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An interspecific graft between two Japanese species of *Acetabularia*[1)]

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Interspecific grafting experiments using *Acetabularia* first performed by HÄMMERLING (1940) were duplicated using two Japanese species of *Acetabularia*, *A. ryukyuensis* OKAMURA et YAMADA and *A. calyculus* QUOY et GAIMARD. In their natural habitats, both species produced caps whose cap-rays (sporangia) were arranged evenly in a disk. However, under different culture conditions, caps of irregular, loose rays consisting of detached sporangia were frequently formed. Ninety per cent of caps produced on enucleate stalks consisted of irregular loose rays. Whereas HÄMMERLING used two types of cap shape, as a marker of gene expression, the results of this study indicate cap shape cannot be used as a characteristics of Japanese *Acetabularia* species.

The number of rays in each cap was found to be stable under different culture conditions and the average number of rays in each species was notably different. Therefore the number of cap-rays was used as the marker of gene expression in this experiment. The caps produced in the early stages after graft treatment had the number of cap-rays characteristic of the enucleate apical fragment, while in the later stages, the number of cap-rays were characteristic of the nucleate basal fragment. The caps regenerated on the decapitated stalks had the cap-ray number of the nucleate basal fragments. These results agree with those of HÄMMERLING, who stated that the nucleus produces caps characteristic of the species by the production of nuclear-controlled morphogenetic substances.

Key Index Words: Acetabularia calyculus; Acetabularia ryukyuensis; *cap shape*; *cap-ray number*; *graft*.

Interspecific grafts between 2 species of the genus *Acetabularia* were first carried out by HÄMMERLING and the interaction between the cytoplasm and transplanted cell nucleus was observed (HÄMMERLING 1940). The results of the experiments were later interpreted by HÄMMERLING (1953, 1963) as that the chemical compounds (morphogenetic substances) synthesized within the nucleus were released into the cytoplasm of the stalk and controlled differentiation of species specific feature of the cap. A biochemical explanation of these events has been attempted rather recently (SCHWEIGER 1976), though some problems still remained unsolved.

Grafting experiments using *Acetabularia* have interested Japanese biologists, but confirmative studies have rarely been attempted. In the course of morphogenetic studies on Japanese *Acetabularia*, the authors performed graft experiments using two Japanese species

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of *Acetabularia*, *A. ryukyuensis* OKAMURA et YAMADA and *A. calyculus* QUOY et GAIMARD. The results of the experiments will be given in the present paper.

Material and Method

Acetabularia calyculus growing on shell fragments was collected from Noto Peninsula in Ishikawa prefecture (SANO *et al.* 1980) in August, 1978 and 1979. Gametes liberated from cysts in the cap-rays (sporangia) were isolated. Uncalcified stalks were produced by the germination of zygotes derived from fusion of these gametes. A month later, cells which had detached from the culture dishes were observed floating. They acquired a length of 3-4 cm about 10 weeks after gamete liberation. The cells used for the experiments were the descendants of this material, which was maintained in the authors' laboratory for 2-3 generations under unialgal culture conditions.

Acetabularia ryukyuensis was collected from Ishigaki Island in Okinawa (ARASAKI *et al.* 1979) in March, 1979 and 1980. Stalks growing on dead coral pieces or on Nylon fishing wire were cut off at their bases. After elimination of attached organisms, the substrata was cut into small fragments. Fresh, uncalcified stalks regenerated from the rhizoids which had remained on the substratum. Single cells on each fragment, which acquired a length of 3-4 cm in several weeks, were used for the experiments. Since the liberation of gametes in *A. ryukyuensis* was never observed, isolation of cells was not achieved.

All cultures, before and after graft treatment, were maintained at 23°C in sterilized Jamarin artificial sea water*, which was renewed once every 2 weeks, under 2000 lux illumination of fluorescent lamps, with 12 hours illumination daily.

