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Disappearance of centrioles derived from female gametes in zygotes of *Colpomenia bullosa* (Phaeophyceae)*

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Isogamous fertilization and zygote development of *Colpomenia bullosa* Yamada were studied with electron microscopy. Chloroplasts and mitochondria from male and female gametes remained in zygotes. Whereas two pairs of centrioles (=flagellar basal bodies) are derived from both male and female gametes, only one pair remains within about four hours after plasmogamy. Disappearance of one pair of centrioles occurred irrespective of karyogamy. Since morphological differences between male and female gametes of *C. bullosa* could not be detected, it was impossible to determine which one pair of centrioles disappeared. But observations on polyspermic zygotes (two or three male gametes to one female gamete) showed that only one pair of centrioles disappeared even in these zygotes. As a result, it was strongly suggested that centrioles from the female gamete disappeared.

Key Index Words: *brown algae—centrioles*—Colpomenia bullosa—*fertilization—isogamy—paternal inheritance.*

Sexual reproduction in almost all algal groups (with the exception of the red algae) is conducted by motile female and male gametes. In the brown algae, three types of sexual reproduction have been confirmed, i.e., isogamy, anisogamy and oogamy (Wynne and Roiseaux 1976). Ultrastructural studies on brown algal fertilization have been carried out in detail on *Fucus* and *Laminaria* (Brawley *et al.* 1976a, b, Motomura 1990). But sexual reproduction in these genera is oogamous, so there have not been any similar studies on the fertilization of isogamous and anisogamous groups in the brown algae.

Motomura (1990) reported in detail the fertilization of *Laminaria angustata* using complete serial sections. The results indicated that chloroplasts and mitochondria in zygotes were originated from eggs, while centrioles were originated from sperms by egg centrioles disappearing after plasmogamy. Paternal inheritance of centrioles has been well known in animal fertilization (oogamy) (Schatten *et al.* 1988, Sluder *et al.* 1989, Luykx 1991). Subsequently, by comparing the development of zygotes and parthenogenotes using immunofluorescence microscopy, it became clear that this paternal inheritance of centrioles had a crucial role in normal development of zygotes, especially in normal spindle formation (Motomura 1991).

In this study, it was found that the selective disappearance of centrioles from female gametes occurs even in isogamous brown alga.

Materials and Methods

Culture. Mature gametophytes of *Colpomenia bullosa* Yamada were collected in January–April, 1988–1990, at Charatsunai, Muroran, Hokkaido, Japan. The plants were washed with autoclaved seawater, wiped with paper towels, put in Petri dishes one by one and incubated in a refrigerator overnight. The next day, cold PESI medium (Tatewaki 1966) was poured into these Petri dishes under illumination. After several minutes, many

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gametes were liberated and sexuality was determined by mixing gametes derived from different individuals. Firstly, only female gametes were inoculated into Petri dishes. Many female gametes settled down within 20-30 min and female gametes still swimming were washed out with the medium. Next, male gametes were inoculated into these dishes, and plasmogamy was synchronous. Cultures of zygotes were maintained in PESI medium, 10°C or 14°C, under continuous illumination with fluorescent lamps (55 $\mu Em^{-2}s^{-1}$ photon flux density).

TEM preparation. Zygotes were fixed at regular intervals of time. They were fixed with a solution containing 3% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.2), 2% NaCl, 1% caffeine and 0.1% $CaCl_2$ for 2 hr at 4°C. Samples were detached from Petri dishes with a soft paint brush, and the fixative solution containing samples was transferred into centrifuge tubes. Fixed zygotes were washed with 0.1 M cacodylate buffer (pH 7.2), 2% NaCl, 1% caffeine and 0.1% $CaCl_2$, pelleted by centrifugation and finally embedded in 1% agar. They were post-fixed for 2 hr in 2% OsO_4 or overnight in 1% OsO_4, 0.1 M cacodylate buffer (pH 7.2), 2% NaCl and 0.1% $CaCl_2$. Samples were stained *en-bloc* with 0.5% uranyl acetate for 15 min at 4°C, dehydrated gradually with acetone and finally embedded in Spurr's resin (Spurr 1969). Serial sections were cut with a diamond knife on a Porter-Blum MT-1 ultramicrotome and mounted on formvar-coated slot grids. Sections were stained with uranyl acetate and lead citrate or only with lead citrate, and observed with a Hitachi H-300 electron microscope. Results in this paper were obtained from zygotes which were completely serial-sectioned.

