

The life history of some species of *Gracilaria* (Rhodophyta) from Brazil

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OLIVEIRA, E.C. DE and PLASTINO, E.M. 1984. The life history of some species of *Gracilaria* (Rhodophyta) from Brazil Jap. J. Phycol. 32: 203-208.

Gracilaria cervicornis, *G. crassissima*, *G. debilis* and *Gracilaria* sp. (aff. *G. verrucosa*) were cultivated in enriched seawater. Erect fronds developed from isolated spores, or from fusion of sporelings. The life history of *Gracilaria* sp. and *G. debilis* was completed *in vitro* and is of the "Polysiphonia-type". Tetraspores of *G. cervicornis* gave rise to male and female gametophytes which produced cystocarps; the carpospores developed into plants with the typical morphology of the species, but remained infertile for two years. Tetraspores of *G. crassissima* gave rise to infertile plants with narrow, terete branches, quite different from the specimens collected in the field. *In situ* germination of tetraspores was observed for both *Gracilaria* sp. and *G. debilis*, *in vitro*, and in natural populations. The resulting gametophytes produced spermatangia, carpogonia and cystocarps when only 2-3 cm in length, being more precocious than the gametophytes growing free from the parent plants. The causes for the occurrence of mixed phase reproductive structures are discussed, stressing the importance of sporeling fusions and *in situ* germination of tetraspores.

Key Index Words: Rhodophyta; *Gracilaria cervicornis*, *G. debilis*, *G. crassissima*; life-history; mixed reproductive phase.

The genus *Gracilaria* GREVILLE has about one hundred species (KYLIN 1956) and is particularly well represented in Brazil (OLIVEIRA 1977), where it has been exploited as raw material for the agar industry (OLIVEIRA 1981). Considering the economical potential of the genus *Gracilaria* in Brazil, an effort is being made to gather more informations about the local species. Life history provide basic information for understanding the natural population behaviour as well as a rational support for mariculture activities. This paper presents observations on the life histories of four species of *Gracilaria* occurring in Brazil and discuss the existing hypothesis to explain the occurrence of mixed phase reproductive structures in the genus.

Materials and Methods

Gracilaria debilis (FORS.) BOERG., *G. cervicornis* (TURN.) J. AG., *G. crassissima* CROUAN ex J. AG., and *Gracilaria* sp. were studied (Table 1). The last mentioned species has morphological and reproductive features that have been broadly attributed to *G. verrucosa* (HUD.) PAP. However, considering the uncertainties about the real delimitation of *G. verrucosa* we preferred not to name it in this paper.

Fertile specimens were transported in insulated box to the laboratory, brushed in sterile seawater, and put in membrane filtered seawater (0.45 μ m) where spores were liberated and settled onto cover glasses. The cover slips with attached spores were transferred to petri dishes containing 50 ml of von STOSCH medium (VON STOSCH 1969) at one

Table 1. Reproductive phase, places and date of collection of the plants studied

Species	Tetrsp.	Cystoc.	Locality	Date
<i>Gracilaria cervicornis</i>	+	+	Monsuaba, RJ	Nov. 79
<i>Gracilaria crassissima</i>	+	-	Natal, RN	Feb. 80
<i>Gracilaria debilis</i>	+	+	Natal, RN	Oct. 79
<i>Gracilaria</i> sp.	+	+	Natal, RN	Oct. 79

Table 2. Minimum, mean and maximum sizes (μm) of spores of *Gracilaria*. N=30

Species	Tetraspores				Carpospores			
	(Min.)	Mean	(Max.)	Sd.	(Min.)	Mean	(Max.)	Sd.
<i>G. cervicornis</i>	(19.0)	21.5	(26.6)	2.7	(15.2)	20.4	(26.6)	2.0
<i>G. crassissima</i>	(19.2)	22.6	(26.8)	1.3				
<i>G. debilis</i>					(22.8)	27.1	(30.4)	2.6
<i>Gracilaria</i> sp.	(19.0)	21.9	(30.4)	2.7	(15.2)	20.1	(22.8)	2.1

half strain. After attaining 5 mm in length unialgal sporelings on cover glasses were moved to 250 ml erlenmeyer flasks containing 200 ml of the same medium and bubbled with air; the medium was changed weekly; the temperature was $26^{\circ}\text{C}(\pm 3)$ and illumination provided by fluorescent lamps (Philips "cool-light") giving $60 \mu\text{E.m}^{-2}.\text{s}^{-1}$ under an 18:6 hours photo-regime; germanium dioxide were used to suppress diatom growth when necessary.

