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Studies on the morphotype alternation in *Monoraphidium* sp. (Selenastraceae, Chlorophyta)

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Abstract – The green alga *Monoraphidium* sp., isolated from a pond at Changhua city in Taiwan, was examined for its morphological responses to different environmental factors. The alga exhibited fusiform and spherical cells when cultivated in the laboratory. Three different temperatures (20°C, 25°C and 30°C), adjustments in the initial pH of medium from 6.22 to 9, and replacement of the medium at late-stationary phase on day 25, were tested to determine the factors that affected the morphotype alternation in this green alga. In these physiological experiments, fusiform cells grew flourishingly in fresh medium and transformed into spherical cells in aged medium. Spherical progeny were observed in all physiological experiments. In the temperature experiments, the size of spherical cells at 30°C was significantly larger than that of those at 20°C and 25°C ($P < 0.05$). Through time, medium alkalization occurred naturally in the temperature experiments. However, temperature and pH were observed to have no significant influence on morphotype-alternation pattern ($P > 0.05$). Replacing the medium at late-stationary phase resulted in drastic alternation between fusiform and spherical morphotypes. This result suggests that medium content is the major factor influencing morphotype alternation. Based on these experiments and detailed observations, a schematic model of morphotype alternation in *Monoraphidium* sp. is suggested.

Chlorophyta / *Monoraphidium* / morphotype alternation / nutrient / pH / Selenastraceae / temperature

Résumé – Etudes de l'alternance de morphotypes chez *Monoraphidium* sp. (Selenastraceae, Chlorophyta). Les réponses morphologiques à différents facteurs environnementaux ont été étudiés chez l'algue verte *Monoraphidium* sp., isolée d'un étang à Changhua city à Taiwan. L'algue présente des cellules fusiformes et sphériques quand elle est cultivée en laboratoire. Des températures différentes (20 °C, 25 °C et 30 °C), des modifications du pH initial du milieu (de 6.22 à 9), et des remplacements du milieu au 25e jour, dernière phase stationnaire, ont été testées pour déterminer les facteurs qui affectent l'alternance de morphotype chez cette algue verte. Durant ces expériences physiologiques, les cellules fusiformes ont proliféré dans le milieu frais et se sont transformées en cellules sphériques dans le vieux milieu. Des lignées sphériques sont observées dans toutes les expériences physiologiques. Lors des tests de température, d'une part, les cellules sphériques sont significativement plus larges à 30 °C qu'à 20° ou 25 °C ($P < 0.05$), d'autre part, le milieu s'alcalinise naturellement. Cependant, la température

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et le pH n'ont pas d'influence significative sur le processus d'alternance de morphotype ($P > 0.05$). Le remplacement du milieu lors de la dernière phase stationnaire, entraîne une alternance drastique entre les morphotypes fusiformes et sphériques. Ces résultats suggèrent que le contenu du milieu est le principal facteur influençant l'alternance de morphotype. En se basant sur ces expériences et sur ces observations détaillées, il est proposé un modèle schématique de l'alternance de morphotype chez *Monoraphidium* sp.

Alternance de morphotype / Chlorophyta / *Monoraphidium* / nutriement / pH / Selenastraceae / temperature

INTRODUCTION

In aquatic systems, algae are exposed to rapid fluctuations in nutrient levels, light intensity, temperature, and water motion (Fisher *et al.*, 1996; Sommer *et al.,* 1986; Zirbel *et al.,* 2000). Depending on environmental conditions, many algae are notoriously plastic in morphology, growth, and physiology. Different environmental conditions may favor certain morphotypes. For example, the freshwater green alga, *Scenedesmus*, exhibits extreme phenotypic plasticity (Egan & Trainor, 1989; Trainor & Egan, 1991; Trainor, 1998; Lürling & Donk, 1999). Such phenotypic plasticity is defined as the capability of a genotype to produce several phenotypes under changing environmental conditions. In *Scenedesmus* spp. in culture, unicellular organisms are produced at the beginning of the population growth curve, becoming gradually replaced by bicellular coenobia, long-spined quadricaudate coenobia, and short-spined quadricaudate coenobia toward the completion of the exponential phase. At the end of the population growth curve, spineless coenobia possessing heavy ridges are produced (Morales & Trainor, 1997). All such morphological changes are regarded as either cyclomorphosis, polyphenism, or polymorphism (Black & Slobodkin, 1987; Morales & Trainor, 1997; Gibson, 1975; Trainor, 1992a, 1992b), and such phenotypic plasticity has been attributed to changes in nutrients and temperature (Trainor, 1992a, 1992b).

In addition to morphological changes, microalgae can increase rates of nutrient uptake by vertical migration, thereby protecting cells from nutrient limitation (Pasciak & Gavis, 1975; Fenchel, 1988; Lazier & Mann, 1989; Karp-Boss *et al.,* 1996). Many algae also change their surface area to volume (SA/V) ratio to facilitate vertical migration and nutrient uptake (Egan & Trainor, 1989; Lürling & Donk, 1999).

Monoraphidium sp. is a green freshwater planktonic microalga belonging to the Selenastraceae of the Chlorophyta (Krienitz *et al.,* 2001). This green alga exhibited two different morphotypes when maintained in axenic culture in this study. Such morphotype alternation in *Monoraphidium* has been undocumented until recently (Chang *et al.,* 1999; Krienitz & Klein, 1988; Krienitz *et al.,* 2001), when the genus was shown to exhibit fusiform and spherical cells in suspended culture and agar plate culture, respectively. A change from the fusiform to the spherical cell morphotype in the stationary phase was also observed. However, the causes of the observed morphological variation have remained unidentified until now.