Results and Discussion

Shape and structure of female and male gametes were typical of brown algal swarmers (Clayton 1989, O'Kelly 1989) and it was difficult to distinguish gametes from their ultrastructure. They have a long, mastigoneme-bearing anterior flagellum and a short, non-decorated, posterior flagellum. A nucleus is located above a cup-shaped chloroplast which has an eyespot near the base of the posterior flagellum. One pyrenoid protrudes from the chloroplast at the opposite side of the eyespot.

Plasmogamy in *Colpomenia bullosa* proceeds as in other brown algae (Maier and Müller 1986, Peters and Müller 1986); female gametes first settle down and secrete pheromones, and then male gametes are attracted to them and plasmogamy occurs. Frequently, two or three male gametes fertilize a female gamete (polyspermy), especially when many more male gametes were inoculated than female gametes.

Both nuclei fused (karyogamy) after plasmogamy, but the timing of karyogamy was not constant. When female and male nuclei are close to each other after plasmogamy, karyogamy occurs soon afterward (Figs. 2-4). Karyogamy is delayed when one or two chloroplasts are situated between both nuclei (Figs. 11, 12).

After plasmogamy, zygotes started to develop, and germinate after about 12 hr. The zygote development was examined by serial sections till 24 hr in culture. Cellular organelles, such as chloroplasts and mitochondria from both gametes, continued to exist in the zygote development. On the contrary, one pair of centrioles disappeared during zygote development.

Figures 1-6 show six sections of a one-hour-old zygote after plasmogamy. There are two chloroplasts, each having a pyrenoid and an eyespot. Therefore this zygote was produced by normal plasmogamy, not polyspermy. The outer membranes of both nuclei have just fused (Figs. 2-4). Two pairs of centrioles (Figs. 2, 6) derived from both female and male gametes could be detected in one-hour-old zygote after plasmogamy. Basal plates were observed at the distal end of centrioles but axonemes were detached from them (Fig. 6).

One pair of centrioles disappeared in four-hour-old zygotes. Figures 7-9 show three

sections of a four-hour-old zygote after plasmogamy. This zygote contained one nucleus and two chloroplasts, each containing a pyrenoid and an eyespot. Therefore, clearly the zygote was formed after normal plasmogamy and karyogamy had occurred. In this zygote, only one pair of centrioles could be detected (Fig. 7). Also, Figures 10-12 show three sections of another four-hour-old zygote after normal plasmogamy. The nuclei (N1 and N2) were not fused yet. Similar to the previous example, only one pair of centrioles existed near the nucleus (N1) (Fig. 11), the other pair of centrioles had disappeared. Therefore, it became clear that the presence or absence of nuclear fusion did not affect the disappearance of one pair of centrioles.

Because of the identical ultrastructure of flagellar basal bodies of female and male gametes, it was difficult to determine which pair of centrioles disappeared. However observations on polyspermic zygotes suggest the female gamete's centrioles disappear. Figures 13-18 show six sections of a four-hour-old zygote of polyspermy. This zygote resulted from polyspermy (two male gametes to one female gamete) because three chloroplasts could be detected, each having a pyrenoid and an eyespot. Three nuclei were not fused yet with one another. In this polyspermic zygote, two pairs of centrioles remained (Figs. 16, 17), one pair have disappeared. Disappearance of only one pair of centrioles was not affected by the presence or absence of karyogamy, like normally fertilized zygotes. Disappearance of one pair of centrioles was also observed in polyspermic zygotes which resulted from one female gamete and three male gametes (not shown). Therefore, I believe that centrioles which were derived from the female gamete disappeared and ones derived from the male gamete remained. It means that centrioles in diploid thallus (=sporophyte) cells are originated from basal bodies (=centrioles) of flagella of male gametes. Similar results were obtained from *Scytosiphon lomentaria* (Scytosiphonales) and *Analipus japonicus* (Ralfsiales) in the brown algae (Motomura unpublished data).

