REGULAR CHANGES IN MATING TYPE COMPOSITION IN SELFING CULTURES AND IN MATING TYPE POTENTIALITY IN SELFING CARYONIDES OF *PARAMECIUM AURELIA*¹

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IN *Paramecium aurelia* mating type normally is inherited faithfully in the course of vegetative reproduction. As a result, all of the cells in a clone of *P. aurelia* usually are the same mating type—the same type as was the cell used to initiate that clone. Intracolonial mating normally will not occur because mating involves pairing of cells complementary in mating type (SONNEBORN 1937). Mating does occur, however, within certain exceptional clones (*selfers*). KIMBALL (1939) has shown (1) that the two cells comprising each mating pair found in such clones are, at the time of mating, expressing complementary mating types and (2) that subclones produced by single cells isolated from selfing clones frequently manifest selfing themselves. Selfing, then, seems to be a genetic property in which a single paramecium can produce vegetative progeny of both complementary mating types.

In *P. aurelia*, the mating type of a cell is controlled by its macronucleus (see BEALE [1954] and SONNEBORN [1947] for reviews). The nature of the macronuclear differences, heritable through asexual reproduction, which account for mating type differences is not understood except that they are *not genic* and that normally they arise very soon after the formation of new macronuclei following conjugation or autogamy. Genetic evidence indicates that the macronucleus is a compound structure (SONNEBORN 1947). In light of these facts, and on the basis of his studies with *selfers*, NANNEY (1953, 1954) has suggested that selfing reflects a macronuclear heterogeneity in which a fraction of the macronuclear subunits are determined to control one mating type while the remainder control another.

The purpose of this paper is to describe investigations of selfing clones in syngen 7 of *P. aurelia*. Regular changes in mating type composition within individual selfing, clonal cultures as well as regular changes in overall mating type composition within a series of successive subcultures have been discovered in this material. These findings may shed some light on the problem of the nature of the heritable macronuclear differentiation which controls mating type and on the mechanism of mating type expression. The pattern of mating type inheritance and the mechanism of mating type determination in syngen 7 of *P. aurelia* have been described previously (TAUB 1963).

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MATERIALS AND METHODS

Stocks: Stocks 38 and 227, both of syngen 7, were used in these investigations. Their origins, mating type potentialities and genotypes have been described in detail by TAUB (1963). In brief, stock 38 is a so-called "one-type" stock, cells of which are capable of expressing only mating type XIII. Stock 227 is a "two-type" stock. Cells of this stock can express either of the complementary mating types XIII and XIV. The difference in mating type potentiality between these two stocks has as its basis a difference in genotype at the *mt* (mating type) locus: stock 38 is homozygous for a gene, *mt*^{XIII}, while stock 227 is homozygous for an allele, *mt*^{XIII.XIV}. For this effect on mating type potentialities, *mt*^{XIII} is recessive to *mt*^{XIII.XIV}; heterozygotes can express either mating type XIII or XIV.

TAUB (1963) has shown that syngen 7 is a member of the group B syngens in which the differentiated macronucleus in two-type stocks controls not only the mating type of a cell, but also what conveniently can be described as a cytoplasmic "state" which directs the differentiation of the *new* macronuclei of the next sexual generation (i.e., the new macronuclei produced following the next conjugation or autogamy). The cytoplasmic state of a given parental cell normally is such that new macronuclei developing in that cytoplasm will be directed to differentiate to control the same mating type as did the macronucleus of that parent. For example, new macronuclei which replace the old, and "take over" the cytoplasm in exconjugant cells produced by mating type XIII cells of stock 227 will, under the influence of inherited (mating type XIII-determining) cytoplasm, differentiate very soon after conjugation to control type XIII. In a corresponding manner, mating type XIV cells of stock 227 transmit mating type XIV-determining cytoplasm to their cytoplasmic sexual progeny.

These alternative mating type-determining cytoplasmic states appear not to be autonomous (SONNEBORN 1954; TAUB 1963); rather, mating type determination from one sexual generation to the next within two-type stocks of group B syngens seems to involve a cycle of inherited cytoplasm determining the nucleus at the time of macronuclear differentiation and the macronucleus, or any of its daughter macronuclei, all bearing this heritable differentiation, controlling the mating type-determining state of the cytoplasm to be transmitted to the next sexual generation.

In these terms, which mating type an exconjugant or exautogamous syngen 7 clone homozygous for *mt*^{XIII.XIV} or heterozygous (*mt*^{XIII.XIV}/*mt*^{XIII}) will become depends on the cytoplasmic mating type-determining state controlled by the differentiated macronucleus of the preceding sexual generation. In contrast, exconjugant or exautogamous clones homozygous for *mt*^{XIII} always become mating type XIII regardless of the state of the inherited cytoplasm.

In addition to its effect on mating type potentialities, the *mt*^{XIII} gene also affects, in an unexpected manner, the mating type-determining state of the cytoplasm of any cell which carries it (TAUB 1963). Homozygotes for *mt*^{XIII}, which only express mating type XIII, and heterozygotes, regardless of whether they are expressing mating type XIII or XIV, always transmit mating type XIV-determining cytoplasm to their cytoplasmic progeny. Such progeny, therefore, become mating type XIV, if they are genically competent (i.e., are not homozygous for *mt*^{XIII}), even if their cytoplasmic parents expressed mating type XIII. Because this effect of the *mt*^{XIII} gene manifests itself in heterozygotes as well as in homozygotes, it is a *dominant* effect of *mt*^{XIII}.

Derivation of the caryonides used in this study: Selfers were obtained from among the progeny of two different crosses: (1) stock 227, mating type XIII × stock 227, mating type XIV (both homozygous for *mt*^{XIII.XIV}), and (2) stock 38 (homozygous for *mt*^{XIII} and therefore always mating type XIII) × stock 227, mating type XIV. Crosses were carried out according to the method described by SONNEBORN (1950).

