PARTHENOGENESIS IS RARE IN THE REPRODUCTION OF A SEXUAL FIELD POPULATION OF THE ISOGAMOUS BROWN ALGA *SCYTOSIPHON* (SCYTOSIPHONACEAE, ECTOCARPALES).[1]

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Abstract

Parthenogenetic development of unfused gametes is commonly observed in laboratory cultures amongst various brown algal taxa. There is, however, little information on the contribution of parthenogenesis to the reproduction of field populations. In this study, we investigated whether parthenogenesis is present in a sexual population of the isogamous brown alga *Scytosiphon* with a 1:1 sex ratio. In culture, both female and male gametes showed higher mortality and slower development compared to zygotes. More than 90% of surviving partheno-germlings formed parthenosporophytes irrespective of the culture conditions tested. Therefore, if parthenogenesis occurs in the field, most unfused gametes are expected to form parthenosporophytes. Contrary to this expectation, parthenosporophytes were rare in the field population. We collected 126 sporophytic thalli and isolated and cultured a unilocular sporangium from each of them. We confirmed that cultures of 120 unilocular sporangia produced both female and male gametophytes by the observation of zygotes or amplification of PCR-based sex markers indicating that these sporangia originated from zygotic sporophytes. Only females were detected in cultures from two sporangia and only males from four sporangia suggesting that these sporangia originated from parthenosporophytes. In the *Scytosiphon* population, although parthenogenesis is observable in culture, our results demonstrate that the contribution of parthenogenesis to reproduction is small ($\leq 4.8\%$) compared to sexual reproduction. Unfused gametes may not survive to form mature parthenosporophytes in significant numbers in the field partly due to their higher mortality and slower development compared from zygotes.

Key index words: parthenogenesis; parthenosporophyte; sex marker; sex ratio; sexual population

Abbreviations: GLMM, Generalized Linear Mixed Model; PESI, Provasoli Enriched Seawater with Iodine

Introduction

Parthenogenesis is a form of asexual reproduction in which an unfertilized gamete develops into a new individual. In laboratory culture, this asexual process has been commonly observed in various taxa of Phaeophyceae and Ulvophyceae (Brawley and Johnson 1992, Luthringer et al. 2014 and references therein). In these taxa, gametes form zygotes if they encounter gametes of the opposite sex or will otherwise undergo parthenogenesis. If such parthenogenesis also functions in the field, it would increase reproductive efficiency because it would avoid some cost of sexual reproduction in that unfused gametes are not wasted. However, there is little information regarding whether the parthenogenesis observed in culture conditions contributes to the reproduction of field populations (De Wreede and Klinger 1988).

In brown algae, the frequency of parthenogenesis seems to vary from population to population. In some populations in which sexual reproduction is unlikely to occur, parthenogenesis undoubtedly functions as the main method of reproduction. For example, populations showing a significant female-biased sex ratio have been reported in *Colpomenia peregrina* and *Scytosiphon* (Yamagishi and Kogame 1998, Hoshino et al. 2018b). In these populations, most of the field sporophytes are unisexual females originating from parthenogenesis of female gametes (Yamagishi and Kogame 1998, Hoshino et al. 2018b). Female-dominant populations which are likely maintained by parthenogenesis have been also reported in *Mutimo cylindricus* (Kitayama et al. 1992).

Parthenogenesis seems to be rare in populations in which sexual reproduction likely occurs (such as populations showing 1:1 sex ratio). In *Lessonia nigrescens* (Laminariales), unfertilized eggs form parthenosporophytes under culture conditions. However, in the field, only one out of 45 sporophytic thalli was determined as a parthenosporophyte (Oppliger et al. 2007). In *Dictyosiphon foeniculaceus* (Ectocarpales), although parthenogenetic development of both female and male gametes

was observed in culture, no parthenosporophytes were found among 34 field sporophytic thalli collected (Peters and Müller 1985). Parthenogenesis is also prevalent in *Ectocarpus* (Ectocarpales) in the laboratory. However, no parthenosporophytes were detected among nearly 200 field sporophytic thalli examined (Couceiro et al. 2015). Therefore, we hypothesized that parthenogenesis rarely contributes to reproduction in sexual populations, even if it can be observed under culture conditions.

The isogamous brown alga *Scytosiphon lomentaria* is distributed in cold and warm waters worldwide (Lüning 1990). It has macroscopic dioicous isomorphic gametophytes. Sexual reproduction is nearly isogamous: male gametes are slightly smaller than female gametes. Zygotes develop into microscopic discoid sporophytes (Nakamura and Tatewaki 1975). The sporophytes produce unilocular sporangia in which meiosis occurs. In laboratory cultures, both female and male gametes undergo parthenogenesis and develop into either parthenosporophytes (discoid or filamentous thalli) or gametophytes depending on temperature and daylength (Nakamura and Tatewaki 1975, Dieck 1987). Populations of *S. lomentaria* in Japan include at least five cryptic species (species Ia–Va; Hoshino et al. 2018a). Species Ia includes parthenogenetic female-dominant populations in addition to populations consisting of both female and male gametophytes (sexual populations; Kogame et al. 2015, Hoshino et al. 2018b). In species IIa, IIIa and Va, only sexual populations have been found.

In this study, to test the hypothesis that parthenogenesis is a rare event in sexual field populations, we focused on a population of *S. lomentaria* species IIa in Oshoro Bay, Japan. In this population, we found a sex ratio of approximately 1:1 and gamete fusion is observable in the laboratory. Using this population, we investigated the parthenogenesis of female and male gametes compared to development of zygotes in culture and the frequency of female and male parthenosporophytes and zygotic sporophytes in field.

Materials and Methods

Sex ratio of the field gametophyte population. To examine the sexuality of the population, gametophytes of *S. lomentaria* species IIa were collected in Oshoro Bay, Otaru, Hokkaido, Japan (43°21’ N, 140°85’ E) on 21 March 2018 (Table S1). Each individual served to make a herbarium specimen and total genomic DNA was extracted from a fragment for molecular experiments as described in Hoshino et al. (2018a). The sex of each gametophyte was determined using PCR-based sex markers. Partial sequences of the sex-specific non-recombining regions in the female and male sex-determining regions (Lipinska et al. 2017) were amplified and used as the sex markers (Hoshino et al. 2018b). The presence/absence of PCR products was determined by 1% agarose-gel electrophoresis.

