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Mating System and Morphology of the Temperate Form of *Dictyostelium purpureum* Olive

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Abstract Thirty-four strains of *Dictyostelium purpureum* isolated from Japan and Korea were paired with mating test strains of the temperate form of *D. purpureum*. Pairings of the 22 strains resulted in macrocyst formation. These heterothallic strains belonged to a single mating group, in which 7 mating types were recognized and a strain of each mating type was compatible with strains of all other mating types. Such a mating system is multipolar and has not been reported in dictyostelids. The 22 strains comprised in the single mating group closely fit the original description of the temperate form except for the spore shape.

Key words: dictyostelids, *Dictyostelium purpureum*, heterothallic, macrocysts, multipolar mating system.

Dictyostelium purpureum Olive is a globally ubiquitous species at lower latitudes (Cavender, 1973; Swanson *et al*., 1999). Japanese isolates of *D. purpureum* were grouped into two forms, the temperate form and the subtropical form, based on a combination of four morphological characters of the sorocarps; namely, sorus color, sorophore length, supporter size and spore shape (Hagiwara, 1992a). The temperate form was originally described by Hagiwara (1989). This form produces large sorocarps with purple sori that are pale to dark, but not blackish. The sorocarps have comparatively small supporters when prostrate and bear elliptical spores with a more than 2.1 ratio of length to width (Hagiwara, 1989, 1992a).

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Dictyostelid species are classified on the basis of the morphological characters of sorocarps produced in the asexual stage. The mating systems of dictyostelids have been previously investigated in order to help establish a biological concept in the taxonomy of dictyostelids (Kawakami & Hagiwara, 1999, 2002; Hagiwara, 1992b, 2003, 2004). The sexual stage of dictyostelids is represented by macrocyst formation. The macrocysts have three surrounding walls. The outermost primary wall is loose and fibrillar in structure, the secondary wall is comparatively rigid because it is rich in cellulose, and the tertiary wall is membranous and pliable (Erdos *et al*., 1972, 1973a; Raper, 1984). It has been shown that there are heterothallic and homothallic mating systems in dictyostelids (Raper, 1984). In *D. purpureum*, homothallic, heterothallic and non-sexual strains have been reported (Clark *et al*., 1973; Nickerson & Raper, 1973).

In the course of our investigation, macrocyst formation was discovered in the temperate form

Table 1. Strains of *Dictyostelium purpureum* examined.

