

DETERMINATION AND INHERITANCE OF MATING TYPE IN *PARAMECIUM CAUDATUM*¹

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MATING type determination and inheritance in *Paramecium caudatum* has been reported only for syngen 12 (HIWATASHI 1958). Inheritance was usually synclonal, i.e. the two clones derived from the two members of a conjugating pair are identical in mating type expression, indicating direct genic control of mating type. However the breeding analysis was incomplete and some of the observations, as will be pointed out below, were difficult to reconcile with simple direct genic control. In the hope that another syngen of this species might be more amenable to analysis and might help understand the difficulties in syngen 12, attention was directed to syngen 3 with the results here to be set forth. As will appear, the needed clarification has been obtained and the main features of the genetics of mating types in both syngens can now be confidently formulated.

Special genetic interest attaches to the analysis of mating type genetics in *Paramecium* and certain other Ciliates because of certain remarkably regular and puzzling deviations from a one to one correspondence between phenotype and genotype (SONNEBORN 1937, 1947; BUTZEL 1955; TAUB 1963; BLEYMAN 1967; NANNEY and CAUGHEY 1953; NANNEY, CAUGHEY and TEFANKJIAN 1955; BARNETT 1966). While one gene may limit expression to one mating type, an allele may permit expression of this and the complementary type in species that have only two mating types per syngen. In species with multiple mating types per syngen, one gene may permit expression of any one of a certain array of mating types while an allele permits expression of a different array of types, with much overlap between the two arrays. In both kinds of species, determination of which mating type will be expressed by cell lineages of identical genotype may occur soon after fertilization by some ununderstood events which "differentiate" the developing macronucleus. Yet in other Ciliates differences of mating type appear to be strictly correlated with differences of genotype, e.g. in *Euplotes* (KIMBALL 1942; POWERS 1943; SONNEBORN 1947; HECKMANN 1961, 1964), in *P. bursaria* (SIEGEL and LARISON 1960), in syngen 13 of *P. aurelia* (SONNEBORN 1966), and in syngen 8 of *Tetrahymena pyriformis* (ORIAS 1963). Both of the latter two species include other syngens that show the peculiarities mentioned above. Some of these peculiarities are found in *P. caudatum*. This encourages the hope that light may be thrown on them by studies of the system of mating type

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genetics in this species. The present paper provides a basis for further contributions to that objective.

MATERIALS AND METHODS

Stocks: Five stocks belonging to syngen 3 of *P. caudatum* and some clones derived from them were used. Stock Ma1 and Ga1 of mating type V, collected in the United States, were furnished by L. C. GILMAN and K4 of mating type VI by S. KOIZUMI. Stocks Nd3 and Ys3, mating type V and VI respectively, were collected in Japan and maintained by the author.

Culture methods: Culture methods and other experimental techniques were almost identical to those described for *P. aurelia* (SONNEBORN 1950). In later stages of the work, however, lettuce juice in phosphate buffer or in Dryl's solution (DRYL 1959) was used for the culture medium instead of ordinary baked-lettuce infusion. This was made as follows: fresh lettuce leaves were washed and immersed in boiling water for a few seconds to inactivate oxidases and other enzymes and then juice was obtained from them with an ordinary juicer or Waring blender. Two liters of juice were obtained from 1 kg of fresh lettuce leaves. The juice was sterilized at 100°C for a few minutes on three successive days and then stored in a refrigerator. Conventional autoclaving before dilution coagulated the juice rendering it unsuitable for use. For use as medium, the juice was diluted 1:20 or 1:30 with phosphate buffer or Dryl's solution and inoculated with *Aerobacter aerogenes*. This method is especially convenient when various kinds of buffer or salt solution are desired in the culture medium.

Cytological methods: Cytological observations during and for some period after conjugation were made using DIPPELL's (1955) temporary acetic orcein and fast green stain. For permanent preparations and detailed observation of micronuclear chromosomes, DIPPELL's (personal communication) variation of TJIÖ's aceto-orcein technique was employed.

Conjugation: For genetic work, true cross-fertilized conjugants must be distinguished from false conjugants. The latter include conjugants that separate precociously without undergoing meiosis or fertilization, conjugants that undergo self-fertilization or cytogamy (WICHTERMAN 1940), and exconjugants whose macronuclei regenerate from fragments of the parental macronucleus (macronuclear regeneration, SONNEBORN 1947) instead of developing from a product of the fertilization nucleus. The first and third types of false conjugation can be eliminated by rejecting clones that fail to show an immature period of a month or more after conjugation. Only conjugants that undergo fertilization and develop macronuclei from a product of the syncaryon yield clones with an immature period. To eliminate cytogamy, genic markers are required and they also serve to eliminate all kinds of false conjugation. In most experiments, no genic marker was available but in some crosses serotypic alleles (HIWATASHI 1967) were used as markers.