In order to explain the morphotype alternation observed in the planktonic alga *Monoraphidium* sp., we manipulated culture conditions by using combinations of different temperatures, adjustments in the initial pH of the

medium to 9, and medium replacement at late-stationary phase, in order to determine which factors are possible influences on the observed morphotype alternations. Based on a series of experiments that monitored the changes in the different morphotypes (and size of spherical cells) of *Monoraphidium* sp. under different culture conditions, we identified the critical point for morphotype alternation in *Monoraphidium* sp.. We suggest a schematic model of morphological alternation of *Monoraphidium* sp. based on results obtained in this study.

MATERIALS AND METHODS

Unialgal culture

The green alga *Monoraphidium* sp. was first isolated from a population growing in a pond at Changhua city (E120°33.510', N24°04.919') in Taiwan and kept in axenic culture. Isolation was carried out by agar plate purification. After performing purification using serial agar plate cultures, spherical cells were transferred into a 150 ml Erlenmeyer flask filled with 100 ml *Chlorella* medium at room temperature for mass culture. The culture medium was as originally suggested by Kuhl (1962), with some modifications according to Chen & Lorenzen (1986). The composition of the modified medium is shown in Table 1. Subsequently, 100 ml algal solution was transferred to 400 ml culture tubes containing 300 ml *Chlorella* medium in a climate-controlled chamber. The algal population grown under controlled climate conditions was used as the stock algal solution for the physiological experiments. Algal cells were cultured at 25°C under a light-dark (L: D) period of 12:12 hours and aerated with air filtered through a 0.2 μm membrane filter. The photon flux was 80-100 μol photons m^{-2} s^{-1}, provided by five fluorescent

Table 1. The composition (mg l^{-1}) of modified *Chlorella* medium.

Nutrient	*Concentration (mg l^{-1})*
Major nutrient	
KNO_3	1011
$NaH_2PO_4 \cdot 2H_2O$	621
$ZnSO_4 \cdot 7H_2O$	287
$MgSO_4 \cdot 7H_2O$	246
$MnSO_4 \cdot H_2O$	169
$Na_2HPO_4 \cdot 2H_2O$	71
EDTA (Ethylenediaminetetraacetic acid)	18.6
$FeSO_4 \cdot 7H_2O$	13.9
Trace nutrient	
H_3BO_3	0.62
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.129
$CaCl_2 \cdot 2H_2O$	0.03
$CuSO_4 \cdot 5H_2O$	0.025

tubes. The initial pH of the medium was determined by using a pH meter (SUNTEX, SP-701) and maintained at pH 6-7 by addition of 1% HCl or 1% NaOH solution. Algal populations were maintained in late-exponential phase or early-stationary phase by transferring algal solution into fresh medium upon reaching late-stationary phase, and harvested prior to the experiments. The exponential and stationary phases were distinguished according to the algal population growth curve. Because two doublings of the population are needed to produce new progeny, population growth curves are presented as log to the base 2, where an increase of one unit is equivalent to one doubling of cell numbers.

Physiological experiments

Algal cells at the early stationary phase were centrifuged at 3000 rpm for 15 min and transferred into 400 ml culture tubes containing 300 ml fresh *Chlorella* medium. Experimental manipulations involving three different temperatures (20, 25, and 30°C), adjustment of the initial medium pH value to 9 by addition of 1% NaOH solution, and medium replacement at stationary phase by centrifuging the culture and replacement of old medium with 300 ml fresh medium on day 25 were combined to determine the factor that influences morphotype alternation. Initial cell densities (ICDs) of 2×10^6 cells/ml were maintained at the start of temperature experiments. The experiments involving medium adjustment to pH 9 and medium replacement at day 25 were maintained at 25°C and at similar ICDs. Prior to testing the response of individual morphotypes, the percentage of each morphotype was controlled at 60% spherical cells and 40% fusiform cells at the beginning of each experiment. In the experiment involving medium replacement at stationary phase, the percentage and cell numbers of each morphotype before and after medium replacement were carefully monitored and maintained to prevent the introduction of errors as a result of physical damage. Batch cultures were run in triplicate. Cell numbers and pH values were recorded every 24 h for 24-45 d. The diameter of spherical cells was also measured throughout the experiment.

Morphological observations

When a series of physiological experiments was performed to examine morphological responses, an alternation of two morphotypes was also observed using light microscopy. Morphotype alternation was further followed throughout the physiological experiments. We again examined morphotype alternation, and division conditions. Fusiform and spherical mother cells producing fusiform or spherical progeny cells (Figs 1-9) were distinguished by using light microscopy under 400×, 1000× (Zeiss Axioskop 2). Such detailed observations lasted for 30 d. The algal genus was determined using the key provided by Yamagishi (1992). Although our material showed clear pyrenoids with starch envelopes, the pyrenoid region was further discerned by staining with Acetocarmin G (1% in acetic acid) (Krienitz *et al.*, 2001). Photographs were taken on a Zeiss light microscope with a camera (Zeiss MC80) or through a cool CCD system (Pixera Penguin 600CL with Automontage Software). Based on these physiological experiments and further morphological examinations, the life cycle and the pattern of morphotype alternation were proposed.

Statistical analysis

A one-way ANOVA design was used to analyze the effect of temperature on growth rate, morphotype alternation pattern and spherical cell size. Paired T

test design was used to analyze the initial pH effect (low and high pH) at 25°C on morphotype alternation pattern, and the effect of replacing medium at stationary phase on morphotype alternation.

