THE GENETIC CONTROL OF MATING TYPE DIFFERENTIATION IN PARAMECIUM¹

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REGULAR changes in nuclear function or morphology occurring during normal developmental processes have been described in many different organisms (KING and BRIGGS 1956; JACOB and MONOD 1961; BEERMAN 1961). The term *nuclear differentiation* has been used in reference to many of these cases. There seems to be little doubt that this phenomenon (or group of phenomena) is a fundamental aspect of normal ontogeny (SONNEBORN 1960).

Nuclear differentiation is involved in mating type inheritance in *Paramecium* (SONNEBORN 1942) and in other ciliates (NANNEY 1956; ALLEN and NANNEY 1958). Considerable research with these organisms, therefore, has been aimed at elucidating the genetic basis for this phenomenon. The purpose of the present paper is to describe and discuss two mutant genes which affect the pattern of mating type inheritance in one kind of *Paramecium aurelia* (syngen 7 [SONNEBORN 1957]). Both of these mutations cause a restriction in mating type potentialities in homozygotes. In addition, one of the mutant genes has a dominant effect on a cytoplasmic mechanism of mating type determination. Some of these results have been reported in preliminary communications (TAUB 1958, 1959, 1962).

MATERIALS AND METHODS

Stocks: Three of the stocks of syngen 7, *Paramecium aurelia*, used in the work reported here were collected from nature by L. C. GILMAN and obtained from T. M. SONNEBORN by the author. Stock 38 was collected at Fort Lauderdale, Florida, in February 1939, stock 227 in Illahaw, Florida, in February 1956, and stock 253 in Taylor county, Florida, in April 1956.

Stock d7-1 was derived by a series of backcrosses of stock 227 to stock 38. It carries the *mt*^{XIII, XIV} allele at the mating type locus (see RESULTS AND CONCLUSIONS, section B) but otherwise is essentially co-isogenic with stock 38. Stock m7-1 was obtained as a mutant following ultraviolet irradiation of mating type

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XIV of stock d7-1. The method by which this mutant stock was selected is to be described below.

Two kinds of stocks occur in syngen 7. *One-type* stocks contain only one mating type. Stock 38 is such a stock because it expresses only type XIII. *Two-type* stocks contain both mating types XIII and XIV. Stocks 227 and 253 are examples of the latter.

Culture methods: The paramecia were grown according to the methods described by SONNEBORN (1950). An infusion of baked lettuce inoculated with *Aerobacter aerogenes* the day before use was employed as culture medium. Its pH was adjusted to 6.5-7.0 with sterile $\text{Ca}(\text{OH})_2$. The progeny obtained in genetic experiments were maintained by the method of "daily reisolation" (SONNEBORN 1950). The cultures were kept at 27°C.

Conjugation, autogamy and mating type inheritance: These processes have been described in detail elsewhere (SONNEBORN 1947; BEALE 1954). They will be only briefly outlined here. Mating, or conjugation, normally can occur only between paramecia of complementary mating type. As a consequence of conjugation, the two co-conjugant clones produced by any given conjugant pair become genically identical. These two clones (which comprise a unit called a *syncclone* [SONNEBORN 1957]) therefore become the unit of Mendelian inheritance in *P. aurelia*. The usual Mendelian ratios occur within any large group of syncclones obtained from a cross. For example, 75 percent of the syncclones will show the dominant phenotype and 25 percent, the recessive phenotype following a cross between two heterozygous parent clones. During normal conjugation only nuclear genetic material is exchanged between the mates. Any non-nuclear (cytoplasmic) differences which may exist between mates, therefore, normally will remain as differences between the resulting co-conjugant clones.

Autogamy is a process of self-fertilization which can be induced in cells after they have undergone a certain minimal number of fissions (about 35 to 70 in syngen 7 [TAUB unpublished]) since their last fertilization. The genetic consequence of autogamy is to produce only completely homozygous exautogamous progeny. Among the exautogamous clones produced by a heterozygote, 50 percent will be homozygous for one allele at any given originally heterozygous locus and the remaining 50 percent, homozygous for the other allele.

Two genically identical, new macronuclei are produced from the fertilization nucleus in each conjugant or autogamous cell. These segregate one to each daughter cell produced by the first fission of such a cell but then divide at every subsequent fission. Each exconjugant or exautogamous clone, therefore, consists of two sub-clones (called *caryonides*), each of which originated from a cell with a single new macronucleus.

All the cells in a clone usually are the same mating type as that of the cell used to initiate that clone. Occasionally, however, clones are found within which conjugation is observed. The latter clones are referred to as *selfers*.

The mating type of a cell is controlled by its macronucleus. Two cells of identical genotype may, however, differ in mating type. The macronuclear differences which account for mating type differences arise within the first few hours of the

development of the new macronucleus into a mature macronucleus. These differences usually are inherited throughout asexual reproduction. While mating type changes, therefore, usually will not occur during asexual reproduction, the formation of *new* macronuclei following sexual reproduction permits mating type changes to occur at these times. Whether such changes actually will occur, however, depends upon the mechanisms involved in directing the differentiation of the new macronuclei.

