COMMITMENT TO AUTOGAMY IN PARAMECIUM BLOCKS MATING REACTIVITY:

IMPLICATIONS FOR REGULATION OF THE

SEXUAL PATHWAY AND THE BREEDING SYSTEM

SHAMSA RAHEMTULLAH

B.Sc., University of British Columbia, 1985

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Zoology)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

February 1990

© Shamsa Rahemtullah, 1990

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of ___Zoology___

The University of British Columbia
Vancouver, Canada

Date ___April 20, 1990___

ABSTRACT

The ciliate *Paramecium tetraurelia* exhibits two major developmental pathways - the vegetative cell cycle and the sexual pathway. The latter manifests itself in two forms - autogamy (self- fertilization) and conjugation (cross-fertilization) between cells of complementary mating types. In the normal life history young cells are immature for autogamy, but enter conjugation readily. Autogamy first occurs normally at about 20 fissions of age and conjugation disappears by 25. This study documents and analyzes the two major phenomena underlying this unusual life history. It shows how their interaction produces the observed pattern of immaturity, maturity, and senescence (Fig. 1).

There are two principal findings of this study. First that commitment to autogamy leads to rapid loss of mating reactivity and second, that there are different starvation thresholds for initiation of mating reactivity and autogamy. The starvation threshold for initiation of mating reactivity is constant, while that for initiation of autogamy decreases progressively as clonal age increases. During the immature period for autogamy the lag between onset of mating reactivity and induction of autogamy decreases with increasing clonal age. The progressive decrease in the starvation threshold required for induction of autogamy brings about the end of the mature period for conjugation. As autogamy is initiated at progressively earlier

stages in the growth of a culture, fewer and fewer cells reach the level of starvation required for initiation of mating reactivity prior to induction of autogamy. When the threshold for induction of autogamy becomes so low that no cells develop mating reactivity prior to entering autogamy, the period of maturity for conjugation is over and autogamy becomes the sole sexual process for the remainder of the life history.

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

This thesis would not have seen the light of the day but for the constant direction, critique, and valuable guidance given me throughout the duration of this study by Dr. J.D. Berger. It would be remiss of me if I did not also gratefully acknowledge my appreciation to Dr. T. Grigliatti and Dr. D. Holm for their help in preparing this thesis. Finally, I owe a deep debt of gratitude to my family for their love, support and encouragement over the years.

INTRODUCTION

The two major developmental pathways in the ciliate *Paramecium tetraurelia* are the vegetative cell cycle and the sexual pathway. The sexual pathway, which includes mating, meiosis, fertilization, and macronuclear development, is entered by two different routes. One is conjugation or reciprocal cross-fertilization, which is initiated by surface interactions between sexually reactive cells of complementary mating type. The other is the internally initiated process of self-fertilization called autogamy which takes place in non-paired mature cells. Since autogamy is entered from the vegetative cell cycle, without the external stimulus of interacting with a reactive cell of complementary mating type, preparation for each cell cycle necessitates that the organism must determine whether the next cell cycle will be vegetative or whether it will enter meiosis and proceed through autogamy.

Both autogamy and conjugation are induced by starvation in *Paramecium*. When cells are unable to enter either autogamy or conjugation, for example when grown continuously in the presence of excess food, the fission rate gradually decreases and the line dies out after a characteristic number of cell cycles. Both autogamy and conjugation lead to fertilization and formation of a new macronucleus, the events critical for initiation of a new life cycle and restoration of full vigor. However, each process has a different pattern of occurrence in the life cycle of the

organism. Age is defined as the number of fissions completed since the last fertilization. In general, young cells readily enter conjugation but not autogamy while older cells undergo autogamy but cannot initiate conjugation. Unlike many other ciliates which show an immature period for conjugation of significant length (50 to 100 cell cycles) during which young cells do not develop mating reactivity and thus cannot enter conjugation, *P. tetraurelia* shows no immature period for conjugation. That is, cells can enter conjugation even during the first cell cycle after fertilization (Sonneborn, 1957, 1974). However, the period of maturity for conjugation lasts only for 25 cell cycles following fertilization. This interval is about ten percent of the entire life span of approximately 240 fissions (Sonneborn, 1957, 1974).

In conjugation, two cells each with a macronucleus and two micronuclei meet to form a conjugant pair. Both micronuclei in each cell then undergo meiosis (two of three prezygotic divisions) to produce eight haploid nuclei. Seven of the eight micronuclear products degenerate and the remaining micronucleus proceeds through a third prezygotic (mitotic) division to yield two haploid gamete nuclei or pronuclei. One of these, the migratory pronucleus of each individual, moves into the coconjugant and fuses with the stationary pronucleus to form a synkaryon. Two mitotic postzygotic divisions of the synkaryon then follow to yield four nuclei, two of which become macronuclear anlagen (destined to become new macronuclei) and two

micronuclei. Conjugants separate during the postzygotic divisions. Beginning at the end of meiosis, the old parental macronucleus breaks up to form about 35 rounded fragments. A distinctive coarse, rope- like conformation of the macronucleus called a skein marks the early stage of this process. Finally, each exconjugant containing two micronuclei and two macronuclear anlagen divides, segregating to each daughter cell a macronuclear anlage while the two micronuclei divide by ordinary mitosis to restore the normal nuclear condition (1 macronucleus and 2 micronuclei).

Autogamy is like conjugation except that it occurs within single unpaired cells. It is an entirely endogenous event; external factors other than nutrient level do not play a role in invoking its occurrence. The result of autogamy is that the genome of Paramecium becomes completely homozygous owing to the union of two genetically identical haploid pronuclei in the same cell. Since the new macronucleus is a derivative of the homozygous synkaryon or zygote nucleus, it is also homozygous. In conjugation (cross- fertilization), the synkaryon of both partners is identical, but it may be heterozygous.

Commitment to autogamy is a two- step process. The initial commitment takes place at a point in the cell cycle, about 90 minutes prior to fission, at which cells become committed to division (Berger, 1986). If cells are subjected to nutrient downshift prior to the point of commitment to division, they enter autogamy (meiosis) immediately following the next fission.

If, however, starvation is induced after commitment to division, the next cell cycle will be vegetative, regardless of the nutrient level. This initial commitment to meiosis is conditional. If cells are returned to growth medium, the commitment is reversed. Final commitment to meiosis occurs within 20 minutes following the pre- autogamous fission. If cells are returned to medium with excess food after this point, meiosis proceeds regardless.

The patterns of occurrence of autogamy and conjugation differ. Cells are immature for autogamy during the first 15 to 20 cell cycles following fertilization. In these cells autogamy does not occur following brief starvation as it does in mature cells. During the latter part of this immature period, however, less and less starvation is required for the cells to enter autogamy (Sonneborn, 1974). The clonal age at which cells can no longer develop mating reactivity, but directly enter autogamy, marks the end of the period of maturity for conjugation and the beginning of senescence (Sonneborn, 1957, 1974). "Senescence" is marked by the loss of the capability to mate. This period is by far the longest component of the organism's life history and may persist for more than 200 fissions. Finally, death of the clone marks both the end of senescence and the end of the life cycle (Sonneborn, 1957, 1974).

This thesis examines the control of the sexual pathway of Paramecium in order to ascertain the nature of the relationship between autogamy and conjugation. The question examined here is

whether there exists a hierarchy with respect to autogamy and conjugation in mature cells. In other words, if mature cells are permitted to become mating reactive before commitment to autogamy, does conjugation still proceed or does autogamy block conjugation? And, conversely, does mating reactivity ever develop once mature cells have become committed to autogamy?

The preference of one sexual pathway over the other in *P. tetraurelia* has been suggested in earlier studies. Beisson and Capdeville (1966) have shown that abrupt starvation first induces mating reactivity and then autogamy in cells that are old enough to enter either pathway at approximately the same time given suitable conditions. Mature cells become mating reactive about three hours after being washed in exhausted medium. They then rapidly lose their mating reactivity as autogamy sets in. The addition of protein synthesis inhibitors appears to block autogamy by preventing the synthesis of specific proteins needed for the nuclear reorganization process. Mating reactive mature cells continue to remain reactive in the presence of such inhibitors (Beisson and Capdeville, 1966).

This study shows that mating reactive mature cells destined to undergo autogamy loose their reactivity at some point between commitment to division and fission, which occurs 90 minutes later. One of the aims of this thesis is to determine precisely where shut off of mating reactivity occurs within this 90 minute interval. More importantly, this thesis examines the hierarchical regulation of the sexual pathway in *P. tetraurelia*,

i.e., how the relationship between autogamy and conjugation changes as fission age increases and cells pass from maturity to senescence, leaving autogamy as the sole sexual process in the latter portion of the organism's life history. This information enhances our knowledge of the ecology of *P. tetraurelia*, its breeding strategy, and its relationship with other members of the *P. aurelia* species complex and other members of the genus.

MATERIALS AND METHODS

Stocks and cultures of Paramecium

Paramecium tetraurelia Sonneborn 1975 wild type stock 51-S

and stock d4-1001 carrying the *ccl* mutation (Peterson and Berger,

1976; Rasmussen and Berger, 1984) were grown in phosphate-

buffered Cerophyl medium (Sonneborn, 1970) with *Klebsiella*

pneumoniae as the food organism. Standard methods used were

those of Sonneborn (1970). Cultures were maintained at 27 C.

Establishment of isolation lines and induction of autogamy

Several cells were transferred from a stock culture of one

mating type to a depression slide well containing Cerophyl

medium. They were then washed by being transferred, one by one

from one well to the other, through five more wells containing

medium. The cells were left in the last well for one hour after

which three cells were individually transferred by micropipette

through three additional wells containing medium and were

assembled in the last well. Finally, each of the three cells was

transferred to a separate well with medium in a single depression

slide.

Daily isolation lines of each mating type were maintained

separately by transferring a cell from the previous day's well to

a new well containing fresh medium. Cells were washed every two

weeks. Autogamy was induced by starvation (Sonneborn, 1950).

Third- day- leftover depression well cultures that had reached

stationary phase were used as sources of autogamous cells. The cells were tested for the presence of autogamy with Dippell's stain (Sonneborn, 1970). A well was judged to be autogamous if more than 90% of a sample of 30 stained cells exhibited macronuclear fragmentation.

Preparation of tester cultures

Lines were expanded by transferring a three day old depression slide well culture to a tube with 10 ml. of fresh medium. The next day, the single tube culture was distributed equally among four fresh tubes and refed with 15 ml. medium to achieve a high number of cells with maximum growth rate. On the third day, the top 2-3 cm. of culture from each tube were transferred to a fresh tube. Each of the four new tubes was then fed approximately 20, 15, 10, and 5 ml. of food to produce dilutions of approximately 1:4, 1:3, 1:2, and 1:1 respectively of the original culture with fresh medium. 24 hours later, cells of each mating type from tube cultures having similar amounts of food were mixed together and tested for mating reactivity (see below). Alternatively, several flask cultures (see page 12) of each mating type were diluted with fresh medium to obtain a range of densities (500-2000 cells/ml.) by the next morning. Cultures of each mating type were separately filtered and washed twice in Dryl's buffer when they reached a minimum density of 2000 cells/ml. Approximately 3.5 hours after washing in Dryl's buffer the cultures became mating reactive and were used as a source of

reactive testers as long as high mating reactivity was present (8-16 h). Availability of testers that were 40-50% mating reactive throughout the day was ensured by setting up a dilution series on the previous day.

Determination of cell densities

Population density was estimated by manually counting cells. Samples of 0.05 ml. were removed with an Eppendorf pipetter from a thoroughly stirred culture. The cells were ejected into 1 ml. of medium in a depression slide well. Formalin (0.05 ml.) was added to kill the cells, and the dead cells were then counted as they were withdrawn with a micropipette. Each count was the mean of three samples (at least 100 cells/sample). The count was multiplied by the appropriate dilution factor to give the number of cells/ml. As a standard medium was used throughout these experiments, cell density was used as a measure of the degree of starvation and the availability of food.

Mating reactivity tests

Approximately 0.5 ml. of culture of each mating type was placed in the middle well of a depression slide. The same volume of each was added separately to each of the two end wells. These cultures served as controls for possible contamination of the tester cultures. Mating reactivity was scored. Three procedures were used in various experiments to estimate the degree of mating

reactivity.

(1) Mating reactivity was scored 10 minutes after the tester
strains were mixed together. The degree of mating
reactivity was estimated using a ranking scale ranging from
0 to 5. Zero corresponded to no observable mating
reactivity (absence of pairs or clumps) while five
corresponded to the highest degree of mating reactivity
observed in equivalent mixtures of tester strains.

(2) As the arbitrary scale described above gave only a relative
measure of mating reactivity, a single cell mating
reactivity test was designed to measure the percentage of
mating reactivity. A drop of Dryl's buffer was placed in
each of 42 depression slide wells. A single experimental
cell was placed in each depression well. An excess of
reactive testers of complementary mating type prepared in
Dryl's buffer was added to each well. A positive mating
reaction was noted if a pair or clump was observed one hour
after testers were added. The time of the test was taken
to be the time at which the testers were added to the
experimental cells. The percentage mating reactivity was
taken as the fraction of the 42 experimental cells mating.

(3) A known number of experimental cells (30-42) was placed in
0.5 ml. Dryl's buffer in a depression well. An excess of
reactive testers of complementary mating type was added to
the well. The number of pairs observed one hour after the
testers were added was taken to be the fraction of

experimental cells added that were mating reactive at the time of addition of testers.

Determination of cell cycle duration

a. Control cells

The cell cycle duration of control cells was determined by transferring approximately 25 dividers from a Petri plate culture (see page 12) in normal medium to a single depression well containing normal medium within a brief (2- 3 min.) interval. Four to five hours after collection of the dividers, a single cell was placed in each of 42 wells containing normal medium. The wells were then checked periodically (every 20 min.) for the presence of dividers or divided cells starting about 5.5 hours after fission. The cumulative number of cells having reached division was recorded at each interval until more than 75% of the cells had divided. The number of divided cells was then plotted as a function of time. The median time of division was estimated by extrapolation.

b. Cells subjected to nutrient downshift

Approximately 25 dividers were transferred from a Petri plate culture in normal medium to a depression well containing normal medium. Some time later (2-4 h), the dividers were washed through three wells containing Dryl's buffer. Single cells were placed in wells containing Dryl's solution four to five hours

after nutrient downshift. The median time of division was determined as described above.

