Inheritance pattern of chloroplast DNA is correlated with gamete types based on sex-specific arrangement of the cell fusion site in Caulerpa (Ulvophyceae, Chlorophyta)

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SUMMARY
Using field emission scanning electron microscopy (FE-SEM) and fluorescence microscopy, the respective relationships between the arrangement of the gamete cell-fusion site and the inheritance pattern of chloroplast DNA (cp-DNA) were studied for Caulerpa brachypus Harvey, C. okamurae Weber-van Bosse, C. racemosa (Forsskål) J. Agardh var. laete-virens (Montagne) Weber-van Bosse, and C. serrulata (Forsskål) J. Agardh var. serrulata f. lata (Weber-van Bosse) Tseng. The eyespot of the biflagellate gamete was visualized using FE-SEM. The female gamete, but not the male, has one eyespot on the cell body posterior. In most mating pairs, the female gamete is fused at the anterior left side of the eyespot and the male gamete at a cell surface that is perpendicular to the plane of the flagellar beat when both gametes are mixed. Then, the inheritance pattern of cp-DNA was observed using fluorescence microscopy after staining with 4′,6-diamidino-2-phenylindole. Male and female gametes have one cell nucleus and one chloroplast each. Chloroplasts of the female gamete usually contain 1–11 spherical or rod-shaped nucleoids. In contrast, nucleoids are not usually detected in the male gamete’s chloroplast. After mixing male and female gametes, the male gamete without nucleoids and female gametes with nucleoids are always associated at the lateral side and become planozygotes. Such a correlation between the arrangement of the cell fusion site and the inheritance pattern of cp-DNA was found in another member of Caulerpales, Bryopsis maxima Okamura. These results suggest the possibility that the arrangement of the cell fusion site in the gamete is not determined randomly regardless of sex, but is rather correlated with specific mating types. The relation of these results to those for Chlamydomonas is discussed.

Key words: Caulerpa, cell fusion, chloroplast DNA, fertilization, field emission scanning electron microscopy, gamete, maternal inheritance, sex.

INTRODUCTION
During fertilization of green algae, two gametes come together and become attached by their anterior ends. In most cases, they subsequently become laterally opposed to one another, thereby engendering cell fusion and, ultimately, zygote formation. The cell fusion sites of gametes in some green algae have been observed using electron microscopy: it appears to be differentiated to specialized cell membranes (Chlamydomonas reinhardtii Dangeard, Friedmann et al. 1968; Cavalier-Smith 1975; Goodenough & Weiss 1975; Ch. moewusii Gerloff, Triemer & Brown 1975; Hydrodictyon reticulatum (L) Lagerh., Marchant and Pickett-Heaps (1972). In Ch. reinhardtii, this portion of plasma membrane is designated as a ‘mating structure’ (Goodenough & Weiss 1975). This structure has been reported in subsequent studies in other green algae (e.g. Melkonian 1980, 1981; O’Kelly et al. 1984; Hori 1988), although it has not been observed in some green algae (Gaffal & Schneider 1978; Roberts et al. 1981).

Recently, studies using light and electron microscopy have shown that the mating structure and/or cell fusion sites of the gametes occupy different positions between the opposite mating types in several green algae ranging from Prasinophyceae to Chlorophyceae and Ulvophyceae (Ch. reinhardtii, Holmes & Dutcher 1989; Collinsiella cava (Yendo) Printz, Nakayama and Inouye 2000; Ulva arasakii Chihara, Miyamura et al. 2003; U. compressa L., Miyamura 2003; Bolbocelion piliferum N. Pringsheim, O’Kelly et al. 2004); Nephroselmis olivacea Stein, Suda et al. 2004. For example, in biflagellate isogametes of Ch. reinhardtii, the mating structure occupies a fixed location. The mating type minus (mt’) structure is located at the cell apex on the same side of the flagellar basal bodies as the

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eyespot, whereas the mating type plus (mt+) structure is located in the opposite position (Holmes & Dutcher 1989, 1992). The microtubular roots that extend from the basal bodies presumably define both positions (Goodenough & Weiss 1978). According to the numbering system for flagella and roots proposed by Moestrup and Hori (1989), the mt+ structure is associated with the 2 d root, whereas the mt– structure is related with the 1 d root, creating the asymmetric localization of the mating structures (Holmes & Dutcher 1989, 1992). Therefore, we have suggested that the mt+ locus specifies that the mt+ mating structure will form in association with the 2 d root, whereas the mt– locus specifies the 1 d root (Goodenough 1991).

