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Short Communication

Macrocyst formation in three dictyostelid species, *Dictyostelium monochasioides*, *Polysphondylium candidum*, and *P. pseudo-candidum*

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Macrocyst formation in the sexual cycle was found in three dictyostelid species: *Dictyostelium monochasioides*, *Polysphondylium candidum*, and *P. pseudo-candidum*. Mating tests suggested that *D. monochasioides* and *P. pseudo-candidum* were heterothallic and *P. candidum* was homothallic. The primary walls of macrocysts had partially or fully degenerated, while the inner walls, believed to be tertiary walls, showed an undulate structure.

Key Words——cellular slime molds; dictyostelids; macrocyst; mating system; taxonomy.

About 70 species of dictyostelids or cellular slime molds belonging to the Dictyosteliomycota have been hitherto described. They are classified on the basis of the morphological characters of fruiting bodies made in the asexual stage. Aspects of sexual reproduction have been sparingly used in their taxonomy (Raper, 1984). We have investigated macrocyst formation, representing the sexual stage, in order to help establish the biological species concept in the taxonomy of dictyostelids.

Macrocysts have three surrounding walls: the primary outermost wall is loose and fibrillar in structure, the secondary wall is more finely fibrillar and very rigid, and the tertiary wall is trilaminar and pliable (Erdos et al., 1972, 1973a). Macrocyst formation is known only in 11 species. Five are heterothallic: *Dictyostelium giganteum* Singh (Erdos et al., 1975), *D. lacteum* van Tieghem (J. C. Cavender, personal communication), *D. mexicanum* Cavender, Worley et Raper (Raper, 1984), *D. rosarium* Raper et Cavender (Chang and Raper, 1981), and *Polysphondylium tenuissimum* Hagiwara (Hagiwara, 1989). Four are both heterothallic and homothallic: *D. discoideum* Raper (Erdos et al., 1973b), *D. purpureum* Olive (Clark et al., 1973; Nickerson and Raper, 1973), *P. pallidum* Olive (Eisenberg and Francis, 1977), and *P. violaceum* Brefeld (Clark et al., 1973; Nickerson and Raper, 1973). Two are homothallic: *D. minutum* Raper and *D. mucoroides* Brefeld (Nickerson and Raper, 1973).

