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Variability of Autogamy-Maturation Pattern in Genetically Identical Populations of *Paramecium tetraurelia*

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ABSTRACT—Autogamy in *Paramecium tetraurelia* is a form of sexual reproduction in a single cell that results in homozygosity in every genetic locus. Autogamy becomes inducible by natural starvation several fissions after the previous autogamy, and percent autogamy increases gradually with clonal age to reach 100%. We here report the degree of variability of the autogamy-maturation pattern, and how it is inherited through autogamous generations. We assessed the autogamy-maturation pattern by monitoring percent autogamy at the ages of 9, 18 and 27 fissions in the wild-type stock 51. To determine how the autogamy-maturation pattern is inherited, clones that showed the lowest and the highest percent autogamy at age 18 in a given autogamous generation (Gn) were examined for their percent autogamy in the next autogamous generation (Gn+1). This procedure was repeated through successive autogamous generations. We found that percent autogamy at ages 9 and 27 was rather stable (low and high, respectively), while it was extremely variable at age 18 ranging from 3% to 100%. We also found that percent autogamy at age 18 in the progeny clones was variable irrespective of percent autogamy at age 18 in the parental clones; there was no regular rule such as producing progeny with higher (or lower) percent autogamy from parents with lower (or higher) percent autogamy.

Key words: autogamy-immaturity, sexual maturation, clonal age, inheriting pattern, variability

INTRODUCTION

Paramecium tetraurelia has a clonal life cycle initiating with autogamy, and its life span is measured by the number of fissions (cell divisions). When autogamy occurs, the macronucleus collapses and a new one is formed from the micronucleus. All genetic loci become homozygous and the number of fissions of the clonal age is reset to time zero (Sonneborn, 1954, 1957). Autogamy is induced by starvation, but after autogamy there is a period called autogamy-immaturity during which natural starvation cannot induce it. Percent autogamy inducible by natural starvation increases gradually with clonal age and finally reaches a plateau. This stage, called autogamy-maturity, is followed by senescence terminated with clonal death (Sonneborn, 1970, 1974; Smith-Sonneborn, 1981, 1990; Takagi, 1988, 1999).

We have attempted to isolate mutants with modified life-cycle features to anatomize the unidirectional time-passing process in *P. tetraurelia*. We have isolated a mutant with extremely short clonal life span, which was also short in the length of autogamy-immaturity (Takagi *et al*., 1987b, 1989). Mutants with short lengths of sexual immaturity (post-conjugational mating-inability period) have been isolated in *Tetrahymena thermophila* (Bleyman, 1971) and in *P. caudatum* (Myohara and Hiwatashi, 1978). But there are no mutants with long lengths of sexual immaturity or with long clonal life span in ciliates. We are aimed at isolating mutants with longer autogamy-immaturity in *Paramecium*.

For screening such mutants, we need to know the range of variation of the autogamy-maturation pattern with clonal age in a wild-type stock in order to tell the length of autogamy-immaturity of a mutagenized clone being lengthened. We studied the autogamy-maturation pattern in the wild-type stock 51 through 7 successive autogamous generations by examining percent autogamy at ages 9, 18 and 27 in each generation. The clones that showed the maximum and the minimum values at age 18 in a given generation were examined for percent autogamy at ages 9, 18 and 27 in the next generation. We found that the autogamy-maturation pattern in the wild-type was unexpectedly variable. We also found that the autogamy-maturation pattern in progeny clones was independent of that in parental clones. These results suggest that mutants with longer length of autogamy-

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immaturity should be screened for such clones as showing ~0% autogamy at age 27.

MATERIALS AND METHODS

Cells

Paramecium tetraurelia, wild-type stock 51, was used.

Culture

Cells were grown in 0.5% Wheat Grass Powder (Pines Int., Inc., Lawrence, KS) medium that had been inoculated with *Klebsiella pneumoniae* 2 days before use (Tokusumi and Takagi, 2000). All cultures were kept at 25°C.

