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Seasonal variation of desmids at a small marsh in Hiroshima, Japan*

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Seasonal changes in cell size and occurrence of desmids were studied from April 1984 to February 1985 in a small marsh in Hiroshima-ken, western Honshu, Japan. In this study, 23 species of desmids belonging to 10 genera were observed. Only *Netrium digitus* and *Euastrum montanum* showed a tendency of periodicity of occurrence. In *Netrium digitus*, the periodicity was considered to be a result of the formation of zygospores in the autumn and their germination in the spring. In *Netrium digitus*, *Closterium costatum*, *C. pseudolunula* and *Hyalotheca dissiliens*, cell width was considered to be the more reliable character for classification than cell length. Concerning the seasonal variation in cell length, all species except *Closterium costatum* showed rather large variations without any correlation to the seasonal changes, while cell width was rather stable throughout the whole year in all the species examined.

Key Index Words: *Cell size*; *Chlorophyceae*; *desmids*; *seasonal variation*.

The variation of vegetative cell characters in desmids has been studied by many investigators. However, most of them have dealt with the qualitative characters such as those of spines, warts and radiations (RAYNOLDS 1940, ROSENBERG 1944, TEILING 1957, BICUDO and CARVALHO 1969, GERRATH 1979, SOUTH 1984). Although the cell size is much used as a taxonomic criterion, the variation of cell size has been poorly studied in the laboratory (ICHIMURA and WATANABE 1976, WATANABE 1978) and still more poorly in the field. DUTHIE (1965b) and RŮŽIČKA (1971) showed that cell size of desmids was influenced by temperature under culture conditions. Therefore, it is expected that desmids may show periodical variation in cell size according to the seasonal change in temperature. In order to study this, periodical observations of desmid populations at a fixed site in a marsh were carried out from April 1984 to February 1985. The marsh is at about 770 m altitude and is located in Iiyama, Saeki-cho, Saeki-gun, Hiroshima-ken, which is situated in the western part of Honshu, Japan. This marsh has been used as a paddy field but it has been left without cultivation of the crop for about 20 years. The vegetation at present is mainly composed of *Scirpus wichrae*, *Carex dispalata*, *Cirsium sieboldii*, *Eriocaulon iskokianum*, *Thuidium glaucinum*, etc. In August heights of the vegetation attained up to about 1 m, but the sunlight reached the surface of the water through the vegetation. During the months of January and February in 1985 this marsh was covered with snow. To show the climate of this region, monthly mean atmospheric temperatures at Yoshiwa-cho, which is located about 8 km northeast of the studied site are shown in Table 1.

The present study deals mainly with variations of vegetative cell size in natural popu-

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Table 1. Seasonal changes of atmospheric temperature at Yoshiwa-cho, which is located about 8 km northeast of the studied site.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Mean temp.	-0.5[1]	0.2	4.0	10.0	14.5	18.3	23.1	23.5	19.2	12.8	7.4	2.5
Maximum temp.	3.8	4.9	9.6	16.4	20.9	23.7	27.8	28.8	24.6	19.1	13.4	7.4
Minimum temp.	-4.8	-4.4	-1.7	3.5	8.0	12.9	18.3	18.2	13.8	6.5	1.3	-2.6

[1] Degrees Celsius (°C); all data are mean values during the past 20 years.

lations of several desmids along with their seasonal fluctuation in abundance, but also with the occurrence of zygospores in the field.

Materials and Methods

Samples (ca. 20 ml) were collected by glass pipette once a month from a definite spot of about 100 cm^2 in the marsh. At the time of collection, pH and water temperature were measured by a pocket pH meter (Model PH51, Yokogawa Electric Works) and the data are shown in Table 2. Cells were taken out from each sample to study the morphology of chloroplasts and pyrenoids in living condition. After this, the rest of the samples were preserved in 5 percent formalin and used for the measurement of cell size. For each measurement, 25 cells per species, except for some cases when fewer cells were available, were observed under the light microscope using the micrometer. All the samples studied were deposited in the Herbarium of Hiroshima University (HIRO).