Strain	Date of isolation	Morphology of sorocarps****			
		Sorophore	Sorus	Supporter	Spore
Japanese strains					
FNN 2	Fukushima Pref., Nishigou-mura, 1998, by HH*	M	M	T	M
FNN 6	Fukushima Pref., Nishigou-mura, 1998, by HH	M	M	M	M
Hagiwara 5	Tokyo Pref., Mt. Takao, 1970, by HH	T	T	T	T
IrU 11	Okinawa Pref., Iriomote Isl., 1994, by SK**	T	T	T	T
IrU 21	Okinawa Pref., Iriomote Isl., 1994, by SK	T	T	T	T
IrU 42	Okinawa Pref., Iriomote Isl., 1994, by SK	T	T	T	T
IrU 151	Okinawa Pref., Iriomote Isl., 1994, by SK	M	T	M	M
IrU 174	Okinawa Pref., Iriomote Isl., 1994, by SK	T	T	T	T
IsB 11	Okinawa Pref., Ishigaki Isl., 1994, by SK	S	T	S	M
IY 71	Ibaraki Pref., Mt. Yamizo, 1994, by HH	S	T	T	T
JKS 50	Wakayama Pref., Wakayama-shi, 1998, by HH	S	S	S	S
JKS 56	Wakayama Pref., Wakayama-shi, 1998, by HH	M	T	S	M
JKS 80	Hyogo Pref., Awaji Isl., 1998, by HH	S	T	S	T
JKS 143	Yamaguchi Pref., Oobatake-cho, 1998, by HH	T	T	T	T
JKS 156	Yamaguchi Pref., Yuu-cho, 1998, by HH	T	T	T	T
JKS 161	Hiroshima Pref., Miyajima Isl., 1998, by HH	T	T	T	T
JKS 162	Hiroshima Pref., Miyajima Isl., 1998, by HH	T	T	T	T
JKS 168	Hiroshima Pref., Higashihiroshima-shi, 1998, by HH	M	M	M	M
JKS 274-1	Wakayama Pref., Wakayama-shi, 1999, by HH	T	T	T	T
JKS 274-2	Wakayama Pref., Wakayama-shi, 1999, by HH	S	M	S	S
JKS 275	Wakayama Pref., Wakayama-shi, 1999, by HH	S	S	S	S
OH 7	Okinawa Pref., Hateruma Isl., 1986, by O. Yamazaki	S	S	S	S
TI 49	Tokyo Pref., Mikura Isl., 2002, by HH	T	T	T	T
TI 110	Tokyo Pref., Hachijo Isl., 2002, by HH	S	M	S	M
TI 115	Tokyo Pref., Hachijo Isl., 2002, by HH	S	M	S	S
TI 130	Tokyo Pref., Hachijo Isl., 2002, by HH	T	T	T	T
TNY 14	Tochigi Pref., Nasu-cho, 1999, by HH	T	T	T	T
Korean strains					
KMM 1	Masan, Mt. Muhak, 2000, by JH***	S	S	S	M
KMM 2	Masan, Mt. Muhak, 2000, by JH	M	T	T	M
KMM 4	Masan, Mt. Muhak, 2000, by JH	T	T	T	T
KMM 6	Masan, Mt. Muhak, 2000, by JH	M	M	M	M
KMM 8	Masan, Mt. Muhak, 2000, by JH	M	M	S	M
KMM 9	Masan, Mt. Muhak, 2000, by JH	T	T	T	T
KMM 10	Masan, Mt. Muhak, 2000, by JH	T	T	T	T

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of *D. purpureum*. Its mating system was heterothallic, but different from the heterothallic mating system known in *D. purpureum*. We report here the mating system and new morphological characteristics of the temperate form of *D. purpureum*.

Materials and Methods

Thirty-four strains of *Dictyostelium purpureum* were used in this study (Table 1). They consisted of 27 Japanese strains and 7 Korean strains. All strains were maintained at 20°C on non-nutrient agar with *Escherichia coli* (Migula) Castellani & Chalmers as a food bacterium.

Morphological characters of sorocarps were macroscopically observed under a dissecting microscope (×16). Spores were mounted in distilled water and measured with a Kogaku digital micrometer (×1000). Twenty spores per strain were used for calculating the mean ratio of length to width. The mean ratio is abbreviated as L/W in the following text.

Procedures of the mating test closely followed

Table 2. Four morphological characters of sorocarps used for grouping the strains into the temperate and subtropical forms of *Dictyostelium purpureum*. T: Typical of the temperate form. S: Typical of the subtropical form. M: Mediate between both forms.

	Sorophore	Sorus	Supporter	Spore
T	usually not prostrate, and mostly more than 5 mm in length	purple	small and poorly developed	L/W≧2.1
S	usually prostrate and the upright parts less than 2 mm in length	blackish purple	large and well-developed	L/W≦1.9
M	often prostrate and usually the upright parts more than 2 mm in length	dark purple	usually small, but sometimes large	1.9＜L/W＜2.1

Kawakami & Hagiwara (1999). To test the mating competence, spores of each pair of strains were inoculated into small colonies of *E. coli* on 0.1% lactose/0.1% proteose peptone agar plates. For underwater cultures, 5 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 3 weeks incubation.

Macrocysts were mounted in distilled water and the outside of the secondary wall (Fig. 2E) was measured with a Kogaku digital micrometer (×1000). Fifty macrocysts per pair of strains were used for calculating the mean diameter.