Because of asymmetric localization of the mating structures and/or cell fusion sites in the gametes, it is presumed that two eyespots, each from the opposite mating type, lie on the same side of the quadriflagellate planozygote. They might cooperate in shading the photoreceptor for phototaxis (Holmes & Dutcher 1989) (Fig. 1A,B). Furthermore, in cases where only the female gamete has an eyespot, such as those of anisogametes of caulerpalean algae *Bryopsis maxima* Oka-mura (Hori 1988; Miyamura et al. 2005) and *Caulerpa racemosa* var. *laete-vires* (Miyamura 2005), the cell fusion site of the female gamete also occupies the specialized position. In these algae, cell fusion usually occurs between the anterior left side of the eyespot in the female gamete and one cell surface bisecting the flagellar beat of the male gamete (Fig. 1C).
applicable to many green algae, it would be possible to address the question of whether specific mating types specify the arrangement of the cell fusion site in the gamete or not. One such characteristic is the inheritance pattern of the chloroplast genome. In most species, it is derived from a single parent: uniparental inheritance of chloroplast genomes (Correns 1909; Kirk & Tilney-Basset 1978; Sears 1980; Whatley 1982; Kuroiwa 1991; Mogensen 1996). This inheritance pattern is linked to mating types and is controlled by the mating type locus in *Ch. reinhardtii* (Armbrust 1998), in which the chloroplast DNA (cp-DNA) of the mt’ gamete is transmitted preferentially to the next generation. In contrast, that of mt is digested by nuclease within 30–40 min after mating (Kuroiwa et al. 1982; Nishimura et al. 1999). In anisogamous and oogamous algae and land plants, cp-DNA is generally transmitted from the female gamete (maternal inheritance), except for some gymnosperms and angiosperms (Sears 1980; Whatley 1982; Mogensen 1996). In maternal chloroplast inheritance, the cp-DNA of male gametes disappears during gametogenesis (*Bryopsis maxima*, Kuroiwa & Hori 1986; *B. plumosa* (Hudson) C, Agardh, Ogawa (1988); *C. brachyphus* Kuroiwa and Hori (1990); *C. okamurae*, Miyamura and Hori (1997); *Derbesia tenuissima* (Moris et De Notaris), Crouan frat., Lee et al. 2002; *Chara corallina* Wildenow, Sun et al. (1988); *Pteris vittata* L., Kuroiwa et al. (1988); angiosperms, Miyamura et al. 1987; Corriuveau and Coleman 1988; Kuroiwa 1991; Nagata et al. 1999) or immediately before and during fertilization (*Volvox carteri* Stein f. *kawasakiensis*, Kuroiwa et al. 1993); consequently, the cp-DNA is usually derived from the female gamete. Therefore, these studies indicate that the inheritance pattern of cp-DNA is a sex-specific characteristic in anisogamous and oogamous species also.

Among previously investigated algae, the relationship between the inheritance pattern of cp-DNA and arrangement of the cell fusion site has remained unknown for most species except for *Ch. reinhardtii* and *B. maxima*. *Caulerpa* is a common inhabitant of intertidal and infratidal tropical and semitropical marine waters (Bold & Wynne 1985; Lee 1999). The ecomictic thallus has a creeping rhizome with rootlike rhizoids and erect shoots. The cytoplasm becomes reticulate at the time of sexual reproduction and undergoes gamete formation. Zygotes derived from the fusion of gametes produce germlings that form new thalli directly (Price 1972; Ishiwara et al. 1981; Enomoto & Ohba 1987). In *Caulerpa*, although the inheritance pattern of cp-DNA and arrangement of the cell fusion site have been investigated separately in different species, their relationship within species has not been elucidated. Therefore, this study elucidates the relationships of these features in *Caulerpa* using fluorescence microscopy and field emission scanning electron microscopy (FE-SEM).