Grafting treatments were performed under a dissecting microscope using sterilized opthalmologic scissors. The cells of each species were put in sterilized sea water in a petri dish and were cut at a region 5 mm from the rhizoids. Immediately after cutting, the nucleate and enucleate cell fragments from the different species were gently put together and the more slender stalk was inserted into the larger one (Fig. 1). The connected stalks were left overnight in a stationary dish in dim light. Morphogenetic observation took place after transferring these cells into fresh sea water.

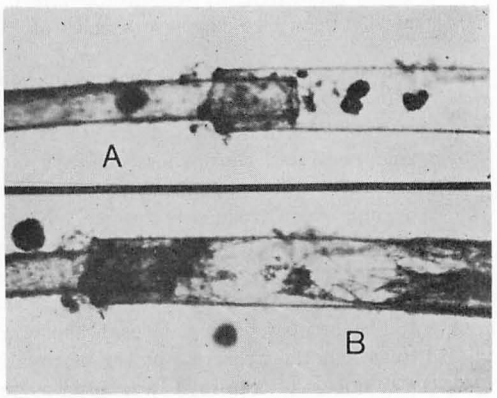

Fig. 1. Connection between apical and basal fragments. A. Directly after insertion. Some lumps of protoplasm appear at the junction site. ×25. B. 6 hours later. Protoplasm extends from untreated area to injured portion. ×25.

To obtain enucleate cell fragments, the stalks were tied with Nylon thread before cutting to avoid excess leaking of cytoplasm.

Results

1. Problem of morphological markers:

a. Shape of cap and cap-ray.

The appearances of caps varied according to environmental conditions (YAMAOKAYANO 1980). In the laboratory, cells frequently produced abnormal caps with an abnormal arrangements of cap-rays (Fig. 2). The shape of the cap-ray was also observed to be affected by culture conditions. Table 1 shows the acceleration of abnormal caps by higher temperature and higher light intensity. Enucleate cell parts produced a high number of abnormal caps under 2000 lux

* Commerciel medium manufactured by Jamarin laboratory.

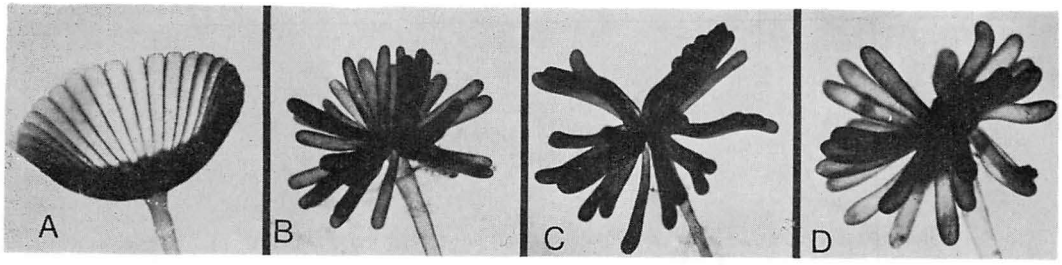

Fig. 2. Shape of caps. A. Normal cap; ×10. B, C and D. Abnormal caps. ×10.

Table 1. Acceleration of abnormal cap formation.

	Temperature	
	32°C	22°C
Under 5000 lux illumination:		
nucleate cell	100* (100)**	67 (92)
enucleate cell fragment	100 (100)	71 (98)
Under 2000 lux illumination:		
nucleate cell	80 (93)	0 (0)
enucleate cell fragment	58 (92)	60 (90)

* Number shows the percentage of abnormal cap.