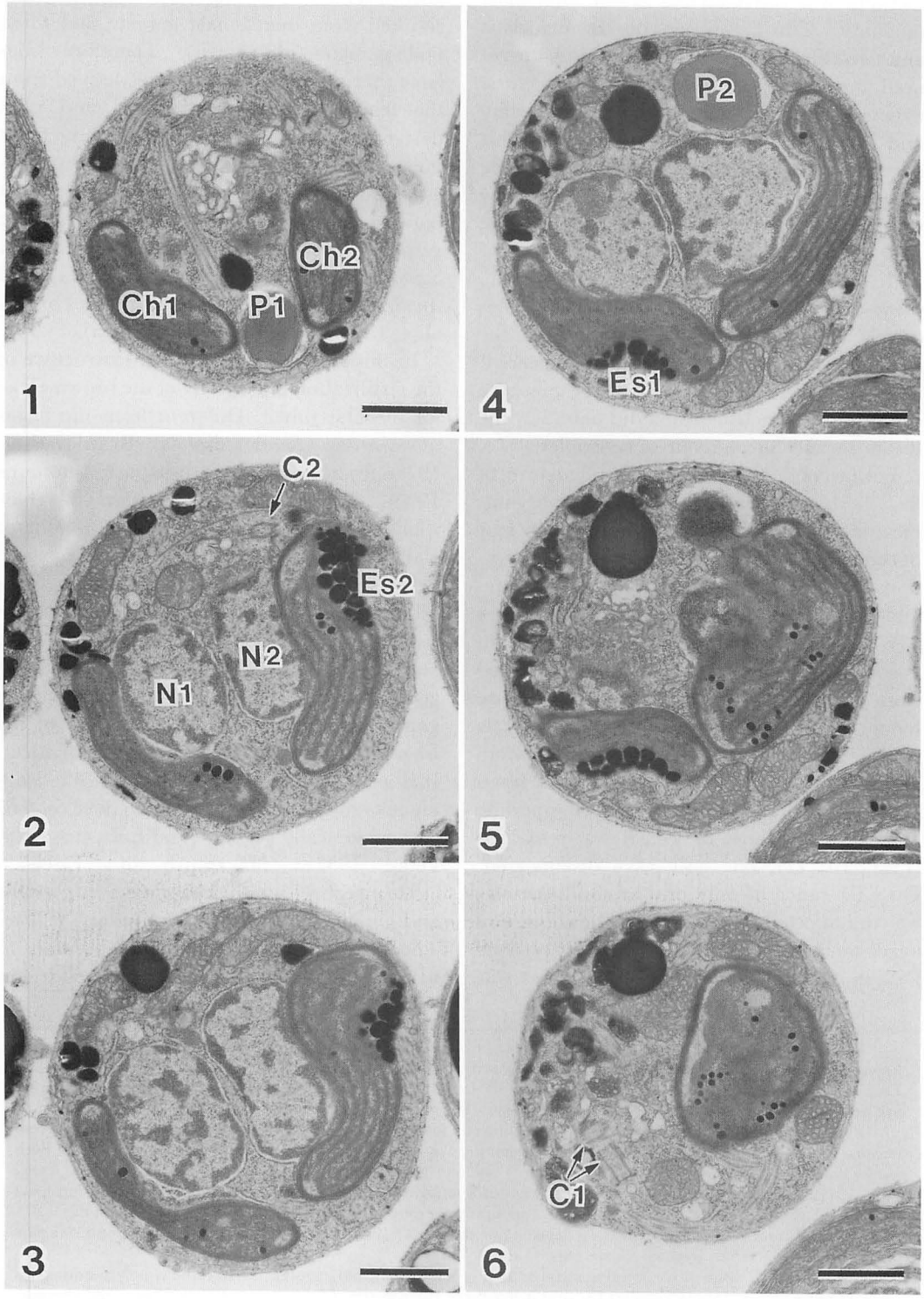
In this study, I report the ultrastructure of the fertilization in isogamy of the brown algae for the first time. Different from the oogamous group, *Fucus vesiculosus* (Brawley *et al.* 1976a, b) and *Laminaria angustata* (Motomura 1990), degradation or digestion of chloroplasts and mitochondria of male gametes was not observed in *Colpomenia bullosa* zygote development. In the oogamous groups, these cellular organelles of sperms, especially chloroplasts, are smaller than those of the eggs, and the sperms can not develop parthenogenetically. On the contrary, cellular organelles in female and male gametes of the isogamous brown algae are almost identical. It is well known that the gametes of the isogamous group of brown algae can develop parthenogenetically (Wynne and Loiseaux 1976, Peters 1987). Nakamura and Tatewaki (1976) reported parthenogenesis of female and male gametes of *Colpomenia bullosa*. Therefore, monoparental or biparental inheritance of chloroplasts and mitochondria in

Figs. 1–6. Six non-consecutive serial sections of an one-hour-old zygote. Both nuclei (N1 and N2) are just fusing their outer nuclear membranes. There are two chloroplasts (Ch1 and Ch2) containing eyespots (Es1 and Es2) and pyrenoids (P1 and P2). Two pairs of centrioles (C1 and C2) exist in this zygote and note that axonemes are detached from the centrioles having a basal plate (Fig. 6 C1). Scale bars=1 μm.