Cross breeding analysis in this species was plagued by poor survival after conjugation, especially in F_2 generations. Very few combinations of stocks yielded sufficient F_1 and backcross generations, thus severely limiting the number of crosses that could be used. In some crosses, survival after conjugation was greatly increased by isolating the exconjugants in the same culture fluid that the animals were living in when they were mated.

Temperature for growth was 27°C and that for experimental procedures and observations was 23°C, unless otherwise mentioned.

RESULTS

Mode of inheritance: As different syngens of the same species are known to exhibit different modes of mating type determination and inheritance (e.g. NANNEY and CAUGHEY 1953 and ORIAS 1963 on *Tetrahymena pyriformis* and SONNEBORN 1947, 1966, on *P. aurelia*), HIWATASHI's (1958) report of synclonal uniformity of mating type in syngen 12 of *P. caudatum* left open the question of whether the same would hold for other syngens of this species. This question was answered for syngen 3 by the results of two crosses (Table 1). In the first cross,

TABLE 1

Mating type distribution among F₁ clones and synclones

Cross V VI	Synclones		Clones		Synclones and clones		Clones died or underwent MR [†]
	V	VI	V	VI	V	VI	
Nd3 × Ys3	0	22	0	0	0	22	6*
Ma1 × Ys3	0	14	0	4	0	18	8
Ga1 × Ys3	0	50	0	13	0	63	113
					Total	0 103	
Nd3 × K4	8	10	2	2	10	12	4
d-43b × K4	8	6	2	1	10	7	7
					Total	20 19	

* Also includes those discarded (see text).

† MR = macronuclear regeneration; same symbol used in all tables.

between stock Nd3, mating type V, and stock Ys3, mating type VI, all eight caryonides (Figure 1) for each of 25 conjugating pairs were isolated. Three pairs were rejected because one or more of the isolates from them underwent macronuclear regeneration and produced progeny lacking an immaturity period. All 176 of the caryonides of the remaining 22 pairs became mature in 30–35 days (60–70 cell generations) after a preceding period of immaturity and all were mating type VI. In the second cross, between Nd3 (type V) and K4 (type VI), only 18 of the 22 pairs yielded a full set of eight viable caryonides that underwent normal nuclear reorganization (and passed through a period of immaturity). Of these 18 sets of caryonides, all eight from each of 10 pairs developed mating type VI and all eight from each of 8 pairs developed mating type V. Thus, both crosses agree in showing that the mode of inheritance of mating type in syngen 3 is synclonal, the clone (four caryonides) from one conjugant developing the same mating type as the clone (four caryonides) from its mate.

Although the final character of the clones and caryonides was consistent and readily interpretable, their earlier character included a puzzling feature: some caryonides failed to become immature immediately after conjugation; they remained sexually reactive, usually for about 10 days (about 20 cell generations) but in one case for more than 20 days, the remainder of their history paralleling that of caryonides that did not exhibit early maturity, i.e. they became immature and remained so until 30–35 days after conjugation, when they finally became persistently mature. For example, among the 176 caryonides in the first cross mentioned above, five—each from a different conjugant pair—exhibited early transient “maturity”. It will be recalled that all of the 176 caryonides were mating type VI during the later persistent period of maturity. However, during the early period of transient maturity only one of the five caryonides was mating type VI, the other four were mating type V. The possible significance of these puzzling observations and their relation to observations and interpretations in the literature will be considered in the DISCUSSION section.