Cells were cultured in 2 ways; daily reisolation culture for preventing the occurrence of autogamy, and mass culture for inducing autogamy. For daily reisolation culture, single cells were isolated in 100 μl of fresh culture medium in each well of depression slides, cultured for one day and isolated in fresh culture medium after counting the number of cells. The number of fissions was calculated from $\log_2 N$, where N is the number of cells. For mass culture, cells left over after reisolation were cultured until the food bacteria were exhausted. Food exhaustion was judged by transparency of the culture due to decrease in bacterial number and growth of paramecia. This method is reliable to make the starvation level appropriate for the induction of autogamy, since almost 100% autogamy is induced invariably by this method for the fully autogamy-mature cells.

To count percent autogamy, about 100 cells were sampled from the food-exhausted culture, stained with Dippell's stain (Dippell, 1955), and the percentage of cells with macronuclear fragments was examined under a microscope. To minimize the variation of percent autogamy due to the duration of the starvation period, autogamy counts were carried out on 2 consecutive days and the higher rate was adopted as percent autogamy of that culture.

Examination of autogamy-maturation patterns

From a 100% autogamous culture, 12 cells were isolated in 100 μl of fresh culture medium and kept in 12 clones of daily reisolation cultures. The cultures remaining after the first isolation were kept in mass cultures. These mass culture conditions allowed a single cell to divide ~9 times before starvation ready for the autogamy test. Therefore, we regarded the age of the first autogamy test as 9 fissions. In order to examine percent autogamy at age 18, a 9-fission-old cell was monitored in each clone in the daily reisolation cultures and the left over cells were allowed to divide 9 fissions in mass culture. In the same way, percent autogamy at age 27 was studied in each of 12 clones of a given generation.

When the autogamy tests at age 18 were over for all of the 12 clones, the average age of these clones in the daily reisolation cultures was about 30 fissions. Two of these clones that showed the maximum and the minimum values of percent autogamy at age 18 were each expanded to more than 10 subclones in 100 μl of fresh culture medium and mass cultured to induce autogamy. A 100% autogamous culture, usually at about the age of 39 fissions, was used to initiate the next autogamous generation. This procedure allowed us to study both the variability and the mode of inheritance of percent autogamy at ages 9, 18 and 27 through successive autogamous generations.

RESULTS

Variability of percent autogamy at age 18 in the wild-type stock 51

We examined percent autogamy at age 18 in the wild-type stock 51 through 7 successive autogamous generations (Fig. 1). In the first generation (G1), 11 clones showed 78.1% autogamy on average ranging from 40% to 100%. The 2 clones that showed the minimum (40%) and maximum percent autogamy (100%) were examined at age 18 in the next autogamous generation (G2). The progeny derived from the parental clones that had shown the minimum and the maximum values showed an average of 84.3% ranging from 50% to 99% and an average of 91.3% ranging from 73% to 100%, respectively. We repeated this procedure until the fourth generation (G4). One of the G4 populations, in which a clone with the lowest value (7%) was included, was selected and continued to subsequent autogamous generations (G5–G7). Percent autogamy at age 18 in a total of 345 clones through 7 autogamous generations was 53.0±25.9% on average and varied from 3% to 100%. High variability was observed not only as a whole but also in clones of the same population. For example, a range of variation from 7% to 98%, occurring in one of the 4 populations of G3, was almost comparable to the range of overall variation. It should be stressed that all of the clones studied here are thought to be genetically identical because all genetic loci become homozygous after autogamy.

Instability of percent autogamy at age 18 through successive autogamous generations

The results described in the preceding section included information about how the autogamy-maturation pattern was inherited through autogamous generations. This is shown in a different way in Fig. 2B in which values of percent autogamy at age 18 of the progeny generations are dotted on the vertical axis at each point of the horizontal axis indicating percent autogamy at age 18 of parental generations. The data are from those shown in Fig. 1 and other additional experiments. For example, the values of percent autogamy at age 18 of 12 clones in G2 (50, 62, 76, 84, 88, 88, 89, 90, 92, 94, 98 and 99%) derived from the clone showing 40% in G1 were marked on the vertical axis standing at 40% on the horizontal axis, and those of 12 clones (73, 85, 86, 87, 88, 89, 94, 97, 98, 98, 100 and 100%) derived from the clone showing 100% were marked on the vertical axis standing at 100% on the horizontal axis (Fig. 2B). The overall average of percent autogamy at age 18 was 57.9±24.2% (n=541).

