# A NATURALLY OCCURRING DEVELOPMENTAL SYNERGISM BETWEEN THE CELLULAR SLIME MOLD, DICTYOSTELIUM MUCOROIDES AND THE FUNGUS, MUCOR HIEMALIS[1]

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## ABSTRACT

We here report the second record of a developmentally aberrant strain of a cellular slime mold from natural populations and demonstrate that this *Dictyostelium mucoroides* variant is capable of undergoing normal morphogenesis in the presence of the phycomycete fungus, *Mucor hiemalis*. The synergism is induced by an extracellular product(s) which is diffusable through thin agar membranes and is released by the fungus. The presence of the fungus not only induces stalk formation in this stalkless variant, but also increases the rate of sorocarp formation in 3 of 5 additional species of cellular slime molds assayed.

CELLULAR SLIME MOLDS of the genus *Dictyostelium* have become a model system for the study of development (Bonner, 1967; Loomis, 1975). Accordingly, considerable effort has been expended in the artificial induction of mutant strains exhibiting one or more developmental anomalies. A number of investigators have noted that two such deficient strains grown in combination are often capable of producing normal fruiting bodies, despite the inability of one or both of the strains to fruit in isolation (Sussman, 1954, 1955; Sussman and Lee, 1955; Sussman and Sussman, 1956; Ennis and Sussman, 1958; Filosa, 1962; Kahn, 1964b; Yanagisawa, Loomis and Sussman, 1967; Weber and Raper, 1971; Yamada et al., 1973; Darmon, Brachet and da Silva, 1975). This interaction has been called developmental synergism by Sussman (1954). All known cases of synergism in cellular slime molds are intraspecific interactions. The role of developmental synergism in natural populations of cellular slime molds is unknown, as developmentally aberrant forms described from natural populations are rare (Cavender, 1980).

In the course of a field-sampling program, a typical fruiting specimen of *Dictyostelium mucoroides* was found growing within the mycelium of the fungus, *Mucor hiemalis*. Subsequent isolation of the slime mold revealed that, in the absence of the fungus, the cellular slime mold fruited but did not produce the characteristic cellular stalk. We here report this phenomenon in detail and demonstrate that the fungus produces an extracellular product(s) which both induces stalk formation in *Dictyostelium mucoroides* and influences the rate of sorocarp formation in several species of *Dictyostelium*.

METHODS AND MATERIALS—A field sampling program was initiated in a second-growth forest in Mt. Carmel, CT, during June 1980. The sampling method followed that of Eisenberg (1976). Briefly, single plastic soda straws (6 mm diameter) were pressed into the leaf litter to a depth of 2 cm. The cores were extruded, weighed, suspended in 10 ml of sterile water, and agitated in a vortex mixer. A 0.5 ml aliquot of this suspension was inoculated, along with 0.4 ml of dense *Escherichia coli* (strain b/r) suspension, onto a sterile 100 × 15-mm petri dish containing 25 ml of 0.1% lactose-peptone (L/P) agar (Raper, 1951). Three replicates were prepared from each soil core and each was incubated at 22 C for 4 days. Slime mold aggregations were mapped as they became visible and were thereafter examined every 3–4 hr until fruiting had occurred.

Soil cores typically contain a large number of microorganisms, predominantly fungi and bacteria. Cellular slime molds were isolated from plates by carefully removing slime mold sorocarps and suspending them in sterile water. The suspended spores were counted in a Levy chamber, diluted to an appropriate density, and plated on 0.1% L/P agar with 0.4 ml of *E. coli* as a food source to generate single spore isolates.

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In one set of soil cores, an apparently typically stalked strain of *D. mucoroides,* was found within the mycelium of the fungus, *Mucor hiemalis.* Upon isolation, all spores of this strain produced a developmentally aberrant form which lacked the characteristic cellular stalk. To determine whether the presence of the fungus had induced stalk formation in this strain, an additional 200 plates were inoculated with a single-spore suspension of the stalkless variant and a weak suspension of *E. coli.* One half of these plates were inoculated with 0.88 mg of the fungal mycelium suspended in 1 ml of sterile water and the remainder with 1 ml of sterile water alone. All plates were incubated at 22 C for 3 days and examined for the frequency of stalked and stalkless individuals.