Segregation of mating types: As pointed out in the preceding section, one cross

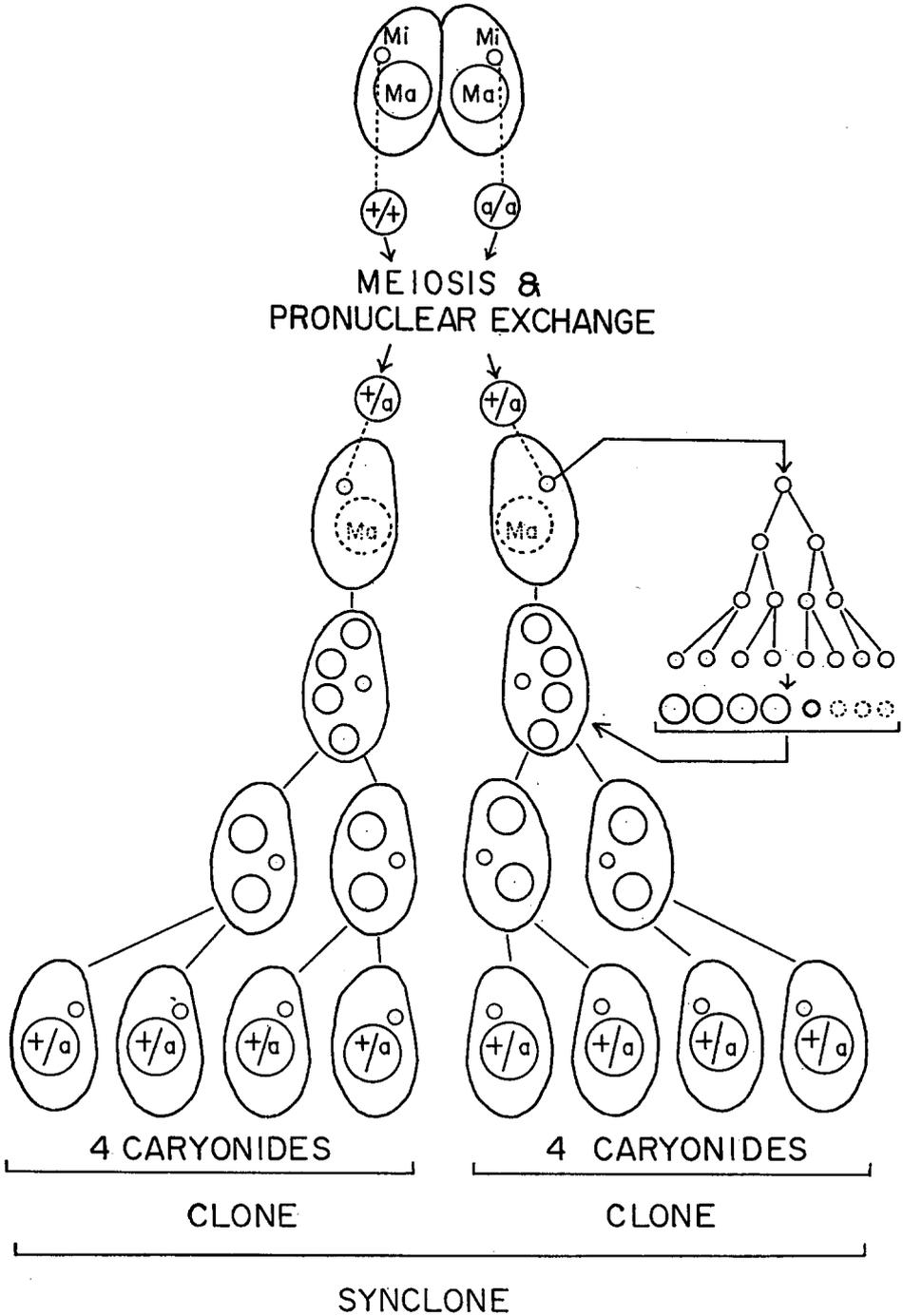


FIGURE 1.—Diagram showing nuclear reorganization in conjugation of *Paramecium caudatum*. Dotted circles in diagram represent degenerating macronuclei (Ma) and degenerating postzygotic division products (small circles). Ma, macronucleus; Mi, micronucleus.

yielded all synclones of type VI and the other yielded 8 type V and 10 type VI, i.e. apparently a 1:1 ratio. Of the five crosses listed in Table 1, three gave the first result and two gave the second. These five crosses were the only stock combinations, among more than 20 tried, that gave enough F_1 survival to tabulate. In most of these crosses only one caryonide from each conjugant was grown and tested since the results in the preceding section show that the four caryonides of a clone regularly agree in mating type during the normal mature period. The synclonal mode of inheritance also requires testing only one clone of a synclone; hence, such data are included in Table 1.

This table further shows that the cross of type VI stock Ys3 to all three type V stocks gives in each case 100 percent type VI in F_1 (total of 103 pairs tested); but that one of these type V stocks, Nd3, gives the 1:1 ratio (10:12) when crossed to a different type VI stock, K4. These results suggest that type V is homozygous for a recessive mating type gene, which will be designated mt^v , while type VI can be heterozygous ($mt^v/+$) or homozygous ($+/+$) for a dominant allele determining type VI. Ys3 then would be a homozygous type VI and K4 heterozygous. The results from the cross of K4 ($mt^v/+$) \times type V stock d-43b (mt^v/mt^v), namely a 1:1 ratio, are in agreement with this hypothesis. Although only 39 pairs were tested in segregating crosses, the segregation numbers were 20:19.

