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Dependence of the carposporophyte on the maternal gametophyte in three ceramiacean algae (Rhodophyta), with respect to carposporophyte development, spore production and germination success

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The reproductive performance of carposporophytes with various lengths of attached female branch was compared among three ceramiacean red algae, *Antithamnion nipponicum, Ceramium boydenii* and *C. japonicum*. The length of time prior to initiation of spore discharge was comparatively stable, but the number of spore discharge events fluctuated, regardless of the branch length attached to the carposporophyte. The total number of discharged spores and their size were both drastically reduced in *A. nipponicum* carposporophytes with female branches < 1 mm long, whereas in the two *Ceramium* species the size and number gradually decreased in carposporophytes with branches < 4 mm long. Carposporophytes that had been completely removed from the vegetative branches generally liberated the smallest and fewest carpospores and discharged spores only once; the accompanying young gonimolobes never grew larger. This conspicuous reduction in spore size was observed even if carposporophytes were excised the day before spore discharge. The size of spores released from the excised carposporophytes was significantly reduced in the three different strains of *C. japonicum,* but the ratio of the total spore number to control decreased by 53–88%. Germlings from large spores discharged from an unexcised carposporophyte showed faster growth rates than germlings from small spores from an excised carposporophyte. Our results suggest that nutrients are continuously supplied from gametophyte to carposporophyte until spore discharge and that carposporophytes cannot maintain the normal size and number of carpospores without remaining attached to a critical length of female branch, the length varying among taxa. The pathway for metabolite translocation and the significance of the association of carposporophyte with gametophyte are discussed.

INTRODUCTION

The carposporophytic stage is one of the unique reproductive features of florideophycean red algae. After syngamy, the diploid zygote develops into a multicellular carposporophyte, while remaining connected to the female thallus. Short filamentous gonimoblasts, which comprise carposporophyte tissue, generate carpospores that develop into diploid tetrasporophytes after discharge. The developmental patterns of the carposporophyte are diverse within the florideophycean algae and have been used as key systematic characters for generic or higher taxonomic rank. In contrast, the physiology, ecology and genetics of the carposporophytes are still for the most part unknown. Carposporophytes of some red algae discharge many carpospores for several weeks, with the total numbers of spores released reaching into the thousands (Boney 1960; Wilce & Sears 1991; West & McBride 1999). Because of their tiny size and low content of photosynthetic pigments, carposporophytes have long been thought to take up nutrients from the associated female gametophytic cells, even though there is little experimental evidence demonstrating this dependence (Turner & Evans 1978).

In the florideophycean algae, adjacent daughter cells are generally linked by a primary pit connection, which is closed by a proteinaceous pit-plug. Much more expanded pit-plugs, or pit-plugs that lack cap membranes on their cytoplasmic sides, are found between the basal cells of the carposporophyte and adjacent female cells (Wetherbee 1979, 1980; Broadwater & Scott 1982; Tsekos & Schnepf 1985; Hommersand & Fredericq 1990). Though the function of pit-plugs remains debatable (Pueschel 1980, 1990), active nutrient flow via pit-plug to the carposporophyte from vegetative tissue has been presumed on the basis of such cytological evidence.

Recently, West & McBride (1999) reported that carposporophytes excised with minimal associated vegetative branches showed much reduced spore production, as gauged by the total number of released spores, the number of spore discharge events, and the spore size. The productivity of the carposporophyte itself is still unknown.

In this study the reproductive performance of carposporophytes – comprising the period prior to the start of spore discharge, spore size, total number of discharged spores, number of spore discharge events and growth rate of discharged spores – was examined in the excised carposporophytes of three ceramiacean red algae: *Antithamnion nipponicum* Yamada & Inagaki, *Ceramium boydenii* Gepp and *C. japonicum* Okamura. Based on the protologue of *A. nipponicum,* Athanasiadis (1996) suggested that there was every indication that it was conspecific with the earlier *A. pectinatum* (Montagne) Brauner. However, because a type of specimen of *A. nipponicum* has not been designated, there remains some doubt about this. We chose to identify the Japanese species as *A. nipponicum*

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Table 1. Studies of red algae used in this study.

Species	Collection site	Collection date	Culture no.
A. nipponicum	Hachinohe, Aomori, Japan	21 Mar. 1995	1078
C. boydenii	Awaji Island, Hyogo, Japan	28 May 1996	1074
C. japonicum	Muroran, Hokkaido, Japan	12 Jul. 1999	1221
C. japonicum	Shimoda, Shizuoka, Japan	6 Apr. 1996	1076
C. japonicum	Kasumi, Hyogo, Japan	10 Oct. 1999	1249

until this problem has been sorted out. Specifically, the following questions were addressed: (1) how is the reproductive performance of carposporophytes influenced by varying the length of branch left attached to them; (2) do all species show similar diminished productivity in the excised carposporophytes; and (3) if carposporophytes take up and store nutrients from the parental gametophyte tissues, does this long-term connection between them cause a higher productive performance?

Most red algae have carposporophytes that are embedded in the female thalli or covered by multilayered pericarps derived from the female vegetative tissues (or both), and such enclosures make it difficult to isolate carposporophyte tissues from female thalli. In most ceramiacean algae, including the present three species, the carposporophytes are loosely surrounded by several involucres, which proliferate from peripheral vegetative cells after fertilization, and this feature allows the investigation of carposporophytes that have been entirely excised from the female thalli.

