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Gamete surface and attachment during fertilization of *Palmaria* sp. (Palmariales, Rhodophyta)

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Attempts to characterize the gamete surface structures of *Palmaria* sp. (Palmariales, Rhodophyta) were made. The colorless covering of the living and chemically fixed spermatium was degraded by proteolytic enzymes added to the spermatial suspension without affecting cell structure and viability. The trichogyne cell wall was coated with a fibrous structure containing vicinal glycol residues which were destroyed by periodic acid oxidation followed by sodium borohydride reduction. A brief pretreatment of spermatia by proteolytic enzymes significantly inhibited the gamete attachment as well as the degradation of vicinal glycols of the trichogyne coat. These results suggest that the specific gamete attachment during fertilization of this alga is principally governed by these adhesive surface structures.

Key Index Words: cell surface—fertilization—gamete attachment—Palmaria—*proteolytic enzymes—Rhodophyta—spermatium—trichogyne—vicinal glycols.*

In the life history of red algae, syngamy is achieved by fusion between a non-flagellated spermatium liberated from a spermatangium and a trichogyne, a specialized process of a carpogonium formed on the thallus. Though amoeboid movement has frequently been noted in red algal spermatia (Dixon 1973), the initial attachment of the passively moving spermatia to the trichogyne is established immediately after spermatium inoculation to trichogynes (Mine and Tatewaki 1994). Therefore, adhesive gamete surfaces may play critical roles in the recognition and attachment between these, essentially non-motile gametes.

There have been a number of ultrastructural and cytochemical studies on the spermatial surface structure in red algae (e.g. Scott and Dixon 1973, Kugrens 1974, 1980, Peel and Duckett 1975, Fetter and Neushul 1981, Magruder 1984, Cole *et al.* 1985, Broadwater *et al.* 1991, Kim and Fritz 1993a, b, Mine and Tatewaki 1994). Some of these studies on bangiacean and ceramiacean algae have shown specialized spermatial appendages concerned with the attachment to trichogynes (Magruder 1984, Cole *et al.* 1985, Broadwater *et al.* 1991, Kim and Fritz 1993a, b). The ultrastructure (Broadwater and Scott 1982, Mine and Tatewaki 1994) and histochemical nature (Cole *et al.* 1985) of the receptive surface of female gametes have also been reported in some species.

Experimental studies on the attachment of spermatia to trichogynes have also existed in several red algae. Comparisons of gamete attachment between intraspecific and intergeneric fertilization have been made in ceramiacean (Magruder 1984) and palmariacean (Mine and Tatewaki 1993) algae. Kim and Fritz (1993a) developed an experimental system for quantitative assays of gamete attachment using a uniseriate ceramiacean alga. They demonstrated the inhibitory effect of a lectin and a specific sugar on the attachment.

The palmariacean algae have a unique life history (van der Meer and Todd 1980, van der Meer 1981, Mitman and Phinney 1985, Deshmukhe and Tatewaki 1990, Mine and Tatewaki 1993) providing a sufficient number

of naked, sessile carpogonia and good opportunities for observations and experiments of the fertilization processes (Mine and Tatewaki 1993, 1994). We have previously shown the morphology of the spermatial covering and trichogyne coat of *Palmaria* sp., both of which apparently mediate the gamete attachment during fertilization of this alga (Mine and Tatewaki 1994). In this paper, we report a part of the cytochemical feature of these gamete surface structures and the inhibition of gamete attachment by chemical and enzymatic pretreatment of the gamete surfaces.

Materials and Methods

Preparation of male and female gametes, and comparative spermatium inoculation

Mature sporophytes and male gametophytes of *Palmaria* sp. were collected from January to June of 1992 and 1993 at Charatsunai, Muroran, Hokkaido. Tetraspore germlings containing mature female gametophytes growing on coverslips, and spermatial suspensions from male gametophytes were prepared as described previously (Mine and Tatewaki 1994). Artificial seawater (ASW) was 450 mM NaCl, 30 mM $MgCl_2$, 16 mM $MgSO_4$, 10 mM KCl, 10 mM $CaCl_2$, and 8.25 mM Tris-HCl (pH 7.8).