In certain syngens (group A), the differentiation of each new macronucleus occurs independently of the mating type of the parent or of the mating type controlled by other macronuclei developing in the same cytoplasm. In these syngens, therefore, the mating types of "sister" caryonides (caryonides originating from the same exconjugant or exautogamous cell) often differ in mating type.

In another group of syngens (group B), the cytoplasm transmitted by the parent is strongly influential in directing the differentiation of new macronuclei. In these syngens, therefore, there is a high correlation in the mating type of sister caryonides.

The differences between the two alternative mating type-determining states of the cytoplasm in an exconjugant or exautogamous cell of a group B syngen are the result of the activities of the macronucleus of the previous sexual generation. In most stocks of group B syngens, the macronucleus of a cell of a given mating type controls the production of such a cytoplasmic state that the *same* mating type will be expressed in the next sexual generation. This results in a correlation in mating type between parent and cytoplasmic progeny.

Selection of mutants following ultraviolet irradiation: The following procedure was designed to induce, and to permit the recovery of, mutations which restrict cells of syngen 7 to mating type XIII. It is based on the principle that when such a mutant would arise in a population of cells of mating type XIV, it would readily find a mate and proceed to conjugate. Any such mutations, therefore, could be retrieved by removal of the resulting conjugant pairs.

Single cells of stock d7-1, mating type XIV, were maintained by the method of daily reisolation until they were old enough to undergo autogamy. At this time, cells from the main line of descent, which always had been in the presence of excess nutrient and, therefore, had not yet undergone autogamy, were isolated each into 30 ml of fresh medium in a 20 × 150 mm culture tube. They were permitted to grow for two days at 27°C. The resulting clones were irradiated with ultraviolet light just before autogamy was induced by exhaustion of the medium. Ten-ml samples were pipetted into 100 mm Petri dishes and placed under a 15 watt General Electric germicidal lamp. They were exposed for one minute at a dose rate of 60 ergs/mm²/sec as measured by a Latarjet dosimeter. A 10-ml sample was retained as an unirradiated control. Each 10-ml sample was then put into a sterile 20 × 150 mm tube where the cells were permitted finally to exhaust the medium and undergo autogamy. Any mutations which occurred in the micronuclei would, therefore, be expressed by the macronuclei of the exautogamous generation.

Samples were removed from the tubes two and three days after irradiation and examined cytologically to ascertain the percentage of autogamous cells (SONNEBORN 1950). The control cells always reached their maximal percentage of autogamy about a day before the irradiated cells. Control cultures which reached 100 percent autogamy and irradiated cultures which reached at least 90 percent autogamy were examined as follows for the occurrence of mutations: Eight ml of culture were removed from the tube and added to 120 ml fresh medium. One hundred twenty-six 1-ml samples of each culture were then placed in depression slides and grown at 27°C. Two days later in the case of the controls, and three days in the case of the irradiated cells, sexual reactivity obtained and the depression slide cultures were examined for mutants as indicated by the presence of conjugant pairs. No pairs were ever found in the unirradiated controls, but at least one conjugant pair was obtained from each of 30 separate depression cultures originating from irradiated cells. The pairs were isolated into fresh medium and each of the resulting exconjugant clones was maintained by the method of daily reisolation. Any pair which was formed by the mating of a bona fide mutant with a wild-type cell would produce heterozygous exconjugants. These, in turn, should produce a 1:1 genotypic ratio following autogamy. If the exconjugant clones are mating type XIV, the expected 1:1 genotypic ratio would be reflected in a 1:1 ratio for mating type XIII to mating type XIV. Exautogamous progeny obtained from ten such clones were tested for mating type. Nine of the clones gave no indication of being the expected heterozygotes; they produced only type XIV exautogamous progeny. The remaining clone produced both type XIII and type XIV progeny. A type XIII exautogamous caryonide produced by this clone was investigated further and is described below (RESULTS AND CONCLUSIONS, section D).

RESULTS AND CONCLUSIONS

A. The pattern of mating type inheritance in two-type stocks of syngen 7: Although the first stock (38) belonging to syngen 7 was collected in 1939, it was not then possible to ascertain directly the pattern of mating type inheritance in this syngen because stock 38 expressed only one mating type, type XIII. Stocks containing both mating types (XIII and XIV) of this syngen were not available until almost 20 years later. In 1946, however, SONNEBORN and DIPPILL, on the basis of indirect evidence, classified syngen 7 in group A. They based their conclusion on the following observations: (1) Stock 38, in a manner analogous to a mutant stock (16, formerly designated P) in syngen 1, a group A syngen, always expressed only one and always the same mating type; and (2) Stock 38 would give definite mating reactions when mixed with certain mating types belonging to Group A syngens, but not when mixed with any mating types of group B syngens.

In 1956, two stocks, 227 and 253, both containing mating types XIII and XIV were collected, and direct genetic analysis then became possible. Contrary to expectations, the results of crosses between mating types XIII and XIV of stock

TABLE 1

Frequency of mating type combinations in sister caryonides obtained from exconjugant cells of stock 227. The line marked "Expected" gives the values expected on the assumption of a random distribution of mating type among caryonides

	XIII and XIII	XIII and Selfer	Selfer and Selfer	XIV and Selfer	XIV and XIV	XIII and XIV
Observed	83	11	18	12	82	0
Expected	38.0	25.4	4.2	25.2	37.6	75.6

Assuming random distribution of mating type: $\chi^2=233.7$, $P < 0.001$ (5 d.f.).