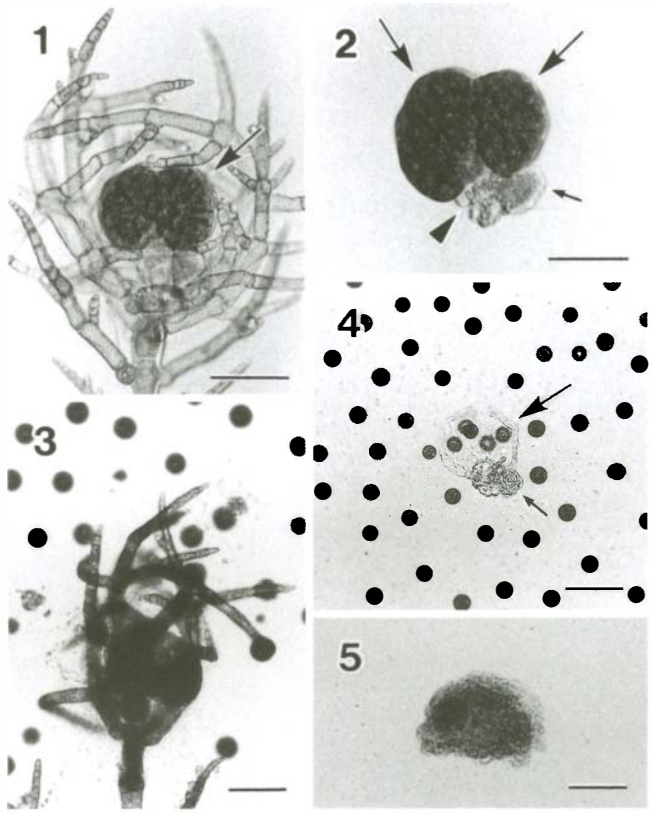
MATERIAL AND METHODS

The algal cultures used are listed in Table 1. The strains were incubated in 120 ml disposable plastic cups (Clean-cup™, Risu Pack Co., Tokyo) containing 50 ml Provasoli's enriched seawater medium (Starr & Zeikus 1993), occasionally supplemented with 1 mg l^{-1} GeO_2 to suppress diatom contaminants. These cups were placed at 20°C under 40 μm m^{-2} s^{-1} from cool-white fluorescent tubes, with a photoperiod of 14 h:10 h light–dark. Male and female gametophytic strains were established by isolating tetraspores from tetrasporophytes.

In a preliminary test to verify that the carposporophyte depends on its attachment to the gametophyte, rather than on exudates released from the gametophyte to the culture medium, excised carposporophytes were cultured together with excised vegetative branches: a carposporophyte and a branch were placed together in the same well of a 48-well culture plate (Falcon® 1178). In all three species, there was no improvement in the productivity of the excised carposporophytes cultured with separate gametophyte branches, compared to the excised carposporophytes cultured alone.

For excision experiments, several 3 cm–long female shoot tips with procarps and a 1 cm–long male shoot tip with spermatangia were transferred to a new plastic cup containing 15 ml fresh medium. They were cultured on a reciprocating shaker at 20 cycles min^{-1} for 5 days, and then the female branches were transferred into fresh medium. When carposporophyte tissues became visible under a stereomicroscope, about 1 week after fertilization, female branches were cut off, each bearing a single carposporophyte. The branchlets were trimmed to a specific length, comprising the total length of main branch, lateral branchlets and involucres. Each fragment was placed individually in a well of a 48-well culture plate. All carposporophytes were transferred to new culture plates containing fresh medium twice a week before carpospores were discharged and daily after spore discharge. The number and size of the spores released were measured by direct observation with an inverted research microscope (Olympus IX70). The experiment was stopped 23 days after fertilization.

In order to determine the growth rate of spores, batches of carpospores discharged from the test carposporophytes with long and short vegetative branches were placed separately in 120 ml disposable plastic Petri dishes (Iwaki SH90-20) containing 50 ml of medium. These dishes were sealed by parafilm and cultured in the conditions specified above. The



Figs 1–5. Carposporophytes of *A. nipponicum.*

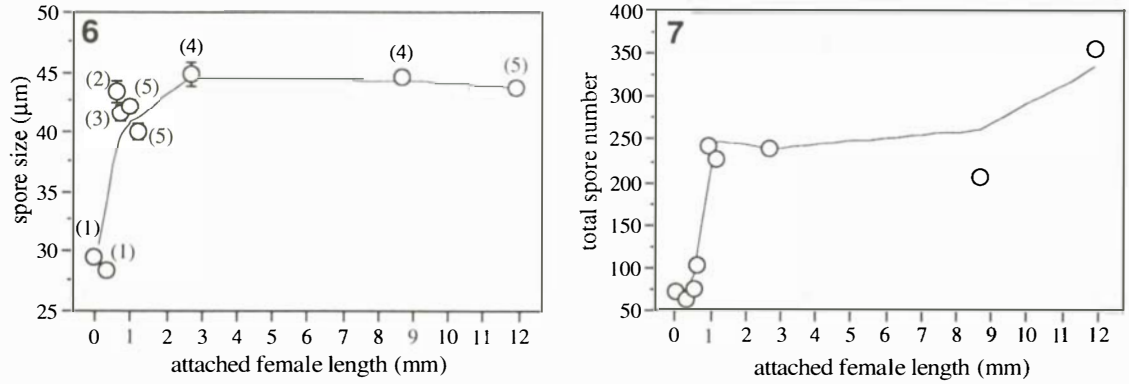
Fig. 1. Eight-day-old carposporophyte attached to a gametophyte branch. A coloured gonimolobe (arrow) has been produced at the apex. Scale bar = 100 μm.

Fig. 2. Eight-day-old carposporophyte completely excised from the gametophyte branch. Large coloured gonimolobes (large arrows) and a young small one (small arrow) are attached to the colourless gonimoblast tissue (arrowhead). Scale bar = 100 μm.

