Journ. Fac. Sci., Hokkaido Univ. Ser. V (Botany), **14** (1) : 11-38, 1987.

Life history studies in culture of a *Mastocarpus* species (Rhodophyta) from central Japan

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Mastocarpus plants (= *Gigartina mammillosa* sensu MIKAMI) were collected at 9 localities covering its whole geographical range in Japan. A total of 286 plants were studied in laboratory culture using unialgal strains each derived from individual plants, inoculating carpospores or vegetative tips of blades. Two types of life history were found among the strains : a heteromorphic type with the alternation of foliose dioecious *Mastocarpus*-phase gametophytes and a crustose *Petrocelis*-phase tetrasporophyte ; and a direct type involving only cystocarpic *Mastocarpus* plants. The former type was found throughout the range. The latter, however, was present only at a single locality, where a mixed pattern, in which carposporelings from a cystocarpic plant developed into either *Petrocelis*-like crusts or *Mastocarpus* blades, was also observed. In addition, 4 strains from tetraspores of *Petrocelis*-phase plants sampled at 2 localities were investigated. The tetraspores grew into dioecious *Mastocarpus* gametophytes. Hybridization experiments with 14 female and 9 male plants from 8 localities indicated that interbreeding is potentially free among the populations studied. Heteromorphic type plants showed a short day response in reproduction, but direct type plants did not show such a photoperiodic response. These features agreed with respective reproductive phenologies in the field.

The red algal genus *Mastocarpus* KÜTZ. (Petrocelidaceae, Gigartinales) was recently reinstated for *Gigartina* STACKH. subgenus *Mastocarpus* (KÜTZ.) SETCH. et GARDN. (GUIRY *et al.*, 1984). At present 4 species are referred to this genus (GUIRY *et al.*, 1984). Life history studies of these species have been undertaken in the following areas : *M. stellatus* (STACKH. in WITHER.) GUIRY in the north Atlantic (GUIRY and WEST, 1983), *M. papillatus* (C. AG.) KÜTZ. in Pacific North America (POLANSHEK and WEST, 1977), *M. jardinii* (J. AG.) J. A. WEST in Pacific North America (WEST *et al.*, 1978) and *M. pacificus* (KJELLM.) PEREST. in northern Japan (MASUDA *et al.*, 1984). These studies have shown that each of the species has two types of life history : a heteromorphic type involving the alternation of foliose dioecious *Mastocarpus*-phase gametophytes with a crustose *Petrocelis*-phase tetrasporophyte ; and a direct type involving only cystocarpic *Mastocarpus* plants.

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An alga* which has been known as "*Gigartina mammillosa* (GOOD. et WOODW.) J. AG." in Japan is also, however, considered to belong to *Mastocarpus*. This alga has been reported from several localities on the Pacific coast of central Honshu, ranging from Chiba to Shizuoka Prefecture (YENDO, 1916; CHIHARA, 1958; MIKAMI, 1965). *Gigartina mammillosa* is a nomenclatural synonym of *Mastocarpus stellatus* (GUIRY *et al.*, 1984). *M. stellatus* is found from northern Russia to Mauritania in the eastern Atlantic and from Newfoundland to North Carolina in the western Atlantic (GUIRY and WEST, 1983). Its occurrence in Japan is an only record outside the Atlantic. There is a long pending question concerning the taxonomic relationship between the aforementioned alga that is narrowly restricted to the Pacific coast of Japan and *M. stellatus* (= *G. mammillosa*) that is widely distributed in the Atlantic. Vegetative and female reproductive features of the Japanese alga were described by MIKAMI (1965) and an outline of the life history was reported by MASUDA and KUROGI (1985). In this paper we present detailed information on the algal life history in culture and the results of hybridization experiments among the heteromorphic type strains. The taxonomic relationship between the alga and *M. stellatus* is discussed.

Materials and Methods

Mastocarpus and *Petrocelis* plants for culture experiments were collected from 1980 to 1983 at 9 localities listed in Table 1 and shown in Fig. 1. Additional collections of *Mastocarpus* and *Petrocelis* plants were made between 1979 and 1985 at these localities in order to obtain data on their reproductive phenologies. The materials were packed on ice in an insulated chest for transport to the laboratory or were sent by mail. Cultures were established 2-10 days after collection. Carpospores of *Mastocarpus* plants and tetraspores of *Petrocelis* plants were isolated into unialgal culture according to the methods described earlier (MASUDA and KUROGI, 1981; OHNO *et al.*, 1982). Reproductively sterile apices of *Mastocarpus* blades were surface sterilized and cultured (POLANSHEK and WEST, 1975). Spores or apices from individual plants were cultured separately from each other. Thus, each strain represents a single individual plant sampled. The cultures were placed in plant growth chambers illuminated with cool-white fluorescent lamps [2500-3500 lux (30-40 μE/m^2/s)]. The temperatures and photoperiods were regulated in the following combinations: 10°C, 16:8 LD

* Taxonomic treatment of this alga awaits further comparative studies with *Mastocarpus papillatus* to which the alga is most similar in morphology. See Discussion.

(light-dark cycle); 10° C, 8 : 16 LD; 15° C, 16 : 8 LD; 15° C, 8 : 16 LD; 15° C, 8 : $7\frac{1}{2}$: 1 : $7\frac{1}{2}$ LDLD; 20° C, 16 : 8 LD; 20° C , 8 : 16 LD; and 20° C, 8 : $7\frac{1}{2}$: 1 : $7\frac{1}{2}$ LDLD. The cultures were chiefly maintained at 15° C, 16 : 8 LD and were transferred to other conditions as occasion arose.

Using selected strains the effects of different temperatures and photoregimes on gametogenesis were examined under all the culture conditions mentioned above. Prior to each experiment excised blades derived from one (either female or male) or 2 (both female and male) plants of each strain were transferred to 10° C, 16 : 8 LD under which, after a month culture, these blades ceased forming procarps or spermatangia. They were then divided into 8 groups and subjected to each of the culture conditions. After 2 months the numbers of procarps just below the papilla apex were counted in surface view in grid squares totaling 1 mm^2. Similarly, after 2 months spermatangial production was examined by scraping from the male blade surface or serial longitudinal sections. Cultured fertile female and male plants were crossed to determine interfertility among the strains. The procedures were de-

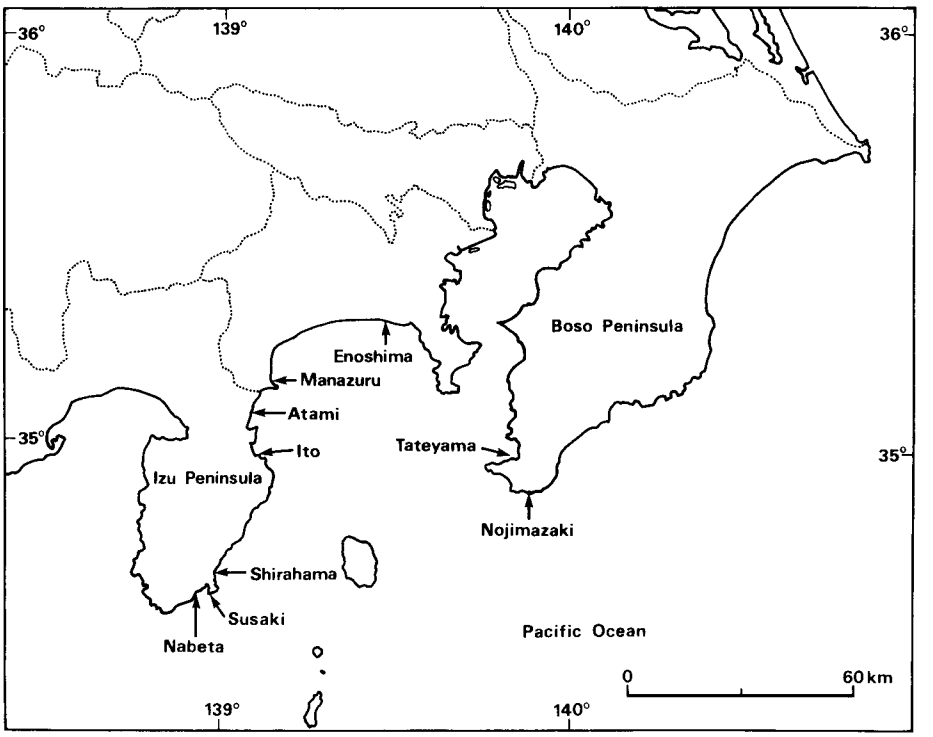

Fig. 1 Map of the Pacific coast of central Japan indicating collection sites.

scribed previously (POLANSHEK and WEST, 1975,1977). Crosses were undertaken on a Taiyo R-II Rotary Shaker at 90-100 rpm in 20° C, 8 : 16 LD. Numbers of cystocarpic and non-cystocarpic papillae and length of time required for carpospore discharge were recorded in each cross as indicators of the degree of interfertility. Plants derived from carpospores were cultured for 3-12 months to test their viability and fertility.

