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Mating Type Inheritance at Conjugation in Variety 4 of *Paramecium aurelia**

DAVID L. NANNEY†
Zoology Departments, Indiana University and University of Michigan

SUMMARY. The pattern of mating type distribution observed at conjugation under different conditions in stock 51, variety 4, of *Paramecium aurelia* is presented. When the two mating types (VII and VIII) found in this variety are crossed, each pair member usually gives rise to two caryonides of its own mating type. Either parent may, however, give rise to progeny unlike itself. When cytoplasmic fusion is induced by antiserum treatment, the two members of a pair characteristically yield progeny of the same mating type, and the progeny are usually mating type VIII. The direction of change can be influenced to some extent by the temperature at which conjugation occurs; changes from VII to VIII are more frequent at the higher temperatures.

A modification of mating type distribution is observed when conjugation occurs in the unstable clones designated as "selfing caryonides". Changes are frequent under these circumstances, but are predominantly from type VIII to type VII—precisely the reverse direction observed when pure clones are crossed. The relative frequencies of the changes in the two directions are again influenced by the temperature at which conjugation occurs. These observations indicate a separation of two normally correlated macronuclear functions—the activity in determining the phenotype (mating type) of the cell and the activity in determining the cytoplasmic conditions responsible for nuclear differentiation in the following generation. An interpretation of these results, based on macronuclear heterogeneity, is suggested.

TWO PATTERNS of mating type determination have been described for *Paramecium aurelia*(7,12), the so-called Group A pattern found in varieties 1, 3, 5, 7 and 9 and the Group B pattern occurring in varieties 2, 4, 6, and 8. Considerable data have been published documenting the Group A pattern(4,10,11), but the Group B pattern has been discussed only in general terms(6,7,12,14). One purpose of this report is to make available to those not working in Paramecium genetics specific information on the inheritance of mating types at conjugation in Group B. It should be understood that some of the data to be presented are derived from experiments similar to those performed much earlier by Sonneborn, but not published. The use of Sonneborn's unpublished data is gratefully acknowledged. A second and more significant purpose is to present an analysis of the inheritance pattern at conjugation in the clones of unstable mating type in the Group B varieties. Unstable clones occur in both Group A and Group B, but the Group A clones have been discussed in detail in publications(5) and the Group B clones, which show additional features of interest, have been only briefly mentioned(7).

MATERIALS AND METHODS

The methods used in these studies correspond in detail to those described by Sonneborn(13) and need to be discussed only in relation to special features introduced in the course of the experiments. The essential cytogenetic phenomena have also been presented elsewhere(12) and will not be elaborated here. The only stocks used in this study were stocks 29 and 51, both of variety 4.

RESULTS

1. *The normal pattern of mating type inheritance in variety 4.* Sonneborn(11) has reported that the usual result of conjugation in a Group B variety is the perpetuation of the mating type of the cytoplasmic parent among its progeny, but that occasional diversities arise either at the first fission following conjugation or continuously during vegetative growth. This differs from the situation in the Group A varieties where no correlation is observed between the mating type of the cytoplasmic parent and the mating types of the progeny, and where the mating types are distributed at random among the first fission products at conjugation. It is this latter situation which was first termed "caryonidal inheritance". Caryonidal inheritance derives its name from the correlation between the distribution of new macronuclei and the distribution of new mating types following conjugation. The progeny of the first fission products, each

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of which receives an independently developed macronucleus, are termed caryonides; caryonides are usually pure for a single mating type, but—in Group A—sister caryonides are no more often alike than caryonides from different pairs. Caryonidal inheritance suggests that the macronuclei control the mating types and that macronuclei with the same genes can control different mating types. This implication of the macronucleus is further supported by mating type distribution under certain unusual conditions(12). In certain stocks more than two new macronuclei are formed at conjugation and these are distributed to the daughter cells over several fissions; under these conditions the separation of diverse lines of pure mating type also occurs over several fissions. Secondly, when new macronuclei are aborted and the fragments of the old macronuclei regenerate, no change in mating type is observed at conjugation. There can be no reasonable doubt, therefore, that the macronuclei control the mating types in the Group A varieties.

The "caryonidal" character of mating type determination in the Group B varieties is not so readily apparent and is based not so much on the mating type distribution normally observed at conjugation, as on the mating type distribution under certain experimental conditions(14). We will return to these crucial experiments after surveying the more typical results of conjugation. When clones of types VII and VIII are mixed, pairs are isolated, and the exconjugants and caryonides are separated and grown into cultures, results of the sort shown in Table I are obtained. In the majority of pairs no change of mating type occurs; the type VII pair member yields two caryonides of type VII; the type VIII member produces two caryonides of type VIII. In some pairs, however, the type VII pair member may yield two type VIII caryonides or the type VIII pair may produce two type VII caryonides. In these cases the exconjugants produce pure clones of a mating type, but change has occurred; these pairs are classified as showing "clonal" changes. In other cases the change

TABLE I. The distribution of mating types among caryonides arising at conjugation in stock 51.