To estimate the effect of gamete pretreatment on the gamete attachment, comparative spermatium inoculation experiments were conducted. Pretreatment procedures were as described below and spermatium inoculation was carried out as described previously (Mine and Tatewaki 1994) using pretreated and control gametes. After inoculation, coverslips with tetraspore germlings were then washed by pipetting 10 times with ca. 3 m*l* of calcium-depleting ASW (ASW without $CaCl_2$ added with 1 mM ethylene glycol-bis[beta-aminoethyl ether] N,N,N′,N′-tetraacetic acid) to remove indirectly co-aggregated spermatia (Figs. 1, 2). Samples were then fixed in 1% glutaraldehyde (GA) in ASW for 1-3 h at 4°C. After rinsing with ASW, samples were mounted in a 1 : 1 mixture of ASW and glycerol, and the numbers of trichogynes and attached spermatia were determined under light microscopy.

A gamete attachment was estimated by counting the number of attached spermatia divided by the number of trichogynes grown on the examined coverslip (designated 'spermatia/trichogynes'). In an experiment, tetraspore germlings derived from the same tetraspore suspension were used in both control and experimental duplicate coverslips and they were inoculated simultaneously with

Figs. 1–14. Light and transmission electron micrographs of *Palmaria* sp. Figs. 1, 2. Light micrographs of trichogynes inoculated with untreated spermatia. Living material. Scale bar=50 μm. Fig. 1. Washed in ASW. Numerous indirectly co-aggregated spermatia (arrowhead) remain around a trichogyne (arrow). Fig. 2. Washed in calcium-depleting ASW. Spermatia (arrowheads) attached directly to the trichogynes (arrows) remain. Figs. 3–7. Light micrographs of spermatia prepared in India ink/seawater. Scale bar=10 μm. Figs. 3–5. Living materials. Fig. 3. Untreated. Colorless, ca. 3 μm thick covering (arrowhead) excluding carbon particles of India ink is observed around the cell (arrow). Fig. 4. Treated with 1% Pronase E for 30 min. The spermatial covering (arrowhead) is thinner than that of untreated cells. Fig. 5. Treated with 1% Pronase E for 120 min. The spermatial covering cannot be detected. Figs. 6, 7. Spermatia fixed in 1% GA in seawater attached on coverslips coated with poly-L-lysin. Fig. 6. In 90% ethanol after gradual dehydration. Shrunken spermatial coverings (arrowheads) are outlined by sediment of carbon particles of India ink. Fig. 7. In water after dehydration followed by gradual rehydration. Spermatial coverings (arrowheads) have recovered in thickness. Figs. 8–10. Transmission electron micrographs. Median section of a spermatium. SC indicates a spermatial covering and an arrowhead indicates plasma membrane. Scale bar=0.5 μm. Fig. 8. Untreated spermatium. Fibrous reticulated spermatial covering is observed around plasma membrane. Fig. 9. Treated with 0.1% Pronase E for 90 min. The spermatial covering has thinned moderately. Fig. 10. Treated with 1% Pronase E for 90 min. The spermatial covering is no longer detected. Figs. 11–14. PATAg test on an oblique section of trichogyne. An arrowhead indicates a trichogyne coat. Scale bar=1 μm. Figs. 11, 12. Untreated trichogyne. Fig. 11. Section oxidized with periodic acid. The trichogyne coat is stained positively. Fig. 12. Section oxidized hydrogen peroxide as a control. The trichogyne coat is stained negatively. Figs. 13, 14. Trichogyne treated by oxidation and sodium borohydride reduction before preparation for PATAg test. Section was oxidized with periodic acid in PATAg test. Fig. 13. Trichogyne oxidized with periodic acid. The trichogyne coat is very slightly PATAg-positive. Fig. 14. Trichogyne oxidized with hydrogen peroxide. The trichogyne coat is significantly PATAg-positive.

spermatia of the same spermatial suspension.