Microscopic preparations for anatomical examinations were made as described previously (MASUDA *et al.*, 1984). Voucher specimens and slides are deposited in the herbaria in Hokkaido University at Sapporo (SAP) and University of California at Berkeley (UC). Stock cultures of heteromorphic and direct type strains are maintained at the Center for Experimental Plants and Animals of Hokkaido University and in the Department of Botany, University of California, Berkeley.

Results

Carpospore cultures

Reproductive phenology of *Mastocarpus* plants is summarized in Table 2. Female plants with cystocarps were found throughout the year at only one locality, Shirahama in Shimoda. The female plants collected at this locality could be divided into two groups according to procarp abundance. The numbers of procarps were counted as described for cultured plants (see Materials and Methods). Female plants with procarps fewer than 100 per mm^2 of papilla tip surface were observed throughout the year. Their cystocarps and carpospore release were also found throughout the year. These plants grew in the lower intertidal zone and were not associated with male plants and *Petrocelis* crusts. On the other hand, female plants with abundant procarps (more than 300 per mm^2 of papilla tip surface) were found from September to February. Their developing cystocarps were observed from October onward and their mature cystocarps were found from November to February. These plants grew in the more upper intertidal zone than did the former plants and were always associated with male plants and *Petrocelis* crusts. Male plants with spermatangia appeared in September and persisted until February. They became greenish and heavily epiphytized in February, suggesting that the spermatangial production was in a terminal phase. Plants with spermatangia were not found from April onward. At the other localities female plants with abundant procarps and male plants with spermatangia were observed only from September onward as far as can be determined from our collections. Plants with mature cystocarps were restricted during late autumn and spring months.

Table 1. Collection data and results of carpospore, blade tip and tetraspore cultures from *Mastocarpus* sp.

Locality	Date	Plants Sampled	Isolation material	Culture number[1)]	Life history types: Heteromorphic ⊕	Heteromorphic ♀	Heteromorphic ♂	Direct	Mixed
Chiba Pref.									
Nojimazaki	9 v 82	3	B	1399, 1400, 1402			3		
	24 ix 82	2	B	1570, 1581		2			
		1	B	1582			1		
	19 xi 82	7	C	1654-1660	7*				
		3	B	1662-1664		3			
		2	B	1665, 1666			2		
	9 xii 83	3	C	2315-2317	3				
Tateyama	2 iv 81	5	C	795-799	5*				
		8	B	801-806, 808, 810		8			
		9	B	807, 809, 811-817			9		
	30 i 82	11	C	1122-1132	11*				
	30 iii 82	8	C	1353-1360	8				
	9 v 82	3	B	1403-1405		3			
	23 ix 82	2	B	1583, 1584			2		
		2	B	1585, 1586		2			
	21 iii 83	6	C	1818-1823	6				
Kanagawa Pref.									
Enoshima	2 viii 80	4	B	663, 665, 669, 670		4			
		3	B	664, 668, 672			3		
	10 i 82	12	C	1050-1061	12*				
		3	B	1062-1064		3			
		1	B	1065			1		
	21 ii 82	5	C	1212-1216	5				
Manazuru	2 iv 80	3	B	649, 650, 652			3		
		1	B	651		1			
	7 viii 80	3	B	673-675		3			
		1	B	676			1		
	6 ii 81	1	B	755			1		
		1	B	758		1			
		5	C	760-764	5*				
	11 i 82	6	C	1106-1111	6				
Shizuoka Pref.									
Atami	9 i 83	24	C	1740-1763	24*				
		3	B	1764-1766		3			
		2	B	1767, 1768			2		
Ito	4 iv 81	6	B	818, 819, 822, 823 830, 835		6			
		12	B	820, 821, 824-829, 832-834, 836			12		
	9 i 82	1	B	1077			1		
		1	B	1078		1			
		5	C	1080-1084	5*				
		2	T	1118, 1120		2			

Table 1. Continued

Locality	Date	Plants Sampled	Isolation material	Culture number[1)]	Life history types ⊕	Hetero-morphic ♀	Hetero-morphic ♂	Direct	Mixed
Ito	18 ix 82	1	B	1566		1			
Shimoda									
Shirahama	16 i 80	2	C	597, 598	2				
		1	C	599					1***
		9	C	600-602, YO 52-57				9**	
		2	T	603, 604		2			
	3 iv 80	7	C	628-634				7**	
		2	B	646, 647			2		
		1	B	648		1			
	2 viii 80	1	C	677				1	
		3	B	678-680				3**	
		5	B	681, 683, 684, 687 688		5			
	2 x 80	1	B	689		1			
		3	C	690-692				3	
		2	B	693, 696			2		
		3	B	697-700				3**	
	5 ii 81	3	C	726-728	3*				
		4	B	729, 733, 735, 736		4			
		4	B	730-732, 734			4		
		8	B	738-742, 744-746				8**	
	3 iv 81	3	B	849, 850, 852				3**	
	17 ix 82	5	C	1548-1552				5	
Shimoda									
Susaki	12 i 82	13	C	1032-1037, 1041, 1042, 1044-1048	13*				
		2	B	1038, 1049		2			
Shimoda									
Nabeta	5 ii 81	3	B	748, 751, 753		3			
		2	B	749, 750			2		
	13 i 82	1	B	1086		1			
		1	B	1087		1			
		18	C	1088-1105	18*				
Total		290	B: 127 C: 159 T: 4			247		42	1

1) Culture numbers refer to MM other than YO. Each strain represents single individual plants sampled. B, blade tip; C, carpospore; T, tetraspore; ⊕, formed *Petrocelis* crusts (*, bore tetrasporangia); ♀, developed procarpic papillae; ♂, formed spermatangia; **, released carpospores; ***, *Mastocarpus* blades released carpospores, although *Petrocelis*-like crusts did not sporulate. For the majority of blade tip cultures, the development of cystocarps in the absence of spermatangial blades was used as a criterion to recognize direct type life history, and dioecious gametophytes were considered part of a heteromorphic type life history. See the text for mixed type of life history and other details.

Mastocarpus plants had flattened upright blades with inrolled margins. The size and morphology of fertile blades were highly variable. The blades were 5-25 cm high and 0.2-6.5 cm wide. They were sometimes simple but usually they were dichotomously to palmately branched (Fig. 2). Female blades produced procarps and cystocarps in specially-developed papillae formed on their surface and margin. The number of papillae varied according to blade width: broad blades produced abundant papillae, whereas narrow blades produced a few papillae.

Liberated carpospores (Figs. 3A, 5A) were light red in color and averaged 17.7 μm (range 15-20 μm; 500 spores measured) in diameter. Carpospores from 159 individual plants were first cultured at 15° C, 16 : 8 LD. They germinated in a manner similar to that described for *M. pacificus* (MASUDA and UCHIDA, 1976; MASUDA and KUROGI, 1981; MASUDA *et al.*, 1984) and grew into crustose sporelings (Figs. 3B-E, 5B-E) or spherical masses of cells (Fig. 5G). These sporelings subsequently developed differently according to strains as follows. (1) All grew into *Petrocelis* crusts (Fig. 3F-I). (2) All developed directly upright *Mastocarpus* blades (Fig. 5F, I, J). (3) Either *Petrocelis*-like crusts or *Mastocarpus* blades were developed from sporelings derived from a single cystocarpic plant.

The first pattern was observed for many strains from all the localities examined (Table 1). Carpospores released from narrow blade plants (0.2-

Table 2. Summary data of reproductive phenology in *Mastocarpus*-phase gametophytes in the field

Locality	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Nojimazaki					v		v		p, s		p+c, s	p+c, s
Tateyama	p+c		c	c	v		v		p, s		p+c, s	
Enoshima	p+c, s	c	v		v			v	p, s		p+c, s	
Manazuru	p+c	c	v	v		v		v				
Atami	p+c, s							v				
Ito	c, s		v	v			v		p, s	p+c		
Shirahama[1]	p+c, s	p+c, s		v	v	v		v	p, s	p+c	p+c, s	p+c, s
	p+c	p+c		p+c	p+c	p+c		p+c	p+c	p+c	p+c	p+c
Susaki	p+c, s				v					p+c, s		
Nabeta	p+c, s	c		v	v	v		v	v		p, s	p+c, s

The data are based on Table 1 and additional collections made during 1979 and 1985. c, plants with cystocarps; p, plants with procarps; p+c, plants with both procarps and cystocarps; s, plants with spermatangia; v, plants without reproductive organs; blank, collections not attempted.

1) The data in the upper half are for plants growing at the upper to middle intertidal zone and those in the lower half for plants growing at the lower intertidal zone.