Results

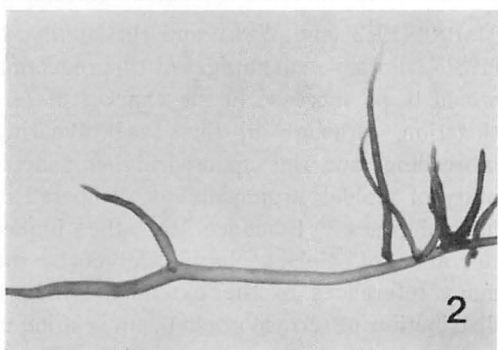
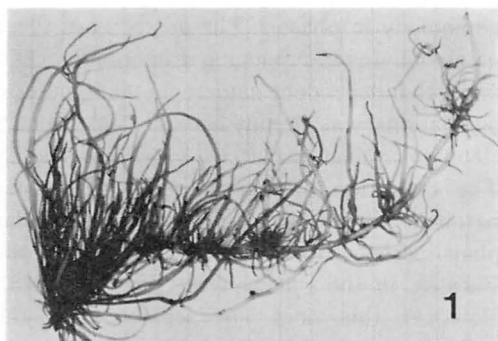
Spore size and development: The size of the spores of the species studied varied continuously. The mean diameter of the spores measured soon after liberation in seawater was 20-22 μm for carpospores and tetraspores, except for *G. debilis* which had larger carpospores. The total variation for spores produced from a single plant is greater than the mean variation among the different species (Table 2). Germination and subsequent development of the sporelings was as described for the genus since 1878 by THURET. After the formation of the multicellular disc, an erect frond is produced, which becomes conspicuous in culture one

month old. Fusions of adjacent discs are common, especially in dense cultures, where, according with the number of fusions two or more erect shoots are produced. The early stages were very similar to each others species studied except for *G. cervicornis*, whose erect shoots very soon become flattened and produced dentiform lateral branches; long hyaline hairs were common on developing sporelings of *G. debilis* and *Gracilaria* sp. Growth of erect fronds is made from an apical meristem.

Life-history: Carpospores of both *G. debilis* and *Gracilaria* sp. gave rise to tetrasporophytic plants whose tetraspores developed into male and female gametophytes, the latter soon produced cystocarps. The life history of *G. debilis* was completed in about 5 months, and in about 9 months for *Gracilaria* sp. In both species gametophytic thalli in culture tend to be more branched than the tetrasporophytic ones. The tetraspores of *G. cervicornis* gave rise to male and female plants, the latter produced cystocarps in 4 months; the carpospores developed normally and gave rise to plants with the morphological characteristics of this species but remained infertile for 24 months of culture. Tetraspores of *G. crassissima* produced erect

fronds scarcely branched, similar in morphology to the "*G. verrucosa* type of thallus" and very different from the field collected plants. *G. crassissima* was maintained in culture for 22 months without becoming reproductive.

In *Gracilaria* sp. and in *G. debilis* there was a high incidence of germination of tetraspores on the tetrasporophytic branches (Figs. 1 and 2). However we did not find any evidence for the development of tetraspores or tetrasporangia within the thallus of the tetrasporophytes. In the two species referred to young gametophytes that germinated on the tetrasporophytes can be distinguished from normal branches by a basal expansion of the frond at the point of contact with the tetrasporophyte (Fig. 3). Beyond a certain stage of development the fusion is completed and the partially "endophytic" gametophytes become indistinguishable while vegetative, from the normal branches of the tetrasporophyte. In *Gracilaria* sp., gameto-



Figs. 1-2. *Gracilaria* sp. Tetrasporophytic plant with many gametophytic "branches" resulting from the germination of tetraspores *in situ*. Fig. 1 $\times 0.6$. Fig. 2 $\times 1.2$.



Fig. 3. *Gracilaria* sp. Longitudinal section of a young gametophyte developing on a tetrasporophyte, showing partial fusion of tissues. $\times 130$.

phyte branchlets being only 2 cm long were already fertile, with normal production of spermatangia or carpogonia and cystocarps. The same situation was later noted on plants collected in the field. The precocious production of gametes from the gametophytes growing on the tetrasporophytic phase suggests an influence of the mature tetrasporophyte on the reproductive maturation of the gametophytes.

Discussion

An examination of the sporelings and plantlets showed that at no stage of development could a typical apical cell be found as described by KILLIAN (1914) and OZA and KRISHNAMURTHY (1967) for *G. verrucosa*. In the case of *G. cervicornis*, whose branch tips are pointed, a terminal cell can be recognized in some cases; however the thallus structure is obviously not uniaxial.

The life history of *Gracilaria* sp. and *G. debilis* is of the "*Polysiphonia*-type" as expected, and has been documented in the literatures for other three species (OGATA *et al.* 1972, MCLACHLAN and EDELSTEIN 1977 and BIRD *et al.* 1977). Our failure to com-

plete the life history of *G. cervicornis* is most likely a problem of finding the right conditions to trigger the production of tetrasporangia, as tetrasporic plants are found commonly in natural populations. The development of the tetraspores in *G. crassissima*, giving rise to infertile plants with a general morphology similar to *G. verrucosa*, is unexpected and should be further studied under different incubation conditions, especially considering that we did not find sexual plants in nature.