Fig. 3. Carpospores discharged from an 11-day-old carposporophyte attached to a gametophyte branch. Scale bar = 200 μm.

Fig. 4. Carpospores discharged from an 11-day-old carposporophyte completely excised from its gametophyte branch. An empty gonimolobe (large arrow) and a small colourless gonimolobe (small arrow) are visible. Note that the spore size is obviously smaller than in Fig. 3. Scale bar = 200 μm.

Fig. 5. Eighteen-day-old carposporophyte completely separated from the parental branch. About 60 carpospores were released from this carposporophyte at 3 days after excision, but no additional spores were produced. Scale bar = 200 μm.



Figs 6, 7. *Antithamnion nipponicum:* the productivity of nine carposporophytes with various lengths of vegetative branch left unattached. The solid lines represent LOWESS-smoothed (tension = 0.6) estimates (Cleveland 1979), which were calculated with the Statview 5.0 (SAS Institute, North Carolina, USA) statistical program.

Fig. 6. The mean diameter (μm) and standard error (bar) of spores discharged during the experiment (measured 23 days after fertilization). The number in parentheses represents the number of spore discharge events; 10 spores were measured at each event.

Fig. 7. The total number of spores discharged during the experiment.

lengths of 10 randomly selected germlings were measured every second day with the inverted microscope.

RESULTS

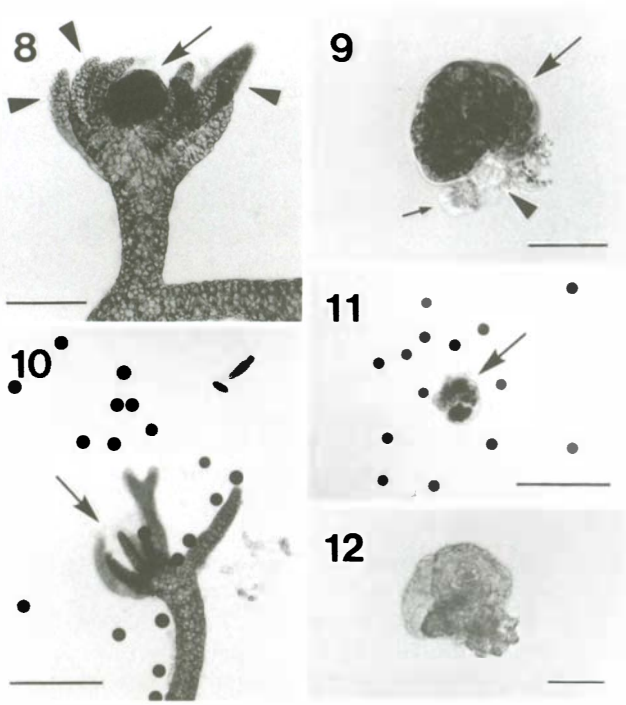
Productivity of carposporophytes with various vegetative branch lengths

In *A. nipponicum,* eight branches with carposporophytes were cut into 11.9, 8.6, 2.6, 1.1, 0.9, 0.6, 0.5 and 0.3 mm lengths, respectively, 8 days after fertilization, and on the same day three carposporophytes were completely separated from their female branches (Figs 1, 2). All the carposporophytes began to release spores at 10 or 11 days (Figs 3, 4). All three completely excised carposporophytes (Fig. 5) discharged spores once (58–87 were formed per carposporophyte), but did not produce any additional ones. The mean diameter of the discharged spores was relatively stable in carposporophytes attached to vegetative branches ≥ 0.5 mm long, but was much less in carposporophytes attached to very short branches < 0.5 mm long (Fig. 6). Carposporophytes attached to female branches discharged spores once to five times during the experiment, those with shorter branches tending to release spores less frequently (Fig. 6). Occasionally the spore size was significantly different ($P < 0.001$) between spore discharge events within the same carposporophyte, regardless of the branch length, and the same tendency was observed in the other two species examined. The interval between spore discharges varied from 1 to 9 days, with most of the carposporophytes releasing spores every 3 or 4 days. An extreme reduction in the total number of discharged spores was observed when the attached branch length was below 0.9 mm (Fig. 7).

In *C. boydenii,* eight female branches with carposporophytes were cut into 11.6, 9.2, 6.4, 4.6, 2.6, 1.4, 0.8 and 0.6 mm lengths 8 days after fertilization, and on the same day three carposporophytes were excised entirely from their female branches (Figs 8, 9). All the carposporophytes began to release spores at 10 or 11 days after fertilization (Figs 10, 11). All three completely excised carposporophytes liberated spores once (20–38 per carposporophyte), but no more spores were produced subsequently (Fig. 12). The diameter of discharged spores was less in carposporophytes with branches < 4.6 mm long (Fig. 13). Unlike the fully excised carposporophytes, those with female branches attached discharged spores once to four times during the experiment, but there was no correlation between the number of discharges and the attached branch length (Fig. 13). The interval between spore discharges varied from 2 to 6 days, with most carposporophytes releasing spores every 3 or 4 days. The total number of discharged spores decreased progressively with decreasing branch length when the carposporophytes were attached to branches < 2.6 mm long (Fig. 14).