To further assess the influence of *M. hiemalis* on its associated strain of *D. mucoroides,* various concentrations of the fungus were inoculated with 30 *D. mucoroides* spores/plate and the rate of fruiting of the slime mold observed. Each plate was inoculated with 1 ml of sterile water to which one of four concentrations of the fungal mycelium had been added (0 mg, 0.044 mg, 0.44 mg, 4.4 mg). For each treatment, 15 replicate plates were prepared. As slime mold aggregations became visible, plates were examined periodically and the stage of development of the slime mold was noted. This procedure was repeated using a typically stalked strain of *D. mucoroides,* as well as strains of *D. purpureum, D. rosarium, D. discoideum,* and *Polysphondilium pallidum.* All strains except *D. discoideum* (strain NC-4) were recently isolated from natural populations.

The following experiment was performed to determine whether alterations in atmospheric conditions could produce effects similar to those induced by *M. hiemalis.* Twenty plates were inoculated with 30 spores each of the stalkless *D. mucoroides* variant and 0.4 ml of *E. coli* as a food source. These plates were divided into two groups: ten plates had approximately 6 gm of activated charcoal added to them, and the remaining plates did not. All plates were incubated in inverted position for 4 days and observed for evidence of stalk formation.

Two preliminary experiments were performed to determine whether the effect of *M. hiemalis* on *D. mucoroides* stalk formation was mediated by extracellular product(s) released by the fungus. In the first experiment, 0.44 mg of the fungal mycelium was inoculated in 500 ml of liquid culture medium (Robinson, 1978) and incubated for a period of 4 days. The culture medium was then filtered through a 0.22-μm millipore filter at a suction of less than 0.3 atm. Four volumes of this filtrate, 0, 0.01, 0.1,

1 ml, were then incorporated in 25 ml of 0.1% L/P agar. For each concentration, ten plates were inoculated with 30 *D. mucoroides* spores, incubated for 4 days at 22 C, and examined for stalk formation. In the second experiment, 0.22-μm millipore filters (13 mm diameter) were coated on both sides with a thin layer of agar. Each filter was carefully examined for air bubbles within the agar and those found to have bubbles were discarded (Sussman and Lee, 1955). On opposite sides of 12 of these filters, a wire-loop streak of *M. hiemalis* and of *D. mucoroides* was inoculated. As a control, an additional six filters were inoculated with only *D. mucoroides* spores. All filters were suspended with alligator clips within a 17 × 12 × 6-cm tissue culture dish at high humidity, incubated for 4 days at 22 C, and examined for stalk formation.

RESULTS—The field-sampling program of 12 soil cores yielded a total of ten *D. mucoroides* isolates. All but one of these forms, both in the initial isolate and in subsequent single spore isolates, were found to produce a normal fruiting structure composed of a sorus atop a cellular stalk. The sole exception was an isolate found within a large mycelium of *M. hiemalis.* In the initial isolate from the field this form produced a typical fruiting structure, but in each of the single spore isolates (i.e., in the absence of the fungus and other microorganisms), this strain failed to produce a stalk ($n = 15$). This stalkless form aggregates normally but does not produce a slug, nor does it migrate. The aggregating mass rounds up into a spore mass directly on the surface of the agar. Sections of this mass showed that it was composed of spores and dead amoebae, and that it lacked stalk cells. This naturally occurring stalkless isolate appears similar to several stalkless forms artificially produced in (or isolated from) laboratory populations (e.g., the S-2 fruitless-1 strain of Sussman and Sussman, 1953; the GV strain of Filosa, 1962).

Results of the test for synergism between the fungus and the stalkless slime mold are presented in Table 1. In the presence of the fungus, all slime mold aggregation centers yielded stalked fruiting structures; in the absence of the fungus, all aggregation centers yielded stalkless forms.

