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LIGHT-DEPENDENT MORPHOGENESIS OF UNICELLULAR STAGES IN SYNCHRONIZED CULTURES OF SCENEDESMUS QUADRICAUDA (TURP.) BRÉB. (CHLOROPHYCEAE)

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SUMMARY

Cells of *Scenedesmus quadricauda* are synchronized by light-dark cycles. During the photoperiod they build up the capacity to divide. First division into 2- and 4-celled coenobia is induced, then during the second half of the photoperiod the induction of division into 8 unicells takes place. Division itself and the subsequent liberation of daughter cells occur in the dark period.

By giving a definite photoperiod the formation of either coenobia or unicellular stages is determined. The formation of both coenobia and unicells is followed using a light microscope. In both cases only the pattern of cytokinesis is similar. After cytokinesis the unicells become ovoid in shape and form two spines at each pole. They are released from the parental wall as separate cells.

The occurrence of both unicellular and coenobial stages of *Scenedesmus* in nature as well as under artificial conditions has been clearly established (TRAINOR & HILTON 1963, SWALE 1967). This morphological variability has its taxonomic implications, as the unicells could be confused with members of the genus *Chodatella* (FOTT 1968).

Apparently nutrients have a role in determining whether unicells or coenobia arise. SHUBERT & TRAINOR (1974) proved that the phosphorus concentration governs the production of unicells. We call attention for the role of light in this respect. By selecting a definite photoperiod in synchronized cultures of *S. quadricauda* we are able to induce the development of either one or the other form.

The clone of *S. quadricauda* was isolated from Tjeukemeer (Friesland, the Netherlands) in May 1974 and kindly placed at our disposal by Mrs. Marianne Blaauboer. Cultures are grown in a simple mineral medium in 1000 ml glass vessels at 30 °C and continuously stirred and aerated with 400 ml air minute^{-1} enriched with 3.0 vol% of CO_2. Synchronization is achieved by exposing the cultures to light-dark changes of 14 hours of light and 10 hours of darkness (LD:14,10). A light intensity of 1.5×10^5erg cm^{-2}sec^{-1} from fluorescent tubes (Philips TL 33) is used.

When permanent synchronization is required the cultures are diluted daily to a standard cell number with fresh nutrient medium (STEENBERGEN 1968). In a synchronized culture all cells pass through the same stages of the life cycle

Table 1. Percentage of unicells, 2-celled, 4-celled and 8-celled coenobia in a synchronized culture of *S. quadricauda* at the start of 4 successive LD-cycles. At the start of the third LD-cycle the culture is diluted for the first time and the LD regime is shifted from LD:14,10 to LD:11,13

	unicells	2-celled	4-celled	8-celled
cycle 1 (inoculum)	0.2	8.4	90.4	1.0
cycle 2	6.2	19.0	69.9*	
cycle 3	62.8		1.8	35.4
cycle 4	97.2	0.1	1.2	1.5

* 4.9% of the population in the second cycle consisted of mothercells just before liberation of daughter cells.

more or less simultaneously. Trophic processes are restricted to the photoperiod, whereas cytokinesis and release of daughter cells occurs in the dark (cf. SETLIK et al. 1972). The inoculum, which is maintained at 15 °C under a LD:14,10 regime and a light intensity of 0.05×10^5 erg $cm^{-2}sec^{-1}$, contains 2- and 4-celled coenobia. In the first and second LD-cycles after the inoculation unicells and 2-, 4- and 8-celled coenobia are produced (see *table 1*). At the start of the third LD-cycle the synchronized culture is diluted for the first time and the cell density is adjusted to c. 2×10^4 cells ml^{-1}. If in the third cycle, instead of the usual LD:14,10 regime, a regime of LD:11,13 is used predominantly unicells are formed *(table 1)*. The unicells are elliptical or oval and possess two spines at each pole, occasionally a unicell bearing one or two additional spines is seen (*fig. 2* D). The ratio length to width of young unicells is c. 1.4, whereas the coenobial cells are more elongated, the young ones being 2-2.3 times longer than wide. The unicells show remarkable similarity to the *Chodatella*-like cells described by SWALE (1967) and FOTT (1968).

The life cycle of *S. quadricauda* cells during a unicell-yielding LD:11,13 cycle is studied in more detail (see *fig. 1* A). Young cells, produced in the previous LD-cycle, start their life cycle at the onset of light. After a certain photoperiod some cells have grown sufficiently to be able to divide after being transferred to darkness. First a few but gradually, at a given photoperiod, all cells have attained the capacity to divide. The period of the life cycle during which the cells attain the capacity to divide is determined in the following manner. Starting at the third hour of the LD-cycle hourly samples are removed from the parent culture and incubated in the dark at 30 °C until the induced divisions are terminated. Then the division products i.e. young 2- and 4-celled coenobia and unicells are recorded separately in the samples and their percentage in the population is plotted against the respective sampling times. By this method the curves (a), (b) and (c) in *fig. 1* A are composed. They indicate how a progressively increasing proportion of the cell population first is able to divide into 2-celled coenobia, then into 4-celled coenobia and finally into unicells. It is clear from the position of the curves (a), (b) and (c) in *fig. 1* A that if the photoperiod is interrupted at the sixth hour of the LD-cycle (i.e. a LD:6,18 regime) the cells are able to pro-

duce mainly 2- and 4-celled coenobia. This case is represented in *fig. 1* B.

