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Do Volvocal Algae Form Their Cultures as Autonomous Systems?

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Abstract. A low reproducibility of some characteristics in the life cycle of the volvocal alga *Chlamydomonas geitleri* was observed in repeated experiments. The sets of the cultures were very similar in the number of the studied characteristics, but some of them differed significantly in one or several characteristics whereas the others agreed in a whole set. This is documented by the growth in a nitrogenless medium, the formation of zygotes, and their maturation and germination. The results of a small representative set of experiments were treated by an analysis of variance. Some possible explanations are discussed.

Unexpected responses are sometimes met within repeated experiments with populations of microorganisms. They are often difficult to explain, especially if the cultures grew parallel with several other cultures responding according to expectation. This may concern one, several or all of the followed characteristics of a culture.

Several hypotheses may be taken into account, but which of them will satisfy the given case is very difficult to predict, if no additional information is available. At least some of them are:

1. An unregistered induction impulse by a short-term change of an environmental factor concerning only some of the compared cultures causes an experimental artifact. If we do not know this, we cannot use it for an explanation and exclude such a culture from the experiment.
2. Such an unregistered environmental impulse may cause chaos of a certain degree in one or some of the populations. The spontaneous settlement of this chaos in the affected population is the less regular, the lower the degree of chaos induced (TOMITA 1982, VANDERMEER 1982).
3. The unexpected behaviour of the population may also be caused by spontaneous or induced mutations (SIMCHEN 1978, VAN WINKLE-SWIFT and BAUER 1982, NEČAS *et al.* 1983).
4. The phylogenetically conditioned inclination to such autonomous behaviour cannot be excluded either (especially in some *Volvocales*). The genetic drift obviously may play an important role here.

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5. The culture may represent a cryptic autonomous population which sometimes suddenly responds in a different way to the same conditions in which the cultures under comparison are growing. However, the behaviour of most such cultures may differ only moderately. These differences then may fall into the corresponding experimental error. Such a cryptic autonomy might be the case of the volvocal alga dealt with in this paper.

Unicellular and colonial types occur in volvocal algae. In nature, a clear-cut border line can only seldom be drawn between those types which are found either in colonies or as unicells (KIKUCHI 1978, NAKAMURA *et al.* 1978).

Many peculiarities of the cultures of *Volvocales* my be expected from this fact (FRITSCH 1965, HOEK 1978, ETTL 1980). Taking all this into account, I am of the opinion that at least some of the cases in which the cultures of volvocal algae behave quite differently, might be explained on the basis of this fact.

Nevertheless, similar phenomena in the behaviour of the populations of chlorococcal algae, as regards the responses to stress conditions caused by various mutagens, were noted some time ago (NEČAS 1973, 1979a). The present paper contains some cases of surprisingly different results, which were obtained in the very carefully repeated experiments and, therefore, a simple experimental artifact is excluded,

MATERIAL AND METHODS

Material

The homothallic isogamous volvocal alga *Chlamydomonas geitleri* ETTL strain 1966/3 was chosen for the present work. Two clone cultures from zygote progenies 2 and 9 were prepared for this purpose. Clone cultures 2/2 and 9/5 were recloned once more as 2/2 I, 2/2 II and 9/5 I, 9/5 II. They were maintained in our collection of algal strains on slants under daylight and laboratory conditions. The mineral medium according to ZACHLEDER and ŠETLÍK (1982) with 1/2 concentration of nitrogen was solidified by 2% of agar. The agar cultures were many times reinoculated in order to maintain their good physiological state. Afterwards no further recloning was performed.

Methods

The experimental cultures were started by transferring the agar cultures to the same liquid medium as mentioned above. They were adapted to nearly optimal conditions (TETÍK and NEČAS 1977) during approximately five cell cycles (temperature 23 °C, irradiance 52 W m^{-2} PhAR, pH *ca.* 6.5, flow of 100 l h^{-1} air containing 4% of CO_2 passing through 4 cultivation vessels of 0.5 l volume). A laboratory cultivation device equipped with plan-parallel vessels of 4 cm width, was used. The cultures grew in continuous light and were bubbled through by the mentioned gas mixture.