The influence of the different concentrations of *M. hiemalis* on the time until the appearance of the first fruits and on the time required for 100% fruiting is presented in Fig. 1 for all strains tested. In all slime molds tested, fruiting began significantly earlier in treatments with fungus added (Table 2). The time required for 100%
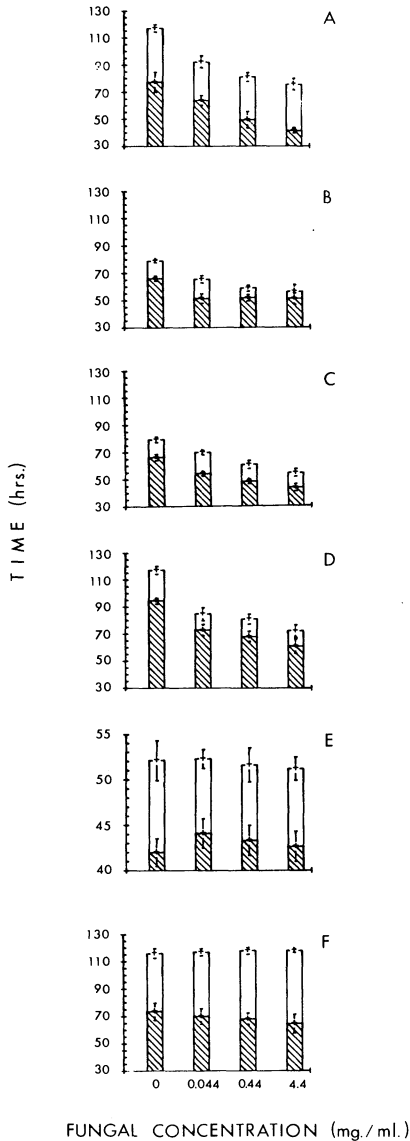
Fig. 1. The time to appearance of first fruiting body (crosshatched region) and the time to 100% fruited at four different concentrations of *Mucor hiemalis* mycelium for (A) *Dictyostelium mucoroides*, stalkless form, (B) *D. mucoroides*, stalked form, (C) *D. purpureum*, (D) *D. rosarium*, (E) *D. discoideum*, and (F) *Polysphondilium pallidum*.

TABLE 1. *Test for synergism*

| Treatment | $N^a$ | N.E.[b] | P.E.[c] | Stalked | Stalk-less |
|---|---|---|---|---|---|
| *M. hiemalis* +. *D. mucoroides* | 86 | 100 | 86% | 86 | 0 |
| *D. mucoroides* alone | 162 | 100 | 162% | 0 | 162 |

[a] $N$ = number of aggregation centers.
[b] N.E. = expected number of aggregations centers.
[c] P.E. = plating efficiency (N/N.E.).

contrast, fungal filtrate incorporated into agar induced stalk formation in the stalkless *D. mucoroides* strain in all replicates at all concentrations of fungal extracts. In the absence of fungal extract, all replicates failed to produce stalks. Although the low-concentration treatments induced stalk formation, the stalks in these replicates were noticeably shorter than those formed at higher concentrations. Results were similar in the test for a diffusable extracellular product by the growth of the fungus and slime mold on opposite sides of an agar-coated filter. All replicates with the fungus added induced stalk formation, whereas no stalks were formed on control filters.

DISCUSSION—This is the second report of a naturally occurring slime mold strain exhibiting developmental abnormalities typical of those induced artificially in laboratory populations (Cavender, 1980). The results further demonstrate that this variant produces typical stalked fruiting structures in the presence of the fungus, *M. hiemalis,* with which it occurs sympatrically. The synergism between the fungus and the slime mold is induced by an extracellular product(s) released by the fungus, which diffuses through agar and increases the rate at which various species of cellular slime molds fruit.

Several investigators have induced or accelerated normal fruiting in cellular slime molds using activated charcoal to adjust relative humidity or to remove gases in vitro (Raper, 1956; Wittingham and Raper, 1957; Bonner and Dodd, 1962; Kahn, 1964a; Raper and Cavender, 1968). Particularly striking is the demonstration that the fungus *Dematum nigrum* can accelerate fruiting in *D. polycephalum* and that similar effects can be induced in this species by the addition of activated charcoal to culture vessels (Wittingham and Raper, 1957). Our results provide no evidence that activated charcoal mimics the effect of *M. hiemalis* on this variant of *D. mucoroides.*

Fungi, however, produce an enormous variety of metabolites which can be recovered in

fruiting was significantly related to fungal concentration for all strains except *D. discoideum* and *P. pallidum* (Table 2).

