Journal of Applied Phycology **10:** 253–260, 1998.

Intraspecific reproductive variation in *Gelidium pusillum* (Stackh.) Le Jol. (Gelidiales, Rhodophyta) from Europe

Jan Rueness & Stein Fredriksen
Department of Biology, Section for Marine Botany, University of Oslo, P.O. Box 1069 Blindern, Oslo, Norway

(*Author for correspondence)

Received 1 December 1997; in revised form 30 April 1998; accepted 4 May 1998

Key words: Gelidium, reproductive biology, chromosome number, life history, female sterility, sporeling survival

Abstract

Interfertility has been demonstrated *in vitro* between isolates of *G. pusillum* from Norway, France and the British Isles, but anomalies in reproductive behaviour were observed in the two Norwegian isolates. In one of the latter (Fedje), female gametophytes were sterile. Carpogonia and nutritive filaments were differentiated, but further development was always disrupted and carposporangia never formed. On one occasion, bilocular 'pseudo-cystocarps' were formed in a self-cross, but no carpospores were produced. Male reproductive structures were functional and used in crossability tests. In the other Norwegian isolate (Solund), only a small percentage of the released tetraspores survived, and most of these had an aberrant dwarfed growth habit. Only a few of several thousand spores produced functional male and female gametophytes of normal appearance. In quantitative experiments, significantly higher sporeling survival was found in one of the French isolates (Cancale) than in the Solund isolate. Stages of the first meiotic division were observed and a haploid chromosome number of approximately $n = 15 - 20$ was counted for the Solund isolate. During the second meiotic division, failure was frequently observed in that cytokinesis took place without completion of nuclear divison. In the French isolates of the same species (Cancale and Wimereux isolates), a haploid chromosome number of $n = 20$ or 21 was determined during meiosis in tetrasporocytes.

Introduction

The European *Gelidium* species have their centre of distribution in the warm temperate region, where the individual species are fully developed and both haploid and diploid plants become reproductive, producing gametangia and tetrasporangia, respectively. At the northern limits of distribution, field observations suggest that the sexual reproductive potential cannot be expressed, and that populations are maintained by perennation and vegetative propagation (Rueness & Fredriksen, 1989). Two species of *Gelidium* are known from Scandinavian waters, where both reach their northern limit on the Norwegian west coast. *Gelidium latifolium* (Greville) Born. et Thur. is the commoner of the two species and has the northernmost distribution. It is usually recorded in the vegetative state; tetrasporangia have occasionally been seen from July to September, but never gametophytes (Rueness & Fredriksen, 1989). *Gelidium pusillum* (Stackh.) Le Jol. is reported to have a worldwide distribution and forms minute turfs on intertidal rocks. Recent studies have shown that several morphologically similar entities belong to distinct species that might earlier have been included in the *G. pusillum* complex (Maggs & Guiry, 1987; Guiry & Womersley, 1993; Fredriksen et al., 1994; Freshwater & Rueness, 1994; Rico & Guiry, 1997). Field plants of *G. pusillum* from Norway have invariably been recorded in the vegetative state, and the species is only known with certainty from the two localities where material used in the present study was originally isolated (Fedje and Solund). Cultures of *G.*

pusillum were established from vegetative tips, and tetrasporangia were induced in culture. Tetraspores from both isolates have developed into mature dioecious gametophytes. In a previous paper (Fredriksen et al., 1994), we reported on crossability tests between male plants of the Fedje isolate and a number of isolates of *G. pusillum* from the British Isles and France. However, the female plants from Fedje were apparently sterile and never produced cystocarps, even when self-crossed. In the present study we examine details of the reproductive development of the aberrant female plants from the Fedje isolate. In addition we report on the results obtained from the other Norwegian isolate (Solund). Here gametophytes of both sexes were functional, but quantitative experimens showed the survival rate of released tetraspores to be very much lower than that of an isolate from France (Cancale). We therefore examined details of sporogenesis, and used karyological observations during meiosis to detect any failure that might help to explain the phenomena observed.