The hypothesis was further tested by obtaining selfing in some of the stocks. Selfing does not normally occur in clones of type V, but they can be induced to self by mixing them with killed cells of type VI (HIWATASHI 1950). Table 2 shows that all viable selfing pairs (total 124) induced in this way in three stocks of type V produced clones of type V only, as required by the hypothesis. On the other hand, many stocks of type VI do self naturally (see below, p. 379). All clones from 31 selfing pairs obtained from type VI stock Ys3 were type VI, in agreement with the results of crosses (see above) that indicated it was a homozygous ($+/+$) stock. The critical type VI stock, K4, which behaved in crosses as if heterozygous, gave low survival (4/34) after natural selfing, but among the four survivors, two were type V and two were type VI. The hypothesis requires that a sufficient sample of survivors from selfing in stock K4 ($mt^v/+$, type VI) should include 1 mt^v/mt^v (type V): 2 $mt^v/+$ (type VI): 1 $+/+$ (type VI). The fact that the very small sample included clones of each mating type is in qualitative agreement with this requirement. Moreover, further crosses showed that

TABLE 2

Mating type distribution among clones from the selfings of parental stocks

Stock	Mating type	Synclones		Clones		Synclones and clones		Clones died or underwent MR
		V	VI	V	VI	V	VI	
Nd3	V	42	0	11	0	53	0	19
Ga1	V	16	0	1	0	17	0	7
Ma1	V	28	0	26	0	54	0	66
Ys3	VI	0	24	0	7	0	31	7
K4	VI	2	2	0	0	2	2	60

TABLE 3

Mating type distribution among progenies of the cross, Nd3 × K4s15b

Experiment No.	Synclones		Clones		Synclones and clones		Clones died or underwent MR
	V	VI	V	VI	V	VI	
1	0	32	0	3	0	35	7
2	0	12	0	5	0	17	5
3	0	18	0	0	0	18	6
Total	0	62	0	11	0	73	18

one of the type VI clones, K4s15b, was a homozygous recombinant: this clone was crossed three times to stock Nd3 (mt^V/mt^V), producing a total of 73 type VI progeny and none of the type V (Table 3). All of these type VI clones should be heterozygous ($mt^V/+$). Three of them were test-crossed to stock Nd3 (mt^V/mt^V), with the results shown in Table 4. Each yielded the expected 1:1 ratio, altogether including 83 type V and 92 type VI.

All of the preceding results clearly support the hypothesis proposed. However, quantitative data are given only for the following types of crosses: V (mt^V/mt^V) × V (mt^V/mt^V); V (mt^V/mt^V) × VI (+/+); V (mt^V/mt^V) × VI ($mt^V/+$); and VI (+/+) × VI (+/+). They gave the required results: 1:0; 0:1; 1:1; and 0:1, respectively. The remaining possibility, VI ($mt^V/+$) × VI ($mt^V/+$), which should give the 1:2:1 (phenotypically 1:3) result was attempted several times, but always gave too few viable progeny by normal nuclear reorganization to obtain a statistically significant result. One such example was given in the preceding paragraph. It and the other attempts gave, however, the required qualitative

TABLE 4

Mating type distribution among progenies of the test-cross

Cross P(V) × F ₁ (VI)	Synclones		Clones		Synclones and clones		Clones died or underwent MR
	V	VI	V	VI	V	VI	
Nd3 × d-143a							
Observed	22	32	9	8	31	40	93
Expected	27	27	8.5	8.5	35.5	35.5	..
Chi square	1.85		0.06		1.14		
P value	0.2 > P > 0.1		0.9 > P > 0.8		0.3 > P > 0.2		
Nd3 × d-6a							
Observed	9	10	0	0	9	10	6
Expected	9.5	9.5	..
Chi square		0.05		
P value		0.9 > P > 0.8		
Nd3 × d-2b							
Observed	38	34	7	8	43	42	47
Expected	36	36	7.5	7.5	42.5	42.5	..
Chi square	0.22		0.07		0.01		
P value	0.7 > P > 0.5		0.8 > P > 0.7		0.95 > P > 0.90		

result, namely, progeny of each mating type. Hence, as far as the analysis could be carried, all results were as required by the hypothesis. The recessive allele (mt^v) restricts homozygotes to the expression of mating type V, and presence of either one or two doses of the dominant allele (+) results in the expression of mating type VI. These results and this hypothesis do not imply that no other locus plays a role in the mating type system of syngen 3, but only that the difference between the mating types in the stocks examined is due to the differential action of the two alleles mt^v and +.

Instability in the expression of mating type: Most clones of genotype mt^v/mt^v express only mating type V and never show natural selfing, but most clones of mating type VI can self naturally. Apparently selfing is due to the presence of cells of type V (see DISCUSSION); but the clone remains predominantly type VI. Some type VI stocks (e.g. Kn6) or clones, but not all, show a more extreme form of mating type instability, like that found earlier in only one clone of syngen 12 (HIWATASHI 1960). This extreme instability is brought to expression by controlling fission rate by varying the amount of food supplied. When provided daily with enough food to permit rapid fission rate (e.g. 2 fissions per day or more), the clones are prevalingly type VI although selfing may occur; when provided daily with enough food to permit only a slow fission rate (e.g. 0.5 fission per day), the cells are all type V and no selfing occurs. The changes are reversible. Clones capable of this extreme instability do not show it during early maturity; it usually develops first about one month after attaining maturity. Further details will be given in a forthcoming paper.