Two genically identical, new macronuclei arise in each exconjugant cell as a result of a differentiation of two division products (macronuclear Anlagen) of the fertilization nucleus. These segregate one to each daughter cell at the first fission following conjugation, restoring the exconjugant progeny to the normal condition of a single macronucleus. (The two subclones produced by the first fission products of an exconjugant cell are referred to as "sister caryonides"; each caryonide consists of cells whose macronuclei all have descended from one of the two macronuclear Anlagen formed in an exconjugant. The four caryonides produced by a pair of co-conjugant cells constitute a unit known as a "syncclone".)

Culture medium: Sterile baked lettuce extract saturated with CaCO_3 was inoculated with *Aerobacter aerogenes* and incubated at 37°C for 12 to 24 hours before use. The pH was then adjusted to 6.5 to 7.0 with sterile $\text{Ca}(\text{OH})_2$. Details regarding the preparation of this medium have been given by SONNEBORN (1950).

Culture methods: Exconjugant caryonides all were maintained at 27°C by the method of daily isolation (SONNEBORN 1950). A thorough understanding of the consequences of this method is essential in interpreting the results presented in this communication. These methods, therefore, are now described in detail. Each of the two first fission products of each exconjugant cell were isolated into 0.2 ml medium in a depression slide, thus initiating the caryonidal lines of descent. The next day, for each culture, one randomly chosen cell was removed from among the vegetative progeny of the original isolated cell and transferred to another depression slide to initiate a 0.2 ml subculture. Eight-tenths ml of fresh medium were then added to the first culture to bring its final volume up to 1.0 ml. The first culture was referred to as "discard culture 1" or "D1". On the day following this, from the second culture (D2) a single cell would be chosen at random to initiate D3 and D2 would then be brought up to 1.0 ml with the addition of 0.8 ml fresh medium. This procedure would be repeated every day for a total of six days, producing seven successive subcultures (discard cultures) for each caryonide. The first discard culture grew more slowly at first than did any of the succeeding ones. On the first day following initiation of D1, therefore, it usually contained fewer animals (ca. 2 to 8) than did, at the corresponding time, any of the later cultures (ca. 16 to 32).

Within each discard culture (except for the first, which exhibited a slight lag), growth would proceed at an initial rate of about 4 to 5 doublings per day but eventually would slow down as the medium became exhausted. Growth usually ceased between two and three days after initiation of a discard culture, when the medium was exhausted. At this point the 1 ml culture normally would contain about 1000 to 1500 cells.

Mating type tests and observations for selfing: Throughout the terminal phase of growth and part of the stationary phase (*viz.*, between 56 hours and 96 hours after initiation) each culture periodically was tested to ascertain the mating type(s) present and examined for the appearance of selfing pairs. This 40-hour period was long enough to include essentially all of the "sexually reactive" phase of the culture during which time it is possible to ascertain whether a culture is a selfer and, if not, which of the two mating types, XIII or XIV, is being expressed.

Mating type tests were performed according to the recommendations of SONNEBORN (1950). A small sample (ca. 0.1 ml) of a culture to be tested was mixed with about 0.1 ml of a "tester" culture containing sexually reactive cells known to be mating type XIII. A second sample was mixed with a "tester" known to be type XIV and a third sample was used as a control. A culture would be classified as mating type XIII only if mating was observed in the mixture with the mating type XIV testers and not in the control nor in the mixture with type XIII testers. If mating occurred in the mixture with type XIII testers but not in the other mixture nor in the control, the culture was classified as mating type XIV. If mating was observed in the original culture, in the control sample and/or in both mixtures, it was classified as a selfer.

When mating type tests were carried out on selfing cultures, very often the mating reaction observed in a mixture with one of the tester cultures was more or less equal in intensity to the reaction in the corresponding control sample while the reaction in the mixture with the other tester culture was much more intense. Whether the difference in intensity of the mating reaction between the two different mixtures was due to differences in the inherent reactivity of the tester cultures themselves often could be ascertained by comparisons of the behavior in this respect of several different selfing cultures all tested with the same pair of tester cultures. When differences between the tester cultures could be ruled out, the difference between the two mixtures was interpreted to reflect the presence in the selfing culture being tested of one mating type in excess over the other. The excess type, of course, would be the one complementary to the mating type of the tester culture which the stronger mixture contained.

Except for the first discard culture, which did not regularly become sexually reactive, all discard cultures up to and including the seventh were examined and tested according to the following schedule: (a) Approximately 56 hours after initiation each culture was examined for the presence of mating pairs as an indication of selfing. (b) At approximately 72 hours, each

TABLE 1

Typical patterns of change of mating type composition within selfing discard cultures

Culture	Time of observation				Summary
	56 hours	72 hours	85 hours	96 hours	
39 F1-1A1-D2		XIII		XIII	XIII
39 F1-1A1-D4		XIII	Selfed	Selfed	XIII → Selfer
39 F2-3B2-D7		Selfed	Selfed	Selfed	Selfer
39 F1-14A1-D4		Selfed	Selfed	XIV	Selfer → XIV
39 F1-12B2-D2		XIV		XIV	XIV

culture was subjected to a mating type test as described above. (c) At about 80 to 85 hours each culture was again simply examined for selfing. (d) A final mating type test was carried out at about 96 hours.

This set of observations permitted each culture to be classified not only as to whether it was purely mating type XIII, purely type XIV or a selfer, but also, if it selfed during any one or more of the four observations, whether during some other part of the sexually reactive phase, only one mating type was being expressed.