Preparation of immature and mature mass cultures (for mass culture experiments)

a. *Production of mature cultures*

Cells were washed and maintained in isolation line cultures. Two days later, a plate (14 depression slides, 42 wells) of single cell isolation cultures was made from a single healthy well. The initial slide was saved as an autogamy control (i.e. to determine whether the parent cells were mature as indicated by autogamy in a high percentage of the control culture). Mature cells undergo autogamy when food is exhausted. The next day cells from six well cultures were transferred to a separate Petri plate. At least one depression slide well culture was saved as an autogamy control. Finally, on the next day, the Petri plate cultures were pooled in a flask and diluted as desired to obtain mature cultures of various densities.

b. *Production of immature cultures*

To obtain an immature culture, a mature culture in which more than 95% of cells were in autogamy was diluted with fresh medium.

Determination of fraction of cells committed to division

   Paramecium cells become committed to division about 90

minutes prior to fission (Ching and Berger, 1986; Berger and

Ching, 1988). This is readily observed in cells carrying the *ccl*

mutation which results in rapid cell cycle arrest following

transfer of the cells to the restrictive temperature (34.4 C).

However, if cells have already reached the point of commitment to

division, they continue to fission after transfer to restrictive

conditions. Synchronous samples of 42 *ccl* cells were placed in

0.5 ml. of fresh medium in 1.5 ml. tubes and placed in a

waterbath at 34.4 C. One hour after control cells had completed

fission, the fraction of cells dividing under restrictive

conditions was determined. This value was used as an estimator

of the fraction of cells committed to division at the time of

transfer to restrictive conditions.

Determination of fraction of cells in autogamy

   To determine the fraction of cells completing meiosis, 100

experimental cells were killed by drying on albumen- coated

slides at each time of collection. The slides were stained with

Azure A and the number of cells in each sample that had reached

the stage of macronuclear fragmentation in autogamy (skein stage

or later) was scored. The skein stage, a thick, coarse rope-

like conformation of the macronucleus is a convenient marker

because it is easily identifiable in stained cells at low

magnification. It appears just after completion of meiosis, at

4.5 hours after the pre- autogamous fission (Berger, 1986).

RESULTS

A. *Mating reactivity tests*

The mating reaction is the first step in the conjugation pathway in *Paramecium*. Consequently, assessment of mating reactivity is a key requirement for analysis of initiation of the sexual pathway in conjugants. Three procedures were developed to estimate the degree of mating reactivity in experimental cells (Methods, page 9). Of these, measurement of mating reactivity using a ranking scale was the most subjective. This method is quick and easy, but not quantitative. There is no assurance that the spacing between levels in the scale is uniform. Consequently, quantitative single cell and multiple cell mating reactivity tests were also developed (Methods, page 9). These tests gave quantitative, repeatable estimates of the fraction of the population entering conjugation. Wherever possible, the latter two procedures were employed.

The optimum timing for scoring mating reactivity tests was examined. Single cell mating reactivity tests (Methods, page 9) were set and the percentage mating reactivity was scored at five minute intervals (Fig. 2). The percentage of cells finding mates reached a stable maximum value by 30 to 45 minutes after testers were added. Therefore, subsequent mating reactivity tests were scored 60 minutes after addition of testers to provide a margin of assurance.

B. Onset of mating reactivity

1. Asynchronous cultures of unknown age

The initial task was to establish the conditions necessary for inducing mating reactivity. 5 ml. of culture was diluted with various amounts of fresh medium (Methods, page 8). 24 to 30 hours after feeding, the 1:1 tube showed strong mating reactivity and the 1:2 tube a much lower level. This provided a source of mating reactive cells.

Since nutrient limitation plays a major role in inducing mating reactivity, cell density and mating reactivity were examined as a culture entered stationary phase. The initial cell density was about 2800 cells/ml. The rate of population growth declined over a period of four to six hours (Fig. 3). Mating reactivity first appeared at a density of 3400 cells/ml., but it did not gain strength until several hours after the growth rate declined to low levels. By this time the cell density had stabilized at 4000 cells/ml.

2. Mating reactivity onset in Dryl's buffer

To determine the time of onset of mating reactivity in cells washed into Dryl's buffer, exponential growth phase cultures of complementary mating type (density 2000 cells/ml.) were washed twice by gentle centrifugation in Dryl's buffer, resuspended in buffer and tested at various times for mating reactivity using the ranking scale method (Methods, page 8). Mating reactivity consistently appeared between 3.25 and 3.50 hours following

washing and persisted for at least three hours (Fig. 4). This observation made it possible to reliably obtain reactive testers from mass cultures.

3. *Initiation of mating reactivity with respect to cell cycle stage*

Initiation of mating reactivity is not cell cycle stage dependent. The 3.3 hr interval observed between the time that a culture was washed into Dryl's buffer and the onset of mating reactivity did not change with cell cycle stage when sets of synchronous cells were washed into Dryl's buffer at different positions within the cell cycle (Fig. 5).

C. *Persistence of mating reactivity in immature and mature cells*

The persistence of mating reactivity after its initial onset was then examined. I first examined the pattern of mating reactivity in immature cells which are unable to undergo autogamy. This was important owing to possible interference of autogamy with the pattern of mating reactivity.

1. *Naturally starved, immature cells*

Cell density and the percentage of mating reactive cells in a naturally starved immature culture were scored over a period of 20 hours (Fig. 6). In this experiment, mating reactivity first appeared when cell density was about half of its final level (2300 cells/ml.), increased rapidly for the next six hours and

then decreased slowly, persisting at low levels throughout the remainder of the 20 hour observation period. Thus, there is extremely long persistence of mating reactivity in at least some cells in the culture.

2. Abrupt downshift, immature cells

Similar results were obtained when cells were subjected to abrupt nutrient downshift by being washed into Dryl's buffer (Fig. 6). The main difference between this experiment and that with naturally starved cells was the slightly more accelerated loss of mating reactivity such that mating reactivity disappeared by 18 hours after washing.

3. Naturally starved, mature cells

The cell density, percentage of cells completing meiosis (Methods, page 13), and percentage mating reactivity of a mature culture was followed over time (Fig. 7). The fraction of cells completing meiosis followed the pattern of increase in cell number. Throughout this period, no mating reactivity was observed.

The preautogamous fission takes place 4.5 hours prior to macronuclear skein formation (Berger, 1986). A preautogamous fission curve can thus be constructed by shifting the curve showing macronuclear fragmentation back 4.5 hours. Since cells traverse the final point of commitment to autogamy within minutes following the preautogamous fission, the preautogamous fission

curve can be taken as a good indicator of the kinetics with which cells become committed to autogamy. The curve shows that the cell density at which mature cells become committed to autogamy is far lower than that at which mating reactivity usually appears (about 1000 cells/ml. vs. 3500 cells/ml.).

The foregoing suggests that two different starvation thresholds exist, one for autogamy and one for mating reactivity. As a mature culture enters stationary phase, autogamy appears to occur well before mating reactivity could develop. In other words, cells do not have to be nearly as starved to become committed to autogamy as they do to develop mating reactivity. The results also indicate that entry into autogamy blocks development of mating reactivity.

4. *Abrupt downshift, mature cells*

A mature culture with an approximate starting cell density of 2000 cells/ml. was washed in Dryl's buffer. As in previous experiments, mating reactivity appeared approximately 3.5 hours after downshift (Fig. 8). Thereafter it decreased rapidly and disappeared by nine hours following downshift. In Figure 8 mating reactivity is shown as the percentage of control reactivity (45%). In most of the experiments, the controls had a percent mating reactive score of approximately 40 percent. In all cases the control value was taken to be 100 percent. The rapid loss of mating reactivity was already underway as cells completed the preautogamous fission. Moreover, the initial

mating reactivity was 80% of the control value which corresponds to the estimated 20% of cells that had completed the preautogamous fission at this point. This implies that reactive mature cells loose capability to remain mating reactive some time before the preautogamous fission, presumably soon after they traverse the initial point of commitment to autogamy. Therefore, as cells become committed to autogamy their entry into the conjugation pathway seems to be blocked.

5. *Naturally starved, mature and immature cells, mixed*

If different starvation thresholds for autogamy and conjugation do indeed exist, then a naturally starved mixed culture should show onset of autogamy in mature cells well before mating reactivity develops in immature cells.

Mature and immature cells were grown separately in Petri plates. On the day of the experiment, two plates containing immature cells and three containing mature cells (about 1000 cells/ml.) were combined in a flask. This cell density was chosen to ensure that mature cells had not yet begun meiosis (Fig. 9). The cell density, percentage completing meiosis, and percentage of mating reactive cells were followed.

The median point of onset of mating reactivity occurred at a cell density of 3500 cells/ml. (Fig. 9). If initiation of mating reactivity was triggered 3.5 hours earlier, as indicated by the abrupt downshift experiments, the cell density at the point of initiation of mating reactivity would be 2500 cells/ ml. At this

point (3.6 h), more than a third of the mature cells had reached the stage of macronuclear fragmentation. Initial commitment to autogamy occurs 6 hours prior to macronuclear fragmentation and would have taken place at a cell density below 1000 cells/ml. That initial commitment to autogamy occurs several hours before mating reactivity is triggered and occurs at a much lower cell density indicates that different starvation thresholds exist for autogamy and conjugation (less than 1000 cells/ml. and about 3500 cells/ ml., respectively). Commitment to autogamy also appears to override entrance into the conjugation pathway in mature cells.

D. Loss of mating reactivity

The preceding experiments strongly suggest that autogamous cells are not mating reactive. They not only confirm earlier observations that commitment to autogamy overrides entrance into the conjugation pathway in mature cells, but they also suggest that mating reactive mature cells, having traversed the initial point of commitment to autogamy, must lose sexual reactivity prior to the preautogamous fission since final commitment to autogamy occurs within 20 minutes after the preautogamous fission (Berger, 1986). Loss of mating reactivity was determined by measuring mating reactivity during the latter part of the first cell cycle after nutrient downshift. When nutrient downshift occurs prior to the point of commitment to division meiosis begins soon after the following fission (Berger, 1986). I

therefore examined the pattern of mating reactivity in synchronous cell samples during both the first cell cycle (in which the nutrient downshift occurred) and in the second cell cycle (during which autogamy occurs) in mature cells subjected to abrupt nutrient downshift.

1. Immature cells (control)

Immature cells do not undergo autogamy. Therefore, cells that develop mating reactivity in the first cell cycle should retain reactivity through at least a portion of the second cell cycle. To demonstrate this point dividers were collected from immature Petri plate cultures and washed in Dryl's buffer 2 hours after fission, early enough so that they could become mating reactive well before fission. Mating reactivity appeared about 18 hours before fission and continued steadily until at least 50 percent of the cells had divided and had entered the next cell cycle (Fig. 10). Therefore, mating reactivity does not appear to be blocked in immature cells completing the first cell cycle. Repeat experiments gave similar results. Mating reactivity in immature cells proceeds without interruption from the time of its onset in the first cell cycle through to at least several hours following the first fission after downshift.

To examine the persistence of mating reactivity in immature cells during the second cell cycle after downshift, dividers were collected from Petri plate cultures of immature cells and washed in Dryl's buffer approximately three hours later to allow onset

of mating reactivity before fission. Sets of synchronous

dividers were collected at the next fission. Mating reactivity

tests were then carried out on the sets of newly synchronized

cells. Mating reactivity was maintained at approximately the

same level as in the first cell cycle for about two hours and

then gradually declined during the next five hours (Fig. 10).

Mating reactivity persisted at low levels after this five hour

period.

2. Mature cells

If autogamy blocks conjugation, then mature cells that

develop mating reactivity in the first cell cycle after nutrient

downshift and subsequently become committed to autogamy should

show loss of mating reactivity. To this end, the pattern of

mating reactivity was examined in both the first and second cell

cycles of mature cells (30 to 50 fissions old).

Using the same procedure that was employed for the immature

cell experiments, mature cells were collected and washed. They

become mating reactive slightly more than three hours before the

mean time of the preautogamous fission (Fig. 10). The first

mating reactivity test was carried out three hours before fission

at which time 40% of the cells were reactive. This corresponds

to the control value (40%). Mating reactivity then declined

rapidly. No mating reactivity was present 30 minutes prior to

the preautogamous fission in any experiment and complete loss by

one hour prior to fission occurred in one of three experiments.

As mature cells traverse the initial point of commitment to autogamy they lose mating reactivity during the following 30 to 60 minutes.

Mature cells that have become committed to autogamy and lose mating reactivity by the end of the first cell cycle remain non-reactive during the second cell cycle while undergoing autogamy (Fig. 10). In this experiment mature cells, which developed mating reactivity in the first cell cycle, were resynchronized at fission. Mating reactivity tests were then carried out at 15 minute intervals for several hours during the second cell cycle after downshift. There was no mating reactivity through the entire observational period. I conclude that initiation of autogamy blocks the development of mating reactivity.

E. Effect of clonal age on starvation thresholds for initiation of autogamy and mating reactivity

The preceding experiments show that initiation of autogamy blocks mating reactivity and that different starvation thresholds exist for conjugation and autogamy in mature cells. The observation that autogamy does not occur readily in young cells but is initiated readily at low starvation levels in mature cultures implies that the starvation threshold required for the initiation of autogamy decreases as cells mature. I therefore examined the association between starvation levels, onset of mating reactivity, and completion of autogamy in cultures of different clonal age. Cultures of known clonal age were prepared

by sequential dilution of an autogamous culture. Initially, four dilutions were made and grown in separate flasks (1:1, 1:4, 1:16, and 1:64) to give cultures that were one, three, five, and seven fissions old, respectively, on reaching full stationary phase density. A four- fold dilution of the 1:64 culture was used to obtain a culture that would reach an age of nine fissions on entering stationary phase. Subsequent four- fold dilutions were made from the immediately preceding culture in the regimen when it had reached a density of about 500 cells/ml. This ensured that the newest culture had not yet become committed to autogamy at the time of dilution. This process was continued until a clonal age of 41 cycles was reached.