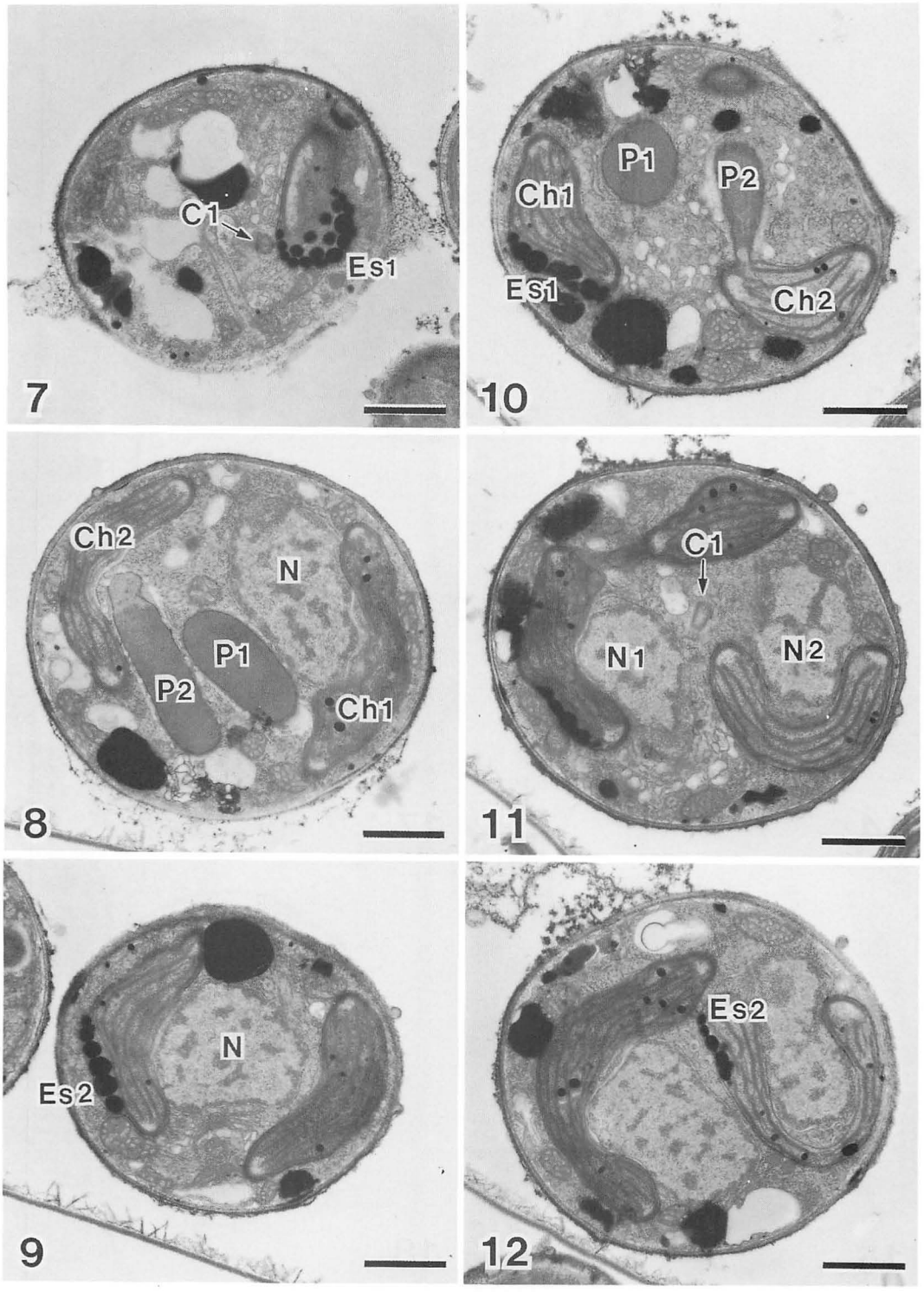
Figs. 7–9. Three non-consecutive serial section of a four-hour-old zygote. This zygote is normally fertilized because it has two chloroplasts (Ch1 and Ch2) containing eyespots (Es1 and Es2) and pyrenoids (P1 and P2). Both nuclei had already fused into one (N). Note only one pair of centrioles (C1) in this zygote. Scale bars=1 μm.