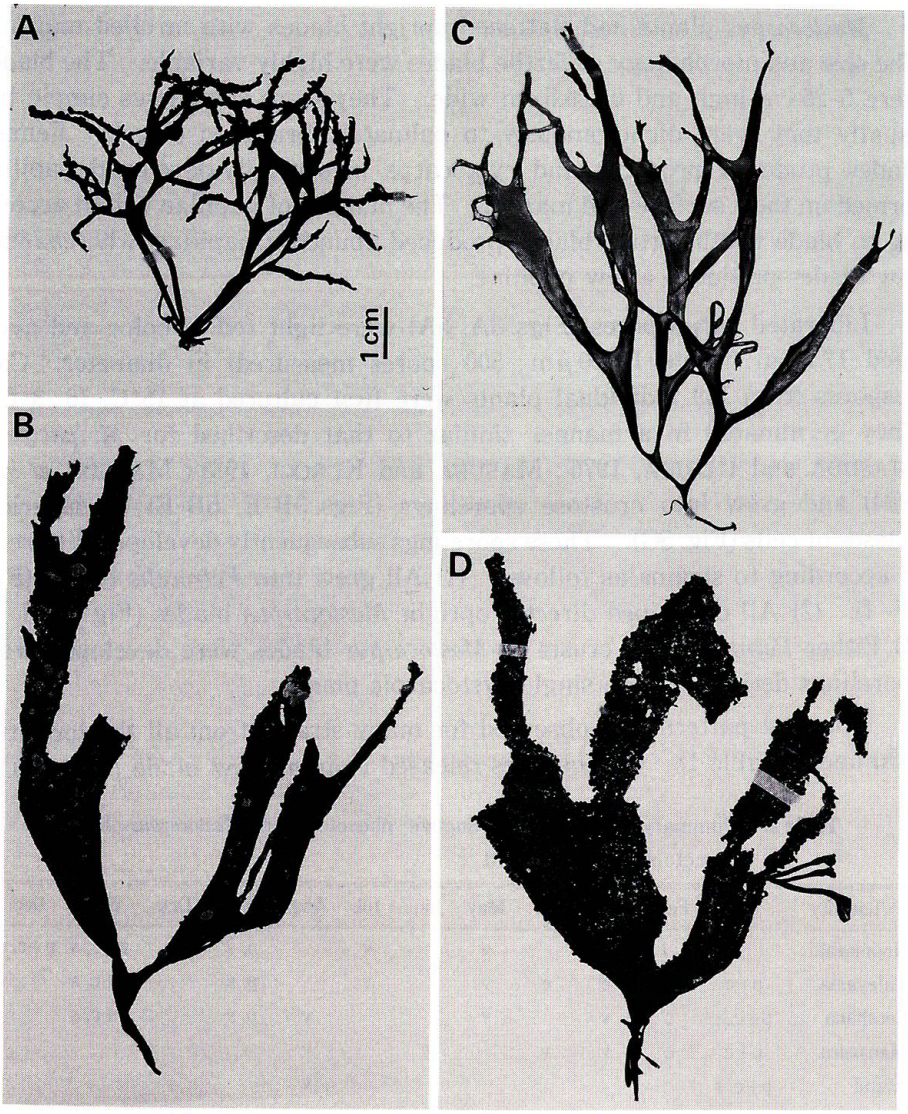

Fig. 2. Field-collected specimens of *Mastocarpus* sp. which were used in culture experiments. All specimens are dried. A : Cystocarpic plant for 598 (heteromorphic), Shirahama in Shimoda on January 16, 1980 (SAP 032239). B : Cystocarpic plant for 1744 (heteromorphic), Atami on January 9, 1983 (SAP 032297). C : Vegetative plant for 809 male, Tateyama on April 2, 1981 (SAP 032271). D : Cystocarpic plant for YO 52 (direct), Shirahama in Shimoda on January 16, 1980 (SAP 032240). Scale in A applies also to B-D.

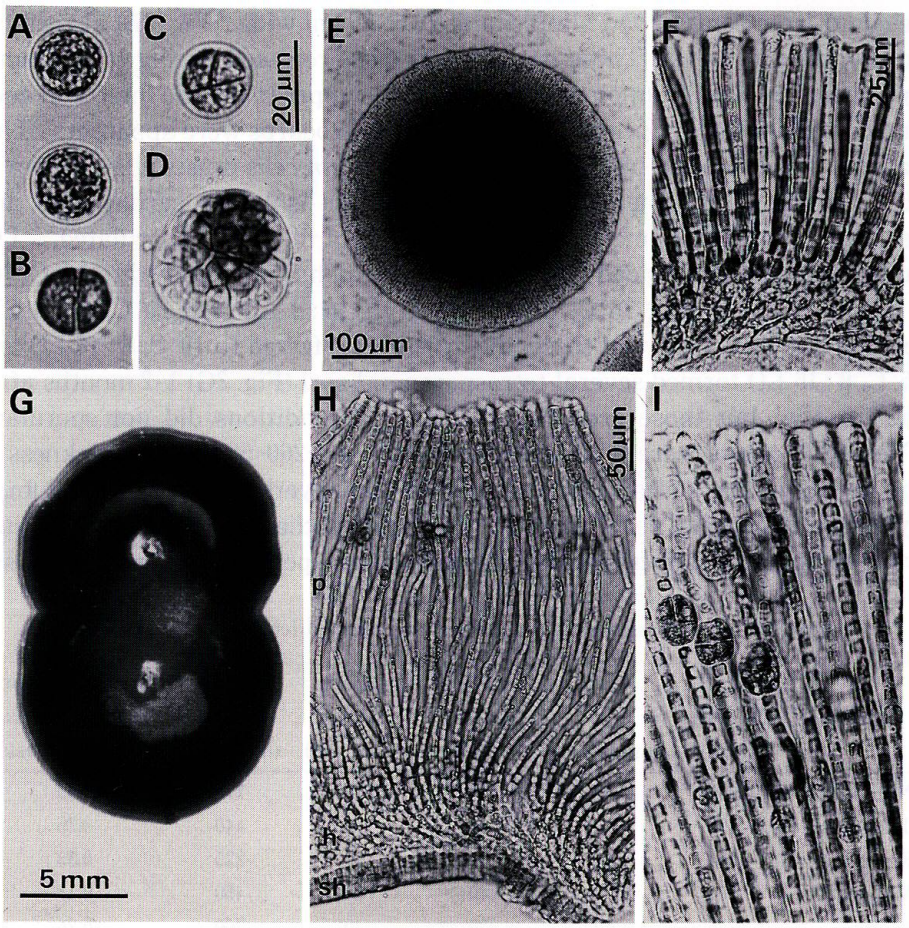

Fig. 3. Carpospore culture of *Mastocarpus* sp. with heteromorphic type life hisory. All photographs from living material. A-E : Carpospores and their early development at 15° C, 16 : 8 LD (Shirahama 598) ; A, carpospores ; B, one-day-old germling ; C, two-day-old one ; D, seven-day-old one ; E, one-month-old one. F : Radial section of a 2-month-old crust, showing *Petrocelis* anatomy, grown at 15° C, 16 : 8 LD (Enoshima 1051). G-I : Ten-month-old fertile tetrasporophytic crusts grown at 15° C, 16 : 8 LD for the first 8 months and then moved to 15° C, 8 : 16 LD (Manazuru 761) ; G, two coalescent crusts, note that the paler parts indicate tetrasporangial sori just after spore release ; H, radial section of a crust, showing the subhypothallium (sh), hypothallium (h) and perithallium (p) ; I, radial section of a crust, showing the upper part with nearly mature tetrasporangia. Scale in C applies also to A, B and D ; scale in F applies also to I.

0.5 cm wide; Fig. 2A) always showed this developmental pattern, but those released from some broad blade plants (0.6-3.0 cm wide; Fig. 2B) also showed the same pattern. These carpospores were released during late autumn and spring months from plants growing in the upper to middle intertidal zone and bearing abundant procarps so far as their procarps were apparent. This pattern was exhibited by 133 of 159 strains. *Petrocelis* crusts were grown at 15° C, 16 : 8 LD for the first 6-8 months after isolating carpospores and reached 7-9 mm in diameter. However, none of the crusts sporulated. The cultures were then divided into 6 groups of which 5 were moved to 10° C, 16 : 8 LD, 10° C, 8 : 16 LD, 15° C, 8 : 16 LD, 20° C, 16 : 8 LD and 20° C, 8 : 16 LD, leaving one as it was. Of these, the crusts transferred to 10° C, 8 : 16 LD and 15° C, 8 : 16 LD formed several tetrasporangial sori (Fig. 3G) 1-2 months after the transfer, but those grown in the other 4 conditions did not sporulate. The fertile crusts were 9-12 mm in diameter and 260-585 μm in thickness in the region of the sorus. They consisted of 3 layers : a subhypothallium, a hypothallium and a perithallium. The subhypothallium and hypothallium were composed of strongly coherent cells, but the perithallium was composed