TABLE 2

Mating type distribution among co-conjugant clones of stock 227

	Mating types of co-conjugant clones		
	"XIII" and "XIII"	"XIII" and "XIV"	"XIV" and "XIV"
Number of syncrones	0	83	2

"XIII" = both caryonides were mating type XIII or one caryonide was type XIII and the other was a selfer.

"XIV" = both caryonides were mating type XIV or one caryonide was type XIV and the other was a selfer.

There were no clones containing one mating type-XIII caryonide and one mating type-XIV caryonide. There were 18 syncrones which could not be classified because both caryonides of at least one exconjugant were selfers.

227 do not support the hypothesis that syngen 7 is a member of group A. There is a high positive correlation in the mating types of sister caryonides (Table 1). This result is strongly diagnostic of group B syngens. It indicates that the cytoplasm received from the previous sexual generation influences mating type determination. Co-conjugant clones almost always differ in mating type (Table 2). This feature, too, is typical of Group B syngens and, by analogy with syngen 4, a well studied group B syngen, suggests that, in these crosses, the mating type XIII exconjugant clones should be the cytoplasmic descendants of the type XIII parent and the type XIV exconjugant clones the descendants of the type XIV parent. Studies involving the use of antigen markers immediately following conjugation to ascertain the cytoplasmic parentage of exconjugant cells (SONNEBORN 1950) revealed that the expected positive correlation in mating type between cytoplasmic parent and progeny does occur.

Results similar to those presented for stock 227 have been obtained also for stock 253.

All of the features of mating type inheritance in stocks 227 and 253 are characteristic of the group B pattern of mating type inheritance and justify classifying syngen 7 in that group. The positive correlation in mating type between sister caryonides and between parent and cytoplasmic progeny which is characteristic of group B syngens is due to the effect of cytoplasm transmitted from the previous generation. In stocks 227 and 253, therefore, the cytoplasm transmitted by mating type XIII parents will be designated *XIII-determining* and that from type XIV parents as *XIV-determining*.

B. Genetic basis for the differences between the one-type stock 38 and the two-type stocks in syngen 7: Stock 38 has been cultivated in the laboratory for almost 25 years. During this time, this stock has never been observed to self and, whenever tested, has expressed only one and always the same mating type, *viz.*, type XIII. By analogy with the one-type stock 16 of syngen 1 (SONNEBORN 1939) and on the basis of indirect evidence obtained by BUTZEL (1953), experiments were designed to test the working hypothesis that a one-gene difference is responsible for the difference with respect to mating type potentialities between these two different kinds of stocks in syngen 7.

A cross of stock 38 to mating type XIV of stock 227 and to type XIV of stock 253 produced an exconjugant F_1 generation. The data describing the phenotypes of this generation are presented in Table 3. In contrast to the results obtained within either stock 227 or stock 253, at least one and usually both of the caryonides derived from each parent were mating type XIV in 40 of 44 F_1 synclones. All non-XIV caryonides were selfers. When studied in detail, these selfing caryonides proved to contain high initial proportions of cells expressing type XIV. In crosses made within stock 227, selfing caryonides which are cytoplasmically descendant from the type XIV parent show similar, high initial proportions of type XIV whereas selfing caryonides descendant from the type XIII parent do not (TAUB unpublished). In the crosses described in Table 3, therefore, the mating type XIII, stock 38, parent produced cytoplasmic progeny which resemble those derived from type XIV parents of two-type stocks; they differed markedly from progeny derived from type XIII parents of these stocks. The explanation of these F_1 data is postponed to the next section (C) because they are best understood in the light of additional evidence bearing on the hypothesis presently in question.

Thirty-five exautogamous F_2 clones were obtained from two different mating type XIV F_1 caryonides produced by the cross between stocks 38 and 227. Eighteen of the clones were mating type XIII and 17 were type XIV.

The 1:1 ratio for mating type in the F_2 suggests that a single gene difference between stocks 38 and 227 accounts for the difference in mating type potentialities.

TABLE 3

Mating type distribution among co-conjugant clones produced by crosses of stock 38 to stock 227 and to stock 253

	Mating types of co-conjugant clones					
	"XIII" and "XIII"	"XIII" and "Selfer"	"Selfer" and "Selfer"	"XIV" and "Selfer"	"XIV" and "XIV"	"XIII" and "XIV"
Number of synclones from						
cross of stock 38 to:						
stock 227	0	0	0	1	17	0
stock 253	0	0	0	3	23	0

"XIII" = both caryonides were mating type XIII or one caryonide was type XIII and the other was a selfer.

"XIV" = both caryonides were mating type XIV or one caryonide was type XIV and the other was a selfer.

"Selfer" = both caryonides were selfers.