The foregoing procedure led to the discovery of patterns of change in mating type composition of selfing cultures which are described below. Examples of typical observations for several of the more common patterns observed *within single discard cultures* are presented in Table 1. Patterns of change also are evident *from one discard culture to the next* when a comparison is made among the six successive discard cultures studied (D2-D7) in each caryonide (Table 2).

Cytoplasmic parentage: Depending on the cross involved, one of two methods was used to ascertain whether a selfing caryonide (one in which selfing occurred in at least one of the six successive discard cultures studied) was a cytoplasmic descendant of the mating type XIII or of the type XIV parent. In the cross of mating type XIII of stock 227 with type XIV of the same stock, cytoplasmic parentage was ascertained for selfing exconjugant caryonides by consideration of the mating types of the other three caryonides (its sister caryonide plus the two caryonides of the co-conjugant clone) belonging to the same synclone and by the knowledge that this cross *normally* results in a pair of sister caryonides both expressing mating type XIII and a co-conjugant pair both expressing type XIV (TAUB 1963). For example, a selfing caryonide was assumed to be descended from a mating type XIII cytoplasmic parent if its sister caryonide was type XIII or if both of the caryonides of the co-conjugant clone were type XIV.

The cytoplasmic parentage of selfing exconjugant caryonides produced by the cross of stock 38 to mating type XIV of stock 227 could not be ascertained by the method described above because normally this cross produces synclones in which all four caryonides are mating type XIV. In this case, advantage was taken of differences between the parents with respect to the specificity of their immobilization antigens (or serotypes) (see SONNEBORN 1950 for details regard-

TABLE 2

Typical patterns of mating type composition from one discard culture to the next in selfing caryonides

Caryonide	Discard culture number						Summary
	2	3	4	5	6	7	
39 F1-11A1	XIII	XIII	XIII	XIII	XIII	XIII	XIII
39 F1-12A2	XIII	XIII	XIII	XIII	Selfed	Selfed	XIII → Selfer
39 F2-4B2	Selfed	Selfed	XIV	XIV	XIV	XIV	Selfer → XIV
39 F2-3A1	XIV	XIV	XIV	XIV	XIV	XIV	XIV

ing this method). Parental immobilization serotype is retained in cytoplasmic progeny for several fissions following conjugation. In the 38×227 cross, therefore, it was possible to ascertain cytoplasmic parentage by observing the difference in effect on the two exconjugant cells of antisera homologous to one of the differing parental serotypes.

RESULTS

Frequency of selfing among exconjugant caryonides: In a large number of crosses of mating type XIII of stock 227 or stock 38 with type XIV of stock 227, the frequency with which at least one of the two caryonides of an exconjugant clone is a selfer averaged about 20% both for the descendants of the mating type XIII parent as well as for those of the type XIV parent. In slightly less than half of these exconjugant clones (or in about 8 to 10% of all such clones), both sister caryonides were selfers. In the remaining clones, only one of the two caryonides selfed while the other remained pure for the parental mating type.

Regular changes in the apparent mating type composition within selfing discard cultures: Within 392 exconjugant caryonides produced by crosses between mating types XIII and XIV of stock 227 and examined from D2 to D7 according to the procedures described in MATERIALS AND METHODS, a total of 277 discard cultures were found which selfed during at least one of the four observations made during the sexually reactive phase. These selfing cultures were distributed among 81 of these caryonides. Forty-six of these cultures selfed throughout their sexually reactive phase but the remaining 231 cultures tested as pure mating type XIII or pure type XIV during at least one observation and selfed during at least one other. The frequencies of the various observed patterns of change are summarized in Table 3. Cytoplasmic parentage was ascertained as described previously. Only rarely were dividing cells seen in these cultures during the sexually reactive phase (and even then only very early in the reactive phase) indicating that the changes recorded in Table 3 were occurring in the absence of significant growth. Note that in the 227×227 crosses more than 80% of all selfing discard cultures

TABLE 3

Changes of mating type composition within selfing discard cultures

Summary of change of mating type composition of culture	Number of discard cultures, classified according to cross and mating type of cytoplasmic parent				
	227 $\times$ 227			38 $\times$ 227	
	XIII	XIV	Not ascertainable	XIII	XIV
XIII $\rightarrow$ Selfer	92	35	13	4	1
XIII $\rightarrow$ Selfer $\rightarrow$ XIII	2	0	1	0	0
XIII $\rightarrow$ Selfer $\rightarrow$ XIV	0	3	0	1	2
Selfer $\rightarrow$ XIII	2	0	1	0	0
Selfer $\rightarrow$ XIV	5	61	13	74	80
XIV $\rightarrow$ Selfer	0	1	0	3	3
XIV $\rightarrow$ Selfer $\rightarrow$ XIV	0	2	0	4	3
No change; i.e., culture remained a selfer	9	37	0	10	16
Total	110	139	28	96	105

belonging to caryonides cytoplasmically descendent from the mating type XIII parent tended to test as pure type XIII early in the sexually reactive phase but to self later (XIII $\rightarrow$ Selfer). In contrast to this, only 25% of selfing discard cultures belonging to caryonides which descended from the type XIV parent in this cross showed this pattern. Rather, if they showed a change, such cultures tended to self early in the sexually reactive phase and tested as pure for mating type XIV later (Selfer $\rightarrow$ XIV). The majority of all changes observed, regardless of cytoplasmic parentage, tended to involve some part of the sequence: XIII $\rightarrow$ Selfer $\rightarrow$ XIV.