We examined percent autogamy also at ages 9 and 27 through successive autogamous generations (Fig. 2A, C; respectively). The average rate of autogamy at age 9 was 8.3±8.9% ranging from 0% to 55% (n=333), and the average rate of autogamy at age 27 was 97.3±5.1% ranging from 62% to 100% (n=208).

The panels were divided into 4 sections with lines of 50% autogamy to show the distribution pattern of the dots. The dots of percent autogamy at age 9 were clustered at lower sections (Fig. 2A). Percent autogamy at age 9 was mostly in the range under 30% irrespective of percent autogamy at age 18 in the parental generation. In contrast, the dots of percent autogamy at age 27 were concentrated in upper sections (Fig. 2C), indicating that percent autogamy

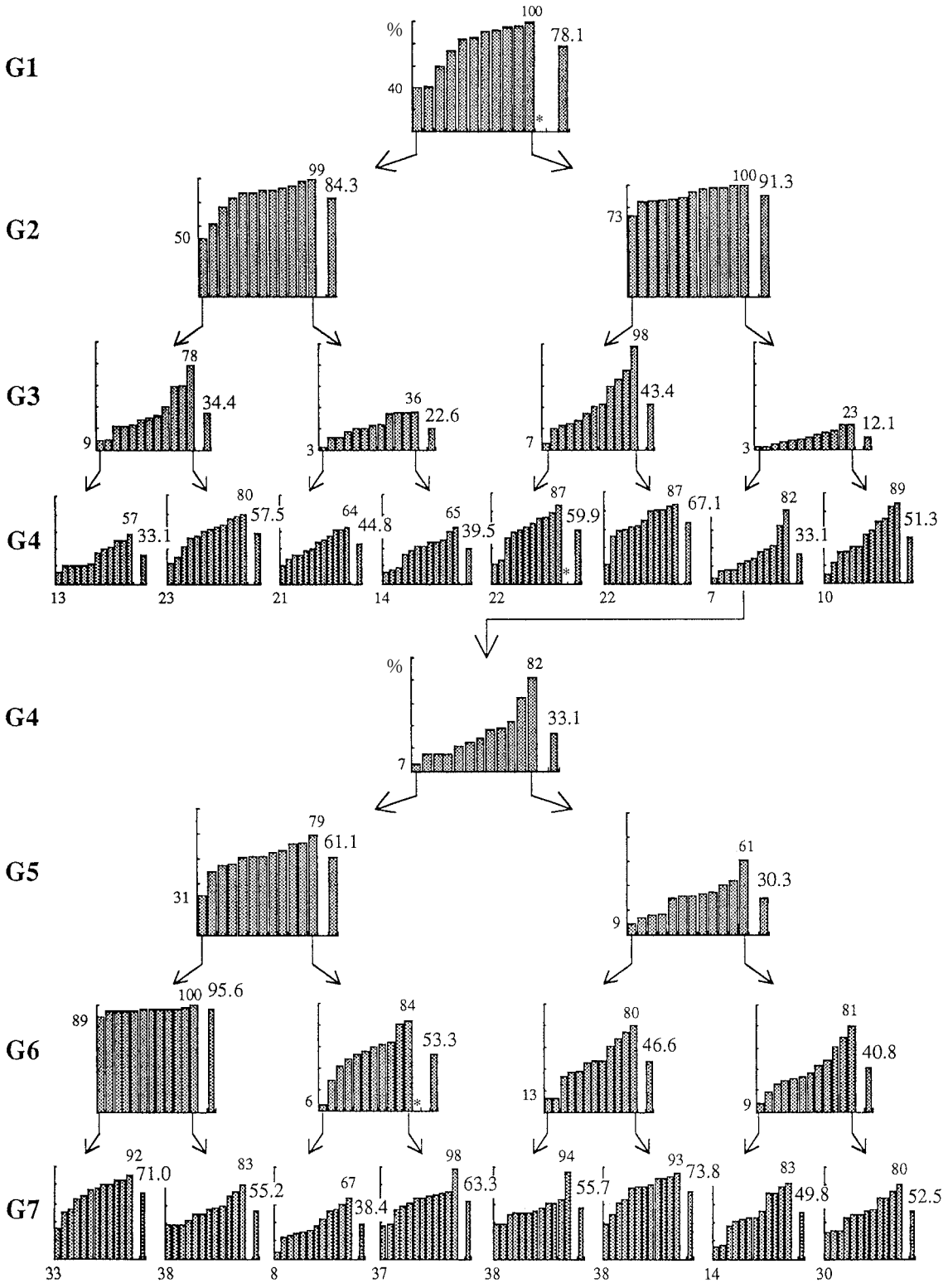

Fig. 1. Percent autogamy at age 18 in 29 populations belonging to 7 autogamous generations in the wild-type stock 51. In each population, 12 clones (11 in some cases) were arranged in order from minimum (left end) to maximum (right end) with the average (extreme right). From a 100% autogamous culture, 12 cells were isolated, kept in daily reisolation culture and percent autogamy was examined at age 