Parthenogenetic development in laboratory culture. To examine the parthenogenetic capacity of gametes, parthenogenetic development was observed in culture. The culture isolates used are listed in Table 1. Culture experiments were conducted using plastic Petri dishes (90 × 20 mm) and PESI medium (Tatewaki 1966) with fluorescent lighting of 30–50 μmol $m^{-2}s^{-1}$ photon flux density. Gametophytes and their gametes were cultured in two conditions: 3°C short day (9.5:14.5 LD) and 10°C long day (16:8 LD). These culture conditions roughly correspond to the water temperatures and the daylengths of February and May in Oshoro Bay, respectively, where mature gametophytes were observed during these months.

Gametes were cultured at 3°C for 12 weeks and at 10°C for eight weeks. Zygotes were cultured at 3°C for four weeks and at 10°C for two weeks. The cell numbers of each four-week-old germling (gametes/zygotes) at 3°C and one-week-old germling at 10°C were recorded (30–100 germlings were observed for each gametophyte strain and five germlings were observed for each zygote strain). For the statistical analysis of the cell number of the germlings among experimental groups (female/male/zygote), a generalized linear mixed model (GLMM) was adopted with a

Poisson distribution. In this model, the experimental groups (female/male/zygote) were considered as fixed effects and the identity of each strain was considered as a random effect to deal with non-independence of the data from the same culture strain. The GLMM analyses were conducted using the lme4 package (Bates et al. 2015) in R ver. 3.3.1. (R Core Team 2016).

Subsequent to making the above observations, we examined whether the germlings of unfused gametes develop into gametophytic or sporophytic thalli. For each culture strain, 80–400 germlings were examined for 12-week-old thalli at 3°C and eight-week-old thalli at 10°C.

Parthenosporophytes in the field. Sporophytic thalli (Fig. 1A) were collected in Oshoro Bay from August to December of 2016 and 2017 (Table S2). Zygotic sporophytes (originating from sexual reproduction) produce both male and female gametophytes in the offspring from a unilocular sporangium (Fig. 2). On the other hand, parthenosporophytes (originating from parthenogenesis of gametes) produce unisexual gametophytes (Fig. 2; Nakamura and Tatewaki 1975). Therefore, gamete fusion (zygotes) should be observed among the gametophytes derived from a sexually formed sporophyte, but not observed among the gametophytes derived from a parthenosporophyte.

Single unilocular sporangium (Fig. 1B) was isolated from each sporophytic thallus and was cultured at 10°C in short-day condition. Spores from a unilocular sporangium grew up to gametophytes (Fig. 1C) and produced gametes in four months. The presence or absence of gamete fusion or numerous zygotes (settled cells showing two eyespots; Fig. 1E) was checked in each culture from a single unilocular sporangium using a light microscope. For cultures in which the presence of gamete fusion or zygotes could not be confirmed (e.g., due to insufficient fertility/growth of gametophytes or a small number of zygotes), the PCR-based sex markers were used to determine whether the culture includes both sexes. In unisexual samples, PCRs were repeated once to

verify reproducibility.

Results

Sex ratio in the field population. The sex of 27 gametophytes was identified with the sex markers. The ratio between female and male gametophytes was 12:15 (Table S1) and not significantly different from 1:1 (G test, $G = 0.334$; df = 1, $P = 0.563$).

Parthenogenetic development in laboratory culture. Unfused gametes showed higher mortality and slower development than zygotes (Fig. 3). The GLMM predicted that the cell numbers of germlings of female gametes would be significantly larger than those of male gametes and significantly smaller than that of zygotes (Table 2). We followed the development of 20 zygotes (four zygote strains) at 3°C and 15 zygotes (three zygote strains) at 10°C. Except for one zygote of strain Z2, which died at 3°C, they developed into discoid sporophytes (which were 20–100-celled after four weeks culture at 3°C and 9–15-celled after one week culture at 10°C; Fig. 3). In female strains, most gametes showed slower development compared to zygotes although in some exceptions the gametes developed at the same rate (see non-outlier ranges and outliers in Fig. 3). In male strains, most gametes stayed 1–3-celled both at 3°C (four weeks) and at 10°C (one week; Fig. 3).

The parthenogenetic development was different between female and male gametes. Although the germination rate of female gametes was lower than male gametes, survival rates seemed to be higher in female gametes than male gametes. In all female strains, 20–60% (varied across the strains) of gametes swelled unusually without germination in both 3°C and 10°C conditions (Figs. 4B and 5) and these swollen gametes frequently bursted and died. However, female gametes rarely died once they successfully germinated (Fig. 4A). In male strains, swollen gametes were rarely observed, except for strain M4 at 3°C where nearly 30% of gametes were swollen (Fig.

5). Male gametes showed higher germination rates than female gametes (Fig. 5); however, 94–97% of them stopped their development before the 5-celled stage (Figs. 4C and 5) and died in several weeks.

In both 3°C and 10°C conditions, surviving gametes usually developed into parthenosporophytes (discoid or filamentous thalli) and rarely formed erect thalli (gametophytes). The ratio of thalli that formed erect thalli were 0.8 ± 0.3 (Avg. ± SD) % in female strains and 0.4 ± 0.4% in male strains at 3°C, and 2.9 ± 4.1% in female strains and 1.6 ± 0.9% in male strains at 10°C (Table S3).

Parthenosporophytes in field. In total, 126 unilocular sporangia were isolated from 126 different field-collected sporophytic thalli and were cultured. The presence of gamete fusion or numerous zygotes (Fig. 1D, E) was confirmed in 104 cultures of unilocular sporangia. The remaining 22 cultures were the followings: 1) two cultures in which spores did not develop into gametophytes; 2) seven cultures in which some gametes had multiple eyespots and more than two flagella (possibly twins; Fig. 1F) and confirmation of zygotes was impossible since these zoids were hardly distinguishable from zygotes; and 3) 11 cultures in which neither gamete fusion nor zygotes were not observed. In the sex marker PCRs, out of these 22 cultures, both female and male markers were amplified in 16 cultures, only female markers were amplified in two cultures, and only male markers were amplified in four cultures (Table S2).