Table 3 also records the results obtained from the studies of selfing caryonides produced in crosses between stock 38 and mating type XIV from stock 227. The total of 201 selfing discard cultures found were distributed among 78 exconjugant caryonides. As in the previously described crosses, definite patterns of change were observed during the sexually reactive phase of most of these cultures. In contrast to the 227 $\times$ 227 crosses, however, there was no difference in the predominant pattern of change observed between cultures belonging to caryonides descended from the type XIII parent (stock 38) and those belonging to caryonides descended from the type XIV parent (stock 227). About 75% of all selfing discard cultures, regardless of cytoplasmic parentage, selfed early in the sexually reactive phase and tested as pure type XIV later (Selfer $\rightarrow$ XIV). The majority of the remaining cultures tended to self throughout their sexual reactivity.

In selfing discard cultures of both the 38 $\times$ 227 crosses and the 227 $\times$ 227 crosses the changes in mating type composition described above, usually tending towards an increase in the proportion of type XIV, often could be detected in more detail when data concerning the excess mating type present at the time of selfing were taken into account. For example, in cultures which had undergone a change from type XIII to Selfer, the excess type often was XIII throughout the selfing phase or, more rarely, XIII early in the selfing phase and XIV later. In cultures which selfed throughout the sexually reactive phase, the excess type usually remained constant but a few cultures were found in which the excess type was XIII early in the reactive phase and XIV later. In cultures which changed from Selfer to type XIV, the excess type present during the selfing phase usually was type XIV.

Regular changes in apparent mating type composition during the course of vegetative reproduction in selfing exconjugant caryonides: The selfing discard cultures produced by crosses between mating types XIII and XIV of stock 227 were distributed among 81 caryonides. Of these caryonides, 26 selfed in every discard culture from D2 to D7 during at least one of the four observations of each culture. In the remaining 55 caryonides, at least one discard culture (usually more) tested as pure for either mating type XIII or XIV throughout all four observations. These changes in apparent mating type composition from one discard culture to the next within a given caryonide seemed to follow regular patterns. In some selfing caryonides, the earlier discard cultures, D2 through D4, for example, would self during at least one of the four observations of each of these cultures but would behave as a pure type XIV caryonide in the remaining cultures examined (D5–D7 in this example). Other caryonides were pure for

TABLE 4

Changes of mating type composition of successive discard cultures

Summary of change of mating type composition from one discard culture to the next	Number of caryonides, classified according to cross and mating type of cytoplasmic parent				
	227 × 227			38 × 227	
	XIII	XIV	Not ascertainable	XIII	XIV
XIII → Selfer	25	3	5	0	0
XIII → Selfer → XIII	4	0	0	0	0
Selfer → XIII	2	0	0	0	0
Selfer → XIV	1	11	3	25	32
Selfer → XIV → Selfer	0	0	1	3	2
XIV → Selfer	0	0	0	2	1
XIV → Selfer → XIV	0	0	0	2	1
No change; i.e., caryonide remained a selfer	8	17	1	5	5
Total	40	31	10	37	41

mating type XIII in all of the earlier discard cultures but selfed during at least one of the four observations of each of the later discard cultures. These results are summarized in Table 4. Of interest here is the fact that the majority of selfing caryonides cytoplasmically descendant from the mating type XIII parent tended to test as pure type XIII in the earlier discard cultures but selfed in the later ones (XIII → Selfer) while those caryonides which descended from the type XIV parent, if they showed a change, tended to self in the earlier discard cultures and behave as pure type XIV in the later cultures (Selfer → XIV).

Regular changes in apparent mating type composition from one discard culture to the next also were observed among the selfing caryonides produced in the crosses between stock 38 and mating type XIV of stock 227. These results are summarized in Table 4. Among these selfing caryonides, unlike those produced in the 227 × 227 crosses, the cytoplasmic descendants of both the type XIII and the type XIV parent behaved in the *same* fashion: most of these caryonides, regardless of cytoplasmic parentage, tended to self in the earlier discard cultures but to test pure for type XIV in later cultures (Selfer → XIV). Furthermore, these caryonides underwent the change from Selfer to type XIV much more rapidly, i.e., in the course of fewer successive discard cultures, than did those selfing caryonides in the 227 × 227 crosses which changed from Selfer to type XIV.

In both the 227 × 227 crosses and the 38 × 227 crosses a definite correlation was observed between the particular pattern of change manifested by a selfing caryonide and the patterns of change observed within the selfing discard cultures of such a caryonide. The selfing discard cultures of XIII → Selfer caryonides usually tested pure for type XIII early in the sexually reactive phase and selfed later (i.e., showed the XIII → Selfer pattern of change in mating type composition). Occasionally, selfing cultures in these caryonides underwent a change from Selfer to type XIV but, in these cases, such cultures were the last of a

sequence of three to five selfing cultures, the earlier cultures of which changed from type XIII to Selfer or which selfed throughout the entire reactive phase. Selfing discard cultures of Selfer $\rightarrow$ XIV caryonides almost always selfed early in the sexually reactive phase and tested pure for type XIV later or selfed throughout the entire reactive phase.

DISCUSSION

The problem: Two distinct kinds of change in apparent mating type composition have been reported in this communication. They differ not only in how they are observed, which determines the nature of the time scale against which these changes are characterized, but probably also in the kinds of basic cellular phenomena which they reflect. Both kinds of change follow regular patterns and tend to involve some part of the sequence: XIII $\rightarrow$ Selfer $\rightarrow$ XIV. In the 227×227 crosses, the segment of this sequence manifested by a selfing caryonide and within the selfing discard cultures of such caryonides *appears* to be a function of parental mating type (XIII $\rightarrow$ Selfer if the cytoplasmic parent is type XIII and Selfer $\rightarrow$ XIV if the cytoplasmic parent is type XIV). In contrast, in the 38×227 crosses, most selfing caryonides and their selfing discard cultures, regardless of parental mating type, behave in the same fashion, *viz.*, as do the selfing cytoplasmic descendants of the mating type XIV parent in the 227×227 crosses (Selfer $\rightarrow$ XIV).