Enzymatic treatment of spermatial covering

A spermatial suspension was diluted with ASW into 200 spermatia per μl suspension, mixed rapidly with an equal volume of ASW containing a proteolytic enzyme and kept at room temperature. Proteolytic enzymes used were Pronase E (Protease Type XIV; Sigma Chemical Co., St. Louis), trypsin (Type IX; Sigma), and papain (Wako Pure Chemicals, Tokyo). At the time of measurement, a small part of the mixture was mixed with 1/5-1/20 volume of India ink (Pellikan AG, Hanover) and the outer diameter of the transparent covering which excluded India ink was measured on 10 cells under light microscopy within 5 min. The thickness of the covering was calculated by subtracting the average cell diameter (5 μm) from the meas-

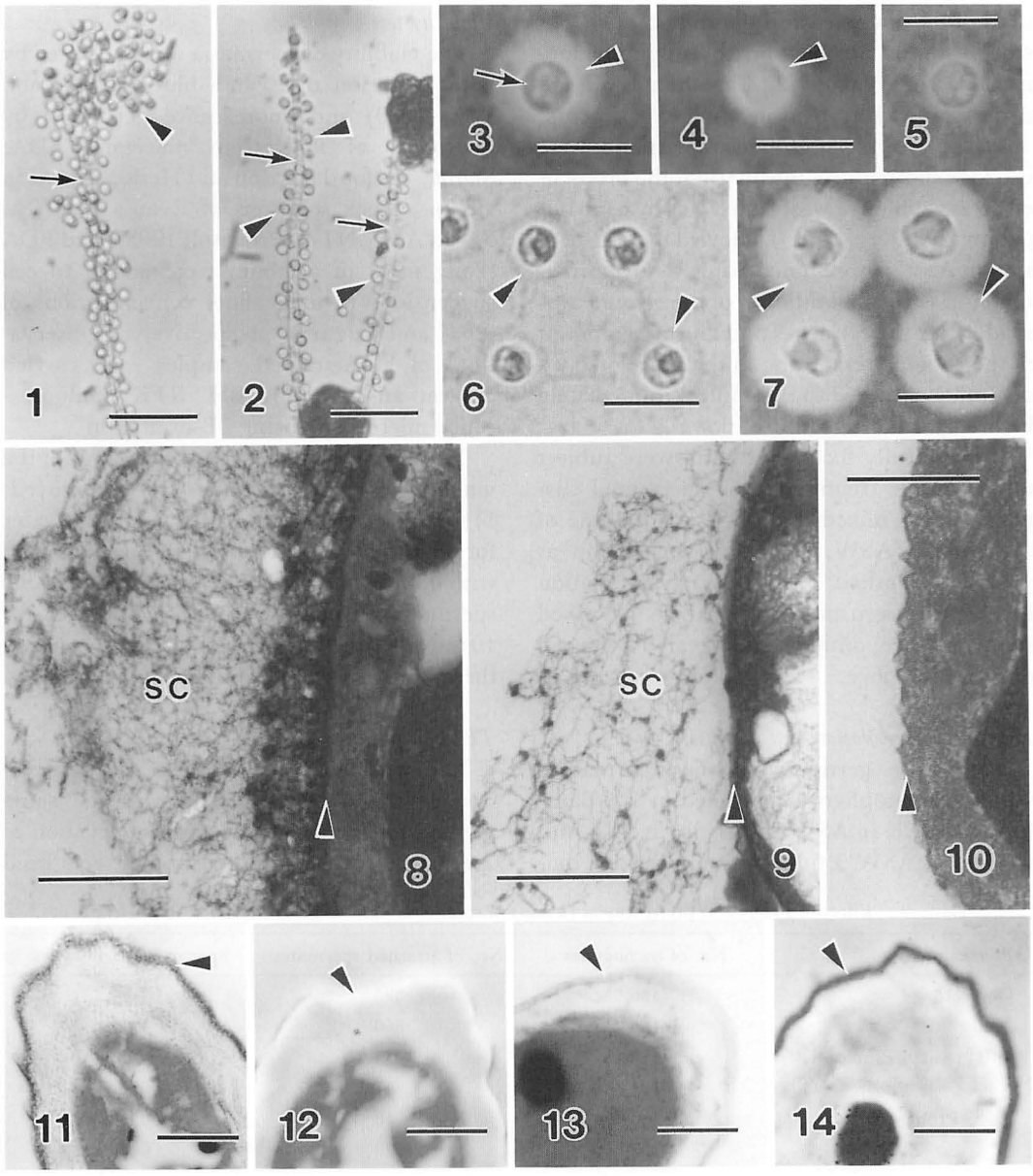


Table 1. Viability test of spermatia after complete degradation of covering.