Unicells of *Scenedesmus* arise from coenobial cells and vice versa. Using non-synchronized cultures SWALE (1967) could not follow the cell division in detail due to a shortage of intermediate stages. Synchronized cultures are most suit-

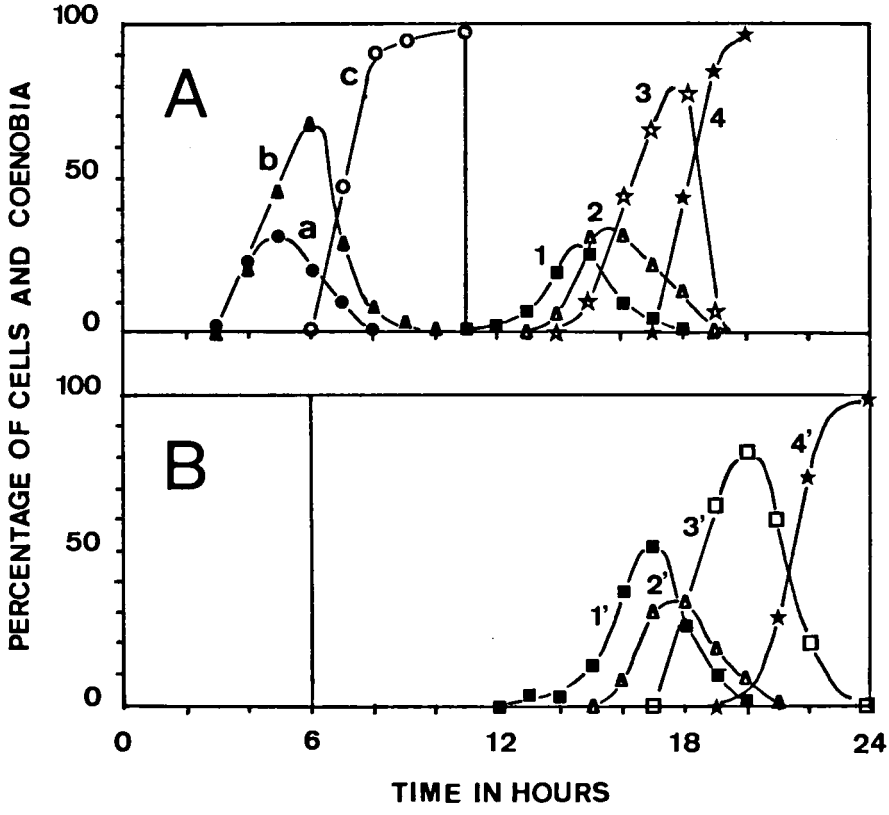

Fig. 1 A. Third LD-cycle after inoculation of a synchronized culture of *S. quadricauda* under a LD:11,13 regime yielding 97% unicells. In the curves (a), (b) and (c) the duration of exposure to light is plotted against the percentage of respectively young 2-celled and 4-celled coenobia and unicells in samples which have been incubated in the dark (see text). Therefore the curves represent the course of the capacity of the synchronized cells to produce the successive stages. In the curves 1, 2 and 3 the progress in time is plotted against the percentage of cells in the population which have completed respectively the primary and secondary cleavage and the arrangement of daugther cells just before emergence. Curve 4 indicates the percentage of unicells in the total cell population during the emergence.

Fig. 1 B. Third LD-cycle after inoculation of a synchronized culture of *S. quadricauda* under a LD:6,18 regime yielding a population consisting of 2% unicells, 14.3% 2-celled coenobia, 80.3% 4-celled coenobia, 2.2% 8-celled coenobia, 1.2% undivided cells. The curves 1′, 2′ and 3′ indicate the course of respectively the completion of the primary and secondary cleavage and the arrangement of daughter cells after their elongation. Curve 4′ indicates the course of the liberation of the young coenobia. As no secondary cleavage occurs in mothercells yielding 2-celled coenobia curve 2′ refers only to 82.5% of the population.