The experiments testing for the influence of atmospheric conditions on stalk formation in the stalkless variant produced no evidence for such an effect. The presence of charcoal in the absence of *M. hiemalis* failed to produce any evidence of stalk formation ($n = 24$ fruits). In

TABLE 2. *Analysis of variance*

| Slime mold | | Time to first fruit | | | | | Time to 100% fruit | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | df | MS | F | P< | SS | df | MS | F | P< |
| D. mucoroides | E[a] | 1,185 | 3 | 3,952 | 159 | 0.0001 | 15,721 | 3 | 5,240 | 300 | 0.0001 |
| stalkless | R[a] | 1,387 | 56 | 25 | | | 771 | 56 | 13 | | |
| | T[a] | 13,242 | 59 | | | | 16,492 | 59 | | | |
| D. mucoroides | E | 2,504 | 3 | 835 | 87 | 0.0001 | 4,878 | 3 | 1,626 | 164 | 0.0001 |
| stalked | R | 534 | 56 | 9 | | | 556 | 56 | 10 | | |
| | T | 3,038 | 59 | | | | 5,434 | 59 | | | |
| D. purpureum | E | 4,485 | 3 | 1,495 | 350 | 0.0001 | 5,443 | 3 | 1,814 | 298 | 0.0001 |
| | R | 239 | 56 | 6 | | | 341 | 56 | 4 | | |
| | T | 4,724 | 59 | | | | 5,784 | 59 | | | |
| D. rosarium | E | 9,397 | 3 | 3,132 | 197 | 0.0001 | 17,480 | 3 | 5,827 | 342 | 0.0001 |
| | R | 890 | 56 | 16 | | | 955 | 56 | 17 | | |
| | T | 10,287 | 59 | | | | 18,435 | 59 | | | |
| D. discoideum | E | 29 | 3 | 10 | 4.2 | 0.0101 | 11 | 3 | 4 | 1.3 | 0.277 |
| | R | 122 | 56 | 2 | | | 155 | 56 | 3 | | |
| | T | 151 | 59 | | | | 166 | 59 | | | |
| P. pallidum | E | 636 | 3 | 212 | 6.7 | 0.001 | 39 | 3 | 13 | 1.3 | 0.269 |
| | R | 1,773 | 56 | 32 | | | 536 | 56 | 10 | | |
| | T | 2,410 | 59 | | | | 574 | 59 | | | |

[a] E = explained, R = residual, T = total.

extracellular preparations (Turner, 1971), some of which are known to play important functions in symbiotic relationships between fungi and other plants (Cooke, 1977). The identity of the extracellular substance(s) responsible for the results presented here is unknown. Unfortunately, the stalkless strain was lost in laboratory culture before this problem could be addressed. We suspect that the product may be cyclic-AMP, the chemotactic agent responsible for normal slime mold aggregation and morphogenesis. Cyclic-AMP is known to induce stalk formation in stalkless mutants of *D. discoideum* (Bonner, 1970; Chia, 1975; Town, Gross and Kay, 1976; Kay, Garrod and Tilly, 1978). The three species in which an acceleration in the time required to fruit was observed are species for which the chemotactic agent is known to be cAMP (Bonner et al., 1972). In the two cases in which no such acceleration was noted, one, *D. discoideum,* is a cAMP species and the other, *P. pallidum,* is not (Bonner et al., 1972). The failure of *D. discoideum* to fruit quickly in the presence of the fungus, however, does not negate the possibility that the fungal product responsible is cAMP. In contrast to the other species tested, the *D. discoideum* strain was a laboratory strain which has been in near continuous culture for over 45 yr. It is typical for such strains to fruit far more rapidly than field isolates (see Fig. 1). It is likely that this laboratory culture has been selected over the years to display its maximum capacity for increase. This issue can be at least partially addressed by further experimentation with artificially created mutants.

The collection of this stalkless variant from a field population does not necessarily indicate a significant ecological interaction between the slime mold and the fungus. This slime mold variant may have simply undergone a mutation to a stalkless form that was fortuitously collected before selection eliminated it in nature. This explanation, however, seems unlikely. The rate of mutation to such forms in laboratory cultures is on the order of $10^{-4}$ to $10^{-8}$ per generation (Demerec, 1946; Sussman and Sussman, 1953) and in a collection of only ten field isolates, the probability of such a collection seems exceedingly low. A more parsimonious explanation is that after the initial mutation to the stalkless condition, this strain germinated in the presence of the fungus and was dispersed with it to new locations by soil invertebrates. The fruiting bodies of the slime mold are intimately interwoven within the mycelium of the fungus, thus, passing soil invertebrates are unlikely to disperse one form without the other. This interaction, rather than representing a fortuitous collection event, likely attests to the subtlety of ecological interactions in the soil.

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