In *C. japonicum,* eight branches with carposporophytes were cut into 11.0, 5.0, 4.1, 2.3, 1.1, 0.6, 0.5 and 0.3 mm lengths 5 days after fertilization, and six carposporophytes were fully separated from their female branches at 11 days (Figs 15, 16), when it was easiest to remove vegetative branches from the bases of the carposporophytes. All the carposporophytes left attached to female branches began to release spores at 12 or 13 days (Figs 17, 18). One of the six carposporophytes without vegetative tissues discharged 94 spores at 15 days, but then produced no further carpospores, even though it had a few young gonimolobes, whose size and colour remained unchanged during the experiment. The other five excised carposporophytes released either none or only a few spores during the experiment (Fig. 19). The size of the discharged spores was relatively constant if the attached female branch was ≥ 2.3 mm (Fig. 20); the total number of spores discharged varied greatly in carposporophytes with branches ≥ 4.1 mm (Fig. 21). Where the attached branch length was < 2.3 mm, the spores were smaller and fewer, the size and number being in proportion to the length. The number of spore discharge events varied from one to six, and was not strictly correlated with the branch length (Fig. 20). The interval between spore discharges in this species (2–3 days) was shorter than that of the other two species.

The productivity of carposporophytes with either long or very short female branches (mean lengths = 7.2 and 0.8 mm, respectively) was compared for 19 days between strains of *C. japonicum* collected from three distant Japanese populations,



Figs 8–12. Carposporophytes of *C. boydenii.*

Fig. 8. Eight-day-old carposporophyte attached to a gametophyte branch. A coloured gonimolobe (arrow) is surrounded by several involucres (arrowheads) derived from the vegetative tissue. Scale bar = 200 μm.

Fig. 9. Eight-day-old carposporophyte completely excised from its gametophyte branch. A large coloured gonimolobe (large arrow) and a young colourless one (small arrow) are attached to the colourless gonimoblast tissue (arrowhead). Scale bar = 100 μm.

Fig. 10. Carpospores discharged from an 11-day-old carposporophyte attached to the gametophyte branch. An empty gonimolobe (arrow) is visible. Scale bar = 400 μm.

Fig. 11. Carpospores discharged from an 11-day-old carposporophyte completely excised from its gametophyte branch. The gonimolobe (arrow) still contained carpospores inside, which were not released during the experiment. Note that the spore size is obviously smaller than in Fig. 10. Scale bar = 400 μm.

Fig. 12. Eighteen-day-old carposporophyte completely excised from the parental branch. About 30 carpospores were released from this carposporophyte at 2 days after excision, but no additional spores were released. Scale bar = 200 μm.

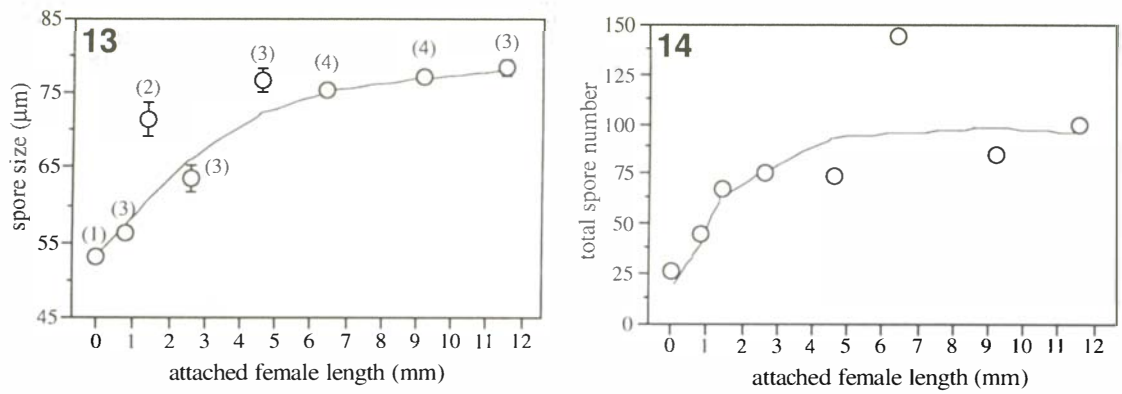
at Muroran (42°18′N, 140°58′E), Shimoda, (34°40′N, 138°56′E) and Kasumi (35°38′N, 134°37′E) (Tables 1, 2). According to preliminary hybridization tests, these three strains were reproductively compatible with each other (data not shown). In all three strains, the period before spore discharge began was nearly the same in carposporophytes with long branches as in those with short branches (Table 2). The strains from Muroran, Shimoda and Kasumi exhibited reductions of 16, 11 and 14% in spore size and 58, 88 and 53% in total spore number as a result of the removal of the vegetative branches from the carposporophytes. The excised carposporophytes in the Kasumi strain discharged slightly but significantly ($P < 0.001$) smaller spores than those from the other two strains. Although excision did not have an influence on the number of spore discharge events in the Muroran and Kasumi strains, the number was reduced in the strain from Shimoda (Table 2).