Table 3. Thicknesses of cultured and field-collected fertile *Petrocelis* crusts

Locality	Culture Number (MM)	Crust (max. μm)	Subhypothallium (max. μm)	Hypothallium (max. μm)	Perithallium (max. μm)	Subhypothallium/hypothallium : crust thickess
Nojimazaki	1654	260	45	45	170	0.35
Tateyama	799	585	85	60	440	0.25
Enoshima	1061	300	25	50	225	0.33
Manazuru	761	550	40	60	450	0.18
Atami	1755	270	30	40	200	0.26
Ito	1080	265	20	30	215	0.18
	field[1)]	585	75	60	450	0.23
	field[2)]	525	75	50	400	0.24
	field[3)]	555	50	75	430	0.23
	field[4)]	505	40	45	420	0.17
Shirahama	726	375	50	75	250	0.33
	field[5)]	380	50	50	280	0.26
	field[6)]	320	30	40	250	0.22
Susaki	1033	340	35	55	250	0.26
Nabeta	1099	360	40	60	260	0.28

1, 2, 5, 6) Parent crusts of tetraspore cultures (1118, 1120, 603 and 604)
3, 4) These crusts were collected on January 9, 1982.

of loosely coherent filaments (Fig. 3H). The subhypothallium was derived from lowermost cells of the hypothallium and not found in young crusts (Fig. 3F). The thickness of the hypothallium was almost uniform throughout individual crusts, although it was somewhat variable among different crusts, ranging from 30 to 75 μm (Table 3). However, the thicknesses of the perithallium and subhypothallium varied according to the parts of a single plant. The perithallium was 170-450 μm thick in the central part and became gradually thinner toward the margin. The subhypothallium was 25-85 μm thick in the central part but it was not evident at the margin. The subhypothallium and hypothallium constituted 0.18-0.35 of the total crust thickness in the region of the sorus (Table 3). A single tetrasporangium was borne in an intercalary position on each vertical filament of the sorus (Fig. 3I). Tetrasporangia were cruciately divided and measured 30-35 μm long $\times$ 20-25 μm broad. Liberated tetraspores (Fig. 4A) were similar in many respects to carpospores and averaged 17.3 μm (range 12.5-20.0 μm ; 500 spores measured) in diameter. Tetraspores were first cultured at 15° C, 16 : 8 LD. The early germination pattern (Fig. 4B) was similar to that of carpospores and resulted in discoid germlings. After one and a half months discs reached 400-600 μm in diameter and began to produce upright terete axes (Fig. 4C). The discs were composed of closely appressed filaments of thick-walled cells similar to those of the basal discs of field-collected *Mastocarpus*. A subhypothallium (Fig. 4D, arrow) was formed in these basal discs similar to that of the tetrasporangial crusts. The upright axes became flattened apically and branched dichotomously. Upright blades reached 2.5-3.5 cm long, showing 2-4 dichotomies, but they did not become reproductive 5 months after germination. At this time upright blades were detached from basal discs of 16-20 individual plants in each strain and were transferred to 4 subcultures and grown at 15° C, 16 : 8 LD, 15° C, 8 : 16 LD, 20° C, 16 : 8 LD and 20° C, 8 : 16 LD. After 2 months the blades began to produce procarps and spermatangia in separate plants (Fig. 4E, male, H, female). Procarps were formed in papillate outgrowths borne on the surfaces and margins of female blades. The production of procarps usually took place under both long and short day regimes at 15° C and 20° C, although the number varied according to photoregimes and strains as shown later in a photoperiodic experiment (Tables 4, 5). Female blades of some strains produced procarps only under short day regimes. Each procarp consisted of a large supporting cell, a 3-celled carpogonial branch, and a 2-celled sterile branchlet borne on the first cell of the carpogonial branch (Fig. 4J). Procarpic papillae continued to grow, bearing many branchlets and formed carpogonial branches when female

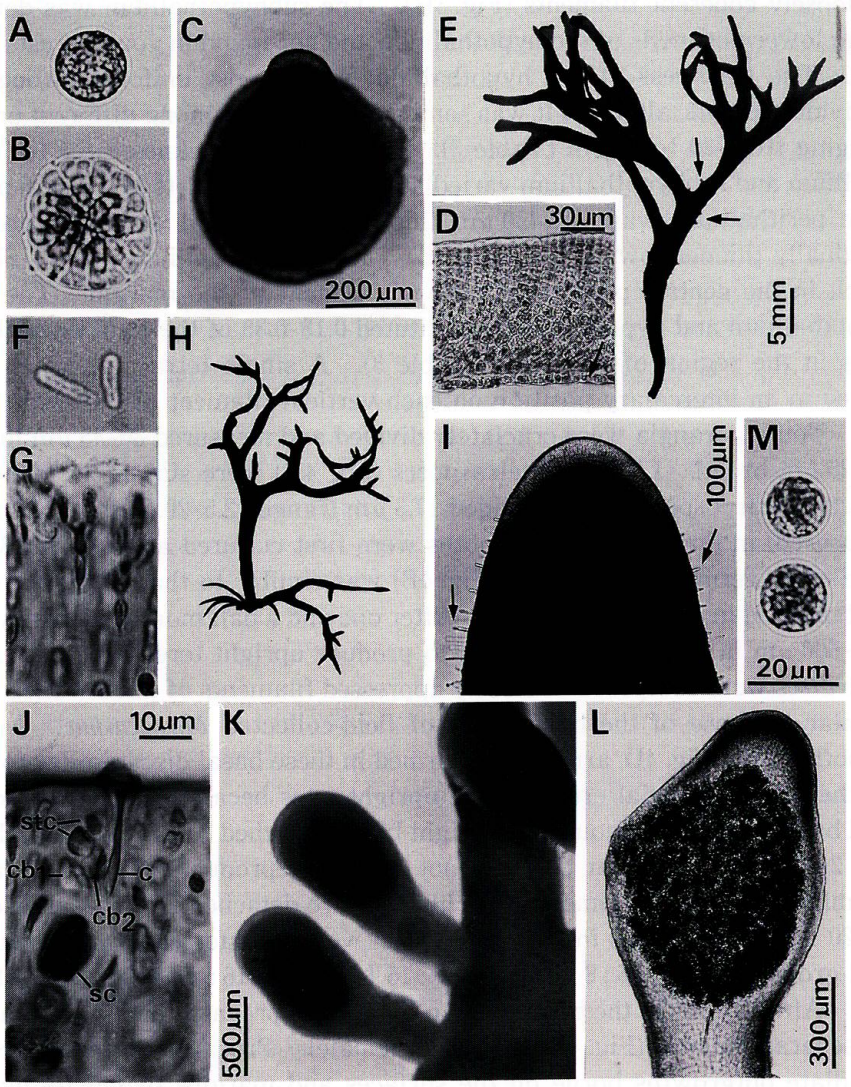

Fig. 4. Tetraspore culture in Manazuru 761. Photographs from living material unless otherwise indicated. A : Tetraspore. B-D : Tetrasporelings grown at 15° C, 16 : 8 LD ; B, five-day-old one ; C, one and a half months old one issuing an upright axis ; D, radial section of the basal disc with the subhypothallium (arrow) of a 4-month-old one. E : Seven-month-old spermatangial plant grown at 15° C, 16 : 8 LD for the first 5 months and then moved to 20° C, 8 : 16 LD (arrows indicate young proliferations). F : Released spermatia from the plant shown in E. G : Longitudinal section of the plant shown in E through a spermatangial sorus (stained with cotton blue). H : Seven-month-old procarpic plant grown at 15° C, 16 : 8 LD for the first 5 months and then moved to 20°

blades were cultured separately from male blades. Spermatangial production was easily recognized when white clouds of released spermatia were present on the surface of the plants. Such production was observed only on the blades grown at 15° C, 8 : 16 LD and 20° C, 8 : 16 LD. Neither released spermatia nor spermatangia were found under the other conditions. Spermatangial blades lacked papillate outgrowths, but they formed proliferations with indeterminate growth from the margins (Fig. 4E) and sometimes from the blade surfaces. Young proliferations were similar to papillae borne on female plants. Spermatangia were first formed on the upper portions of blades and later on proliferations. One or two elongated spermatangia were produced from each spermatangial parent cell (Fig. 4G). Released spermatia (Fig. 4F) were cylindrical and 10–16 μm × 2.5–3.8 μm. At 15° C, 8 : 16 LD and 20° C, 8 : 16 LD the spermatangial blades of most strains continued active growth and formed spermatangia continuously, but those of some strains did not produce further spermatangia after the initial release of numerous spermatia.

Female blades bearing procarps were mixed in single dishes with male blades releasing numerous spermatia derived from the same strain. These were placed on a rotary shaker at 20° C, 8 : 16 LD (the most suitable condition for the production of procarps and spermatangia). Slightly swollen papillae with rounded apices were observed on the female blades 14 days after the initiation of crosses. They developed cystocarps (Fig. 4K, L) which released carpospores (Fig. 4M) 23–34 days afterward. The resulting carpospores were similar to those from fields and measured 15–20 μm in diameter. Thus, for many strains this heteromorphic type of life history was completed within 16–19 months in the laboratory. After carpospore release, most strains were not investigated further, however, for some strains (Tateyama 796; Enoshima 1055, 1061; Manazuru 761, 765; Nabeta 1095, 1099) it was confirmed that all carpospore cultures formed *Petrocelis* crusts which sub-

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C, 8 : 16 LD. I : Surface view of a papilla formed on the plant shown in H, note that many trichogynes are evident (arrows). J : Longitudinal section of a papilla formed on the plant shown in H, showing a young procarp that consists of a supporting cell (sc), a 3-celled carpogonial branch (cb_1, cb_2,c) and 2 sterile cells (stc). K : Cystocarpic papillae formed on the plant shown in H which was later crossed with a spermatangial plant (preserved specimen). L : Longitudinal section of a mature cystocarpic papilla formed on the plant shown in H. M : Released carpospores from the plant shown in H. Scale in E applies also to H ; scale in J applies also to F and G ; scale in M applies also to A and B.

sequently produced tetrasporangia as described above.