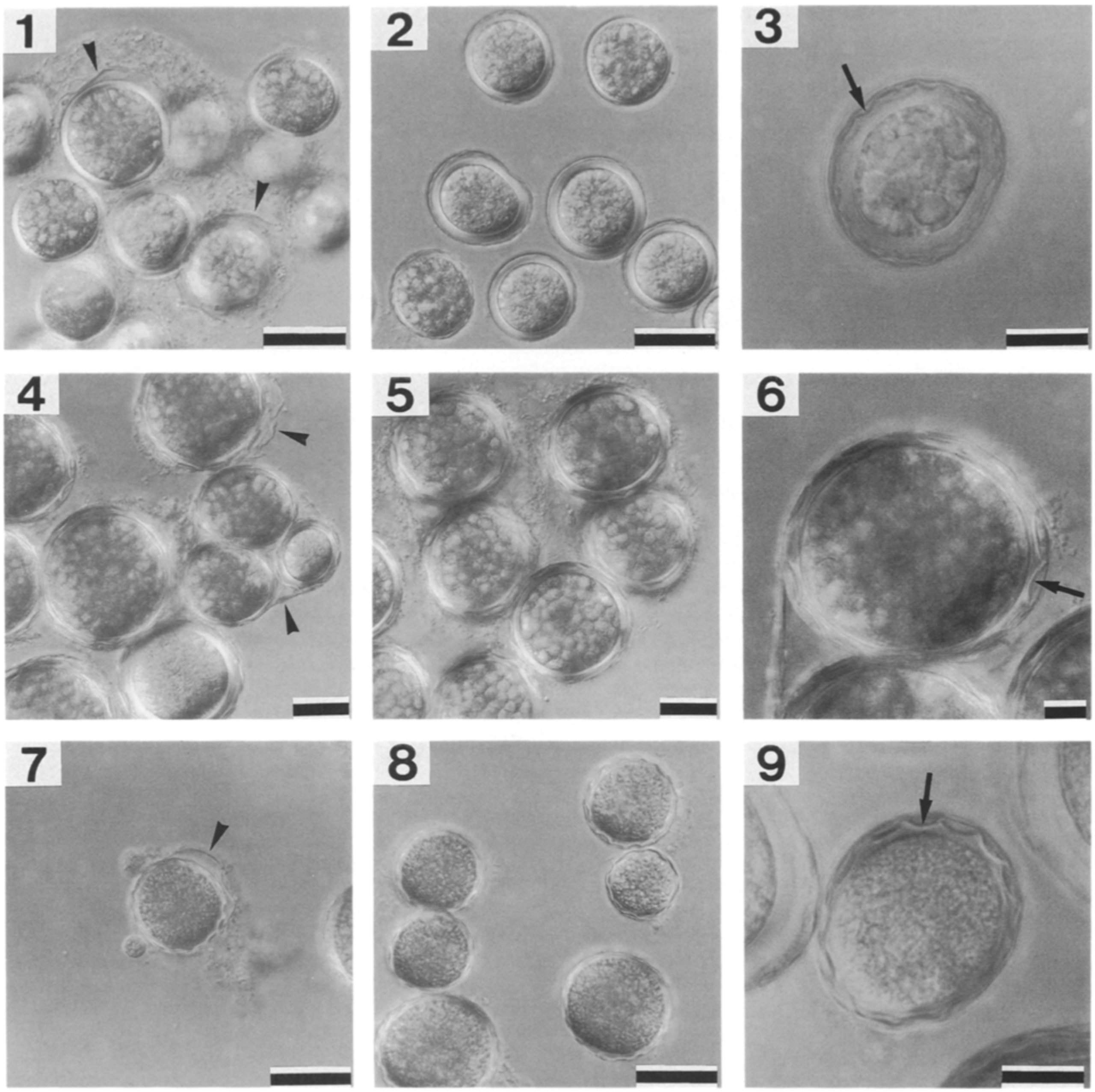
In the course of our investigation, macrocyst formation was discovered in *D. monochasioides* Hagiwara, *P. candidum* Hagiwara, and *P. pseudo-candidum* Hagiwara. We report here the mating systems of these species and the morphological features of their macrocysts.

All the strains examined were maintained on non-nutrient agar with *Escherichia coli* as the food source at 20°C. To test the mating competence, spores of each pair of strains were inoculated into small colonies of *E. coli* on 0.1% lactose/proteose peptone agar plates (6 cm in diameter). For underwater cultures, 6 ml of sterile Bonner's salt solution (Bonner, 1947) was added to each plate after the spores had germinated. Cultures were incubated at 25°C in the dark and observed after 25 d incubation. Micrographs were taken using a microscope (Zeiss Axiophoto) equipped with Nomarski differential interference contrast optics. The diameters of macrocysts were measured excluding the thickness of the primary wall. The tester strains for mating tests had been subcultured in the Department of Botany, National Science Museum, Tsukuba, Japan.

Nineteen strains isolated from soil in Okinawa Pref., Japan, in June 1994, were morphologically identified as *D. monochasioides*. The preliminary mating test showed that two strains, IrU202 and IrU302, were cross-compatible. The former was arbitrally assigned to mating type A1 and the latter to A2. These strains were used as the testers for mating tests. Seventeen other strains produced macrocysts with one or the other of the two testers (Table 1). Therefore, all the compatible strains segregated into two mating types. The macrocysts were 15 to 40 μm in diam. Their primary walls had partially (Fig. 1) or fully (Fig. 2) degenerated, and an undulate structure was occasionally detected in an inner wall (Fig. 3).

Three strains identified morphologically as *P. candidum*, IK53, IT22 and IT42, were isolated from soil in Ibaraki Pref., and one strain, CT21, was isolated from soil in Chiba Pref., Japan, in April 1994. Mating tests with various combinations revealed that two strains, IT42 and CT21, produced macrocysts by themselves, viz., they were self-compatible. The macrocysts were 20 to 68 μm in diam. Their primary walls were partially (Fig. 4) or considerably (Fig. 5) degraded. An undulate structure was constantly detected in an inner wall (Fig. 6).