Effect of excision time on carposporophyte productivity

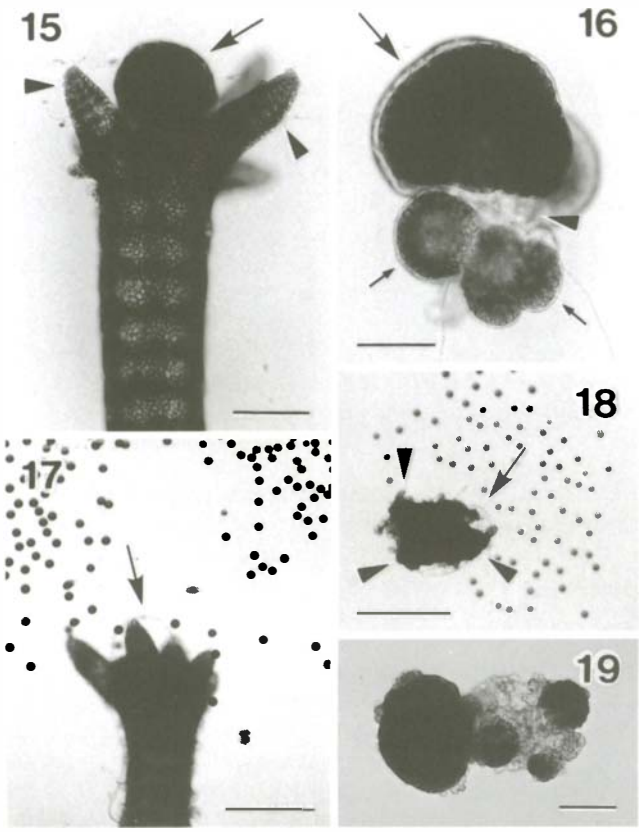
The ability to produce carpospores was compared between carposporophytes excised at three different times after fertilization (Table 3). Carposporophytes were accompanied by small amounts of vegetative tissue (0.3–0.6 mm) because it was difficult to remove them completely when the carposporophytes were at early developmental stages. Most carposporophytes discharged spores only once, a few twice or more, and none demonstrated long-term spore production during the experiment (23 days after fertilization). Carposporophytes of *A. nipponicum* excised at 5 days after fertilization discharged much fewer spores and took a longer time (6–11 days) to release initial carpospores than those excised at 8 or 13 days. Although the carposporophytes isolated at 13 days released spores only 1 or 2 days after excision, their spore size was significantly reduced ($P < 0.001$), compared with unexcised carposporophytes (control in Table 3). The effect of excision time was much greater in *C. japonicum* than in *A. nipponicum,* but in *C. boydenii* the period before spore release, the spore size, and the total spore number were scarcely influenced by the different excision time.

Comparison of growth rates between carpospores of different sizes

The growth rates of released carpospores were studied in relation to the differences in spore size brought about by dif-



Figs 13, 14. *Ceramium boydenii:* the productivity of eight carposporophytes with various lengths of vegetative branch left attached. See the caption to Figs 6, 7 for further details.



Figs 15–19. Carposporophytes of *C. japonicum.*

Fig. 15. Eleven-day-old carposporophyte attached to a gametophyte branch. A coloured gonimolobe (arrow) is surrounded by several involucres (arrowheads) derived from the vegetative tissue. Scale bar = 200 μm.

Fig. 16. Eleven-day-old carposporophyte completely excised from the gametophyte branch. A large coloured gonimolobe (large arrow) and young ones (small arrows) are attached to the colourless gonimoblast tissue (arrowhead). Scale bar = 100 μm.

Fig. 17. Carpospores discharged from a 13-day-old carposporophyte attached to its gametophyte branch. An empty gonimolobe (arrow) is visible. Scale bar = 400 μm.

Fig. 18. Carpospores discharged from a 13-day-old carposporophyte with only several involucres (arrowheads) left attached. An empty gonimolobe (arrow) is visible. Note that the spore size is much smaller than in Fig. 17. Scale bar = 400 μm.

Fig. 19. Twenty-eight day-old carposporophyte completely separated from the gametophyte branch. This carposporophyte failed to release any spores in spite of the normal size and colour of the gonimolobes. Scale bar = 100 μm.

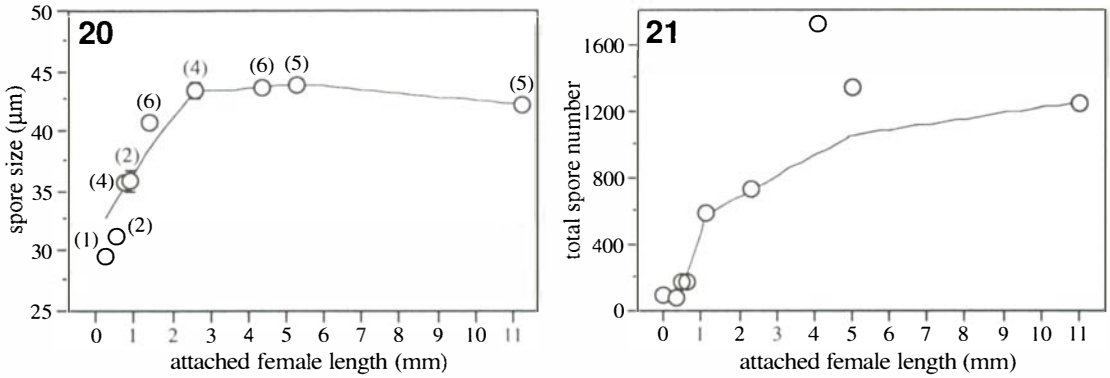
ferences in the length of the branch left attached to the carposporophyte (Figs 22, 23). More than 40 carpospores were isolated from each carposporophyte of *A. nipponicum,* one with a 6.3 mm branch attached and the other with a 0.2 mm branch, and from each of two carposporophytes of *C. japonicum,* with 11.0 and 0.3 mm branches attached. The comparison was not carried out in *C. boydenii* because we could not obtain enough spores. In *A. nipponicum* the difference in mean spore diameter was < 10 μm at the beginning (Figs 22, 24, 25), but the length difference between the germlings reached > 100 μm after 10 days (Figs 22, 26, 27). There was also < 10 μm difference in mean spore diameter in *C. japonicum* (Figs 23, 28, 29); the length difference between germlings became larger day by day, until it reached more than 70 μm at 18 days (Figs 23, 30, 31). In both species, the differences between germlings were caused by a change in axial cell numbers, not by differences in the size of the axial cells (Figs 22, 23).