Of the two kinds of change in apparent mating type composition, the first kind, observed within individual discard cultures, probably reflects changes in the cell's physiology concomitant with the change from the nonreactive to the sexually reactive state; i.e., these are changes reflecting the process of *expression of mating type*. The second kind of change, observed when comparing successive discard cultures, may reflect regular changes in the *mating type potentialities* of the individual cells of the caryonide occurring along with, and very likely as a consequence of, growth and cell division. The arguments supporting these conclusions and their implications are developed below. It is necessary first to point out the nature of the difference, with respect to time scale, between the two kinds of change observed.

The time scales of the observations: The changes occurring within individual discard cultures take place during and after the beginning of the stationary phase of the growth cycle of the culture and in the absence of any significant amount of cell division. In these cases, therefore, the same population of cells is behaving as a selfer at one time but as pure for mating type at another. In contrast to this situation, the changes occurring from one discard culture to the next can only occur as cell division proceeds within the caryonide because a succession of discard cultures represents a periodic sampling of a continuously growing and dividing line of descent. For this kind of change, therefore, selfing must occur at some time within a given population of cells representing one point in a caryonidal line of descent but not at all in a different population of cells representing another point in the same line of descent. Briefly, then, the first kind of change

occurs in the absence of growth and division, the second, concurrent with, and perhaps as a consequence of, growth and division.

The cellular basis for the changes in mating type composition within individual discard cultures: By isolating, from selfing cultures, pairs of cells in stages of the mating reaction early enough to permit easy separation of the cells and ascertaining their mating types by observing their reactions when mixed with cells of known type, KIMBALL (1939) has shown for syngen 1 that each selfing pair consists of one cell of mating type I and one of type II. He never found any evidence to indicate that selfing involves the occurrence of any cells capable of reacting both with mating types I and II. Similar studies of selfing cultures in syngen 7 (TAUB, unpublished) have shown, in a manner analogous to the situation in syngen 1, that selfing pairs consist of one mating type XIII cell and one type XIV cell. Therefore, any hypothesis designed to explain the changes in mating type composition observed in selfing cultures of syngen 7 need not presume the presence of "nonspecific" cells capable of reacting both with types XIII and XIV.

The changes observed in apparent mating type composition within individual selfing discard cultures of syngen 7 necessarily represent changes only in the composition of the sexually reactive fraction of the whole population. (This fraction may vary from a few percent of the cells present in the culture to almost 100%.) At the level of the individual reactive cells in selfing cultures, the occurrence of either one or both of the following phenomena may account for the observed changes:

(1) There may be two subpopulations among those cells destined to become sexually reactive: (a) a subpopulation which will express only mating type XIII, and (b) a subpopulation which will express only mating type XIV. Changes in mating type composition would occur in cultures containing two such differing subpopulations if the two tended to become sexually reactive at different times. For example, if the type XIII cells became reactive before the XIV's, the culture would change from one which tested as type XIII (i.e., gave a reaction only when type XIV testers were added) to one which tested as type XIV (i.e., reacted only with type XIII testers). If the sexually reactive phases of the two subpopulations overlapped, a selfing phase would occur between the time a culture tested as pure type XIII and the time it tested as pure type XIV. If one subpopulation contained many more cells than did the other, or was reactive for a longer period of time than the other, the sexually reactive phase of the first population might be sufficiently long to overlap completely the reactive phase of the other resulting in a culture in which a selfing phase is not interposed between pure type XIII and type XIV phases, but rather is either preceded or followed by only one of these phases. This situation could account for the majority of changes observed (XIII $\rightarrow$ Selfer and Selfer $\rightarrow$ XIV) if the type XIII subpopulation usually became reactive before the type XIV subpopulation. Sufficient overlap of the reactive phases of the two subpopulations could result in cultures which self throughout the entire reactive period.

(2) The changes in mating type composition which occur within individual selfing discard cultures may represent changes in mating type specificity occurring within individual cells. Some or all of the cells in such cultures may express one mating type at one time and the other later. For example, in a culture originally testing as type XIII, a change in some fraction of the reactive cells from this type to type XIV would result in the frequently observed XIII $\rightarrow$ Selfer pattern. A similar change, but in a large proportion of the cells, or in a culture which contained some type XIV cells from the onset of the sexually reactive phase, would result in the other common pattern of change, Selfer $\rightarrow$ XIV. The patterns of change in mating type composition actually observed would require that most, if not all, cells changing type would be changing from XIII to XIV and not *vice versa*.

It is not yet known for syngen 7 whether single cells can change type as required by the second hypothesis. Work in progress is designed to test this possibility. However, BLEYMAN (1963) has reported comparatively brief, transient changes in mating type of individual cells in selfing cultures of syngen 5, *P. aurelia*, from type X to IX and back to X again and SONNEBORN (1957) and BARNETT (1961, 1966) have reported periodic (circadian), reversible changes in the mating type of individual cells in syngen 2 of *P. multimicronucleatum*.

It is interesting to note in reference to the selfing cultures of syngen 7, that regardless of whether there are two populations of cells or whether single cells can change mating type, type XIII tends to precede type XIV. This sequence is suggested by the fact that the predominant changes observed were XIII $\rightarrow$ Selfer and Selfer $\rightarrow$ XIV and by the evidence which indicates that selfing is the result of the simultaneous presence of cells expressing type XIII and cells expressing type XIV. It is also consistent with the notion, based on genetic evidence, that the mating type XIII substance is a precursor of the type XIV substance (TAUB 1963).