Material and methods

Vegetative fronds of *Gelidium pusillum* were collected from rocks in the littoral zone under the *Ascophyllum nodosum* canopy at two sites on the west coast of Norway. The Fedje isolate is the same as that used in an earlier study (Fredriksen et al., 1994). Solund is a site about 40 km north of Fedje where vegetative specimens were isolated into unialgal culture from a collection on 4 October 1993. Isolates of *Gelidium pusillum* from France and the British Isles were the same as those listed in Fredriksen et al. (1994). The culture medium and methods were the same as those in Fredriksen & Rueness (1990). Stock cultures were maintained at 17 EC under a 16: 8 light: dark regime at ca 10 μmol photon m^{-2} s^{-1}. To induce the formation of tetrasporangia, cultures were transferred to a short-day regime (8:16, 40 μmol photon m^{-2} s^{-1}) and tetrasporangia usually formed after about 4 weeks. To examine the germination and survival of tetrasporelings, 6 mature stichidia of each isolate tested (Solund, Cancale) were placed in each of 12 wells containing culture medium on multi-well plates (4×6 wells). For 4 consecutive days, the stichidia were transferred to new wells, and the number of relased spores was counted. The number of survivors as a percentage of the number of spores released was calculated from examinations of the sporeling populations every 4 d for a period of at least 3 weeks. The results are presented as survivorship curves.

Wittmann's aceto-iron-haematoxylin-chloral-hydrate (Wittmann, 1965) was used as a chromosome stain after fixation in 3:1 absolute ethanol: acetic acid for at least 6 h (material fixed in 1:1 absolute ethanol/acetic acid as used by Maggs & Rico (1991) gave similar results). Reproductive axes were hand-sectioned with a razor blade on the slide and then stained and gently squashed under the coverslip. To study details of sporeling morphogenesis, tetraspores settled on cover slips were fixed and stained with Wittmann's stain or mounted in Lactophenol-Wasserblau (Chroma).

Results

Life history and reproductive structures

Stock cultures were established from vegetative apices derived from both the Norwegian populations (Fedje and Solund). The two isolates were morphologically indistinguishable in culture and produced tetrasporangia after a period of 3–4 weeks when incubated under a short-day regime (8:16) at 17 ∘EC and a photon fluence rate of 40 μmol photon m^{-2} s^{-1}. Male and female gametophytes of both isolates were produced from tetraspores and maintained as cloned cultures. These also became reproductive when incubated under short-day conditions. In a previous study we demonstrated interfertility between males of the Fedje isolate and 5 different isolates of the same species from France and the British Isles (Fredriksen et al., 1994). In the present study we tested a cross between the male of the Fedje isolate and a female of the Solund isolate. The cross resulted in carposporophyte development (Figure 4) and we conclude that both the Norwegian entities and those from France and the British Isles belong to the same biological species.

The tetrasporangial stichidia are formed terminally in flattened tips of lateral axes. Tetrasporangia mature acropetally and tetrasporangia are shed continuously for several weeks. At the end of dehiscence the stichidia may decay and eventually be shed, but vegetative growth is resumed from initials below the stichidium (Figure 8). The tetrasporangia develop from the cortical filaments and the tetrasporangium initials are easily distinguished in cross-section, where various stages of sporangium maturation can be seen (Figure 7). In the Solund isolate, many sporangium initials remain undivided or divide only once to form two spores (see below).