Results and Discussion

All 34 strains were examined for four morphological characters of the sorocarps, namely, the length of the upright parts of the sorophores, color of sori, size of supporters and shape of spores. Each character was typologically grouped into three categories which were indicated by T, S and M as shown in Table 2. Hagiwara 5, IrU 11, IrU 21, IrU 42, IrU 174, JKS 143, JKS 156, JKS 161, JKS 162, JKS 274-1, TI 49, TI 130, TNY 14, KMM 4, KMM 9 and KMM 10 were typical of the temperate form of *Dictyostelium purpureum*, and JKS 50, JKS 275 and OH 7 were typical of the subtropical form (Table 1).

From the 16 typical strains of the temperate form of *D. purpureum*, Japanese strain JKS 156 and Korean strain KMM 4 were selected as mating test strains. Both the test strains were paired with all strains examined. The results of the pairings are shown in Table 3.

JKS 156 produced macrocysts with 7 strains including KMM 4, but KMM 4 made no macrocysts with these 7 strains. On the other hand, KMM 4 made macrocysts with 5 strains including JKS 156, but JKS 156 made no macrocysts with these 5 strains. This fact suggests the mating type of JKS 156 is different from and compatible with the mating type of KMM 4 and that the 7 strains compatible with JKS 156 had the same mating type as KMM 4, while the 5 strains compatible with KMM 4 had the same mating type as JKS 156.

In Table 3, ten strains were shown to make macrocysts with both JKS 156 and KMM 4. This suggests that these strains are homothallic. Then they were tested and shown not to be homothallic. This suggests that these 10 strains are included in a single mating group with JKS 156 and KMM 4, but they had other mating type(s) different from those of JKS 156 and KMM 4. JKS 161 was selected as the third mating test strain and paired with each of the other 9 strains. As a result, 7 strains produced macrocysts with JKS 161 (Table 3). Therefore, the 3 strains incompatible with JKS 161 had the same mating type as JKS 161. Next, one of the 7 strains compatible with JKS 161, Hagiwara 5, was selected as the fourth mating test strain and paired with each of the other 6 strains. By such repeated selections, IrU 42 was left as only one strain compatible with 6

Table 3. Macrocyst formation from pairings of 34 strains of *Dictyostelium purpureum*.

	JKS 156	KMM 4				
IrU 11	+	−				
JKS 143	+	−				
JKS 274-1	+	−				
TI 130	+	−				
KMM 4	+	−				
KMM 6	+	−				
KMM 9	+	−				
IrU 21	−	+				
IY 71	−	+				
JKS 156	−	+				
JKS 162	−	+				
KMM 10	−	+	JKS 161	Hagiwara 5	JKS 168	FNN 6
FNN 2	+	+	−			
FNN 6	+	+	+	+	+	−
Hagiwara 5	+	+	+	−		
IrU 42	+	+	+	+	+	+
IrU 174	+	+	−			
JKS 161	+	+	−			
JKS 168	+	+	+	+	−	
TI 49	+	+	+	−		
TNY 14	+	+	+	+	−	
KMM 2	+	+	+	−		
IrU 151	−	−				
IsB 11	−	−				
JKS 50	−	−				
JKS 56	−	−				
JKS 80	−	−				
JKS 274-2	−	−				
JKS 275	−	−				
OH 7	−	−				
TI 110	−	−				
TI 115	−	−				
KMM 1	−	−				
KMM 8	−	−				

mating test strains (Table 3).

The final results of mating tests are summarized in Table 4. Seven mating types were recognized and a strain from each mating type was compatible with strains of all other mating types. Such a mating system is a variation of the heterothallic mating system and is called multipolar and known to occur in a protozoan *Paramecium bursarina* Focke (Hausmann *et al*., 2003). The temperate form of *D. purpureum* appears to have a heterothallic and multipolar mating system. Although the multipolar mating system has not been reported in dictyostelids, a similar system has been found in *D. discoideum* Raper, *D. giganteum* Singh and *D. rosarium* Raper & Cavender in which 3, 4 and 3 mating types were respectively identified (Erdos *et al*., 1973b, 1975; Chang & Raper, 1981).