**MATERIALS AND METHODS**

**Algal materials**

The thalli of *Caulerpa brachyphus* and *C. okamurae* were collected at Shichirigahama, Kanagawa Prefecture, Japan, in June and July during 1989–2005. *C. serrulata var. serrulata f. lata* was collected at Komesu, Okinawa Prefecture, Japan, in April 2003 and at Ayamarumisaki, Amami Island, Kagoshima Prefecture, Japan, in June 2005. In addition, *C. racemosa var. laete-virens* and *C. webbiana* Montagne f. *tomentella* Weber-van Bosse were collected at Tomori, Amami Island, Kagoshima Prefecture in June 2003–2005. Each thallus except for that of *C. webbiana* f. *tomentella* was placed in a separate vessel containing seawater and was maintained for a few days under natural daylight conditions and then moved to a 14:10 LD regime, 15–58 µmol photons m$^{-2}$ s$^{-1}$ at 25°C until liberation of the gametes. When thallus of *C. webbiana* f. *tomentella* was collected, it had already undergone gametogenesis and released male and female gametes early the next morning.

**Scanning electron microscopy**

One volume of suspension of male or female gametes was mixed with an equal volume of 6% glutaraldehyde comprising 3% NaCl in 0.1 M cacodylate buffer, pH 7.1, on a Nuclepore polycarbonate membrane (Whatman Japan KK, Tokyo, Japan) which was coated with 0.1% poly L-lysine (Sigma Chemical Co., St. Louis, MO, USA). For fertilization experiments, one volume of suspension of male gametes was mixed with one volume of female gametes on a Nuclepore polycarbonate membrane and fixed with two volumes of fixative 30 min after mixing. The cells were fixed at room temperature. After removing the supernatant, the cells were further fixed with 2.5% glutaraldehyde, 3% NaCl, 0.05 M cacodylate buffer, pH 7.1, for 6 h at room temperature. They were washed in a series of 0.05 M cacodylate buffer solutions containing 3.00, 2.25, 1.50, 0.75 and 0% NaCl, each step taking 15–20 min. Post-fixation was made in 1% OsO$_4$ dissolved in 0.05 M cacodylate buffer, pH 7.1, overnight at 4°C. After dehydration through a graded series of ethanol, the cells were infiltrated with t-butyl alcohol, freeze-dried at 4°C and coated with platinum-palladium in a Hitachi E-1030 or a Hitachi E-102 sputter-coating unit (Hitachi High-Technologies Corp., Tokyo, Japan). Observations were made using a JSM 6330F field-emission scanning electron microscope (JEOL, Tokyo, Japan) at 5 kV.
Fluorescence microscopy

One volume of the specimens was mixed with one volume of 3% paraformaldehyde dissolved in 0.05 M cacodylate buffer, pH 7.1, containing 3% NaCl, and one volume of 1 µg/mL 4′,6-diamidino-2-phenylindole (DAPI) dissolved in a modified S buffer containing 20 mM Tris-HCl, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 0.28 M sucrose, 1.2 mM spermidine, 7 mM 2-mercaptoethanol and 0.4 mM phenylmethysulfonyl-fluoride (Kuroiwa & Suzuki 1980). After 2–10 min, the cover slip was pressed gently against the specimens. All observations were made using an epifluorescence microscope (BHS-RFC type; Olympus Optical Co. Ltd, Tokyo, Japan) that was equipped with phase contrast optics. Photographs were taken using monochrome film (Neopan 400PRESTO; Fuji Photo Film Co. Ltd).

RESULTS

In Caulerpa brachypus, C. okamurae, C. serrulata var. serrulata f. lata and C. racemosa var. laetevirens, the cytoplasm of all parts of the thallus, except most of the rhizoidal portion, began to accumulate and formed a network within one month of collection. This network underwent gamete production and was provided with narrow discharge tubes. Viscous material containing biflagellate male and female gametes was released through the discharge tubes and precipitated on the bottom of the vessel. In dioecious gametophyte of C. brachypus, each male and female thallus released male and female gametes, respectively, while in other monoecious species thallus discharged a mixture of opposite sex type gametes.