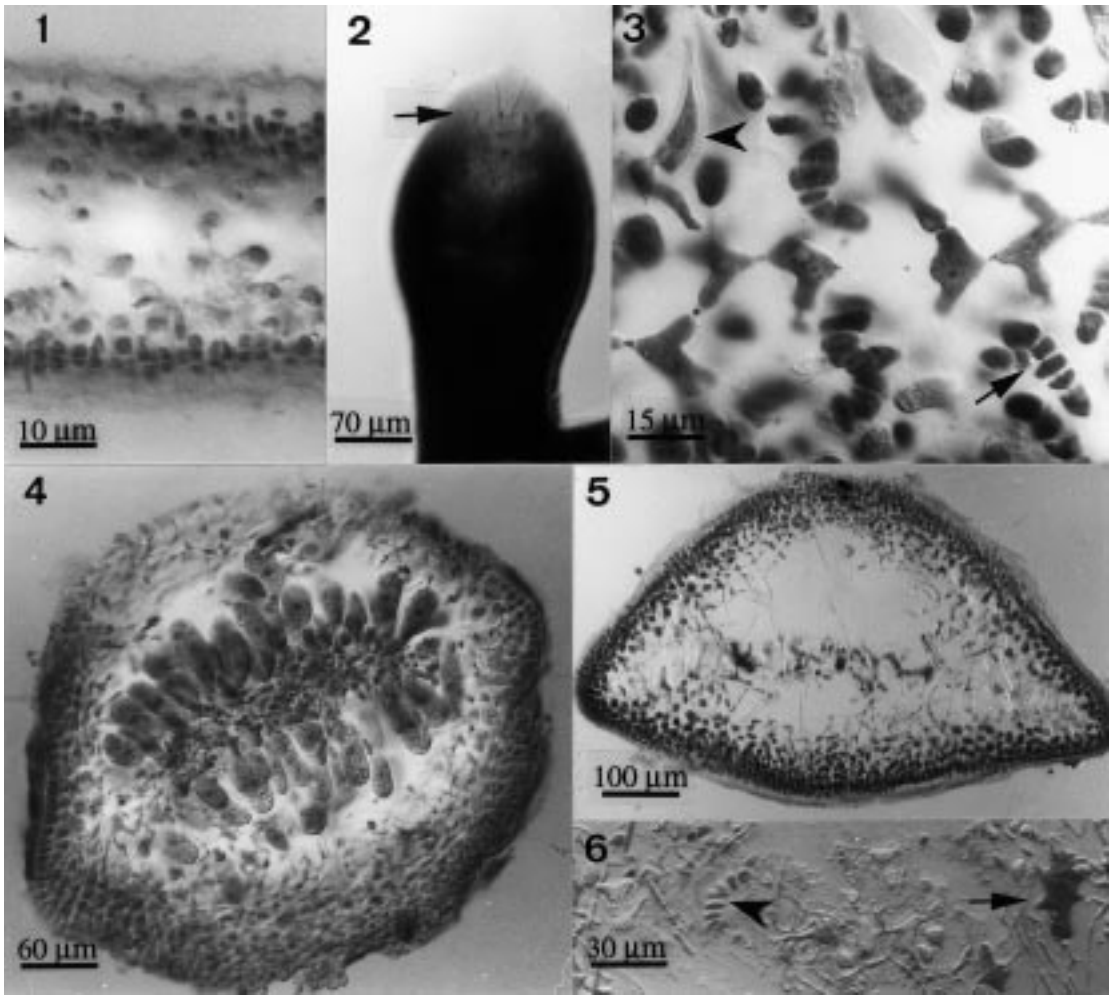

Figure 1–6. Reproductive development in *Gelidium pusillum* from Norway in culture.
1. Section of male plant showing transverse divison of spermatangia.
2. Apical portion of female branch of the Fedje isolate, showing trichogynes (arrow).
3. Section of reproductive female of the Fedje isolate, showing carpogonia (arrow head) and nutrient filaments (arrow) and second order filaments joined by secondary pit connections.
4. Transverse section of mature bilocular cystocarp resulting from the cross beteween Fedje male × Solund female.
5. Transverse section of 'pseudo-cystocarp' resulting from a self- cross of the Fedje isolate. Note nutritive filaments and enlarged carpogonia or fusion cells.
6. Same as in Figure 5. Detail of 'pseudo-cystocarp' to show nutrient filament (arrow head) and enlarged carpogonium or fusion cell (arrow).

Gametangial plants of the Solund isolate were similar to that described and illustrated by Fredriksen et al. (1994) for the Wimereux isolate. A cross section of a male pinnule of the Fedje isolate is illustrated in Figure 1. The external morphology of the non-functional female plants of the Solund isolate was indistingushable from that of functional females of the other *G. pusillum* isolates. Fertile female branchlets are distinguished from vegetative tips by having slightly notched, pale apices with several trichogynes visible on either side of the pinnule (Figure 2). In section it can be seen that nutritive filaments and carpogonia begin differentiation close to the apex (Figure 3). Usually there is no further development of gonimoblast tissue. In one self-cross of the Fedje isolate, biconvex swelling of the fertile pinnules took place and a bilocular cystocarp-like structure formed with ostioles on one or both sides. In transverse section a placenta and long cortical filaments could be seen, but no carposporangia (Figure 5). Some large cells were irregularly swollen, and these are interpreted as aborted carpogonia or fusion cells (Figures 5, 6). The sterile apiculate