There were no clones containing one mating type-XIII and one mating type-XIV caryonide.

ties. It is postulated that a two-type stock such as 227 carries a gene called $mt^{XIII,XIV}$ which permits the expression of either mating type XIII or XIV, the type expressed depending on the kind of cytoplasm transmitted by the parent. The one-type stock 38 carries the allele mt^{XIII} which causes this stock, or any other homozygote for mt^{XIII} , to express mating type XIII regardless of the kind of cytoplasm transmitted from the previous generation. F_1 hybrids formed between stocks 38 and 253 would be genotype $mt^{XIII}/mt^{XIII,XIV}$. Because these heterozygous F_1 's are capable of expressing mating type XIV, the mt^{XIII} allele would have to be recessive for its effect on mating type potentialities.

Conclusive evidence for the hypothesis that a single gene restricts the mating type potentialities of stock 38 to type XIII was obtained from backcrosses of the F_1 to stock 38. If the F_1 were heterozygous for the postulated pair of alleles, then backcrossing it to the one-type parent should result in some synclones homozygous for the mt^{XIII} allele and therefore uniformly mating type XIII, and an equal number of synclones heterozygous and therefore capable of expressing either mating type (see Figure 1). Twenty-five synclones were obtained in backcrosses of the F_1 to stock 38. Fourteen of these synclones were uniformly type XIII. In the remaining 11 synclones, both exconjugant clones contained only type XIV or selfing caryonides. According to the hypothesis presented above, the uniformly type XIII synclones were homozygous for the mt^{XIII} allele, while the remaining synclones were heterozygous. It should be noted that stock 38 again produced only type XIV or selfing progeny caryonides when these were, according to the above hypothesis, genically competent to be type XIV.

In order to obtain a stock homozygous for the postulated $mt^{XIII,XIV}$ allele but otherwise isogenic with stock 38, a type XIV caryonide produced in the first backcross to stock 38 was again crossed to stock 38. Results similar to those

FIGURE 1.—Backcross of F_1 to one-type parent.

obtained from the first backcross were obtained in this generation. This process of repeatedly crossing a presumed heterozygote of mating type XIV to stock 38 always resulted in about 50 percent uniformly type XIII syncrones and about 50 percent of syncrones in which each of the four caryonides was type XIV or, occasionally, a selfer. From a series of nine backcrosses, 38 uniformly type-XIII syncrones and 49 syncrones in which all four caryonides were competent to express type XIV were produced. A chi-square value of 1.15 ($P > 0.25$) is obtained when this 38:49 result is compared to the expected 1:1 ratio for the single-locus hypothesis presented above.

These data confirm the hypothesis that the difference in mating type potentialities between stock 38 and stock 227 is due to a single gene difference. The $mt^{XIII, XIV}$ allele present in stock 227 permits the expression of either mating type XIII or XIV; which type is expressed depends on the kind of cytoplasm transmitted by the parent. The mt^{XIII} allele restricts homozygotes to type XIII and does so regardless of the kind of cytoplasm transmitted by the parent. The mt^{XIII} allele is recessive to $mt^{XIII, XIV}$ for its effect on mating type potentialities.

As the result of the genetic analysis described above, it was discovered that cytoplasmic progeny of any homozygote for mt^{XIII} become type XIV when these progeny are genically competent to do so (i.e., contain $mt^{XIII, XIV}$). This observation is in contrast to the pattern of mating type determination observed in crosses within two-type stocks (where type XIII parents produce type XIII cytoplasmic progeny) and is the subject of the investigations now to be described.

C. Mating type determination in cytoplasmic progeny of parents which carry mt^{XIII} . One possible explanation for the results obtained in the F_1 , and obtained following other crosses in which a homozygote for mt^{XIII} is a parent, is that the heterozygous genotype $mt^{XIII}/mt^{XIII, XIV}$ results directly in the expression of mating type XIV. This possibility could easily be excluded if heterozygotes of mating type XIII can be produced and if mating type determination in heterozygotes can be shown to be influenced by parental cytoplasm in the same way as in crosses within two-type stocks. The following genetic analysis was designed to test this point (see Figure 2).

Mating type XIV, F_1 caryonides produced by the cross between homozygotes for mt^{XIII} and stock 227 were crossed to mating type XIII of stock 227. In the majority of the resulting syncrones, both caryonides of one exconjugant clone were mating type XIV, and both caryonides of the other clone were type XIII. A few syncrones contained a selfing caryonide in one or the other exconjugant clone.

Groups of exautogamous progeny were obtained from 35 type XIV caryonides of the generation produced by this cross of heterozygotes to stock 227. Each of these 35 caryonides could be placed into one of two classes (1 or 2) depending on the kind of exautogamous progeny it produced. Caryonides of Class 1 produced only type XIV exautogamous progeny. There were 18 of these. The remaining 17 caryonides fell into the second class because they produced both type XIII and type XIV progeny, and each did so in approximately a 1:1 ratio. For these 17, the overall proportion of XIII:XIV was 144:154.

FIGURE 2.—Genetic analysis designed to obtain a heterozygote of mating type XIII.

These results imply the following: After the cross of the type XIV heterozygote to stock 227, type XIII, two kinds of progeny syncrones are expected in equal numbers: homozygotes for $mt^{XIII, XIV}$, and heterozygotes. The 18 caryonides of Class 1 must have come from the homozygous syncrones, whereas the 17 of Class 2 were from the heterozygous syncrones. Because the two clones of a syncrone are genically identical, the type XIII caryonides of the Class 2 syncrones must

also have been heterozygous. Genetic analysis of these syncrones revealed that this was the case. Heterozygotes, therefore, can express mating type XIII as well as XIV.