The second pattern was observed for 25 of the 159 plants. All these plants had broad blades (2.0-6.5 cm wide ; Fig. 2D) and were collected at one locality, Shirahama in Shimoda (Table 1). Carpospores of this pattern were released throughout the year from plants growing in the lower intertidal zone. The carpospores were similar in dimensions to those of plants of the first pattern mentioned above and showed the same initial developmental pattern at 15° C, 16 : 8 LD (Fig. 5A-E) as them. However, these sporelings grew into discs composed of strongly coherent cells and directly formed upright axes after one and a half months culture (Fig. 5F). These upright axes grew into dichotomously divided blades similar in morphology to *Mastocarpus* plants from the field. Some sporelings grew into spherical masses of cells which did not form a marginal meristem (Fig. 5G). They developed single upright axes which later became easily dislodged from the substrate by the slightest mechanical disturbance. These axes also formed dichotomously divided *Mastocarpus* blades. Ten-month-old, attached or detached plants did not show any reproduction at 15° C, 16 : 8 LD. Individual plants were then isolated into separate culture dishes. Subcultures of several strains (600, 601, 602, YO 52, 53, 56, 57) were transferred to 15° C, 8 : 16 LD, 20° C, 16 : 8 LD and 20° C, 8 : 16 LD. Two months after the transfer plants grown at 20° C, 16 : 8 LD formed numerous surface and marginal papillae (Fig. 5I, J). These papillae produced small numbers of procarps (approximately 30-80/mm^2) (Fig. 5H) and developed cystocarps in the absence of male blades (Fig. 5K). These cystocarps subsequently discharged numerous viable carpospores (Fig. 5L). Plants of the other subcultures and those maintained at 15° C, 16 : 8 LD also formed cystocarps in the absence of male plants later. Neither spermatia nor spermatangia were not observed on these plants. Carpospores from 601, YO 52 and YO 56 strains were cultured at 20° C, 16 : 8 LD. These sporelings also grew into basal discs from which upright blades issued.

One strain (599) showed the third pattern. Carposporelings derived from a single cystocarpic plant from Shirahama in Shimoda grew into either basal discs which formed upright *Mastocarpus* blades (54 of 72 sporelings) or *Petrocelis*-like crusts (18 of the 72 sporelings). Of these plants, 16 *Mastocarpus* plants and 8 *Petrocelis*-like crusts were cultured for 18 months. Individual *Mastocarpus* plants isolated into separate dishes and cultured at 15° C, 16 : 8 LD became reproductive 14 months after inoculation. These plants produced many papillae on the surfaces and margins which bore about 40-70 procarps per mm^2 on their apices and developed cystocarps in the absence of

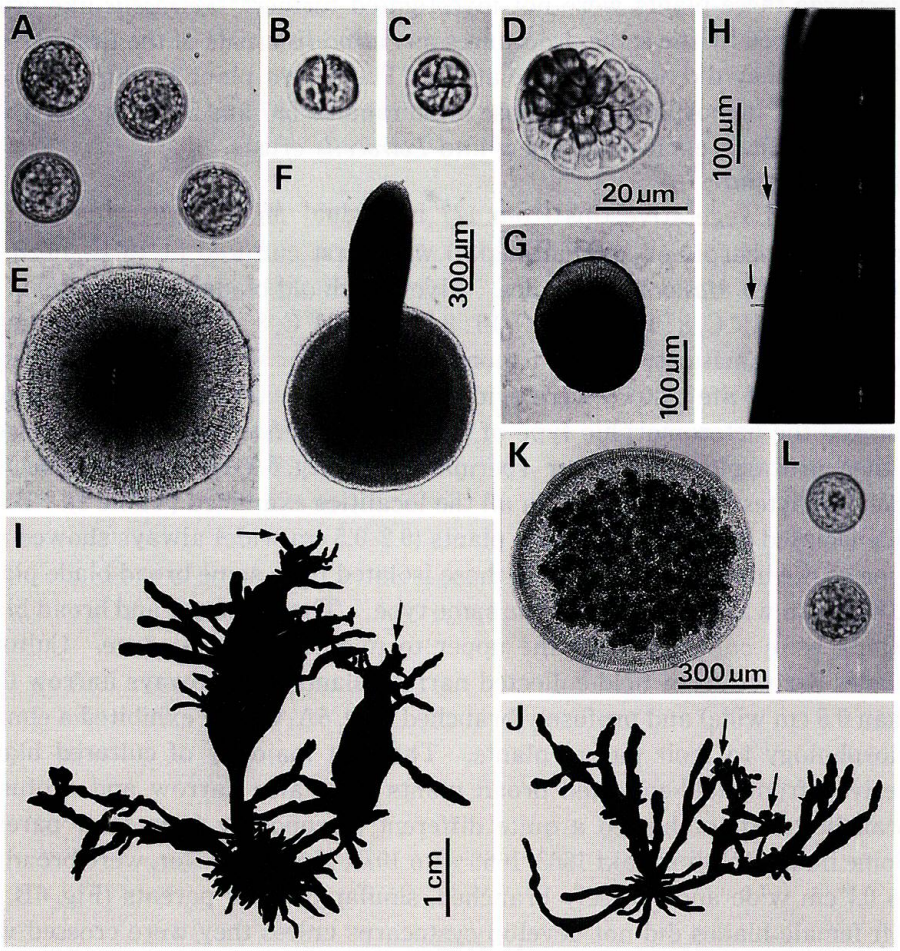

Fig. 5. Carpospore culture with direct type life history. All photographs from living material. Shirahama strains (A-H, K, L, 600 ; I, J, YO 52) grown at 15° C, 16 : 8 LD. A-G : Carpospores and their early development ; A, carpospores ; B, one-day-old germling ; C, two-day-old one ; D, seven-day-old one ; E, G, one-month-old ones, note that the spherical germling in G has no marginal meristem ; F, two-month-old one issuing an upright axis. H : Surface view of a procarpic papilla formed on a 12-month-old plant, showing two trichogynes (arrows). I, J : Fifteen-month-old cystocarpic plants (arrows indicate cystocarpic papillae). K : Cross section of a cystocarpic papilla formed on the plant shown in J. L : Released carpospores from the plant shown in J. Scale in D applies also to A-C and L ; scale in G applies also to E ; scale in I applies also to J.

male plants as did those of plants of the second pattern. After carpospore discharge these plants were not investigated further. *Petrocelis*-like crusts were cultured by the same procedures as *Petrocelis* crusts of the first pattern. However, they did not sporulate at all. The parent plant of Shirahama 599 was similar in morphology to those of Shirahama 597 and 598 (Fig. 2A) whose carposporelings exclusively grew into *Petrocelis* crusts.

Blade tip cultures

Excised vegetative tips from 127 individual *Mastocarpus* plants (vegetative, cystocarpic or spermatangial) were first cultured at 15° C, 16 : 8 LD and grew into *Mastocarpus* blades. Five-month-old blades were grown at 15° C, 16 : 8 LD, 15° C, 8 : 16 LD, 20° C, 16 : 8 LD and 20° C, 8 : 16 LD. They reached reproductive maturity after 2–5 months under all or some of these conditions. Two types of life history were evident as follows. (1) 110 of the 127 strains showed the heteromorphic type of life history. *Mastocarpus* blades formed either procarpic papillae or spermatangia (Fig. 6A–D). These dioecious gametophytes were observed in all the localities examined (Table 1). Blade tips isolated from narrow blade plants (0.2–0.5 cm wide) always showed this type of life history. In addition, those isolated from some broad blade plants (0.6–3.0 cm wide) also showed the same type. These narrow and broad blade plants were collected from the upper to middle intertidal zone. Cultured blades derived from field-collected narrow plants were always narrow (less than 0.5 cm wide) and profusely branched (Fig. 6A, C) and exhibited a similar morphology to their parent plants. The vast majority of cultured blades derived from field-collected broad plants were also narrow and profusely branched and so showed a quite different morphology from their parents. Some blades (Nojimazaki 1663, 1665 ; Ito 1078, 1566), however, were broad, up to 0.7 cm wide and sparsely branched, similar to their parents (Fig. 6B, D). All female blades did not develop cystocarps unless they were crossed with male blades. Resulting carposporelings exclusively grew into *Petrocelis* crusts. (2) 17 of the 127 strains showed the direct type of life history. All the strains were obtained from the lower intertidal zone at one locality, Shirahama in Shimoda (Table 1). *Mastocarpus* blades produced cystocarps, releasing carpospores, in the absence of male blades. Blade tips isolated from plants with 2.0–6.5 cm width showed this type of life history. Cultured blades (Fig. 6E) were up to 0.8 cm wide and narrower than their parent plants. After carpospore release, most of them were not investigated further, but carpospores from 698, 738, 850 and 852 were cultured. These carpospores germinated and grew into *Mastocarpus* blades again.