Discussion

We demonstrated that the contribution of parthenogenesis to reproduction is small in the sexual population of *S. lomentaria* species IIa, being consistent with our hypothesis that parthenogenesis is a rare event in sexual field populations. Out of 126 field sporophytic thalli, only six (4.8%) showed no evidence of a zygotic origin, but rather a parthenogenetic origin (Table 3). In culture, more than 90% of partheno-germlings that survived and grew developed into sporophytic thalli irrespective of the

culture conditions examined. This suggests that if parthenogenesis occurs in the field, most of them would form parthenosporophytes. Therefore, the rarity of field parthenosporophytes in our results indicates that parthenogenesis seldom occurs compared to sexual reproduction in the field.

A dominance of zygotic sporophytes (i.e., rare parthenosporophytes) has also been reported in the sexual populations of *Laminaria digitata*, *Lessonia nigrescens*, *Dictyosiphon foeniculaceus* and *Ectocarpus* (Peters and Müller 1985, Oppliger et al. 2007, 2014, Couceiro et al. 2015). Several causes can be considered for explaining the rare occurrence of parthenogenesis. High fertilization success (i.e., few unfused gametes) has been proposed as the cause of the rare parthenosporophytes (Peters and Müller 1985, Couceiro et al. 2015). It is well known that brown algae have various mechanisms that enhance the probability of gamete encounter, such as synchronous gamete release, phototaxis, and attraction of male gametes by sexual pheromones (Brawley and Johnson 1992). Although the fertilization rates of natural populations are largely unknown, it is noteworthy that high fertilization values (70–100%) have been reported in fucoids (reviewed in Santelices 2002). Another possible cause is the reduced viability of parthenogenetic germlings (Peters and Müller 1985). We showed that the survival rate and growth rate of unfused gametes were much lower than those of zygotes. In the field, biotic interactions should also be considered as a contributing factor to the rarity of parthenosporophytes. For instance, gametes may have various predators in the field. Additionally, unfused gametes are probably more vulnerable to grazing or predation than zygotes which rapidly start development and are protected by a cell wall and body size. Unfused gametes need to stay naked for some time to wait/look for their mating partner and even if they started parthenogenetic development, its development is much slower than that of zygotes.

Previous reports of parthenogenesis under culture conditions should be treated with caution. Most studies described only the occurrence of parthenogenesis

and did not compare its survival rate and development with those of zygotes. The studies, which compared the development of unfused gametes and that of zygotes, often show that unfused gametes have high mortality and slow/abnormal development compared to zygotes (e.g., Berthold 1881, Kemp and Cole 1961, Nakahara 1984, Peters and Müller 1985, Hoshino et al. 2018b). In such cases, parthenogenesis is unlikely to function in the field. Interestingly, female gametes from the parthenogenetic populations of *S. lomentaria* species Ia show parthenogenetic development that is as rapid as zygotic development in culture (Hoshino et al. 2018b).

It has been considered that both female and male gametes are capable of parthenogenesis in brown algal isogamous taxa (Oppliger et al. 2007, Luthringer et al. 2014). However, in our culture experiments, parthenogenetic capacity (i.e., germination rate and survival rate) was significantly different depending on sex. Such sex-based differences in parthenogenetic development have also been reported in the other isogamous taxa including *Ectocarpus siliculosus* and several *Scytosiphon* species (Berthold 1881, Han et al. 2014, Hoshino et al. 2018b). Differences between the sexes in parthenogenetic capacity, despite their isogamous nature, may be attributable to differences in subcellular components such as mitochondria and centrioles (Nagasato et al. 1998, Kimura et al. 2010, Han et al. 2014). In *Scytosiphon*, to detect the high mortality of male gametes, observation for several weeks was essential since most male gametes normally develop up to the 4-celled stage (Han et al. 2014, Hoshino et al. 2018b). Thus, the high mortality of male gametes may have been overlooked in previous studies. In spite of the high mortality of male gametes in culture, we detected more male parthenosporophytes than female ones in field. Although it may have been accidentally caused owing to such a small number of parthenosporophytes detected (six individuals), we cannot offer any explanation for this phenomenon in the current study.

In brown algae, both sexual populations and parthenogenetically maintained populations have been found in a single species (e.g., Peters 1987, Kitayama et al, 1992,

Hoshino et al. 2018b). In *S. lomentaria* species Ia, sexual populations showing 1:1 sex ratios are found in warm waters while female-dominant parthenogenetic populations are found in cold waters (Kogame et al. 2015, Hoshino et al. 2018b). The 1:1 sex ratio populations in species Ia likely reproduce mainly by sexual reproduction similar to the species IIa populations examined in this study. Considering these facts, parthenogenesis seems to function mainly in cold environments in species Ia. A similar geographic distribution pattern between parthenogenetic populations and 1:1 sex ratio populations has been reported in the anisogamous *Mutimo cylindricus* (Kitayama et al. 1992, Kogishi et al. 2010). In anisogamous taxa, only female gametes are usually capable of parthenogenesis (Luthringer et al. 2014). Therefore, if parthenogenesis of female gametes functions in anisogamous taxa, the sex ratio will be biased to females. It allows for the prediction that parthenogenesis is not being performed in 1:1 sex ratio populations of anisogamous species. Therefore, parthenogenesis likely functions only in cold environments for *M. cylindricus* as well. Unfortunately, it is unclear as to why parthenogenesis is preferred in cold environments in the above two species. Interestingly, in the green alga *Ulva mutabilis*, abortive mating reportedly increased with decreasing temperature and it has been theoretically predicted that populations in cold environments tend to quit sexual reproduction and rely on parthenogenesis (Løvlie and Bryhni 1976, 1978). Investigation on the relationship between fertilization success and temperature is required for *Scytosiphon* species Ia and *M. cylindricus*. To further understand the function of parthenogenesis in brown algae, more details on the reproductive mode of populations across various localities and species is essential.

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Table 1. Culture strains used in experiments at 3°C and 10°C, respectively. -: not used.. Cultures of female and male gametophytes were established from sporophytes collected in Oshoro on 6 October 2017.