** Number in parenthesis represents the percentage of abnormal caps when partially abnormal caps were included with abnormal caps.

illumination at 22°C, although nucleate whole cells usually produced normal caps in this culture condition. Some of the abnormal caps consisted of similarly shaped cap-rays, and other abnormal caps had differently shaped cap-rays. The change of cap shape was accompanied by a change in hair number on the corona superior. This change was used by Hämmerling as one of the specific markers, as well as the cap shape. Even in normal caps of *A. calyculus*, the shape of the cap-rays of laboratory grown material was usually different from that of thalli collected from their natural habitat. The tips of the cap-rays in the latter were flat and concave, while in the former they were convex (Fig. 3). Accordingly, it appears that the shape of cap is too variable to use as a morphological marker of species.

b. The number of cap-rays.

More than 200 caps from each species were collected from both laboratory cultures and their natural habitats. The number of rays in a cap were counted and the relationship of this number to the different species and to different environments (Fig. 4) was investigated. The average number of rays in a cap is 34 in *A. calyculus* and 72 in *A. ryukyuensis*. There appears to be high constancy in the number of cap-rays for each species, especially for *A. calyculus*.

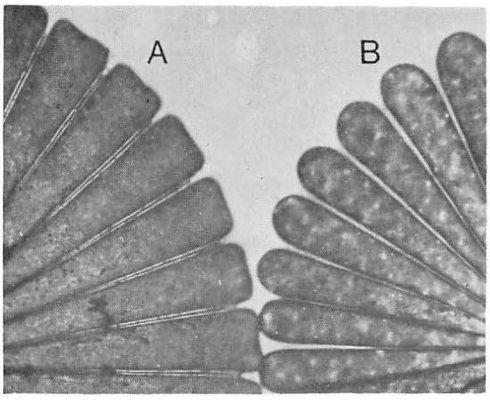

Fig. 3. Different appearance of cap edges. A. Cap produced at natural habitat; ×30. B. Cap produced in laboratory. ×30.

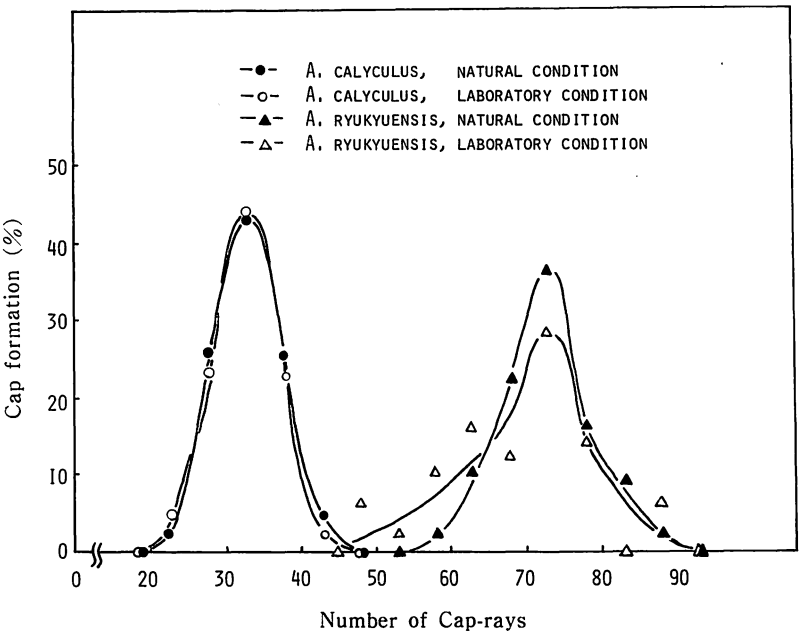

Fig. 4. Distribution of cap-ray numbers in a cap.

Table 2. Interspecific grafts of *A. ryukyuensis* and *A. calyculus*. Combination of the basal fragment of *A. calyculus* and the apical fragment of *A. ryukyuensis*.

days	number of cap-rays	species specific appearance of caps
6	52	RYU
6	48	RYU
6	46	RYU
7	52	RYU*
8	61	RYU*
8	52	RYU
9	36	CAL
10	38	CAL
12	34	CAL
14	36	CAL
15	34	CAL
16	30	CAL
18	35	CAL
19	36	CAL
21	35	CAL
33	30	CAL

* These cells were used in Experiment 3.

