CLONAL AGE AND THE PROPORTION OF DEFECTIVE PROGENY AFTER AUTOGAMY IN *PARAMECIUM AURELIA*

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ABSTRACT

The relation of mortality and the proportion of progeny with reduced fission after autogamy to the clonal age in *Paramecium aurelia* was investigated. This relation is not linear but the proportion of defective progeny increases stepwise. The observations are in agreement with those expected from the calculations of the number of deleterious mutations in the micronucleus.

THE modern concept of clonal aging in paramecium was established in SONNEBORN (1954). In *P. aurelia*, for example, a clone is destined to perish after about 200–300 fissions with daily reisolation culture without fertilization, and the clone decreases in fission rate, in the frequency of survivals at autogamy and in the ability to yield phenotypically normal daughter cells with the increase of age. These phenomena have been considered to be the results of the accumulation of deleterious events in the nucleic or in the cytoplasm (RAFFEL 1932; MITCHISON 1955; FUKUSHIMA 1974) or as programmed process subsequent to the development of the zygote (SIEGEL 1967).

In a previous paper, the author (FUKUSHIMA 1974) showed that X-irradiation reduced the clonal life-span and the fission rate and suggested that deleterious mutations accumulated in the macronucleus might be responsible for the limited life-span of a clone. In the present experiment, the relation of the proportion of defective progeny after autogamy to the clonal age was investigated in relatively younger stages during which the clone still maintained a high fission rate. The results showed that the aging described by the increase of the mortality at autogamy proceeded randomly rather than regularly among the isogenic lines and proceeded stepwise rather than linearly.

MATERIALS AND METHODS

Stock 51 of *Paramecium aurelia* (syngen 4), which had undergone repeated autogamy until the mortality after autogamy was zero, was used.

An exautogamous animal was maintained in daily reisolation culture for 3 days. Seven cells were randomly isolated from the population of the 3rd day and each of them was maintained in multiple daily reisolation culture to ensure that the line continued vegetative reproduction. These seven lines were designated as lines A–G.

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For the induction of autogamy, the remaining cells after an isolation were allowed to divide 8-9 times and then fully starved. More than 20 cells from the resulting population, which was called the surplus culture, were stained with acetocarmine fast green (DIPPELL 1955) to detect the presence of the fragmented macronuclei characteristic of autogamy.

For testing survival after autogamy, fourteen, forty or eighty animals from the surplus culture (autogamous culture) were isolated into a new culture medium sufficient for 10 fissions. The exautogamous cells that exhausted the culture medium within 3 days were judged as fully viable (N). Those that did not exhaust the medium but were still dividing on the 4th day were judged as reduced viable (R). Those that did not divide at all or stopped dividing for 4 days were judged as nonviable (D).

The general techniques were the same as those described by SONNEBORN (1950, 1970) with the exception of the culture medium (MIYAKE 1968). All the procedures in the present experiment were carried out at 25°.

RESULTS AND DISCUSSION

Lines A, B, C, D and E were respectively tested for mortality after autogamy at the ages of 48, 115, 130, 139 and 196 fissions. Line F was tested for mortality at the age of 45 fissions and at the age of 57 fissions. Line G was divided into two sister lines at the age of 41 fissions. One was tested for mortality at the ages of 49, 141 and 166 fissions, and the other at the age of 112 fissions. All the lines except B and E maintained high fission rates of over 3 fissions per day until they were induced to undergo autogamy. The frequency of autogamy was always 100% in the present study.

The results of mortality tests are shown in Figure 1. In these data three points are worthy of notice. (1) Defective progeny after autogamy increased stepwise

FIGURE 1.—Diagram of the experimental design and results. The results of the tests for survival after autogamy at the fission ages shown in parentheses are indicated by the numbers of practically observed exautogamous animals; N: fully viable, R: reduced viable, D: nonviable.

(see D, F and G). (2) Such increases are observed even in the young stage of cell lines (F and G). On the other hand, line C was fully viable even after one hundred fissions. (3) The increased proportion of defective progeny was maintained in the same line through several divisions (F and G).

The most likely mechanism for these phenomena is the accumulation of mutations in the micronuclei. If we assume the increase in the proportion of defective progeny after autogamy is caused by mutations in the micronuclei, the theoretical proportion of such progeny increases stepwise first 25% (one mutation), then to 37.5% or 50% (two mutations), and then to 44%, 50%, 62.5% or 75% (three mutations) and so on because *Paramecium aurelia* has two diploid micronuclei and autogamy renders the genes at all loci homozygous. Figure 2 briefly shows the theoretical relation between the combination of chromosomes and the frequency of defective progeny after autogamy. The observed results are not inconsistent with this theory, as mentioned below.