Table 2. Water temperature and pH of the samples studied.

Sample No.(HIRO)	Collection date	Time	Water[1] temp.	pH
so-934	27 iv '84	12:30	23.8	5.5
so-944	1 vi	10:10	20.5	5.7
so-949	5 vii	19:40	23.2	5.6
so-951	5 viii	12:30	32.3	5.6
so-956	5 ix	11:20	19.9	5.8
so-962	6 x	13:00	15.3	5.4
so-969	4 xi	11:00	3.9	5.8
so-974[2]	4 xii	13:00	2.5	5.9
so-980[2]	11 i '85	12:00	1.7	5.2
so-983	7 ii	13:30	3.0	-

[1] Degrees Celsius (°C). [2] Marsh was covered with snow, and samples were collected from another site near the study point.

The experiment of zygospore germination of *Netrium digitus* was carried out at about 20°C. Light was provided by cool white fluorescent tubes on a cycle of 12 hr light and 12 hr dark. Light intensity was about 2500 lux. Samples containing zygospores were kept in a refrigerator until the time of use. When the sample was set on this condition, a great number of zygospores germinated within a few days.

Results

1) Seasonal changes in desmid population

During the present study 23 species of desmids belonging to 10 genera were found and their monthly occurrences are shown in Table 3. Associated algae with these desmids were mainly diatoms, blue-green algae and other groups of green algae. Some species of diatoms were abundant during the winter, and *Eremosphaera viridis* (Chlorellales) was always dominant throughout the period of this study.

Among the desmids found in this marsh, *Micrasterias denticulata* var. *angulosa* (Fig. 1j) was always dominant throughout the year. Even during the winter, many vegetative cells of this species were observed, but no zygospores were found. *Netrium digitus* (Fig. 1a), however, showed a marked contrast to this. The vegetative cells were abundant from April to October, but sudden-

Table 3. Occurrence of desmids from April 1984 to February 1985.

Species	Apr.	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
Spirotaenia condensata	rr	rr	—	—	rr	rr	rr	r	rr	rr
S. obscura	rr	rr	—	—	rr	rr	rr	rr	r	rr
Cylindrocystis brebissonii	—	—	—	—	—	—	rr	—	—	—
Netrium digitus	c	cc	c	c	cc	cc[1]	r[1]	r[1]	rr[1]	—[1]
Closterium closterioides var. *intermedium*	rr	rr	c	+	r	r	—	rr	—	rr
C. costatum	r	r	+	+	c	r	+	+	+	+
C. dianae var. *minus*	—	—	rr	—	—	—	—	—	—	—
C. intermedium	—	—	—	—	rr	—	—	—	—	—
C. (cf.) *kuetzingii*	—	—	rr	rr	rr	rr	rr	—	—	—
C. lunula	r	rr	r	rr	rr	rr	r	rr	—	r
C. moniliferum	—	—	rr	—	—	—	—	—	—	—
C. pseudolunula	r	+	+	+	+	+	r	+	r	r
C. striolatum	—	—	—	—	rr	—	rr	—	rr	—
Tetmemorus granulatus f. *minor*	—	—	—	rr	—	rr	rr	rr	—	—
Euastrum luetkemuelleri var. *carniolicum*	—	—	—	rr	rr	rr	—	—	—	—
E. montanum	cc	cc	+	+	+	+	rr	rr	rr	r
E. oblongum	rr	—	—	—	—	—	—	—	—	—
Micrasterias denticulata var. *denticulata*	+	+	r	r	r	r	r	r	r	rr
M. denticulata var. *angulosa*	c	c	c	c	c	c	c	c	c	c
Actinotaenium cucurbita	—	—	rr	—	—	—	—	—	—	—
Cosmarium quadratum	rr	rr	rr	—	—	—	—	—	—	rr
C. westii	rr	rr	rr	—	rr	—	—	—	—	rr
Hyalotheca dissiliens	cc	c[2]	+[2]	r[2]	r	+	+	+	c	+

cc: Very abundant; c: Abundant; +: Common; r: Rare; rr: Very rare. [1] Zygospores were observed. [2] Parthenospores were observed.

