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Preliminary Study of Sexual Segregation in Dictyostelids

Hiromitsu Hagiwara

Department of Botany, National Museum of Nature and Science,
Amakubo 4–1–1, Tsukuba 305–0005, Japan
E-mail: h-hagiwa@kahaku.go.jp

Abstract Mixed cultures comprising pairs of strains belonging to two compatible mating types, tentatively designated *mat A* and *mat a*, were prepared from four heterothallic dictyostelid species: *Dictyostelium discoideum*, *D. giganteum*, *D. purpureum*, and *Polysphondylium pallidum*. Most of the 100 sorocarps randomly sampled from each mixed culture belonged to *mat A*, *mat a*, or a mixture of *mat A* and *mat a*. However, these mixed cultures yielded many deformed pseudoplasmodia. This was probably due to the independent aggregations of amoebae belonging to *mat A* and *mat a* in the pseudoplasmodia since each aggregation bore a small but normal sorocarp. These observations suggest that the dictyostelids employ a sexual segregation mechanism.

Key words: cellular slime molds, deformation of pseudoplasmodia, dictyostelids, intraspecific mixed culture, sexual segregation.

Dictyostelids, or dictyostelid cellular slime molds, are microorganisms living in soils and on rotting plant material. Dictyostelid amoebae are mononuclear and haploid in the vegetative stage. They feed on bacteria and propagate by binary fission. In the asexual cycle, propagated amoebae aggregate and form sorocarps. On the other hand, in the sexual cycle of heterothallic species, a mixture of amoebae belonging to compatible mating types aggregates to giant amoebae (corresponding to zygotes) and subsequently produce macrocysts.

Historically, the first experiment with interspecific mixed cultures of dictyostelids was carried out by Olive (1902). Since then, only a few investigations on mixed cultures have been carried out (see Bonner, 1982; Mizutani *et al.*, 1990; Hagiwara, 1992a). Based on these investigations of mixed cultures, three different phenomena were identified; namely, overlapping of pseudoplasmodia, deformation of pseudoplasmodia, and killing of amoebae (Hagiwara, 1992a).

In the course of a study on interspecific mixed cultures, the deformation of pseudoplasmodia was also found in a mixed culture of two compatible mating strains of *Dictyostelium discoideum* Raper, NC-4 and V-12, used as a control culture (Hagiwara, unpublished). This observation suggests that the phenomenon of pseudoplasmodium deformation not only provides a convenient means to distinguish between different species, but also between different strains of the same species. Therefore, preliminary examinations of intraspecific mixed cultures were carried out in two heterothallic species, *D. discoideum* and *Polysphondylium pallidum* Olive. Two strains per species belonging to opposite mating types were used in order to avoid the possibility of using the same clone. As a result, the deformation of pseudoplasmodia was sometimes found in *D. discoideum* but not in *P. pallidum*. In addition, by examining the mating types of 20 sorocarps randomly sampled from the sorocarps produced in the *D. discoideum* mixed culture, it was suggested that the deformed pseudoplasmodia were due to imperfect segregation between the amoebae of the two mating types (Hagiwara, 1996). This suggestion meant that the deformation of pseudoplasmodia was a phenomenon associated with the sexual segregation in *D. discoideum*. In order to confirm that sexual segregation is common in dictyostelids, more detailed examinations

Table 1. Dictyostelid species and strains examined.

Species and strain	Mating type	Data of strain	Reference
D. discoideum			
NYA62	*mat A*	Nagano Pref., Japan, 2005, by HH*	
WS656	*mat a*	Wisconsin, USA, by M. A. Wallace	
D. giganteum			
NG16	*mat A*	Bagmati, Nepal, 1980, by HH	Hagiwara (1992b); *D. magnum* in Hagiwara (1983)
KPA25	*mat a*	Kathmandu, Nepal, 1988, by HH	Hagiwara (1992b); *D. magnum* in Hagiwara (1990)
D. purpureum			
JKS 274-2	*mat A*	Wakayama Pref., Japan, 1999, by HH	Hagiwara (2005)
JKS 275	*mat a*	Wakayama Pref., Japan, 1999, by HH	Hagiwara (2005)
P. pallidum			
CK8	*mat A*	Chiba Pref., Japan, 1972, by HH	Mizutani *et al.* (1990); *Hagiwara* 226 in Hagiwara (1989)
CK9	*mat a*	Chiba Pref., Japan, 1972, by HH	Mizutani *et al.* (1990); *Hagiwara* 227 in Hagiwara (1989)

* H. Hagiwara

were carried out in the present study.