Arrangement of gamete’s cell fusion site in Caulerpa

To determine whether cell fusion always occurs at the specific site of gametes in Caulerpa, the gamete cell fusion in C. brachypus, C. okamurae, C. serrulata var. serrulata f. lata and in C. webbiana f. tomentella were studied using FE-SEM. In these four species, male and female gametes’ morphology and their behavior during fertilization were similar to those observed in C. racemosa var. laetevirens (Fig. 1, Miyamura 2005).

We describe fertilization of C. serrulata var. serrulata f. lata as a representative case. The gametes are pear-shaped and their anterior end displays a papilla, from whose opposite sites two isokont flagella arise (Figs 2,3). The female gamete has an eyespot (Fig. 3), but not the male (Fig. 2). The eyespot is situated on the side of a cell posterior of the female gamete that corresponds to the plane of the beat of the flagella.

They begin to fuse immediately when sediments containing male and female gametes are mixed in a culture-vessel (Fig. 4). The gamete pairs lay side-by-side with their longitudinal axes nearly parallel and become fused, as reported previously for other species of Caulerpa using light microscopy (Miyake & Kunieda 1937; Goldstein & Morrall 1970; Enomoto & Ohba 1987). Figures 5–10 show the planozygotes viewed from various perspectives. For all mating pairs (Figs 4–10), the female gamete is always fused at the anterior left side of the eyespot and the male gamete at one of the anterior cell surfaces that is perpendicular to the plane of the flagellar beat, when the cells are viewed from the surface containing the eyespot and are oriented in such a way that the no. 1 and no. 2 flagella point to the left and right of the photograph, respectively (Fig. 4), c. 30 min after mixing the sediments. This fusion pattern was observed in 60 mating pairs (n = 60). In order to confirm this fusion pattern in other species (Fig. 11), mating pairs of C. okamurae were shown at low magnification and corresponding high magnification images were superimposed (Fig. 12). All mating pairs (Fig. 12a–j) exhibit the same fusion pattern as described above. This fusion pattern was observed in 52 mating pairs in total (n = 59), although in seven mating pairs we could not determine whether they exhibit this fusion pattern or not. These results suggest that the sex-specific cell fusion pattern observed in C. serrulata var. serrulata f. lata is probably conserved in the genus Caulerpa.

Inheritance pattern of chloroplast DNA in Caulerpa

The cp-DNA inheritance pattern was studied using fluorescence microscopy after staining with DAPI. Figures 13–21 show the phase contrast, bright field and epifluorescence images of gametes and planozygotes of C. serrulata var. serrulata f. lata. Female gametes were distinguished from male ones in the mixture of both gametes using the eyespot as the female gamete marker (Fig. 17). Male and female gametes have one cell nucleus and one chloroplast each (Figs 13–18). The cell nuclei, mitochondrial nucleoids (mt-nucleoids) and chloroplast nucleoids (cp-nucleoids) of the gamete emitted a strong blue-white fluorescence after DAPI staining. Chloroplasts of the female gamete usually contained 1–11 spherical or rod-shaped cp-nucleoids. In contrast, cp-nucleoids were not observed in the chloroplast of male gametes in most cases (99.4 ± 0.1%) (Table 1). Both male and female gametes contained 1–8 spherical mt-nucleoids. After mixing male and female gametes, male gametes without cp-nucleoids were always associated at the left side of the female gamete when the cells were viewed from the surface containing the eyespot (Figs 19–21). Mt-nucleoids derived from both gametes were still apparent in planozygotes 30 min after mixing.
Table 1 summarized the inheritance pattern of cp-DNA in four species of *Caulerpa*. Disappearance of cp-nucleoids was also observed in male gametes of *C. brachypus*, *C. okamurae* and *C. racemosa* var. *laete-virens*, whereas chloroplasts of female gametes always contained cp-nucleoids. As the chloroplast of vegetative cells contained 6–23 (*C. brachypus*), 1 (*C. okamurae*), 1–21 (*C. serrulata var. serrulata f. lata*) and 2–18 nucleoids (*C. racemosa* var. *laete-virens*), it is suggested that the disappearance of cp-nucleoids occurred during the male gametogenesis. However, frequency of the male gametes that have no cp-nucleoids varied from 81.8 to 99.4% among four species. When the male gametes contained cp-nucleoids, 1–14 and 1–6 nucleoids were present in the chloroplast of *C. brachypus* and *C. okamurae*, respectively, and a few nucleoids in *C. racemosa* var. *laete-virens* and *C. serrulata var. serrulata f. lata*. Furthermore, the male gametes both with and without cp-nucleoids were observed in the mating pairs of these species.