The genetic basis of this extreme instability has not yet been analysed, but some possibilities can be excluded by the following results. Stock K4s15b is type VI (+/+) and does not exhibit extreme instability, nor did the F_1 obtained by crossing it to stock Nd3 (mt^v/mt^v) although all F_1 clones were type VI (Table 3). However, after two backcrosses to Nd3, more than half of the type VI clones belonged to the extremely unstable class. Therefore if this class has a genic basis, the decisive gene or genes appear to have been derived from stock Nd3. The gene mt^v of stock Nd3 could hardly be a decisive one because it was present in the F_1 in the same dosage and accompanied by the same + allele as in the backcross generations; yet the F_1 did not contain extremely unstable clones. Hence, it would appear that a genic basis would have to be referred to another locus or other loci; but this remains to be investigated.

An exceptional case of abnormal segregation: One of the extremely unstable clones gave aberrant mating type ratios both after selfing and backcrossing. This clone was an F_1 from the cross of stock Ma1, mating type V, by stock Ys3, mating type VI and genotype +/+. The F_1 consisted exclusively of type VI clones (Table 1). Among the 32 clones from 18 pairs of conjugants (8 clones died or underwent macronuclear regeneration), 24 became unstable in mating type and 14 of these were shown to undergo mating type reversibility in dependence on growth rate; the other 10 did not. One of the 14 extremely unstable clones was the one that gave the aberrant ratios. Like all of the F_1 , it should have been heterozygous both for a serotype marker (f^{Ma1}/f^{Ys3}) and for the mating type alleles

TABLE 5
Abnormal distribution of mating types and normal segregation of serotype F classes among progenies of the test-cross and of selfing in the F₁ from the cross, Ma1 × Ys3

Cross	Synclones		Mating type		Synclones and clones		Serotype marker*		Clones died or underwent MR	
	V	VI	V	VI	V	VI	f^{Ys3}/f^{Ys3}	f^{Ma1}/f^{Ma1}		
Test-cross (f^{Ys3}/f^{Ys3} , mt^V/mt^V × f^{Ma1}/f^{Ys3} , $mt^V/+$)	Observed	0	25	2	16	2	41	23	20	54
	Expected	12.5	12.5	9	9	21.5	21.5	21.5	21.5	..
	Chi square	35.37	...	0.21
	P value	P < 0.001	...	0.7 > P > 0.5
Selfing of F ₁ (f^{Ma1}/f^{Ys3} , $mt^V/+$ × f^{Ma1}/f^{Ys3} , $mt^V/+$)	Observed	3	46	4	23	7	69	17	59	91
	Expected	12.3	36.7	6.7	20.3	19	57	19	57	..
	Chi square	10.1	...	0.28
	P value	0.01 > P > 0.001	...	0.7 > P > 0.5

* Tests were made with anti-K4 serum which gave titers of 1/1600 for f^{Ma1}/f^{Ma1} and f^{Ma1}/f^{Ys3} , 1/100 for f^{Ys3}/f^{Ys3} respectively.

($mt^V/+$) because the two parents were homozygous for different alleles at both loci: stock Ma1 was mt^V/mt^V , f^{Ma1}/f^{Ma1} and stock Ys3 was $+/+$, f^{Ys3}/f^{Ys3} . As shown in Table 5, the serotype markers segregated normally in the F_2 (by selfing) and in the testcross to stock d-42a (mt^V/mt^V , f^{Ys3}/f^{Ys3}); but the mating type alleles did not. In both crosses, clones of type VI were excessively frequent. Nevertheless, synclonal inheritance was the rule without exception. This implies a nuclear basis for the abnormal ratio, perhaps an aberration of the mt chromosome. Solution of this problem obviously requires further experimental analysis.

DISCUSSION

Early transient maturity: As pointed out on p. 375, some caryonides exhibited early transient maturity with "maternal" inheritance of mating type; this was followed by immaturity and then by synclonal uniformity in mating type, i.e. zygotic, not maternal, inheritance. HIWATASHI (1958) reported comparable observations in some clones of syngen 12 when the F_1 was obtained and interpreted them as cytoplasmic (phenomic) lag, i.e. the cytoplasm of the mother cell was believed to determine mating type expression for about 20 cell generations or more in some clones. This interpretation is now clearly untenable because the present paper indicates that early maturity and maternal inheritance of mating type are not clonal but seemingly caryonidal characters, although the possibility of subcaryonidal differences in these respects was not excluded. In the work on syngen 12, only one caryonide from each exconjugant was studied, so the subclonal aspect was missed and unsuitable interpretations were proposed.