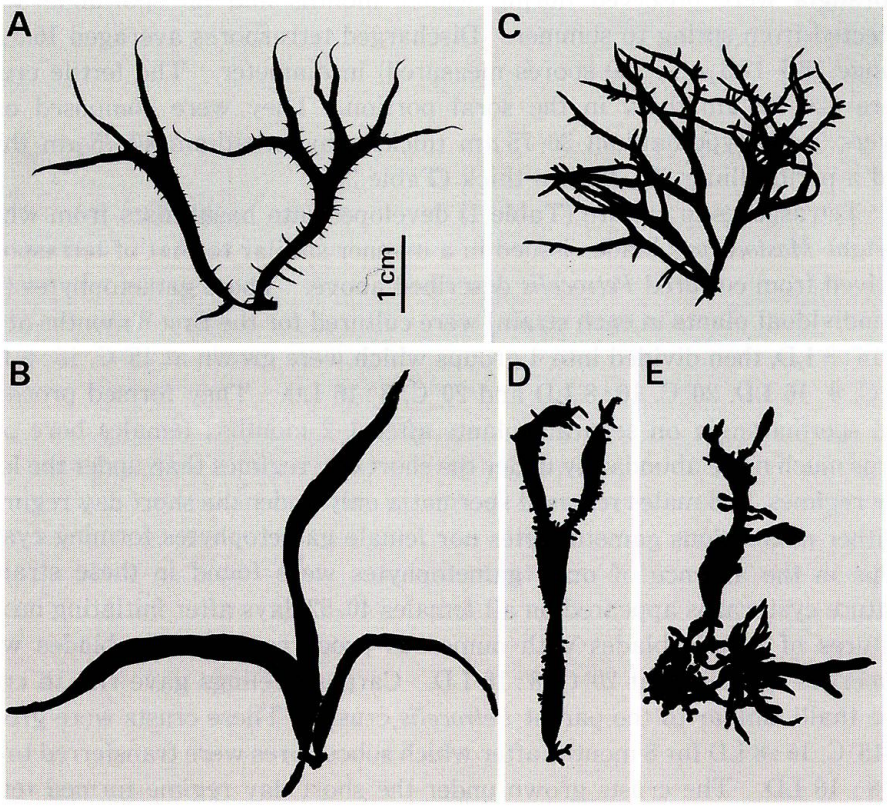

Fig. 6. Blade tip culture grown at 15° C, 16 : 8 LD for the first 5 months and then moved to other conditions. All photographs from living specimens. A : Seven-month-old spermatangial plant moved to 15° C, 8 : 16 LD (Enoshima 1065). B : Ten-month-old spermatangial plant move to 20° C, 8 : 16 LD (Nojimazaki 1665). C : Ten-month-old procarpic plant moved to 20° C, 8 : 16 LD (Tateyama 808). D : Ten-month-old procarpic plant moved to 20° C, 8 : 16 LD (Nojimazaki 1663). E : Ten-month-old cystocarpic plant with direct type life history moved to 20° C, 16 : 8 LD (Shirahama 741). Scale in A applies also to B-E.

Tetraspore cultures

Petrocelis crusts grew on rocks in the upper to middle intertidal zone associated with *Mastocarpus* plants at the localities examined. Reproductive phenology of *Petrocelis* crusts was examined at Shirahama in Shimoda. The crusts were found throughout the year. They were vegetative from April to September. Tetrasporangial primordia were observed in October when the daylength was about 11 hr. Mature, cruciately divided tetrasporangia were found from December to February. At the other localities mature tetra-

sporangia were observed during winter months and no sporangia were detected from spring to summer. Discharged tetraspores averaged 15.3 μm (range 12.5-17.5 μm; 100 spores measured) in diameter. The fertile crusts were 320-585 μm thick in the soral portion. They were composed of 3 layers: a subhypothallium 30-75 μm thick, a hypothallium 40-75 μm thick and a perithallium 250-450 μm thick (Table 3).

Tetraspores in culture (Table 1) developed into basal discs from which upright *Mastocarpus* blades issued in a manner similar to that of tetraspores derived from cultured *Petrocelis* described above. These gametophytes (16-20 individual plants in each strain) were cultured for the first 8 months at 15° C, 16 : 8 LD, then divided into 4 groups which were grown at 15° C, 16 : 8 LD, 15° C, 8 : 16 LD, 20° C, 16 : 8 LD and 20° C, 8 : 16 LD. They formed procarps and spermatangia on separate plants after 1-2 months; females bore procarps much more abundantly under the short day regimes than under the long day regimes, and males released spermatia only under the short day regimes. Neither monoecious gametophytes nor female gametophytes forming cystocarps in the absence of male gametophytes were found in these strains. Mature cystocarps appeared on all females 40-52 days after initiating mixed cultures of female blades with numerous procarps and male blades with numerous spermatia at 20° C, 8 : 16 LD. Carposporelings gave rise to crustose thalli similar to the parent *Petrocelis* crusts. These crusts were grown at 15° C, 16 : 8 LD for 8 months after which subcultures were transferred to 15° C, 8 : 16 LD. The crusts grown under the short day regime formed tetrasporangia and released viable tetraspores 1-2 months after the transfer, whereas control cultures at 15° C, 16 : 8 LD remained vegetative.

Photoperiodism

The effects of different temperatures and photoregimes on gametogenesis were examined using 12 female plants (Table 4) and 8 male plants (Table 5). These plants were preconditioned as described in Materials and Methods. No procarps were formed at 10° C; at this temperature papillae became flattened apically and grew into vegetative branches. Nojimazaki 1662, Manazuru 673, Atami 1765 and Nabeta 1090 female plants produced procarps only under short day regimes at 15° C and 20° C. All these female plants formed more abundant procarps at 20° C than at 15° C (Table 4). The other female plants, however, formed procarps under all photoregimes attempted at 15° C and 20° C. These plants also formed more abundant procarps at 20° C than at 15° C, but under the long day and night-break regimes only one half to one seventh the number of procarps borne under the short day regimes were formed (Table 4). The interrupted-night regimes showed

Table 4. Number of procarps per mm² papilla surface in female strains of *Mastocarpus* cultured at different temperatures and photoregimes

Locality	Culture number (MM)	10°C		15°C			20°C		
		S	L	S	B	L	S	B	L
Nojimazaki	1662	0	0	356±40	0	0	920±78	0	0
Tateyama	796	0	0	374±80	148±60	150±50	740±90	303±77	240±60
Enoshima	665	0	0	838±120	372±54	380±60	1216±107	620±100	400±100
	670	0	0	740±100	300±44	350±50	1308±150	600±90	428±80
	1055	0	0	514±65	259±40	260±42	1047±180	267±65	258±80
Manazuru	673	0	0	344±70	0	0	534±76	0	0
	761	0	0	670±80	333±80	320±60	1163±82	602±38	436±98
Atami	1764	0	0	415±80	73±28	768±74	112±38	108±43	
	1765	0	0	450±65	0	0	903±109	0	0
Ito	1118	0	0	414±84	65±16	59±15	763±103	229±46	225±30
Shirahama	648	0	0	415±93	182±48	127±42	745±88	383±111	337±64
Nabeta	1090	0	0	532±105	0	0	1195±103	0	0

B, 8 : $7\frac{1}{2}$: 1 : $7\frac{1}{2}$ LDLD; L, 16 : 8 LD; S, 8 : 16 LD. Ten papillae of 5 blades (2 from each blade) were counted for each set (S, B, L) and mean and standard deviation were shown.

entirely or almost the same effect as the long day regimes. In this experiment only a single plant of each strain was examined. In each of Tateyama 796, Enoshima 1055, Manazuru 761, Ito 1118 and Nabeta 1090 strains 8-12 individual female plants derived from the same *Petrocelis* crust were grown under short day and long day regimes at 15° C and 20° C (see the sections of carpospore cultures and tetraspore cultures). All plants of each strain showed the same photoperiodic response as the plant shown in Table 4. Female plants thus showed a short day response in the formation of procarps. The response is either absolute or quantitative according to the strains examined.