	Culture strain	3°C	10°C
Female	F1: Oshoro-171006-Cr2B	used	used
	F2: Oshoro-171006-Cr2E	-	used
	F3: Oshoro-171006-Cr3D	-	used
	F4: Oshoro-171006-Cr5A	used	used
	F5: Oshoro-171006-Cr5C	used	used
Male	M1: Oshoro-171006-Cr1B	-	used
	M2: Oshoro-171006-Cr3A	used	used
	M3: Oshoro-171006-Cr5B	used	used
	M4: Oshoro-171006-Cr5D	used	used
Zygote	Z1: Oshoro-171006-Cr5A×Oshoro-171006-Cr5D	-	used
	Z2: Oshoro-171006-Cr2B×Oshoro-171006-Cr5D	used	used
	Z3: Oshoro-171006-Cr5C×Oshoro-171006-Cr5B	-	used
	Z4: Oshoro-171006-Cr3A×Oshoro-171006-Cr5A	used	-
	Z5: Oshoro-171006-Cr5A×Oshoro-171006-Cr5B	used	-
	Z6: Oshoro-171006-Cr5C×Oshoro-171006-Cr5D	used	-

Table 2. The effects of the experimental groups (female/male/zygote) on the cell numbers of germlings at 3°C and 10°C. Coefficients of the experimental groups (female/male and female/zygote) indicate that cell numbers of germlings from female gametes are larger than that of male gametes, but smaller than that of zygotes at a 5% significance level.

	Cell number of germlings							
	3°C 4 weeks after				10°C 1 week after			
	Coef.	SE	z value	*p*	Coef.	SE	z value	*p*
Intercept	1.71	0.28	6.14	< 0.0001	1.03	0.07	14.11	< 0.0001
Group(female/male)	-1.01	0.39	-2.56	0.0103	-0.76	0.11	-6.72	< 0.0001
Group(female/zygote)	2.11	0.37	5.75	< 0.0001	1.48	0.13	11.23	< 0.0001

387 Table 3. The percentage of zygotic sporophytes, unisexual female sporophytes, and
388 unisexual male sporophytes in field sporophytic thalli.

Total number of unilocular sporangia (sporophytes) examined	Discrimination result		
	Zygotic	Female	Male
126	120 (95.2%)	2 (1.6%)	4 (3.2%)

389

Fig. 1. Field sporophytic thalli and observations of the cultures established from their unilocular sporangia. (A) Field sporophytic thallus on a rock. (B) Cross-section of a sporophytic thallus showing a unilocular sporangium (arrow). (C) Gametophytes derived from an isolated unilocular sporangium. (D) Fertilization observed in a culture. Multiple male gametes (arrow head) gather around a female gamete (arrow). (E) Zygote observed in a culture, two eyespots (e) are visible. (F) Abnormal gamete showing multiple eyespots (e) and more than two flagella (f).

Fig. 2. Schematic figure of the life cycle of *Scytosiphon* in culture. Fusion of female and male gametes (F!) produces a zygote which develops into a zygotic sporophyte. The zygotic sporophyte produces unilocular sporangia (US) in which meiosis (M!) occurs and zoids (meiospores) develop into female or male gametophytes. Unfused gametes can undergo parthenogenesis (P!) and mainly develop into parthenosporophytes, not gametophytes. Unilocular sporangia of parthenosporophytes produce unisexual gametophytes.

Fig. 3. Cell numbers of germlings (4-week-old germlings at 3°C and 1-week-old germlings at 10°C) of gametes and zygotes. The gametes/zygotes that failed to germinate and aborted during development are also included (bursted gametes are excluded since they were impossible to count). Codes of culture strains are listed in Table 1. The boxes and whiskers represent the interquartile range and the non-outlier ranges. The horizontal band in the box is the median. Black dots represent outliers.

Fig. 4. Two-week-old germlings of gametes and zygotes at 10°C. (A) Germling of a female gamete forming discoid thalli. (B) Unusually swollen female gamete. (C) Germlings of male gametes, which stopped their development at 2-celled-stage (arrow). Large one (arrowhead) is a germling that successfully grew (filamentous). (D) Germling

417 of a zygote having discoid part.

418

419 Fig. 5. Percentage of four phenotypes (non-germinated and non-swollen; non-
420 germinated and swollen; germlings less than 5-celled; germlings more than 4-celled)
421 observed in four-week-old gametes at 3°C. Bursted gametes are not included.

422

423 Table S1. PCR-based sexing of gametophytes. f: female; m: male.

424

425 Table S2. Observation of zygotes and amplification of the sex markers in the cultures
426 from unilocular sporangia. +: zygotes were observed; -: no zygote was observed; tw:
427 gametes having multiple eyespots and more than two flagella (possibly twins) were
428 observed; f,m: both female and male markers were amplified; f,-: only female marker was
429 amplified; -,m: only male marker was amplified.

430

431 Table S3. The ratio of partheno-germlings which formed erect thalli to the total
432 partheno-germlings (erect, filamentous, and discoid thalli).

Table S1. PCR-based sexing of gametophytes. f: female; m: male.

	Code of gametophytes	Sex check	Voucher specimen
1	Oshoro180321 sl1	m	SAP115421-1
2	Oshoro180321 sl2	m	SAP115421-2
3	Oshoro180321 sl3	f	SAP115421-3
4	Oshoro180321 sl4	m	SAP115421-4
5	Oshoro180321 sl6	m	SAP115421-6
6	Oshoro180321 sl7	m	SAP115421-7
7	Oshoro180321 sl8	f	SAP115421-8
8	Oshoro180321 sl9	f	SAP115421-9
9	Oshoro180321 sl10	f	SAP115421-10
10	Oshoro180321 sl11	m	SAP115421-11
11	Oshoro180321 sl13	m	SAP115421-13
12	Oshoro180321 sl14	f	SAP115421-14
13	Oshoro180321 sl15	f	SAP115421-15
14	Oshoro180321 sl16	f	SAP115421-16
15	Oshoro180321 sl17	m	SAP115421-17
16	Oshoro180321 sl18	f	SAP115421-18
17	Oshoro180321 sl19	m	SAP115421-19
18	Oshoro180321 sl20	m	SAP115421-20
19	Oshoro180321 sl21	f	SAP115421-21
20	Oshoro180321 sl22	m	SAP115421-22
21	Oshoro180321 sl23	f	SAP115421-23
22	Oshoro180321 sl24	m	SAP115421-24
23	Oshoro180321 sl25	f	SAP115421-25
24	Oshoro180321 sl26	m	SAP115421-26
25	Oshoro180321 sl27	m	SAP115421-27
26	Oshoro180321 sl28	m	SAP115421-28
27	Oshoro180321 sl30	f	SAP115421-30

Table S2. Observation of zygotes and amplification of the sex markers in the cultures from unilocular sporangia. +: zygotes were observed; -: no zygote was observed; tw: gametes having multiple eyespots and more than two flagella (possibly twins) were observed; f,m: both female and male markers were amplified; f,-: only female marker was amplified; -,m: only male marker was amplified.