Treatment	FDA	Evan's blue
None	+	−
1% Pronase E, 180 min	+	−
1% trypsin, 180 min	+	−
1% GA, 30 min	−	+
0.05% saponin, 30 min	−	+
70°C, 10 min	−	+

ured outer diameter and dividing by two. A stock solution of phenylmethylsulphonylfluoride (PMSF; Wako; 0.2 M in ethanol) was added along with trypsin in some experiments.

When enzyme-pretreated spermatia were used for spermatium inoculation, the pretreated spermatia were washed 3 times by centrifugation (1,500 × g, 5 min) followed by resuspension in ASW before inoculation. An approximately 3×10^5-fold dilution of the enzyme was made after these washes and an equal amount of the enzyme remaining in the pretreated inoculum was added to the control immediately before spermatium inoculation.

If chemically fixed spermatia were subject to enzymatic treatment, the spermatial suspension was mixed with an equal volume of 2% GA in ASW, incubated for 30 min at 4°C, and washed 3 times by centrifugation followed by resuspension in ASW. Washed samples were diluted and treated with an enzyme as above.

Periodic acid oxidation and reduction

Tetraspore germlings containing mature female gametophytes were fixed in 1% paraformaldehyde in ASW for 30 min at 4°C, and washed in ASW 3 times. Samples were oxidized in 10 mM periodic acid in ASW containing 8.25 mM maleic acid (pH 4.0) for 5 min in the dark at room temperature, washed in ASW 3 times, and reduced in 0.1% sodium borohydride in ASW (pH 9.4) for 10 min in the dark. Two different controls were employed (Table 4). One percent hydrogen peroxide was used in place of periodic acid for non-specific oxidation, and, in the second control, reduction alone was used.

Viability test

The viability of spermatia was examined by both exclusion of Evan's blue (Taylor and West 1980) and fluorescence generated by hydrolysis of fluorescein diacetate (FDA; Sigma; Heslop-Harrison and Heslop-Harrison 1970). Stock solutions of Evan's blue (ca. 5% in $ASP_{12}NTA$ (Provasoli 1963)) and FDA (2 mg ml^{-1} in acetone) were added to cell suspensions to make final concentrations of 0.5% and 4 μg ml^{-1}, respectively. Observations of FDA-stained samples were carried out on an Olympus BH2-RFK epifluorescence microscope using "B-excitation".

To kill the cells for negative controls of the viability test, three methods were employed; 1) spermatia were fixed in 1% GA in seawater for 30 min at 4°C, 2) spermatia were treated with 0.05% saponin for 30 min at 4°C, 3) the spermatial suspension was placed at 70°C for 10 min. Attachment did not occur between these killed spermatia and trichogynes.

TEM preparation and cytochemistry

The method for preparation and observation of the TEM specimen was the same method for enhancement and preservation of spermatial covering and trichogyne coat used

Table 2. Effect of Pronase E pretreatment (5 min) of spermatia on attachment to untreated trichogynes.

Pretreatment	No. of trichogynes	No. of attached spermatia	Spermatia/trichogynes
Control	156	525	3.36
	165	660	4.00
0.01% (w/v) Pronase E	118	73	0.62
	137	41	0.29
0.1% Pronase E	168	18	0.11
	187	20	0.11

Table 3. Effect of trypsin pretreatment (5 min) of spermatia on attachment to untreated trichogynes.

Pretreatment	No. of trichogynes	No. of attached spermatia	Spermatia/trichogynes
Control	256	966	3.77
	271	894	3.30
0.05% (w/v) trypsin	311	483	1.55
	336	473	1.41
0.5% trypsin	257	15	0.06
	354	12	0.03

in Mine and Tatewaki (1994). Compounds containing vicinal glycol residues were cytochemically localized by a periodic acid—thiocarbohydrazide—silver proteinate (PA-TAg) test according to Roland and Vian (1991). Specimens for cytochemistry were fixed in 3% GA, 2% NaCl in cacodylate buffer (pH 7.2), washed in buffer containing NaCl, rinsed in water, dehydrated in ethanol and embedded in LR white (London Resin, Hampshire).