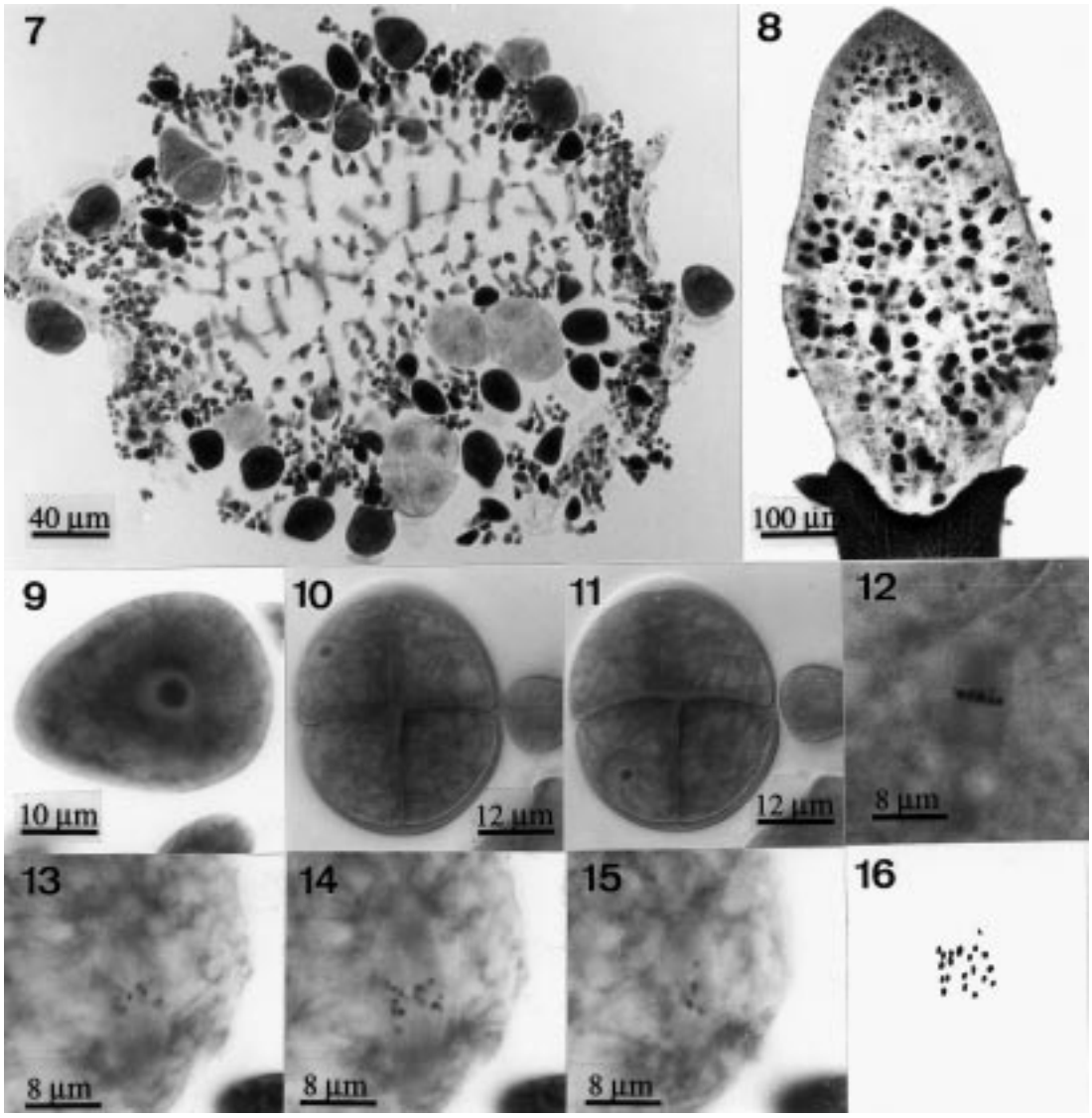

Figure 7–16. 7. Transverse section through tetrasporangial stichidium of the Solund isolate showing several sporangium initials. A few sporangia are divided in two uninucleate cells and a few others into four cells, only two of which contain a nuclei (see Figures 10, 11).
8. Surface view of tetrasporangial stichidium with many emptied sporangia. Note regeneration from apical cells below the stichidium.
9. Tetrasporangial initial cell with enlarged nucleus and heavily stained nucleolus.
10–11. Tetrasporangium of the Solund isolate in two focal planes to show that only two nuclei are visible/present.
12. Nuclear division at the metaphase stage during tetrasporogenesis of the Solund isolate.
13–16. Nuclear division at late meiotic prophase during tetrasporogenesis of the Cancale isolate. Three focal planes and a composite drawing showing 20 chromosomes.

tip continued vegetative growth, and in a few fertile branches two 'pseudo-cystocarps' were produced along the midline of the same pinnule.