Materials and Methods

Eight strains of four heterothallic species, *D. discoideum*, *D. giganteum* Singh, *D. purpureum* Olive and *P. pallidum*, were used for the examination of sexual segregation (Table 1). Each species was represented by two sexually-compatible strains or mating types. One mating type was tentatively designated *mat A*, the other *mat a*. All the strains were maintained on non-nutrient agar at 20°C with *Escherichia coli* (Migula) Castellani et Chalmers as the food source.

In order to test mating competence, the spores of each pair of strains were inoculated into small colonies of *E. coli* on 0.1% lactose/0.1% proteose-peptone agar plates (6 cm diameter). For underwater cultures, 5 ml of sterile Bonner's salt solution (Bonner, 1947) was added to each plate after the spores had geminated. The cultures were incubated at 25°C in the dark and were observed after 21 days in order to determine the presence of macrocysts on the plates.

The sequence of the test for sexual segregation is shown in Fig. 1. A suspension of *E. coli* cells together with the spores of both compatible mating types was inoculated onto two plates, one of which was the control. After 7 days at 20°C, 105 sorocarps were randomly sampled from the sorocarps produced on the plates. Each of the 105 sorocarps was transplanted to a set of three plates, which were previously inoculated with spores of one mating type (*mat A*) and *E. coli* cells, spores of the other mating type (*mat a*) and *E. coli* cells, and *E. coli* cells only, respectively. After 1 day at 25°C, all the plates were examined for germination and if there was a plate exhibiting no germination, it and the other two plates in the set of three were discarded. After 20 days at 25°C in the dark underwater, the plates were examined for macrocyst formation. One hundred sets of three plates were selected from the former and were scored for segregation ratios.

For the examination of the deformation of pseudoplasmodia in intraspecific mixed cultures, JKS274-2 (*mat A*) and JKS275 (*mat a*) of *D. purpureum* and CK8 (*mat A*) and CK9 (*mat a*) of *P. pallidum* were used. The spores of 10–15 sorocarps of one strain (*mat A*) were mixed with those of the other strain (*mat a*) in a suspension of *E. coli* cells. The suspension was inoculated onto a non-nutrient agar plate which was incubated at 20°C. The plate was observed under the microscope after 2 days. The process of pseudoplasmodium formation was observed