The linear phase of growth in the culture was maintained by daily dilution during the adaptation. In order to induce gametogenesis, the culture was centrifuged and washed once with distilled water and transferred then to a nitrogenless medium. The washed cells were resuspended in such a volume of the nitrogenless medium as to reach the required absorbance.

The growth of the culture was expressed by absorbance (750 nm) and by the number of cells per volume unit (Bürker's chamber). The formation of the zygotes was expressed by their frequency from 300 to 500 vegetative cells and gametes. The sample of the culture for this determination was fixed by Lugol's solution and before counting the zygotes differentiated by sodium hydrogensulfite so that only the zygotes maintained their colour. The zygotes were separated from the other cells either by chloroform vapour or by a three-day passage through distilled water. This killed the vegetative cells and gametes. After separation, the zygotes were left to mature at 23 °C in the dark either on the surface of an unlimited agar medium or in the same liquid medium for three weeks. Then they were transferred to the cultivation device (NEČAS 1979b) and made to germinate under continuous light at 23 °C and 20 W m^{-2} PhAR. The germination was followed for five days and expressed by the maximum frequency of the germinated zygotes and the dynamics of their daily increments.

RESULTS

Culture Growth and Formation of Zygotes

The parallel cultures started and further on were growing at very similar absorbance. Under mutual comparison of the experiments (Fig. 1A), the starting numbers of the cells per volume unit were less homogeneous (Fig. 1B). Even small differences in the starting cell numbers could become more

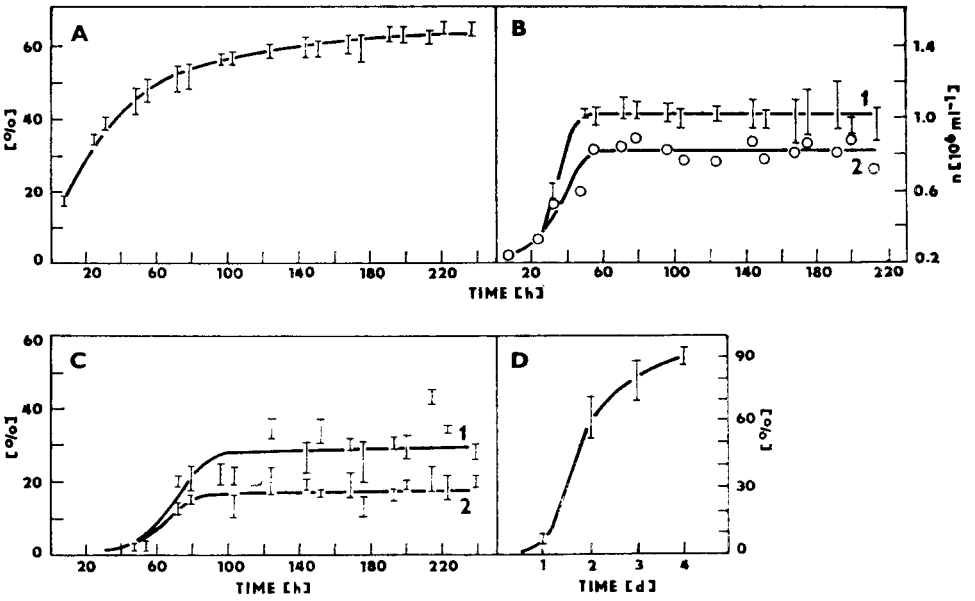

Fig. 1. Concordance and discordance in the responses of a set of four parallel growing cultures.

A — all cultures agree in absorbance; B — one culture differs in growth and homeostasis level (2); C — two cultures differ in zygote formation (2); D — all cultures agree in zygote germination. The bars on the curves represent the range of measured values (in all Figs.).

significantly pronounced during two or three days of growth in the nitrogenless medium (Fig. 1B). The differences, indeed, in no case reached such a level that the dependence of the zygote formation on the culture density was a deciding factor. The pronounced dependence of zygote formation on the culture density is convincing only if the same culture is divided into several parts that sufficiently differ in the starting absorbance (NEČAS 1981).