In Table 3, twelve strains were shown to make no macrocysts with either JKS 156 or KMM 4. Three of them, JKS 50, JKS 275 and OH 7, clearly belonged to the subtropical form of *D. purpureum* based on morphology (Table 1). Among the other strains, there may be some non-sexual strains of the temperate form. This possibility remains to be investigated.

Twenty-two strains comprising a single mating group of the temperate form of *D. purpureum* in

Table 4. Macrocyst formation from pairings of seven different mating types in the temperate form of *Dictyostelium purpureum*.

	A1 IrU 11	A2 JKS 156	A3 JKS 161	A4 Hagiwara 5	A5 JKS 168	A6 FNN 6	A7 IrU 42
A1 IrU 11	−	+	+	+	+	+	+
A2 JKS 156	+	−	+	+	+	+	+
A3 JKS 161	+	+	−	+	+	+	+
A4 Hagiwara 5	+	+	+	−	+	+	+
A5 JKS 168	+	+	+	+	−	+	+
A6 FNN 6	+	+	+	+	+	−	+
A7 IrU 42	+	+	+	+	+	+	−

Mating type A1: IrU 11, JKS 143, JKS 274-1, TI 130, KMM 4, KMM 6, KMM 9. A2: IrU 21, IY 71, JKS 156, JKS 162, KMM 10. A3: FNN 2, IrU 174, JKS 161. A4: Hagiwara 5, TI 49, KMM 2. A5: JKS 168, TNY 14. A6: FNN 6. A7: IrU 42.

this study were morphologically observed in detail. They closely fit the original description of the temperate form of *D. purpureum* (Hagiwara, 1989), except for the ratio of length to width of spores. Hagiwara (1989) reported the spores were usually 2.1–3.0 times longer than broad (L/W: 2.2–2.8). However, 5 of these 22 strains, FNN 2, FNN 6, JKS 168, KMM 2 and KMM 6, produced comparatively thick spores (Fig. 1C). Therefore, the ratio of length to width of spores was amended as follows: Spores usually 1.8–3.0 times longer than broad (L/W: 1.9–2.8).

Morphological features of macrocysts were appended to the description of the temperate form of *D. purpureum* as follows: Macrocysts usually globose to subglobose or ovoid, but sometimes irregular in shape, mostly 21–64 μm in diameter (Min.: 13 μm. Max.: 99 μm. Range of the mean diameters: 30–44 μm).

The following morphological characters were newly identified in the temperate form of *D. purpureum*: Sorophore tips were fan-shaped or invertedly triangular in a strict sense (Figs. 1D & 1E), though Hagiwara (1989) stated that they were capitate; Dark yellowish brown granules were sometimes found on sorophore tips in JKS 161, KMM 2, KMM 4 and KMM 10 (Fig. 1E & 1G), and such granules have not been reported in other dictyostelids; Cells of supporters were clearly smaller than those of sorophores (Fig. 1H), and this size difference was quite obvious in contrast to that of the subtropical form of *D. purpureum*; Finally, macrocysts sometimes had vacuolated cells in the primary wall (Fig. 2D).