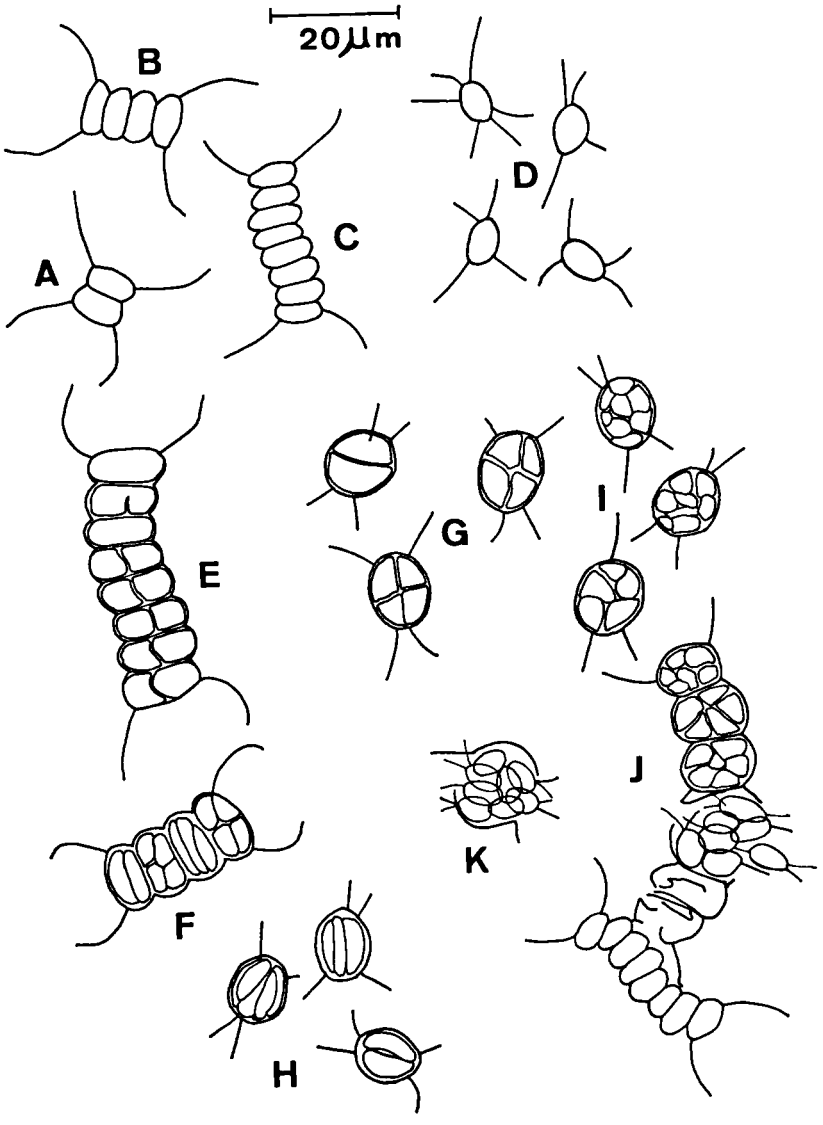

Fig. 2. Forms of *S. quadricauda* occurring in synchronized cultures. A-D, young coenobia and unicells; E-K, division stages of unicells and coenobial cells; E, first cleavage; F, second cleavage and arrangement of elongated daughter cells; G, first and second cleavage; H, arrangement of elongated daughter cells; I, arrangement of ovoid daughter cells; J, arrangement of ovoid daughter cells and emergence of mature unicellular daughter cells, furthermore emergence of 8-celled coenobium; K, Unicellular daughter cells just before their emergence. (Camera lucida drawings with a Zeiss Standard RA microscope, phase contrast, 800 ×).

able to study this problem. At the twelfth hour of the LD-cycle the first signs of cell division become visible. Then in hourly samples the percentages of division stages are determined. The results are plotted in *fig. 1* A and *fig. 1* B. The first cleavage is transverse (*fig. 2* E, G) and the second cleavage is longitudinal (*fig. 2* F, G) in cycles which yield either unicells (*fig. 1* A, curves 1, 2) or coenobia (*fig. 1* B, curves *1'*, 2'). Usually 8 unicells per mother cell are produced, so a third cleavage occurs which, however, could not be clearly followed. After cytokinesis in the unicell-yielding cycle the daugther cells become ovoid in shape. They are arranged within the parental wall more or less radially as shown in *fig. 2* I, J (see *fig. 1* A, curve 3). Contrary to the observations of FOTT (1968) that coenobia after being liberated disintegrate into unicells which then develope into *Chodatella*-like stages, we observed that the unicells emerge from the parental wall as separate cells bearing spines (*fig. 2* J, K). After cytokinesis in the coenobia-yielding cycle the daughter cells elongate and become arranged with their axis parallel to the axis of the mother cell (*fig. 2* F, H and *fig. 1* B, curve 3) (see SMITH 1914). Afterwards the daughter cells adhere to one another at specific sites and cell-wall layers and spines are formed (PICKETT-HEAPS & STAEHELIN 1975). Young coenobia emerge, flatten out and unfold their spines within a few seconds.

Having thus shown that the formation of unicells is light-dependent further experiments on the effect of intensity and amount of light are in progress. At the relatively low light intensity of 0.19×10^5erg $cm^{-2}sec^{-1}$ and under a LD: 14,10 regime unicells (c. 94%) arise in synchronized cultures of *S. quadricauda*. At this light intensity the growth rate of the cells is greatly reduced and consequently the curves indicating the division capacity of the cells are postponed (cf. SETLIK et al. 1972). They occur between the seventh and thirteenth hour of the LD-cycle. Our tentative conclusion is that the production of unicells is linked to the trophic effect of light rather than to a specific photochemical process.

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