ly decreased their numbers after October, when zygospores (Fig. 1b) of this species were first observed. Although a small number of living vegetative cells were seen with zygospores from November to January, no living vegetative cells were observed in February. Zygospore germination was observed in the sample collected in April 1984. *Euastrum montanum* (Fig. 1h) also showed a marked fluctuation in vegetative cell number. It was abundant from April to June, and commonly seen from July to October, but rarely from November to January and very rarely in February. No zygospores were observed throughout the year. *Closterium pseudolunula* (Fig. 1e), *C. costatum* (Fig. 1f), *C. closterioides* var. *intermedium* (Fig. 1g), *Micrasterias denticulata* var. *denticulata* (Fig. 1i) and *Hyalotheca dissiliens* (Fig. 1c) were observed throughout the period studied. In these four species, however, neither seasonal fluctuation in vegetative cell number nor zygospore formation were detected. But from June to August only parthenospores of *Hyaloteca dissiliens* (Fig. 1d) were observed. The remaining species were rare or occurred sporadically, and no zygospores were observed.

2) Variation of cell size

As to the most frequently appearing six species, *Netrium digitus, Closterium costatum, C. pseudolunula, Micrasterias denticulata* var.

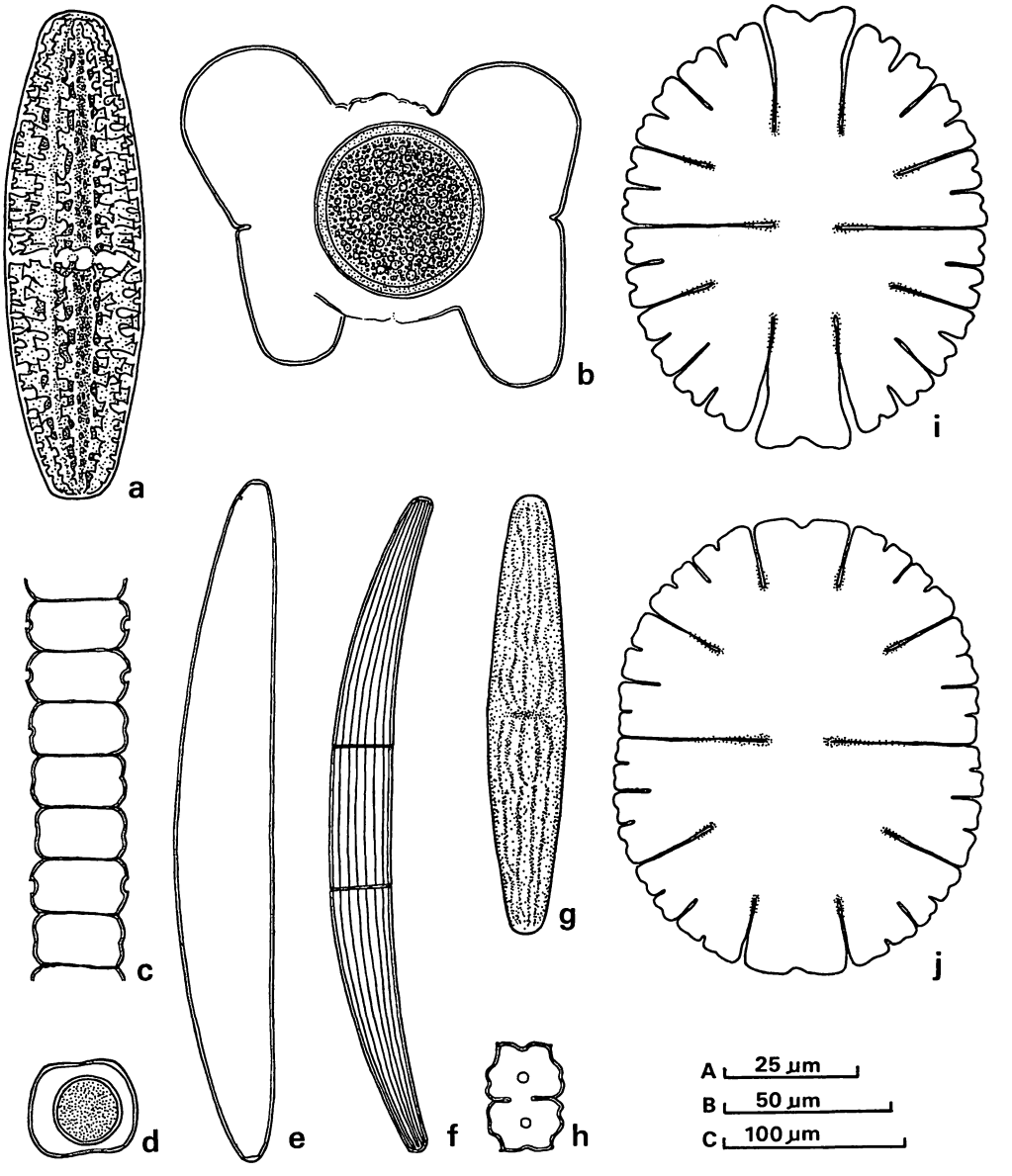