The fusion of sporelings during early germination was observed in the species studied here. This is especially common among carpospores which tend to be closer to each other because of the mechanism of spore liberation. This has also been observed for other species of *Gracilaria* (SEGAWA *et al.* 1955, RAO and THOMAS 1974). In agreement with BIRD *et al.* (1977), but in contrary to that described by JONES (1956), we observed that erect fronds are equally produced from discs originated from isolated spores as from those produced by fusion of sporelings. Fusion of sporelings and germination of tetraspores *in situ* are mechanisms that are certainly responsible for the occurrence of mixed reproductive phases in the plants studied. This could also be an explanation for several reported cases in the literatures (CHURCH 1919, OHMI 1958, KIM 1970), although generalizations can not be made by lack of details. In this connection, other possible mechanisms for mixed reproductive phase in *Gracilaria* have been suggested. CABIOCH (1972) produced evidence that in *G. verrucosa* the tetrasporangial contents develops directly into a sexual structure that is either a male crypt or a presumed female gametangium when cystocarps were found. A quite different explanation was given by VAN DER MEER (1977) for *Gracilaria* sp. (= *G. tikvahiae* MCLACHLAN) who attributed the presence of male and female structures on the same plant, to a failure in cytokinesis in some tetrasporangia evidenced by the production of tetraspores of three different size classes, and by the segregation of mutant

genes. In another paper, on the same species, VAN DER MEER and TODD (1977) indicated that mitotic recombination is the mechanism responsible for the production of male and female gametes on tetrasporophytes. Thus, with respect to reproduction in species of *Gracilaria*, it seems that at least 5 different mechanisms leading to mixed-phase reproduction may be operating:

1. fusion of developing sporelings;
2. germination of tetraspores *in situ*, on the tetrasporophytes, giving rise to normal, though somewhat reduced gametophytes;
3. direct development of the sporangial contents into gametangia;
4. failure of cytokinesis during meiosis (tetraspores with 2 or more haploid nuclei), and
5. mitotic recombination.

The last process corresponds, in essence, to a form of apospory, well known in mosses and ferns, although here controlled by a different mechanism. Processes 2 and 3 are only different stages in the reduction of the gametophytic phase. The precocity of these gametophytes in producing gametangia, compared to independent gametophytes in natural populations, was already noted by OSTERHOUT (1896) for *Rhabdonia tenera* (= *Solieria tenera*). The reduction of the gametophytic phase parallels the situation known for vascular plant series (BOWER 1908), and for the Fucales, in the Phaeophyta (FRITSCH 1945). However this does not seem to be the general trend in the Rhodophyta as seen in MAGNE (1972) and WEST and HOMMERSAND (1981). Possible advantages of this reduction would be an increase in the chances of fertilization, although in this case favouring inbreeding, and the supposed higher adaptability of diploid organisms as compared to haploid ones. Evidence for the higher tolerance of diploid phase is given by the many references to the extended northern distribution of tetrasporophytes in relation to gametophytes (but see DIXON 1965).

In conclusion, it remains to be demonstrated in what extent each of the mentioned mechanisms operate in the natural popula-

tions and what is their possible evolutionary significance for the genus *Gracilaria*.

Acknowledgements

Drs. J. MCLACHLAN, J. VAN DER MEER and L. CHEN criticized the manuscript and gave valuable suggestions. The research was carried on during a scholarship from the FAPESP to E. M. P. The final version of the manuscript was completed under a cooperative program between the National Research Councils of Canada (NRC) and Brazil (CNPq) to E. C. O.

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Gracilaria cervicornis, *G. crassissima*, *G. debilis* および *Gracilaria* sp. の培養を行った。分離した孢子および発芽体の癒合体から直立体が生じた。*Gracilaria* sp. と *G. debilis* とでは培養で生活史が完結された。生活史はイトグサ型であった。*G. cervicornis* の四分孢子は雄性および雌性の配偶体を生じ、雌性の配偶体は嚢果を形成した。果孢子は典型的な形態をもつ藻体になったが、2年間未成熟のままであった。*G. crassissima* の四分孢子は細い、尖った枝をもち野生のもの異なる未熟の藻体を生じた。*Gracilaria* sp. と *G. debilis* の四分孢子の体内からの発芽を培養した自然条件下で観察した。それから生じた配偶体は蔵精器、造果器および嚢果を葉長 2~3 cm になったときに形成し、母藻から独立して生じた配偶体より著しく早熟であった。雌性と雄性の生殖器官の混生の理由について論議し、四分孢子の体内からの直接発芽と発芽体の癒合の重要性を強調したい。(Departamento Botanica, Universidade de Sao Paulo, Caixa Postal 11461, Sao Paulo, Brasil)