18. The clones that showed the minimum and maximum values were monitored for percent autogamy at age 18 in the next generation by inducing autogamy at about the age of 39 fissions in their sibling lines cultured in daily reisolation. One of the populations in the fourth generation was selected to produce the next generation. Asterisks (*) indicate that percent autogamy was not examined because the cell did not grow to a population sufficient for autogamy test.

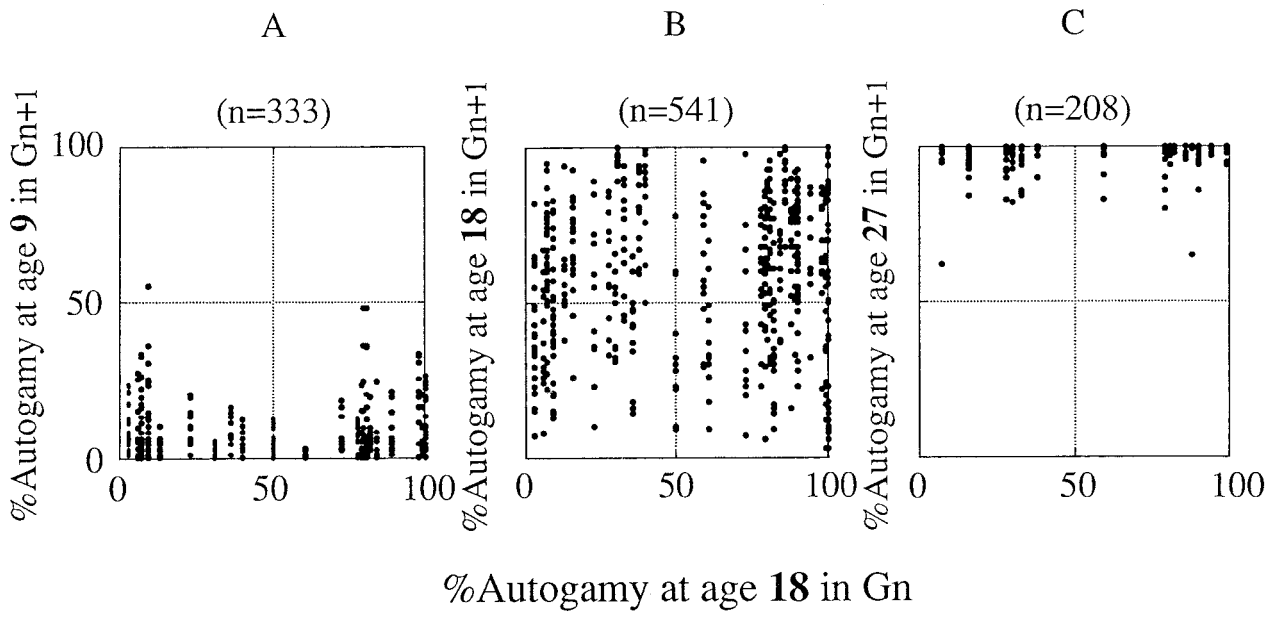

Fig. 2. The pattern of inheritance of percent autogamy at ages 9 (A), 18 (B) and 27 (C) through successive autogamous generations in the wild-type stock 51. The values of percent autogamy in 12 progeny clones (Gn+1) are marked on the vertical line at the point of the abscissa indicating the value of percent autogamy at age 18 in the parental clones (Gn).

at age 27 was stably high over 90% irrespective of percent autogamy at age 18 in the previous generation. The dots of percent autogamy at age 18 were scattered all over the plots (Fig. 2B), indicating that percent autogamy at age 18 was highly unstable without any association with percent autogamy at age 18 in the previous generation.

