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Karyology and sex determination in *Aglaothamnion oosumiense* Itono (Ceramiaceae, Rhodophyta)

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Abstract

A cytogenetic investigation on male and female reproductive cells of *Aglaothamnion oosumiense* Itono indicates that the sexuality of this species might be determined by a sex chromosome. Chromosome counts in female and male gametophytes gave 37 and 36, respectively. Sex ratio of gametophytes was 1:1. Both male-derived and female-derived bisexual plants were observed. Bisexual plants were different in gross morphology and position of carpogonial branches from normal unisexual gametophytes. The chromosome number of female-derived bisexual plants was $N = 37$ and male-derived bisexual plants was $N = 36$. Some male plants developed parasporangia in addition. The paraspore germlings showed the same chromosome number as the male plants. The fertilized carpogonium and gonimoblast cells had $2N$ = ca. 70 chromosomes.

Introduction

Chromosome number and morphology can be used as critical taxonomic features in red algal taxonomy (e.g. Kim et al., 1999). Cytogenetic details, however, other than chromosome numbers are unavailable for most marine red algae because of the small size of nuclei, difficulty in obtaining adequate nuclear staining, and rarity of nuclear divisions in collected material (Kapraun, 1989). Recently, improved cytogenetic techniques and use of periodically fixed material through culture have made karyological studies more rewarding (Kapraun, 1989, 1993; Cole, 1990).

Some irregular sequences in the life history of red algae such as mixed phases or bisexuality have been reported for more than a century, and new reports appear almost every year (Chah & Kim, 1998). Although the cause and meaning of the occurrence of bisexuality in normally dioecious species are still under debate (Hawkes, 1990; Choi & Lee, 1996), these irregular sequences provide a good opportunity for studying sex determination mechanism.

So far, the only satisfactory explanation for the sex determination mechanism in red algae is that reported in *Gracilaria tikvahiae* McLaclan (Van der Meer & Todd, 1977; Van der Meer, 1981, 1986). From genetic experiments, they demonstrated that the sexuality of this species is controlled by a pair of alleles rather than a pair of sex chromosomes, and heterozygosity for mating type rather than the diploid state triggers development of the tetrasporophytic phase (Van der Meer & Todd, 1977). They further suggested that the bisexuality could arise by a recessive mutation of a gene other than the primary sex determining locus (e.g., Van der Meer, 1990).

Drew (1955) suggested that segregation of sex chromosomes during meiosis is responsible for the production of equal numbers of male and female gametophytes in *Antithamnion spirographidis* Schiffner. The presence of sex chromosomes in red algae was first reported by Rao (1970, 1971) in *Wrangelia argus* Mont. Observing heteropycnosis of four prophase I chromosomes during tetrasporogenesis, he suggested that they might be sex chromosomes. There appears

to be no other reports of sex chromosomes in the red algae (Cole, 1990).

In this study, we examined the life history and karyology of *Aglaothamnion oosumiense* and tried to elucidate the sex determining mechanism from crossing experiments between bisexual plants.

Materials and methods

Tetrasporic plants of *Aglaothamnion oosumiense* were collected from 5 to 10 m depth at Auchungdo, the south-western coast of Korea and were maintained in modified f/2-enriched seawater (Kim & Fritz, 1993). The plants were kept at 15 °C under a 16:8 h LD cycle with 10 μmol·m^{-2}·s^{-1} cool-white fluorescent light. Tetraspores were released and developed into male and female plants four weeks after germination. The sexual plants were maintained separately.

To observe the effects of environmental factors on vegetative morphology and sex ratio of the species, some isolates were maintained under the following combinations of temperature, LD cycle and photofluence rate; 15 °C + 8:16 h LD, 15 °C + 12:12 h LD, 15 °C + 16:8 h LD at 5–10 μmol·m^{-2}·s^{-1}, 20 °C + 8:16 h LD, 20 °C + 16:8 h LD at 10–30 μmol·m^{-2}·s^{-1}, and 25 °C?8:16 h LD at 25 μmol·m^{-2}·s^{-1}. Procedures for crossing experiments were the same as those described by Kim et al. (1996).