The type XIII exconjugant clones of all 35 syncrones derived their cytoplasm from stock 227, mating type XIII. This parent carries type XIII-determining cytoplasm. All the type XIV clones descended from the type XIV heterozygous parent, which has type XIV-determining cytoplasm. Mating type determination in $mt^{XIII,XIV}/mt^{XIII}$ cells, therefore, is under the control of cytoplasm transmitted from the previous generation, just as in crosses involving only two-type parents.

Because mating type determination in $mt^{XIII,XIV}/mt^{XIII}$ cells is under the control of inherited cytoplasm, homozygotes for mt^{XIII} must transmit to their progeny type XIV-determining cytoplasm if these progeny, when heterozygous, are to become type XIV, as did, for example, the F_1 described above. In addition to its effect on mating type potentialities, mt^{XIII} has, therefore, a second, unexpected effect which causes the cytoplasm to be type XIV-determining.

Although the effect of mt^{XIII} on mating type potentialities is recessive, the preceding evidence does not reveal whether the mt^{XIII} gene also is recessive for its effect on the mating type-determining state of the cytoplasm. If the cytoplasmic effect of mt^{XIII} is recessive, then $mt^{XIII,XIV}/mt^{XIII}$ cells should be capable of possessing either type XIII- or XIV-determining cytoplasm, which kind depending on the kind of cytoplasm transmitted from the previous generation, as in crosses within two-type stocks. On the other hand, if the mt^{XIII} gene is dominant for its cytoplasmic effect, heterozygotes always should have type XIV-determining cytoplasm regardless of their mating type (i.e., regardless of the kind of cytoplasm they inherited). To test this point, mating type XIII heterozygotes were obtained from a cross of a type XIV heterozygote to type XIII of stock 227. As in crosses described above, half of the resulting syncrones should have been homozygous for $mt^{XIII,XIV}$ (Class 1) and the other half, heterozygous (Class 2) (see Figure 3). Crosses were then made between type XIII caryonides and their co-conjugant type XIV caryonides in 14 randomly chosen syncrones. Eight of these 14 crosses produced a total of 108 progeny syncrones, all of which were capable of expressing mating type XIV. In the majority of these progeny, the two caryonides in one exconjugant clone were mating type XIII and the two in the other were type XIV. In the remaining progeny, one or more of the caryonides were selfers. The eight parent syncrones which produced these progeny must have been homozygous for $mt^{XIII,XIV}$ (Class 1); if they had been heterozygous they would have produced 25 percent mt^{XIII}/mt^{XIII} progeny, all four caryonides of which would have been type XIII.

The other six crosses, however, did produce some progeny syncrones in which all four caryonides were type XIII; of a total of 82 progeny, 14 contained only mating type XIII caryonides. In the majority of the remaining 68 progeny, the two caryonides of *both* exconjugant clones were type XIV. A few of these 68 progeny syncrones contained both mating type XIV and one or more selfing caryonides. The approximate 3:1 ratio (68:14; $\chi^2 = 2.34$ when compared to a 3:1 ratio, $P > .10$) of progeny capable of expressing type XIV to progeny re-

FIGURE 3.—Genetic analysis demonstrating the dominance of the cytoplasmic effect of the mt^{XIII} gene.

stricted to type XIII indicates that the parents of these 82 synclones were heterozygous (Class 2). The 14 progeny which were uniformly type XIII must have been homozygous for mt^{XIII} . The remaining 68 progeny must have been homozygous for $mt^{XIII, XIV}$ or heterozygous. Because both exconjugant clones in these 68 progeny tended strongly to be type XIV, both of their parents—the type XIII as well as the type XIV parent—must have carried type XIV-determining cytoplasm. The mt^{XIII} gene, therefore, must be dominant for its effect on the mating

type-determining state of the cytoplasm; otherwise, the type XIII, heterozygous parent would not have produced type XIV exconjugant progeny.

This conclusion was confirmed by exautogamous progeny obtained from three type XIII heterozygotes. They produced the following proportions of types XIII: XIV—8:5, 9:12, and 43:37. Half of their exautogamous progeny should have been mt^{XIII}/mt^{XIII} and genically restricted to type XIII. These would account for the 60 type-XIII progeny produced by the three parent caryonides. The remaining 54 caryonides must have been homozygous for $mt^{XIII,XIV}$ and capable, therefore, of expressing either type XIII or XIV. The fact that they became type XIV indicated that their heterozygous type XIII parents transmitted type XIV-determining cytoplasm.

The effects of the mt^{XIII} gene are summarized as follows: (1) It has a recessive effect which restricts homozygotes to mating type XIII. Cells which carry its allele $mt^{XIII,XIV}$ can express either type XIII or XIV, the type expressed depending on the cytoplasm transmitted by the previous generation. (2) It has a dominant effect which causes the cytoplasm of any cell which carries it to be type XIV-determining, regardless of the mating type of such a cell.