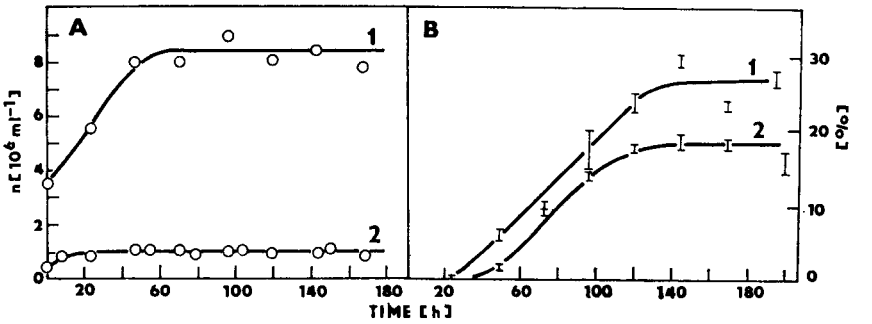

Fig. 2. Variability of the growth and zygote formation in a set of ten cultures growing under the same conditions but not simultaneously and not from the same starting absorbance.
A — fan of the growth curves framed by those of two extreme cultures; B — curve of the zygote formation in two extreme cultures from A (1) and curve of the average frequencies of zygotes in ten cultures of the clones 2/2 I, II and 9/5 I, II (2).

A random set of the cultures growing under the same environmental conditions (but not simultaneously), which were transferred to a nitrogenless medium at various absorbance, displayed a wide fan of growth curves (Fig. 2A). It was also possible to observe on the curves a wide range of slopes and progresses to the homeostatic equilibrium as well as different levels of its stabilization. But the frequency curves of the formed zygotes, corresponding to these growth curves, did not fulfil our expectation with regard to the divergence of the curves (Fig. 2B). The cultures, rather different in their growth, showed very similar frequencies of the zygotes (Table 1). This also gives evidence that it is not only the growth in a nitrogenless medium, but also the transition to homeostasis and the yields of zygotes that are not

TABLE 1
Some characteristics of the life cycle of the clone 2/2 in its several cultures chosen by chance

Culture number	Cell number n [10^6 ml^{-1}]		Zygote frequencies [%]		Zygote germination+ [% after 5 d]
	start	homeostat. equilibrium	maximum	homeostat. equilibrium	
1	4.18	10.03	22.59	17.22	91.38
2	0.96	1.11	21.73	20.63	76.47
3	0.68	1.08	26.01	20.13	78.00
4	1.10	1.26	23.53	20.73	92.98
5	1.17	1.21	22.67	18.65	88.98

+ Maturation three weeks.

TABLE 2
The average characteristics of the zygote formation and their germination of two compared clones in ten cultures chosen by chance

Clone	Zygote frequencies [% homeostat. equilibr.]	Zygote germination+ [% after 5 d]
2/2	19.52	90.39
9/5	21.68	89.07

+ Maturation three weeks.

conditioned by the environment only. Endogenous factors in the culture, besides the inherited ones, are apparently important for the behaviour of the cultures. The set of the experiments presented showed that the share of the inherited factors in the whole variability was almost negligible because of the genetic similarity of both clones under comparison (Tables 2, 3).

The triggering of the expression of the autonomy in the culture behaviour as the population system is not yet known in detail. Such an inner regulation of the culture may manifest itself in a single or several characteristics. Fig. 3B indicates that one culture behaved quite differently in the zygote formation in comparison with the other three cultures which grew under the same conditions.

