MATING TYPES IN CELLULAR SLIME MOLDS

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SUMMARY

We have discovered mating types in three species of cellular slime mold. One of these species is Dictyostelium discoideum, hitherto the subject of extensive biochemical investigations, and now amenable to genetic studies.

The cellular slime molds have been used by many workers to investigate the biochemistry of cell differentiation. Unfortunately, progress in this direction has been hindered by the absence of a practical system of genetic analysis. Raper et al. (2), however, have recently reported the existence of a true sexual phase in the life cycle of a cellular slime mold, Polysphondylium violaceum. Their cytological evidence shows a zygote forms during development of the macrocyst, a multicellular, thick-walled structure, and that meiosis occurs before germination of the macrocyst.

Very few of the many strains of cellular slime molds which have been isolated from nature actually form macrocysts (3,4). This fact led us to suppose that these might be rare homothallic strains of basically heterothallic organisms. We report here the results of a search for mating types in five species of cellular slime molds.

Methods

Cellular slime molds were isolated from soils of woodlands

near Newark, Delaware using the technique of Cavender and Raper (5), except that Cerophyll agar (6) was routinely used as a substrate instead of hay agar. A total of 11 Dictyostelium discoideum, 8 D. purpureum, 25 D. mucoroides, 10 Polysphondylium violaceum and 9 P. pallidum clones were isolated. Each isolate was tested individually for its ability to make macrocysts by growing it on Escherichia coli on a cerophyll plate, then flooding the plate with sterile Bonner's salt solution (7) and incubating for 3-4 days in the dark (2,4). These procedures are standard. Most isolates are self sterile when tested in this way. Self-sterile isolates of each species were then mixed in all possible pairs and again tested for macrocyst formation. These mixtures were made in two ways: by inoculating spores of the two isolates together on E. coli growing on cerophyll agar as described above; and by mixing in depression slides amebae which had been pregrown in flask culture by Gerisch's (8) method. In the latter case, approximately 2×10^6 amebae of each member of the pair were mixed in 0.25 ml of Bonner's salt solution. The two methods usually work equally well. The mixtures were scored for the presence of macrocysts after 3-4 days in the dark at 22-27°C. Two or more independent repeats of each experiment were performed.

Results and Discussion

Dictyostelium purpureum. Results of mixtures with this species are shown in Table 1. Macrocysts form only in mixtures of DEP2 with any of isolates DEP1, DEP6 or DEP7. The 4 remaining isolates form no macrocysts alone or in any combination.

Since the formation of macrocysts is the result of a sexual union, these strains may be classified in two mating types:

Table 1. Formation of macrocysts in mixtures of D. purpureum isolates

	DEP2	DEP1	DEP6	DEP7
DEP2	0	⊕	+	+
DEP1		0	0	0
DEP6			0	0
DEP7				0

0 - no macrocysts observed
⊕ - macrocysts sparse or irregular
+ - macrocysts numerous

Isolates DEP3, DEP4, DEP5, and DEP8 made no macrocysts in any combination.

mating type I (isolate DEP2) and II (isolates DEP1, DEP6, DEP7).

D. discoideum. The pattern of interaction among 11 isolates of this species indicates that two mating types exist here, as in D. purpureum. One isolate, De5, forms macrocysts only with each of 6 others (De1, De3, De6, De7, De11, De8). Three other isolates (De2, De4, De9) never make macrocysts. These macrocysts are much larger than those of D. purpureum, but are of similar internal structure (Fig. 1).

Shortly after we had communicated our early (and in fact, erroneous) results to Professor K. B. Raper, similar experiments with D. discoideum were begun in his laboratory. He has very kindly sent us his findings, which extend ours in indicating that a third mating type may be present in D. discoideum (9).