What then might be the explanation of irregularly occurring early, transient maturity during which the mating type of the cytoplasmic parent is expressed? Macronuclear regeneration in the form now known could account for early maturity and expression of the "maternal" mating type, but it cannot account for the transiency of this maturity or for the change from the "maternal" to the zygotically determined mating type during the later stage of normal, persistent maturity. If it is assumed, however, that a fragment of the prezygotic macronucleus becomes incorporated in a developing macronuclear anlage and reproduces as a part of the macronucleus, all of the observations could be accounted for. Since the incorporated old part had been and should continue to be active (as in macronuclear regeneration), the cell-lineage should express the mating type of the cytoplasmic parent. The eventual disappearance of this phenotypic effect and correlated appearance of a new phenotypic effect (immaturity and, later, the zygotic mating type) due to activity of the other part of the macronucleus, would be expected as the result of distribution of macronuclear subunits to daughter macronuclei during asexual reproduction (compare ALLEN and NANNY 1958). This hypothesis leads to a number of other predictions, all of which could be tested in future investigations.

Mating type determination in syngen 12 of P. caudatum: HIWATASHI (1958) reported results on mating type inheritance in syngen 12 of *P. caudatum* that parallel those here presented on syngen 3 except for one apparent contradiction.

Aside from that, the results accord with the hypothesis that mating type XXIII is homozygous for the recessive gene mt^{XXIII} while the dominant allele, +, determines mating type XXIV. The apparent contradiction concerned stock K₃2 of mating type XXIV. When this stock was crossed to type XXIII, the F₁ segregated 1 type XXIII: 1 type XXIV, as if stock K₃2 were a heterozygote ($mt^{XXIII}/+$); but when stock K₃2 selfed, all of the progeny were type XXIV, as if stock K₃2 were a homozygote (+/+). The present study suggests a possible interpretation. Macronuclear regeneration (MR) now appears to be of common occurrence in *P. caudatum* and it regularly results in retention of maturity and the "maternal" mating type. If MR had occurred after selfing in stock K₃2, but not in the cross to type XXIII, the apparent contradiction in results would disappear. There are two reasons to believe it did occur in all of the surviving selfed pairs. First, they all lacked an immature period. At that time, not knowing that MR can occur with high frequency in *P. caudatum*, HIWATASHI (1960) supposed that clones from selfing pairs of some stocks lacked a period of immaturity, though there was no evidence to support this and the present results show that this is not true in syngen 3. Second, as pointed out in the RESULTS section (see also Table 2, last line), selfing in hybrids of syngen 3 gives very few viable clones that have reorganized their nuclei normally after conjugation; nearly all of the progeny that survived had undergone macronuclear regeneration. This now makes it reasonable to conclude that 100 percent type VI resulting from selfing in stock K₃2 of syngen 12 was due to the exclusive occurrence of macronuclear regeneration in every survivor; and that the inference as to the hybridity of this stock ($mt^{XXIII}/+$), based on crosses, was correct.

An attempt was therefore made to test this interpretation by reinvestigating selfing in stock K₃2. Keeping preconjugalts, conjugalts and exconjugalts in the same medium without transfer to fresh medium, only 12 of 36 clones (18 pairs) obtained by natural selfing died or underwent MR. The remaining 24 clones (12 synclones) went through a typical period of immaturity; then 4 of the 12 synclones developed mating type XXIII and 8 developed mating type XXIV. This qualitatively confirms the interpretations in the preceding paragraph: lack of a period of immaturity is not due to selfing; and the clone K₃2 was a heterozygote ($mt^{XXIII}/+$). This resolution of the initial apparently contradictory results leaves all of the results consistent with the conclusion that mating types in the stocks of syngen 12, like those in the stocks of syngen 3, are determined by a pair of allelic genes: mating type XXIII develops in homozygotes mt^{XXIII}/mt^{XXIII} ; type XXIV develops when the dominant allele, +, is present. However, the breeding analysis in syngen 12 was less extensive than that presented in the present paper for syngen 3.

Comparison of the genetics of mating types in P. caudatum with that in other Ciliates: The chief features of the genetics of mating types in *P. caudatum* are: (a) the mating type in the stocks examined and their progeny is determined by alleles at one locus, the *mt* locus; (b) the recessive allele restricts homozygotes to the expression of one mating type, the one designated by an odd number (type V in syngen 3 and type XXIII in syngen 12); (c) presence of either one or two

doses of the dominant allele permits expression of the mating type designated by an even number (types VI and XXIV, respectively, in these two syngens); (d) when the dominant allele is present, the even mating type is expressed exclusively or almost so during about the first month of the mature period, but thereafter some of these clones can produce some cells of type V, others produce only type V cells when grown slowly by limiting the food supply, but some produce no type V cells at all; (e) preliminary results suggest that these differences among clones possessing the dominant allele are due to recessive genes at a locus or loci other than the *mt* locus.

