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Conditions for the induction of the mating process and changes in contents of carbohydrates and nitrogen compounds during the mating process of *Spirogyra*

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Induction of the mating process in *Spirogyra* sp. was done successfully by the following procedures: the vegetatively growing cells were precultivated on a solid medium or in a liquid medium containing glucose (0.5 to 1.0%) and an extremely small amount of nitrate at 15°C for four weeks under continuous illumination, then the cells were incubated in a liquid mating medium without nitrogen source at 15°C under continuous illumination and gentle aeration.

During the mating process, intracellular carbohydrates, mainly starch, increased markedly, while nitrogen compounds decreased. A high C/N ratio produced by depletion of nitrogen compounds seemed to be needed for the induction of the mating process.

It has been reported that in several algae the induction of the mating process is depended on light and nitrogen depletion (FÖSTER, 1957 and 1959; ICHIMURA, 1971; LEVIN, 1954 and 1956; SAGER and ORANICK, 1954; UENO and SASAKI, 1978). Some strains of *Spirogyra* conjugated in a definite season of the year under natural conditions (SASAKI, 1977). In *Spirogyra* cells, artificial induction of a synchronous mating process is very difficult. Although there have been a few reports of artificial induction of the mating process in some strains of *Spirogyra* (CZURDA, 1933), reliable conditions for the repetition of the induction of the synchronous mating process are not yet known. To succeed in artificial control of the cell cycle or the life cycle in *Spirogyra* or other algae, is necessary, first, for further studies of the physiological and biochemical properties related to sexual differentiation and the mating process in green plants.

This paper will report the conditions needed for the induction of the sexual process and the quantitative changes in carbohydrates and nitrogen compounds in *Spirogyra* cells during the mating process.

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Materials and Methods

Plant materials: *Spirogyra* sp. (cell width, approx. 75 μm) was collected from a pond on the campus of Hokkaido University at different stages of the life cycle. Under natural conditions, this material conjugated almost synchronously in June (SASAKI, 1977). The mating process was conveniently classified into 6 stages: (1) pairing, (2) swelling, (3) budding and conjugation tube-forming, (4) conjugation tube-connecting, (5) protoplasm-translocating (gamete-migrating), and (6) new zygote stages.

Culture conditions: Basal culture medium consisted of KNO_3 (0.2 g), $MgSO_4 \cdot 7\ H_2O$ (10 mg), KH_2PO_4 (20 mg), $FeSO_4 \cdot 7\ H_2O$ (5 mg), and distilled water (*l*), and pH of the medium was adjusted to 6.6, 7.4, 7.8, or 8.4 (CZURDA, 1933). The solid medium contained agar (3%) and the basal medium. The liquid glucose medium contained the same components as the basal medium except glucose (0.5−1.0%) and KNO_3 (0.0002%). The liquid mating medium was made by elimination of nitrogen source from the basal medium. For artificial induction of the mating process, vegetatively growing cells were precultivated on the surface of the solid medium, or in the liquid glucose medium under gentle aeration, at 15°C under continuous illumination for 7, 20, 30, 36, 40, or 45 days, then the cells were incubated in the liquid mating medium at 15°C under continuous illumination and gentle aeration until they reached the zygote stage. The light source was four 100 watt Toshiba white lamps to get approx. 8000 lux.

Assays: Reducing sugar was determined by the Bertland method. Cells were dried and homogenized in distilled water. The amount of free reducing sugar in the soluble fraction separated from the insoluble fraction of the homogenate was indicated as glucose. The total amount of carbohydrates in the homogenate was determined after hydrolysis with 0.5 N HCl at 100°C for 4 hr and indicated as glucose. The amount of starch in the homogenate was indicated as a difference between the amounts of the total and soluble free carbohydrates.

Total nitrogen content in the dried cells was determined by the Kjeldahl method.

Free amino acids in soluble fraction of the cells were assayed by paper chromatography. The filter paper used was Toyo-filter paper No. 50, the solvent system was a mixture of *n*-butanol, acetic acid, and water (9 : 1 : 7), and the indicator was ninhydrine (0.1% in *n*-butanol).

Endogenous respiratory activity, Q_{O_2}, and RQ were measured manometrically with 0.2 g fresh weight of the cells in 2.0 m*l* of 0.02 M phosphate buffer (pH 7.2) at 25°C in the dark (SASAKI, 1977).