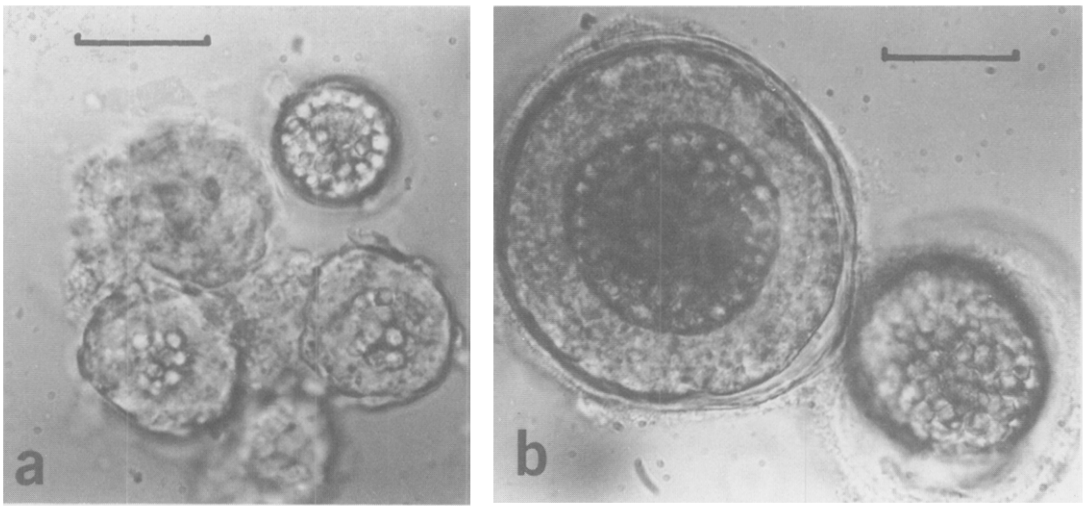

Fig. 1. Macrocysts.
a. After four days in D. purpureum (DEP1xDEP2).
b. After four days in D. discoideum (De1xDe5).
Scale = 30 μ.

D. mucoroides. Exhaustive studies with this species have so far failed to demonstrate mating types. Five of 25 isolates form abundant macrocysts in 3 days when grown alone. Nineteen make macrocysts more or less irregularly, sparsely, and slowly when grown in isolation and are not induced to quicker or more extensive development in any combination (Table 2). One strain has never made macrocysts. Further, the more nearly self-sterile isolates inhibit macrocyst formation when mixed with self-fertile isolates, and a hierarchy exists with respect to this inhibitory ability (Table 2). Details of this interaction will be published elsewhere.

Polysphondylium violaceum. In this species a pair of mating types were found in each of two groups of isolates (Table 3). No cross-reactions occur between members of different groups. This pattern of reactions indicates that the single morphological species P. violaceum consists of two syngens, or breeding species.

Table 2

Formation of macrocysts in mixtures of *D. mucoroides* isolates

Group	a	b	c	d	e	f	g	h	i
Isolate No.	12	4	3	9	2	25	21	22	20
12	+	+	+	+	+	+	+	+	+
4		⊕	+	+	+	+	+	+	0
3			+	+	+	+	+	0	0
9				+	+	+	0	0	0
2					+	0	⊕	0	0
25						0	0	0	0
21							0	0	0
22								0	0
20									0

group a. - isolates 1, 10, 12, 14, 23, 24

group b. - isolates 4, 5, 6, 19

group c. - isolates 3, 7, 8

group d. - isolate 9

group e. - isolate 2

group f. - isolates 18, 25

group g. - isolates 15, 16, 21

group h. - isolates 17, 22

group i. - isolates 13, 20

Mating may occur within each syngen, but not between syngens, as in the case of *Paramecium* (10). This situation is being investigated more fully.

P. pallidum. No macrocysts have yet been seen in any combination of isolates in this species.

Table 3

Formation of macrocysts in mixtures of P. violaceum isolates

	DF1	DF2	DF4	DF5	DF7	DF6	MAC1	MAC2
DF1	0	+	⊕	+	+	0	0	0
DF2		0	0	0	0	0	0	0
DF4			0	0	0	0	0	0
DF5				0	0	0	0	0
DF7					0	0	0	0
DF6						0	+	+
MAC1							0	0
MAC2								0

Symbols as in Table 1.
Isolates DF3 and MAC3 make no macrocysts in any combination.

Currently under study is the frequency of genetic exchange in nature. That breeding in natural conditions does occur is suggested by the fact that strain De5 of mating type I of D. discoideum was isolated from the same square meter of forest floor as strains De1, De6, De7, De9 and De11 of mating type II. Also, strains DF1 and DF2 of P. violaceum and of opposite mating type were isolated from a single 10 gram soil sample.

We do not yet know the manner of mating type inheritance in these organisms. Genetic control of mating types in fungi and protozoa ranges from single locus - one allele systems to multiple loci - multiple allele systems (10,11). Our results with D. discoideum, D. purpureum, and P. violaceum are consistent with control by 2 alleles at a single locus within each syngen. The large proportion of isolates that do not make macrocysts in any

combination suggests that this simple interpretation is incomplete, however. We continue investigations on this point.

The cellular slime molds, and particularly D. discoideum, have become a major tool in the biochemical analysis of development. Until now, genetic analysis in this organism has been possible only by means of a most cumbersome parasexual system (1). The finding of a complete sexual system in the cellular slime molds will be of great practical value in permitting genetic investigation of cell differentiation.

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