The number of rays is not easily affected by environmental conditions, though the shape of cap and other morphological features are frequently altered. Thus, the number of cap-rays is seemed to be available as an index of species specificity, or of specific gene expression in this experiment.

2. *Interspecific graft*:

Experiment 1.

After the basal fragments (nucleated) of *A. calyculus* and the apical fragments (enucleated) of *A. ryukyuensis* were connected (Table 2), the caps formed in the first 8 days after the treatment had more than 46 cap-rays, while the caps formed after 9 days had less than 38. The cap-ray number of the former is different from the average number of *A. ryukyuensis*. However, caps with such a number of rays as 46 frequently appeared in laboratory culture of this species, and was different for *A. calyculus*. Therefore caps formed in the first 8 days had a ray number consistent with *A. ryukyuensis* wheras caps formed after 9 days were consistent with *A. calyculus*.

Experiment 2.

After the basal fragments (nucleated) of *A. ryukyuensis* and the apical fragments (enucleated) of *A. calyculus* were combined (Table 3), the caps formed in the first 11 days after treatment had less than 37 cap-rays, while the caps formed after 13 days had more than 59. This clear difference

Table 3. Interspecific graft of *A. ryukyuensis* and *A. calyculus*. Combination of the basal fragment of *A. ryukyuensis* and the apical fragment of *A. calyculus*.

days	number of cap-rays	species specific appearance of caps
10	28	CAL
10	37	CAL*
11	37	CAL
13	60	RYU
14	59	RYU
16	59	RYU
18	65	RYU
19	66	RYU
20	75	RYU
22	68	RYU
25	61	RYU

* The cell was used in Experiment 3.

Table 4. Interspecific grafts of *A. ryukyuensis* and *A. calyculus*. Regenerated caps after decapitation of the cells.

basal fragment (nucleated)	apical fragment (enucleated)	First cap formation			Second cap formation		
		days	number of cap-rays	species specific appearance of caps	days	number of cap-rays	species specific appearance of caps
CAL	RYU	7	52	RYU	13	35	CAL
CAL	RYU	8	61	RYU	23	36	CAL
RYU	CAL	10	37	CAL	15	65	RYU

was observed in the caps which were produced before the 11th day and after the 13th day. The former indicated the specificity of *A. calyculus* and the latter indicated that of *A. ryukyuensis*.

Experiment 3.

Three caps which were produced in the first 10 days after treatment (See Table 1 and 2) were cut off and the decapitated cells were successively cultured under the same culture conditions. Regenerated caps appeared 2 to 3 weeks after decapitation. As shown in Table 4, the regenerated caps had the cap-ray number of the species corresponding to their basal fragments, although the first caps had the cap-ray number of the apical fragments.

3. *The terms that the information of cap formation exists in enucleate cell fragments*:

Before cap initiation, 50 thalli of more than 3 cm in length of each species were randomly selected and their rhizoids were removed. These enucleate apical fragments were cultured under the same condition as the graft experiments. They survived more than several months, although cap formation was limited to only the early stages. The last cap initiation took place at the 16th day after enucleation in *A. ryukyuensis* and no more caps were produced after the 17th day (Fig. 5). It seems therefore that information for cap formation persists in cytoplasm

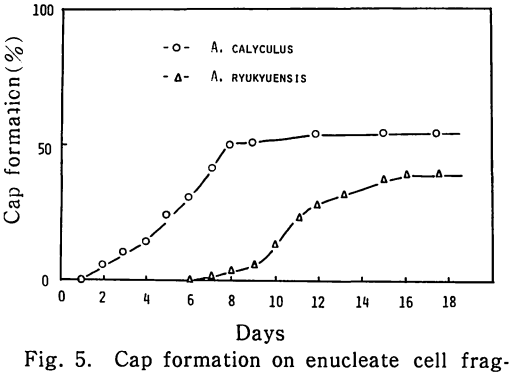

Fig. 5. Cap formation on enucleate cell fragments after removal of rhizoids. No more formation takes place after 17th day.

for around 10 days under the culture condition of these experiments. This length of time is identical to the period when the caps express species specific number of cap rays of apical fragment in Experiment 1 and 2. These results support the fact that in the graft experiments caps produced in several days after treatment express the specific feature of the apical fragment, without expression of the genetic information in the new nucleus.