Spermatangia were formed only under short day regimes at 15° C and 20° C (Table 5). The male plants with equal culture numbers to females shown in Table 4 were derived from the same *Petrocelis* crusts, respectively. These female plants except Nabeta 1090 showed a quantitatively short day response (Table 4). In each of Tateyama 796, Enoshima 1055, Manazuru 761, Ito 1118 and Nabeta 1090 strains 8-12 individual male plants derived from the same *Petrocelis* crust were cultured under short day and long day regimes at 15° C and 20° C. No plants formed spermatangia when they were maintained under long day regimes (see the sections of carpospore cultures and tetra-

Table 5. Spermatangial production in male strains of *Mastoparpus* cultured at different temperatures and photoregimes

Locality	Culture number (MM)	10°C		15°C			20°C		
		S	L	S	B	L	S	B	L
Tateyama	796	−	−	+	−	−	+	−	−
	809	−	−	+	−	−	+	−	−
Enoshima	664	−	−	+	−	−	+	−	−
	1055	−	−	+	−	−	+	−	−
Manazuru	761	−	−	+	−	−	+	−	−
Ito	825	−	−	+	−	−	+	−	−
	1118	−	−	+	−	−	+	−	−
Nabeta	1090	−	−	+	−	−	+	−	−

B, 8 : $7\frac{1}{2}$: 1 : $7\frac{1}{2}$ LDLD; L, 16 : 8 LD; S, 8 : 16 LD. +, spermatangia were produced; −, spermatangia not produced. Five blades were cultured at each condition and 5 branch apices of each blade were examined.

spore cultures). Other male plants than those shown in Table 5 also did not produce spermatangia when they were cultured under long day regimes as described above. Male plants of all strains examined thus showed an absolutely short day response for gametogenesis.

Hybridization experiments

Heteromorphic type strains including both broad and narrow blade forms of 14 females and 9 males were selected to represent 8 localities covering the geographical range and crossed to test interfertility (Fig. 7). Cystocarp development and viable carpospore release were observed for all attempted crosses (Fig. 7). Since no cystocarps were observed in isolated female controls, the results indicate the occurrence of fertilization in these crosses. Percent occurrence of cystocarpic papillae in total papillae formed varied from 11 to 100%; in most crosses it was more than 50% and in a few crosses less than 20%. In the 7 crosses in which fewer than 20% of the papillae formed cystocarps there appears to be no relationship between blade width and percent fertilization; for example, Tateyama 796 female with broad blade × Nojimazaki 1400 male with broad blade, Enoshima 669 female with narrow blade × Nabeta 1099 male with broad blade, Enoshima 1064 female with narrow blade × Manazuru 761 male with narrow blade. Two crosses, Nojimazaki 1581 female × Ito 1118 male and Enoshima 1064 female × Tateyama 796 male were repeated. These crosses showed higher frequency of cystocarp production when the females were crossed with male blades discharging numerous spermatia. For example, in the first cross

between Enoshima 1064 female and Tateyama 796 male only 5 of the 45 papillae formed cystocarps, but in the second cross 25 of the 30 papillae developed cystocarps. This indicates that the low frequency of cystocarp production depended on the amount of spermatia released rather than on any other factors.

The time required for carpospore release varied also from 37 to 99 days; in most crosses carpospores were released in 37-55 days and in a few crosses they were released in 70-99 days. The latter crosses appeared also regardless of blade width. There was a strong correlation between the first appearance of swollen papillae with rounded apices and the number of days required for carpospore release. For example, in a cross between Nojimazaki 1581 female and Nabeta 1099 male swollen papillae appeared 14 days after initiation of the cross and carpospores were released 23 days thereafter, whereas in a cross between Shirahama 648 female and Enoshima 1065 male swollen papillae appeared 70 days after initiation of the cross and carpospores were discharged 29 days thereafter. This indicates that delayed carpospore release was not due to slow development of cystocarps but due to delayed fertilization. The following 3 crosses in which swollen papillae were late in appearing were repeated: Enoshima 669 female × Manazuru 761 male; Ito 823 female × Ito 825 male; and Shirahama 648 female × Enoshima 1065 male. These females formed swollen papillae 14-17 days after initiation of the cross and mature cystocarps released spores 24-30 days thereafter when the females were crossed with males discharging numerous spermatia. The delayed carpospore release may also be attributed to limited spermatial production. Thus, crosses between plants with different blade widths did not show any significant differences as compared with crosses between plants with similar blade widths.

Carposporelings were grown at 20° C, 8 : 16 LD for the first a month and then moved to 15° C, 16 : 8 LD. No significant differences in growth rate and morphological appearance among the sporelings were observed and they gave rise to *Petrocelis* crusts. The crust diameter increased at the rate of 1.0-1.2 mm a month under 15° C, 16 : 8 LD. *Petrocelis* crusts derived from 79 cross-combinations (those labeled with an asterisk in Fig. 7) were tested for tetrasporogenesis, although those derived from other crosses were terminated 3-5 months after germination. The crusts grown at 15° C, 16 : 8 LD for 6-8 months reached 7-10 mm in diameter and were divided into 2 groups; one was moved to 15° C, 8 : 16 LD and the other was retained at 15° C, 16 : 8 LD or transferred to 15° C, 8 : $7\frac{1}{2}$: 1 : $7\frac{1}{2}$ LDLD. The crusts grown at 15° C, 8 : 16 LD formed tetrasporangia 1-3 months after the transfer and subsequently

discharged viable tetraspores. However, the crusts grown during subsequent 4 months under the long day and light-break regimes did not sporulate. Consequently, these cultures were terminated.

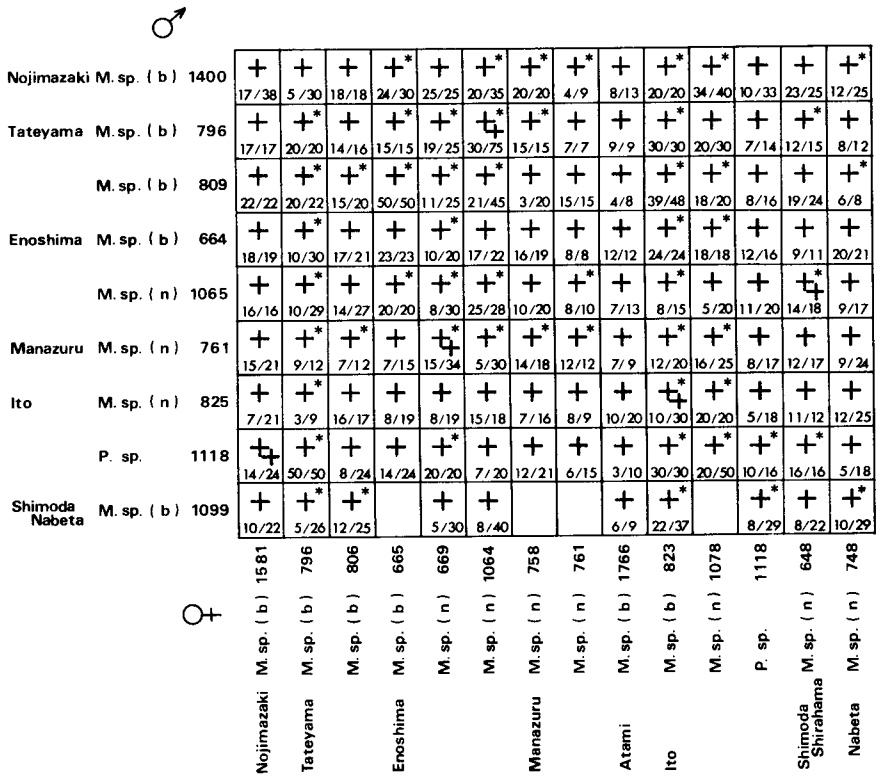

Fig. 7. Results of hybridization between gametophytic plants derived from field *Mastocarpus* sp. (M. sp.) and *Petrocelis* sp. (P. sp.). Each number designates the individual culture number. (b), parent plant of cultured strain with broad blade exceeding 6 mm wide; (n), parent plant of cultured strain with narrow blade less than 5 mm wide; +, carpospores released from female plants; $^{+}_{+}$, cross repeated for the first cross showing either low frequency of cystocarp production or delayed carpospore release; asterisk, *Petrocelis* crusts (cross progeny) tested for sporogenesis and tetraspores released from the crusts; blank, cross not attempted. For each cross attempted the number of papillae with cystocarps and the total number of papillae formed are listed (e. g., 17/38); for repeated crosses the totals of both crosses are listed.

Discussion

The alga under study might be divided into two groups according to the types of life history: the heteromorphic type involving the alternation of foliose dioecious *Mastocarpus*-phase gametophytes with a crustose *Petrocelis*-phase tetrasporophyte; and the direct type involving only cystocarpic *Mastocarpus* plants. Nevertheless, the mixed pattern, in which carposporelings from a single plant grew into either *Petrocelis*-like crusts or basal discs with upright *Mastocarpus* blades, was also observed in one locality. At present we can hardly assess any reproductive significance of this mixed pattern, as neither sporulation in the *Petrocelis*-like crusts nor spermatangial formation on the *Mastocarpus* blades was observed. These two types of life history are also known in *Mastocarpus papillatus* (POLANSHEK and WEST, 1977), *M. jardinii* (WEST *et al.*, 1978), *M. stellatus* (GUIRY and WEST, 1983) and *M. pacificus* (MASUDA *et al.*, 1984). Geographical distribution of heteromorphic and direct type plants was reported previously. Both types are widely distributed in 3 species except *M. jardinii*. In *M. papillatus* and *M. stellatus*, however, the heteromorphic type plants predominate in the southern localities of their geographical range and the direct type plants are frequent in the northern localities. In *M. pacificus* no clear distributional pattern is known. The alga considered in this report shows a distributional pattern similar to *M. jardinii*: heteromorphic type plants occur throughout the geographical range and direct type plants have a narrow range. As pointed out in an earlier paper (MASUDA *et al.*, 1984), direct type plants seem to be derived from heteromorphic type plants, although the mechanism has not yet been elucidated. The sympatric occurrence of heteromorphic and direct type plants within a very restricted area, Shirahama in Shimoda (Fig. 2) may suggest that the direct type plants have recently developed in the alga under study.