In the first of two experiments, the time that autogamy appeared after the onset of mating reactivity was examined. In the one- fission old culture, macronuclear fragmentation first appeared about 240 hours after the onset of mating reactivity. As the cells became older, autogamy appeared progressively earlier and the rate at which cells entered autogamy increased (Fig. 11). At 17 fissions autogamy was initiated at the same time as mating reactivity (Fig. 12). By 21 fissions of age the first cells completed autogamy as early as seven hours *before* the onset of mating reactivity (Fig. 12). In four further dilutions (27, 29, 31 and 33 fissions old) mating reactivity was not observed (data not shown). As the clonal age of the cells increased, autogamy appeared earlier and earlier until it eventually superseded development of mating reactivity

(Fig. 13). That the four oldest cultures entered autogamy without developing mating reactivity suggests the following: As clonal age increases, the starvation threshold required for the initiation of autogamy decreases until all cells become committed to autogamy before any of them reach the mating reactivity threshold. At this point, no mating reactivity occurs.

Experiment 2 examined the cell density at which cells entered autogamy. Flasks were stored overnight at 17 C to slow down growth during unmonitored periods and facilitate accurate cell density measurements during monitored periods. This differed from the first experiment, in which all flasks were kept at room temperature throughout the procedure.

In the youngest cultures (1 through 13 fissions old) autogamy began at the full stationary phase cell density of 5000 to 5500 cells/ml. (Fig. 14). As the cells became older, autogamy began at progressively lower cell densities (Fig. 15). Finally, the oldest cultures (35 to 41 fissions old) showed the first completion of autogamy at a density of 1000 cells/ml. (Fig. 16). Less and less starvation was required for initiation of autogamy as the maturity of the cells increased. Figure 17 compares the cell density at median autogamy (where 50% of the cells have completed autogamy) to the estimated cell density at commitment to autogamy. The latter curve was constructed by using a standard growth curve to calculate the cell density at the point of commitment to autogamy which occurs six hours prior to macronuclear fragmentation. The youngest cultures did not

initiate autogamy until stationary phase (5000 cells/ml.) while the oldest cultures initiated autogamy at a density of approximately 875 cells/ml. The estimated cell density at commitment to autogamy decreased in an approximately linear fashion with increasing clonal age. Figure 17 also summarizes the results of Experiment 1 which showed that mating reactivity appeared at a consistent cell density (3000-3500 cells/ml.) in all cultures through 25 fissions in age, and that no mating reactivity appeared in older cultures.

DISCUSSION

The principal findings of this study are that commitment to
autogamy results in loss of mating reactivity and that the
starvation threshold required for initiation of autogamy
decreases as cells age, while that for initiation of mating
reactivity does not. Together the observations provide an
explanation both for the presence of the immature period for
autogamy and for the loss of mating reactivity at the end of the
period of maturity for conjugation. The discussion will deal
first with regulation of mating reactivity and then with
regulation of autogamy and its implications for the life history
and breeding strategy of the organism.

Regulation of mating reactivity

Development of mating reactivity does not appear to be cell
cycle stage dependent. This hypothesis was suggested in earlier
studies by Miwa and Umehara (1983) who demonstrated that G2 phase
cells (4C micronuclear DNA content) can conjugate with G1 phase
cells (2C micronuclear DNA content) in stock hr^d,
P. tetraurelia. In my experiments, the interval between the time
that a culture was washed into Dryl's buffer and the onset of
mating reactivity did not change with cell cycle stage. However,
the cell cycle stage of cells at onset of mating reactivity 3.3
hours after washing was not known. Nutrient downshift
significantly increases cell cycle length making it difficult to

determine the cell cycle stage of cells at initiation of mating reactivity (Rasmussen, 1967).

The molecular signals that initiate mating and that lead to conjugation in *Paramecium* are not fully understood. In certain ciliates, such as *Blepharisma*, mating- type substances occur as soluble pheromones (gamones) in the cell- free fluid, and have been isolated and characterized (Miyake and Beyer, 1973). In *Paramecium*, however, mating substances are integral proteins of the ciliary membrane (Metz, 1954; Hiwatashi, 1969; Watanabe, 1977; Kitamura and Hiwatashi, 1978). Generally believed to be glycoproteins (Brock, 1965; Crandall et al., 1974; Wiese, 1974; Kitamura and Hiwatashi, 1978), these mating substances mediate an initial recognition event. The initial adhesive interactions initiate subsequent responses which include meiosis and fertilization.

The present experiments show that loss of mating reactivity is a direct consequence of initiation of autogamy. The entire population of cells mature for autogamy (27 fissions of age or older) entered autogamy without ever developing mating reactivity when allowed to exhaust their food supply. However, when these mature cells were subjected to abrupt downshift, mating reactivity developed in most cells but disappeared rapidly thereafter. Analysis of the kinetics of initiation of autogamy in these cells shows that the rapid loss of mating reactivity was associated with commitment to autogamy. Similar observations have been reported by Beisson and Capdeville (1966).

Beisson and Capdeville noted that when the protein synthesis inhibitor puromycin was added to mature cells at the end of the washing process, those cells becoming mating reactive remained mating reactive for up to two weeks while autogamy was completely inhibited. When puromycin was removed and cells were resuspended in exhausted medium, mating reactivity disappeared and autogamy began. This suggests that autogamy requires the synthesis of new proteins which trigger the loss of and subsequent replacement of flagellar membrane containing the mating substances. Addition of puromycin at or after the second hour did not stop the process of autogamy (Beisson and Capdeville, 1966). This transition point probably corresponds to the point of final commitment to autogamy which occurs minutes after the preautogamous fission and about 110 minutes after the point of initial commitment to autogamy (Berger, 1986).

My mating reactivity control values are not as high as those obtained by Beisson and Capdeville (1966) probably because of differences in washing procedure. Beisson and Capdeville washed their testers in exhausted medium and obtained 90% mating reactivity. On the other hand, I washed my testers in Dryl's buffer and achieved lower mating reactivity levels -- typically 40% to 50%. This difference could be due to the deleterious effects of the non- nutrient buffer. The presence of mature cells (30%) in the tester mixture could also account for the low levels of mating reactivity obtained.

Regulation of autogamy and the life history

The two forms of the sexual pathway in *P. tetraurelia*, conjugation and autogamy, occur during different phases of the organism's life history. This study shows that the occurrence of mating reactivity is restricted to the first 10% of the clonal life span and autogamy is virtually the sole sexual process during the remainder of the life history.

The timing of initiation of autogamy following the onset of mating reactivity was examined in cells of increasing clonal age (1 to 33 fissions old). Initially, autogamy did not occur until about 240 hours after onset of mating reactivity at about 3200 cells/ml. in naturally starved cultures. The lag between onset of mating reactivity and initiation of autogamy decreased exponentially and reached zero by the 15th cell cycle. The period between 1 and 15 fissions can thus be regarded as the immature period for autogamy since autogamy does not begin until mating reactivity has first been initiated. In older cells, initiation of autogamy occurs prior to or coincident with the development of mating reactivity.

This study indicates that there is no absolute immature period, but rather a tendency for cells to initiate autogamy at progressively lower levels of starvation as clonal age increases. It was found that until about 17 fissions, the point at which 50% of the cells had completed autogamy was reached no earlier than the point at which full stationary phase cell density was attained (at least 5000 cells/ml.). Concurrently,

the median point of commitment to autogamy does not fall below a cell density of 5000 cells/ml. until about 15 fissions. During this interval conjugation takes place more readily than autogamy since less starvation is required to induce the former than the latter. The duration of the immature period for autogamy has been estimated to be 15 to 20 cell cycles by various observers (Sonneborn, 1957, 1974; Miwa and Hiwatashi, 1980; Bleyman, 1971). This study reveals the basis for much of the variation in the observed duration of the immature period, for cells initiate autogamy at progressively lower levels of starvation as clonal age increases. The observed immature period depends on the time elapsed between onset of starvation and scoring for presence of autogamy.

Sonneborn (1957, 1974) stated that autogamy in P. tetraurelia cannot be induced by starvation during the first 12 to 15 cell divisions after the beginning of a clonal cycle, regardless of whether the clonal cycle is begun by autogamy or conjugation. I have shown that autogamy is inducible even in very young cells. In young cells autogamy occurs only after severe starvation. As cells become older, autogamy occurs sooner and sooner after the onset of mating reactivity. The absence of reports of autogamy in young cells in earlier studies is likely the result of observational periods of inadequate duration.

The consistent exponential decrease in lag between the onset of mating reactivity and initiation of autogamy with increasing clonal age suggests that immaturity for autogamy in

P. tetraurelia is governed by a dilutable factor. Clones of some species of *Paramecium* have, after conjugation, a period of immaturity during which the cells cannot mate (Sonneborn, 1957). Miwa et al. (1975) found that in three different groups of *Paramecium* species, the cytoplasm of immature cells contains a protein of MW 10,000 d. This protein, called immaturin (Haga and Hiwatashi, 1981), represses mating reactivity when injected into sexually mature cells.

Although *P. tetraurelia* has no immaturity period for conjugation, it does respond to immaturity substances from *P. primaurelia* and *P. caudatum* (Miwa, 1979). That is, when the immature cytoplasm of the latter two species is microinjected into mature *P. tetraurelia*, mating reactivity is not developed for a period of 10 to 15 cell cycles. It seems likely, therefore, that *P. tetraurelia* cells share a common immaturin receptor with these species.

If, as Sonneborn (1974) noted, the time lag between the onset of mating reactivity and onset of autogamy decreases as the maturity of the cells increases, it is possible that with each cell cycle immaturin- like substances are progressively diluted so that the period of immaturity for autogamy becomes progressively shorter. If an immaturin- like substance occurs in *P. tetraurelia*, it must control autogamy not conjugation. Since *P. tetraurelia* cells respond to immaturin from *P. caudatum* (Miwa, 1979), *P. tetraurelia* might have immaturin- like substances which act in a different manner than what is observed in *P. caudatum*,

namely to inhibit autogamy.

Interpretation of the pattern of decrease in lag between mating reactivity and autogamy is not a simple matter. Miwa et al. (1975) found that the effect of immaturin often lasted for more than 15 fissions when injected into mating reactive (mature) cells. They reasoned that if simple dilution with each successive cell cycle was responsible for the observed suppression of mating reactivity, then 15 fissions after the injection there should be 2^{-15} of the amount initially injected or an amount far lower than the actual concentration found in an immature cell or one that is unable to mate (Miwa et al., 1975). For these given reasons, they propose that pre- existing immaturity substances stimulate further synthesis of the same substances in each cell cycle. Hence, immaturin levels gradually decrease with succeeding fissions. Miwa et al. suggest that the amount of immaturity substances at various stages of immaturity could be measured to verify the hypothesis laid out in their paper.

Analysis of the kinetics of the results of this study shows a first order exponential decrease in immaturin concentration at a rate of 21% per cell cycle. This is consistent with the idea of autocatalytic activation of immaturin synthesis combined with growth driven dilution. This explanation would account for the plateau eventually reached which likely marks the end point of immaturin- like protein synthesis or the critical concentration of the substance below which autogamy occurs without prior

development of mating reactivity.

The problem presented by the hypothesis of autocatalytic activation of immaturin synthesis is that the first order decay in immaturin concentration suggested by this study should result in a linear decrease in lag with increasing clonal age if a first order decay is assumed to occur during the starvation period. On the other hand, a linear decrease in immaturin concentration results in a first order decrease in the lag period. It is difficult to see how a linear decay pattern of immaturin with increasing clonal age could be obtained.

Kosciusko and Koizumi (1983) have used a different approach to study immaturity for autogamy. They transplanted macronuclear karyoplasm from young clonal age donors to pre- autogamous recipients. Injection of macronuclear karyoplasm had no effect on the rate and timing of autogamy. On the other hand, when macronuclear karyoplasm from pre- autogamous donors was transplanted to young recipients, the duration of the immature period was significantly reduced. Further, removal of part of the macronucleus led to reduction of the immature period suggesting that clonal age is determined by the number of rounds of macronuclear DNA synthesis that have taken place since the last fertilization (Mikami and Koizumi, 1983). These observations are difficult to reconcile with my results which imply that a cytoplasmic factor is responsible for the timing of occurrence of autogamy.

The immature period for autogamy lies wholly within the
mature period for conjugation. The period of maturity for
conjugation begins after fertilization and persists for 25 cell
cycles, about 10% of the total life span. Cells older than 25
fissions do not show any mating reactivity when allowed to starve
naturally. Throughout this interval mating reactivity develops
at a constant level of starvation when cultures reach a density
of approximately 3500 cells/ml. During the interval between 15
and 25 fissions autogamy and conjugation can occur simultaneously
in different cells of the population, marking the end of the
immature period for autogamy. This transitional period is
terminated by total loss of mating reactivity at a clonal age of
about 25 cell cycles.

Senescence begins with the end of maturity for conjugation
at about 25 fissions. From this point on, all cells become
committed to autogamy at a cell density that is lower than that
required for the onset of mating reactivity. The cell cycle
experiments discussed above established that commitment to
autogamy is associated with loss of mating reactivity. Hence, if
cells become committed to autogamy at a cell density that is
lower than that required for the onset of mating reactivity,
conjugation cannot take place. Sonneborn (1974) states that
mating does not occur until after autogamy is completed in these
cells. My experiments show that cells do not become mating
reactive after completion of autogamy unless they are refed.
Autogamous cultures were refed by adding fresh medium in various

amounts (0.5% to 5% of initial volume). Mating reactivity developed in all the refed cultures as the added food was exhausted. No mating reactivity appeared in the unfed control culture.