Figs. 10–12. Three non-consecutive serial section of a four-hour-old zygote. The zygote is normally fertilized because it has two chloroplasts (Ch1 and Ch2) containing eyespots (Es1 and Es2) and pyrenoids (P1 and P2). But two nuclei (N1 and N2) have not fused yet because one chloroplast (CH2) exists between them. Note only one pair of centrioles (C1) in this zygote. Scale bars=1 μm.

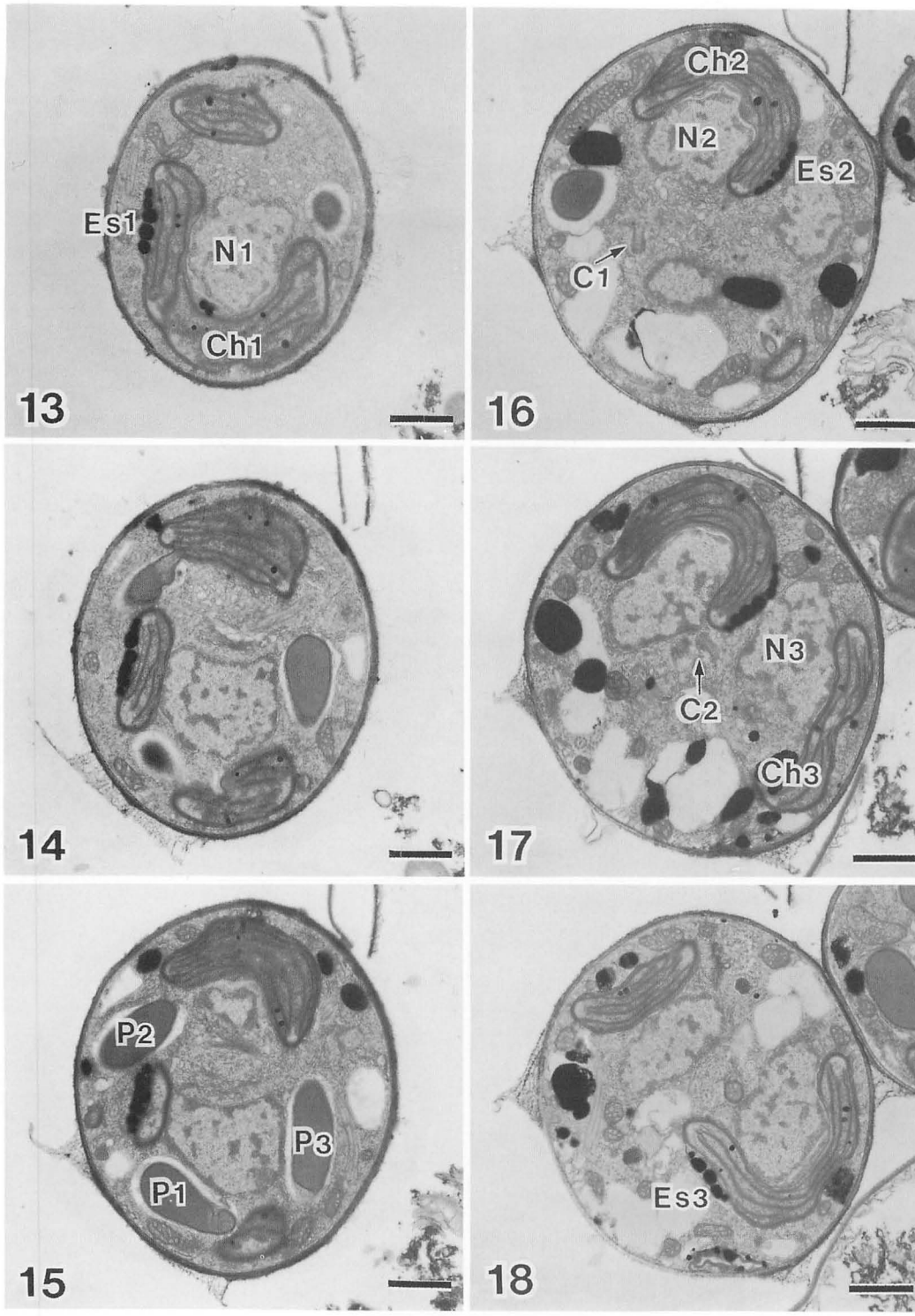
Figs. 13–18. Six non-consecutive serial section of a four-hour-old zygote. The zygote is polyspermic (two male gametes to one female gamete) because it has three chloroplasts (Ch1, Ch2 and Ch3) containing eyespots (Es1, Es2 and Es3) and pyrenoids (P1, P2 and P3). Three nuclei (N1, N2 and N3) have not fused yet. Note two pairs of centrioles (C1 and C2) in the zygote. Scale bars=1 μm.