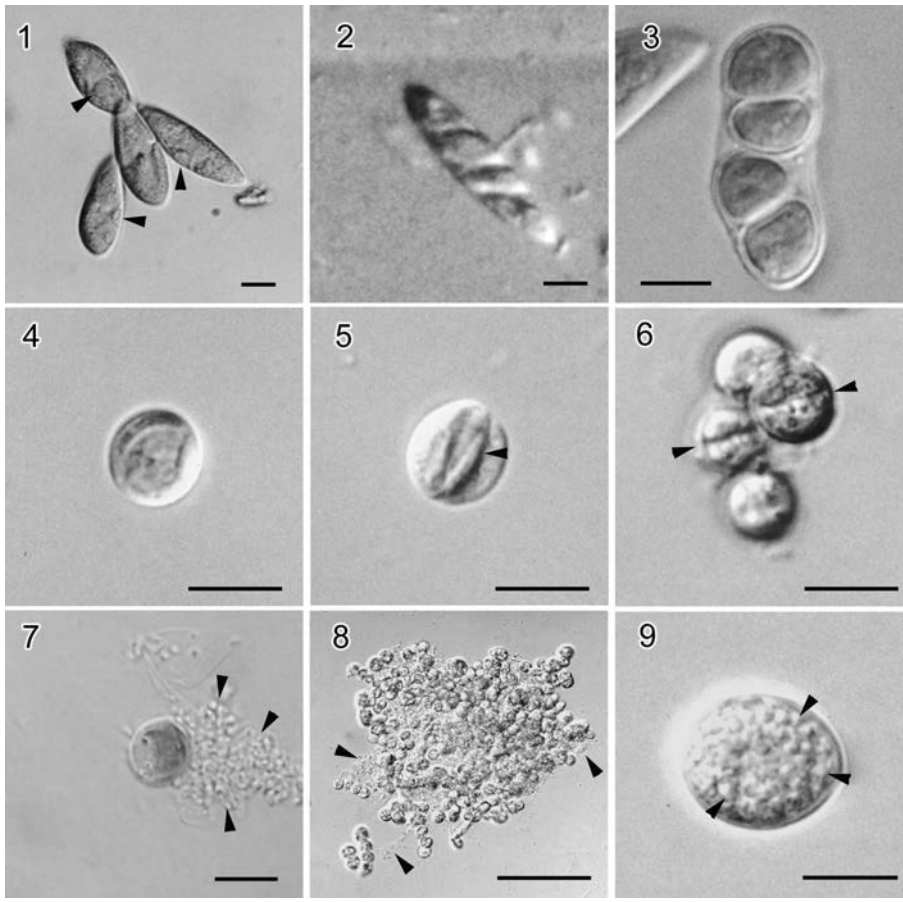
RESULTS

Morphological observations

We were able to distinguish two morphotypes in the cells under observation: fusiform (Fig. 1) and spherical cells (Fig. 4). The fusiform cells closely fit the description of *Monoraphidium* in Yamagishi (1992). Such cells are solitary, elongate cylindrical to fusiform, tapering gradually towards the thin, pointed ends, usually roundly acute; chloroplasts are of the single, parietal, laminate type, with or without a pyrenoid (Fig. 1); 2-5 μm wide, 10-26 μm long. Although the basic morphological features in *Monoraphidium* were found in our materials, morphotype alternation from fusiform to spherical cells has not been described before (Chang *et al.,* 1999; Krienitz & Klein, 1988; Krienitz *et al.,* 2001). First, spherical cells produced 2-4 fusiform progeny (Fig. 5) during the early exponential phase. Then fusiform cells produced 4-8 fusiform progeny (Fig. 2) in the middle/late exponential phase. In the late-exponential or stationary phase, both spherical and fusiform cells produced spherical progeny (Figs 3, 6). Fusiform cells produced 4-8 spherical progeny (Fig. 3), and spherical cells produced 4-8 spherical progeny (Fig. 6). Obviously, the two morphotypes can be observed in different phases of the medium. In addition to the morphological changes, aggregation of spherical cells in late-stationary phase was observed. The spherical cells secreted a gelatinous substance which caused cell aggregation (Figs 7, 8). In the later stationary phase, cyst-like cells resembling resting spores were also observed. These characteristically showed a grainy protoplast (Fig. 9), unlike the smooth appearance found in fusiform and spherical cells (Figs 1, 4).

Physiological experiments

Monoraphidium sp. was cultured at three different temperatures. Growth rate was significantly higher at 20°C (0.192 ± 0.010 doubling d^{-1}) and 25°C (0.168 ± 0.014 doubling d^{-1}) when compared with the growth rate at 30°C (0.101 ± 0.003 doubling d^{-1}) ($P < 0.05$, Figs 10a, 10d, 10g). Apparently, our isolate does not grow well when cultured at high temperatures. At the onset of morphotype change, the delay in the change from spherical to fusiform morphotype at 30°C (*ca* 4 d) was longer than at 20°C (*ca* 2 d), while the shortest delay was observed at 25°C (*ca* 1 d). There is a significant difference in the delay time in the three experiments ($P < 0.05$, Figs 10a, 10d, 10g). These results suggest that moderate temperature was suitable for morphotype transformation from spherical to fusiform cells. Through time, the morphotype alternation occurred again when the population growth curve reached late-exponential or early-stationary phase. At this stage, a change from the fusiform to the spherical morphotype was observed, and the duration of morphotype change appeared to increase with temperature: 18 d at 20°C, 22 d at 25°C, and 24 d at 30°C (Figs 10a, 10d, 10g), though this phenomenon was not statistically significant. Comparing the interval time for morphotype alternation between the earlier phase and the late-exponential/early-stationary phase, the algal populations at the three temperatures showed closely similar morphotype



Figs 1-9. Observations in *Monoraphidium* sp., LM. **(1-6)** Four divisions conditions presents here. Fusiform mother cells and the production of fusiform and spherical progeny. Spherical mother cell and the production of fusiform & spherical progeny. Scale bars, 4 μm. **(1)** Fusiform cells showing the pyrenoid region penetrating the chloroplast (arrowhead). **(2)** Four fusiform progeny cells dividing inside a fusiform mother cell. **(3)** Four spherical progeny cells dividing inside a fusiform mother cell. **(4)** Spherical cell. **(5)** Four fusiform progeny cells dividing inside a spherical mother cell. Arrowhead indicates cell plate formation. **(6)** Spherical progeny formation inside spherical mother cell. Arrowheads indicate cell plate formation. **(7-9)** An amount of hyaline mucilage (arrowheads) was accumulated near the spherical cells and aggregated them together at stationary phase. **(7)** Spherical cell secretes hyaline gelatinous substance. Scale bar, 4μm. **(8)** The gelatinous mucilage causes the spherical cells to aggregate. Scale bar, 20 μm. **(9)** Enlarged cyst-like cell derived from spherical cell appeared in late-stationary phase and granular protoplast (arrowheads) was observed throughout the cell. Scale bars, 4 μm.

alternation times ($P = 0.064$, *ca* 19 ± 1 d). Such results indicate that temperature itself may have no influence on the timing of morphotype alternation.