DISCUSSION

The present research demonstrates the intimate association between the carposporophyte and its parental female gametophyte in three ceramiacean species. The size and number of the discharged carpospores were markedly reduced by cutting off the attached branch, indicating that carposporophytes require a critical length of vegetative branch to achieve normal spore development. We repeated these experiments and obtained similar results. During the experiments, mean spore size remained comparatively steady in carposporophytes with long vegetative branches, whereas in carposporophytes with short branches, the spore size increased little by little (data not shown). The vegetative branches bearing the carposporophytes continued to grow during the experiments: the involucres elongated at the apex and a number of monosiphonous rhizoids appeared from the branch bases. In carposporophytes with short vegetative branches, the size of the carpospores gradually increased as the attached branches grew and, in the end, these changes masked the differential productivity initially observed in carposporophytes with various branch lengths. Excised *Pterosiphonia pennata* (C. Agardh) Sauvageau carposporophytes with minimal vegetative tissues have been observed to continue to release spores for more than a month (West & McBride 1999), and this long-term discharge may have been made possible by the regrowth of attached tissues.

Surprisingly, spores were discharged continuously from those carposporophytes excised with nothing left attached apart from the involucres derived from the peripheral vegetative tissue after fertilization. Perhaps these involucres develop not only to protect the carposporophytes, but also to provide supplementary nutrition for carpospore formation. Even if the carposporophytes were excised from the vegetative tissues only a day before spore discharge began, the spores were obviously reduced in size compared with those produced by unexcised carposporophytes (Table 3). Furthermore, continuous spore production did not occur in the totally excised carposporophytes, regardless of the length of time that they had remained connected to the female branches (Table 3). These results suggest that the gametophyte continues to supply metabolites to the carposporophyte until spore release and that the carposporophyte has no or only a small capacity to store nutrients for continued development.

Although a very much diminished productivity of the excised carposporophytes was observed in all three species examined, the effect of excision differed between them. In *A. nipponicum* the size and total number of discharged spores were drastically reduced when the length of the female branch attached to the carposporophyte was < 0.5–0.9 mm (Figs 6, 7), whereas the other two species showed a relatively small decrease when the length was < 2–3 mm (Figs 13, 14, 20, 21). The differences in vegetative morphology between *A. nipponicum* and the two *Ceramium* species, including cortication, the diameter of branches and the branching pattern,



Figs 20, 21. *Ceramium japonicum:* the productivity of nine carposporophytes with various lengths of vegetative branch left attached. See the caption to Figs 6, 7 for further details.

are perhaps related to the different reactions shown by their excised carposporophytes. Furthermore, the carpospores produced by an unexcised *A. nipponicum* carposporophyte were on an average smaller than those of *C. boydenii* (*c.* 44 μm vs 76 μm) and fewer than those of *C. japonicum* (*c.* 250 vs 1250). This may explain why a long female branch is not necessary for *A. nipponicum* to produce carpospores of normal size and number.

Compared with the unexcised carposporophytes, the totally excised carposporophytes produced spores that were reduced in size by 33, 35 and 30% in *A. nipponicum, C. boydenii* and *C. japonicum,* respectively. In contrast, the decrease in the total number of discharged spores was much greater and more variable (88, 55 and 94%, respectively). The total spore number did not always correlate with the length of branch left attached (Figs 7, 14, 21) and fluctuated even within the same species (Table 2). This number is related to the number of discharge events, the discharge interval and the spore number at each event, and these features were quite variable among the carposporophytes, even those with similar lengths of branch attached (data not shown). The reproductive performance is probably determined not only by the attached branch length, but also by carposporophyte condition, for example, the developmental maturity or nutritional status of spermatia and carpogonia that fuse to form the zygotes. Some of the carposporophytes that had been entirely removed from the parental tissues in *C. japonicum* did not liberate carpospores, but it is uncertain whether this failure resulted from poor nutrition in culture or from physical damage caused during the removal of the vegetative tissues.

The developmental pattern of the carposporophyte varies greatly among florideophycean red algae (Hommersand & Fredericq 1990). Members of the Ceramiales develop only one carposporophyte from a single fertilization, whereas some species of the Gigartinales develop many, through the distribution of divided zygotic nuclei to particular nutritive cells *via* connecting filaments. In the case of the Corallinales, fertilization of one of many carpogonia arranged in a conceptacle is followed by the fusion of the neighbouring unfertilized reproductive cells and the supporting cells, resulting in the formation of a carposporophyte from the extensive fusion cell (Johansen 1981). The degree of carposporophyte dependence can be expected to differ among red algae showing such different postfertilization patterns.

Although nurture and protection of embryo by the female are well known in placental mammals and embryophytes, it is perhaps matrotrophy in bryophytes that provides a particularly good analogy to the relationship between the carposporophyte and the gametophyte in red algae. In bryophytes, as the sporophytes develop, portions of the archegonium divide to form an enclosure – the calyptra – that protects the developing sporophyte. The nutrient flow from gametophyte to sporophyte has been confirmed by several workers, and the sporophyte apparently depends on the gametophyte for sustained growth (Ligrone & Gambardella 1988). Nevertheless, chloroplasts with highly developed systems of thylakoid are present in the sporophyte tissues of many bryophytes, and photosynthesis in these sporophytes contributes part of the assimilate necessary for their growth (Proctor 1977, 1984). Given the limited number of plastids and their immature status

Table 2. Comparison of the productivity between the carposporophytes with long (♀+) and minimal (♀−) female branches in three strains of *Ceramium japonicum.* Spore sizes were measured for each carposporophyte and are given as $\bar{x} \pm s$ (n = 10). The total spore number is recorded as a range and mean ($\bar{x}$).