Fig. 1. Representative species of the desmids studied. a. *Netrium digitus* (RALFS) ITZIGS & ROTHE. b. Zygospore of *N. digitus*. c. *Hyalotheca dissiliens* RALFS. d. Parthenospore of *H. dissiliens*. e. *Closterium pseudolunula* BORGE. f. *C. costatum* RALFS. g. *C. closterioides* (RALFS) LOUIS & PEETERS var. *intermedium* (ROY & BISS.) RŮŽIČKA. h. *Euastrum montanum* WEST & WEST. i. *Micrasterias denticulata* RALFS var. *denticulata*. j. *M. denticulata* var. *angulosa* (HANTZSCH) WEST & WEST. Scale: A for h, B for c, d, g and C for others.

Table 4. Seasonal variation of coefficient of variability of cell size in six species of desmids from Hiroshima.

Month	*Netrium digitus*		*Closterium costatum*		*Closterium pseudolunula*		*Hyalotheca dissiliens*		*Micrasterias denticulata* var. *denticulata*			*Micrasterias denticulata* var. *angulosa*		
	Length[1]	Width[1]	Length	Width	Length	Width	Length	Width	Length	Width	Isthmus[1]	Length	Width	Isthmus
Apr.	13.4	9.2	11.6	2.6	7.4	2.0	8.6	4.2	5.8	3.1	3.3	2.6	3.1	5.0
June	9.8	6.1	8.2	2.6	6.9	2.5	10.0	4.5	4.4	3.6	5.2	4.0	3.6	3.8
July	9.6	2.5	10.6	2.4	6.5	1.8	9.4	3.4	2.9[6]	3.8	5.2	3.1	3.6	2.8
Aug.	8.4	1.9	11.4	2.9	7.0	1.8	7.5	3.8	3.1	2.7	3.7	4.2	3.4	3.3
Sep.	6.7	2.4	11.3	2.9	7.1	1.8	9.3	3.8	3.3	3.4	2.7	3.6	3.6	3.9
Oct.	6.1	1.7	9.1	2.4	8.0	1.6	7.3	3.7	3.2	3.9	3.0	4.5	4.3	2.9
Nov.	8.1[2]	3.1	10.0	2.6	7.3[3]	2.0	8.5	3.4	2.5[7]	3.8	3.9	3.6	4.0	4.2
Dec.	—	—	13.3	2.6	6.8	1.8	6.5	3.8	2.7	2.8	2.7	3.9	3.2	4.0
Jan.	—	—	13.7	1.9	5.0[4]	2.2	11.1	4.1	3.2[8]	1.9	1.8	4.1	4.1	3.0
Feb.	—	—	11.6	2.6	6.8[5]	2.2	8.1	3.8	—	—	—	4.5	3.7	3.4

[1] Coefficient of variability (%).

