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Crossing Experiments between Populations of So-called *Gracilaria verrucosa* (Huds.) Papenfuss from Two Localities, Shinori and Kikonai in Hokkaido*

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Abstract

Crosses between two populations of *Gracilaria verrucosa* from Shinori and Kikonai in southern Hokkaido were carried out.

The hybridizations were successful, and showed that the morphologies expressed in the two populations are of local variation.

The alga called *Gracilaria verrucosa* is widely distributed in Japan (Yamamoto, 1984). Owing to the morphological variability, however, this taxon is difficult to identify, and some confusion exists as to whether this is a single, or a mixture of several species.

The taxonomy of *G. verrucosa* has been reviewed world-wide recently. McLachlan (1979), mainly on the basis of spermatangial pattern (Yamamoto, 1975) and chromosome numbers, gave a new name, *G. tikvahiae*, to the taxon which sometimes had been referred to as *G. verrucosa* on the Atlantic coast of Canada. Bird et al. (1982) confirmed by crossings that an alga with this name from Vancouver Island is sexually incompatible with the plants of the type locality (England) of the species. Abbott (1984) changed "*G. verrucosa*" from California (U.S.A.) to *G. pacifica* based on vegetative features differing from the specimens from the type locality. Bird et al. (1986) renamed the alga from Chile known by both *G. lemaneiformis* and *G. verrucosa* to *G. chilensis*. Zhang and Xia (1985) proposed a new name, *G. asiatica*, for *G. verrucosa* of China, and included "*G. verrucosa*" of a district in Japan, thus suggesting this species in other districts of Japan might also be assigned to their new taxon.

In view of these circumstances, we attempted crossing experiments between individuals from different but geographically close localities (Shinori and Kikonai) of southern Hokkaido. The morphology of this alga is remarkably distinctive between these areas and suggests two distinct species, although the two representatives have been treated as the same species because of their anatomical similarity.

The main axes of the fronds from the Shinori area are thick, 2.2-3.7 mm diam. and may have a few primary branches with short branchlets, while those from

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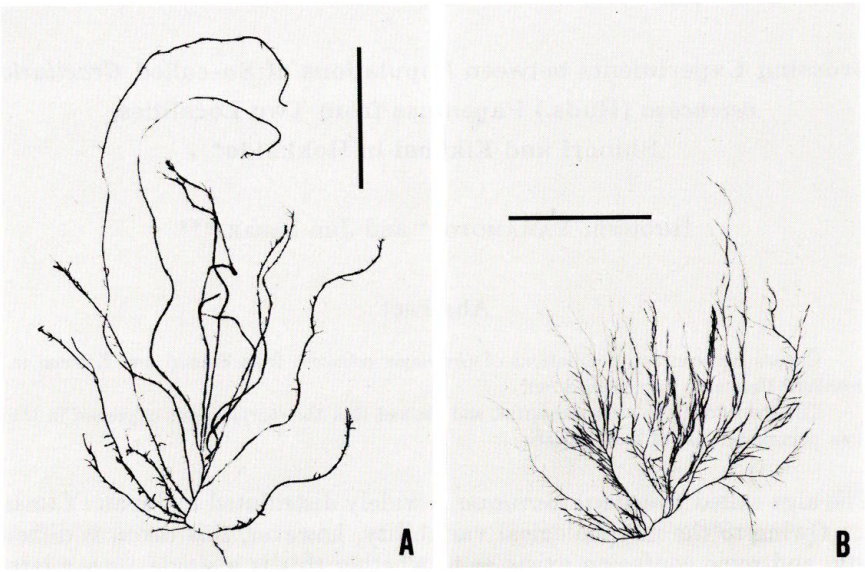

Fig. 1. Typical habits of natural specimens from two localities in southern Hokkaido. A. Tetrasporangial plant from Shinori (June, 1986). B. Tetrasporangial plant from Kikonai (June, 1986). Scale bar : 10 cm.

Kikonai are thin, 0.8–1.4 mm diam. and have many primary branches with comparatively long secondary branches (Fig. 1).

Individuals from both populations have Verrucosa-type spermatangial conceptacles (Yamamoto, 1975) where measurements are nearly the same, with little difference in the depth. Absorbing filaments in a cystocarp are moderate or weak in number, and other anatomical differences are not found between the two.

Materials and Methods

Fertile tetrasporangial fronds were collected from two localities, Shinori and Kikonai, in southern Hokkaido from June to July, 1986. Specimens were transported to the laboratory of the Faculty of Fisheries, Hokkaido University in plastic bags. Several small pieces were immediately excised from these fronds, and shaken with several changes of sterile sand to remove surface contaminants for unialgal culture. Each of the segments was put in a petri dish of approximately 100 ml. with sterile sea water. Released spores were left to settle on the bottom of the dish and incubated.

Tetraspore-derived sporelings cultured to approximately 3 mm in length in about 40 days were detached from the dish and transferred into a 1000 ml flask to be incubated alone. After identifying sex, one female from Shinori and one male frond from Kikonai were co-cultured to cross in flask, and vice versa. Self-crosses were also set as a control. Two sets of each combination were prepared.

The culture was carried out under the conditions of 5000–6000 lux of a white fluorescent lamp, a photoperiod of 14 (light)-10 (dark), a temperature of 20–21°C,

and aeration after detaching the sporelings from the substratum. PES medium without vitamins was changed once a week throughout the incubation. Spores from mature plants in culture were collected and incubated in a similar manner.

Results

Crosses of Shinori (♀)×Kikonai (♂) and Kikonai (♀)×Shinori (♂) were successful. The carpospores from resulting cystocarps developed into fertile tetrasporophytes, from which tetraspores gave rise to both gametophytes. These gametophytes formed normal cystocarps. The cycles were completed in about 200 days from the initial incubation of tetraspores. The process was the same in the controls.

Resulting spermatangial conceptacles were similar to those of the parents in pattern and in size. Absorbing filaments in the cystocarps were as moderate or weak in number as those in the field.

The fronds formed by self-crosses reserved the original external features, but hybrids (F_1 tetrasporophytes) between different populations tended to show Shinori-type morphology, modified by the closed environment of a flask.

Discussion

The variation of external appearance of "*G. verrucosa*" ranges widely in Japan and even in a small district of southern Hokkaido. Successful crossing, however, of two forms shows that both are the same species, and morphological differences are only local variations between these two populations.

Features of specimens collected in Shinori appeared on F_1 tetrasporophytes more or less stronger than those of Kikonai in the cross between the two. Since the Kikonai (♀)×Kikonai (♂) kept its morphology, it is presumed that Shinori features may be dominant in heredity. Detailed observation on heterosis such as Patwary and van der Meer (1983) found will be attempted in the future.

As the almost equal occurrence of both gametangial and tetrasporangial phases in the field suggests, all the crosses demonstrated a typical *Polysiphonia*-type life history and an approximately 1 : 1 ratio of males to females in the culture as many accounts on *Gracilaria* species have shown (Ogata et al. 1972; Bird et al. 1977; Bird et al. 1982, 1986; Oliveira and Plastino, 1984). Females which were in the presence of only males formed fertile cystocarps. Carpogonial branches could be initiated without males, but could not develop further. This differs from the observation of Ogata et al. (1972) who reported that carpogonial branches failed to appear in the absence of males.

Attempted hybridizations have proven useful in reviewing the species and strains of *Gracilaria* (McLachlan and Edelstein, 1977; McLachlan et al. 1977; Guiry and Freamhainn, 1985). We also believe that the crosses between species or local populations are the most reliable approach to confirming taxa which may demonstrate a high degree of morphological variability, in addition to little anatomical differences.

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