In lines A and C that were induced to undergo autogamy at the age of 48 and 130 fissions, all the exautogamous animals were viable. In these lines no mutation which affected the viability of autogamous progeny had occurred in the micronuclei.

FIGURE 2.—The combination of chromosomes and the frequency of defective progeny at autogamy. Two micronuclei of an animal are represented by two neighboring circles. Three pairs of homologous chromosomes are represented. Dots on chromosomes show mutations. As the number of mutations increases from one (the upper row) to three (the bottom row), the frequency of defective progeny increases from 25% to 75% (maximum value). The number shown under micronuclei indicates the theoretical percentage of defective progeny after autogamy in the respective case.

In line B, 7.5% of the autogamous progeny at the age of 115 fissions had reduced vigor. In this line a mutation which reduced the fission rate could have occurred not in the previous isolation cultures but in the final culture in which autogamy was induced.

In line G, 15%, 21% and 22.5% of the progeny had reduced fission rates at 29, 112 and 141 fissions, respectively. In this line a mutation which reduced fission rate after autogamy had perhaps occurred before 38 fissions. Two sister lines preserved this mutation for scores of fissions. In the line in which the proportion of progeny with reduced viability increased to 52.5%, one more mutation perhaps occurred in the other micronucleus or on the homologous chromosome in the same micronucleus in which the previous mutation had occurred.

In line D, a stepwise increase of defective progeny was not clearly shown because autogamy was induced only once. It is, however, reasonable to postulate that three mutations accumulated, that is, a mutation in one micronucleus and the other two on a pair of homologous chromosomes in the other micronucleus.

In line F, 86% of the progeny died at 45 fissions and 71% at 57 fissions. In this line three lethal mutations could have occurred at some time before it divided 39 times. One of them could have occurred in one micronucleus and the other two could have occurred on a pair of homologous chromosomes in the other micronucleus. Although it was not shown in Figure 1, when an animal from the surviving progeny at the age of 57 fissions was maintained in daily reisolation culture and induced to undergo autogamy again, 100% of the progeny were viable. This means that the lethal mutations in the micronuclei were excluded at autogamy.

In line E, more than six mutations could have occurred before autogamy. Because autogamy was induced only once and the combination of deleterious and normal chromosomes becomes very complex as mutations accumulate, we cannot simply identify the set of chromosomes from the datum.

The chi-squares for all the interpretations of the tests for survival after autogamy were summed. The difference between the observed results and the theoretical values with 11 degrees of freedom was not significant ($\chi^2 = 3.96$, $0.95 < p < 0.98$). Macronuclear regeneration was not checked in this experiment. This may make the frequency of defective progeny observed unreliable. The frequency of macronuclear regeneration, however, is usually under several percent in relatively young clones and the genetic interpretation in this study might not be damaged seriously.

RAFFEL (1932) first suggested that mutations in the micronucleus were responsible for the decrease in survival at fertilization. Unfortunately the relation between mortality and occurrence of mutations was not properly managed in his report. It is more difficult to estimate the occurrence of recessive lethal mutations from the mortality after conjugation than to estimate it from the mortality after autogamy.

PIERSON (1938) reported that the mortality at autogamy was directly proportional to the preceding interval without autogamy. The data that she reported

were taken from the average of several lines. As discussed above, the changes of mortality within each cell line seem more important than averages.

MITCHISON (1955) concluded that mutations in the micronuclei are unlikely to be the sole cause of death after fertilization and attached great importance to the effects of cytoplasm and the macronucleus. The present experiment, however, suggests an effect of micronuclei on the changes of mortality after autogamy in clonal aging.

In old age, mortality after fertilization may be affected by deleterious cytoplasm. For example, the accumulation of mutations in the macronucleus (FUKUSHIMA 1974) is postulated to affect mortality after fertilization because a deleterious macronucleus results in deleterious cytoplasm. SONNEBORN and SCHNELLER (1960) actually reported the influence of cytoplasm of old cells at conjugation.

The author emphasizes that mutations in the micronucleus may play an important part in the mortality after autogamy and the increase of mortality with age appears to be mainly caused by mutations in the micronucleus. To prove this hypothesis, conjugation experiments between animals with the postulated recessive lethal mutations and fully viable animals should be carried out.

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