Classes of pairs	Mating types of caryonides	No. observed	Direction of change
No change	VII, VII – VIII, VIII	135	—
"Clonal" change	VII, VII – VII, VII	1	VIII to VII
	VIII, VIII – VIII, VIII	11	VII to VIII
Caryonidal change	VII, VII – VII, VIII	4	VIII to VII
	VII, VIII – VIII, VIII	5	VII to VIII
Sub-caryonidal change	VII, VII – VII, Self.	2	VIII to VII
	VII, Self. – VIII, VIII	5	VII to VIII
	Self., VIII – VIII, VIII	6	VII to VIII
	Self., Self. – VIII, VIII	1	VII to VIII
Total		170	

TABLE II. The distribution of mating types among exconjugant clones in stocks of variety 4.

Stock	Mating types of exconjugants VII VII	VII Self.	VII VIII	Self. Self.	VIII Self.	VIII VIII	Total	Changes to VII	Changes to VIII
29	18	12	133	3	6	18	190	33	27
51	29	8	273	0	14	35	359	37	49

may involve only one of the two caryonides from a particular exconjugant; a type VII cell may produce a pure caryonide of type VII plus a pure caryonide of type VIII. These pairs are classified as showing caryonidal change. Finally pairs occur which produce one or more "selfing" caryonides, i.e., caryonides which contain both mating types and within which conjugation can occur. These selfing caryonides may be associated with either an unchanged or a changed sister caryonide. The *direction* of change can in each instance be determined if one assumes that changes characteristically occur in only a single pair member. It will be observed that changes in both directions occur, but that the changes from type VII to type VIII are more numerous.

Since the two caryonides from a given pair member are so often alike, it is practicable to study mating type distribution without isolating caryonides. This permits the study of larger numbers of pairs and sacrifices relatively little information. The results of some experiments in which the exconjugants were grown as clonal cultures are shown in Table II. The only ambiguity arises in regard to the selfing clones; no basis is available for distinguishing between two pure but different caryonides, one pure and one selfing caryonide, and two selfing caryonides. For most purposes these distinctions are of little significance.

The observations show that differences may occur between sister caryonides in the Group B varieties, but that no precise and convincing correlation is observed between macronuclear distribution and mating type distribution. The belief in a caryonidal pattern is, therefore, based 1) on analogy with the Group A pattern and 2) on certain experiments of Sonneborn to which we will return later.

2. *The effect of cytoplasmic fusion on mating type determination.* Sonneborn(11) has reported that conjugation in the Group B varieties yields different results depending upon whether massive exchanges of cytoplasm occur during the process. His early observations were based on the rare pairs showing "spontaneous" cytoplasmic fusion, but more recently he has developed a technique for inducing fusion by exposing conjugating pairs to specific antiserum(13). Using this technique results of the kind shown in Table III are obtained. The degree of cytoplasmic fusion (as measured by the length of delay in separation of the

TABLE III. The distribution of mating types at conjugation in stock 51 when pairs are treated with specific antiserum.

Antiserum concentration	Hours delay	No. pairs	Pairs with like exconjugants	Like pairs pure type VIII
0	0	54	3 (6%)	3 (100%)
1/12800	0	54	2 (4%)	2 (100%)
1/6400	0– 6	52	7 (13%)	7 (100%)
	6–18	3	1 (33%)	0 (0%)
1/3200	0– 1	2	0 (0%)	0
	0– 4	38	8 (21%)	8 (100%)
	1– 6	15	5 (33%)	5 (100%)
	4–16	10	8 (80%)	8 (100%)
	6–22	25	21 (84%)	21 (100%)
	Doubles	2	2 (100%)	2 (100%)
1/1600	0– 2	4	0 (0%)	0
	0– 5	9	2 (22%)	2 (100%)
	2–10	15	11 (79%)	11 (100%)
	5–15	34	25 (74%)	24 (96%)
	10–25	17	15 (89%)	14 (93%)
	Doubles	24	23 (96%)	21 (91%)

pair members) increases with the antiserum concentration. At the higher concentrations some pairs fail entirely to separate and remain as homopolar doublets (doubles). The probability that the exconjugants will produce like progeny increases with the length of delay and nearly all these changes are in the direction observed most commonly without antiserum treatment, i.e., from type VII to type VIII.