D. The analysis of an ultraviolet induced mutation which affects mating type potentialities: A mutant stock (m7-1) restricted to mating type XIII was derived from mating type XIV of stock d7-1 and selected by the method described in MATERIALS AND METHODS. A genetic analysis similar to that described above for stock 38 was carried out with stock m7-1 to ascertain (1) the genic basis for the restriction in mating type, (2) the cytoplasmic effect, if any, associated with the new mutation and (3) the locus involved, whether the mt locus or otherwise.

Stock m7-1 was crossed to mating type XIV of stock 227. Both exconjugant clones of each F_1 synclone were mating type XIV or, occasionally, selfers. None of the synclones contained one mating type XIV and one mating type XIII clone. Exautogamous F_2 progeny were obtained from eight type XIV F_1 caryonides from different synclones. The mating type distribution of these progeny is summarized in Table 4. It is evident from this analysis that the one-type condition of stock m7-1 is due to a single gene mutation, and, because the F_1 could express

TABLE 4

Exautogamous progeny obtained from eight F_1 caryonides produced by the cross of stock m7-1 to mating type XIV of stock 227

Caryonide no.	Number of exautogamous progeny clones of mating type:	
	XIII	XIV
4A	19	23
5B	23	18
6A	22	18
8B	22	19
12B	16	20
13A	25	17
27B1	79	79
28B1	83	78

type XIV, that the new mutation is not dominant for its effect on mating type potentialities. The new mutation was designated n . Its wild-type allele tentatively was named N . The latter designation was to be dropped if it was discovered that n was at the mt locus, in which case $mt^{XIII, XIV}$ would be its wild-type allele.

FIGURE 4.—Genetic analysis demonstrating the lack of an effect of the n mutation on the mating type-determining state of the cytoplasm.

As in the case with stock 38, the original stock m7-1 produced mainly mating type XIV and no type XIII cytoplasmic progeny when crossed to type XIV of stock 227. A genetic analysis now to be described (see Figure 4) revealed that this result has a different explanation than that derived for this effect in stock 38.

Heterozygous (N/n) mating type-XIV cells were crossed to type XIII of stock 227 (N/N). About half of the resulting progeny synclones were expected to be N/N (Class 1), the rest, N/n (Class 2). Within these progeny, mating type XIII caryonides were crossed to co-conjugant type XIV caryonides. Some of these crosses (Class 1) yielded only synclones in which one mating type XIII and one mating type XIV exconjugant clone occurred (two-type synclones). The other crosses (Class 2) yielded about 25 percent progeny synclones in which both exconjugant clones were mating type XIII (one-type synclones) and 75 percent two-type synclones. In none of these synclones were both exconjugant clones type XIV.

Within the one-type (n/n) synclones, one exconjugant obtained its cytoplasm from a type XIV parent and the other from a type XIII parent. The former parent carried type XIV-determining cytoplasm and the latter XIII-determining cytoplasm as indicated by the fact that each of their two-type (N/n or N/N) progeny synclones contained one mating type-XIII and one mating type-XIV exconjugant clone. One clone of each one-type (n/n) synclone, therefore, obtained its cytoplasm from a parent with type XIII-determining cytoplasm and the other from a parent with type XIV-determining cytoplasm.

The cytoplasmic state of both exconjugant clones of five of the above one-type synclones was ascertained by crossing each of these ten type XIII clones to mating type XIV of stock 227. In all five n/n synclones, one exconjugant clone produced type XIV cytoplasmic progeny and the other produced type XIII cytoplasmic progeny. In each of these five n/n synclones, therefore, one exconjugant clone carried XIV-determining cytoplasm and the other carried type XIII-determining cytoplasm. Because either of the two alternative cytoplasmic states can exist in n/n cells, the n gene, in contrast to mt^{XIII} , must have no effect on the mating type-determining state of the cytoplasm; whether an n/n cell will carry XIII-determining or XIV-determining cytoplasm depends, as in the two-type stocks, on the kind of cytoplasm transmitted by the previous generation.

The original n/n cell, isolated as an exautogamous product of an ultraviolet treated, mating type XIV cell, produced type XIV cytoplasmic progeny when crossed to stock 227. The XIV-determining cytoplasmic state in the original mutant, therefore, must have resulted from the XIV-determining cytoplasm present in the irradiated parent.

To ascertain whether the n mutation occurred in the mt cistron, homozygotes for n with XIV-determining cytoplasm were crossed to $mt^{XIII}/mt^{XIII,XIV}$ heterozygotes of mating type XIV. In all of more than 30 progeny synclones produced by several such crosses, each exconjugant clone was type XIV or a selfer. If the n mutation were in the mt cistron, and had, therefore, affected the identical primary function as does mt^{XIII} , then half of the progeny synclones of these crosses should have been genotype " mt^{XIII}/n " and incapable of expressing mating type

XIV. Because all progeny could express mating type XIV, the *n* mutation is not in the *mt* cistron.

Exautogamous progeny were obtained from six type-XIV caryonides, each from a different synclone, produced by the cross just described. These caryonides were all genotype *N/n*, but, at the *mt* locus, half were expected to be homozygous for *mt^{XIII,XIV}* and the other half, heterozygous. The results are described in Table 5. Three of the caryonides produced a 1:1 ratio of type XIII to type XIV progeny. These caryonides must have been genotype *mt^{XIII,XIV}/mt^{XIII,XIV}, N/n*. The remaining caryonides produced 3:1 ratios of type XIII to type XIV progeny (see Figure 5). These latter caryonides must have been the double heterozygotes. The fact that 25 percent of their progeny were capable of expressing mating type XIV (i.e., were genotype *mt^{XIII,XIV}/mt^{XIII,XIV}, N/N*) indicates that the *mt* and the *n* loci are unlinked (see Figure 6): The frequency of the genotype *mt^{XIII,XIV}/mt^{XIII,XIV}, N/N* represents half of the recombination frequency between the *mt* and the *n* loci, which, therefore, is 50 percent.