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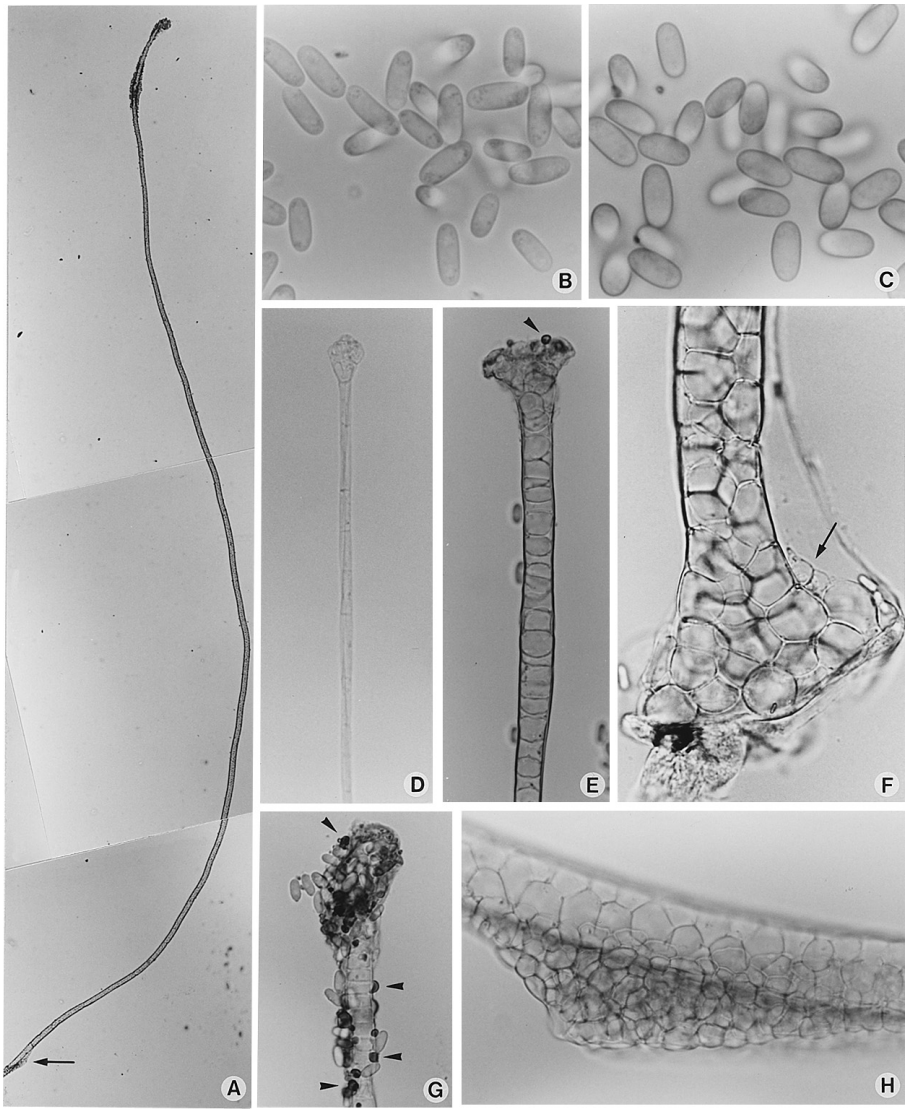

Fig. 1. The temperate form of *Dictyostelium purpureum*. A. Upright part of a prostrate sorophore. Note a small supporter indicated with an arrow. ×45. B. Thin spores, typical of the temperate form. ×1150. C. Comparatively thick spores. ×1150. D. Sorophore tip showing a fan-shaped top. ×460. E. Sorophore tip showing an invertedly triangular top. Note a dark yellowish brown granule indicated with an arrow. ×460. F. Sorophore base expanding conically or with a small disk. Note a few small supporting cells indicated with an arrow. ×460. G. Sorophore tip surrounded by spores and dark yellowish brown granules. Some granules are indicated with arrows. ×460. H. Comparatively large supporter developed on the underside of a prostrate sorophore. Note a clear difference between the cell dimensions of the supporter and sorophore. ×460. Figs. A, D & F, strain Hagiwara 5; Fig. B, strain IrU 174; Fig. C, strain FNN 2; Fig. E, strain KMM 2; Figs. G & H, strain JKS 161.