	Culture code of unilocular sporangia	Zygotes	Sex markers
1	Oshoro160921 cr3	+	
2	Oshoro160921 cr4	-	f,m
3	Oshoro160921 cr5	+	
4	Oshoro160921 cr6	-	f,m
5	Oshoro161102 cr4	+	
6	Oshoro161102 cr5	+	
7	Oshoro161102 cr6	+	
8	Oshoro161102 cr7	-	f,-
9	Oshoro161102 cr8	+	
10	Oshoro161102 cr10	+	
11	Oshoro161102 cr11	+	
12	Oshoro161102 cr12	+	
13	Oshoro161102 cr13	+	
14	Oshoro161102 cr15	+	
15	Oshoro161102 cr18	+	
16	Oshoro161102 cr19	+	
17	Oshoro161102 cr20	+	
18	Oshoro161102 cr21	+	
19	Oshoro161102 cr22	+	
20	Oshoro161102 cr23	+	
21	Oshoro161108 cr1	+	
22	Oshoro161108 cr2	+	
23	Oshoro161108 cr3	+	
24	Oshoro161201 cr3	+	
25	Oshoro161201 cr4	+	
26	Oshoro161201 cr5	+	
27	Oshoro161201 cr6	+	
28	Oshoro161201 cr7	+	
29	Oshoro171006 cr1	+	

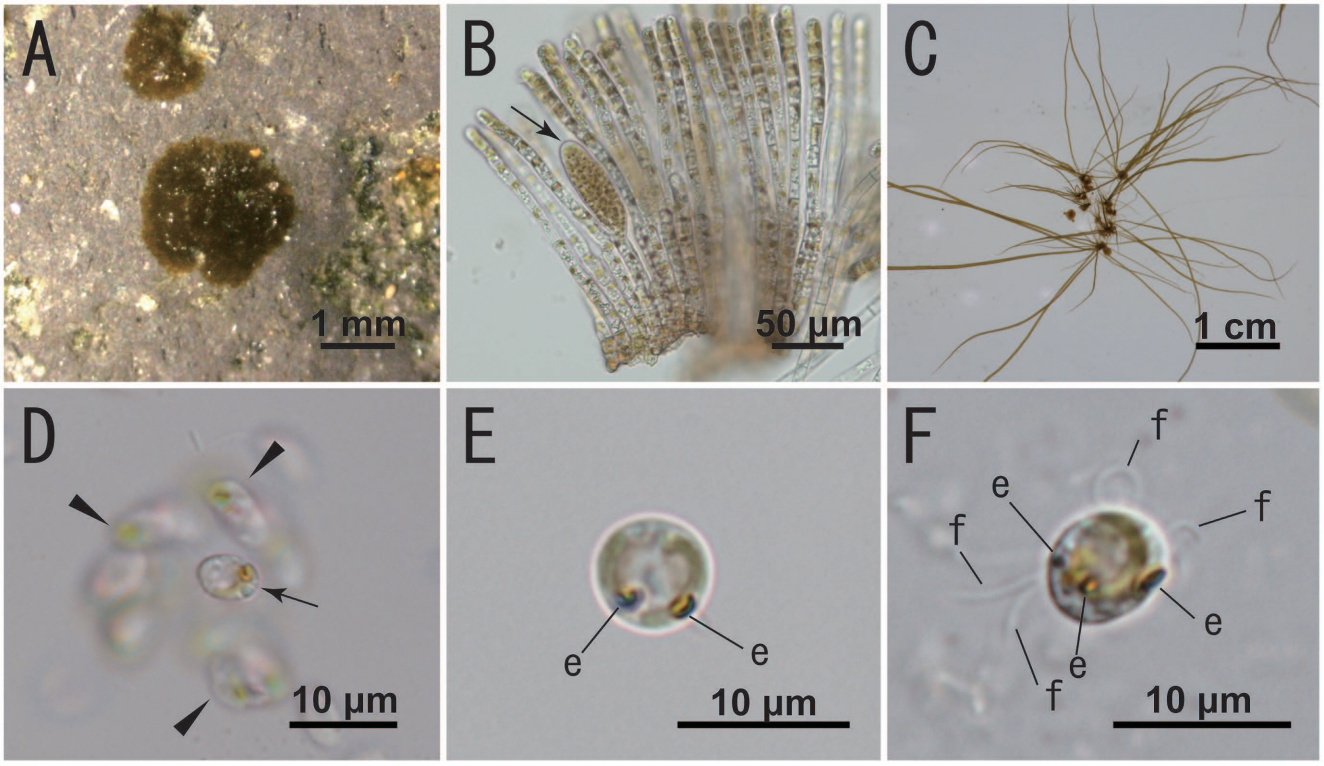
30	Oshoro171006 cr2	+	
31	Oshoro171006 cr3	+	
32	Oshoro171006 cr4	tw	f,m
33	Oshoro171006 cr5	+	
34	Oshoro171006 cr6	-	f,m
35	Oshoro171006 cr7	+	
36	Oshoro171006 cr8	+	
37	Oshoro171006 cr9	+	
38	Oshoro171006 cr10	+	
39	Oshoro171006 cr11	+	
40	Oshoro171006 cr12	-	-,m
41	Oshoro171006 cr13	+	
42	Oshoro171006 cr14	+	
43	Oshoro171006 cr15	+	
44	Oshoro171006 cr16	tw	f,m
45	Oshoro171006 cr17	+	
46	Oshoro171006 cr18	+	
47	Oshoro171006 cr19	+	
48	Oshoro171006 cr20	-	f,m
49	Oshoro171014 cr2	+	
50	Oshoro171014 cr3	-	f,m
51	Oshoro171014 cr4	+	
52	Oshoro171014 cr5	+	
53	Oshoro171014 cr8	+	
54	Oshoro171014 cr9	-	f,m
55	Oshoro171014 cr10	tw	f,m
56	Oshoro171014 cr11	+	
57	Oshoro171014 cr12	-	-,m
58	Oshoro171014 cr13	-	f,m
59	Oshoro171014 cr14	+	
60	Oshoro171014 cr15	+	
61	Oshoro171014 cr16	+	
62	Oshoro171014 cr17	+	
63	Oshoro171014 cr18	-	f,m
64	Oshoro171014 cr20	-	f,m

