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Mating Types and Macrocyst Formation in *Dictyostelium discoideum*

(cellular slime molds/sexuality/self-incompatibility/variation)

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ABSTRACT **Strains of *Dictyostelium discoideum* were paired in various combinations. Certain pairings resulted in the production of macrocysts, which are thought to be the sexual stage in the cellular slime molds. One self-compatible strain was found. Other strains produced macrocysts only when paired with an alternate mating type. Two strains were found to form macrocysts when paired with either mating type but not alone or with each other. Several strains did not form macrocysts under any circumstances. Strains were graded according to their mating competency based on the number of macrocysts produced relative to the aggregated myxamoebae observed in the pairings.**

Macrocysts were first described by Blaskovics and Raper (1) as heavy-walled structures of multicellular origin that developed after cell aggregation in some isolates of *Dictyostelium mucoroides* and *D. minutum* and represented a morphogenetic phase alternative to sorocarp formation. Whether formed singly or in clusters, they noted that development began at the center of each incipient macrocyst and proceeded outward, the constituent myxamoebae seemingly being transformed into cells with semirigid walls, termed endocytes. They speculated that the myxamoebae became walled since they had strongly refractile boundaries. Later, these endocytes disappeared and the contents of the cyst became homogeneous.

Subsequent ultrastructural studies have shown that a large cell, termed a cytophagic cell (2) or a giant cell (3), arises in the population and progressively engulfs the surrounding myxamoebae, which, once enclosed in vacuoles, represent the endocytes. Before this, the associated myxamoebae form a loose, fibrillar wall (primary wall) around the nascent cyst similar to the slime sheath that surrounds the pseudoplasmodium and the developing sorocarp. After engulfment of the myxamoebae is complete, the giant cell secretes a rigid, cellulosic wall (secondary wall) and later a thicker, more flexible, trilaminar wall. Coincident with the formation of this tertiary wall the endocytes are reduced to small fragments and ultimately disappear, giving the cyst a homogeneous appearance. After a variable period of dormancy, depending upon the species, the single large protoplast of the macrocyst undergoes progressive cleavage to give rise to trophic amoebae that emerge upon germination (4).

Factors affecting the formation and germination of macrocysts have been studied (5, 6). In general, macrocyst formation is favored by darkness, very wet cultural conditions, temperature at the higher range of tolerance of the organism, and the absence of phosphate in the medium. The opposite conditions favor sorocarp production. Macrocysts that have undergone the appropriate period of dormancy can be induced to germinate by placing them in the light at low temperature (15–20°).

There is strong evidence to indicate that the macrocyst represents the true sexual stage in the cellular slime molds. In *Polysphondylium violaceum* (3), the giant cell has been shown to be at first binucleate but to be uninucleate by the time it begins to engulf myxamoebae. It is thought that these conditions result from the fusion of two myxamoebae followed by nuclear fusion. Later in the development of the macrocyst, structures resembling synaptonemal complexes, indicators of meiosis, were observed in the nucleus, after which the cyst became multinucleate. Additionally, random assortment of markers from germinated cysts has been shown (unpublished), although evidence of crossing-over is still lacking.

Previous to this report, macrocysts were known to occur in several strains of *Dictyostelium mucoroides*, *D. purpureum*, and *D. minutum* and in single strains of *D. discoideum* and *P. violaceum* (5). Assuming that macrocysts are the sexual stage of the cellular slime molds, we investigated the possibility that mating types exist. The results of such a study of *D. discoideum* are presented here.

MATERIALS AND METHODS

The strains of *D. discoideum* tested were reactivated from lyophilized spores and maintained in association with *Escherichia coli* B/r on 0.1 LP agar at pH 6 (5). The test medium was an unbuffered form of the maintenance medium. Tests of mating competence were performed by placing 5 ml of an aqueous suspension of spores of two selected strains plus the food organism on the surface of the test medium in a petri dish. These paired cultures were then incubated in the dark at 22.5° for 4 days, after which they were scored. They were then incubated three additional days under the same conditions and scored again. Each cross was performed in triplicate and each strain was tested singly for macrocyst formation. Of the strains tested, 16 were from Wisconsin, eight from Illinois, two from North Carolina, two from Vermont, and one each from Virginia, New York, Alabama, and Mexico.