For chromosome observation, plants were fixed in 3:1 absolute ethanol-glacial acetic acid and left overnight (Austin, 1959). Fixed material was stored in 70% ethanol, hydrolysed in 1N HCl for 10 min at room temperature to soften tissue, rinsed in distilled water, and stained in 2% aceto-carmine for 2–3 h prior to squash preparation. Slides were heated until the aceto-carmine solution formed small bubbles under the cover slip, and cells were than squashed with teaspoon. Representative karyotypes were made from various stages of prophase contraction. Chromosome numbers were based on ten or more well-spread mid- to late-prophase nuclei in both vegetative and reproductive cells.

All specimens were examined with an Olympus BX-50 microscope equipped with differential interference optics. Micrographs were taken with Kodak TMAX 100 film.

Results

Culture experiments were initiated to get the material for cytogenetical investigation from excised vegetative apices of field-collected tetrasporophyte of *Aglaothamnion oosumiense.* New sporangia developed after two weeks. Tetraspores grew into male and female gametophytes in a month. The sex ratio was 1:1 regardless of the environmental conditions. After fertilization, two carposporophytes developed at a fertile segment. Carposporophytes released carpospores 1 month after fertilization. Carpospores germinated to form mature tetrasporophytes in 2 months.

Chromosome numbers of haploid and diploid nuclear phases were obtained (Figs 1 and 2). Ten identical counts of $N = 37$ were made for female plants and 18 of $N = 36$ were made for male plants (Fig. 1). Female plants, therefore, had one more chromosome than male. Chromosomes in dividing carpogonial branch cells were generally elongate, sausage-shaped or spherical, and were relatively bigger, 2 μm (small) to 8 μm (large) in length, compared to other red algae (Fig. 1). Centromere position could be discerned in some big chromosomes but not in all. Chromosomes in dividing spermatangial mother cells were similar in shape and size to those of carpogonial branch cells except for having one less (Figs 1C, D, F). The missing chromosome in male cells was not discernible because it belonged to a group of small chromosomes (Figs 1E, F). No heteropycnosis of sex chromosome was observed during mitotic or meiotic divisions. First mitotic division of the fertilized carpogonium was observed (Fig. 2A), and an intercalary cell division was observed during the gonimoblast development (Fig. 2B). The chromosome number was $2N$ = ca. 70 during this post-fertilization process.

Some unusual reproduction was observed during culture (Fig. 3). About 2% of male plants developed parasporangia in addition to spermatangia. The paraspores released and developed into male plants or parasporangiate male plants again. Parasporagium was more irregularly shaped than carposporangium. The paraspore germlings had the same chromosome number as male plants. Bisexual plants were also observed (Fig. 4). Less than 1% of the tetraspore germlings developed into bisexual plants. They never became unisexual again, regardless of environmental conditions. However, parasporangiate plants often did not produce parasporangia any more, and became normal male.