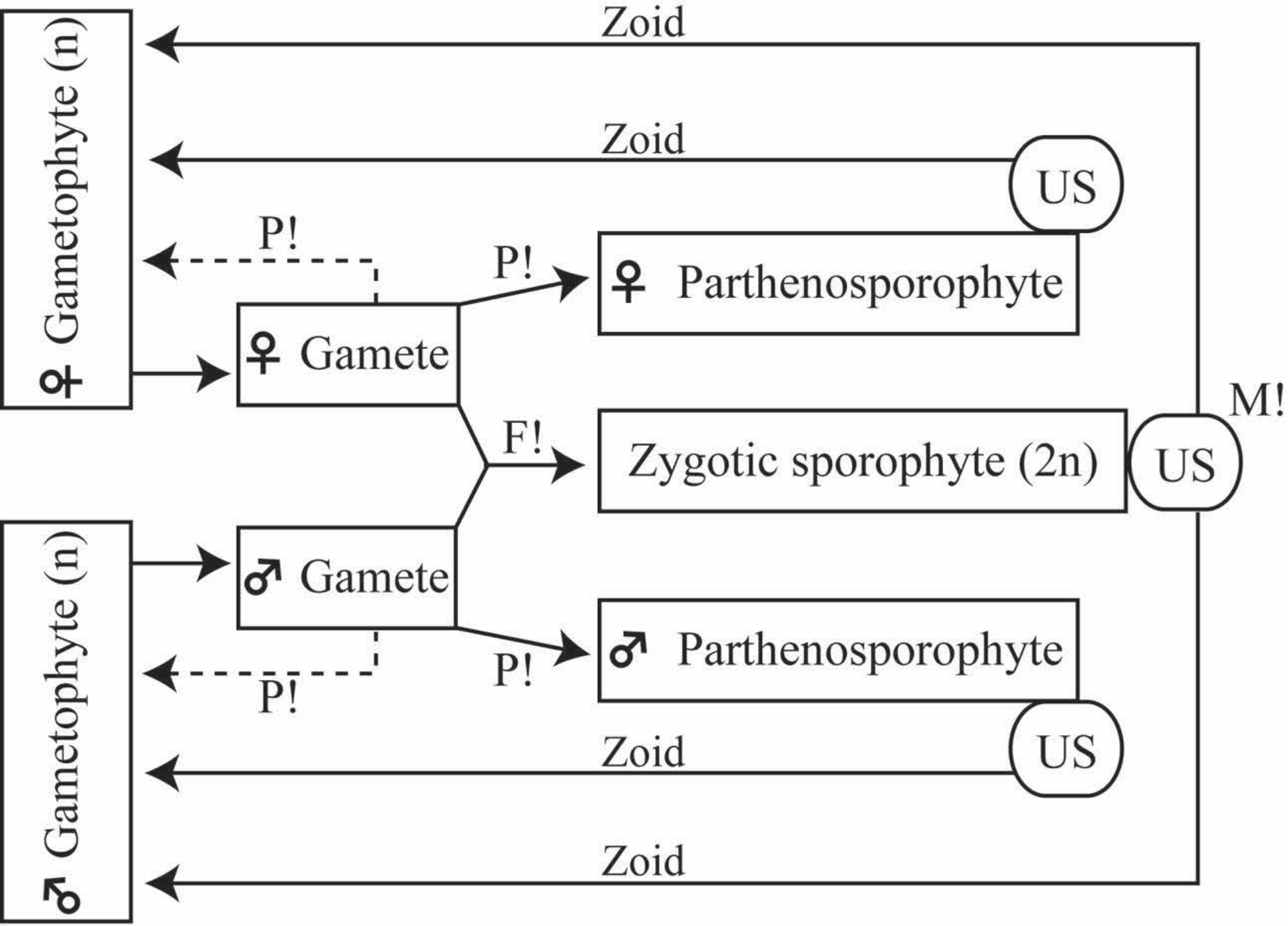
65	Oshoro171014 cr21	+	
66	Oshoro171014 cr22	-	-,m
67	Oshoro171014 cr23	+	
68	Oshoro171014 cr24	+	
69	Oshoro171014 cr25	+	
70	Oshoro171014 cr26	+	
71	Oshoro171014 cr27	+	
72	Oshoro171014 cr29	+	
73	Oshoro171014 cr30	+	
74	Oshoro171014 cr31	+	
75	Oshoro171014 cr32	+	
76	Oshoro171014 cr33	+	
77	Oshoro171014 cr34	-	-,m
78	Oshoro171014 cr35	+	
79	Oshoro171014 cr37	+	
80	Oshoro171014 cr39	tw	f,m
81	Oshoro171014 cr40	+	
82	Oshoro171014 cr41	+	
83	Oshoro171014 cr43	+	
84	Oshoro171014 cr44	+	
85	Oshoro171014 cr45	+	
86	Oshoro171014 cr46	+	
87	Oshoro171014 cr47	tw	f,-
88	Oshoro171014 cr49	+	
89	Oshoro171014 cr50	+	
90	Oshoro171014 cr51	+	
91	Oshoro171014 cr52	+	
92	Oshoro171014 cr53	+	
93	Oshoro171014 cr54	tw	f,m
94	Oshoro171014 cr55	+	
95	Oshoro171014 cr56	+	
96	Oshoro171014 cr58	+	
97	Oshoro171014 cr59	+	
98	Oshoro171014 cr60	+	
99	Oshoro171014 cr62	+	