**DISCUSSION**

**Sex-specific arrangement of cell fusion site of gametes in ulvophycean algae**

Results of the present study using FE-SEM are consistent with previous observations of *C. racemosa* var. *laete-virens* (Miyamura 2005) and *B. maxima* (Miyamura et al. 2005) that the female gamete is always fused at the anterior left side of the eyespot with one cell surface bisecting the male gamete's flagellar beat if the female gamete is oriented such that no. 1 and no. 2 flagella point to the left and right of the image (Fig. 1C). The gamete of *Caulerpa* has a cruciate-type flagellar apparatus; the eyespot situates between 2 s
and 2 d roots (Hori 1981) as in other siphonous green algae (Melkonian & Robenek 1984; Kreimer 2001). Therefore, the cell fusion site of the female gamete might correspond to the flagellar base between the 1 d and 2 s roots. This position almost matches the female gamete’s cell fusion site of *U. arasakii* (Miyamura et al. 2003), *U. compressa* (Miyamura 2003), one of the two mating types in *Co. cava* (Nakayama & Inouye 2000) and *Bo. piliferum* (O’Kelly et al. 2004), the mating type minus the gamete of *Ch. reinhardtii* (Holmes & Dutcher 1989) and the mating type plus the gamete of *N. olivacea* (Suda et al. 2004). In contrast, the cell fusion site of the opposite-sex gamete occupies the opposite positioning in these algae. Nakayama and Inouye (2000), based on the ultrastructure of isogametes and planozygotes of *Co. cava*, proposed that the presence of opposite positioning of the mating structure in each sex gamete is a universal feature of advanced chlorophytes. Results of present and previous studies support and might expand their proposal to anisogametes of ulvophycean algae and isogametes of Ulvophyceae, Chlorophyceae and Prasinophyceae. Furthermore, these observations suggest that the cell fusion sites of ulvophycean female gametes (*Ulvales, U. arasakii* and *U. compressa*; Caulerpales, *B. maxima*, *C. brachypus, C. okamuriae, C. racemosa var. laetevirens, C. serrulata var. serrulata f. lata* and *C. webbiana f. tomentella*) always occupy a common spatial position. Although the number of species examined so far is quite limited, such features might be specific to female gametes in these ulvalean and caulerpalean algae.

**Figs 11,12.** Field emission scanning electron microscope images showing fertilization of male and female gametes of *C. okamuriae*. 11. Mating gametes viewed from the surface containing the eyespot. 12. Gametes and mating pairs (a–j) at low magnification. High magnification images of each mating pair (a–j) were shown in the right columns. Arrows: papilla. In all mating pairs, the female gamete is always fused at the anterior left side of the eyespot and the male gamete at one of the anterior cell surfaces that is perpendicular to the plane of the flagellar beat. E, eyespot; ♂, male gamete; ♀, female gamete; #1, no. 1 flagellum; #2, no. 2 flagellum of female gamete; ♂ F, male flagellum.
Maternal inheritance of chloroplast DNA in Caulerpaceae and Bryopsidaceae