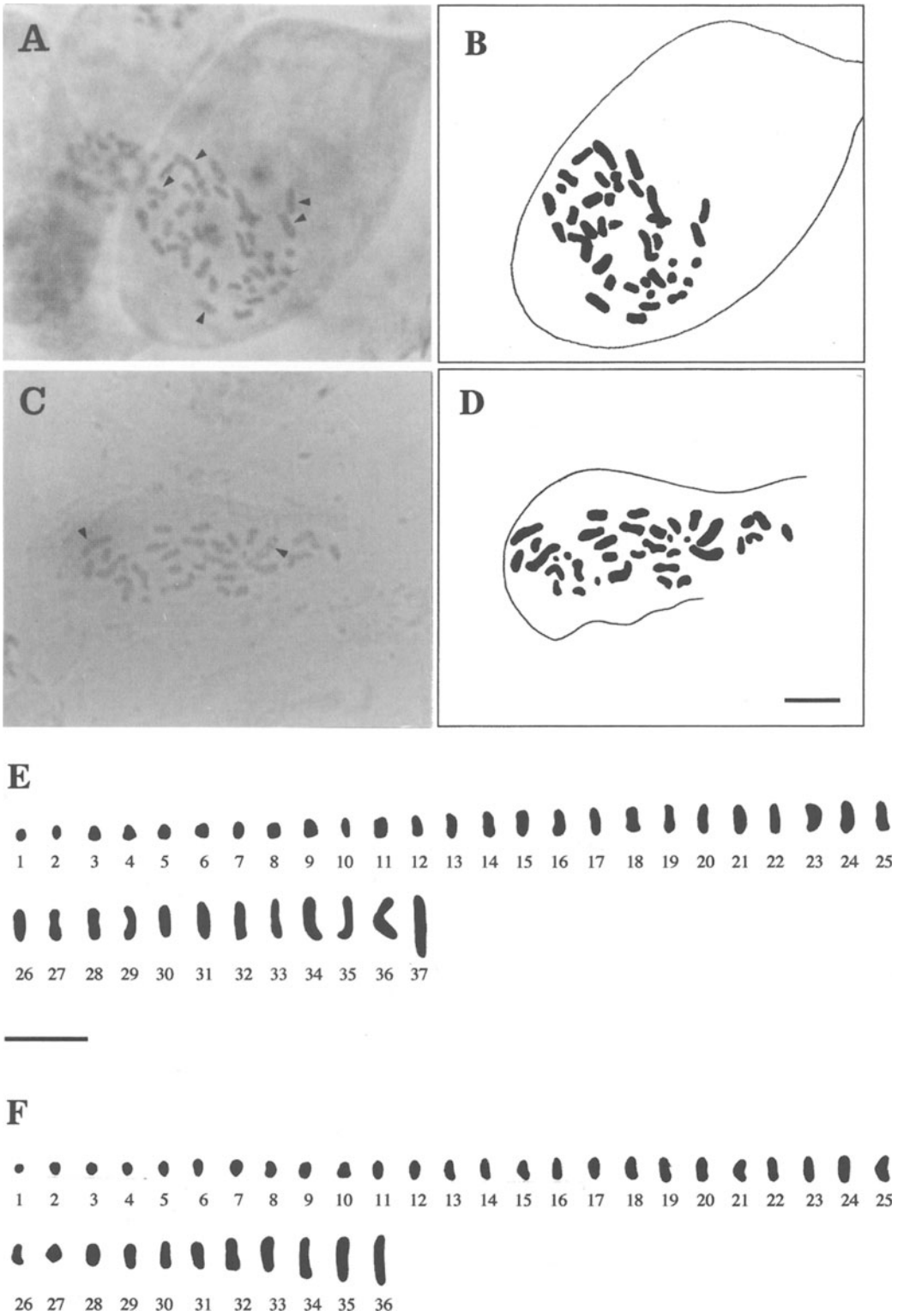

Figure 1. Karyology of *Aglaothamnion oosumiense* Itono. (A, B, E) Chromosomes in carpogonial branch cell of female plant. (C, D, F) Chromosomes in spermatangial mother cell of male plant. Arrow head indicates discernible centromere (scale bar = 10 μm).