The experiments most conclusively establishing the caryonidal nature of mating type determination in the Group B varieties are those of Sonneborn using both cytoplasmic exchange and macronuclear regeneration(14). Regeneration of macronuclear fragments (13) is brought about by heat treatment during the period when the new macronuclei are developing. This treatment either aborts the new macronuclei or delays their development and division so that a fraction of the progeny of an exconjugant fails to obtain a new macronucleus but instead retains a fragment of the old macronucleus which regenerates to normal size. The segregation of the new and the old macronuclei can be followed with reasonable accuracy. Under the circumstances of cytoplasmic fusion and macronuclear regeneration Sonneborn observed that the mating types segregated as the diverse kinds of macronuclei segregated; those sub-lines receiving fragments of the old macronucleus manifested mating type VII, if the parental cell was originally mating type VII. This correlation between the distribution of diverse macronuclei and the distribution of mating types is the same *kind* of correlation observed in the Group A varieties and leads to the same conclusion; the macronuclei control the mating types in the Group B varieties as certainly as in the A varieties.

3. *The effect of temperature on mating type determination.* Marked temperature effects on mating type frequencies have been shown in the Group A varieties (11,12), but such temperature effects on the Group B varieties have not been documented. The results obtained at conjugation at different temperatures are shown in Table IV. These data suggest a slight effect of temperature on the direction of change but the frequency of change is unaltered. At higher temperatures the changes are predominantly in the direction of type VIII. In order to determine if a more striking effect of temperature could be achieved under conditions of greater instability, antiserum-induced double animals were obtained at conjugation at different temperatures. Unfortunately, relatively few doubles were obtained at the lower temperatures but an indication of a temperature effect is seen (Table V). These observations suggest that the cytoplasmic conditions responsible for mating type determination are usually temperature stable, but that the mixture of two kinds of cytoplasm is relatively unstable and may be influenced by temperature.

TABLE IV. The distribution of mating types at conjugation in stock 51 at different temperatures.

Temperature	Mating types of exconjugants VII VII	VII Self.	VII VIII	VIII Self.	VIII VIII	Total	Changes to VII	Changes to VIII
12°C.	5	2	61	3	3	74	7	6
27°C.	29	8	273	14	35	359	37	49
32°C.	1	0	66	3	8	78	1	11

4. *Mating type determination at conjugation in selfing caryonides.* Kimball(5) has shown that occasional caryonides in the Group A varieties contain both mating types and continue to produce both mating types from single cells over long periods of time. He found, however, that following conjugation in these selfing caryonides no greater frequency of selfing progeny is produced than in crosses of pure types. In view of the differences between the Group A and Group B systems, it appeared profitable to examine the consequences of conjugation in equivalent unstable cultures in Group B. The results, shown in Table VI, were most unexpected. A very large fraction of the pairs showed change, and the observed changes—unlike those obtained in crosses of pure cultures—were predominantly from type VIII to type VII. This interpretation of the direction of change presupposes that all pairs consist initially of one type

TABLE V. Temperature effects on mating type determination at conjugation in antiserum induced double animals.

Temp.	No. doubles	Pure VII	Mixed	Pure VIII
13°C.	3	1	1	1
21°C.	8	1	1	6
27°C.	34	3	3	28
32°C.	66	3	5	58

TABLE VI. The distribution of mating type at conjugation in unstable caryonides.

Crosses	Mating types of exconjugants VII VII	VII Self.	VII VIII	VIII Self.	VIII VIII	Total	Changes to VII	Changes to VIII
6	64	19	7	2	0	92	83	2

VII and one type VIII cell and not of two type VII cells. Although this is difficult to demonstrate in these selfing caryonides, several reasons exist for the belief. First, true conjugation between cells of the same type has never been observed under conditions which would permit its detection. Secondly, both mating types occur in selfing cultures and the large majority of the cells in the cultures studied were mating type VIII. This conclusion is based on the matings observed when selfing cultures are mixed separately with pure type VII and pure type VIII cultures. Usually very few pairs are formed in the mixture with type VIII, or in the unmixed selfing culture. When the selfer is mixed with type VII, on the other hand, many pairs are formed, nearly as many as when the two pure types are mixed. Such tests provide evidence for mating type diversities and a rough indication of the relative proportions of the two types in a selfing caryonide.