[2-7] Cell number measured. [2] N=15. [3] N=19. [4] N=15. [5] N=16. [6] N=13. [7] N=14. [8] N=7. Others N=25.

denticulata, its var. *angulosa* and *Hyalotheca dissiliens*, variations in vegetative cell size were statistically analyzed.

a) Variation of cell size in each sample

In *Netrium digitus*, *Closterium costatum*, *C. pseudolunula* and *Hyalotheca dissiliens*, CV (Coefficient of variability: standard deviation/mean) of cell width varied in a smaller range than that of cell length (Table 4). For example, in *Closterium costatum*, CV of cell width ranged from 1.9 to 2.9, while that of cell length was from 8.2 to 13.7. Thus, the cell width is rather stable and can be considered to be a more reliable taxonomic character than the cell length. Nevertheless in *Micrasterias denticulata* var. *denticulata* CV of cell width, that of cell length and that of width of isthmus were in the range from 1.9 to 3.9, from 2.5 to 5.8 and from 1.8 to 5.2, respectively. Therefore, these three characters seem to vary about equally in this desmid. The same is true for *M. denticulata* var. *angulosa*.

b) Seasonal variation of cell size

In *Netrium digitus*, CV of cell length in April and CV of cell width in April and June were considerably larger than those of other months. These higher values resulted from the presence of many small cells in the samples collected in these months. These small cells are considered to be juvenile cells appeared from germination zygospores, because all the developmental stages from small young to fully developed cell were observed with several germination vesicles in these samples (Fig. 2). It is clear from Fig. 3 that cell size variation in July, when no zygospore germination was observed, is restricted to the normal moderate range, while that in April is more widely scattered, especially toward smaller cell size. Mean values of cell size of both months were somewhat smaller than those of other months (Fig. 4a). In *Closterium costatum* (Fig. 4c),

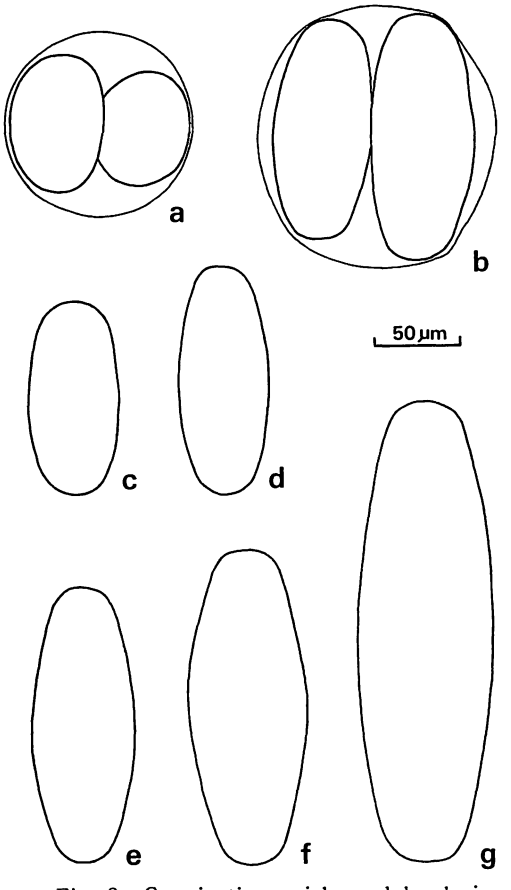

Fig. 2. Germination vesicles and developing cells of *Netrium digitus* in a sample so-934 (April). a, b. Two gones within a vesicle. c-f. Young developing cells of various sizes. g. Fully grown vegetative cell.