DISCUSSION

Since classical observations by Johannsen and others (for review, see Serra, 1965), a wide range of phenotypic variability among genetically uniform organisms has been reported. Cell size (Wichterman, 1953; Berger, 2001), serotype (Preer Jr, 1986; Bleyman, 1996) and clonal life span (Takagi *et al.*, 1987a) are examples in *Paramecium*. The present study of the autogamy-maturation pattern added another example. If the autogamy-maturation pattern has a definite curve, the length of autogamy-immaturity can be defined by the age at which autogamy is first induced, and by the age at which 50% autogamy is induced, etc. Until now, including our previous study (Ishikawa *et al.*, 1998), this assumption has been thought valid. However, the present study revealed that the autogamy-maturation pattern was highly variable in genetically identical populations under environmentally identical conditions. The autogamy rate was relatively low around the age of 9 fissions and high around age 27, with marked variation ranging from 3–100% around age 18. This indicates that autogamy may first be induced as early as age 6 in some clones and as late as age 17 in other clones.

Percent autogamy is known to be variable depending on the starvation level. Berger and Rahemtullah (1990) have shown that autogamy can be induced at any age by intensifying starvation level. In this study, autogamy was induced by natural starvation. Autogamy counts were performed twice on 2 consecutive days, and starvation level was more advanced in the second test than in the first test. The average of percent autogamy at age 18 was 47.4±25.6% (n=365) in the first test and 51.0±25.7% (n=369) in the second test: this difference was not significant. Therefore, the wide variation revealed in this study was not likely to be due to the level of starvation.

This study also revealed that the pattern of increasing percent autogamy is independent of the pattern in the parental autogamous generation. The length of autogamy-immaturity measured by the number of fissions until the first detection of autogamy was reported to be shortened when parental clones at older clonal ages were used to yield ex-autogamous clones (Iizima *et al.*, 1997). The length of autogamy-immaturity measured by the pattern of increase in percent autogamy was reported to be shortened when parental clones at advanced cultural ages were used to yield ex-autogamous clones (Ishikawa *et al.*, 1998). In this study, these factors were kept uniform by inducing parental autogamy at the clonal age of about 39 fissions and at the cultural age of about the second day of the stationary phase. Therefore, the independent pattern of autogamy-maturation from the parental pattern revealed in this study was not due to clonal aging or cultural aging.

The increase of percent autogamy with age may indicate sequential expression of autogamy-associated genes and/or increases in the expression level of an autogamy-controlling gene(s). The sequential expression of conjugation-associated genes was suggested for the maturation

process after conjugation in *P. multimicronucleatum* (Takagi, 1971) and in *Euplotes octocarinatus* (Kuhlmann and Heckmann, 1989). Recently, the *MS2* gene of *P. tetraurelia*, which has been reported to be expressed in an age-associated manner (Tanabe *et al*., 2002), was found to also be expressed in an autogamy-associated manner (Tanabe, personal communication). Previous experiments have also indicated the presence of temporal controlling genes for sexual maturation in ciliates; in *P. bursaria* (Siegel, 1967), *P. caudatum* (Myohara and Hiwatashi, 1978), and *Tetrahymena pyriformis* (Bleyman and Simon, 1967; Bleyman, 1971).

The *jumyo* mutant with an extremely short clonal life span also has a short length of autogamy-immaturity (Takagi *et al*., 1987b, 1989). The shortened autogamy-immaturity was first assessed by the shortened interval of 0% autogamy. It was found recently, however, that some clones of the *jumyo* mutant completely lack autogamy-immaturity as shown by a constant induction of 20–50% autogamy during the early period until the age of 7 fissions (Maruyama and Takagi, unpublished data). Based on the present study, we have started to isolate mutants with longer length of autogamy-immaturity by screening clones showing 0% autogamy at age 27. We have so far isolated one such mutant, the genetic analysis of which is in progress.

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