Because the definition of male and female is generally based on cell size, the smaller gametes are regarded as ‘male’, whereas the larger are ‘female’; it is apparently difficult to distinguish sex exactly in the case of isogamy and slight anisogamy. This study adopted the inheritance pattern of cp-DNA as the sex-specific characteristic. Because maternal inheritance of cp-DNA is dominant in many green plants (Whatley 1982; Kuroiwa 1991), it is possible to use maternal inheritance of cp-DNA as one criterion to distinguish the two sexes irrespective of gamete size. Results of this study show that in two species of Caulerpa in addition to C. brachypus (Kuroiwa & Hori 1990) and C. okamurae (Miyamura & Hori 1997), the cp-DNA of the male gamete disappears before fertilization whereas that of the female is present in the zygote. Although we did not follow the fate of zygotes, it is expected that they could develop into mature thalli, because their development under culture conditions has been shown in C. serrulata (Price 1972), C. okamurae (Ishiwara et al. 1981) and C. racemosa var. laete-virens (Enomoto & Ohba 1987). Therefore, it is indicated that the cp-DNAs of the female gametes are transmitted preferentially to the offspring; although it is difficult to deny the possibility that male cp-DNA may be occasionally transmitted to some zygotes. These results are consistent with the other species of Caulerpales belonging to Bryopsidaceae, such as B. maxima (Kuroiwa & Hori 1986; Kuroiwa et al. 1991), B. plumosa (Ogawa 1988) and Derbesia tenuissima (Lee et al. 2002). Consequently, it is suggested that maternal inheritance of cp-DNA might be a common trait in the Caulerpaceae and Bryopsidaceae.

Although DAPI staining is so sensitive that it can detect one particle of T4 phage whose molecular weight and GC content is very similar to chloroplast DNA (Kuroiwa & Nakamura 1986), it is very difficult to deny...
the possibility that the dispersal of cp-DNA molecules, presumably caused by the digestion or relaxation of the proteins that form the cp-nucleoids, might lead to the disappearance of fluorescent cp-nucleoids. Accuracy of the observations using DAPI staining has been confirmed in B. maxima and Ch. reinhardtii. Kuroiwa et al. (1991) extracted DNA from male and female thalli and gametes of B. maxima and then separated chloroplast, mitochondrial and nuclear DNA using CsCl density gradient centrifugation. The profiles after CsCl density gradient centrifugation of DNAs supported the cytological data. Digestion of cp-DNA during the disappearance of cp-nucleoids has also been confirmed by more precise cytological and biochemical analysis in Ch. reinhardtii (Nishimura et al. 1999). They labeled separately the cp-DNA molecules of mt+ and mt− gametes by using bacterial aadA (aminoglycoside adenyl transferase) sequences. Single zygotes were obtained by using optical tweezers and were subjected to highly sensitive nested PCR analysis of aadA sequences. These results demonstrated that cp-DNA molecules are digested completely during the preferential disappearance of mt− cp-nucleoids, whereas mt+ cp-DNA is protected from the digestion. Taking these previous observations together with the results of this study, it is likely that the cp-DNA was digested in the chloroplast of the DAPI-negative male gamete of Caulerpa. However, precise determination of whether a trace amount of cp-DNA molecules are present in some DAPI-negative male gametes of Caulerpa requires further investigation.

Relationship between inheritance patterns of chloroplast DNAs and arrangement of cell fusion sites of gametes

Considering these results of the inheritance pattern of cp-DNA and the arrangement of cell fusion site, we examined whether specific mating types specify the arrangement of a cell fusion site in both isogametes and anisogametes (Table 2). We designated the two gamete types as ‘α’ and ‘β’, respectively, based on the arrangement of cell fusion site and/or mating structure of mt+ and mt− of Ch. reinhardtii. Type α gametes fuse along the side away from the eyespot or the anterior right side, whereas type β gametes fuse along the same side as the eyespot or the anterior left side. The male and female gametes always correspond to types α and β, respectively, when this designation is applied to anisogametes of Ulva, Bryopsis and Caulerpa. Although the cell fusion sites of male gametes of Bryopsis and Caulerpa could not be determined, they might correspond to type α because: (i) the eyespot of the male gamete might be lost during evolution of Bryopsis and Caulerpa because both male and female gametes of other ulvophycean algae have an eyespot; and (ii) the male gamete is fused at the opposite side of the eyespot in these algae (Fig. 1A, B).