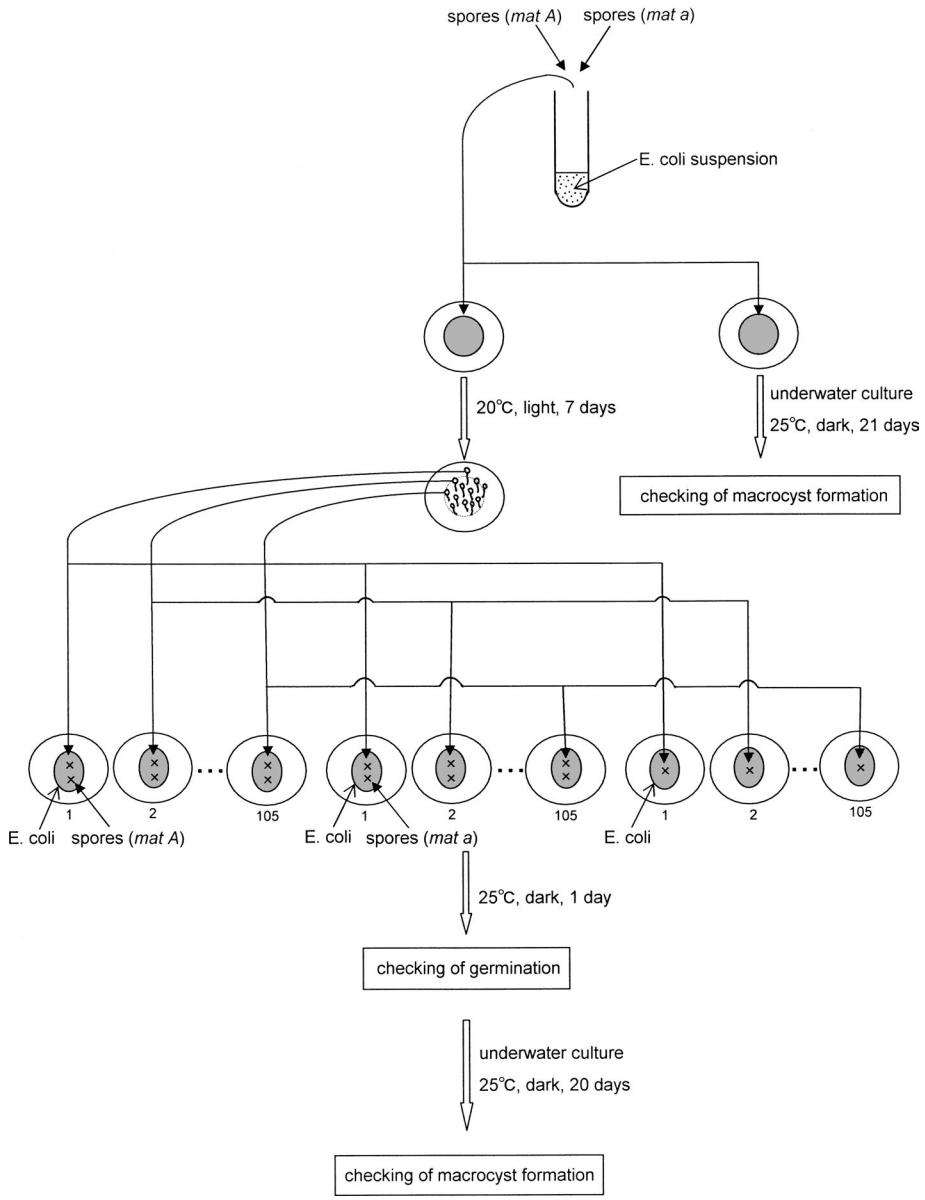

Fig. 1. Flowchart of the test for sexual segregation.

for 2 or 3 days in order to detect the development of deformed pseudoplasmodia.

Results

The results of the tests for sexual segregation are shown in Table 2. In *D. discoideum*, 89 % of the sorocarps independently yielded macrocysts, 9% of sorocarps belonged to the same mating type (*mat a*) as WS656, but no sorocarps belonged to the same mating type (*mat A*) as NYA62. The other 2 sorocarps bore a few macrocyst-like structures in pairing of WS656 and/or by themselves, though they certainly yielded macrocysts in pairing of NYA62. In contrast to *D. discoideum*, in *D. giganteum*, 99 % of the sorocarps belonged to either one mating type (*mat A*) or the other (*mat a*), and only one

Table 2. Results of the tests for sexual segregation in four dictyostelid species.

Species	Mating type	Segregation ratio* (%)
D. discoideum	*mat A*	0
	mat a	9
	Mixture of *mat A* and *mat a*	89
	Unknown**	2
D. giganteum	*mat A*	42
	mat a	57
	Mixture of *mat A* and *mat a*	1
D. purpureum	*mat A*	35
	mat a	35
	Mixture of *mat A* and *mat a*	29
	Unknown	1
P. pallidum	*mat A*	61
	mat a	19
	Mixture of *mat A* and *mat a*	16
	Unknown	4

* Number of sorocarps belonging to *mat A*, *mat a*, or a mixture of *mat A* and *mat a*/Total number of the sorocarps examined ×100.