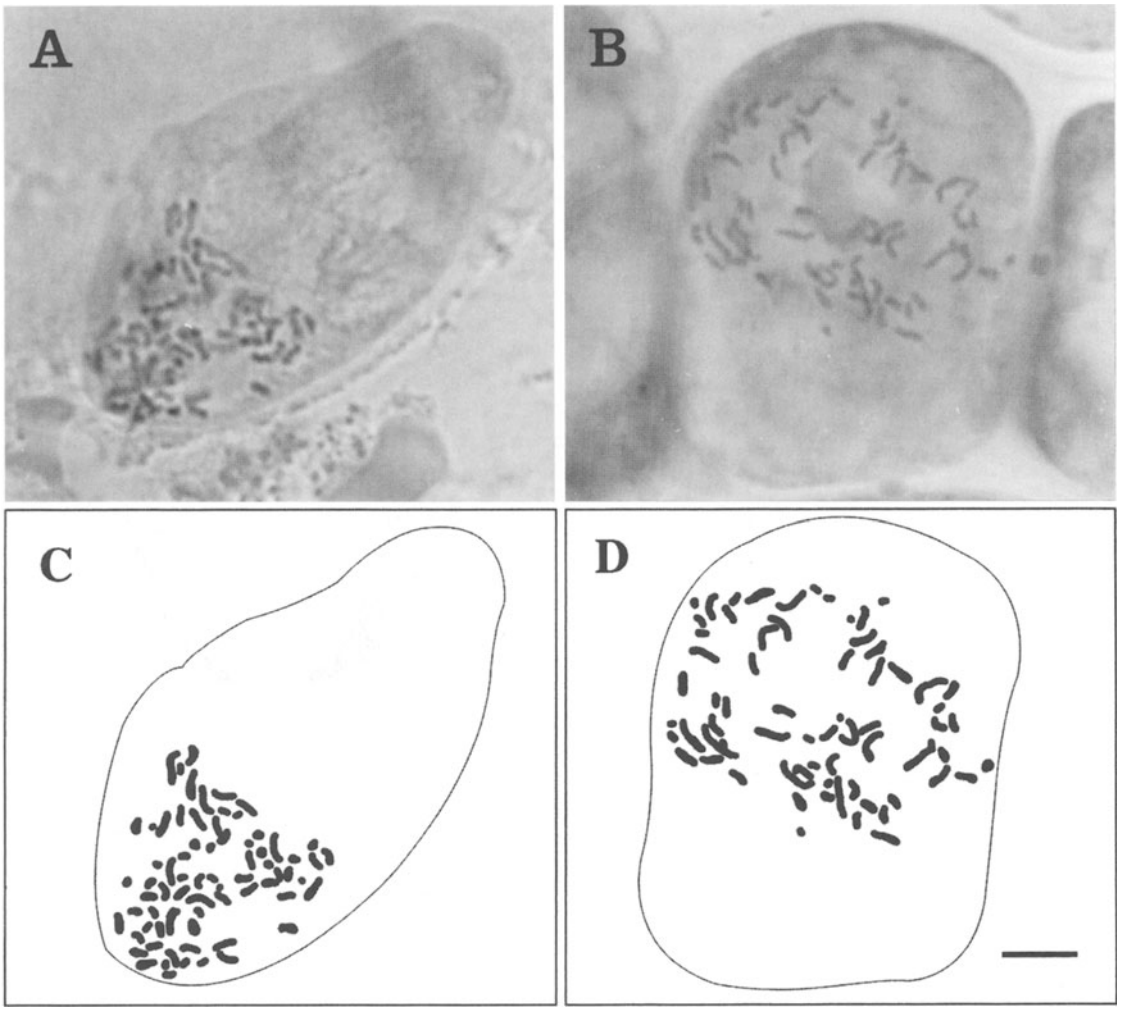

Figure 2. Chromosomes of *Aglaothamnion oosumiense* Itono. (A, C) First cell division in the fertilized carpogonium. (B, D) Intercalary division of the gonimoblast cell (Scale bar = 15 μm).

Table 1. The results of crossing experiment among the isolates of *Aglaothamnion oosumiense* Itono

Crossing experiment			Carposporophyte development (%)
Male plants (1010)	×	female plants (1011)	94
Female-derived bisexual plants (2010)	×	female-derived bisexual plants (2010)	70
Female-derived bisexual plants (2010)	×	female plants (1011)	80
Male-derived bisexual plants (3010)	×	male-derived bisexual plants (3010)	85
Male-derived bisexual plants (3010)	×	female plants (1011)	75

There were two types of bisexual plants; female-derived and male-derived (Fig. 3). They were easily distinguished from normal gametophytes in gross morphology, abundance of sexual reproductive structures, the position of carpogonial branches, and spermatangial clusters (Fig. 3). Eight identical counts of $N = 37$ chromosomes were made for female-derived bisexual plants and seven of N = 36 were