Figs. 1–6.


C1
Es1
7
P1
P2
Ch1
Es1
Ch2
10
Ch2
N
P1
P2
Ch1
8
C1
N1
N2
11
N
Es2
9
Es2
12

Figs. 7–12.



Figs. 13–18.

zygotes might be related to the ability of both female and male gametes to grow parthenogenetically.

Even though flagellar apparatuses of female and male gametes of *Colpomenia bullosa* were identical, one pair of centrioles disappeared during the development of zygotes. Based on the observations on polyspermic zygotes, it would be proper to conclude that centrioles which were introduced from female gametes disappeared. In the case of *Laminaria angustata*, it was possible to distinguish female from male basal bodies (=centrioles) by their arrangement and connecting structures (Motomura and Sakai 1988, Motomura 1989). Motomura (1990) reported that sperm centrioles remained but egg centrioles disappeared in the zygote development of *L. angustata*. Also, in *Fucus evanescens*, liberated unfertilized eggs do not have centrioles, and sperm centrioles are introduced into the egg after plasmogamy (Motomura unpublished data). Therefore, irrespective of isogamy and oogamy, it could be considered that centrioles of the female gamete disappear and ones of the male gamete remain during brown algal fertilization and zygote development. Afterward, the centrioles from male gametes will begin to function as a component of centrosomes in vegetative cells of the diploid sporophytic generation.

Paternal inheritance of centrioles might be universal in brown algal fertilization, based on this study and previous work (Motomura 1990). The occurrence of paternal inheritance of centrioles is well known in animal fertilization (Schatten *et al.* 1988, Sluder *et al.* 1989, Luykx 1991). It is significant that paternal inheritance of centrioles, in other words, regulation of mitotic spindle pole formation in the first division of zygotes, would be a common phenomenon between the brown algal and animal fertilization.

In this experiment, I report the behavior of centrioles, which are one component of the centrosome, using electron microscopy of *Colpomenia bullosa* fertilization. However the behavior of centrosomal material (pericentriolar material), which is the actual microtubule organizing center (Robbins *et al.* 1968, Gould and Borisy 1977), in the isogamous brown algal fertilization is still obscure. Motomura (1991) reported that centrioles in *Laminaria angustata* were derived from the sperm but centrosomal material might be present already or synthesized *de novo* in the egg.

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本村泰三：褐藻ワタモ受精過程での雌性配偶子由来セントリオールの消失

同型配偶子接合を行う褐藻ワタモ（*Colpomenia bullosa*）の受精・発生過程を電子顕微鏡を用いて観察した。受精後約4時間経過した接合子では，雌・雄性配偶子から持ち込まれた二組のセントリオールのうち一組が消失していた。多精した接合子においても一組のセントリオールだけが消失することから，雌性配偶子由来のセントリオールが消失すると結論した。受精時にセントリオールが父性遺伝することは広く動物細胞（卵生殖）において知られており，また褐藻ミツイシコンブ（卵生殖）の受精過程においても同様な現象が最近明らかになった。今回の観察から同型配偶子接合を行う褐藻類においてもセントリオールは父性遺伝することが確かめられた。褐藻植物ではセントリオールは核分裂時において紡錘体の両極に一組ずつ存在することから，同型配偶子接合・卵生殖において接合子の第一回目の核分裂の分裂装置形成に共通した制御機構が存在している可能性を強く示唆する。（051 室蘭市母恋南町1-13　北海道大学理学部附属海藻研究施設）