The size of spherical cells (initial, 6.3 μm) was monitored over the course of the experiments. Cell diameter rapidly increased to 7.6 μm at 20°C on day 10

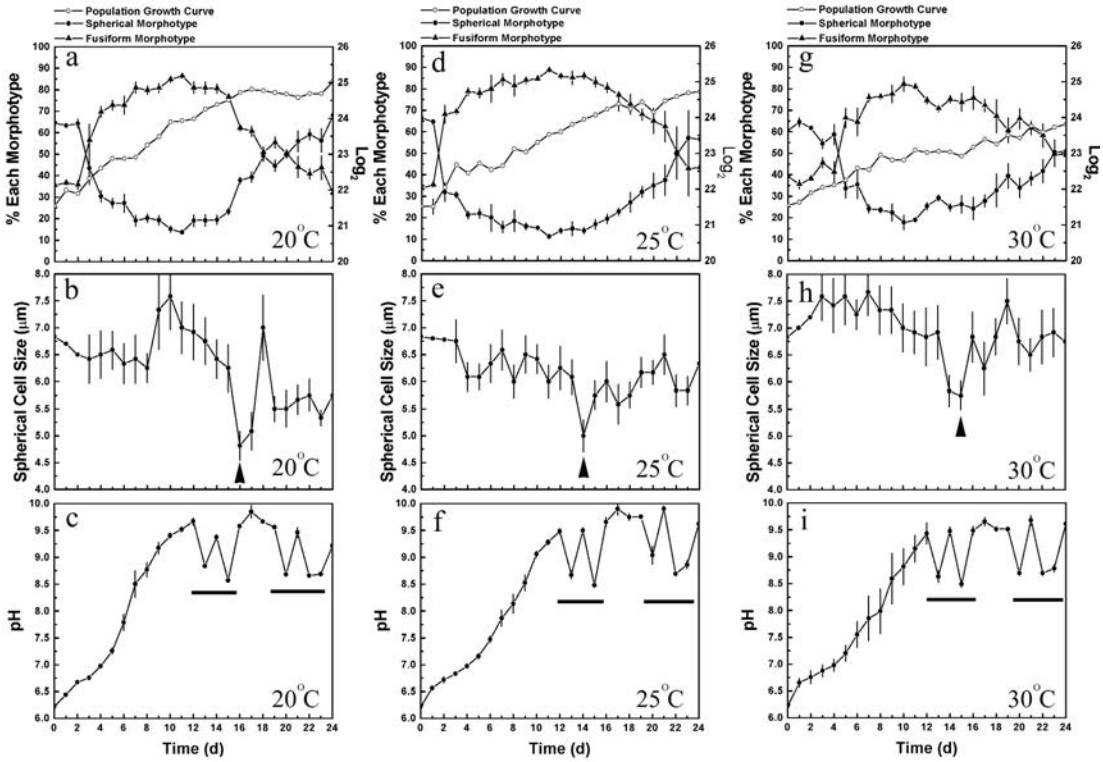


Fig. 10. Three temperature experiments. **(a-c)** Cultivated at 20°C. **(a)** Population Growth (Log_2 cell density) and percentage of each morphotype was tracked for 24 d. Bars are SE (n = 3). **(b)** Spherical cell size fluctuation shows a progeny releasing event (arrowhead). Bars are SE (n = 18). **(c)** Medium became alkaline over time. There is pH variability over short time periods (black bold bars). Bars are SE (n = 3). **(d-f)** Cultivated at 25°C. **(d)** Population Growth (Log_2 cell density) and percentage of each morphotype was tracked for 24 d. Bars are SE (n = 3). **(e)** Spherical cell size fluctuation shows a progeny releasing event (Arrowhead). Bars are SEM (n = 18). **(f)** Medium became alkaline over time. There is pH variability over short time periods (black bold bars). Bars are SE (n = 3). **(g-i)** Cultivated at 30°C. **(g)** Population Growth (Log_2 cell density) and percentage of each morphotype was tracked for 24 d. Bars are SE (n = 3). **(h)** Spherical cell size fluctuation shows a progeny releasing event (arrowhead). Bars are SE (n = 18). **(i)** Medium became alkaline over time. There is pH variability over short time periods (black bold bars). Bars are SE (n = 3).

(Fig. 10b) but decreased to 4.8 μm on day 16 at 20°C (Fig. 10b). From an initial 6.3 μm diameter, cells cultured at 25°C decreased to 5.0 μm on day 14 (Fig. 10e). On day 15, cells decreased to 5.8 μm when cultured at 30°C (Fig. 10h). All cell diameters rose again and remained so at all three different temperatures, showing similar cell size fluctuations (Figs 10b, 10e, 2h). At 30°C, the size of spherical cells was significantly larger than at the other temperatures ($P < 0.05$, 6.2 μm at 20°C, 6.1 μm at 25°C, and 7.0 μm at 30°C; Figs 10b, 10e, 2h). At all three temperatures, medium alkalization during algal grown was observed. The pH values increased with cell growth up to day 12, and stabilized at *ca* pH 8.5~9.5 at all three tem-