** A few macrocyst-like structures were detected in one or two plates of a set of three plates, but they could not be identified with macrocysts because they were too small in size.

sorocarp independently yielded macrocysts. In both *D. purpureum* and *P. pallidum*, 100 sorocarps were divided into three groups, namely, one group comprising one mating type (*mat A*), another group comprising the other mating type (*mat a*), and the third group comprising a mixture of both mating types (*mat A* and *mat a*); however, the scattering of their percentages was biased to the same mating type (*mat A*) with CK8 in *P. pallidum* (Table 2).

The intraspecific mixed cultures of both *D. purpureum* and *P. pallidum* yielded deformed pseudoplasmodia (Figs. 2–4). Further, in both *D. purpureum* and *P. pallidum*, small aggregations were often produced along the streams of amoebae in the deformed pseudoplasmodia. However, such aggregations bore small but normal sorocarps.

Discussion

The result of the tests for sexual segregation indicated that most of the sorocarps examined could be classified into one of three groups: *mat A*, *mat a*, or a mixture of *mat A* and *mat a* (Table 2). This observation suggested that sexual segregation occurred in all the species examined, although the segregation was imperfect rather than perfect. Based on these observations, it is suggested that heterothallic dictyostelid species possess a sexual segregation mechanism.

Deformed pseudoplasmodia were found in intraspecific mixed cultures prepared from *D. purpureum* and *P. pallidum* (Figs. 2–4). Such deformation is probably due to the fact that in a pseudoplasmodium, the aggregation of amoebae belonging to one mating type proceeds independently of the aggregation of amoebae belonging to the other mating type. The results of the tests for sexual segregation and those of the examination for intraspecific mixed cultures indicate that dictyostelids employ a sexual segregation mechanism that operates during the pseudoplasmodium formation of mixed amoebae of both compatible mating types.

Dictyostelid amoebae aggregate in response to a self-generated chemical substance that was named “acrasin” (Bonner, 1947). Bonner (1982) suggested that the overlapping of pseudoplasmodia in a mixed culture of different species was due to the totally different chemotactic signals of acrasins. However, the deformation of pseudoplasmodia in an intraspecific mixed culture is not due to the nature of acrasin because a pair of strains belonging to a single species possesses a common chemotactic signal. Deformed pseudoplasmodia have been found in interspecific mixed cultures (Hagiwara, 1992a). They are apparently the same as those found in the intraspecific mixed cultures examined in this study. Therefore, the deformation of pseudoplasmodia in interspecific mixed cultures is probably not due to the different natures of the acrasins in the two species examined.

Recently Kaushik *et al.* (2006) and Mehdiabadi *et al.* (2006) reported that pairwise mixing experiments using 5 strains of *D. giganteum* and 20 strains of *D. purpureum*, respectively, exhibited evidence of strong kin discrimination. However,