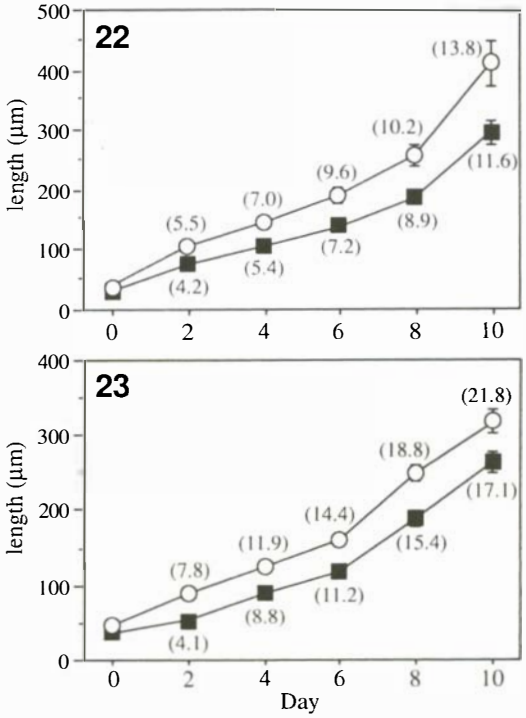
	N[2]		Initiation of spore discharge (days)[3]		Spore size (μm)		Total spore number		Spore discharge events	
Culture[1]	♀+	♀−	♀+	♀−	♀+	♀−	♀+	♀−	♀+	♀−
Muroran (1221)	4	5	13–16	13-14	40.9 ± 1.6	34.4 ± 1.7	530–1170 (802)	270–408 (338)	2–3	2–3
Shimoka (1076)	4	4	11–12	11–12	40.2 ± 2.6	35.7 ± 5.0	806–1411 (1126)	40–195 (135)	4–5	1–3
Kasumi (1249)	3	6	11	10–12	38.8 ± 6.4	33.2 ± 1.8	677–1025 (856)	269–460 (403)	3–4	3–4

[1] Strain information in Table 1.
[2] The number of carposporophytes.
[3] The period prior to initiation of spore discharge.

Table 3. The productivity of carposporophytes excised at three different dates. Spore sizes were measured for each sporophyte and are given as $\bar{X} \pm s$ ($n = 10$). The total spore number is recorded as a range and mean ($\bar{X}$).

Species	Excision date[1] (days)	*N*[2]	Period (days)[3]	Spore size (μm)	Total spore number
A. nipponicum	—[4]	3	—	44.2 ± 3.6	236–497 (342)
	5	4	6–11	31.6 ± 8.1	17–38 (26)
	8	3	2–3	33.1 ± 7.7	56–72 (64)
	13	3	1–2	32.0 ± 1.4	62–84 (71)
C. boydenii	—[4]	4	—	75.0 ± 5.2	74–145 (101)
	8	3	2–3	53.5 ± 5.0	7–52 (32)
	13	3	3	50.4 ± 4.6	29–36 (33)
	18	6	1–2	56.7 ± 5.2	19–50 (33)
C. japonicum	—[4]	3	—	43.2 ± 2.1	1248–1714 (1434)
	5	2	14–16	31.7 ± 2.4	12–72 (42)
	11	2	4–9	34.5 ± 5.1	13–98 (56)
	18	4	1–2	38.5 ± 2.7	97–249 (197)

[1] The data after fertilization when the carposporophytes were excised.
[2] The number of carposporophytes examined in this experiment.
[3] The period prior to initiation of spore discharge.
[4] Controls, in which the carposporophytes were left attached to vegetative branches.



Figs 22, 23. Growth rate of sporelings derived from spores discharged from carposporophytes with different branch lengths. The horizontal axis presents the day from the spore discharge and the vertical one indicates the lengths ($x \pm s$, $n = 10$) of the germlings. Numbers in parentheses indicate the mean numbers of axial cells.

Fig. 22. *Antithamnion nipponicum:* carpospores released from the carposporophytes with 6.3 mm–long (-○-) or 0.2 mm–long (-■-) vegetative branches.

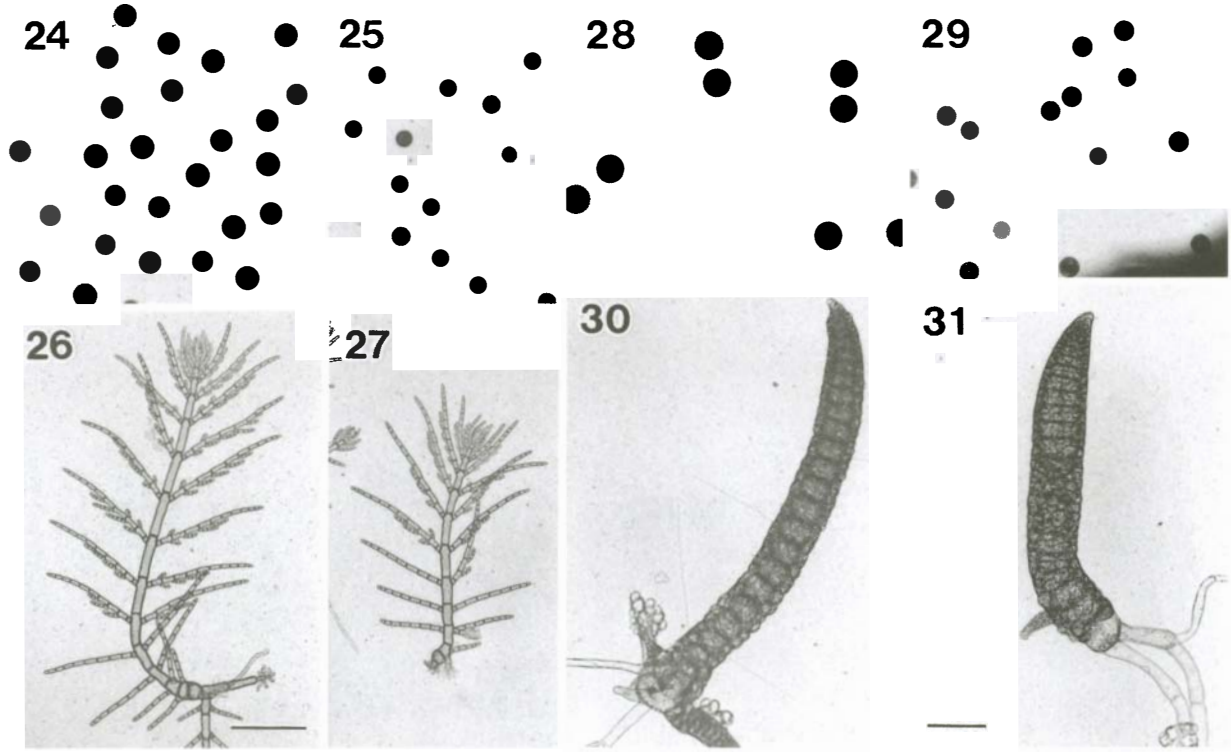
Fig. 23. *Ceramium japonicum:* carpospores released from the carposporophytes with 11.0 mm–long (-○-) or 0.3 mm–long (-■-) vegetative branches.

