminal region and the size of the sorus. And in fructifications where the sorophore terminates in a single row of cells, the shape of these cells varies with the volume of the spore mass. Harper (*2*) has made a detailed study of this matter in *D. mucoroides*, and the writer's observations on *D. discoideum* agree closely with his. If the sorus is very small the cells are narrow and elongated, 15μ to 18μ by 3μ to 3.5μ, or with the long and short diameters in the ratio of 5 to 1; if larger, the cells are more nearly isodiametric; and if still larger, the cells are horizontally flattened, 3μ to 4μ by 9μ to 10μ, or with the long and short diameters in the ratio of 1 to 3. In cases where the sori are especially large, the sorophores are 2 or rarely 3 cells wide and the cells are of approximately equal width and length. Thus it would seem that the size of the sorophore is the essential thing; it must be of a size and strength sufficient to support the spore mass, and the cells that go into the making of it become modified in such a way as to attain this objective.

Sorocarps regularly develop at right angles to the substratum or other support to which they are anchored. In the usual culture they are produced upon the agar surface and are built up vertically. If grown in inverted plates they are built vertically downward. If cultures are grown in uneven light and as a result migrating pseudoplasmodia climb up the side of the culture dish, the resulting sorocarps develop outward horizontally. In cultures grown in uneven light or temperature there is no pronounced inclination of the sorophores toward either light or warmer temperature.

True branching has not been observed in *Dictyostelium discoideum*. On several occasions structures have been seen which, under low magnification, appeared to be branched sorophores. But in each case, on closer examination, the "branched" structure was seen to be composed of two sorocarps complete in every detail, one of which was anchored to the substratum while the other was anchored to the upright sorophore of the first. The "branch" possessed a typical expanded base, which was somewhat curved around the supporting sorophore and was securely anchored to it by an envelop of slime. In regard to such compound structures the question arises as to whether the supporting and the supported sorocarps arise from the same colony of aggregating myxamoebae. The evidence indicates that this is not the case but that the branch originated in a later pseudoplasmodium which climbed up an established sorophore, just

as it would have climbed up the side of the culture dish, and then proceeded to form a sorocarp.

SUMMARY

A new species of *Dictyostelium* is described, for which the name *Dictyostelium discoideum* is proposed.

Following the aggregation of the myxamoebae, the pseudoplasmodium becomes a compact cylindrical mass and moves for a greater or less distance over the culture plate before developing a sorocarp. This migratory stage, which has not been previously reported, is termed the migration pseudoplasmodium.

The mature sorocarp differs from that of the more common species in possessing a cellular basal disk, which surrounds and supports the base of the sorophore. In addition, the sorophore is more rigid and tapers more evenly than in other species.

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