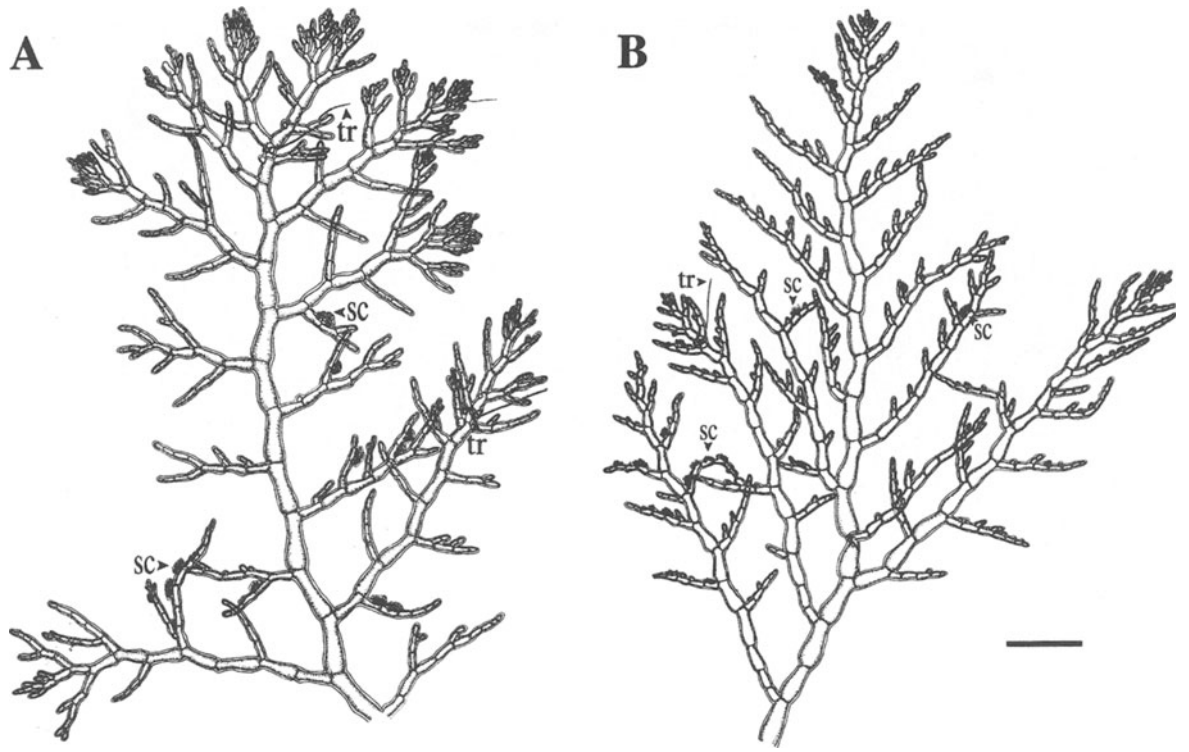

Figure 3. Two types of bisexual plant of *Aglaothamnion oosumiense* Itono. (A) Female-derived bisexual plant. (B) Male-derived bisexual plant (sc; spermatangial cluster, tr; trichogyne, scale bar = 390 μm).

made for male-derived bisexual plants (Figs 4A, B). Female-derived bisexual plants, therefore, had one more chromosome than male-derived ones. No significant difference in shape and size of chromosomes was observed in the both bisexual plants (Figs 4C, D).

The results of crossing experiment between bisexual and unisexual plants are summarized in Table 1. There was no significant difference in the success of fertilization. However, the fate of offspring differed a lot according to the combination of crossing experiments (Fig. 5). Self-crossing of the male-derived bisexual plants resulted in diploid male plants (Fig. 5A). Cross between normal female and male-derived bisexual plants resulted in tetrasporophyte, and the released tetraspores developed into male and female plants (Fig. 5B). The sex ratio was 1:1. Self-crossing of the female-derived bisexual plants resulted in tetrasporophyte, while the released tetraspores developed into female or bisexual plants (Fig. 5C). Cross between normal female and female-derived bisexual plants gave the same result as self-crossing of the female-derived bisexual plants (Fig. 5D).

Discussion

Our data suggest that the sexuality of *Aglaothamnion oosumiense* could be determined by a sex chromosome. Female plants of this species had one more chromosome than male. Male and female gametophytes showed a 1:1 Mendelian segregation regardless of the environmental conditions. The result of crossing experiment and cytogenetical investigation on bisexual plants indicates that there are two types of bisexual mutants in this species, one male-derived and the other female-derived. Both the bisexual plants are haploid. Tetrasprophyte is derived only when the crosses including female or female-derived bisexual plants, implying that genetic loci of tetrasporangia might be associated with the sex chromosome, the extra chromosome observed in the female plant.

So far, there appears to be only one report of sex chromosomes in red algae (Cole, 1990). Using standard Feulgen staining of early and late meiotic stages in tetrasporangia of *Wrangelia argus*, Rao (1970, 1971) observed heteropycnosis of four prophase I chromosomes that formed a ring-like configuration indicative of a translocation of heterozygote. Three of