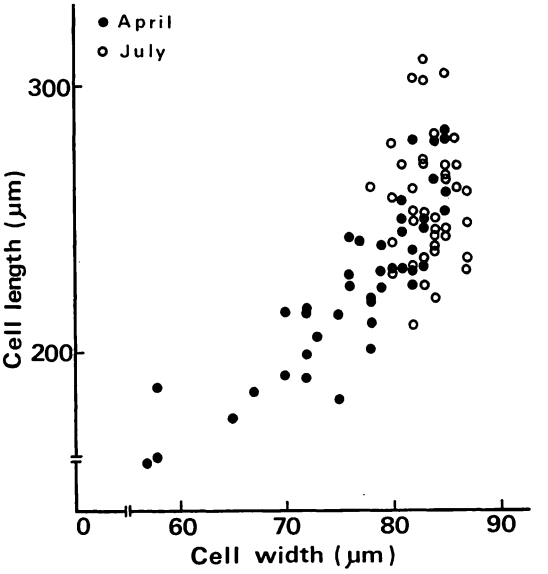

Fig. 3. Cell size variation of *Netrium digitus* in April (●) and July (○).

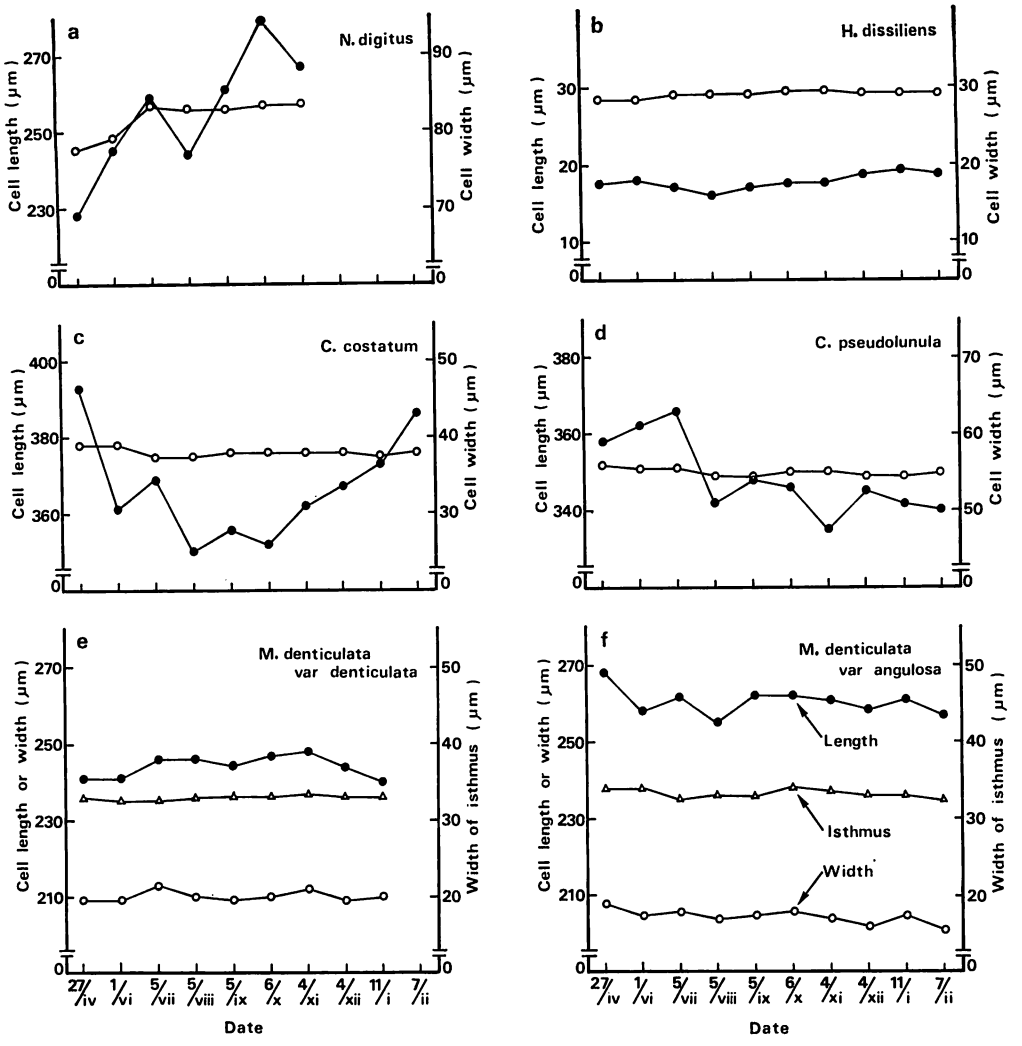

Fig. 4. Seasonal variation of cell size in six species of desmids. a. *Netrium digitus*. b. *Hyalotheca dissiliens*. c. *Closterium costatum*. d. *C. pseudolunula*. e. *Micrasterias denticulata* var. *denticulata*. f. *M. denticulata* var. *angulosa*. ●: Cell length; ○: Cell width; △: Width of isthmus.

a marked seasonal fluctuation in the cell length was observed. The cell length was significantly diminished from April to August, and increased again from August to February. In contrast, the cell width was considerably stable throughout the year. In *Micrasterias denticulata* var. *denticulata* (Fig. 4e) and its var. *angulosa* (Fig. 4f), however, both the cell width and length considerably varied throughout the year and no meaningful change was detected, while the width of isthmus was considerably stable throughout the year. In *Closterium pseudolunula* (Fig. 4d) and *Hyalotheca dissiliens* (Fig. 4b), the cell length varied to a large extent without any correlation with the seasons, but cell width was always rather stable.

Discussion

WEST and WEST (1912) showed the clear seasonal fluctuation in planktonic desmids such as *Staurastrum jaculiferum* and *S. lunatum* var. *planctonicum*. These species were abun-

dant during the summer and the autumn, but diminished in numbers during the winter months, while some other desmids were not rare or rather abundant during winter months (WEST and WEST 1912, DUTHIE 1965a). In the present study, *Netrium digitus* and *Euastrum montanum* showed the seasonal fluctuation, while *Micrasterias denticulata* var. *angulosa* did not show such a tendency, abounding throughout the year.

It is probable that, in *Netrium digitus*, the marked changes in vegetative cell number are caused by the formation of zygospores in the autumn and their germination in the spring. The zygospores of *N. digitus* seem to be resistant to the winter coldness. COESEL (1974) reported that a shallow water habitat appeared to be favorable for the formation of zygospores in certain species of desmids and considered that the formation of zygospores could be explained as a means of survival when desiccation threatens. In the present