Discussion

Many types of inter- and intra-specific grafts have been performed by HÄMMERLING and other workers during the last 40 years for the purpose of analyzing nucleocytoplasmic interactions (BONOTTO *et al.* 1980). *A. mediterranea* was the most generally used for these experiments. A widely known experiment (HÄMMERLING 1940) is the graft between *A. mediterranea* and *A. crenulata*. The cap shape of both species seems to be stable at least under normal culture condition. Acceleration of the production of abnormal caps by higher temperature and higher light intensities has not been reported in these species. The first difficulty encountered, when Japanese species of *Acetabularia* were used for graft experiment in authors' laboratory, was the unreliability of the cap shape. The variability of the cap shape is observed in the arrangement of cap-rays as well as in the size and shape of each cap-ray. Both *A. ryukyuensis* and *A. calyculus* harvested from natural habitats are similar to *A. mediterranea* in the arrangement of cap-rays, which adjoin each other and form a disk. Very often the stalks of above two species produce detached cap-rays whose appearance closely resembles *A. crenulata*. This abnormal cap formation is induced by an unusual environment and also by enucleation of young cells. It is not uncommon to incorrectly identify the Japanese species of *Acetabularia* when the algae is exposed to different environ-

mental conditions. Regulation of morphogenesis by environmental factors, especially cap formation in *Acetabularia* is under investigation.

It was found that the number of cap-rays was comparatively stable in spite of the environmental change and that the distribution curves of their numbers (Fig. 4) never overlap each other. Due to these facts, the number of cap-rays serves as an adequate species marker in the graft experiments using these species, whereas morphological features such as the cap-ray arrangement are not suitable for distinguishing these two Japanese species.

By use of the number of cap-rays as the sole marker of specific gene expression, the present study obtained the same physiological results as HÄMMERLING's study (1953) which used cap shape as the main marker of gene expression. Grafting experiments using the Japanese Acetabularian cell appear to be useful for research about nuclear gene control.

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石川依久子，ジルセ・ミチコ・ヤマオカ・ヤノ，今堀宏三：日本産カサノリの種間接木

ヘマリングによって開発された方法にもとづき，日本産カサノリ，*A. ryukyuensis* OKAMURA et YAMADA（カサノリ）と，*A. calyculus* QUOY et GAIMARD（ホソエガサ）の種間接木を試みた。ヘマリングは平板型カサと立体型カサをそれぞれ表現形質として異った種の接木実験をおこなったが，上記2種の日本産カサノリでは，カサの形態を形質の指標とすることはできなかった。両種のカサはいずれも天然では平板型であるが培養条件下では立体型であることが多い。カサの形態は温度や光の強度に依存し，更に，リゾイドを除去した無核藻体片には多くの立体型カサが生じることがみいだされた。

両種には，カサ当りの子嚢数にあきらかな相違があり，しかもその相違は培養条件による変動が少かったのでこれを指標として接木実験をおこなうことができた。接木処置後，約10日以前に生じるカサは無核藻体片の種の特徴である子嚢数をもち，それ以後に生じるカサは有核藻体片の種の特徴である子嚢数をあらわした。また，前者のカサを除去し，同一茎上に再びカサをつくらせると後者のカサになった。これらの結果は，ヘマリングの実験結果を再確認するものであった。（560 大阪府豊中市待兼山町 1-1，大阪大学教養部生物学教室）