The role of parental cytoplasm in determining the patterns of change observed in selfing discard cultures and caryonides: Almost all of the selfing caryonides and their individual selfing discard cultures described in the RESULTS section which showed any changes could be classified either as XIII $\rightarrow$ Selfer or Selfer $\rightarrow$ XIV. Which of these two patterns a particular discard culture would manifest was very strongly correlated with the kind of pattern of change displayed by the caryonide to which that culture belonged. For example, the selfing discard cultures of a caryonide which displayed the XIII $\rightarrow$ Selfer pattern most often changed from XIII to selfers themselves.

Among the progeny of the 227×227 crosses, the particular pattern of change which characterized a selfing caryonide and its selfing discard cultures was correlated strongly with parental mating type. In these crosses, the type XIII cytoplasmic parent tended to produce the XIII $\rightarrow$ Selfer progeny and the type XIV cytoplasmic parent, the Selfer $\rightarrow$ XIV progeny. In the 38×227 crosses there was no such correlation; regardless of parental mating type, all of those selfing progeny of this cross which did show a change tended to change from selfer to XIV. The apparent difference in the results of these two crosses can be

understood by a consideration of the relationships between the patterns of change manifested by the progeny and the cytoplasmic mating type-determining states of the parents. Mating type XIII of stock 227 transmits type XIII-determining cytoplasm to its progeny while both stock 38 (mating type XIII) and mating type XIV of stock 227 transmit type XIV-determining cytoplasm (TAUB 1963). It appears, therefore, that the mating type-determining state—not the mating type itself—of the cytoplasmic parent plays an important role in determining the pattern of change which the progeny will manifest. Selfing progeny descendent from a cytoplasmic parent which transmits type XIII-determining cytoplasm (mating type XIII of stock 227) tend to show the XIII → Selfer pattern while such progeny descendent from a cytoplasmic parent which transmits type XIV-determining cytoplasm (stock 38 or type XIV of stock 227) tend to show the Selfer → XIV pattern. The possible mechanisms by which parental cytoplasm might determine these patterns is discussed below.

The question of macronuclear heterogeneity and its relation to the changes observed in selfing cultures and in selfing caryonides: On the basis of several lines of evidence indicating that the macronucleus is a compound structure composed of many subnuclei, NANNEY (1953, 1954) suggested that selfing reflects a macronuclear heterogeneity in which a fraction of the subnuclei control one mating type while the remainder control the other. Random segregation of these subnuclei into daughter macronuclei could result in a corresponding heterogeneity among the cells of a clone, such heterogeneity being expressed either as selfing within a culture or as a change in mating type composition from one subculture to the next. Furthermore, such segregation could often result in daughter macronuclei uniform with regard to the mating type controlled by their subnuclei. The hypothesis of random assortment during division of differently determined subnuclei in selfers has been given strong support by a report of ALLEN and NANNEY (1958) in which detailed kinetic data concerning the rate of production of subclones pure for mating type by selfing caryonides of syngen 1 of *Tetrahymena pyriformis* have been shown to fit several critical predictions based on this model.

Many of the details as set forth earlier in this communication concerning selfing in syngen 7 of *P. aurelia* readily can be explained in terms of macronuclear heterogeneity. Several observations may deviate sufficiently from the most likely expectations based on the assumption of such heterogeneity, however, to justify a consideration of additional or alternative mechanisms. In terms of the details of selfing in syngen 7, the following discussion will first show to what extent the assumption of heterogeneity for predetermined macronuclear subunits can explain the observations and will then briefly point out a modification of this assumption possibly required to explain the situation in syngen 7.

Individual selfing discard cultures in syngen 7 are heterogeneous with respect to their mating type compositions in the sense that such cultures contain some cells expressing mating type XIII and others expressing type XIV (TAUB, unpublished). Such heterogeneity may reflect differences among the cells of the culture in terms of the subnuclear compositions of their macronuclei which, in

turn, may have resulted from segregation of differently determined subnuclei in the macronucleus of the cell used to initiate that culture. Because of the possibility (described above) that single cells in selfing cultures can change mating type from XIII to XIV, however, it is conceivable that there are no macronuclear differences among the cells of these cultures but rather that their mating type heterogeneity is due simply to a lack of synchrony (itself not due to macronuclear heterogeneity) within a population of cells all of which express type XIII first but change to type XIV in time. This possibility can be ruled out if it can be shown that selfing cultures contain at least some cells which do not change type.

While discussing mating type inheritance at conjugation in selfers of syngen 4 of *P. aurelia*, NANNEY (1957) raised the possibility that a threshold exists in terms of the fraction of macronuclear subnuclei controlling a given mating type which must be exceeded for the cell to express that type. If selfing cultures of syngen 7 contain cells which differ in terms of the subnuclear compositions of their macronuclei, then those cells whose macronuclei contain subthreshold proportions of subnuclei differentiated to control type XIV will express type XIII and those with suprathreshold proportions will express type XIV. (If such cultures do contain cells which can change type from XIII to XIV, these may be cells whose macronuclei contain proportions of subnuclei at or near the threshold value.)

Turning now to the caryonides which the selfing cultures represented, many of the selfing caryonides described in this communication displayed a progressive change in overall mating type composition from one discard culture to the next. These differences in the mating type compositions of different discard cultures in the same caryonide must reflect differences in the cells used to initiate these cultures. Within most caryonidal lines of descent which showed changes in mating type composition from one discard culture to the next, the single cells used to initiate each of the successive discard cultures seemed to change, from one isolate to the next, in such a way that resulted in an increasing ability to produce daughter cells capable of expressing mating type XIV. Assuming that selfing reflects macronuclear heterogeneity, the successive isolates representing these caryonidal lines of descent must have contained macronuclei with increasing proportions of subnuclei differentiated to control mating type XIV. Such an apparent unidirectional change during the course of cell division in the caryonide may come about as the result either of (1) the random assortment of subnuclei in a caryonidal line of descent which was initiated by a cell whose macronucleus contained a very large proportion of subnuclei predetermined to control mating type XIV and a small minority of subnuclei predetermined to control type XIII, or (2) the persistence through a number of cell divisions of *undetermined* subnuclei which, under the later influence of the same cytoplasmic factors known to direct early macronuclear differentiation in syngen 7, eventually become fixed to control mating type XIV.