In four species of Caulerpa, cp-DNA is always transmitted from type β gametes (female) to the zygotes. Such a correlation is apparent in other members of Caulerpales, including B. maxima. This correlation suggests the possibility that the arrangement of cell fusion sites in the gamete is not determined randomly regardless of sex, but is rather controlled by the specific mating types. Although the inheritance pattern of cp-DNA remains unknown in U. arasakii and U. compressa, it is expected that cp-DNA of the type β gamete (female) might be inherited preferentially to the offspring because the chloroplast from the male gamete of closely related species U. mutabilis has been shown to disintegrate in the zygote (Bråten 1971, 1973). Nevertheless, because the presence of both maternal and biparental inheritance of cp-DNA in a genus has been shown to exist in angiosperms (Liu et al. 2004), we cannot exclude the possibility that both type α and

Table 2. Relationship between the gamete types based on the arrangement of cell fusion site and the transmission pattern of chloroplast DNA in Caulerpa, Bryopsis and Chlamydomonas

<table>
<thead>
<tr>
<th>Species</th>
<th>Presence or absence of chloroplast DNA in the gamete</th>
<th>Sources</th>
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<tbody>
<tr>
<td></td>
<td>Type α</td>
<td>Type β</td>
</tr>
<tr>
<td>Caulerpa brachypus</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>C. okamurae</td>
<td>−</td>
<td>+</td>
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<tr>
<td>C. racemosa var. laete-virens</td>
<td>−</td>
<td>+</td>
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<tr>
<td>C. serrulata var. serrulata f. lata</td>
<td>−</td>
<td>+</td>
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<tr>
<td>C. webbiana f. tomentella</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Bryopsis maxima</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Chlamydomonas reinhardtii</td>
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+, presence of chloroplast DNA in the gamete; −, absence of chloroplast DNA in the gamete; ND, not determined. Types α and β are shown in Fig. 1.
β gametes inherit cp-DNA to the zygotes. Further study is necessary to elucidate the relationship between the arrangement of the cell fusion site and the inheritance pattern of cp-DNA in "U. arasakii" and "U. compressa."

In contrast to these caulerpalean algae, the cp-DNA of the type α gamete (mt+) is transmitted to the next generation in "Ch. reinhardtii." This pattern is opposite to that of "Caulerpa" and "Bryopsis." It remains unclear why this discrepancy occurs between these algal species. However, at least two possibilities exist. One is that the transmission of cp-DNA is opposite for caulerpalean algae ("Caulerpa" and "Bryopsis") and "Ch. reinhardtii." Actually, transformation of the chloroplast inheritance pattern from maternal to paternal is apparent during the evolution of land plants (Mogensen 1996). Although most land plants show maternal chloroplast inheritance and the remainder exhibit potential biparental inheritance (Miyamura et al. 1987; Corriveau & Coleman 1988; Nagata et al. 1999; Zhang et al. 2003), some families of gymnosperms (e.g., Pinaceae) show paternal inheritance of cp-DNA (review in Mogensen 1996).

A second possibility is that numbering of flagellar and basal bodies in "Bryopsis" and "Caulerpa" might be incorrect and actually opposite to that which was first postulated. Numbering of the flagella is based on flagellar transformation, which is widespread among eukaryotes and which offers the possibility of identifying homologous flagella in different organisms (Wright et al. 1980; review in Beech et al. 1991; Moestrup 2000). During flagellar transformation in a biflagellate cell, one flagellum (the immature state) changes in the next generation to another state (the mature state). This process enables determination of the mature state of any flagellum, which will not change its interphase characteristics through subsequent generations. The mature flagellum is inferred to be the no. 1 flagellum and the immature flagellum as the no. 2 in biflagellate cells (Heimann et al. 1989; Moestrup & Hori 1989). This numbering system is based on flagellar transformation during cell division. Therefore, we could not apply this nomenclature precisely to the gamete. In this study therefore we used the eyespot as the positional marker. However, if the eyespot position changed during the evolution of "Caulerpa" and "Bryopsis," it is possible that the flagellum close to the eyespot becomes no. 1 and the other becomes no. 2. In this case, cp-DNA of the type α gamete would be transmitted to the next generation in "Caulerpa" and "Bryopsis," as in "Ch. reinhardtii."

Other sex-specific markers, which are useful in both caulerpales and "Ch. reinhardtii," seem to be necessary to assess those possibilities. The cp-DNA of type β gamete (female) is transmitted to the next generation in "Caulerpa" and "Bryopsis," whereas cp-DNA of type β gamete (mt+) in "Ch. reinhardtii" is not. The reason remains totally unknown. Further study is necessary to elucidate this problem.

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