Maturation and Germination of Zygotes

Different autonomous responses in the mentioned characteristics were recorded, though the manifestation of the other characteristics agreed in all cultures under comparison. The growth of the compared cultures was concordant, but the formation of zygotes did not correspond to expectation (Fig. 4A, B). The different responses of the cultures to the procedure of zygote separation (Fig. 4C, D) could be caused by the mentioned autonomy of the cell populations in individual cultures. As the whole curves of the

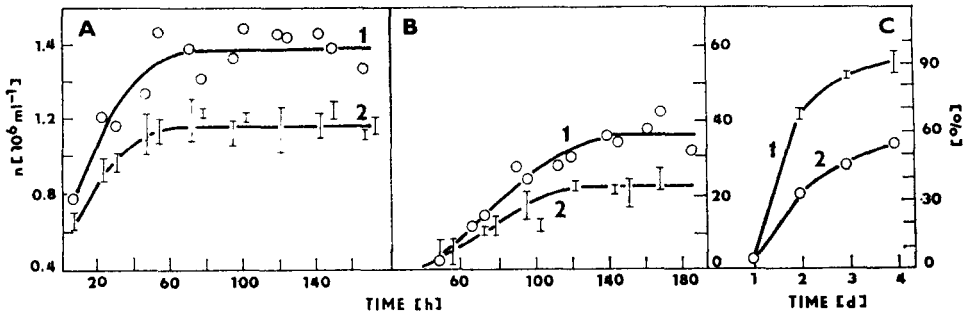

Fig. 3. Concordance and discordance in the responses of a set of four cultures growing under the same conditions but not simultaneously.
A — one culture differs in growth and homeostasis level (2); B — one culture differs in zygote formation (2); C — zygotes of one culture differ significantly in germination (2).

zygote germination kinetics were significantly shifted (Fig. 4C, D), the response of the culture has to be considered as a whole from the beginning of germination. No such surprising differences between the same above mentioned cultures were noted in the germination of zygotes, if both matured in a liquid medium containing nitrogen (Fig. 4E). As the significant influence

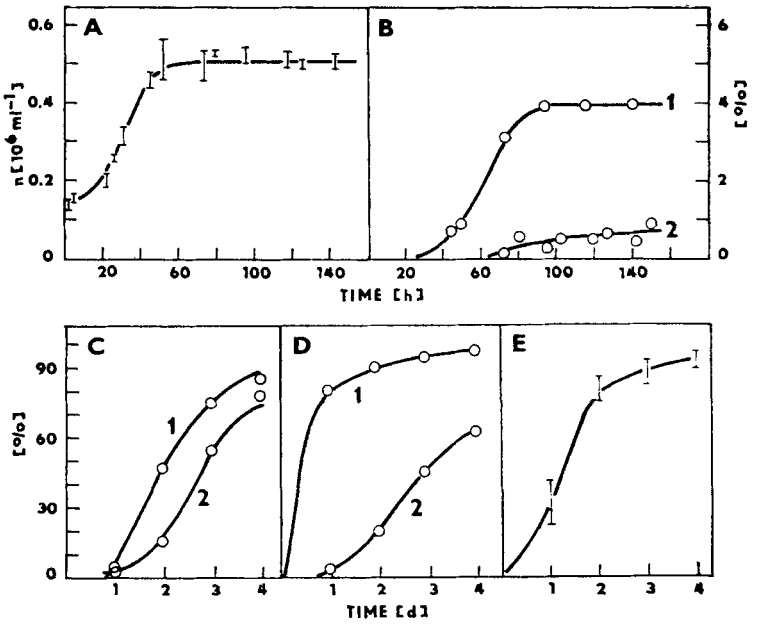

Fig. 4. Concordance and discordance in the responses of two cultures growing under the same conditions but not simultaneously.

A — both cultures agree in growth and homeostasis level; B — both cultures differ significantly in zygote formation (1, 2); C — zygotes from both cultures differ in germination after treatment by chloroform vapour (1, 2); D — zygotes from both cultures differ in germination after treatment by distilled water (1, 2); E = zygotes from both cultures germinated concordantly after maturation in a liquid medium.

of the maturation and germination conditions for the zygotes of *Chlamydomonas reinhardii* on the behaviour of its organellar genetics systems has been convincingly proved (SEARS 1980), we must also take this fact into consideration in our *Chlamydomonas geitleri*.