Results and Discussion

Cell size of conjugating cells: Short cells paired and formed the conjugation tube shortly after the final cell division (Fig. 1). After the pairing, further cell growth and cell division were repressed and the cell form sequentially changed. Cell division prior to conjugation may be a sort of sexual

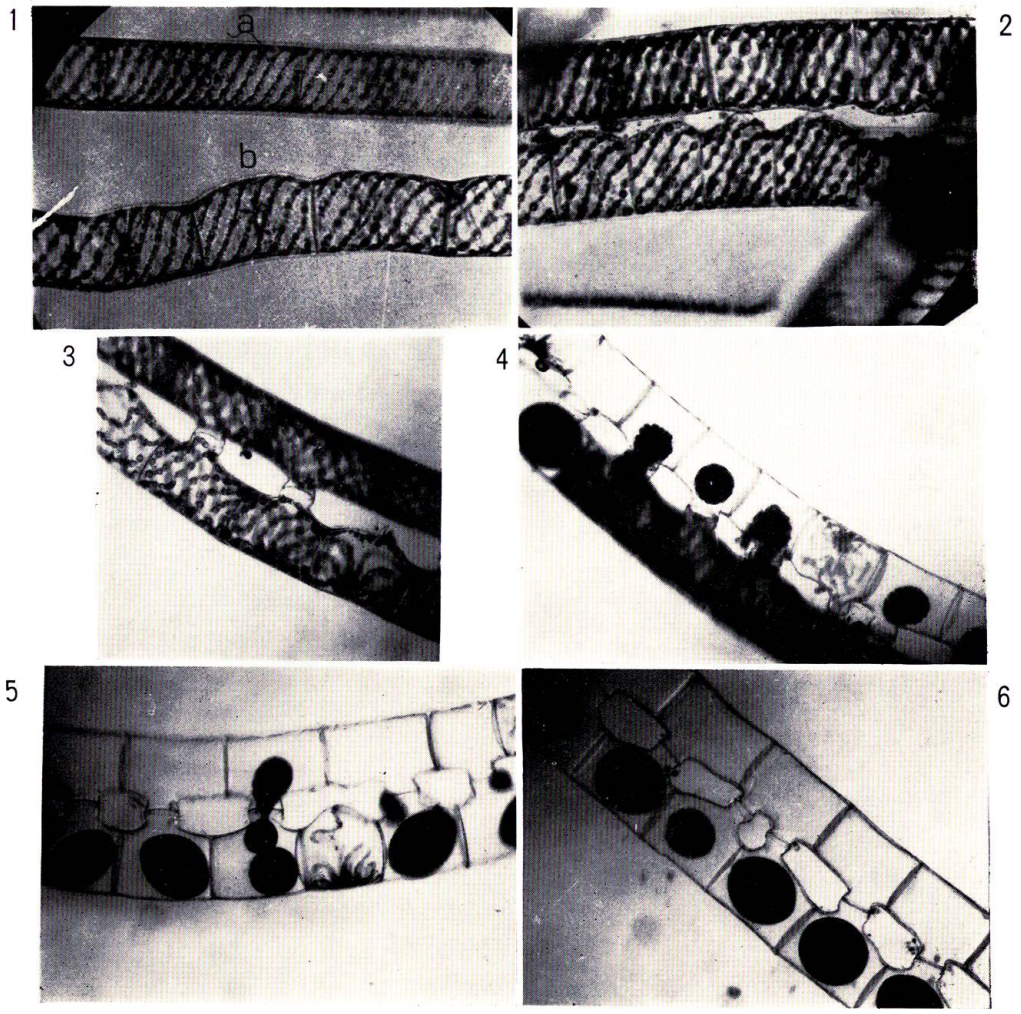

Fig. 1. Morphological changes of *Spirogyra* sp. during natural conjugation process. 1-a, vegetative L-cells or gametangial mother cells; 1-b, swelling cells; 2, pairing and budding cells; 3, conjugation tube-connected cells; 4, aggregating, migrating and fusing gametes; 5, fusing gametes and new zygotes; 6, new zygotes.

cell division as shown in *Closterium* (ICHIMURA, 1971; UENO and SASAKI, 1978) because further cell division was stopped and the cells transformed to gametes. Before the cell division, sexual differentiation may be occured, then gametangial mother cells which have long forms may be formed. Shortly after the cell division, short cells may have an ability to change to gametes but may not have the ability to divide, while long cells, just before the cell division, may not have the ability to change to gametes. At the definite conjugation period under natural conditions, a lot of the cells almost synchronously conjugated. A few cells missed conjugation, grew into abnormally elongated cells, and finally died, as further cell division was repressed (SASAKI, 1977). These results suggest that after sexual cell division, vegetative growth and further cell division were repressed, thereafter the sexual process was induced via metabolic changes. Nitrogen deficiency was necessary conditions for the induction of the sexual process in *Closterium* under illumination. Therefore we assumed that in *Spirogyra* sexual differentiation from vegetative cells to gametangial cells and gametes may be induced by nitrogen deficiency in the light.