Results

Spermatial covering

The spermatial covering could be detected under the light microscope as ca. 3 μm thick colorless covering which excludes carbon particles of India ink (Fig. 3). The spermatial covering exhibited semisolid, or a gel-like character. The appearance of the covering was not changed even after fixation by GA in seawater. Upon gradual dehydration in ethanol of GA-fixed spermatia, the apparent thickness of the covering decreased (Fig. 6). After a gradual rehydration in water, the covering thickness almost completely recovered (Fig. 7).

The spermatial covering was degraded by proteolytic enzymes. As observed under the light microscope, non-fixed spermatial coverings became thinner in both concentration- and time-dependent manner (Figs. 4, 5, 15, 16). In the presence of 1 mM PMSF, the decrease in the covering thickness by trypsin was partially repressed (Fig. 17). After prolonged enzymatic treatment, e.g. 1-2 h in 1% Pronase E, the coverings were no longer detected around spermatial cell surfaces (Figs. 5, 15). The viability of spermatia after deletion of the coverings by enzymatic treatment was confirmed (Table 1).

In the TEM specimen, a fibrous, reticulated substructure of the spermatial covering was seen on the plasma membrane of spermatia (Fig. 8). The decrease in the covering thickness (Fig. 9) or disappearance of the covering (Fig. 10) by proteolytic enzymes was also observed. In addition, the decrease in the covering thickness by Pronase E (Fig. 18) and trypsin (not shown) also occurred in GA-fixed spermatia.

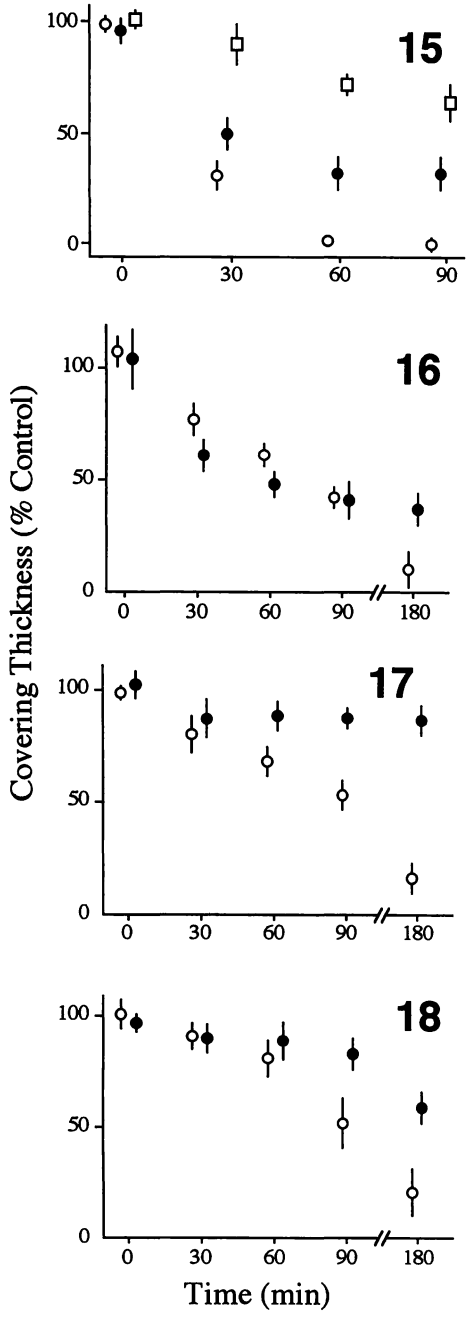
Trichogyne coat

The cell wall surface of the trichogyne was uniformly coated with fibrous structures

Table 4. Effect of degradation of vicinal-glycols of trichogyne coat on gamete attachment.

Oxidative agent used in trichogyne pretreatment	No. of trichogynes	No. of attached spermatia	Spermatia/trichogynes
Periodic acid	185	58	0.31
	76	24	0.32
Hydrogen peroxide	126	570	4.52
	200	671	3.36
None (pH 4.0)	126	977	7.75
	239	387	2.78

(Mine and Tatewaki 1994), and this trichogyne coat was composed of a PATAg-positive material (Figs. 11, 12). This PATAg-positive reaction mostly disappeared after periodic acid oxidation followed by sodium borohydride reduction of chemically fixed trichogynes (Fig. 13), but not after oxidation by hydrogen peroxide and reduction (Fig. 14). The periodic acid-sodium borohydride treatment thus appeared to specifically destroy the PATAg-positive structure, i.e., vicinal glycol residues, of the trichogyne coat.