Because of the established precedence for a mechanism involving the random assortment of predetermined subnuclei, the first possibility requires serious consideration. The observed changes in mating type composition from one discard

culture to the next in syngen 7 could be explained by assuming (1) that all subnuclei are predetermined to control one or the other of the two mating types, XIII and XIV, (2) that a threshold exists which, in terms of the relative proportions of type XIV subnuclei, must be exceeded for a cell to express mating type XIV, (3) that a cell will express type XIII when this threshold is not exceeded, (4) that subnuclei replicate true to type and randomly segregate at cell division, and (5) that the threshold level required for the expression of type XIV is so high that any culture initiated by a cell whose macronucleus contained (a) less than perhaps 90% type XIV subnuclei will be very unlikely to contain any type XIV cells and would, therefore, be pure for type XIII and (b) less than close to 100% but more than 90% type XIV subnuclei would be very likely to contain, as a result of segregation, some cells with subthreshold levels of type XIV subnuclei and some with suprathreshold levels and would, therefore, self. Such an asymmetry with respect to the actual threshold value required for the expression of a given mating type, although not necessarily as extreme, was suggested by NANNEY in his discussion (1957) of mating type inheritance in exconjugant clones derived from selfers in syngen 4.

The assumptions listed above permit an interpretation of the observed changes in mating type composition from one discard culture to the next in syngen 7 in terms of the random assortment of differently determined subnuclei in the course of the series of cell divisions which represent the caryonidal lines of descent. These assumptions imply that, in *selfing caryonides*, the subnuclear input ratio (XIV subnuclei/XIII subnuclei) would have to be so high as to make very likely stabilization in the direction of mating type XIV. This, of course, is what is observed, in the sense that selfing caryonides tend very strongly to manifest an increase in the proportion of type XIV cells from one discard culture to the next.

It was pointed out above that mating type-determining factors transmitted by the cytoplasmic parent play an important role in determining whether a selfing caryonide will show the XIII $\rightarrow$ Selfer or the Selfer $\rightarrow$ XIV pattern of change in mating type composition. If selfing in syngen 7 is due to macronuclear heterogeneity for predetermined subnuclei, then which of these two patterns will be manifested by a selfing caryonide should be a function of the input ratio of type XIV to type XIII subnuclei. This input ratio would be fixed very early in the life of the caryonide, presumably by the time macronuclear differentiation is completed (before the first division of the exconjugant cell from which the caryonide descended), and, to account for the role of parental cytoplasm, would be determined by inherited cytoplasmic factors. Specifically, selfing caryonides descendent from cytoplasmic parents which transmit type XIII-determining cytoplasm would have lower input ratios of type XIV to type XIII subnuclei than would such caryonides descendent from parents which transmit type XIV-determining cytoplasm.

The preceding analysis was based on the assumption that selfing in syngen 7 of *P. aurelia* could be explained in terms of the random assortment of predetermined subnuclei—an assumption which was very successful in explaining the details of selfing in syngen 1 of *T. pyriformis* (ALLEN and NANNEY 1958).

While there are several differences between the patterns of change observed in these two species, most of these can be resolved by the assumption that the threshold level of type XIV subnuclei required for the expression of mating type XIV is extremely high. Examples of these differences are: (1) Most, if not all, selfing caryonides of syngen 1 of *T. pyriformis* self in early discard cultures but produce pure (nonselfing) cultures later, whereas many selfing caryonides in syngen 7 of *P. aurelia*, in particular, almost all such caryonides derived from the type XIII cytoplasmic parent in the 227×227 crosses are pure for mating type (XIII) in early discard cultures but self in later ones; (2) While different subclones pure for mating type obtained from a single selfing caryonide in syngen 1 of *T. pyriformis* can be any one of several different mating types, pure subclones derived from selfing caryonides in syngen 7 of *P. aurelia* almost always are the same mating type, viz., XIV; (3) In a preliminary experiment (TAUB, unpublished), all of 86 subclones of a selfing caryonide of syngen 7 of *P. aurelia*, in spite of attempts to select for selfing, rapidly (within no more than 15 to 20 divisions) became pure for mating type XIV. While ALLEN and NANNEY did report some cases of initially rapid rates of stabilization, they always were able to obtain, by selection, some persistently selfing subclones.

The first two of these three discrepancies perhaps more readily can be reconciled with the model under question than the third. The need to examine alternative models is also evident, however, from a consideration of differences between certain selfing progeny produced by the 227×227 crosses and those produced by the 38×227 crosses. Of 31 selfing exconjugant caryonides cytoplasmically descendent from the mating type XIV parent in the 227×227 crosses, 17 (55%, Table 4) remained selfers throughout the sequence of six discard cultures studied and the remaining 14 (11 Selfer $\rightarrow$ XIV and three XIII $\rightarrow$ Selfer) showed changes in mating type composition in the direction of increasing proportions of type XIV. Among the 78 selfing exconjugant caryonides produced in the 38×227 crosses, where there were no differences in the patterns of change between the progeny of the two parents, only 18 (23%, consisting of ten clearly persistent selfers and eight probably persistent selfers (Selfer $\rightarrow$ XIV $\rightarrow$ Selfer and XIV $\rightarrow$ Selfer)) remained selfers and 60 manifested increasing proportions of type XIV (57 Selfer $\rightarrow$ XIV and three XIV $\rightarrow$ Selfer $\rightarrow$ XIV). All the progeny under consideration here descended from cytoplasmic parents which transmit type XIV-determining cytoplasm. The increase in frequency (or rate) with which progeny of the 38×227 crosses undergo the change from selfer to type XIV, however, probably is not due to any peculiarity of the type XIV-determining cytoplasm transmitted by the stock 38 parent because the cytoplasmic progeny of the other parent in this cross, mating type XIV of stock 227 (a parent involved in both the 38×227 and 227×227 crosses), manifests this increase as well.