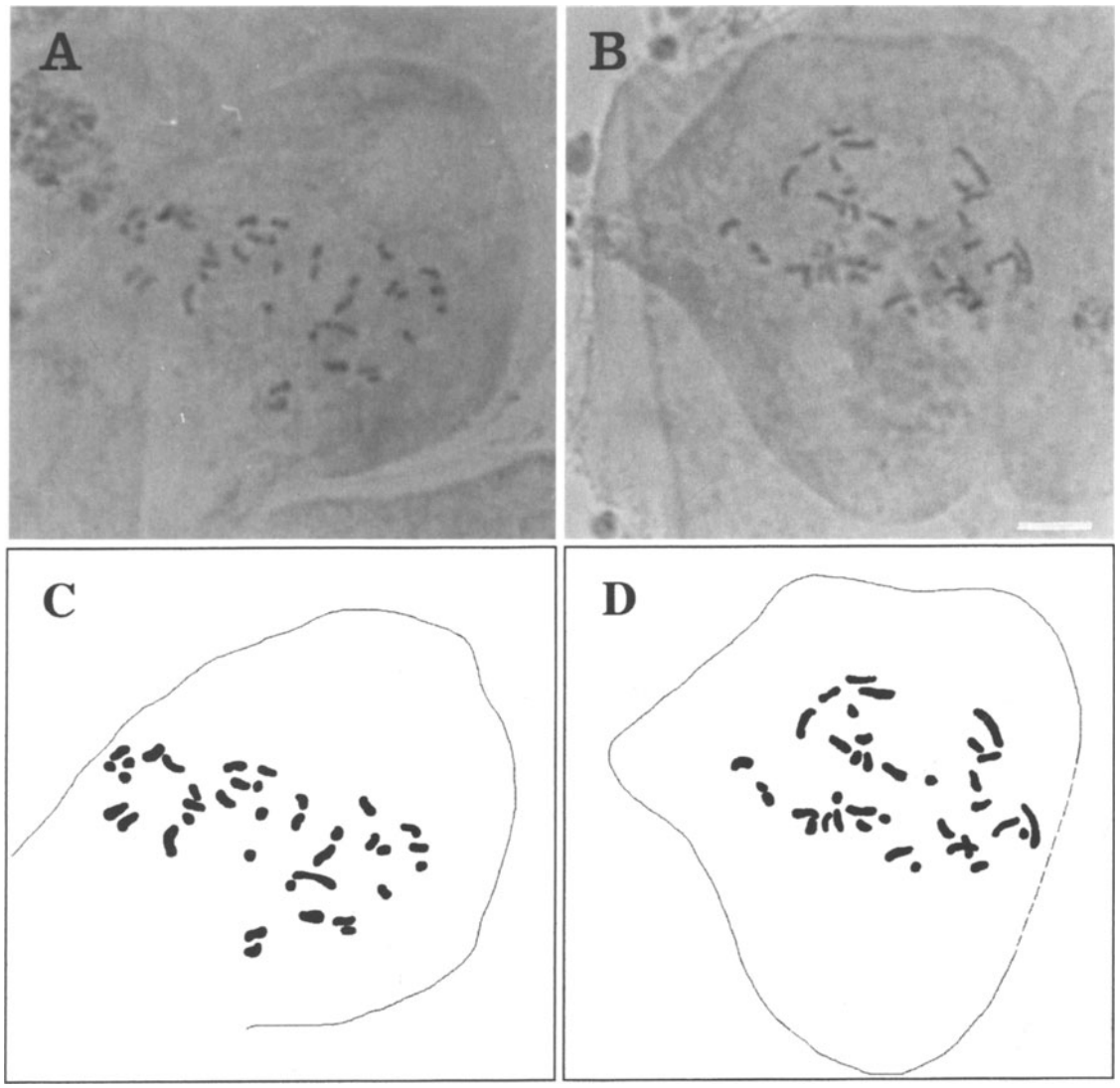

Figure 4. Chromosomes of bisexual plants of *Aglaothamnion oosumiense* Itono. (A) Female-derived bisexual plant. (B) Male-derived bisexual plant (scale bar = 15 μm).

the chromosomes were the largest in the diploid complement, one being particularly long. Each of the four was distinguishable on the basis of length, centromere position, distribution of light and dark staining blocks, and knobs in the chromosome arms (e.g., Cole, 1990). Rao (1971) proposed that these may be sex chromosomes X1, X2, Y1 and Y2, likening the longest to the large heteropycnotic X chromosome present in some laminarialean (Phaeophyta) gametophytes (e.g., Evans, 1965; Yabu & Sanbonsuga, 1981).

In *Aglaothamnion oosumiense* we could identify centromere position and banding pattern in some large chromosomes. However, it was difficult to point out the sex chromosome, because it belonged to a group of the small and spherical chromosomes, and no heteropycnosis was observed. The results of crossing experiment also suggest that the structure and the role of the sex chromosome of *Aglaothamnion oosumiense* may be different from those of *Wrangelia argus*.