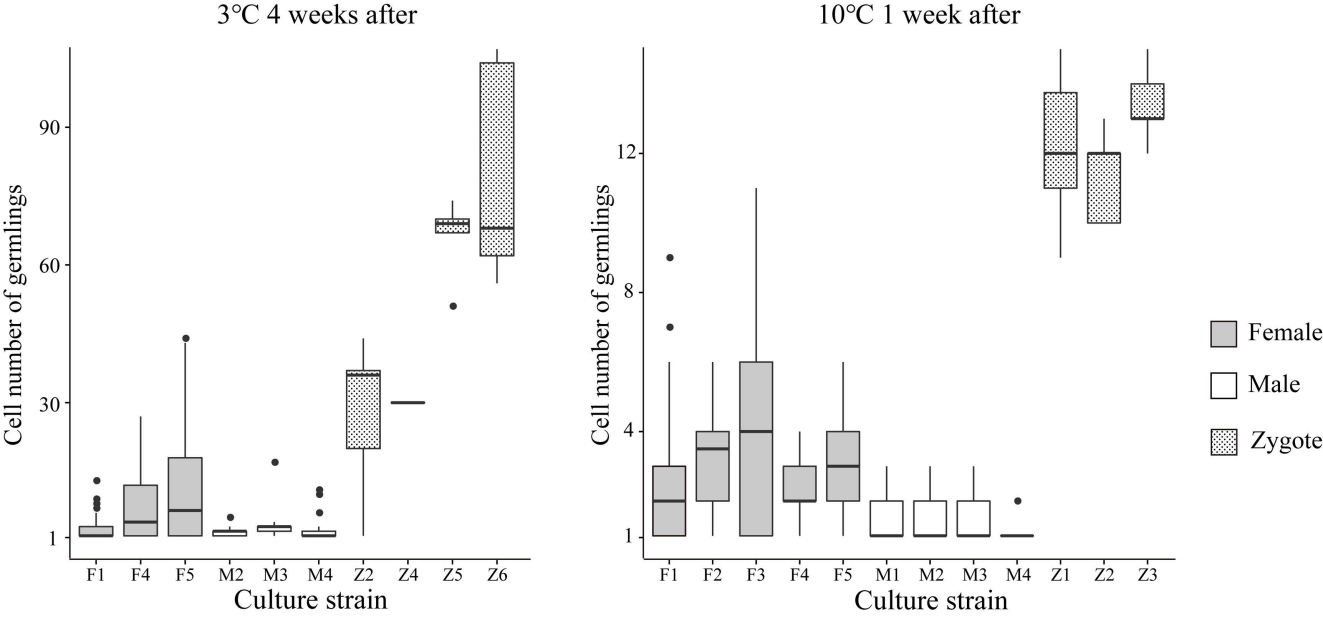
100	Oshoro171014 cr63	+	
101	Oshoro171014 cr69	tw	f,m
102	Oshoro171014 cr70	+	
103	Oshoro171014 cr72	+	
104	Oshoro171014 cr73	+	
105	Oshoro171014 cr74	+	
106	Oshoro171014 cr75	+	
107	Oshoro171014 cr76	+	
108	Oshoro171014 cr77	+	
109	Oshoro171020 cr4	+	
110	Oshoro171020 cr5	+	
111	Oshoro171020 cr6	+	
112	Oshoro171020 cr7	+	
113	Oshoro171020 cr8	+	
114	Oshoro171020 cr12	+	
115	Oshoro171020 cr13	+	
116	Oshoro171020 cr23	+	
117	Oshoro171020 cr24	+	
118	Oshoro171020 cr29	+	
119	Oshoro171020 cr30	+	
120	Oshoro171020 cr33	+	
121	Oshoro171020 cr39	+	
122	Oshoro171020 cr42	+	
123	Oshoro171020 cr43	+	
124	Oshoro171020 cr48	+	
125	Oshoro171020 cr49	-	f,m
126	Oshoro171020 cr54	+	

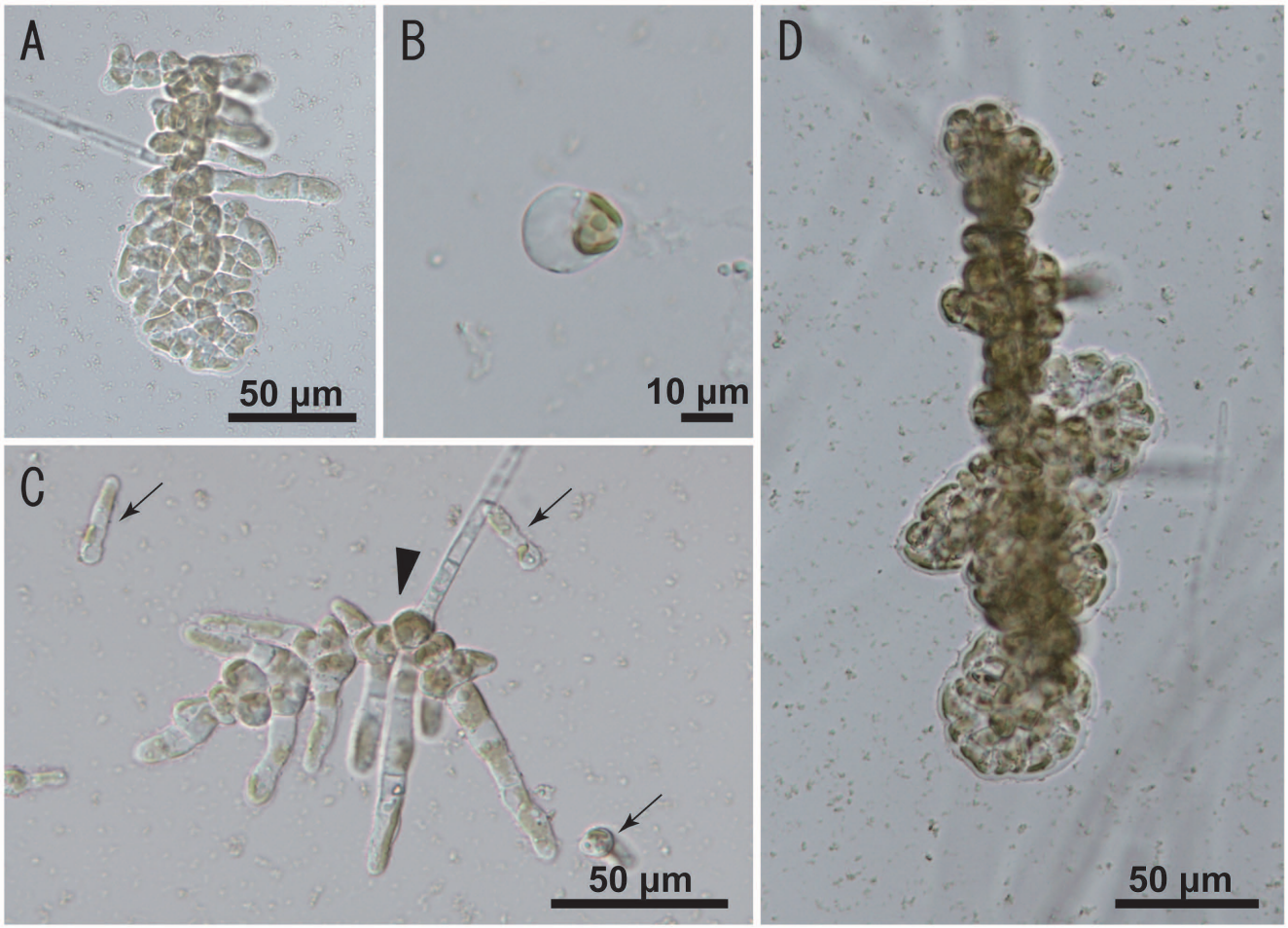
Table S3. The ratio of partheno-germlings which formed erect thalli to the total partheno-germlings (erect, filamentous, and discoid thalli).