TABLE 1. Effects of light and depletion of nitrogen source on the artificial induction of the mating process

Culture conditions				
Preculture		Mating culture		Conjugation*
Medium	Duration (days)	Light	Nitrogen source	
Liquid glucose medium	0	Light	None	−
(0.0002% KNO_3)	7	〃	〃	−
	20	〃	〃	−
	30	〃	〃	+
	36	〃	〃	+
	40	〃	〃	+
	45	〃	〃	+
	0–45	〃	Presence	−
Solid medium	7	Light	None	−
(0.02% KNO_3)	7	Dark	〃	−
	30	Light	〃	+
	30	Dark	〃	−
	40	Light	〃	+
	40	Dark	〃	−

* After 10 to 14 days of the mating culture, zygotes were formed (+) or not formed (−). Zygotes were formed at pH 6.6, 7.4, 7.8 or 8.4.

Conditions for induction of mating process: Sexual process was artificially induced in a strain of *Spirogyra* (cell width, approx. 75 μm) which conjugated in June under natural conditions. The conditions for the artificial induction were as follows: after the preculture of vegetatively growing cells on the surface of solid medium, or in the liquid glucose medium under gentle aeration, at 15°C for 4 weeks under continuous illumination, the cells were incubated in the liquid mating medium in the absence of nitrogen source at 15°C under continuous illumination and gentle aeration. Under the conditions, the cells were conjugated after 10 to 14 days (Table 1). When the vegetative cells were precultivated in the liquid medium without glucose in the presence of nitrate, the cells could not conjugate. The cells precultivated in the dark could not conjugate. Without the precultivation, the vegetative cells could not change to gametes. The cells precultivated could not conjugate in the dark during the mating culture, or in the presence of nitrogen source. Gentle aeration during the preculture and the mating culture was a procedure necessary to support normal growth and conjugation. Vigorous aeration injured the vegetatively growing and conjugating cells. In the culture media used here, carbone dioxide was not supplied. Therefore, it was suggested that aeration may have a role in sufficient supply of CO_2 as a result of the increase in endogenous respiratory activity. Light less 7000 lux could not induce the conjugation. These results indicate that nitrogen deficiency in the light is strict conditions for the induction of mating process in *Spirogyra* as shown in *Chlamydomonas* (FOESTER, 1957; LEWIN, 1954, 1956; SAGER, 1954) and *Closterium* (ICHIMURA, 1971; UENO and SASAKI, 1978). Data on effects of glucose and intensity of light on the zygote formation suggested that light may have a role in accumulation of photosynthetic products for the formation of mature zygote, as seen in accumulation of starch in seeds of higher plants. True role of nitrogen deficiency for the induction of sexual process is uncertain now.

Changes in contents of carbohydrates and nitrogen compounds during conjugation process: Contents of starch and total carbohydrates increased during both the natural (Table 2) and artificial (Table 3) conjugation processes. Of soluble carbohydrates, arabinose and glucose decreased during the natural conjugation process, while polymerized arabinose and glucose increased (YAMASHITA, TAKAHASHI and SASAKI, 1968). These results supported an assumption that a role of the light for conjugation is in photosynthesis for accumulation of large amounts of starch and other polysaccharides.