DISCUSSION

The preceding analysis has revealed the existence of two loci (*mt* and *n*) involved in the determination of mating types in syngen 7. Mutations at either of these loci restrict cells to mating type XIII. One of the two loci (*mt*) apparently also is involved in the establishment of the alternative mating type-determining states of the cytoplasm.

To consider first the effects on mating type potentialities, let us assume that *mt* and *n* are loci both directly involved in the formation of the substance responsible for the mating type XIV phenotype (the "XIV mating type substance" [METZ 1954]). Because a cell must contain both the *mt^{XIII,XIV}* and the *N* genes in order for it to be able to express type XIV, the *mt* and *n* loci probably control different steps in the formation of the XIV mating-type substance.

Mutations at these loci do not detectably alter the ability of the cells to synthesize the XIII mating-type substance. The formation of this substance, therefore, probably does not require any of the specific synthetic pathway(s) which might be affected by the *mt^{XIII}* and *n* mutations.

TABLE 5

Exautogamous progeny derived from six type-XIV caryonides, some of which should have been of genotype mt^{XIII,XIV}/mt^{XIII},N/n (see text)

Caryonide no.	Number of progeny of mating type:		Ratio
	XIII	XIV	
21A2	67	88	0.76:1
27B1	72	77	0.94:1
28B1	82	78	1.05:1
16B3	125	41	3.05:1
20A1	97	30	3.23:1
25A2	112	46	2.44:1

FIGURE 5.—Genetic analysis to ascertain the allelic relationship of the n and mt^{XIII} genes.

Because mating types XIII and XIV normally are alternative phenotypes, if one of the mating type substances were a precursor of the other, the first substance might, in effect, be depleted from those cells producing the second substance. If, therefore, the XIII mating-type substance or one of its specific precursors were itself a precursor of the XIV mating-type substance, mutations such as mt^{XIII} and n would be expected to occur, whereas similar mutations, restricting cells to type XIV, would not. The fact that several attempts to induce mutations restricting cells to type XIV have been unsuccessful (TAUB unpublished), as have been

POSSIBLE RELATIONSHIP OF $\underline{n}$ TO $\underline{mt}$ LOCUS	GENOTYPE OF PARENT	EXPECTED EXAUTOGAMOUS PROGENY			
		GENOTYPE	PERCENT	MATING TYPE	% XIII
$\underline{n}$ AT $\underline{mt}$ LOCUS	$\frac{\underline{n}}{\underline{mt}^{XIII}}$	$\frac{\underline{n}}{\underline{n}}$	50	XIII	100
		$\frac{\underline{mt}^{XIII}}{\underline{mt}^{XIII}}$	50	XIII	
$\underline{n}$ LOCUS LINKED TO $\underline{mt}$ LOCUS BY X MAP UNITS ($0 < X < 50$)	$\frac{\underline{n}}{\underline{N}} \quad \frac{\underline{mt}^{XIII, XIV}}{\underline{mt}^{XIII}}$	$\frac{\underline{n}}{\underline{n}} \quad \frac{\underline{mt}^{XIII, XIV}}{\underline{mt}^{XIII, XIV}}$	$\frac{100-X}{2}$	XIII	$75(100 - \frac{X}{2}) < 100$
		$\frac{\underline{N}}{\underline{N}} \quad \frac{\underline{mt}^{XIII}}{\underline{mt}^{XIII}}$	$\frac{100-X}{2}$	XIII	
		$\frac{\underline{n}}{\underline{n}} \quad \frac{\underline{mt}^{XIII}}{\underline{mt}^{XIII}}$	$\frac{X}{2}$	XIII	
		$\frac{\underline{N}}{\underline{N}} \quad \frac{\underline{mt}^{XIII, XIV}}{\underline{mt}^{XIII, XIV}}$	$\frac{X}{2}$	XIV	
$\underline{n}$ LOCUS UNLINKED TO $\underline{mt}$ LOCUS	$\frac{\underline{n}}{\underline{N}} \quad \frac{\underline{mt}^{XIII, XIV}}{\underline{mt}^{XIII}}$	$\frac{\underline{n}}{\underline{n}} \quad \frac{\underline{mt}^{XIII, XIV}}{\underline{mt}^{XIII, XIV}}$	25	XIII	75
		$\frac{\underline{N}}{\underline{N}} \quad \frac{\underline{mt}^{XIII}}{\underline{mt}^{XIII}}$	25	XIII	
		$\frac{\underline{n}}{\underline{n}} \quad \frac{\underline{mt}^{XIII}}{\underline{mt}^{XIII}}$	25	XIII	
		$\frac{\underline{N}}{\underline{N}} \quad \frac{\underline{mt}^{XIII, XIV}}{\underline{mt}^{XIII, XIV}}$	25	XIV	

FIGURE 6.—Predictions of percentage of mating type XIII, exautogamous progeny based upon the possible alternative allelic relationships of the $\underline{n}$ and $\underline{mt}^{XIII}$ genes.

attempts, in syngen 1, to induce analogous mutations (restricted to type II [SONNEBORN personal communication]) offers further support to this notion.