in the carposporophytes of ceramialean species (Wetherbee 1980; Tsekos & Schnepf 1985; Delivopoulos & Diannelidis 1991), the quantity of nutrient supplied by photosynthesis may be much lower in carposporophytes than in bryophyte sporophytes. Hommersand & Fredericq (1990) proposed that the carposporophytes of primitive florideophycean algae may depend on the attached female thallus less than in advanced groups. For instance, *Nemalion* Duby, thought to be a primitive florideophycean alga, has mature chloroplasts in the gonimoblasts and carpospores that are morphologically similar to those in vegetative cells (Ramm-Anderson & Wetherbee 1982). However, it is unknown whether the carposporophytes of such algae can produce normal carpospores continuously without the vegetative tissue attached. Carposporophyte productivity should be examined in a greater variety of taxa before any further attempt is made to discuss the relationship between red algal evolution and carposporophyte dependence.

A radioactive pulse–chase experiment has demonstrated the flow of photosynthetically fixed carbon from gametophyte to gonimoblast tissue (Turner & Evans 1978), though there is still little evidence concerning the mechanism of this transfer. In many bryophytes, a prominent wall labyrinth is present to the cytoplasm, on adjacent sides of the sporophyte and gametophyte placental cells (Ligrone & Gambardella 1988). Active solute secretion by gametophyte placental cells and active absorption by sporophyte placental cells probably maintains a concentration gradient, along which the solutes diffuse from gametophyte to sporophyte *via* the wall ingrowths (Gunning & Pate 1974). We have confirmed that the excised carposporophytes cannot maintain normal productivity, even if they are placed in close association with the detached vegetative branches. This demonstrates that the transfer of photosynthetic products from the gametophyte to the carposporophyte requires direct cell connection. Wetherbee (1980) argued that pit-plugs function in nutrient transfer and that they become expanded in regions of active nutrient flow. If so, we speculate that the appearance of the pit-plugs is different between excised and unexcised carposporophytes because there is a quantitative difference in the nutrient transfer between them.

This study has shown that spore size has an effect on the growth rate of germlings (Figs 22, 23). Although we do not have any evidence, larger spores may also have a higher germination rate, faster reproductive maturation, and the ability to produce many more reproductive structures on their thalli. Reduction of spore size was induced in our experiment by reducing the length of vegetative branch left attached to the carposporophyte, but it can also be caused by a high density of carposporophytes on the same thallus (West & McBride 1999). Avila *et al.* (1999) observed the greatest cystocarp densities (16–29 cystocarps per square centimetre) of *Gigartina skottsbergii* Setchell & Gardner in summer and found low viability of carpospores discharged from such cystocarps in culture. In the three species examined here, carpospore size is comparatively constant if the length of the vegetative branch attached to the carposporophyte is > 1–4 mm, so this value may indicate the minimum distance between two adjacent carposporophytes on the same thallus for the maintenance of normal spore size and the resultant high-fertility rate.

Spore size was clearly influenced by the length of vegetative branch left attached, whereas the time before the initial



Figs 24–31. Spores and germlings from carposporophytes with long or short gametophyte branches left attached.
Figs 24–27. *Antithamnion nipponicum.* Scale bar = 200 μm (in Fig. 26).
Figs 24–25. Spores discharged from 15-day-old carposporophytes with 6.3 mm–long (Fig. 24) or 0.2 mm–long (Fig. 25) vegetative branches.
Figs 26–27. Ten-day-old germlings from carposporophytes with 6.3 mm–long (Fig. 26) or 0.2 mm–long (Fig. 27) vegetative branches.
Figs 28–31. *Ceramium japonicum* (culture no. 1221). Scale bar = 200 μm (in Fig. 31).
Figs 28–29. Spores discharged from 13-day-old carposporophyte with 11.0 mm–long (Fig. 28) or 0.3 mm–long (Fig. 29) vegetative branches.
Figs 30–31. Eighteen-day-old germlings from carposporophytes with 11.0 mm–long (Fig. 30) or 0.3 mm–long (Fig. 31) vegetative branches.

discharge of spores and the spore discharge interval were comparatively unaffected. This tendency has also been reported in the highly crowded carposporophytes of *Bostrychia moritziana* (Sonder ex Kützing) J. Agardh (West & McBride 1999). These results suggest that, during nutrient limitation, carposporophytes will maintain the same spore discharge interval at the cost of spore size. Frequent and long-term discharge may provide opportunities for spores to encounter better environmental conditions for germination and dispersal, and as a result, increase the chances of survival of offspring and expansion of their habitat.

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