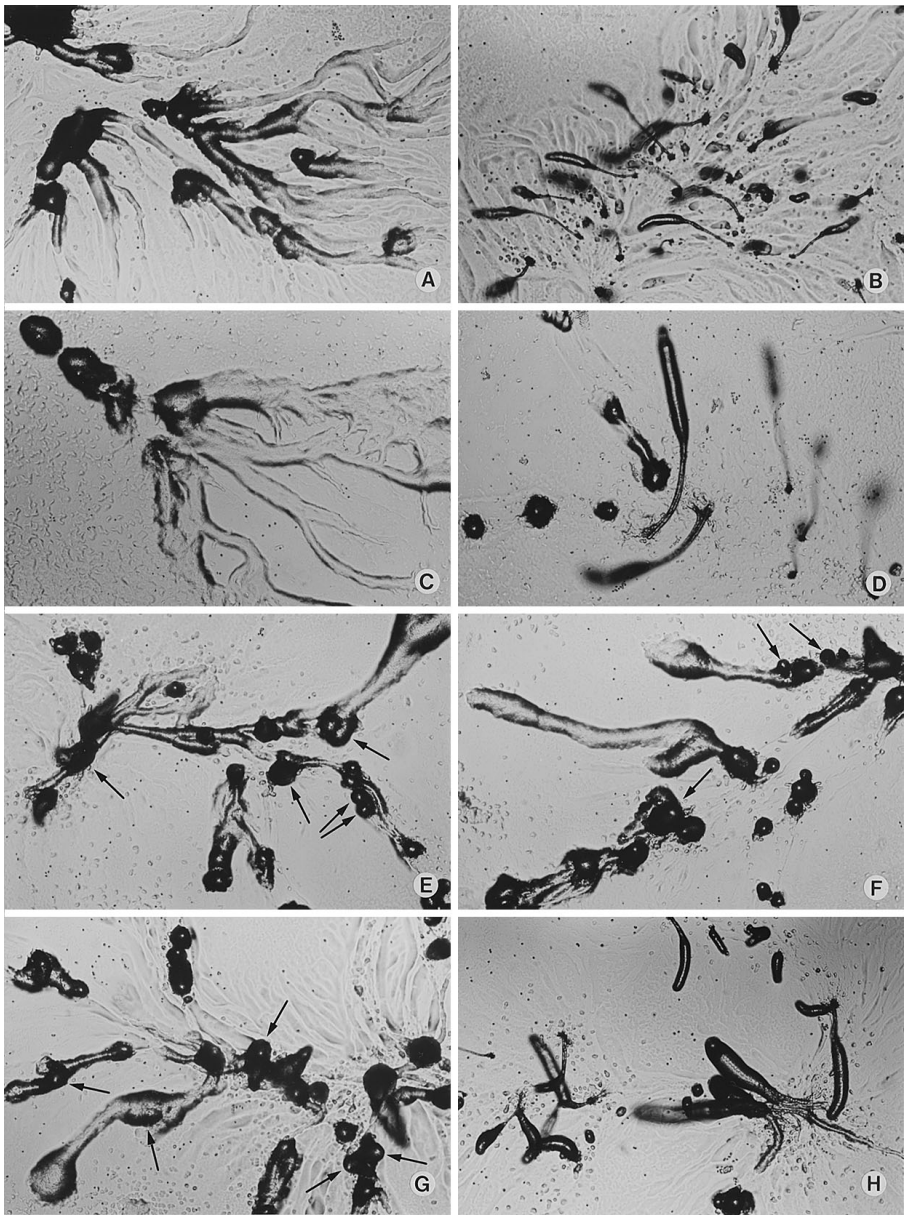

Fig. 2. *Dictyostelium purpureum*. A, B. Strain JKS274-2 (*mat A*). A. Part of a pseudoplasmodium with many small aggregations along the length of its stream; ×46. B. Developing sorocarps originating from a part of a pseudoplasmodium; ×46. C, D. Strain JKS275 (*mat a*). C. Part of a pseudoplasmodium with several small aggregations along the length of its stream; ×46. D. Several small aggregations and developing sorocarps; ×46. E–H. Mixed cultures of JKS274-2 (*mat A*) and JKS275 (*mat a*). E–G. Parts of deformed pseudoplasmodia with many small aggregations. The deformation is due to some aggregations (arrows) attached to streams of the pseudoplasmodia; ×46. H. Developing sorocarps from a part of a deformed pseudoplasmodium. Note that the sorocarps grow in close proximity to one another, are often small in size, but are of normal shape; ×46.