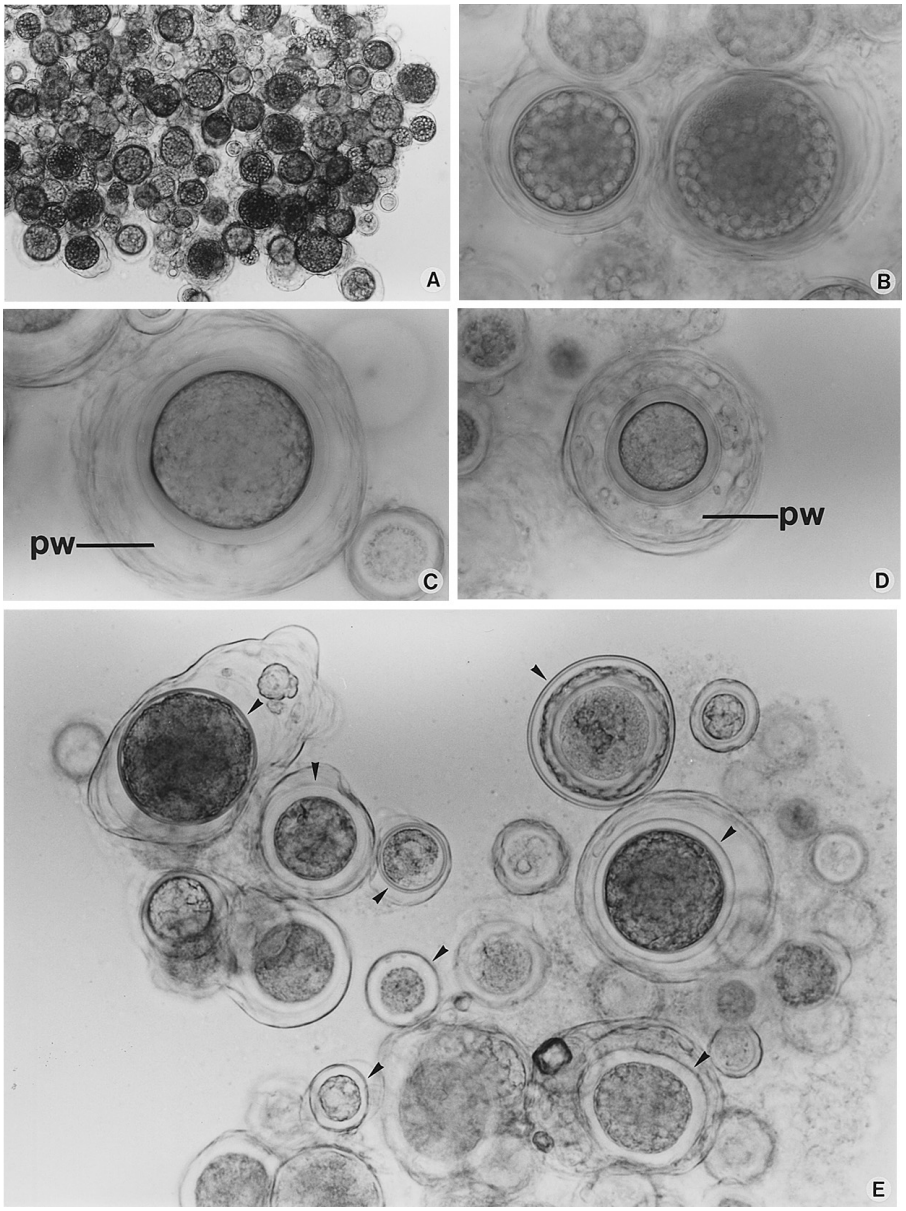

Fig. 2. Macrocysts of the temperate form of *Dictyostelium purpureum*. A. Mass of macrocysts. ×115. B. Endocyte-filled macrocysts. ×460. C. Mature macrocyst. Note a fibrillar thick primary wall (pw). ×460. D. Mature macrocyst. Note many vacuolated cells included in the primary wall (pw). ×460. E. Macrocysts different in morphology and developmental stages. Arrows indicate comparatively sharp outlined secondary walls. ×375. Figs. A–D, a pair of strains Hagiwara 5 and JKS 156; Fig. E, a pair of strains JKS 156 and TI 49.

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