Inhibition of gamete attachment by gamete pretreatment

The pretreatment, which was expected to destroy the gamete surface structures, inhibited the attachment of spermatia to trichogynes. A brief pretreatment (5 min) of spermatia by proteolytic enzymes disrupted the ability of the spermatia to attach to the trichogynes (Tables 2, 3). The number of attached spermatia per trichogyne decreased remarkably according to the concentrations of both Pronase E and trypsin added to the pretreatment mixture. On the other hand, periodic acid oxidation followed by sodium borohydride reduction of fixed trichogynes also inhibited the gamete attachment significantly (Table 4). In contrast to an unclear difference between the controls without oxidation and those with hydrogen peroxide oxidation, a significantly smaller number of spermatia attached to the periodic acid-oxidated trichogynes.

Discussion

Gamete surfaces

In the present study, the viability and ultrastructure of spermatia were not changed after spermatial covering degradation by proteolytic enzymes. The degradation also oc-

Figs. 15–18. Time course of thickness changes in spermatial coverings of *Palmaria* sp. during treatment with proteolytic enzymes. Data were expressed as % of control. Vertical lines indicate ±1/2 standard deviation. Controls were treated with ASW only. Figs. 15–17. Coverings of non-fixed spermatia. Fig. 15. Treated with 1% (white circle), 0.1% (black circle), or 0.01% (white square) Pronase E. Fig. 16. Treated with 1% trypsin (white circle), or 1% papain (black circle). Fig. 17. Treated with 0.5% trypsin (white circle), or 0.5% trypsin along with 1 mM PMSF (black circle). Fig. 18. Coverings of GA-fixed spermatia treated with 0.5% (white circle), or 0.1% (black circle) Pronase E.

curred in chemically fixed spermatia. Therefore, the decrease in the covering thickness during the enzymatic treatment does not seem to be due to the damage of the covering-generating activity, if present, of the living spermatium. Furthermore, since the degradation was inhibited by a specific protease inhibitor, some protein(s) or polypeptide(s) which can be hydrolyzed by the enzymes should be at least one of the constituents of the covering.

Extracellular proteins have been reported in a red alga (Hanic and Craigie 1969), but a proteinaceous nature of spermatial coverings of red algae has not been demonstrated. Cytochemical studies on the spermatial coverings of other red algae have shown that they consist of acidic and neutral polysaccharides (Peel and Duckett 1975, Cole *et al.* 1985) or mannose residues as detected by binding of lectins (Kim and Fritz 1993a, b). Using histochemical techniques to detect proteins, Peel and Duckett (1975) reported a negative staining in the spermatial covering (as 'spermatial coat'). In *Palmaria* sp., we have found no histochemical evidences in the spermatial covering using several procedures for proteins that have shown positive reaction in the spermatial nucleus and cytoplasm (unpubl. observ.). It is otherwise possible that the proteinaceous component is indispensable for maintaining the covering structure but not the major constituent of the covering. Chemical analysis of the lysate during the spermatial covering proteolysis is in progress.

Although controversial results were obtained in a ceramiacean alga (Kim and Fritz 1993b), we have previously showed that the trichogyne of *Palmaria* sp. was stained with calcofluor (Mine and Tatewaki 1994). Cole *et al.* (1985) reported that the receptive surface ("wall coating") of female gamete of *Bangia* contained vicinal glycol residues as detected by a periodic acid-Shiff test. In the present study, we also demonstrated the PATAg-positive nature of the trichogyne coat in *Palmaria* sp. The trichogyne coat was stained by the PATAg test far more strongly than the trichogyne and thallus cell walls that have been reported to consist of vicinal glycol-containing polysaccharides (Turvey and Williams 1970, as *Palmaria palmata*). This indicates that the trichogyne coat material contains a large quantity of vicinal glycol moieties.