Diffusion studies were done in a Belco parabiotic chamber with a Millipore filter barrier with a pore size of 0.45 μm. For such liquid culture, myxamoebae and the food organism were grown in the medium of Sussman (7).

RESULTS

The results of the various pairings are shown in Tables 1 and 2. Only one strain (AC-4) was found to be self-compatible; that is, macrocysts could develop from a population derived from a single spore as has been shown (5). The self-incompati-

TABLE 1. *Macrocyst formation among paired isolates of Dictyostelium discoideum**

	NC-4	WS 582	WS 583	WS 584	WS 5-1	WS 7	WS 10	WS 11-1	WS 28-1	WS 57-6	WS 112b
NC-4	−	+++	−	−	−	+++	−	+	++	−	++
	WS 582	−	+++	−	+++	−	+++	−	−	−	++
		WS 583	−	−	−	+++	−	+	++	−	+
			WS 584	−	−	−	−	−	−	−	−
				WS 5-1	−	++	−	+	+	−	+
					WS 7	−	+	−	−	−	+
						WS 10	−	+	+	−	+
							WS 11-1	−	−	−	−
								WS 28-1	−	−	−
									WS 57-6	−	−
										WS 112b	−

Key: −, no macrocysts; +, less than four macrocysts per aggregate; ++, more than four macrocysts per aggregate and generally on the surface; +++, aggregate entirely converted to macrocysts.

* Results obtained with 11 selected strains paired in all possible combinations.

ble strains fell into three groups. One group consists of those strains that will form macrocysts when paired with an alternate compatible strain. 24 of the strains fell into this category. These strains segregate into two mating types, 16 being type A_1* and eight being type A_2†. A second group of five strains (WS 57-6, WS 269a, WS 380B, WS 526, and WS 584) will not form macrocysts under any of the circumstances investigated. Two self-incompatible strains (WS 216-2 and WS 112b) comprise the third group, which when paired with either type A_1 or A_2 will form macrocysts. When paired with each other no cysts are formed.

The crosses were scored in the following manner: no macrocysts formed (−); fewer than four macrocysts per submerged cell aggregate (+); more than four macrocysts per submerged aggregate, and these generally formed on the surface of the aggregate, but not exclusively in that location (++); submerged aggregates completely converted to macrocysts or nearly so (+++). No crosses were performed with strain AC-4, since it is self-compatible. Based on the mating reactions observed, the various strain were graded according to their sexual competency: strains NC-4, WS 582, WS 7, WS 583, WS 472, V-12, WS 585, WS 195-6, and WS 205 were considered to be fully competent; strains WS 5-1 and WS 10 were called partially competent; the remainder of those that participated in successful crosses were considered minimally competent.

In order to obtain some indication whether cell contact between opposite mating types was required or whether a diffusible substance from one strain could induce macrocyst formation in the opposite strain, cells of the two types were placed in a parabiotic chamber, the types being separated by a barrier that provided free movement of the liquid growth medium but prevented the passage of myxamoebae. Under these conditions, no macrocysts were formed. In a second experiment, cells of one mating type were placed in the filter-sterilized medium that had supported growth of the opposite mating type for 48 hr. No macrocysts were formed.

Among the 32 cultures of *Dictyostelium discoideum* included in our tests are two strains of special interest, NC-4 and V-12. The former, isolated in 1933, represents the type of the species

TABLE 2. *Results of pairings of 20 additional strains of Dictyostelium discoideum against two known mating types*

	NC-4 (A_1)	WS 582 (A_2)	Self
WS 285d	-	++	-
WS 369B	-	+	-
WS 472	-	+++	-
WS 567	++	-	-
WS 568	-	++	-
WS 569	++	-	-
WS 195-6	-	+++	-
WS 200-3	-	++	-
WS 201-3	-	++	-
WS 203-1	-	++	-
WS 205-4	-	+++	-
WS 206-6	-	++	-
WS 209-5	++	-	-
WS 216-2	++	++	-
WS 269a	-	-	-
WS 380 B	-	-	-
WS 390 B	-	++	-
WS 526	-	-	-
V-12	+++	-	-
WS 585	-	+++	-

See Table 1 for key.