Karyological observations during tetrasporogenesis

The uninucleate tetrasporangium initials increase rapidly in size and contain an enlarged nucleus with a distinct darkly staining nucleolus (Figure 9). In the Solund isolate, many sporangial initials remained undivided and had a granular cytoplasm, some sporangia divided into two cells, each with a nucleus (Figure 7), and a few divided a second time to produce four spores, two of which had nuclei, while in the other two no nucleus was visible (Figures 10, 11). In contrast, in

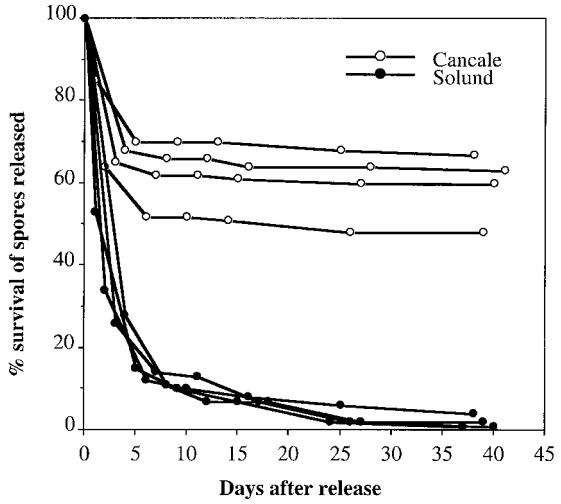

Figure 17. Survival of *Gelidium pusillum* tetrasporelings in isolates from Norway (Solund) and France (Cancale). Each curve represents a tetraspore population from six stichidia and each point the average value for 6 stichidia.

the French isolates from Cancale and Wimereux the nuclei were clearly visible in all phases of sporangial maturation. In some preparations of the Cancale isolate, the chromosome number during the first meiotic division was clearly countable (Figures 12–16). A haploid chromosome complement of n = 20 is most probable. Nuclear divisions were frequently observed in tetrasporocytes of the Solund isolate. No conclusive counts of chromosomes could be made, but observations suggested the same ploidy level (counts in the range of 15–20) as in the Cancale isolate. No chromosome counts were made in mitotic nuclei of vegetative cells either in tetrasporophytes or in gametophytes.

Survival of tetraspores

Survivorship curves for tetrasporelings of the Solund and Cancale isolates are presented in Figure 17. In the former isolate, about half the spore population died or failed to germinate during the first day. After 8 d only about 10% of the spores survived, and after 40 d only 26 of a total of 885 released spores were alive (2.9%). After 80 days only 7 sporelings were alive and these showed an abnormal dwarfed growth form. In the Cancale isolate the germination percentage was significantly higher although variable, and still more than 50% of the sporelings were alive after 40 d and were normal looking and vigourous.

Discussion

Both isolates of *Gelidium pusillum* from Norway exhibited anomalies in their sexual reproductive life cycle, in contrast to the isolates from France and the British Isles, where a regular sequence of gametophytes and sporophytes was found. The Fedje isolate was female-sterile but the males were functional and carposporophyte development resulted in crosses with female isolates from France and the British Isles (Fredriksen et al., 1994) and with the Solund isolate from Norway as shown in this study. The female-sterile plants appear to be very similar to the chemical mutants produced experimentally by van der Meer & Patwary (1991) in the monoecious *Gelidium vagum* Okamura. One of their illustrated mutants (van der Meer & Patwary 1991: Figures 4C, D) produces what we have termed a ‘pseudo-cystocarp’, in which a bilocular swelling of the frond develops, divided by a placenta. In most instances no swelling of the frond occurred, but carpogonia and nutritive filaments were always formed. This phenotype was also included in the range of female-sterile mutants described by van der Meer & Patwary (1991). In *Gelidium pusillum* from Wimereux, Fredriksen (1994) described a morphological mutant that was isolated from a population of hundreds of tetrasporelings. This mutant was minute and had a bushy and later globular form. One of the plants matured and produced spermatangia that were used in crosses with the wild-type. A 1:1 segregation ratio was demonstrated in next tetraspore generation, suggesting a single-gene mutation. Mutants that affect fertility and morphology may be common in *Gelidium* species, and may help to explain the reproductive irregularities and morphological variation observed in the genus. In Norwegian waters, *G. pusillum* is probably maintained as clonal populations by vegetative propagation and perennation with little or no gene flow between populations. The stoloniferous growth form with rhizoidal attachment pads are of importance in vegetative reproduction by fragmentation. Given the environmental constraints at the northern distribution limit, where light and temperature conditions are suboptimal for most of the year, one may speculate whether mutations that reduce or inhibit sexual reproduction can be advantageous from an energetic point of view.