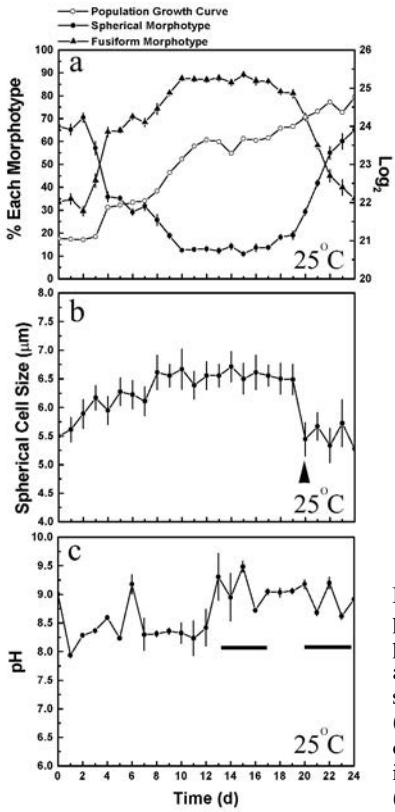


Fig. 11. The experiment involving adjustment of initial pH to 9. **(a)** Population growth (Log_2 cell density) and percentage of each morphotype was tracked for 24 d in alkaline medium. Bar are SE (n = 3). **(b)** Spherical cell size fluctuation. A progeny-releasing event was observed (arrowhead). Bar are SE (n = 18). **(c)** The change in pH over the course of the experiment. There is pH variability over short time periods (black bold bars). Bar are SE (n = 3).

peratures (Figs 10c, 10f, 10i). There was an interesting pattern of high pH variability over very short time periods in the middle of the temperature experiments (black bold bar in Figs 10c, 10f, 10i).

When morphotype alternation from fusiform to spherical cells occurred at late-exponential or late-stationary phase, such morphotype alternation was accompanied by medium alkalization (Figs 10b, 10e, 10h). In order to clarify the effect of medium alkalization on morphotype alternation, an adjustment of the initial medium pH value to 9 was performed in order to examine morphotype alternation at 25°C in an alkaline environment. When compared with morphotype alternation pattern at 25°C and the lower pH in the temperature experiments, a significantly delayed morphotype alternation at the start of experiment was observed (*ca* 3 d, $P < 0.05$, Fig. 11a). Such a phenomenon indicates that medium alkalization did not favor morphotype alternation from spherical to fusiform cells. However, comparing the interval time for morphotype alternation between cultures with an initial medium of pH 6.22 and one of pH 9 at 25°C (Figs 10d, 11a), there was no significant difference ($P > 0.05$). This result suggests that high pH (or medium alkalization) had no influence on the morphotype-altering pattern (Fig. 11a). Cell diameter initially increased from 5.5 μm to 6.5 μm, then fell rapidly to 5.4μm at day 20 (Fig. 11b). The pH dropped from 9 to 7.9 at day 2 and increased

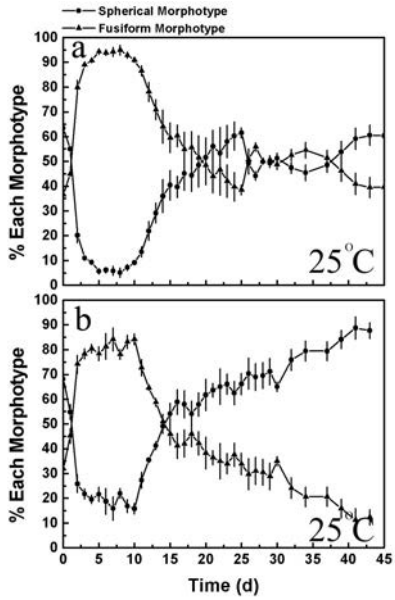


Fig. 12. The effect of medium condition on morphological changes. **(a)** With replacement of fresh medium after day 25. A morphotype alternation from spherical to fusiform cells was observed on day 26. Then morphotype percentage alternation from fusiform to spherical cells was observed on day 38. Arrowhead shows point of medium replacement. Bars are SE (n = 3). **(b)** No replacement of fresh medium after day 25. Bars are SE (n = 3).

to 9.3 at day 13, then kept at *ca* 9 after day 13 (Fig. 11c). There was a tendency for low pH to occur at 0-13 d and higher pH at 13-24 d. Low pH periods from 0 to 13 d showed a peak on day 6 (Fig. 11c). A pattern of erratic pH variability over very short time periods similar to the temperature experiments was also observed (black bold bar in Fig. 11c).

Since temperature and medium pH showed no apparent effect on morphotype alternation, medium composition was examined. When algal growth reached the stationary phase, the culture medium was replaced on the 25th day (Fig. 12a). There were then drastic changes in the percentages of fusiform and spherical cells on the 26th day (Fig. 12a). At *ca* day 38, the percentage of fusiform and spherical cells in the cultures changed again (Fig. 12a). The alternation in morphotype percentage seen after medium replacement was similar to that in the earlier experiments. This phenomenon was observed only in the treatment set (Fig. 12a), not in the control (Fig. 12b). Statistical analysis showed a significant difference between treatment and control ($P < 0.05$).

A schematic model of morphological alternation in Monoraphidium *sp.*

Detailed morphological changes were observed over the course of the experiment, which lasted for 30 d. The fusiform-converting processes were observed at exponential phase. These led to the release of fusiform progeny, whose population rapidly increased. The spherical-converting processes were observed at the onset of stationary phase. These led to the release of spherical progeny, whose population rapidly increased. Fusiform cells were dominant in the fresh medium while spherical cells were dominant in the aged medium (Figs 1-9, 10a, 10d, 10g, 11a). Based on the foregoing experiments and observations, a schematic model of morphotype alternation of *Monoraphidium* sp. is suggested (Fig. 13).