This set of features has not been completely reported for any other species of Ciliate, but various elements of it have been. The parallels to *P. aurelia* appear to be of particular interest and significance. Different syngens of *P. aurelia* are characterized by somewhat different genetics of mating types, certain features of syngens 1, 5, 7 and 13 being of special relevance. The genetic system in syngen 13, the most recent one to be investigated (SONNEBORN 1966) and consequently the least extensively studied, appears to resemble closely the one in *P. caudatum*; mating type expression in the stocks thus far examined is controlled by alleles at a single locus; the odd mating type develops in homozygous recessives (*rr*) and clones possessing the dominant allele (*R*) develop the even mating type; homozygous dominants are exclusively of the even mating type, but heterozygous clones can self and SONNEBORN (personal communication) finds that this is due to development of the odd mating type by some cells for a few hours while a culture is depleting its food supply and becoming sexually reactive. The principal differences from the *P. caudatum* system are that thus far homozygous dominants have never been found to produce cells of the odd mating type and that thus far no evidence has been found for other loci affecting mating type expression in clones bearing the dominant allele. This is perhaps not surprising in view of the relatively limited investigation of syngen 13 and in view of the fact that clones of *P. aurelia* do not normally reach a stage comparable to the one during which selfing appears in *P. caudatum*; instead, their period of maturity is relatively brief, autogamy bringing it to an end by initiating new young clones.

All other syngens of *P. aurelia* differ from those of *P. caudatum* in a fundamental feature: clones or caryonides with identical micronuclear genotype differ in mating type expression and the difference is traceable to an irreversible and therefore hereditary differentiation of the macronuclei occurring before the first cell division after fertilization. In spite of this basic difference, other features of their genetic system have remarkable similarities to the phenomena observed in *P. caudatum* and may help understand them. Thus, in syngen 5 of *P. aurelia*, BLEYMAN (1967) found that caryonides can be restricted to the expression of the odd mating type, but any caryonide that can express the even mating type can also express the odd mating type. In that syngen, no genic differences affecting mating type expression have been discovered, and the different kinds of caryonides have identical micronuclear genotypes. On the other hand, relevant genic differences do occur in syngens 1 and 7 (SONNEBORN 1939; BUTZEL 1955; TAUB 1963). Homozygotes for certain recessive genes are restricted to expression of the

odd mating type. The corresponding homozygous and heterozygous dominants can express both mating types, as in *P. caudatum*. However, macronuclear differentiation restricts the cells of a caryonide to expression of one mating type, as mentioned above. Nevertheless some caryonides of dominant clones are differentiated so as to control hereditary selfing due to the development of both mating types within a caryonide.

BUTZEL (1955) proposed the hypothesis that the odd mating type substance in *P. aurelia* is a precursor of the even mating type substance and that the dominant allele at the *mt* locus controls the conversion reaction. The recessive allele is held to be unable to carry out the conversion. This accounts for the restriction of homozygous recessives to the odd mating type. To account for the potential of dominants to express either mating type, the dominant allele is held to be subject to regulation, i.e. to be active or inactive (see also TAUB 1963).

This hypothesis can be applied directly to features (a), (b), and (c) in the above formulation of the mating type system in *P. caudatum*. Feature (d) would be in conformity with one additional assumption, namely that the probability of repression of the dominant allele increases with clonal age. HECKMAN (1967) has reported such an age effect in the mating type system of *Euplotes*. Feature (e) could be understood as involving loci that operate to control activity of the + allele at the *mt* locus. On the other hand, the possible applicability of BUTZEL's hypothesis to *P. caudatum* is purely formal and neither in this species nor in *P. aurelia* is there any evidence of the nature of the primary function of the genes for mating type expression.

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SUMMARY

Genetics of mating types in syngen 3 of *P. caudatum* includes the following features: (1) mating type potential is determined by alleles at one locus; (2) the recessive allele *mt*^v, restricts homozygotes to mating type V; (3) the dominant allele, +, permits expression of mating type VI; (4) +/+ and +/*mt*^v clones express type VI exclusively or almost so during about the first month of the clonal period of maturity, but thereafter dominant clones differ: some remain pure for mating type VI; some express only mating type V when grown slowly and only or prevaillingly mating type VI when grown rapidly; and some express mating type V only in some cells of the clone; (5) preliminary results suggest that these differences among dominant clones are due to recessive genes at a locus or at loci other than the *mt* locus. One clone gave discordant results, possibly because of a chromosomal aberration. Other rare exceptions are interpreted as due to incorporation of a fragment of the prezygotic macronucleus in a postzygotic macronuclear anlage.—The facts reported by HIWATASHI (1958) for mating type

inheritance in syngen 12 of *P. caudatum* appear to agree with a parallel formulation, homozygotes for the recessive gene *mt^{XXIII}* being restricted to mating type XXIII and the dominants being capable of expressing mating type XXIV, but with variations like those in corresponding dominants of syngen 3. Earlier puzzling results with syngen 12 appear to have been due to undetected macronuclear regeneration.—Parallels between the facts of mating type determination in *P. aurelia* and *P. caudatum* are discussed in relation to the possible applicability to *P. caudatum* of certain interpretations proposed for *P. aurelia*.