* Type A_1: NC-4, WS 5-1, WS 10, WS 285d, WS 369B, WS 472, WS 583, WS 568, WS 585, WS 195-6, WS 200-3, WS 201-3, WS 203-1, WS 205-4, WS 206-6, WS 390B.

† Type A_2: V-12, WS 582, WS 7, WS 11-1, WS 28-1, WS 567, WS 569, WS 209-5.

and is the strain most often used for a wide variety of investigations in our laboratories and elsewhere; the latter, isolated in 1937, has been studied quite extensively by Dr. G. Gerisch and his associates in Tubingen since it was sent to him in 1957. It is considered noteworthy that these two historic strains are both strongly reactive and are of opposite mating type.

DISCUSSION

It is premature to refer to this mating system as heterothallism until the genetics of the mating system is known; however, the evidence presented suggests that a single locus, two-allele system exists. The existence of the two self-incompatible strains capable of mating with either type may represent a situation comparable to that in the water mold *Achlya*, where it was found that certain self-sterile strains could behave as either sex when paired with the opposite sex (8). It is assumed that these strains carry both mating factors but that neither is very strong. This ambivalence in *D. discoideum* could be due to partial or complete chromosomal duplication or to genic duplication on the same chromosome. The duplication may not be total and, therefore, the genetic information available is insufficient to make them self-compatible: however, the presence of one or the other of the mating types may trigger a mating reaction, although in the strains tested the reaction was never very strong. These ambisexual strains may also indicate that a multiallelic system exists similar to that found in some basidiomycetes: that is, that no two specific alleles are required but rather that the alleles need only be different from one another to accomplish a successful mating.

The reduced mating capability observed in some of the crosses may be due to factors other than mating competency. An absence of common aggregation between two strains could also cause such a reaction. If two opposite strains are unable to coaggregate, this would significantly reduce the possibility of mating since the two types would not be in close enough proximity to mate. This could explain why the macrocysts are only found on the surface of the submerged aggregates in poor matings and not inside as they are in the best matings. These surface cysts could arise by chance contact of cells of the opposite mating types, such chances being small if they aggregate separately.

A second factor, other than the mating factor, that could account for the poor matings is competitive inhibition. It has been shown in intergeneric and interspecific pairings that spore germination of one species can be inhibited by the other (9, 10). Horn (11) has shown that in mixed cultures some species are better equipped to compete for the available food supply and that this advantage is not necessarily related to the growth rate of the organisms when cultured separately. It is possible that one organism could inhibit the growth of another, even to its exclusion. These competitive reactions may also occur in interspecific pairings and thus prevent good responses between opposite mating types, although this has not been demonstrated. Additionally, disparate growth rates could give the same result. If these factors, or the inability to coaggregate, are primarily responsible for the poor mating reactions in some of the crosses, then rating the strains according to sexual competency may be invalid.

We have concluded from preliminary experiments that the mating reaction cannot be solely dependent on a diffusible substance since no macrocysts were formed in the diffusion studies. It is probably necessary for cell contact to occur between the mating types before macrocysts can be formed.

More definitive information about the mechanisms involved in macrocyst production in *D. discoideum* must await the successful germination of the macrocysts produced in this study. Current information suggests that a prolonged rest period may be required to secure germination, as has been found in some other species (6). Even so, if this proves to be a workable system it will greatly facilitate genetic studies of *D. discoideum*, since one could be assured that every macrocyst was the product of a heterozygous fusion. Genetic studies to date have had to rely on the occasional parasexual fusions that occur in populations of myxamoebae (e.g., ref. 12), but this is a more circuitous and less efficient means of genetic recombination.

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