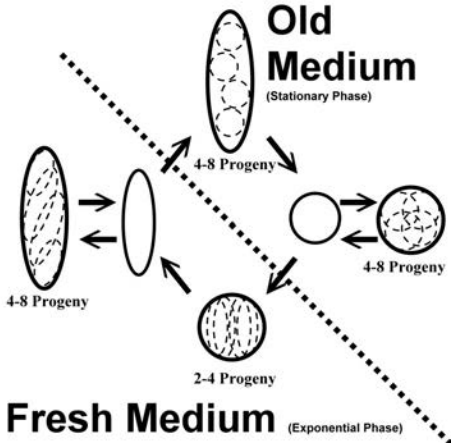


Fig. 13. A schematic model of morphotype alternation in *Monoraphidium* sp. Morphological changes were observed over the course of experiment for 30 d. The fusiform-converting processes were observed at exponential phase, then released fusiform progeny and rapidly increased their population. The spherical-converting processes were observed at stationary phase, then released spherical progeny and rapidly increased their population. Fusiform cells are dominant in fresh medium while spherical cells are dominant in old medium.

DISCUSSION

In cultures subjected to three different temperatures, it was observed that the growth rate of *Monoraphidium* sp. significantly decreased at high temperatures. A possible explanation lies in the temperature of the original habitat condition. Although the study materials were isolated from a tropical region where air/water temperature may reach 30-35°C in summer (Fig. 14), we isolated our materials in the spring, when environmental temperatures were much lower. It is possible that we isolated a strain with a lower optimum temperature (Fig. 14). Although lower and moderate temperatures were suitable for growth of our isolates, a significant delayed morphotype alternation time was observed at 20°C and 30°C, or in high pH medium. Hence, the change from spherical to fusiform morphotype appears to occur at specific T° and pH values. In addition to temperature effects on morphological variation, the spherical cell diameter of *Monoraphidium* sp. was also monitored and found to increase at higher temperature. This phenomenon has also been observed in *Scenedesmus acutus* and other phytoplanktonic species (Reynolds, 1984; Lürling & Donk, 1999). From an ecological point of view, cells may enlarge to endure adverse environments. It is also interesting to note that an increase of the number of spherical cells coincided with the appearance of the smallest cell size at *ca* 14-16 d in the three temperature treatments (arrowheads in Figs 10b, 10e, 10h) and at day 20 of the pH experiment (arrowhead in Fig. 11b). According to our observations, the spherical progeny might have originated from two sources: fusiform cell division into spherical progeny, and division of spherical cells. The initial source of spherical progeny in this study probably came from spherical cell division as these initial progeny were usually smaller in size. Similar observations have also been made in *Scenedesmus* spp. (Egan & Trainor, 1989). In that study, cell size change of one ecomorph of *Scenedesmus armatus* was monitored to show that unicellular progeny was released from a mother unicell ecomorph rather than a mother coenobial ecomorph. Moreover, as pH rose, two groups of cell sizes were seen in the present study. Initially, cell sizes

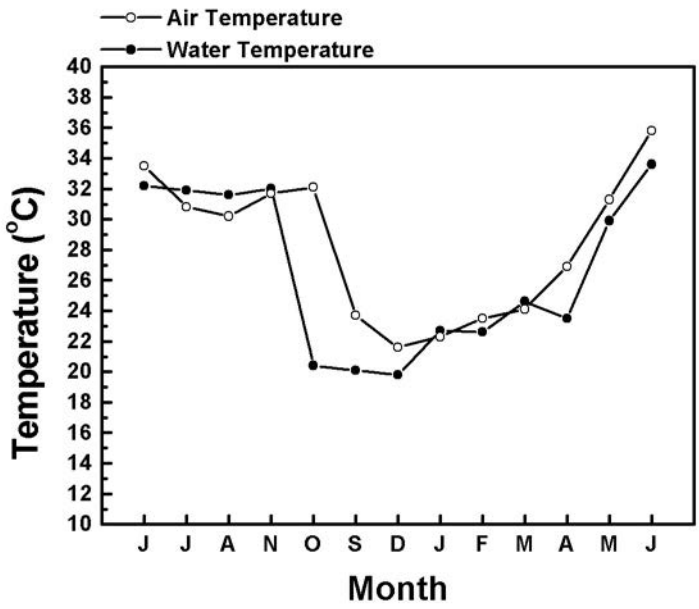


Fig. 14. The seasonal temperature change in the Changhua pond was monitored every month in 2000 and 2001. Our materials were isolated from this pond.

were larger when the medium had lower pH values, while cell sizes became smaller as the medium reached higher pH values (Figs 10b, 10e, 10h, 11b). The two size groups seemed to be affected by progeny-releasing events (arrowheads in Figs 10b, 10e, 10h, 11b). Before progeny release, spherical cells were bigger. After progeny release, spherical cells were smaller. Our results suggest that pH might be the signal for progeny release in our algae. However, these results need to be verified by further investigation and must be treated as preliminary.

In the all physiological experiments, the pattern of pH variability over short time periods shown in the temperature experiments, and in the experiment involving the adjustment of initial medium to pH 9, cannot be explained with available data. We propose two possible explanations. First, the pH variations might be attributable to changes in dissolved CO_2 concentrations. During growth, the algal cells often deplete CO_2, making the main inorganic carbon in medium HCO_3^-, causing the pH rise (Zou *et al.*, 2003). However, during the dark period, respiratory metabolism will cause CO_2 release, reducing the pH again. For instance, samples taken close to the beginning of a light period may have a lower pH as CO_2 produced in respiration will not have been used up, whereas samples taken towards the end of a light period would be strongly CO_2 depleted. However, since the samples were taken at the exact same time each day, such errors could not occur in the present study. Second, the observed pH variability might be derived from the physiological regulation of spherical cells, because pH variability seems to accompany the increase in spherical morphotypes.