Both isolates of *G. pusillum* proved to be tetrasporangial when isolated into culture. The Solund isolate produced numerous spores in culture, but the viability

Table 1. Chromosome numbers in species of Gelidiales.

Species	1N	2N	References
Acanthopeltis japonica Okamura	15	30	Kaneko, 1968
Gelidiella acerosa (Forssk.) Feldmann et Hamel	4	8	Rao, 1974[1]
Gelidiella acerosa	6	12	Subba Rao et al., 1991[2]
Gelidiella acerosa	6		Kapraun et al., 1994
Gelidium amansii Lamouroux	25		Yabu, 1991
Gelidium americanum (Taylor) Santelices	12		Kapraun et al., 1993
Gelidium corneum (Hudson) Lamouroux	4–5	8–9	Dixon, 1955; 1963
Gelidium floridanum Taylor	6	12	Kapraun et al., 1993
Gelidium latifolium (Grev.) Bornet et Thur.	4–5	9–10	Dixon, 1955; 1963
Gelidium latifolium	Ca 18		Boillot, 1963
Gelidium latifolium (Grev.) Bornet et Thur. var. *luxurians* (Crouan) Hamel et Feldmann		25–30	Magne, 1964[3]
Geldium latifolium	29 ± 2	58 ± 4	Maggs & Rico, 1991
Gelidium pristoides (Turner) Kütz.	13–17	28–33	Carter, 1993
Gelidium pusillum (Stackh.) Le Jol.	10	20	Kapraun & Bailey, 1989
Gelidium pusillum	20		Present study
Gelidium serrulatum J. Ag.	10	20	Kapraun et al., 1993
Gelidium vagum Okamura	7–10		Kaneko, 1966
Gelidium vagum	14–15	18–30	D. Renfrew *fide* Cole, 1990
Pterocladia capillacea (Gmel.) Bornet et Thur.	8	16	Kapraun et al., 1993

[1] Material from west coast of India.
[2] Material from southeastern coast of India.
[3] No information of ploidy level.

of sporelings was much lower than in the isolate from Cancale.

The chromosome number of the two isolates was the same as determined during tetrasporogenesis (n = ca 20), but we have no chromosome counts from mitotic nuclei of vegetative cells in gametophytes and tetrasporophytes. The only published chromosome number for *G. pusillum* is that reported by Kapraun & Bailey (1989) in specimens from North Carolina. They found 10 bivalents during meiosis in tetraspore mother cells, and $2n = 20$ chromosomes in dividing cortical cells of the tetrasporophyte. Molecular evidence, based on rbcL sequence data, shows that *G. pusillum* from North Carolina is distinctly different from European *G. pusillum* (Freshwater & Rueness, 1994), and probably belongs to a different species, related to *G. coulteri* Harvey and *G. capense* (Gmelin) Silva. Dixon (1955; 1963) reported chromosome numbers of $n = 4 - 5$ and $2n = 9 - 10$ for two British species of *Gelidium* classified as *G. latifolium* and *G. corneum*. Dixon?s estimate for *G. latifolium* is much lower than the counts of ca $n = 30$ and $2n = 60$ recently published by Maggs & Rico (1991) in the same species from Ireland, and earlier approximate counts by Boillot (1963) and Magne (1964) of n = ca 18 and n or $2n = 25 - - 30$, respectively. We have not been able to examine Dixon's (1955) material of *G. corneum*, but it most probably belongs to the *G. latifolium* aggregate, *sensu* Dixon and Irvine (1977), and not to *G. sesquipedale* (Clemente) Born. et Thur. as listed in Kapraun & Bailey (1989). Kaneko (1968) suggested that the basic chromosome number of the Gelidiales is x = 5 on the basis of the few chromosome counts available at that time, including his own results from *Acanthopeltis japonica* Okamura, $n = 15$, $2n = 30$ and 3 earlier reports on European *Gelidium*. Since then only a few more Gelidialean chromosome counts have been reported (Table 1). Although discrepancies in chromosome numbers exist, all the numbers available are close to 5 or a multiple of five, suggesting that there are polyploid series. Carter's (1993) observations of haploid bispore production instead of tetraspore production is particularly interesting in view of our observations of the Solund isolate showing that in many instances, only two uninucleate spores are formed. Unfortunately, we have no chromosome numbers for the product of these spores due to their low viability. Similarly, in a study of *Gelidium lati-*