It should be noted that extremely different numbers of procarps are formed on the heteromorphic and direct type plants of the alga under study as has been found in *M. papillatus* (POLANSHEK and WEST, 1977), *M. stellatus* (GUIRY and WEST, 1983) and *M. pacificus* (MASUDA *et al.*, 1984). The heteromorphic type females bear 650–1300 procarps per mm^2 of papilla tip surface, but the direct type females produce only 30–80 procarps at most. Because procarps of heteromorphic type females need to be fertilized for cystocarp formation; it is of advantage to the females that they bear numerous procarps in order to receive spermatia released from separate males. On the contrary, the direct type females are apogamous (EDELSTEIN *et al.*, 1974) or

autogamous (GUIRY and WEST, 1983), so that they apparently do not need such large numbers of procarps for cystocarp formation (the species of *Mastocarpus* usually produce a single cystocarp in each papilla).

Reproduction in heteromorphic and direct type plants in the laboratory showed different photoregime responses. The heteromorphic type plants showed a short day response in gametogenesis and tetrasporogenesis, whereas the direct type plants showed a daylength neutral response in the formation of procarps. In the field gametogenesis and tetrasporogenesis of the heteromorphic type plants were restricted during autumn and winter months, but female gametogenesis of the direct type plants was observed throughout the year. Thus, the data obtained in the laboratory agree well with the facts found in the field. This is a first report that shows a clear distinction of reproductive phenology between heteromorphic and direct type plants of *Mastocarpus* species. Year-round gametogenesis is also found in direct type plants of *M. pacificus* (OHNO *et al.*, 1982). Reproductive phenology of heteromorphic type plants of that alga, however, has not been elucidated (MASUDA and KUROGI, 1985). The short day response both in gametogenesis and tetrasporogenesis shown in heteromorphic type plants of the alga under study has not been found for other *Mastocarpus* species. *M. papillatus, M. stellatus* and *M. pacificus* show a short day response only in tetrasporogenesis (POLANSHEK and WEST, 1975, 1977; GUIRY and WEST, 1983; MASUDA *et al.*, 1984). The adaptive significance of a short day response in heteromorphic type plants and a daylength neutral response in direct type plants can be interpreted in relation to their growing zones as follows. In summer months maximum surface seawater temperature is 25° C and maximum air temperature exceeds 30° C (YOKOHAMA, pers. comm.). The growing zone of the heteromorphic type plants becomes strongly exposed to sunshine and desiccation in the low-tide. The following preliminary experiments were conducted at the laboratory using 3-day and 7-day-old carposporelings derived from heteromorphic and direct type plants and their parent plants which were collected at Shirahama in Shimoda on November 17, 1985. Wetted (not immersed in culture medium) and dried (by air at each temperature tested for 1 hr) samples placed in Petri dishes were subjected to 10° C, 15° C, 20° C, 25° C and 30° C (65-72% relative humidity) for 2 hr, then transferred to culture medium, and were grown at 15° C, 16 : 8 LD for 7 days. Wetted sporelings survived at all temperatures tested, but none of dried sporelings survived. No significant differences in percent survival between heteromorphic and direct type sporelings were observed. All parent plants survived. This indicates that well-developed plants have desicca-

tion tolerance but young sporelings are vulnerable. The summer climate may operate as one of environmental pressures on the heteromorphic type plants growing in the upper to middle intertidal zone. It may not strongly affect the direct type plants growing in the lower intertidal zone. The spore release during late autumn and winter in the heteromorphic type plants has adaptive advantage. During this season desiccation stress is less because of the night low-tide. The direct type plants can avoid desiccation during summer months, because they grow in the lower zone. Phenological information on other *Mastocarpus* species is needed in relation to their life history types.

The geographical distribution of the alga in question was confirmed by the present study: the alga is found only in a small region from Boso Peninsula to Izu Peninsula (Fig. 1). YENDO (1916) reported this alga from one isolated locality, Asamushi, in northern Honshu, but we have never encountered the alga in the northern area. In the YENDO Herbarium (TI) we could not find any specimens identified as *Gigartina mammillosa* by YENDO, although a single specimen of this alga collected at Otsu, Kanagawa Prefecture on September 6, 1909 by K. SAKURAI (No. 14) is included in a cover of *Chondrus* as an unidentified alga.

The daylength and seawater temperature differ only slightly within the confirmed range. The climatic conditions, however, may not explain the reason for the narrowly restricted geographical distribution of the alga. Why has not the alga extend its range to adjacent areas where the climatic conditions are similar to those in its present range? Other factors which control its geographical distribution must be present and may be more complex.

The alga under study includes highly variable blade forms (MIKAMI, 1965). Plants with the heteromorphic type life history are generally narrower than those with the direct type life history: blades less than 2 cm wide always showed the former type and those exceeding 3 cm in width showed the latter type. Blade widths of both type plants, however, entirely overlap between about 2 and 3 cm. Among plants with the heteromorphic type life history extremely narrow blades less than 5 mm wide and with frequent branchings appear to be very different from broad blades exceeding 2 cm in width and with infrequent branchings. Our hybridization experiments indicate that plants of different blade forms, as well as similar forms, are freely interfertile. These data and life history studies indicate that a single morphologically variable species is present on the study area. This situation contrasts with that of the Pacific North American species, *Mastocarpus*

jardinii and *M. papillatus*. *M. jardinii* has narrow blades 2–5 mm wide and is distributed along the coast from British Columbia to southern California (ABBOTT and HOLLENBERG, 1976). *M. papillatus* has broader blades and ranges from Amchitka Island, Alaska to northern Baja California (WEST *et al.*, 1983). These species occur in the same habitat within their sympatric area (ABBOTT and HOLLENBERG, 1976). *M. jardinii* is comparable with the narrow-form plants of the alga under study (Fig. 2A) and *M. papillatus* is similar to the broad-form plants (Fig. 2B, D). According to hybridization studies, however, a complete sterility barrier is present between the North American two species (WEST *et al.*, 1978).

GUIRY and WEST (1983) reported detailed morphological and life history studies of the north Atlantic *Mastocarpus stellatus* which synonymizes *Gigartina mammillosa* (GUIRY *et al.*, 1984). In the north Atlantic two breeding groups are present. These are almost allopatric; the southern group is found in Portugal, Spain and France, and the northern one is found in England, Wales, Ireland and France. According to Guiry and West (1983), these groups can be generally distinguished by blade morphology. The southern group has redder (when dried), broader and fan-shaped blades with more dichotomies, rounded apices and a few marginal proliferations, whereas the northern group has more purplish-black, narrower and cuneate blades with fewer dichotomies, pointed apices and many marginal proliferations. As to tetrasporophytic crusts, no differences between the two groups can be found except in dimensions of tetrasporangia and tetraspores; crusts of the southern group have smaller tetrasporangia and tetraspores than those of the northern group. The Japanese alga under study is similar to the northern breeding group of *M. stellatus* rather than the southern group in these respects. However, there is a clear difference between the tetrasporophytic crusts of these two algae. Proportion of thickness of subhypothallium/hypothallium to total crust thickness is 0.17–0.26 for the Japanese alga (Table 2, for field-collected crusts) and 0.43–0.75 for the northern group of *M. stellatus* (GUIRY and WEST, 1983). The Japanese alga is more similar to *M. papillatus* in both gametophytic and sporophytic features, although the latter has thicker crusts up to 2.5 mm (GARDNER, 1917; ABBOTT and HOLLENBERG, 1976). The morphological similarity between the alga under study and *M. papillatus* requires further detailed investigations, including hybridization experiments.

This study was supported in part by grants from the Japan Society for the Promotion of Science and the United States National Science Foundation

as part of the Japan-U. S. Cooperative Science Program, a Grant-in-Aid for Scientific Research (Nos. 57123115 and 58124034) from the Ministry of Education, Science and Culture of Japan, United States National Science Foundation (PCM 79-17342) and United States National Oceanic and Atmospheric Administration Sea Grant (04-6-158-44021). The Center for Experimental Plants and Animals of Hokkaido University provided culture facilities. Y. OHNO provided materials for culture studies and culturing assistance in the early stages of this study. Y. YOKOHAMA, T. HORI, M. YOSHIZAKI, J. TANAKA, M. OHTA and H. MOROHOSHI provided invaluable assistance for sampling. M. D. GUIRY read a draft of the manuscript. To all these organizations and people we wish to express our grateful thanks.

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