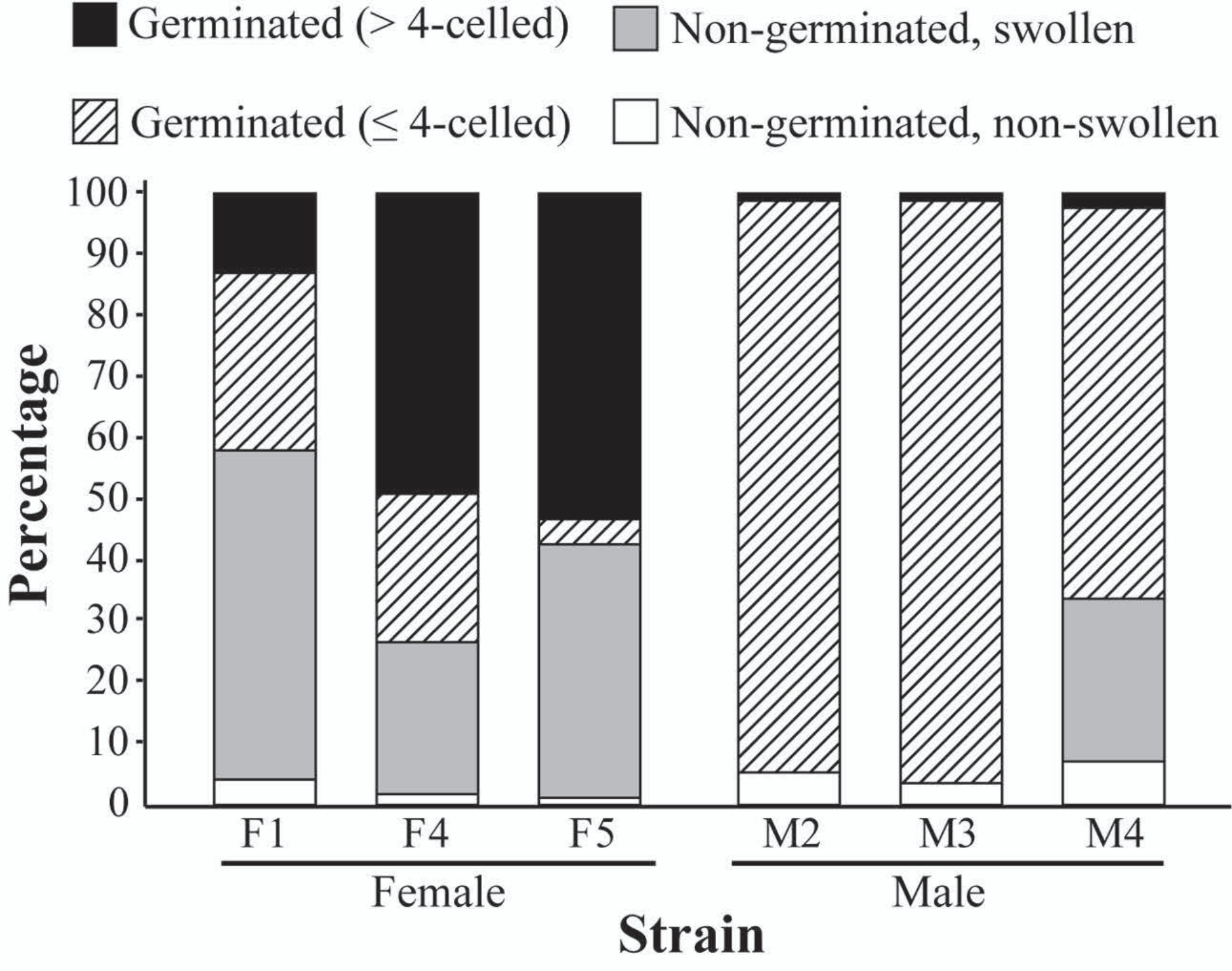
	Strain	3°C 12 weeks	10°C 8 weeks
Female	F1	2/268 (0.7%)	1/139 (0.7%)
	F2	NA	1/154 (0.6%)
	F3	NA	0/110 (0%)
	F4	2/390 (0.5%)	8/82 (9.8%)
	F5	3/278 (1.1%)	5/144 (3.5%)
Female Avg.	Avg. ± SD	0.8 ± 0.3%	2.9 ± 4.1%
Male	M1	NA	4/439 (0.9%)
	M2	0/397 (0%)	3/258 (1.2%)
	M3	3/431 (0.7%)	4/347 (1.2%)
	M4	2/439 (0.5%)	3/103 (2.9%)
Male Avg.	Avg. ± SD	0.4 ± 0.4%	1.6 ± 0.9%


A
1 mm
B
50 µm
C
1 cm
D
10 µm
E
e
e
10 µm
F
f
e
f
f
e
e
f
10 µm


♀ Gametophyte (n)
♂ Gametophyte (n)
Zoid
Zoid
US
P!
P!
♀ Parthenosporophyte
♀ Gamete
M!
F!
Zygotic sporophyte (2n)
US
♂ Gamete
♂ Parthenosporophyte
P!
P!
Zoid
US
Zoid


3°C 4 weeks after
Cell number of germlings
90
60
30
1
F1
F4
F5
M2
M3
M4
Z2
Z4
Z5
Z6
Culture strain
10°C 1 week after
Cell number of germlings
12
8
4
1
F1
F2
F3
F4
F5
M1
M2
M3
M4
Z1
Z2
Z3
Culture strain
Female
Male
Zygote


A
50 µm
B
10 µm
C
50 µm
D
50 µm


Germinated (> 4-celled)
Non-germinated, swollen
Germinated (≤ 4-celled)
Non-germinated, non-swollen
Percentage
100
90
80
70
60
50
40
30
20
10
0
F1
F4
F5
M2
M3
M4
Female
Male
Strain