Gamete attachment

There have been SEM studies on the gamete attachment in red algae (Magruder 1984, Cole *et al.* 1985, Mitman and Phinney 1985, Kim and Fritz 1993a). Three examples given above on the bangiacean and ceramiacean algae showed that the spermatia bore specialized appendages (or 'corns') that were apparently responsible for the initial binding to the receptive surface of female gamete. This was further supported by the inhibition of gamete attachment by a lectin and a localization of the lectin binding to the spermatial appendage in a ceramiacean alga (Kim and Fritz 1993a, b). To the contrary, neither SEM study (Mitman and Phinney 1985) nor TEM study (Mine and Tatewaki 1994) showed any appendages on the spermatia of the palmariacean algae throughout the fertilization processes.

Previous experiments have shown that the attachment and fusion of gametes in the intergeneric fertilization were readily observed in palmariacean algae (Mine and Tatewaki 1993). However, no binding occurred between the gametes of *Aglaothamnion neglectum* and other species of related ceramiacean genera (Magruder 1984). The difference in heterologous gamete affinities may be related to the differences in the morphological and chemical properties of the gamete surfaces between ceramiacean and palmariacean algae.

The inhibition of gamete attachment by the brief pretreatment of *Palmaria* spermatia with proteolytic enzymes was probably due to a degradation of the outermost layer of the spermatial covering. It is otherwise possible that other important proteins were disrupted by the proteolytic enzymes and this had a secondary effect on the spermatial attachment as argued in the gamete adhesion of *Chlamydomonas* (Goodenough 1991). On the other hand, the

PATAg-positive compounds in the trichogyne coat appear to be essential for gamete attachment since the attachment was markedly inhibited by a specific chemical degradation of vicinal glycols of the coat. The PATAg-positive entity can be interpreted in various ways (Roland and Vian 1991), but the great majority of positive compounds are polysaccharides containing 1-4 linkages.

There have been many examples that indicate lectin-polysaccharide interactions in the gamete recognition in animals (Rosati 1985, O'Rand 1988), brown algae (Callow 1985, Schmid 1993) and a red alga (Kim and Fritz 1993a). If the PATAg-positive compounds of *Palmaria* trichogynes are polysaccharides, it is likely that the complementary adhesive substance of the spermatial covering is a lectin that binds to the trichogyne coat polysaccharides. An examination of the effect of lectins or specific glycosidases on the gamete attachment will provide further information on the adhesive trichogyne coat containing vicinal glycols.

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References

Broadwater, S. T. and Scott, J. 1982. Ultrastructure of early development in the female reproductive system of *Polysiphonia harveyi* Bailey (Ceramiales, Rhodophyta). J. Phycol. **18:** 427–441.

Broadwater, S. T., Scott, J. L. and West, J. A. 1991. Spermatial appendages of *Spyridia filamentosa* (Ceramiaceae, Rhodophyta). Phycologia **30:** 189–195.

Callow, J. A. 1985. Sexual recognition and fertilization in brown algae. J. Cell Sci. (supplement 2): 219–232.

Cole, K. M., Park, C. M., Reid, P. E. and Sheath, R. G. 1985. Comparative studies on the cell walls of sexual and asexual *Bangia atropurpurea* (Rhodophyta). I. Histochemistry of polysaccharides. J. Phycol. **21:** 585–592.

Deshmukhe, G. V. and Tatewaki, M. 1990. The life history and evidence of the macroscopic male gametophyte in *Palmaria palmata* (Rhodophyta) from Muroran, Hokkaido, Japan. Jpn. J. Phycol. **38:** 215–222.

Dixon, P. S. 1973. *Biology of the Rhodophyta*. Hafner Press, New York.

Fetter, R. and Neushul, M. 1981. Studies on developing and released spermatia in the red alga, *Tiffaniella snyderae* (Rhodophyta). J. Phycol. **17:** 141–159.

Goodenough, U. W. 1991. *Chlamydomonas* mating interactions. p. 71–112. *In* M. Dworkin [ed.] Microbial Cell-Cell Interactions. American Society for Microbiology, Washington.

Hanic, L. A. and Craigie, J. S. 1969. Studies on the algal cuticle. J. Phycol. **5:** 89–102.

Heslop-Harrison, J. and Heslop-Harrison, Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Stain Technol. **45:** 115–120.

Kim, G. H. and Fritz, L. 1993a. Gamete recognition during fertilization in a red alga *Antithamnion nipponicum*. Protoplasma **174:** 69–73.