study, the zygospores of *N. digitus* were also collected from shallow water. But the sampling site was never completely dried up throughout the period of study and the water containing fresh zygospores was very gelatinous. My colleague, Dr. T. BANDO, and I have collected zygospores of *N. digitus* at several sites including the one reported in this paper during the autumn months (Oct. 30, 1977, Oct. 18, 1982, Nov. 19, 1983, Oct. 6 and 22, 1984) in the same marsh. I could observe the process of zygospore formation in two samples collected on Oct. 18, 1982 and Oct. 22, 1984. Moreover, GRÖNBLAD (1957) reported zygospores on Sep. 20, 1936 in SW Finlnad and BANDO (1981) on Oct. 12-15, 1979 at Kirigamine highland in Japan. On the basis of these results, the formation of zygospores in *N. digitus* seems to occur frequently in the autumn. I consider that the sexual reproduction of *N. digitus* is induced not only by desiccation but also by other factors appearing during the autumn months. Germination of zygospores was not observed from November to February, but was observed in April 1984 in the field. In the laboratory, however, zygospores could germinate at room temperature (ca 20°C) even in January. On the basis of these results of observations in nature and the experiment in the laboratory, I consider the zygospore of *N. digitus* to be a resistant form against low temperature.

Investigating the size variation of some desmids under different temperature conditions in the laboratory, RŮŽIČKA (1971) reported that most species showed a tendency to be smaller in cell size in high temperature (30°C) than in low temperature (10°C). DUTHIE (1965b) also showed similar results on some desmids in two sets of temperature conditions of 4°C and 20°C. Although it was expected that the desmids might show clear seasonal change in cell size, most of the desmids studied did not show such changes, their cell sizes being constant throughout the whole year. RŮŽIČKA (1971) showed that the light intensity also controlled the cell size in desmids. In the field, the combination of temperature, light intensity and other factors probably controls sizes of the desmids. Further studies at different places and on other species will contribute to understanding the problem of the stability of natural populations of desmids.