Based on the results of physiological experiments in the present study, medium content appears to be the most important factor influencing

Monoraphidium sp. transformation. However, the component in the medium that causes morphotype alternation in *Monoraphidium* remains unidentified. Many taxa exhibit morphological changes (*Oscillatoria redenei*, Gibson, 1975; *Scenedesmus* spp., Egan & Trainor, 1989), colony size changes (*Asterionella formosa*, Hayakawa & Kudoh, 1994), and changes in extracellular carbohydrate secretion (estuarine benthic diatoms, Smith & Underwood, 2000) when subjected to nutrient depletion or alteration of other environmental factors. This phenomenon has caused confusion among phycologists in distinguishing genetically similar species in nature (Hegewald & Silva, 1988; Trainor & Egan, 1990; Wood & Leatham, 1992; Hwang *et al.*, 1997; Morales & Trainor, 1997). Such morphological changes in our cultures, from fusiform to spherical cells, may be a manifestation of physiological regulation. A possible explanation for such morphotype alternation in *Monoraphidium* sp. is herein reported. Fusiform cells that possess a small SA/V ratio were dominant in environments with high nutrient content. Over time, nutrients may become depleted as the algae grow, leading to intense intraspecific competition for nutrients. Competition in turn leads cells to transform into morphologies with large SA/V ratios that quickly absorb nutrients, in order to maintain population sizes. This phenomenon has also been observed in *Scenedesmus* spp. and was termed cyclomorphosis (Morales & Trainor, 1997). This may also explain why we first observed rounded cells on the agar-plate culture. In that environment, algal cells with a smaller SA/V (e.g. fusiform cells) would find it harder to absorb nutrients than cells with a larger SA/V (e.g. rounded cells).

In addition to the morphological response from these physiological experiments, spherical cells secrete gelatinous substances during the stationary phase, thereafter forming spherical cell aggregates. These gelatinous substances are thought to be colloidal carbohydrates (Smith & Underwood, 2000). The aggregative behavior helps cells increase their sinking rates to avoid adverse environmental conditions often encountered near the water surface. A similar strategy is widely observed in phytoplankton (Reynolds, 1984; Lürling & Donk, 1999). During the later stationary phase, resting-like cells were also observed. Cells produced sufficient food reserves, making the protoplast look granular. Cells formed cyst-like structures as a protection from adverse environmental conditions. These may germinate under more favorable conditions.

In aquatic environments, many organisms change their appearance when they encounter environments that fluctuate between extremes. The morphological variation observed in such organisms may be attributed to stresses caused by two groups of interactions, environmental, and biological. Many unexpected morphological variations are triggered by environmental changes. A common example is *Scenedesmus*, which has many ecomorphs, including unicells, and spiney multicells. Different ecomorphs are able to endure different environmental factors, permitting survival of the species (Morales & Trainor, 1997). One interesting example of this is the discovery of Zirbel *et al.* (2000) that spine formation in *Ceratocorys horrida* is affected by the water turbulence. High water turbulence will reduce the formation of long spines, facilitating sinking away from the turbulent conditions. On the other hand, biological interactions can also induce the morphological variations extensively observing among aquatic organisms. In *Scenedesmus*, exposure to water containing *Daphnia* will cause colony formation of *Scenedesmus acutus*, thereby conferring an ability to avoid grazing. Such morphological expression is driven by chemical cues from grazers (Lürling & Donk, 1999). In this case, morphological change was due to the relationship between grazers and algae. In some cases biological interactions appear to be necessary. When *Ulva* was cultured in the absence of bacteria, normal morphogenesis was absent (Nakanishi *et al.*, 1996).

Direct contact between *Ulva* and morphogenesis-active bacteria is important for morphogenesis in that genus.

In this study, the morphological alternation of *Monoraphidium* occurred when it experienced changes in medium components during its life span. Cells changed their size and shape, or produced sufficient food reserve to cause the protoplast to become granular. More observations and diagnosis will be helpful in determining the exact cause of these changes, and the relationship between specific morphologies and medium components.

Combining our understanding of the key role of the content medium with the results of our observations of cell transformations yields a schematic model of morphological alternation in *Monoraphidium* sp. Through this model, we hope to understand how *Monoraphidium* sp. switches between fusiform and spherical cells and under what environmental conditions such morphotype alternation patterns may occur. Although we have proposed a model for morphotype alternation in *Monoraphidium* sp., what medium component (or combination of components) affects morphological alternation, as well as the function of the gelatinous substances and resting spores, remain unclear. We also need to improve the taxonomy of our isolates and to compare and assess this against the behavior of other strains. It would also be interesting to investigate changes in metabolism and gene expression as such morphological changes take place. Furthermore, as cultures or algal populations grow, self-shading will become a major environmental stress (Lee & Arega, 1999). A decrease in irradiance led to a rapid decrease in algal biovolume (Thompson *et al.*, 1991; Sciandra *et al.*, 1997). What is the role of light in controlling the morphotype alternation of *Monoraphidium* sp. from large biovolume to small one? As cultures are diluted, both the nutrient concentration and average light intensity will increase. Hence, the effect of light intensity on the morphotype alternation should be clarified using adequate experimental designs in the future study.

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REFERENCES

BLACK R. & SLOBODKIN L., 1987 — What is cyclomorphosis ? *Freshwater Biology* 18: 373-378.

CHANG Y.K., JEON S.L., KIM M.R. & OH H.R., 1999 — Phylogenetic relationships of the genus *Monoraphidium* (Chlorophyta) based on *rbc*L gene sequences. *Algae* 14: 9-21.

CHEN P.C. & LORENZEN H., 1986 — Changes in the productivity and nuclear divisions in synchronous *Chlorella* and circadian rhythm. *Plant Cell Physiology* 27: 1423-1427.

EGAN P.F. & TRAINOR F.R., 1989 — Low cell density: the unifying principle for unicell development in *Scenedesmus* (Chlorophyceae). *British Phycological Journal* 24: 27-83.

FENCHEL T., 1988 — Marine plankton food chains. *Annual Review Ecology and Systematics* 19: 19-38.

FISHER A.E., BERGES J.A. & HARRISON P.J., 1996 — Does light quality affect the sinking rates of marine diatoms? *Journal of Phycology* 32: 353-360.