How the sexuality in red algae is determined will require an explanation through more genetic investigations on an allelic basis (Rueness & Rueness, 1985). At present, however, very little is known about the organization of the mating type locus in red algae. From genetical data on segregation of sex determining elements after mitotic recombination in *Gracilaria*

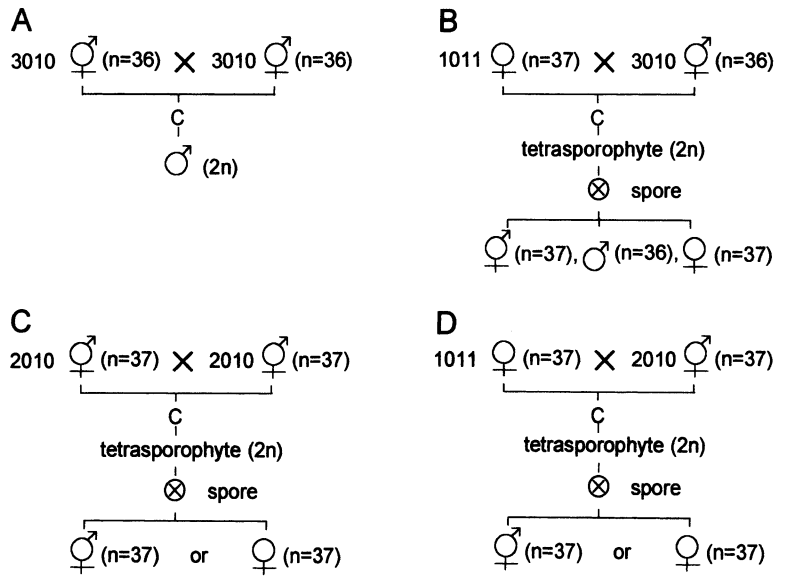

Figure 5. Summarized crossing experiments of *Aglaothamnion oosumiense* Itono.

tikvhiae, Van der Meer & Todd (1977) suggested that sexuality was controlled by a pair of alleles rather than a pair of sex chromosomes, and bisexuality could arise by a recessive mutation of a gene other than the primary sex determining locus. However, the mixed phases shown in many species of Antithamnieae (West & Norris, 1966; Rueness & Rueness, 1973, 1985; Notoya & Yabu, 1981; Kim & Lee, 1989; Kim, 1990) and also Dasyaceae (Choi & Lee, 1996) cannot be explained by mitotic recombination as described by Van der Meer (1990). In regard to sexuality of these species, especially of *Antithamnion tenuissimum* (Rueness & Rueness, 1973), Van der Meer & Todd (1977) suggested that it might be controlled by complex mating type loci like in yeast (Hawthorne, 1963) and *Chlamydomonas* (Gillham, 1969).

The development of equal numbers of male and female plants from tetraspores of *Aglaothamnion oosumiense* demonstrates that the primary control of sex determination is through a single pair of Mendelian factors (e.g., Van der Meer, 1990). The occurrence of bisexuality through a genetic recombination indicates that the sex-determining alleles may be present in a limited region of a chromosome (van der Meer & Todd, 1977; Van der Meer, 1981). The difference between male-derived and female-derived bisexual plants appears to be due to the presence of sex chromosome. The role of sex chromosome in *A. oosumiense* is of interest because both sexes could be expressed even though there was no sex chromosome in male-derived bisexual plants. The sex-determining allele, therefore, appears to be located in an autosome and there may be another regulatory gene in the sex chromosome. The occurrence of two types of bisexual plants may support Van der Meer's suggestion that the bisexuality could arise by a recessive mutation of a gene other than the primary sex determining locus. The difference of self-crossing results of male-derived and female-derived bisexual plants may be due to the presence of sex chromosome in the latter.

From self-crossing experiment of bisexual mutant, Van der Meer & Todd (1977) suggested that heterozygosity for mating type rather than the diploid state triggers the development of the tetrasporophytic phase. Our results support their idea in part; self-crossing of male-derived bisexual plant of *Aglaothamnion oosumiense* resulted in diploid male plants. Although carpospores from self-crossing of female-derived bisexual plants were also a homozygous mating type, they developed into tetrasporophytes, suggesting that the presence of sex chromosome may be more important for the development of tetrasporophyte phase.

More detailed crossing experiments using genetic markers are presently underway to elucidate further allelic basis for gametophytic sex determination in *Aglaothamnion oosumiense*.

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