Kim, G. H. and Fritz, L. 1993b. Ultrastructure and cytochemistry of early spermatangial development in *Antithamnion nipponicum* (Ceramiaceae, Rhodophyta). J. Phycol. **29:** 797–805.

Kugrens, P. 1974. Light and electron microscopic studies on the development and liberation of *Janczewskia gardneri* Setch. spermatia (Rhodophyta). Phycologia **13:** 295–306.

Kugrens, P. 1980. Electron microscopic observations on the differentiation and release of spermatia in the marine red alga *Polysiphonia hendryi* (Ceramiales, Rhodomelaceae). Amer. J. Bot. **67:** 519–528.

Magruder, W. H. 1984. Specialized appendages on spermatia from the red alga *Aglaothamnion neglectum* (Ceramiales, Ceramiaceae) specifically bind with trichogynes. J. Phycol. **20:** 436–440.

Mine, I. and Tatewaki, M. 1993. Life history of *Halosaccion yendoi* I. K. Lee (Palmariales, Rhodophyta) and interspecific spermatium inoculation with *Palmaria* sp. from Hokkaido, Japan. Jpn. J. Phycol. **41:** 123–130.

Mine, I. and Tatewaki, M. 1994. Attachment and fusion of gametes during fertilization of *Palmaria* sp. (Rhodophyta). J. Phycol. **30:** 55–66.

Mitman, G. G. and Phinney, H. K. 1985. The development and reproductive morphology of *Halosaccion americanum* I. K. Lee (Rhodophyta, Palmariales). J. Phycol. **21:** 578–584.

O'Rand, M. G. 1988. Sperm-egg recognition and barriers to interspecies fertilization. Gamete Res. **19:** 315–328.

Peel, M. C. and Duckett, J. G. 1975. Studies of spermatogenesis in the Rhodophyta. Biol. J. Linn. Soc. Lond. **7** (supplement 1): 1–13.

Provasoli, L. 1963. Growing marine seaweeds. Proc. Int. Seaweed Symp. **4:** 9–17.

Roland, J. C. and Vian, B. 1991. General preparation

and staining of thin sections. p. 1–66. *In* J. L. Hall and C. Hawes [eds.] Electron microscopy of plant cells. Academic Press, London.

Rosati, F. 1985. Sperm-egg interaction in ascidians. p. 361–388. *In* C. B. Metz and A. Montroy [eds.] Biology of fertilization. Academic Press, New York.

Schmid, C. E. 1993. Cell-cell-recognition during fertilization in *Ectocarpus siliculosus* (Phaeophyceae). Hydrobiologia **261:** 437–443.

Scott, J. L. and Dixon, P. S. 1973. Ultrastructure of spermatium liberation in the marine red alga *Ptilota densa*. J. Phycol. **9:** 85–91.

Taylor, J. A. and West, D. W. 1980. The use of Evan's blue stain to test the survival of plant cells after exposure to high salt and high osmotic pressure. J. Exp. Bot. **31:** 571–576.

Turvey, J. R. and Williams, E. L. 1970. The structures of some xylans from red algae. Phytochemistry **9:** 2383–2388.

van der Meer, J. P. 1981. The life history of *Halosaccion ramentaceum*. Can. J. Bot. **59:** 433–436.

van der Meer, J. P. and Todd, E. R. 1980. The life history of *Palmaria palmata* in culture. A new type for the Rhodophyta. Can. J. Bot. **58:** 1250–1256.

峯　一朗・舘脇正和：紅藻ダルスの受精における配偶子表面と接着

紅藻ダルス目ダルス *Palmaria* sp. の配偶子表面の構造の性質を調べた。無固定の不動精子の無色の被膜は，細胞懸濁液に加えられた蛋白質分解酵素により，細胞の生存が損なわれずに分解された。化学固定を施した精子の被膜も同様に酵素的に分解された。受精毛細胞壁の表面は，過ヨウ素酸々化・水素化ホウ素ナトリウム還元処理により破壊される，隣接グリコール基を含む繊維状の構造により覆われていることが観察された。精子被膜の短時間の蛋白質分解酵素処理は，受精毛表層の隣接グリコールの破壊と同様に，配偶子接着に有意な阻害効果を示した。このことは本藻の配偶子の特異的接着が，主にこれらの表面構造により支配されていることを示している。

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