The endogenous respiratory activitiy, Q_{O_2}, and RQ values decreased

during the natural (Table 2) and artificial (Table 3) conjugation processes. In a previous study, it was shown that the endogenous respiratory activity increased temporarily in a short period of early conjugation stage, thereafter

TABLE 2. Changes in contents of carbohydrate and nitrogen compounds during natural conjugation process

Assay	Cell stage*						
	V	1	2	3	4	5	6
Cells (mg, dry wt.)	41.5	24.4	34.3	33.3	30.2	34.5	41.8
Carbohydrates (% of dry wt.)							
Soluble	4.1	4.9	5.3	9.3	14.8	11.3	4.8
Starch	25.8	32.9	29.2	38.9	46.2	36.8	56.2
Total	29.9	37.8	34.5	48.2	61.0	48.1	61.0
Nitrogen (% of dry wt.)							
Total	3.9	3.1	—	3.5	3.4	2.3	3.0
C/N ratio (carbohydrates/nitrogen)	7.7	12.2	—	13.8	17.9	20.9	20.3
Qo_2	2.37	1.28	0.41	0.03	0.04	—	1.60
RQ	0.86	0.53	0.37	0.58	—	—	1.08

* Cell stages: V, vegetatively growing stage; 1, pairing stage; 2, swelling stage; 3, budding and conjugation tube-forming stages; 4, conjugation tube-connecting stage; 5, protoplasm-translocating stage; 6, new zygote stage.

TABLE 3. Changes in contents of carbohydrate and nitrogen compounds during artificial mating process

Assay	Cell stage				
	Preculture (days)			Mating culture (days)	
	0	14 (in light)	28	10 — 14 (in dark)	(in light)*
Cells (mg, dry wt.)	16.0	22.0	21.2	14.5	12.0
Carbohydrate (% of dry wt.)					
Soluble	Trace	5.5	11.0	Trace	Trace
Starch	13.2	21.0	14.5	21.0	46.2
Total	13.2	26.5	25.5	21.0	46.2
Nitrogen (% of dry wt.)					
Total	5.5	3.7	3.4	3.3	2.5
C/N ratio (carbohydrates/nitrogen)	2.4	7.2	7.5	6.4	18.4
Qo_2	2.58	1.60	1.06	0.84	
RQ	1.02	1.09	1.14	0.64	

* After 10 to 14 days, conjugation was detected in the light.

decreased sharply (SASAKI, 1977). These results suggest that the accumulation of large amounts of starch in conjugating and new zygote cells can be attributed to the decrease of the activity to consume carbohydrates as an

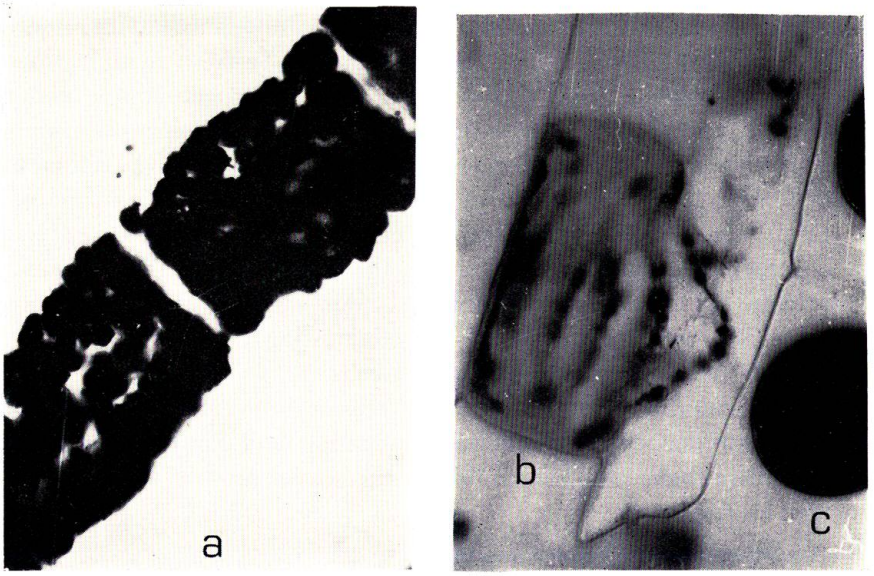

Fig. 2. Starch granules in swelling, conjugating, and zygote cells. Starch was detected by iodo-starch reaction. a, slightly swelled cells; b, conjugation tube-formed cells which missed an opportunity to mate; c, new zygote.