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References

BANDO, T. 1981. Desmid flora of the Kirigamine highland. p. 169-220. *In* H. SUZUKI [ed.] Plants of Kirigamine. The Board of Education of Suwa City, Suwa. (In Japanese with English summary).

BICUDO, C. E. M. and CORVALHO, L. M. 1969. Polymorphism in the desmid *Xanthidium regulare* and its taxonomic implications. J. Phycol. **5**: 369-375.

COESEL, P. F. M. 1974. Notes of sexual reproduction in desmids. I. Zygospore formation in nature. Acta Bot. Neerland. **23**: 361-368.

DUTHIE, H. C. 1965a. Some observations of the algae of Llyn Ogwen, North Wales. J. Ecol. **53**: 361-370.

DUTHIE, H. C. 1965b. Some observations on the ecology of desmids. J. Ecol. **53**: 695-703.

GERRATH, J. F. 1979. Polymorphism in the desmid *Cosmarium taxichondrum* LUNDELL. Br. phycol. J. **14**: 211-217.

GRÖNBLAD, R. 1957. Observation of the conjugation in *Netrium digitus*. Bot. Notiser **110**: 468-472.

ICHIMURA, T. and WATANABE, M. M. 1976. Biosystematic studies of the *Closterium peracerosum-strigosum-littorale* complex. I. Morphological variation among the inbreeding populations and on experimental demonstration for source of the cell size variation. Bot. Mag. Tokyo **89**: 123-140.

REYNOLDS, N. 1940. Seasonal variation in *Staurastrum paradoxum*. New. Phytol. **39**: 86-89.

ROSENBERG, M. 1944. On the variability of the desmid *Xanthidium subhanstiferum* WEST. New Phytol. **43**: 15-22.

RŮŽIČKA, J. 1971. Morphologische Variabilität der Algen, hervorgerufen durch Kultivierungsbedingungen. Arch. Hydrobiol./suppl. 39 Algological studies **4**: 146-177.

SOUTH, G. S. 1984. Taxonomic implications of morphometric variation in a bloom population of *Euastrum didelta* (Chlorophyta: Desmidiaceae). Phycologia **23**: 47-52.

TEILING, E. 1957. Morphological investigations of asymmetry in desmids. Bot. Notiser **110**: 49-82.

WATANABE, M. 1978. A taxonomic study of the *Closterium calosporum* complex (1). Bull. Nat. Sci. Mus., Tokyo. B (Bot.) **4**: 133-154, pls. 1-8.

WEST, W. & WEST, G. S. 1912. On the periodicity of the phytoplonkton of some British lakes. J. Linn. Soc. London Bot. **40**: 359-432, pl. 19.

大谷修司：広島県の小湿地におけるチリモ類の季節変化

広島県北西部に位置する小湿地において，チリモ類の出現個体数および栄養細胞の大きさの季節変化を観察した。1984年4月から1985年2月におよぶ本研究を通して23種類（優占種は *Netrium digitus*, *Closterium costatum*, *C. pseudolunula*, *Micrasterias denticulata* var. *denticulata*, *M. denticulata* var. *angulosa*, *Hyalotheca dissiliens*）のチリモ類が見出された。

Netrium digitus と *Euastrum montanum* において個体数の季節変化が観察された。前者の季節変化は春の接合子の発芽と秋の接合子形成に由来するものであった。上記の優占種6種類について，各月の標本集団中25個体の大きさの測定を行った結果，細胞の幅は長さに比べ概して安定した形質であることが明らかとなった。各月の平均値について見ると上記6種類いずれにおいても，細胞の幅は一年間を通じてほとんど変化が観察されなかったが，長さは大きく変動する傾向にあった。しかし季節と対応した長さの変化は *Closterium costatum* で観察されたにすぎなかった。(730 広島市中区東千田町 1-1 広島大学理学部植物学教室)