The period of senescence comprises almost 90% of the clonal life span. Autogamy occurs readily until about 100 fissions after which the process of self- fertilization becomes harder to induce as the cells approach the end of their clonal life (Sonneborn, 1974). Sonneborn's use of the term "senescence" to describe the portion of the life history of *P. tetraurelia* in which mating does not normally occur may be somewhat misleading. My abrupt downshift experiments and those of Beisson and Capdeville (1966) have shown that when log-phase cultures 30 to 35 fissions old are washed in Dryl's buffer the majority of cells (approximately 70%) develop mating reactivity after 3.0 to 3.5 hours. These cells are beyond the 25 fission period for conjugation, but are able to mate when subjected to conditions that activate both sexual pathways simultaneously. In both sets of experiments, there was a subsequent sharp drop in mating reactivity which was followed by a sharp rise in the percentage of cells completing autogamy, beginning approximately six hours after washing. Estimation of the timing of commitment to autogamy shows that loss of mating reactivity coincides with initiation of autogamy.

The term "senescence" thus seems inappropriate because survival and fertility following both conjugation and autogamy is

100% until long after the start of the "senescent" period.
Therefore, the term might be more suitably assigned to that
period of the organism's clonal life during which autogamy is
difficult to induce and the frequency of lethality increases due
to accumulation of mutations (Sonneborn, 1974). The term "mature
period for autogamy" could replace Sonneborn's "senescence" for
that portion of *Paramecium*'s clonal life history where autogamy
occurs readily.

This study shows that autogamy is the "preferred" sexual
process except in young cells. First, depending on their age,
cells do not need to be as starved to enter the autogamy pathway
as they do to become mating reactive. Second, commitment to
autogamy shuts off mating reactivity. Third, all cells,
regardless of clonal age, are competent to undergo autogamy while
only young cells are competent to participate in conjugation. As
clonal age increases, the time between the onset of mating
reactivity and autogamy decreases, and, in older cells, autogamy
eventually precedes and prevents conjugation.

The results of this study need to be placed in the broader
context of the breeding strategies of the *P. aurelia* species
complex. Some *P. aurelia* species are extremely inbred (Landis,
1986). These populations are characterized as having short
periods of immaturity, if any, and short periods of maturity for
conjugation (Landis, 1986, 1988; Sonneborn, 1957). The short
period of immaturity presumably decreases the probability of
distantly related individuals (cells from very different clonal

populations) mating, since time for dispersal is not allowed
(Landis, 1986). Short periods of maturity for conjugation
encourage conjugation within a population since the time allotted
to this activity is brief (Landis, 1986).

The remainder of the life history is devoted to autogamy,
the primary consequence of which is homozygosity at all loci.
Selection will clump together interacting alleles at different
loci that have become mutually adapted. Inbreeding, in turn,
will maintain such allelic complexes within a clone. Therefore,
over a sufficient length of time, clonal populations become
genetically isolated from each other. For example, conjugation
between stocks of diverse origin results in high lethality in
P. tetraurelia, while conjugation within stocks produces
virtually 100% fertility. The advantage of this phenomenon is
that each population becomes a unique set of highly adapted
individuals. Closely related species can thus cohabit a single
region without competing for the same resource. The disadvantage
is that genetic diversity within stocks of *P. tetraurelia* is very
low due to occurrence of autogamy and lack of conjugation between
populations. On the other hand, the rate of speciation is high
since populations become both genetically and reproductively
isolated from each other thereby becoming incipient biological
species. The *aurelia* complex was likely once a single species
(*P. aurelia*) which now comprises at least 15 separate species.
The complex represents a range of breeding strategies with some
species being less strongly inbreeding than *P. tetraurelia*. The

prevalence of autogamy and the restriction of conjugation to cells within a population were probably significant factors in the development of this sibling species complex.

The *P. bursaria* complex, on the other hand, is highly outbreeding. All species are capable of inbreeding - it is the degree to which this occurs that makes an organism an outbreeder or an inbreeder. Inbreeding and outbreeding are relative terms. Conjugation between populations in *P. bursaria* species is encouraged by the long period of maturity for conjugation, by the long immature period in this species complex, and by the presence of multiple mating types. The long period of immaturity for conjugation presumably allows for dispersal of individuals to areas occupied by different populations before mating. The long period of maturity for conjugation gives ample time for these distantly related individuals to mate when they have met. The multiple mating types of *P. bursaria* increase the chances of mating between cells of different populations. Members of the *P. bursaria* complex, in contrast to the *P. aurelia* complex, also do not undergo autogamy. Consequently, the rate of genetic divergence between populations is lower and fertility in interstock crosses is high. Conversely, autogamy leads to immediate expression of any genetic variability within the population. Newly generated mutant alleles would be expressed, and immediately subjected to selective pressure, resulting in maximum inter- population genetic differentiation with minimum intra- population genetic variation.

In the *P. aurelia* complex, the occurrence of only two mating types restricts mating relative to the multiple mating type system of the *P. bursaria* complex. *P. tetraurelia*, the extreme inbreeder of the *P. aurelia* complex, has carried this trend even further. The great majority of the natural isolates of this species have been of a single mating type (odd) (Landis, 1988). This makes conjugation virtually impossible under natural conditions. The presence of a period of maturity for conjugation and a period of immaturity for autogamy suggest that at some point conjugation must have been useful, and the two mating types probably existed in more equal frequency. The scarcity of the even mating type in the wild may be due to the observed difference (12%, $p<0.001$) in growth rates between the two mating types (Fig. 18).

Even if the two mating types of *P. tetraurelia* existed in equal frequency today, autogamy would still be the primary sexual process since the mature period for conjugation comprises only ten percent of the clonal life span. This implies that *P. tetraurelia* has long been a largely automictic organism (one that relies heavily on selfing to produce progeny). The frequency of conjugation in nature is now near zero suggesting that *P. tetraurelia* has evolved into a wholly automictic organism. As a result, *P. tetraurelia* behaves like a non- mictic haploid species since autogamy enforces complete homozygosity and outcrossing between stocks is nearly impossible.

We must then ask why autogamy retains such a prominent place in the life history of *Paramecium tetraurelia*. The answer is physiological. The organism's short life span requires frequent nuclear reorganization (meiosis, fertilization and macronuclear development) to produce a young macronucleus and the attendant rejuvenation. Aging in ciliates is essentially a macronuclear phenomenon (Aufderheide, 1987). It is associated with decreases in growth rate and general vigor and is accompanied by decrease in DNA repair activity and accumulation of lethal mutations (Fukushima, 1975; Smith- Sonneborn, 1981; Sonneborn and Schneller, 1960). *Paramecium* is thus dependent on autogamy to produce new macronuclei at frequent intervals even when there is little or no genetic diversity within populations. The entire breeding strategy is strongly conservative and suggests that the organism is highly adapted to a closely defined niche in a very stable environment.

CLONAL LIFE HISTORY
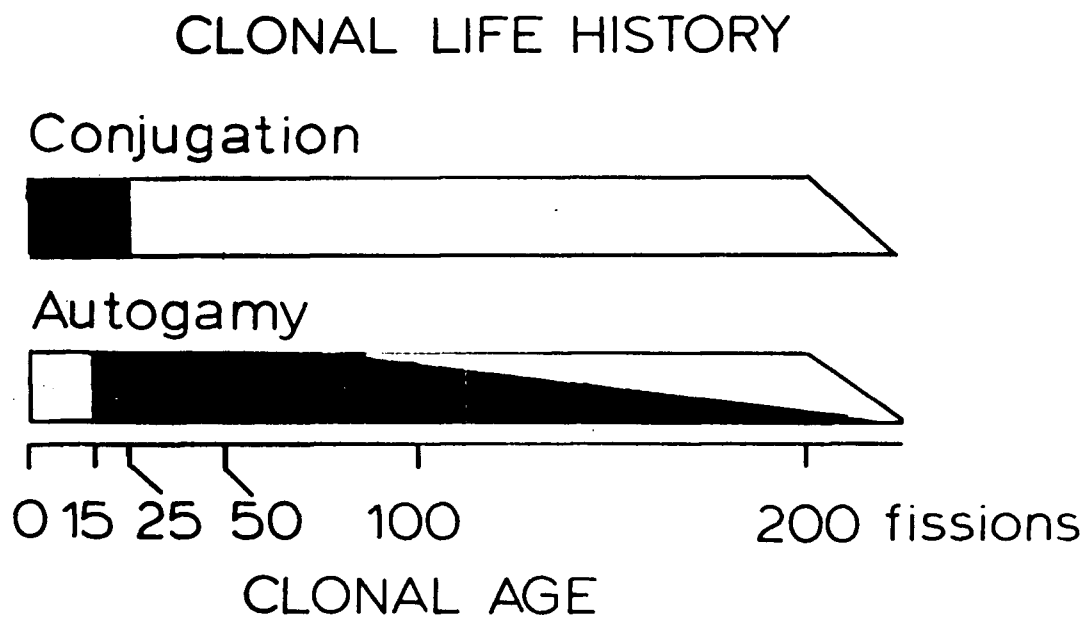
FIGURE 1:

Clonal life history of *Paramecium tetraurelia* showing maturity for conjugation, immaturity for autogamy, and senescence.
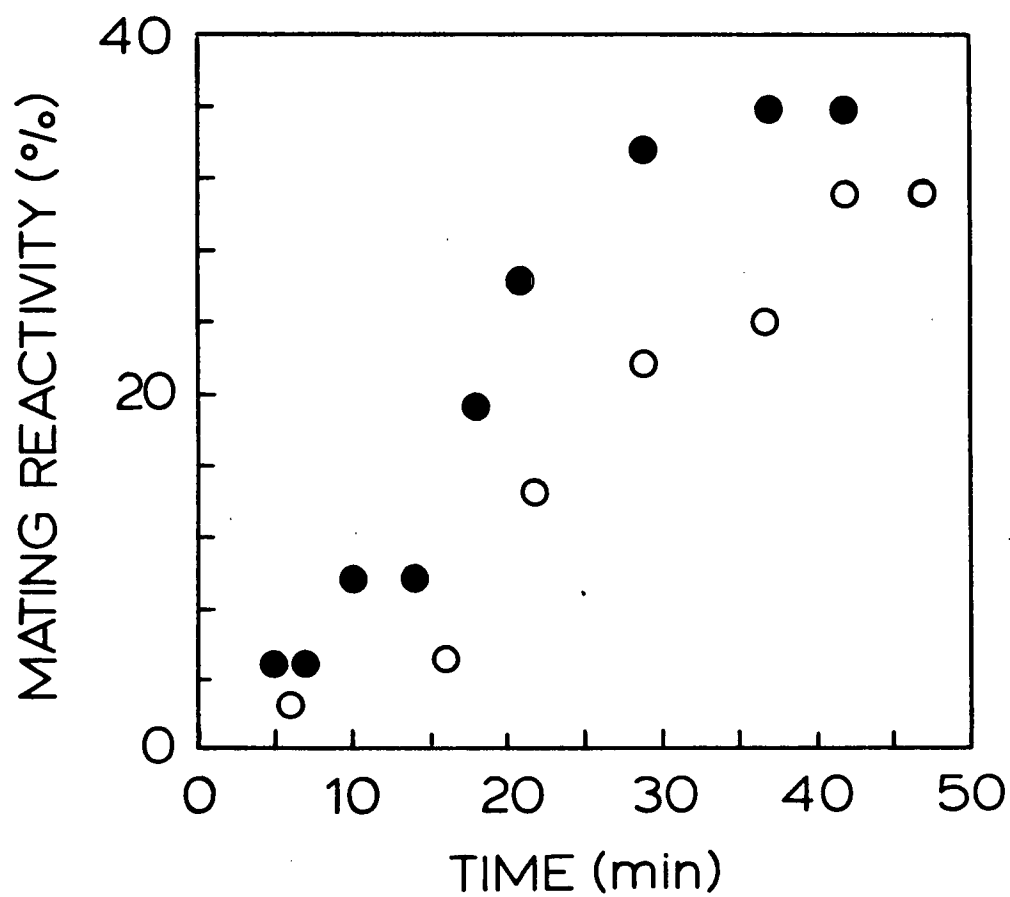
FIGURE 2:

Stabilization of mating reactivity in buffer. Filled circles: first set of observations; open circles: second set of observations.
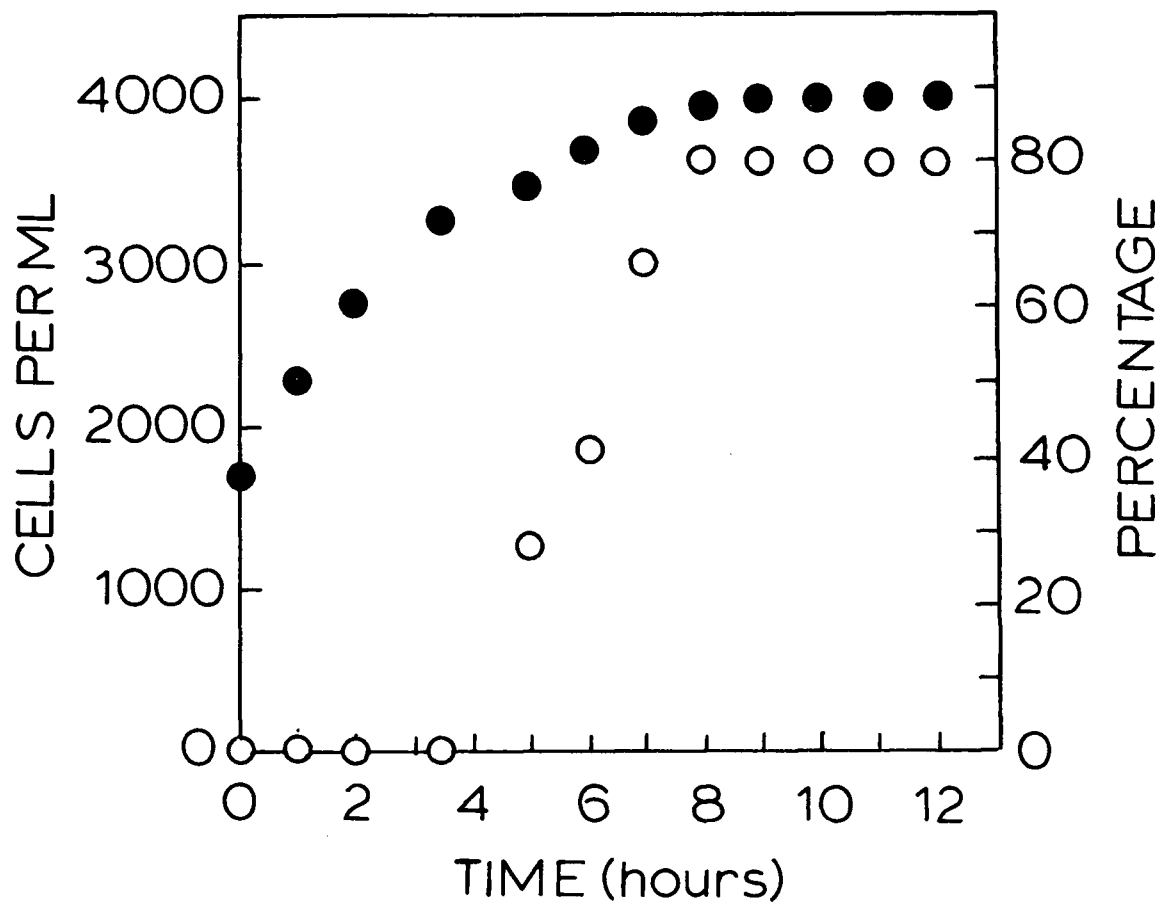
FIGURE 3:

Increase in cell number and onset of mating reactivity in a naturally starved culture of unknown age. Filled circles: cell density; open circles: percentage of cells showing mating reactivity.
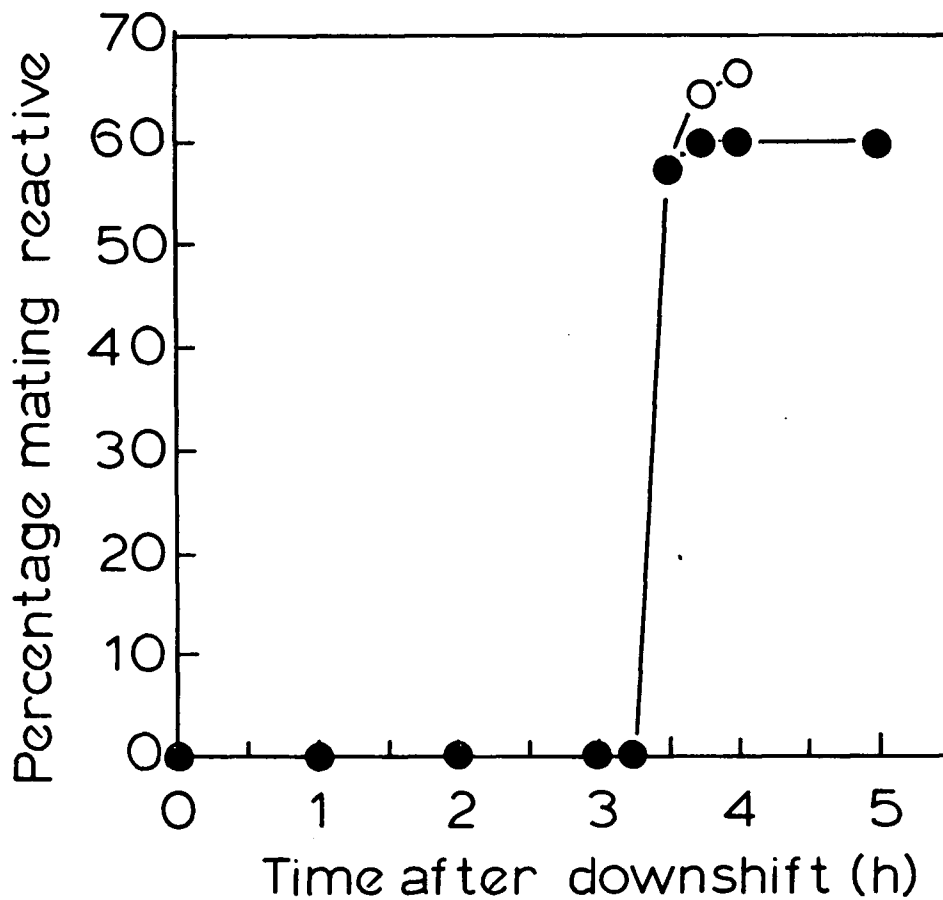
FIGURE 4:

Mating reactivity onset in Dryl's buffer.   Open circles: first set of observations;   filled circles: second set of observations.
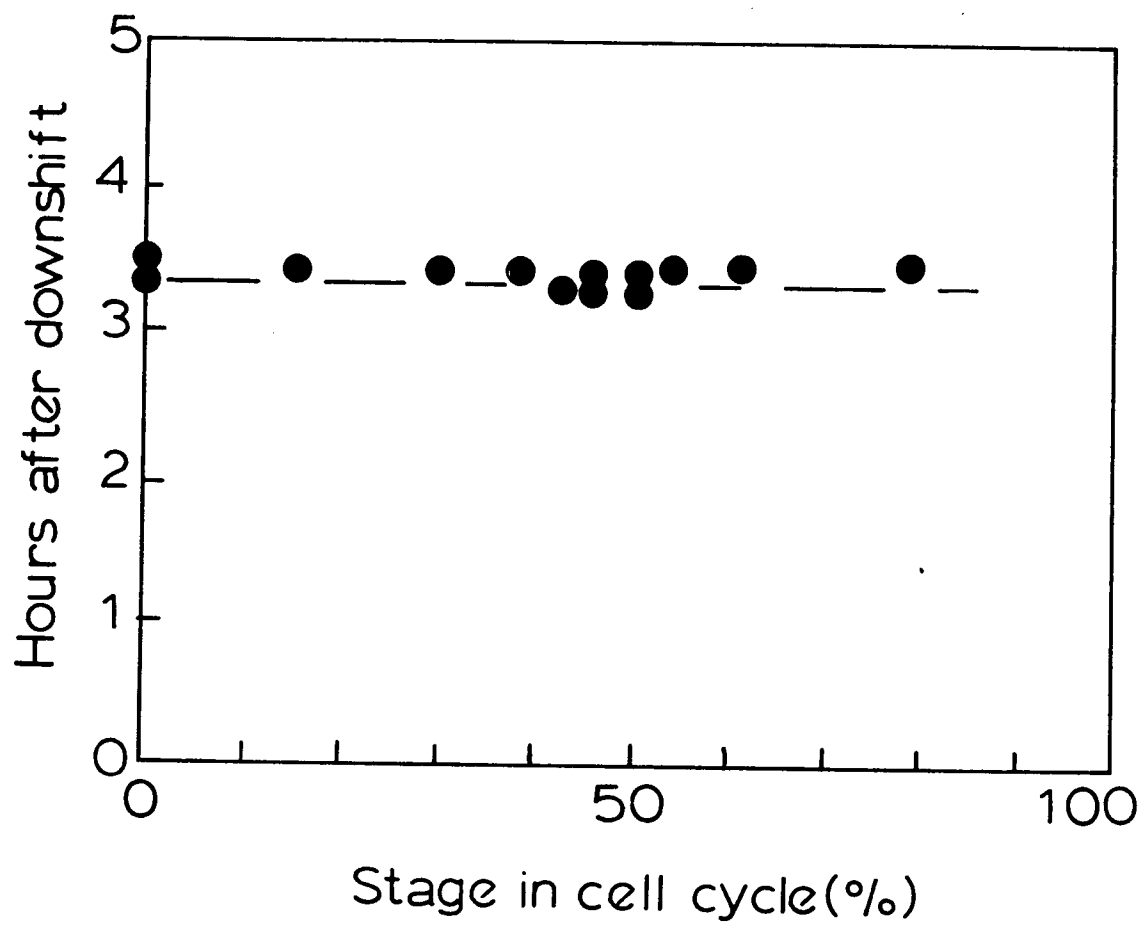
FIGURE 5:

Mating reactivity onset as a function of cell cycle stage occupied by cells upon nutrient downshift.
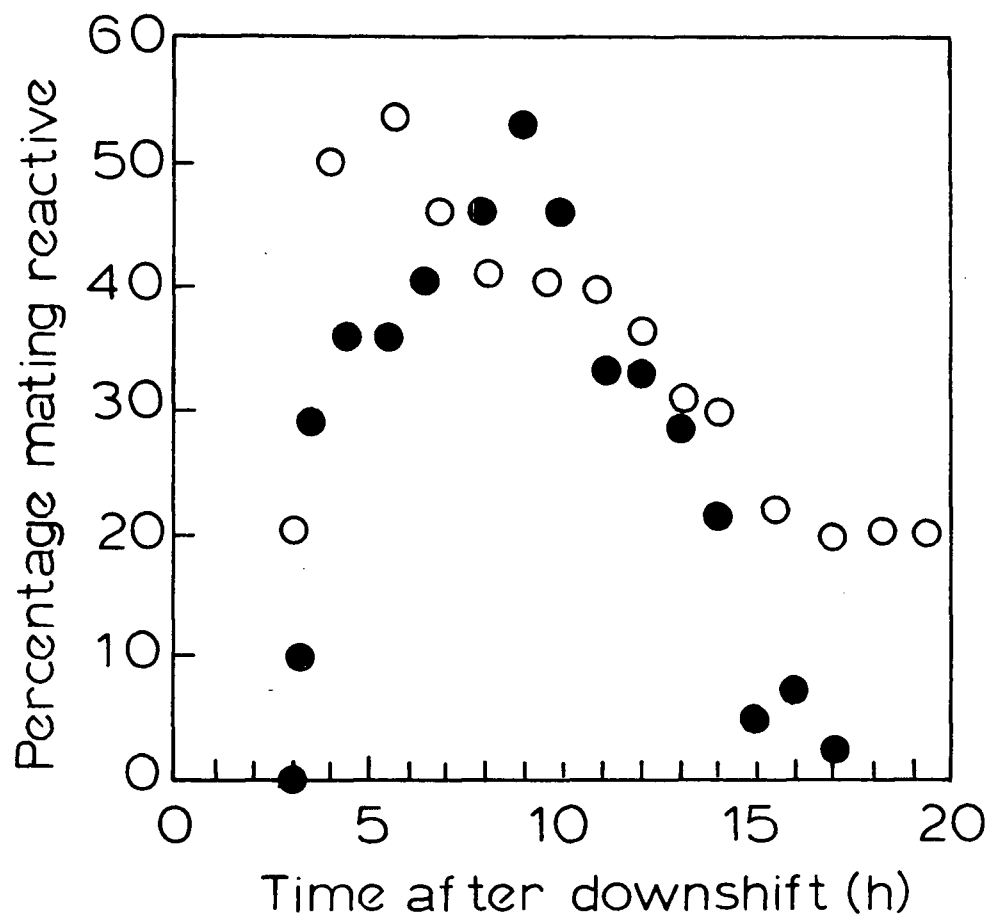
FIGURE 6:

Persistence of mating reactivity in immature cells. Open circles: naturally starved cells; filled circles: cells subjected to abrupt downshift and maintained in buffer.
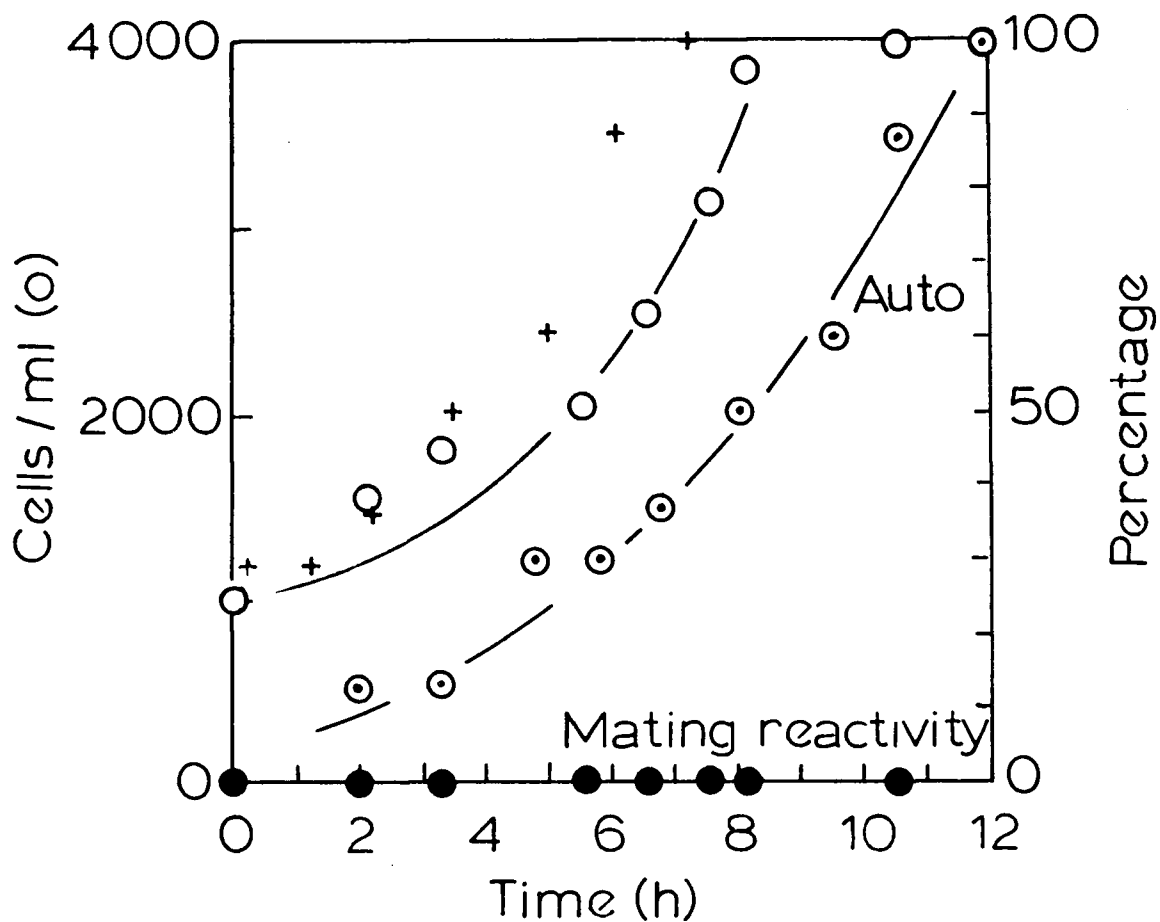
FIGURE 7:

Occurrence of autogamy in naturally starved "senescent" cells, mature for autogamy. Open circles: cell density; circles with central dot: percentage of cells showing macronuclear fragmentation; filled circles: percentage of cells showing mating reactivity; crosses: estimated percentage of cells completing the pre- autogamous fission.
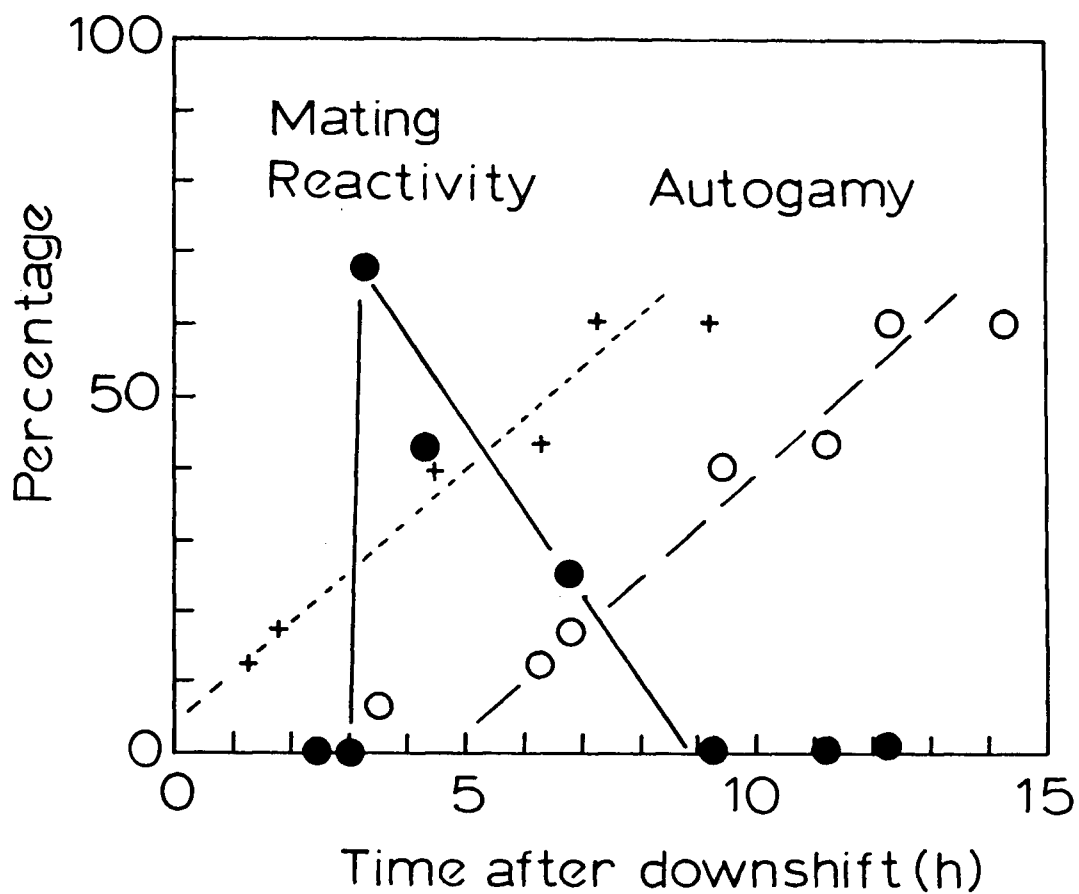
FIGURE 8:

Occurrence of autogamy and mating reactivity in an abruptly starved mature culture. Filled circles: percentage of control mating reactivity; open circles: percentage of cells showing macronuclear fragmentation; crosses: estimated percentage of cells completing the pre- autogamous fission.
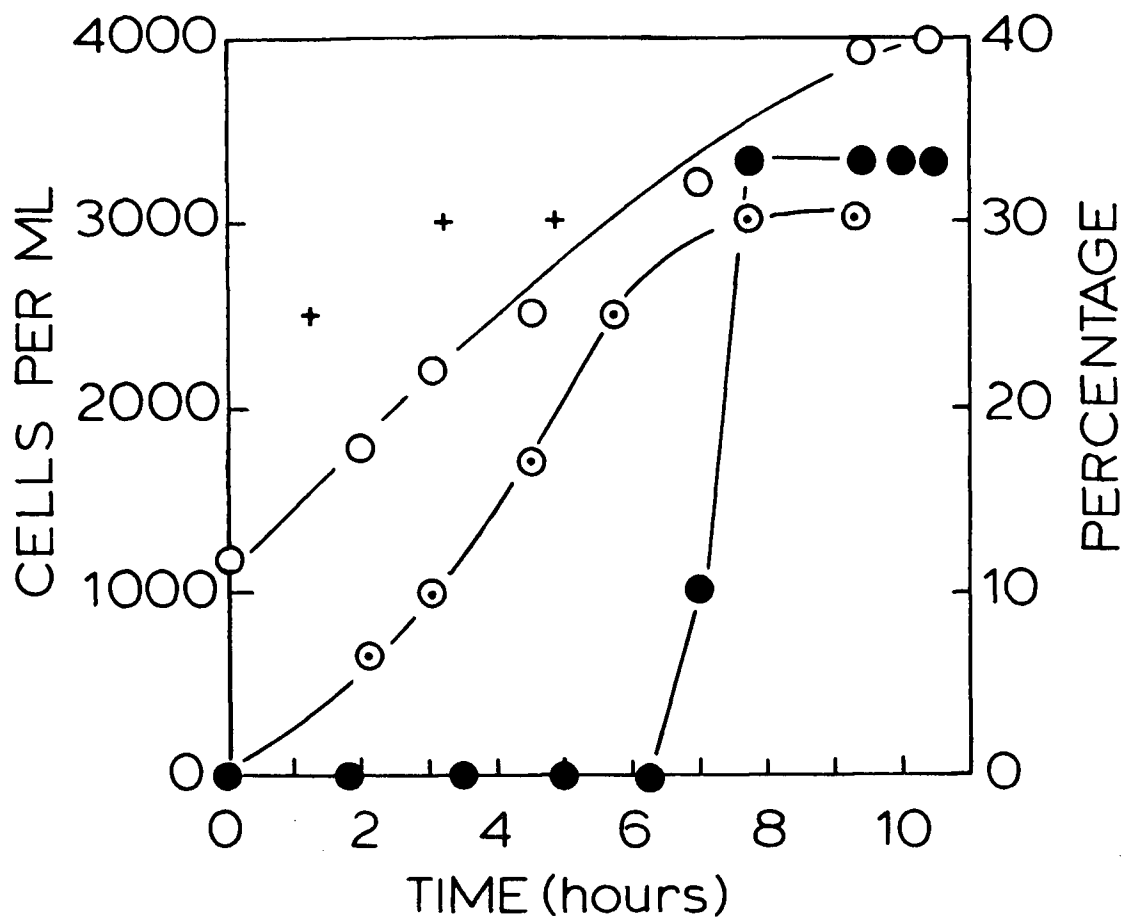
FIGURE 9:

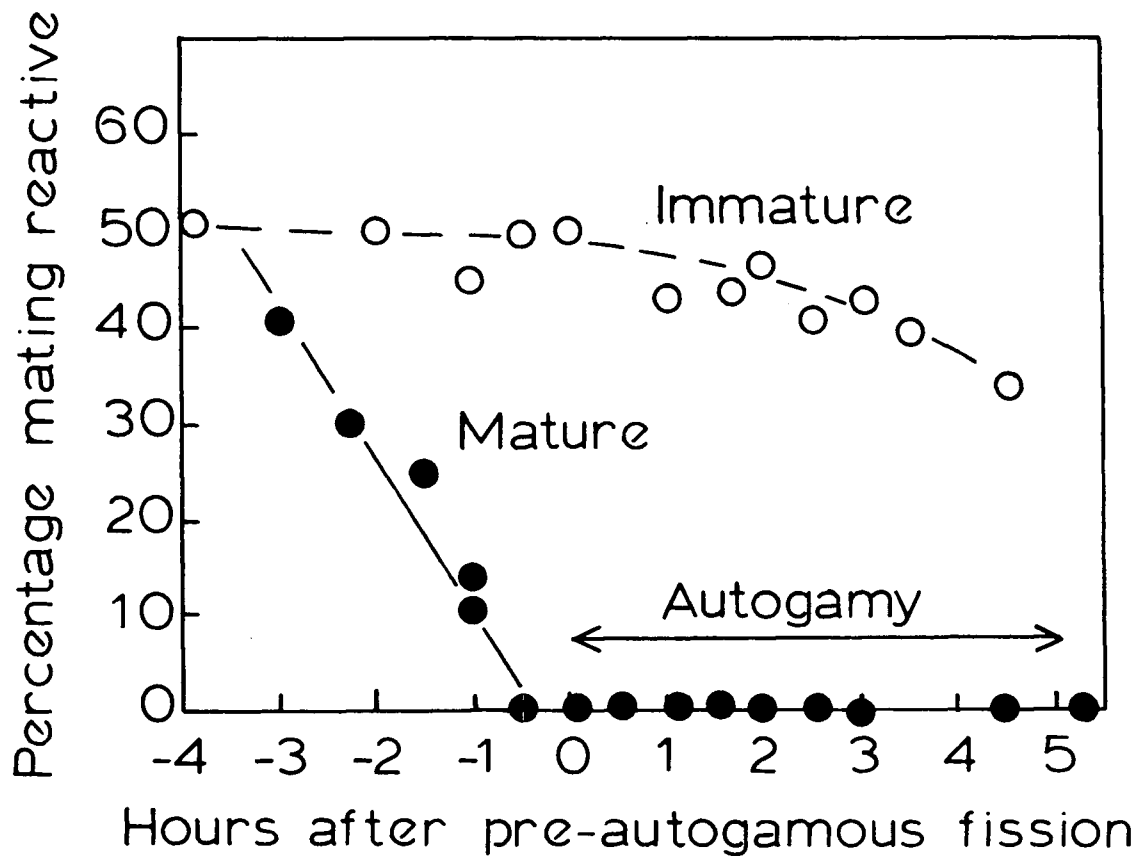Mating reactivity and occurrence of autogamy in a culture
containing both mature and immature cells. Filled circles:
mating reactivity; open circles with central dot: percentage of
cells showing macronuclear fragmentation; open circles: cell
density; crosses: estimated fraction of cells completing the
pre- autogamous fission.

FIGURE 10:

Mating reactivity during the first and second cell cycles after
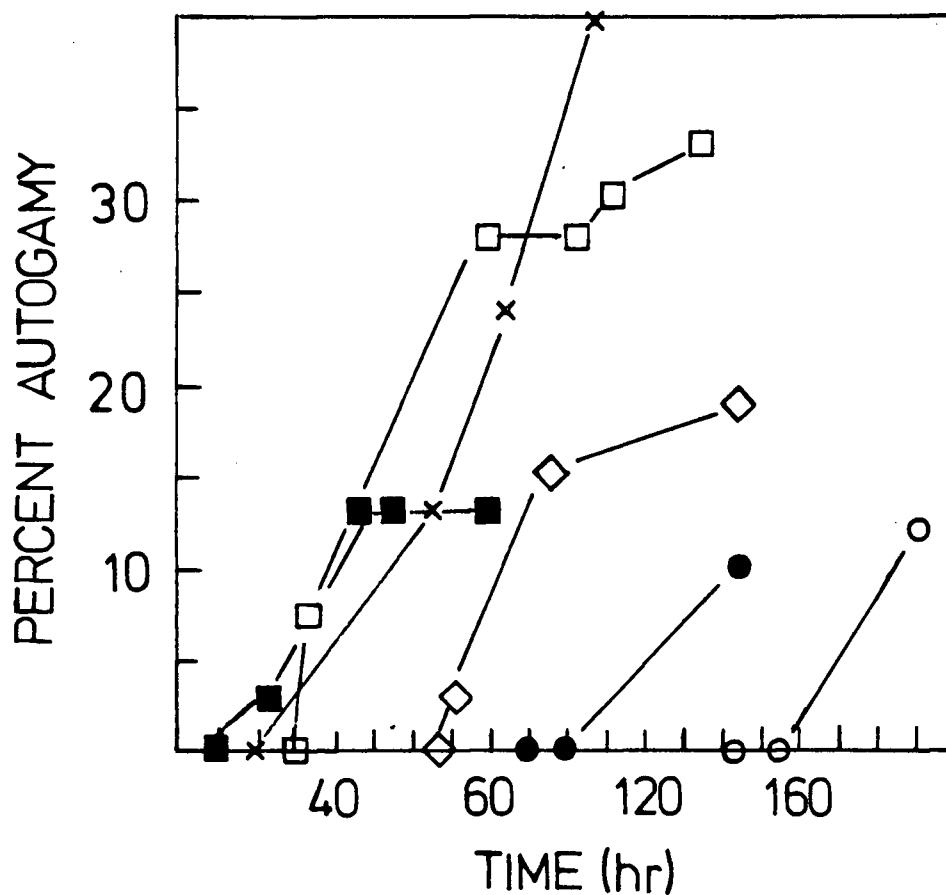nutrient downshift. Open circles: immature cells; filled
circles: mature cells.