folium from Ireland, Maggs & Rico (1991) found that the viablity of tetrasporeling stages was very poor and only one of several hundred grew to maturity. Interestingly, they were able to show that this plant had diploidized spontaneously during development and produced diploid spermatangia. This suggests a mechanism by which polyploids may arise, and indicates that the lack of gametophytes in the local *G. latifolium* population may be a result of the poor viability of haploid tetraspores. In the genus *Gelidiella*, one of the distinguishing features is the lack of gametophytes. In *Gelidiella acerosa*, meiosis in tetraspore mother cells was demonstrated for the first time by Subba Rao et al. (1991), but the fate of the tetraspores is unknown. These authors explain the absence of gametophytes as being a result of germination of undivided tetraspore mother cells.

Intraspecific life history variability is well known in many red algae and has been reviewed by Maggs (1988). There appears to be a pattern of increasing frequency of apomixis and asexual means of reproduction with increasing latitude in many species of red algae (Dixon 1965; Rueness 1968; Guiry & West 1983; Maggs 1988). Although there are many differences between higher plants and red algae, it is interesting that the replacement of sexual reproduction by asexual propagation and the development of polyploid series appear to be common phenomena in arctic and alpine higher plants (Stebbins 1985). At present too little is known about red algae like *Gelidium* species to make generalizations for a species or a region. Rico & Guiry (1996) recently demonstrated latitudinal ecotypes in temperature response for populations of *G. pusillum* from north-eastern Atlantic coasts. The Norwegian isolate used by Rico & Guiry (1996) was the same as the Fedje isolate used in the present study. Our results demonstrate that intraspecific differeces also exist in reproductive behaviour. This is not only of academic interest, but may also have practical implications for strain selection in the commercial agarophyte genus *Gelidium*.

References

Boillot A (1963) Recherches sur le mode de développement des spores du genre *Gelidium* (Rhodophycées, Gélidiales). Rev. Gén. Bot. 70: 130–137.

Carter AR (1993) Chromosome observations relating to bispore production in *Gelidium pristoides* (Gelidiales, Rhodophyta). Bot. mar. 36: 253–256.

Cole KM (1990) Chromosomes. In Cole KM, Sheath RG (eds), Biology of the Red Algae. Cambridge University Press, Cambridge: 73–101.

Dixon PS (1955) Nuclear observations of two British species of *Gelidium*. Br. phycol. Bull. 1: 4.

Dixon PS (1963) Variation and speciation in marine Rhodophyta. In Harding JP, Tebble N (eds) Speciation in the Sea. Syst. Assoc., London: 51–62.

Dixon PS (1965) Perennation, vegetative propagation and algal life histories, with special reference to *Asparagopsis* and other Rhodophyta. Bot. Gothob. 3: 67–74.

Dixon PS, Irvine LM (1977) Seaweeds of the British Isles. Vol. 1. Rhodophyta. Part 1. Introduction, Nemaliales, Gigartinales. Br. Mus. (Nat. Hist.), London, 252 pp.

Fredriksen S (1994) Spore-shedding in *Gelidium pusillum* (Gelidiales, Rhodophyta), with some observation on a morphological mutant. Cryptogamie, Algol. 15: 165–173.

Fredriksen S, Rueness J (1990) Culture studies on *Pterocladia melanoidea* (Schousboe ex Bornet) comb. nov. (Gelidiales, Rhodophyta). Phycologia 29: 182–190.

Fredriksen S, Guiry MD, Rueness J (1994) Morphological and biosystematic studies of *Gelidium pusillum* and *G. pulchellum* (Gelidiaceae, Rhodophyta) from Europe. Phycologia 33: 462–470.