Eleven strains isolated from soil in Okinawa Pref., Japan, in June 1994, were morphologically identified as *P. pseudo-candidum*. The preliminary mating test showed that two strains, IrN43 (mating type A1) and



Figs. 1–3. Macrocysts of *Dictyostelium monochasioides* formed during 25 d incubation.
1. Primary walls (arrowheads) partially remaining. The macrocysts were formed by crossing strains IrU202 and IrU302. 2. No primary walls. The macrocysts were formed by crossing strains IsB52 and IsF72. 3. Undulate structure (arrow) in an inner wall. The macrocyst was formed by crossing strains IrU202 and IrU302. Bars: 1, 2=25 μm; 3=10 μm.

Figs. 4–6. Macrocysts of *Polysphondylium candidum* formed by strain CT21 itself during 25 d incubation.
4. Primary walls (arrowheads) partially remaining. 5. Primary walls considerably degraded. 6. Undulate structure (arrow) in an inner wall. Bars: 4, 5=25 μm; 6=10 μm.

Figs. 7–9. Macrocysts of *Polysphondylium pseudo-candidum* formed by crossing strains IrN 43 and IrO32 during 25 d incubation.
7. Primary wall (arrowhead) partially remaining. 8. No primary walls. 9. Undulate structure (arrow) in an inner wall. Bars: 7, 8=25 μm; 9=10 μm.

Table 1. Results of pairings of nineteen strains of *Dictyostelium monochasioides* against two testers.

	IrU202 (A1)	IrU302 (A2)
IrS72	−	++
IrU61	−	++
IrU92	−	++
IrU113	−	+
IrU193	−	++
IrU202	−	++
IrU274	−	++
IsB111	−	++
IsF72	−	++
IrN33	++	−
IrS54	++	−
IrU32	++	−
IrU52	++	−
IrU71	+	−
IrU81	+	−
IrU175	++	−
IrU212	++	−
IrU302	++	−
IsB52	++	−

Key: −, no macrocysts observed; +, macrocysts sparse; ++, macrocysts numerous.

IrO32 (A2), were suitable as the testers for mating tests. Seven of nine strains examined produced macrocysts with one or the other of the two testers (Table 2). Therefore, all the compatible strains were classified in two mating types. The macrocysts were 19 to 50 μm in diam. Their primary walls had partially (Fig. 7) or fully (Fig. 8) degenerated, and an undulate structure was often found in an inner wall (Fig. 9).

The mating tests suggested that *D. monochasioides* and *P. pseudo-candidum* were heterothallic and *P. candidum* was homothallic. In *P. pseudo-candidum*, however, two strains were not cross-compatible with the testers and in *P. candidum* three strains were neither cross-compatible nor self-compatible. It is possible that these incompatible strains are asexual or other syngens, or produce macrocysts under better conditions. Syngens have been reported for *P. pallidum* (Eisenberg and Francis, 1977) and *P. violaceum* (Clark, 1974).

In the macrocysts of all the species examined, the primary walls had partially or fully degenerated. This was the first clear demonstration of such a phenomenon. An inner wall showed an undulate structure, which has previously been reported only in the macrocysts of *P. violaceum* (Erdos et al., 1972). Ultrastructural study of the macrocysts of *P. violaceum* has shown that the tertiary wall has "an undulating outer boundary" (Erdos et al., 1972). Therefore, the undulate structure detected in our study can be considered to be also formed on the tertiary wall.

Table 2. Results of pairings of nine strains of *Polysphondylium pseudo-candidum* against two testers.

	IrN43 (A1)	IrO32 (A2)
IrN43	−	++
IrU33	−	++
IsB61	−	++
IsBS13	−	++
IrO22	++	−
IrO32	++	−
IsY33	++	−
IrN31	−	−
IrN71	−	−

Key: −, no macrocysts observed; +, macrocysts sparse; ++, macrocysts numerous.

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