The difference in these frequencies between the progeny of the two crosses is more likely related to genotypic differences. Because stocks 38 and 227 are probably quite unrelated, there could be many such differences between them. One difference known to be related to mating type determination is at the *mt* locus where stock 38 is homozygous for *mt*^{XIII} and stock 227, for *mt*^{XIII,XIV}. In terms of

relevant genotypic differences one possible explanation for the difference between the two crosses is that heterozygous macronuclear Anlagen (produced in the 38×227 crosses) may respond to inherited type XIV-determining cytoplasm in such a way that the resulting input ratio of type XIV to type XIII subnuclei in selfers tends to be greater than in homozygous ($mt^{XIII,XIV}/mt^{XIII,XIV}$) macronuclear Anlagen (produced in the 227×227 crosses). A second possibility is that in heterozygotes the threshold level of type XIV subnuclei required for the expression of mating type XIV may be lower than in homozygotes for $mt^{XIII,XIV}$. A third possibility is based on the fact that the mt^{XIII} gene has a dominant effect resulting in the type XIV-determining cytoplasmic state in any cell which carries it, regardless of the mating type of that cell (TAUB 1963). In the selfing progeny of the 38×227 crosses, therefore, *all* subnuclei, regardless of which mating type they control, presumably "produce" type XIV-determining cytoplasm, whereas, in the selfing progeny of the 227×227 crosses, presumably only those subnuclei controlling type XIV would do so. A relatively higher "level" of type XIV-determining cytoplasm in the selfing progeny of the 38×227 crosses could account for the fact that these progeny change from selfer to type XIV more frequently and rapidly than do the selfing progeny of the type XIV parent in the 227×227 crosses if it is assumed that, in selfing caryonides, undifferentiated subnuclei or subnuclei originally controlling type XIII can, under the influence of type XIV-determining cytoplasm, change to control type XIV. Because pure (nonselving) type XIII caryonides bearing type XIV-determining cytoplasm can be obtained (e.g., type XIII heterozygotes at the mt locus, TAUB 1963), the ability of subnuclei originally controlling type XIII to change to XIV would have to be unique to selfing caryonides. The possibility that selfing is due to a prolonged delay or a change in the differentiation of at least some subnuclei might account for the overall pattern of change in mating type composition observed in syngen 7 where cytoplasmic factors are already known to affect early macronuclear differentiation.

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SUMMARY

Regular changes in mating type composition of selfing cultures of syngen 7, *P. aurelia*, have been found. These changes all tend towards an increase in the proportion of type XIV cells. Two main categories of changes were found: (1) XIII $\rightarrow$ Selfer and (2) Selfer $\rightarrow$ XIV. Which of these two kinds of change was manifested by a selfing culture depended on the cytoplasmic mating type-determining state of the parent of the caryonide to which the selfing culture belonged. Parents with type XIII-determining cytoplasm (mating type XIII of stock 227) gave rise to progeny, cultures of which underwent the change from XIII to Selfer, while parents with type XIV-determining cytoplasm (stock 38 and mating type XIV of stock 227) gave rise to progeny, cultures of which underwent the other kind of change, *viz.*, Selfer $\rightarrow$ XIV.—Regular changes also were found in the mating type potentialities of single cells representing a series of points along a

caryonidal line of descent. These changes, too, tended towards an increase in the proportion of mating type XIV and fell into two main categories, (1) XIII $\rightarrow$ Selfer and (2) Selfer $\rightarrow$ XIV. Which pattern of change a given caryonide manifested was related to the cytoplasmic mating type-determining state of the parent of the caryonide in the same fashion as were the changes in mating type composition observed within the selfing cultures of these caryonides.—In general, the pattern of change in mating type composition of a selfing culture tended very strongly to reflect the change in mating type potentiality which was occurring in the caryonide to which the selfing culture belonged.—The selfing caryonides used in these studies were obtained from crosses of mating type XIII of stock 227 to type XIV of the same stock and of mating type XIII, stock 38 to type XIV of stock 227. Stock 38 is homozygous for *mt*^{XIII} and stock 227, homozygous for *mt*^{XIII,XIV}.—The changes in mating type composition of selfing cultures may be due either (1) to the presence of two kinds of cells in selfing cultures—type XIII's and type XIV's—the former becoming sexually reactive before the latter, or (2) to the presence of some cells which express type XIII at one time but are type XIV later. These changes are interpreted to reflect the process of mating type expression, perhaps representing the possibility that the mating type XIII substance is a precursor of the type XIV substance.—The changes in mating type potentiality in the course of growth and cell division within selfing caryonides are interpreted to represent either (1) the random assortment of subnuclei in a caryonidal line of descent which was initiated by a cell whose macronucleus contained a very large proportion of subnuclei predetermined to control mating type XIV and a small minority of subnuclei predetermined to control type XIII, or (2) the persistence through a number of cell divisions of undetermined subnuclei which, under the later influence of the same cytoplasmic factors known to direct early macronuclear differentiation in syngen 7, eventually become fixed to control mating type XIV.

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