Statistical Analysis

To achieve a clearer picture of the effect on the whole variability of results caused by repeatedly prepared experimental culture from the same collection culture, a representative set of such cultures was used for an analysis of variability in the studied characteristics. The analysis of variance (Table 3) indicated a very significant share of the repeated culture preparation in the whole variability. The methodical component of the whole variability was either sufficiently low or at least statistically insignificant. So the significance of the share of the repeated culture preparation in the whole variability was unambiguous. In agreement with the preceding comparisons, the less significant influence of the inheritance of the characteristics followed (genetic

TABLE 3
Values of the F-test from the analyses of variance of several life cycle characteristics in a representative set of 12 cultures

Variability caused by	Cell number start	Cell number homeostat. equilibrium	Zygote frequencies	Zygote germination
Main factors:				
Clones	0.64	0.55	18.26	31.55^{++}
Experiments	153.31^{++}	99.18^{++}	38.59^{+}	57.76^{++}
Replications of the exper.	3.45	0.36	7.25	18.82^{++}
Replications of plates	—	—	—	0.89
Interactions				
Clone × exper.	1.96	1.95	18.52	23.87^{++}
Clone × repl.	0.17	0.17	3.42	0.17
Exper. × repl.	3.06	3.06	1.33	4.75^{+}
Exper. × repl. of plates	—	—	—	0.91

$^{++}$ = significance of $P = 0.01$; $^{+}$ = significance of $P = 0.05$.

component) on the whole variability was confirmed. Only those interaction components of the whole variability were significant, in which the repeated culture preparation was involved. As regards the zygote germination, the mutual ratios of the appropriate F-tests must be first compared for this characteristic and only then with the other characteristics.

DISCUSSION

The low reproducibility of the life-cycle characteristics in our *Chlamydomonas geitleri* made it difficult to prove their genetic basis (NEČAS and PAVINGEROVÁ 1980). To get to the bottom of this low reproducibility of the results in the replicated experiments, we have chosen our algal species whose culture could form an autonomous system. This system mostly follows in the cell-cycle characteristics the known regularities in the behaviour of the cultures under influence of the environment (TETÍK and NEČAS 1977). Yet sometimes we may observe unexpected responses that correspond to an apparent autonomy of the culture system. This seems to contradict the known experience that sufficiently numerous populations of microorganisms repeatedly form cultures consistent in most of the characteristics under unchanged conditions (growth of the culture, biomass production *etc.*). Inconsistent cultures are usually excluded from the experiments, despite the possible cause of their different behaviour.

The life cycle of our *Chlamydomonas geitleri* differs from the life cycle of the more common *Chlamydomonas reinhardii* (NEČAS and PAVINGEROVÁ 1980). A support for the cryptic autonomy of the cell cultures of our alga may also be its ability to form homeostatic cultures (CRAM 1980) in a nitrogenless medium under the induction of gametogenesis (NEČAS and TETÍK 1981). Since endogenous regulations were noted in the cultures of chlorococcal algae under stress conditions (NEČAS 1973, 1979a), they can still more frequently be encountered in the volvocal algae, especially so far as concerns their life cycle. VAN WINKLE-SWIFT and BAUER (1982) have shown that the populations of homothallic volvocal algae may not only contain pure homothallic

clones, but also some conditionally asexual ones. Rarely, self-sterile clones may also be present as mutation products. The hormonal regulation of the behaviour of volvocal algae must also be taken into account, if the life-cycle characteristics are to be studied (KOCHERT 1978, WIESE and WIESE 1978, KOCHERT and CRUMP 1979, DARDEN 1980, MATSUDA 1980). The cases serving for examples in this paper have not yet been analysed in all details, nevertheless, they document well those which may be encountered more frequently.

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