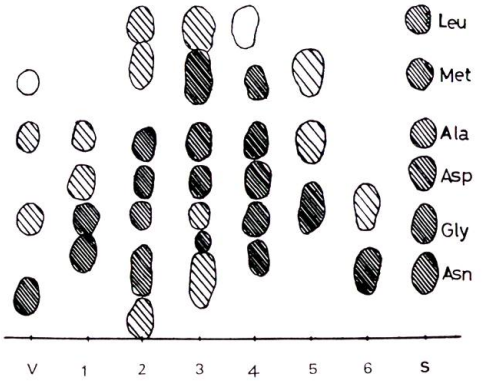

Fig. 3. Paper chromatograms of free amino acids in vegetative cells and in naturally conjugating cells. Asn, asparagine; Asp, aspartic acid; Ala, alanine; Gly, glycine; Leu, leucine; Met, methionine. ▨, large amounts; ▨, medium amounts; ○, small amounts. Cell stages: V, vegetative cells; 1, pairing cells; 2, swelling cells; 3, budding and conjugation tube-forming cells; 4, conjugation tube-connected cells; 5, fusing gametes; 6, new zygote; S, standard.

energy source during the mating process. Conjugation-missed pairing, swelling and conjugating cells could not accumulate large amount of starch (Fig. 2). Starch decreased in aged zygotes.

Total nitrogen contents decreased while soluble amino acids, mainly alanine, aspartate, asparagine, glycine, leucine, and methionine, increased during the natural (Table 2, Fig. 3) and artificial (Table 3) mating processes. These results suggested that the depletion of the nitrogen source under the light induces partial degradation of preexisting intracellular nitrogen compounds, mainly protein, and that such degradation can induce repression of vegetative growth. The ratios of carbohydrates to nitrogen compounds (C/N ratio) was higher in conjugating cells and in newly formed zygotes than in vegetative cells, as a result of the increase of carbohydrates and the decrease of nitrogen compounds. Under intracellular conditions that produce a high C/N ratio as shown in conjugating cells, vegetatively growing cells may can change to gametangial cells.

A more detailed clarification of the reason for induction of the sexual process by nitrogen depletion under illumination and for changes in carbohydrates and nitrogen compounds during the sexual process will be attempted in further studies.

References

CZURDA, V. 1933. Experimentelle Analyse der kopulations-auslösenden Bedingungen bei Mikroorganismen. Beihefte. z. Bot. Cent. **51**: 711–762.

FOESTER, H. 1957. Das Wirkungsspectrum der Kopulation von *Chlamydomonus eugametos*. Z. Naturf. **12 b**: 765–770.

ICHIMURA, T. 1971. Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium*. Proc. VIIth Inter. Seaweed Symp. 208–214.

LEWIN, R. A. 1954. Sex in unicellular algae. *In*: D. WENRICH ed., Sex in microorganisms. pp. 110–133. Amer. Assoc. Adv. Sci., Washington.

LEWIN, R. A. 1956. Control of sexual activity in *Chlamydomonus* by light. J. Gen. Microbiol. **15**: 170–185.

NICOLAI, E. & PRESTON, R. D. 1952. Cell-wall studies in the Chlorophyceae. I. A general survey of submicroscopic structure in filamentous species. Proc. Roy. Soc. B. **140**: 244–274.

SAGER, R. & GRANICK, S. 1954. Nutritional control of sexuality in *Chlamydomonus reinhardi*. J. Gen. Physiol. **37**: 729–742.

SASAKI, K. 1977. Changes in respiratory and photochemical activities during the conjugation process of *Spirogyra*. J. Fac. Sci., Hokkaido Univ., V, **10**: 209–218.

SASAKI, K. & TAKAYA, K. 1972. Suppression of nucleic acid synthesis in mitochondrial and chloroplast fractions of *Spirogyra* during the conjugation process. Plant & Cell Physiol. **13**: 737–740.

SASAKI, K., TAKAYA, K. & ONODA, T. 1966. Metabolism of *Spirogyra* during the conjugation process. Nature **200**: 1042.

SASAKI, K., TAKAYA, K. & UENO, T. 1972. Suppression of nucleic acid synthesis in chromatin of *Spirogyra* during the conjugation process. Plant & Cell Physiol **13**: 601-608.

UENO, T. & SASAKI, K. 1978. Light dependency of mating process in *Closterium acerosum*. Plant & Cell Physiol. **19**: 245-252.

UENO, T. & SASAKI, K. 1978. Synchronous sexual cell division in L_{200}-cells of *Closterium acerosum*. J. Fac. Sci., Hokkaido Univ., V, **11**: 218-224.

YAMASHITA, T., TAKAHASHI, S. & SASAKI, K. 1968. Biochemical, histochemical, and structural changes during conjugation process of *Spirogyra*. Bot. Mag., Tokyo., **81**: 210-218.