Freshwater DW, Rueness J (1994) Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species based upon rbcL sequence analysis. Phycologia 33: 187–194.

Guiry MD, West JA (1983) Life history and hybridization studies on *Gigartina stellata* and *Petrocelis cruenta* (Rhodophyta) in the North Atlantic. J. Phycol. 19: 474–494.

Guiry MD, Womersley HBS (1993) *Capreolia implexa* gen. et sp. nov. (Gelidiales, Rhodophyta) in Australia and New Zealand; an intertidal mat-forming alga with an unusual life history. Phycologia 32: 266–277.

Kaneko T (1966) Morphological and developmental studies of Gelidiales. I. Behaviour of the nucleus in early stages of tetraspore germination in *Gelidium vagum* Okamura. Bull. Jap. Soc. Phycol. 14: 62–70.

Kaneko T (1968) Morphological and developmental studies of Gelidiales II. On *Acanthopeltis japonica* Okamura. Bull. Fac. Fish. Hokkaido Univ. 19: 165–172.

Kapraun DF, Bailey CJ (1989) Karyology and nuclear DNA content of *Gelidium pusillum* (Gelidiales, Rhodophyta) from North Carolina, USA. Jap. J. Phycol. 37: 201–207.

Kapraun DF, Dutcher, JA, Freshwater DW (1993) Quantification and characterization of nuclear genomes in commercial red seaweeds: Gracilariales and Gelidiales. Hydrobiologia 260/261: 679–688.

Kapraun DF, Ganzon-Fortes E, Bird KT, Trono G, Breden C (1994) Karyology and agar analysis of the agarophyte *Gelidiella acerosa* (Forsskål) Feldmann et Hamel from the Philippines. J. appl. Phycol. 6: 545–550.

Maggs CA (1988) Intraspecific life history variability in the Florideophycidae (Rhodophyta). Bot. mar. 31: 465–490.

Maggs CA, Guiry MD (1987) *Gelidiella calcicola* sp.nov. (Rhodophyta) from the British Isles and Northern France. Br. phycol. J. 22: 417–434.

Maggs CA, Rico JM (1991) A karyological demonstration of meiosis in *Gelidium latifolium* (Gelidiaceae, Rhodophyta) from Ireland. Phycologia 30: 487–494.

Magne F (1964) Recherches caryologiques chez les Floridées. Cah. Biol. Mar. 5: 464–671.

Rao PS (1974) Some observations on the cytology of *Gelidiella acerosa* (Forsskål) Feldmann et Hamel. Cytologia 39: 391–395.

Rico JM, Guiry MD (1996) Latitudinal ecotypes in north-eastern Atlantic populations of *Gelidium pusillum* (Gelidiaceae, Rhodophyta). In Keegan BF, O'Connor R (eds) Irish Marine Science 1995. Galway Univ. Press, Galway: 105–116.

Rico JM, Guiry MD (1997) Life history and reproduction of *Gelidium maggsiae* sp. nov. (Rhodophyta, Gelidiales) from Ireland. Eur. J. Phycol. 32: 267–277.

Rueness J (1968) Paraspores from *Plumaria elegans* in culture. Nytt Mag. Bot. 15: 220–224.

Rueness J., Fredriksen (1989) Field and culture studies of *Gelidium latifolium* (Grev.) Born. et Thur. (Rhodophyta) from Norway. Sarsia 74: 177–185.

Stebbins GL (1985) Polyploidy, hybridization, and the invasion of new habitats. Ann. Missouri Bot. Garden 72: 824–832.

Subba Rao PV, Chowdary YBK, Subbaramaiah K (1991) Karyology of *Gelidiella acerosa* (Forsskål) Fedmann et Hamel. Cytologia 56: 187–189.

van der Meer JP, Patwary MU (1991) Genetic alleviation of the self-fertilization complication when hybridizing monoecious *Gelidium vagum*. Hydrobiologia 221: 167–179.

Wittmann W (1965) Aceto-iron-haematoxylin-chloral-hydrate for chromosome staining. Stain Technology 40: 161–164.

Yabu H (1991) Nuclear divisions in the tetrasporangia and tetraspore germlings of *Gelidium amansii* Lamouroux. Jap. J. Phycol. 39: 21–25.