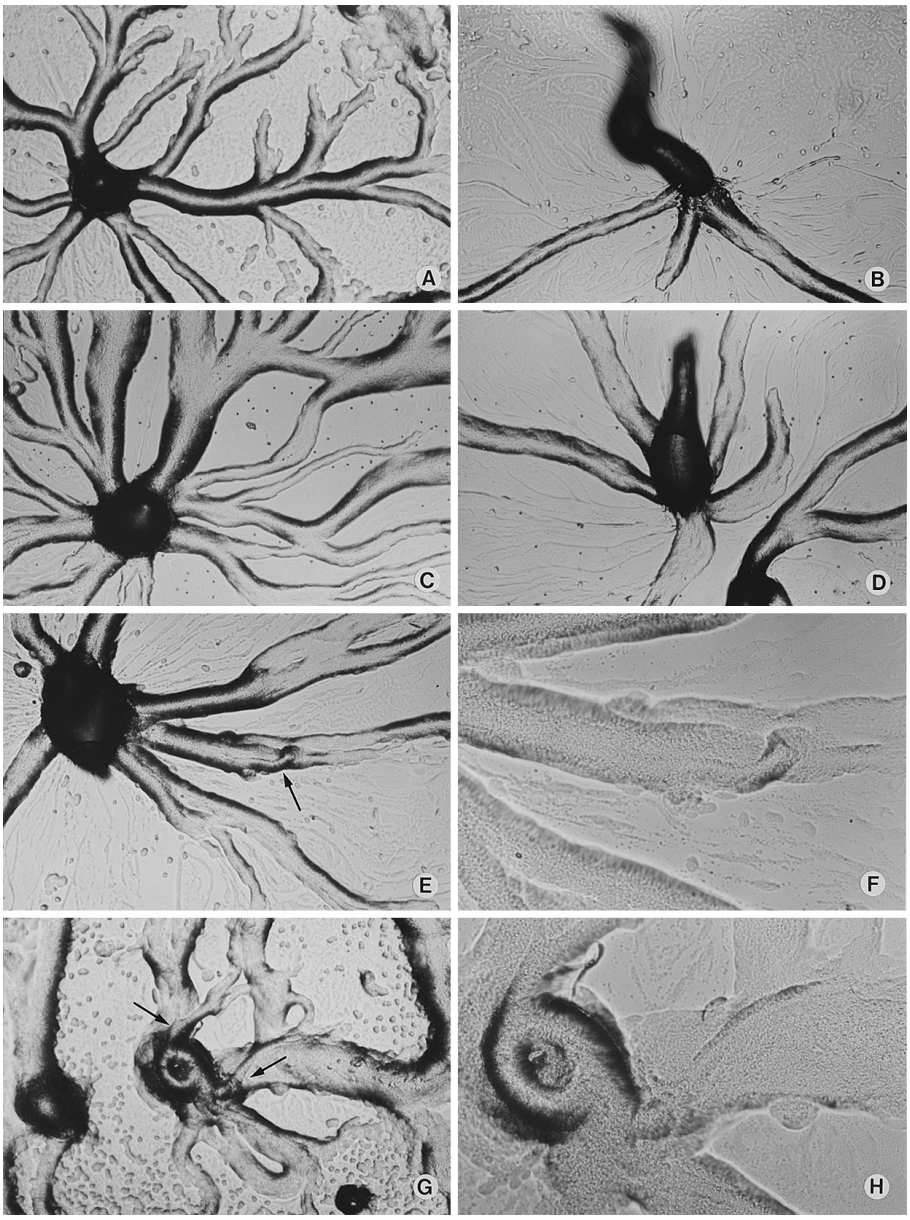

Fig. 3. *Polysphondylium pallidum*. A, B. Strain CK8 (*mat A*). A. Part of a pseudoplasmodium; ×46. B. Developing sorocarp from a pseudoplasmodium; ×46. C, D. Strain CK9 (*mat a*). C. Part of a pseudoplasmodium; ×46. D. Developing sorocarp from a pseudoplasmodium; ×46. E–H. Mixed cultures of CK8 (*mat A*) and CK9 (*mat a*). E, G. Parts of deformed pseudoplasmodia. The deformation is due to small aggregations attached to the streams of the pseudoplasmodia. Typical aggregations are indicated by arrows; ×46. F, H. Higher magnifications of the aggregations indicated by arrows in Figs. E & G, respectively. ×113.