FIGURE 11:

Percent autogamy as a function of time after mating reactivity onset in mature cultures of increasing clonal age. Open circles: 3 fissions old; filled circles: 5 fissions old; open diamonds: 7 fissions old; open squares: 9 fissions old; crosses: 11 fissions old; filled squares: 13 fissions old.
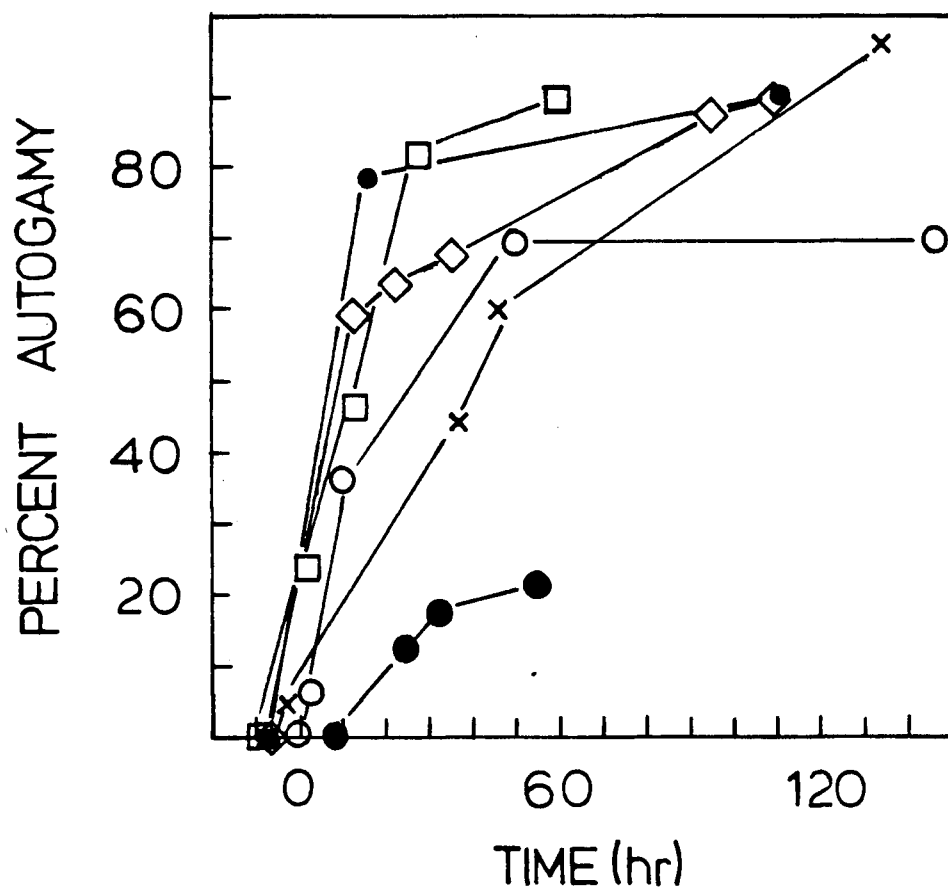
FIGURE 12:

Percent autogamy as a function of time after mating reactivity onset in mature cultures of increasing clonal age. Filled circles: 15 fissions old; open circles: 17 fissions old; crosses: 19 fissions old; open diamonds: 21 fissions old; filled circles: 23 fissions old; open squares: 25 fissions old.
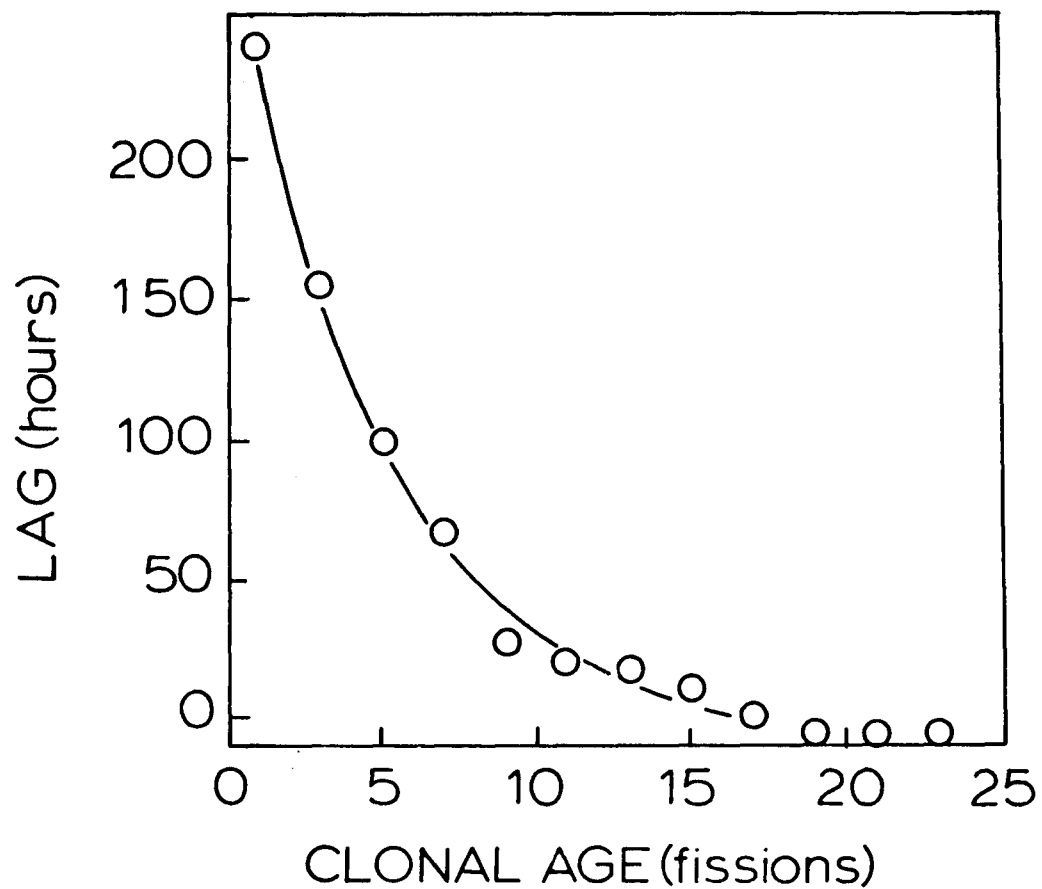
FIGURE 13:

Time between initiation of mating reactivity and initiation of macronuclear fragmentation in stationary phase cultures as a function of clonal age.
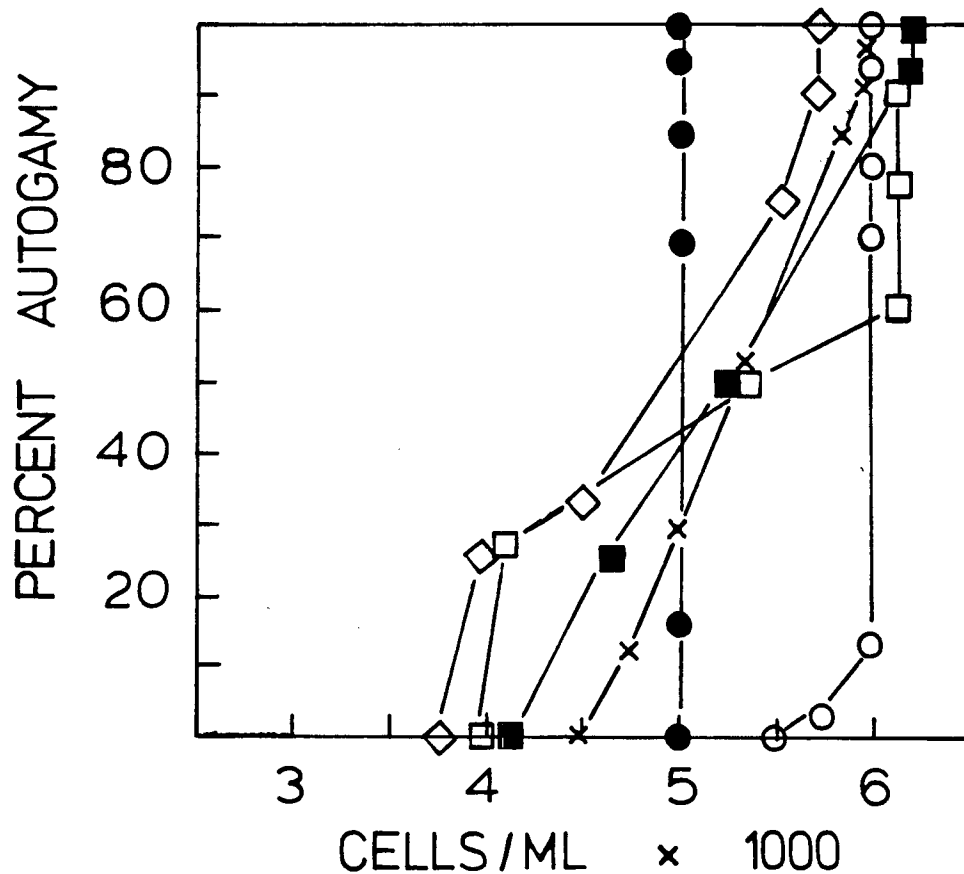
FIGURE 14:

Percent autogamy as a function of cell density in mature cultures
of increasing clonal age. Open circles: 9 fissions old; filled
circles: 11 fissions old; crosses: 13 fissions old; filled
squares: 15 fissions old; open squares: 17 fissions old; open
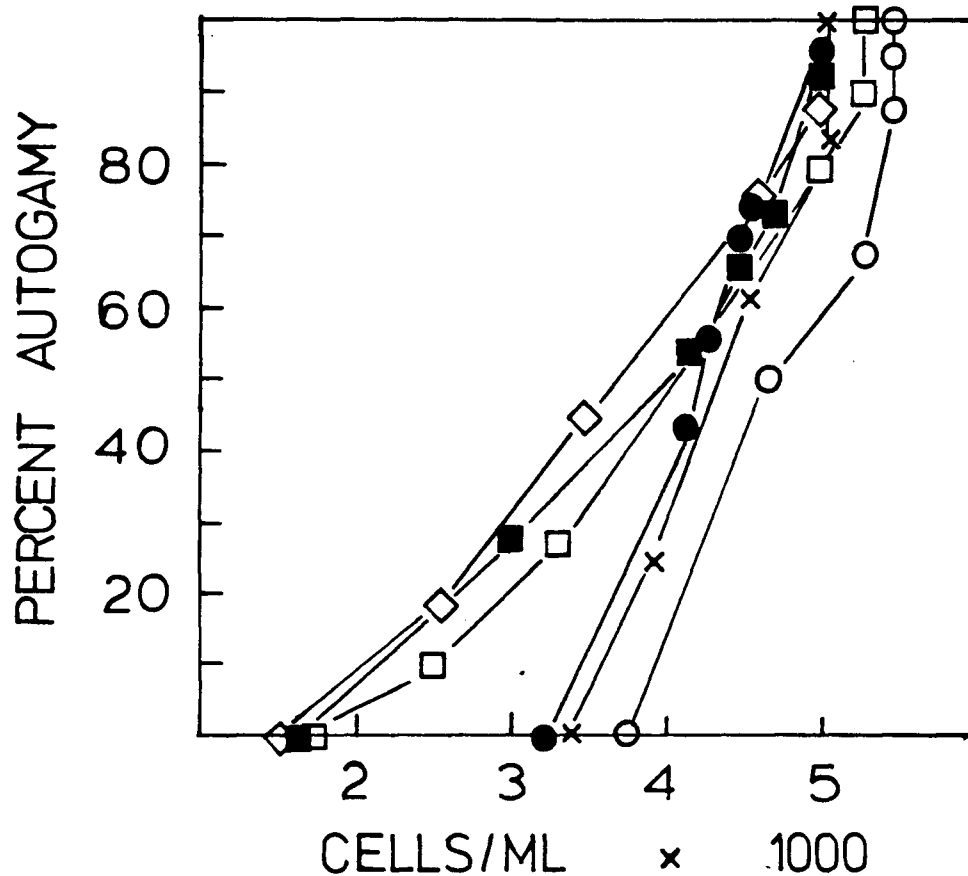diamonds: 19 fissions old.

FIGURE 15:

Percent autogamy as a function of cell density in mature cultures of increasing clonal age. Open circles: 21 fissions old; crosses: 23 fissions old; filled circles: 25 fissions old; open squares: 27 fissions old; filled squares: 29 fissions old; open diamonds: 31 fissions old.
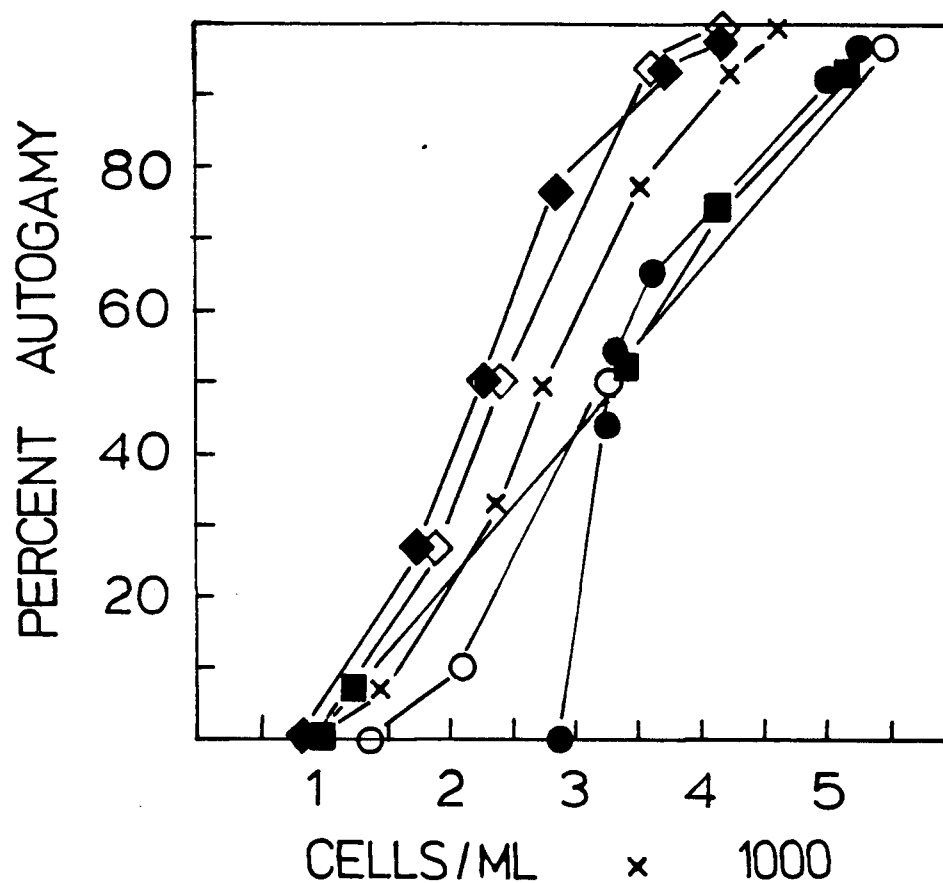
FIGURE 16:

Percent autogamy as a function of cell density in mature cultures of increasing clonal age. Filled circles: 33 fissions old; open circles: 35 fissions old; filled squares: 37 fissions old; crosses: 39 fissions old; open diamonds: 41 fissions old; filled diamonds: 41 fissions old (27 C control).
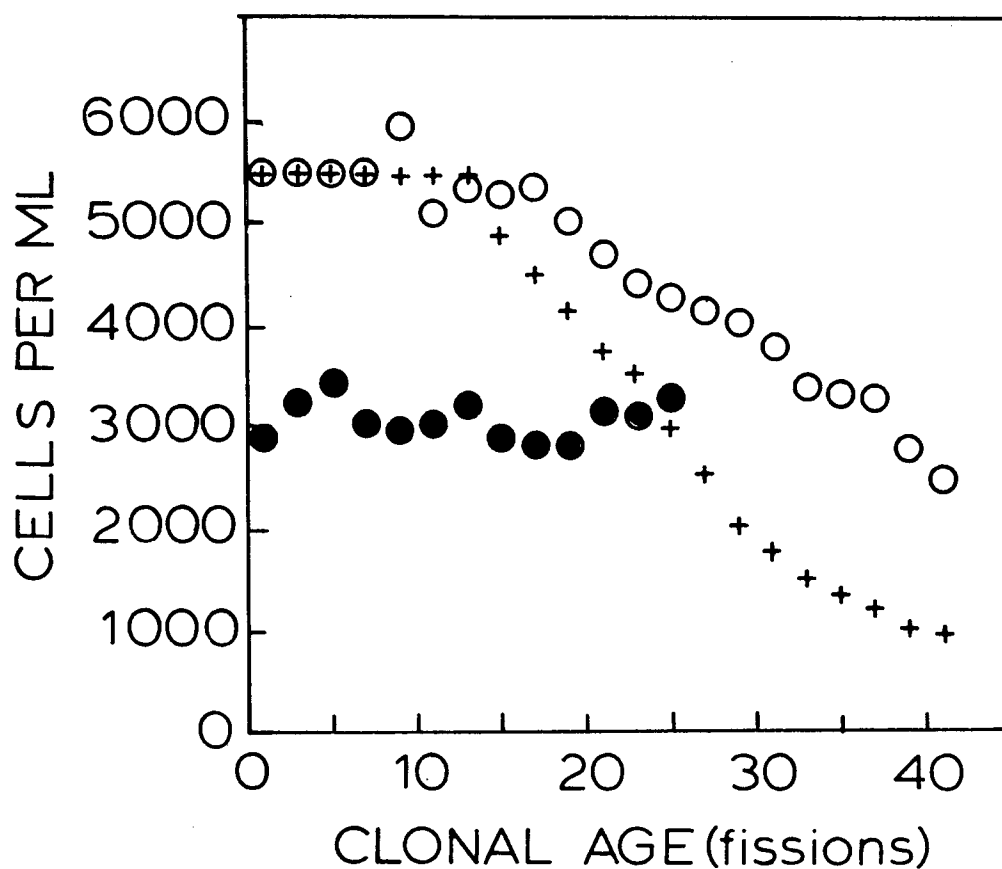
FIGURE 17:

Cell density at autogamy and initiation of mating reactivity as a function of clonal age. Open circles: cell density when 50% of cells showed macronuclear fragmentation; filled circles: cell density at onset of mating reactivity; crosses: estimated cell density at median time of commitment to autogamy.
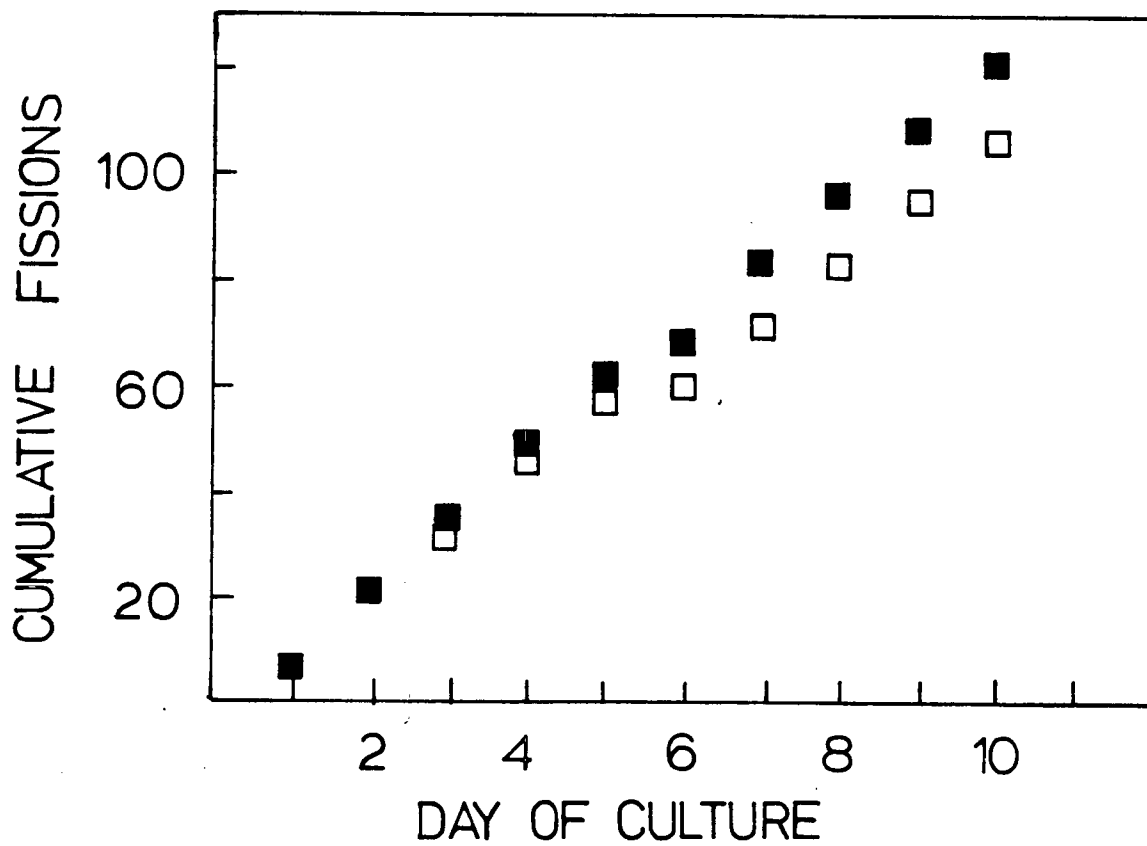
FIGURE 18:

Comparison of growth rates of even and odd mating types. Filled
squares: odd mating type; open squares: even mating type.

BIBLIOGRAPHY

Aufderheide, K.J. 1987. Clonal aging in *Paramecium tetraurelia*. II. Evidence of functional changes in the macronucleus with age. Mech Ageing Develop. 37, 265-279.

Beisson, J. and Y. Capdeville. 1966. Sur la nature possible des etapes de differenciation conduisant a l'autogomie chez *Paramecium aurelia*. C.R. Acad. Sci. 263, 1258-1261.

Berger, J.D. 1986. Autogamy in *Paramecium tetraurelia*: cell cycle stage specific determination of the process. Exp. Cell Res. 166, 475-486.

Berger, J.D. and AS-L Ching. 1988. The timing of initiation of DNA synthesis in *Paramecium tetraurelia* is established during the preceding cell cycle as cells become committed to cell division. Exp. Cell Res. 174, 355-366.

Bleyman, L.K. 1971. Temporal patterns in the ciliated protozoa. *In* Developmental aspects of the cell cycle. T.L. Cameron, G.M. Padilla, and A.S. Zimmerman. eds. Academic Press. New York. 67-91.

Brock, T.D. 1965. The purification and characterization of an intercellular sex- specific mannan protein from yeast. Proc. Natl. Acad. Sci. U.S.A. 54, 1104-1112.

Ching, AS-L and J.D. Berger. 1986. Control of cell division in *Paramecium tetraurelia*: effects of abrupt changes in nutrient level on accumulation of macronuclear DNA and initiation of cell division. Exp. Cell Res. 167, 191-202.

Crandall, M., L.M. Lawrence and R.M. Saunders. 1974. Molecular complementarity of yeast glycoprotein mating factors. Proc. Natl. Acad. Sci. U.S.A. 71, 26-29.

Fukushima, S. 1975. Clonal age and the proportion of defective progeny after autogamy in *Paramecium aurelia*. Genetics. 79, 377-382.

Haga, N. and K. Hiwatashi. 1981. A protein called immaturin controlling sexual immaturity in *Paramecium caudatum*. Nature (London). 289, 177-179.

Hiwatashi, K. 1969b. *Paramecium*. *In* Fertilization. C.B. Metz and A. Monroy. eds. Academic Press. New York. Vol. 2, 255-293.

Kitamura, A. and K. Hiwatashi. 1978. Are sugar residues involved in the specific cell recognition of mating in *Paramecium?* J. Exp. Zool. 203, 99-108.

Klass, M.R. and J. Smith-Sonneborn. 1976. Studies on DNA content, RNA synthesis and DNA template activity in ageing cells of *Paramecium aurelia*. Exp. Cell Res. 98, 63-72.

Kosciuszko, H. and S. Koizumi. 1983. Induction of autogamy by transfer of macronuclear chromatin in *Paramecium tetraurelia*. Exp. Cell Res. 146, 436-438.

Landis, W.G. 1986. The interplay among ecology, breeding system, and genetics in the *Paramecium aurelia* and *Paramecium bursaria* complexes. Progr. Protistol. 1, 225-245.

Landis, W.G. 1988. Ecology. In Paramecium. H.D. Gortz. ed. Springer- Verlag. New York. 419-436.

Metz, C.B. 1954. Mating substances and the physiology of fertilization in ciliates. *In* Sex in microorganisms. D.H. Wenrich. ed. Am. Assoc. Adv. Sci. Washington, D.C. 284-334.

Mikami, K. and S. Koizumi. 1983. Microsurgical analysis of the clonal age and the cell- cycle stage required for the onset of autogamy in *Paramecium tetraurelia*. Dev. Biol. 100, 127-132.

Miwa, I. 1979. Immaturity substances in *Paramecium primaurelia* and their specificity. J. Cell Sci. 38, 193-199.

Miwa, I., N. Haga, and K. Hiwatashi. 1975. Immaturity substances: material basis for immaturity in *Paramecium*. J. Cell Sci. 19, 369-378.

Miwa, I. and K. Hiwatashi. 1970. Effect of mitomycin C on the expression of mating ability in *Paramecium caudatum*. Jap. J. Genet. 45, 269-275.

Miwa, I. and N. Umehara. 1983. Conjugation between G_1 and G_2 phase cells in *Paramecium tetraurelia*. J. Protozool. 30, 271-274.

Miyake, A. and J. Beyer. 1973. Cell interaction by means of soluble factors (gamones) in conjugation of *Blepharisma intermedium*. Exp. Cell Res. 76, 15-24.

Peterson, E.L. and J.D. Berger. 1976. Mutational blockage of DNA synthesis in *Paramecium tetraurelia*. Can. J. Zool. 54, 2089-2097.

Rasmussen, L. 1967. Effects of metabolic inhibitors on
  Paramecium aurelia during the cell generation cycle.
  Exp. Cell Res. 48, 132-139.

Rasmussen, C.D. and J.D. Berger. 1984. A gene function required
  for cell cycle progression during the G$_1$ portion of the cell
  cycle and for maintenance of macronuclear DNA synthesis in
  Paramecium tetraurelia. Exp. Cell Res. 155, 593-597.

Schwartz, V. and H. Meister. 1973. Eine Altersveranderung des
  Makronucleus von Paramecium. Z. Naturforsch. 28c, 232.

Schwartz, V. and H. Meister. 1975. Aging in Paramecim: Several
  quantitative aspects. Arch. Protistenk Biol. 117, 85-109.

Smith-Sonneborn, J. 1981. Genetics and aging in protozoa.
  Int. Rev. Cytol. 73, 319-354.

Sonneborn, T.M. 1950. Methods in general biology and genetics
  of Paramecium aurelia. J. Exp. Zool. 113, 87-147.

Sonneborn, T.M. 1957. Breeding systems, reproductive methods,
  and species problems in protozoa. In The species problem.
  E. Mayr. ed. Am. Assoc. Adv. Sci. Washington, D.C.
  155-324.

Sonneborn, T.M. 1970. Methods in Paramecium research. Methods
  Cell Physiol. 4, 241-336.

Sonneborn, T.M. 1974. Paramecium aurelia. In Handbook of
  genetics II. R.C. King. ed. Plenum. New York. 469-594.

Sonneborn, T.M. and M.V. Schneller. 1960. Physiological basis
  of aging in Paramecium. In The Biology of Aging (American
  Institute of Biological Science Symposium 6).
  B.L. Strehler. ed. Waverly Press. Baltimore, MD.
  283-284.

Takagi, Y. and N. Kanazawa. 1982. Age- associated change in
  macronuclear DNA content in Paramecium caudatum. J. Cell
  Sci. 54, 137-147.

Watanabe, T. 1977. Ciliary membranes and mating substances in
  Paramecium caudatum. J. Protozool. 24, 426-429.

Wiese, L. 1974. Nature of sex specific glycoprotein agglutinins
  in Chlamydomonas. Ann. N.Y. Acad. Sci. 234, 383-395.