In syngen 1, mutations restricting cells to mating type I have occurred independently at least three times (BUTZEL 1955). All of these occurrences were at the same locus. BUTZEL suggested that this locus controls a reaction leading to the formation of the II mating-type substance and that it does not function in wild-type cells expressing type I. To apply this concept to syngen 7, either the $\underline{mt}$ locus, the $\underline{n}$ locus, or both loci would be nonfunctional in type XIII cells, while both of them would be functional in type XIV cells. If such a scheme is correct, the decision as to whether either or both of the two loci will function in any given macronucleus must be made very soon after its origin and the decision must be a heritable one.

The cytoplasm transmitted by the previous sexual generation is the most important factor in determining the path of nuclear differentiation in syngen 7. In terms of the hypothesis discussed above, the effect of the transmitted cytoplasm would be to control the decision as to whether the $\underline{mt}$ locus, the $\underline{n}$ locus or both loci would function. The role of the cytoplasm in making this decision can be imagined to resemble that of the cytoplasmic repressor involved in the control of

the *Lac* locus in *E. coli* (see JACOB and MONOD 1961). If, as seems likely, the specificities of the XIII and XIV mating-type substances are determined by nuclear genes, then, in spite of what their names imply, neither XIII-determining nor XIV-determining cytoplasm need provide any *structural* information to those systems synthesizing the mating type substances. The two alternative cytoplasmic states, therefore, simply may result from variations above or below a threshold in the concentration of some substance, or from the mere presence or absence of a such a substance. This "substance" could be a repressor of, for example, the *mt^{XIII,XIV}* allele. The difference, then, between XIII-determining and XIV-determining cytoplasm would be the presence or absence, respectively, of such a repressor substance.

In the two-type stocks of syngen 7, as in probably all the stocks of other group B syngens, the cytoplasmic state is such as to maintain the same mating type from one sexual generation to the next. For example, mating type XIII of stock 227 carries XIII-determining cytoplasm and mating type XIV carries XIV-determining cytoplasm. One possible explanation for the correlation between mating type and cytoplasmic state is that the mating type substance itself, or one of its specific precursors, determines which mating type an undifferentiated macronucleus will control. This possibility seemed unlikely after NANNEY (1957) had shown in the group B syngen 4 that mating type VIII cells in certain selfing caryonides frequently produce type VII exconjugant progeny. In syngen 7, mating type XIII cells of genotypes *mt^{XIII}/mt^{XIII}* and *mt^{XIII,XIV}/mt^{XIII}* always carry XIV-determining cytoplasm and type XIII cells of genotype *n/n* can do so. The discovery of the complete breakdown of the correlation between mating type and cytoplasmic state in these genotypes provides further evidence that mating type and cytoplasmic state represent two distinct and separable phenomena.

Both of the alternative cytoplasmic states—XIII-determining and XIV determining—can persist from one sexual generation to the next in cells of genotype *n/n* even though such cells always are mating type XIII. None of the experiments described earlier in this paper provides any evidence bearing on the question of whether, in *n/n* cells, the cytoplasmic state remains under nuclear control, as in wild-type cells, or whether it is autonomous. The former possibility implies that macronuclear differentiation still occurs in *n/n* cells and that the course of this differentiation is directed by inherited cytoplasm. Whether this means that macronuclear differentiation with respect to mating type is distinct and separable from such differentiation with respect to cytoplasmic state is not yet clear.

SUMMARY

Within wild-type stocks of syngen 7 of *Paramecium aurelia*, a high positive correlation in mating type occurs between sister caryonides and between parent and cytoplasmic progeny. On the basis of these observations, syngen 7 is classified into group B.

Two mutations are described which affect mating type determination in this syngen. The first, designated *mt^{XIII}*, has a recessive effect causing homozygotes

to become type XIII regardless of the mating type-determining state of the cytoplasm received from the previous generation. The second mutation, designated *n*, has an identical effect. The wild-type alleles of these mutations, designated *mt*^{XIII,XIV} and *N*, respectively, permit the expression of either mating type XIII or XIV, which one depending on the kind of cytoplasm transmitted by the previous generation.

In addition to its effect on mating type potentialities, the *mt*^{XIII} gene has a second effect which causes a carrier's cytoplasm to be type XIV-determining regardless of its mating type. *mt*^{XIII} is *dominant* to *mt*^{XIII,XIV} for this effect.

The *mt*^{XIII} gene was found in a stock collected from nature; the *n* mutation arose following ultraviolet irradiation of wild-type cells. *mt*^{XIII} and *n* are at separate, unlinked loci.

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