GIBSON C., 1975 — Cyclomorphosis in natural populations of *Oscillatoria redekei* van Goor. *Freshwater Biology* 5: 279-286.

HAYAKAWA T. & KUDOH S., 1994 — Temperature-dependent changes in colony size of the freshwater pennate diatom *Asterionella formosa* (Bacillariophyceae) and their possible ecological implications. *Journal of Phycology* 30: 955-964.

HEGEWALD E. & SILVA P., 1988 — An annotated catalogue of *Scenedesmus* and nomenclaturally related genera including original descriptions and figures. *Bibliotheca Phycologicae* 80: 1-587

HWANG M.S., CHUNG I.K. & OH Y.S., 1997 — Temperature responses of *Porphyra tenera* Kjellman and *P. yezoensis* Ueda (Bangiales, Rhodophyta) from Korea. *Algae.* 12: 207-214.

KARP-BOSS L., BOSS E. & JUMARS P.A., 1996 — Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanography and Marine Biology Annual Review* 34: 71-107.

KRIENITZ V.L. AND KLEIN G., 1988 — Morphology and ultrastructure of some species of the genus *Monoraphidium* (Chlorellales) III. *Monoraphidium terrestre* (Bristol) nov. comb. *Algological Studies* 49: 447-463.

KRIENITZ L., USTINOVA I., FRIEDL T. & HUSS V.A.R., 2001 — Traditional generic concepts versus 18S rRNA gene phylogeny in the green algal family Selenastraceae (Chlorophyceae, Chlorophyta). *Journal of Phycology* 37: 852-865.

KUHL A., 1962 — Zur physiologie der speicherung kondensierter anorganischer phosphate in *Chlorella*. *Vorträge aus dem Gesamtgebiet der Botanik N.F.* 1: 157-166.

LAZIER J.R.N. & MANN K.H., 1989 — Turbulence and the diffusive layers around small organisms. *Deep Sea Research* 36: 1721-1733.

LEE J.W. & AREGA F., 1999 — Eutrophication dynamics of Tolo Harbour, Hong Kong. *Marine Pollution Bulletin* 39: 187-192.

LÜRLING M. & DONK E.V., 1999 — Grazer-induced colony formation in *Scenedesmus acutus* (Chlorophyceae): Ecomorph expression at different temperature. *Journal of Phycology* 35: 1120-1126.

MORALES E.A. & TRAINOR F.R., 1997 — Algal phenotypic plasticity: its importance in developing new concepts. *Algae* 12: 147-157.

NAKANISHI K., NISHIJIMA M., NISHIMURA M., KUWANO K. & SAGA N., 1996 — Bacteria that induce morphogenesis in *Ulva pertusa* (Chlorophyta) grown under axenic conditions. *Journal of Phycology* 32: 479-482.

PASCIAK W. J. & GAVIS J., 1975 — Transport limited nutrient uptake rates in *Ditylum brightwellii.* Academic Press, Orlando, Florida, pp. 43-105.

REYNOLDS C.S., 1984 - *The ecology of freshwater phytoplankton.* Cambridge University Press, Cambridge, pp. 40-81.

SCIANDRA A., GOSTAN J., COLLOS Y., DESCOLAS-GROS C., LEBOULANGER C., MARTIN-JÉZÉQUEL V., COPIN-MONTÉGUT C. & AVRIL B., 1997 — Growth-compensating phenomena in continuous cultures of *Dunaliella tertiolecta* limited simultaneously by light and nitrate. *Limnology Oceanography* 42: 1325-1339.

SMITH D.J. & UNDERWOOD G.J.G., 2000 — The production of extracellular carbohydrates by estuarine benthic diatoms: the effects of growth phase and light and dark treatment. *Journal of Phycology* 36: 321-333.

SOMMER U., GLIWICZ Z.M., LAMPERT W. & DUNCAN A., 1986 — The PEG-model of seasonal succession of planktonic events in fresh waters. *Archiv für Hydrobiologie* 106: 433-471.

THOMPSON P.A., HARRISON P.J. & PARSLOW J.S., 1991 — Influence of irradiance on cell volume and carbon quota for 10 species of marine phytoplankton. *Journal of Phycology* 27: 351-360.

TRAINOR F.R., 1992a — Cyclomorphosis in *Scenedesmus comunis* Hegew. Ecomorph expression at low temperature. *British Phycological Journal* 27: 75-81.

TRAINOR F.R., 1992b — Cyclomorphosis in *Scenedesmus armatus* (Clorophyta): An ordered sequence of ecomorph development. *Journal of Phycology* 28: 553-588.
TRAINOR F.R., 1998 - Biological aspects of *Scenedesmus* (Chlorophyceae) phenotypic plasticity. *Nova Hedwigia* 117: 1-307.
TRAINOR F.R. & EGAN P.F., 1990 — The implications of polymorphism for the systematics of *Scenedesmus*. *British Phycological Journal* 25: 273-279
TRAINOR F.R. & EGAN P.F., 1991 — Discovering the various ecomorphs of *Scenedesmus*: the end of a taxonomic era. *Archiv für Protistenkdunde* 139: 125-132.
WOOD M.A. & LEATHAM T., 1992 — The species concept in phytoplankton ecology. *Journal of Phycology* 28: 723-729.
YAMAGISHI T., 1992 — *Plankton algae in Taiwan (Formosa)*. Tokyo, Uchida Rokakuho Press, pp. 98-102.
ZOU D., GAO K. & XIA J., 2003 — Photosynthetic utilization of inorganic carbon in the economic brown alga, *Hizikia fusiforme* (Sargassaceae) from the Soth China Sea. *Journal of Phycology* 39: 1095-1100.
ZIRBEL M.J., VERON F. & LATZ M.I., 2000 — The reversible effect of flow on the morphology of *Ceratocorys horrida* (Peridiniales, Dinophyta). *Journal of Phycology* 36: 46-58.