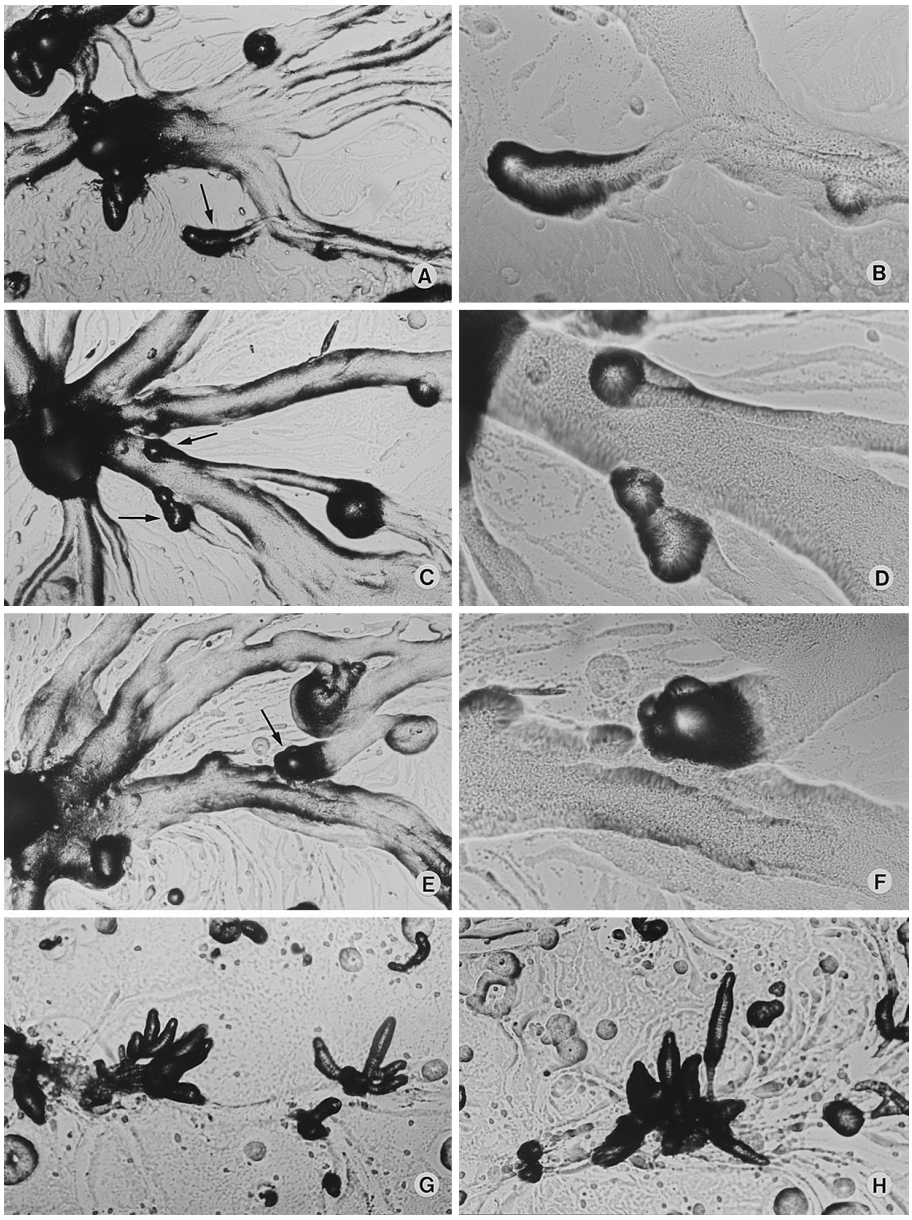

Fig. 4. *Polysphondylium pallidum*. Mixed cultures of CK8 (*mat A*) and CK9 (*mat a*). A, C, E. Parts of deformed pseudoplasmodia. The deformation is due to small aggregations attached to streams of the pseudoplasmodia. Typical aggregations are indicated by arrows; ×46. B, D, F. Higher magnifications of the aggregations indicated by arrows in Figs. A, C, & E, respectively; ×113. G, H. Small sorocarps developing in clusters from parts of deformed pseudoplasmodia. Compare their growth habit and size with those in Figs. 3B & 3D; ×46.

because both the species have a multipolar mating system consisting of 4 or more mating types (Erdos *et al.*, 1975; Hagiwara, 2004, 2005), it will require further investigations in order to determine whether the "kin discrimination" is identical to the sexual segregation.

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