LITERATURE CITED

- ALLEN, S. L., and D. L. NANNEY, 1958 An analysis of nuclear differentiation in the selfers of *Tetrahymena*. *Am. Naturalist* **92**: 139–160.
- BARNETT, A., 1966 A circadian rhythm of mating type reversals in *Paramecium multimicro-nucleatum*, syngen 2, and its genetic control. *J. Cellular Comp. Physiol.* **67**: 239–270.
- BLEYMAN, L. K., 1967 Determination and inheritance of mating type in *Paramecium aurelia*, syngen 5. *Genetics* **56**: 49–59.
- BUTZEL, H. M., 1955 Mating type mutations in variety 1 of *Paramecium aurelia* and their bearing upon the problem of mating type determination. *Genetics* **40**: 321–330.
- DIPPELL, R. V., 1955 A temporary stain for *Paramecium* and other ciliate protozoa. *Stain Technol.* **30**: 69–71.
- DRYL, S., 1959 Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during autogamy and conjugation. *J. Protozool.* **6** (suppl.): 25.
- HECKMANN, K., 1961 Paarungstypen und ihre genetische Determination bei dem marinen Ciliaten *Euplotes vannus* O. F. MÜLLER. *Naturwissenschaften* **48**: 438–439. — 1964 Experimentelle Untersuchungen an *Euplotes crassus* I. Paarungssystem, Konjugation und Determination der Paarungstypen. *Z. Vererb.* **95**: 114–124. — 1967 Age-dependent intraclonal conjugation in *Euplotes crassus*. *J. Exptl. Zool.* **165**: 269–277.
- HIWATASHI, K., 1950 Studies on the conjugation of *Paramecium caudatum*. III. Some properties to the mating type substances. *Sci. Rept. Tohoku Univ. Biol.* **18**: 270–275. — 1958 Inheritance of mating types in variety 12 of *Paramecium caudatum*. *Sci. Rept. Tohoku Univ. Biol.* **24**: 119–129. — 1960 Analyses of the change of mating type during vegetative reproduction in *Paramecium caudatum*. *Japan. J. Genet.* **35**: 213–221. — 1967 Serotype inheritance and serotypic alleles in *Paramecium caudatum*. *Genetics* **57**: 711–717.
- KIMBALL, R. F., 1942 The nature and inheritance of mating types in *Euplotes patella*. *Genetics* **27**: 269–285.
- NANNEY, D. L., and P. A. CAUGHEY, 1953 Mating type determination in *Tetrahymena pyriformis*. *Proc. Natl. Acad. Sci. U. S.* **39**: 1057–1063.
- NANNEY, D. L., P. A. CAUGHEY, and A. TEFANKJIAN, 1955 The genetic control of mating type potentialities in *Tetrahymena pyriformis*. *Genetics* **40**: 668–680.
- ORIAS, E., 1963 Mating type determination in variety 8, *Tetrahymena pyriformis*. *Genetics* **48**: 1509–1518.
- POWERS, E. L., 1943 The mating type of double animals in *Euplotes patella*. *Am. Midland Naturalist* **30**: 175–195.
- SEGEL, R. W., and L. L. LARISON, 1960 The genetic control of mating types in *Paramecium bursaria*. *Proc. Natl. Acad. Sci. U. S.* **46**: 344–349.

- SONNEBORN, T. M., 1937 Sex, sex inheritance and sex determination in *Paramecium aurelia*. Proc. Natl. Acad. Sci. U. S. **23**: 378-385. — 1939 *Paramecium aurelia*: Mating types and groups; lethal interactions; determination and inheritance. Am. Naturalist **73**: 390-413. — 1947 Recent advances in the genetics of *Paramecium* and *Euplotes*. Advan. Genet. 1: 263-358. — 1950 Methods in the general biology and genetics of *Paramecium aurelia*. J. Exptl. Zool. **113**: 87-143. — 1966 A non-conformist genetic system in *Paramecium aurelia*. Am. Zoologist **6**: 589.
- TAUB, S. R., 1963 The genetic control of mating type differentiation in *Paramecium*. Genetics **48**: 815-834.
- WICHTERMAN, R., 1940 Cytogamy: A sexual process occurring in living joined pairs of *Paramecium caudatum* and its relation to other sexual phenomena. J. Morphol. **66**: 423-451.