In regard to the physical basis for this mating type instability at conjugation, three possibilities are suggested. The changes to type VII could result from some peculiarity of the type VII parent, the type VIII parent, or to some interaction between them. These possibilities were explored as follows. A culture of predominantly type VIII cells was mixed with a large excess of normal cells of type VII; nearly all the pairs formed in the mixture should be of the constitution "Selfer VIII" $\times$ pure VII. If the instability were apparent here, its cause could not be attributed to the "Selfer VII's" or to some interaction of Selfer VII and Selfer VIII. The results, shown in Table VII, indicate that the instability at conjugation is a function of the Selfer VIII's; it is detected regardless of the mate with which it is paired. The approximate equivalence of the results of crosses of Selfer VIII $\times$ Selfer VII and Selfer VIII $\times$ pure VII suggests that the Selfer VII is about as stable at conjugation as the pure VII.

TABLE VII. The distribution of mating type at conjugation in crosses of "Selfer VIII's" to normal type VII clones.

Crosses	Mating types of exconjugants VII VII	VII Self.	VII VIII	VIII Self.	VIII VIII	Total	Changes to VII	Changes to VIII
3*	37	5	2	1	1	46	42	2
3†	15	5	20	2	1	43	20	3

* Crosses of predominantly type VIII selfers which showed selfing consistently.

† Crosses of predominantly type VIII selfers which showed selfing only when several sub-cultures were examined.

These data show one further characteristic of the selfing caryonides. Three of the crosses utilized selfing caryonides in which selfing pairs, while not numerous, were consistently found in all subcultures. Another three crosses involved selfing caryonides in which extremely few pairs were found in the unmixed cultures and the selfing was detected only when several sub-cultures were examined carefully. The results of the crosses indicate that the former selfing caryonides—manifesting greater "vegetative instability"—also showed greater instability at conjugation. Whether discrete classes of instability or a continuous spectrum of instability occurs among various selfers is not yet known, but the correlation between vegetative inheritance and inheritance at sexual reproduction is clear.

5. *Temperature effects on mating types at conjugation in selfing caryonides.* Since temperature effects on mating type frequencies in stock 51 were found whenever changes were detected, it appeared likely that temperature effects could also be found at conjugation in selfing caryonides, which regularly show a large amount of change at conjugation. The results, shown in Table VIII, bear out this assumption and show the same direction of effects previously noted; mating type VIII is more prevalent following conjugation at a higher temperature. The temperature effect in conjugation with cytoplasmic exchange was interpreted as due to an instability brought about by mixture of the cytoplasms of two kinds—that normally bringing about the differentiation of type VII nuclei and that normally responsible for type VIII differentiation. It is possible that the temperature response in selfing caryonides reflects a cytoplasmic instability of a similar nature. We will return to this problem later.

6. *Exclusion of certain explanations for the selfers.* Two suggestions regarding the nature of the selfing caryonides require exploration. First it might be suggested that mating type instability at conjugation is reflection of a change of mating type at the previous reorganization, that *any* changed clone requires time before a stable and integrated system of inheritance is developed. This possibility was examined by studying the inheritance of mating types at conjugation in type VIII clones from a variety of sources, many of which had undoubtedly changed from VII to VIII at the previous reorganization, but which had changed—unlike the selfers—from a pure VI to a *pure* VIII. The results (Table IX) show no evidence

TABLE VIII. Temperature effects on mating type determination at conjugation in unstable caryonides.

Crosses	Temp.	Mating types of exconjugants: VII VII	VII Self.	VII VIII	Self. Self.	VIII Self.	VIII VIII	Total	Changes to VII	Changes to VIII
3*	14°C.	15	6	1	1	1	0	24	22	2
	32°C.	10	4	8	0	4	3	29	14	7
2†	14°C.	14	7	3	0	1	0	25	21	1
	32°C.	8	6	10	0	1	0	25	14	1

* Crosses within selfing caryonides.
† Crosses of "Selfer VIII's" to normal VII's.

for more than normal instability and demonstrate that the significant aspect of the selfers is *not* that they have changed, but that they have changed to *selfers*.

A second possibility concerning the instability at conjugation in selfing caryonides is that the growth of two mating types in the same medium brings about instability in the VIII's. Either diffusible substances or chance contacts between the cells might alter the cytoplasmic conditions in the cells. This possibility was examined by studying conjugation in selfing *clones*, derived from single exconjugants. As pointed out above, a certain ambiguity exists in regard to the constitutions of these clones; some contain two pure caryonides of different types; some contain one or more selfing caryonides. The results, shown in Table X, are however unambiguous. Most of the clones show no more instability and no more marked temperature responses than do pure clones mixed at the time of mating. The instability of mating type inheritance in selfing caryonides is not, therefore, due to the continued association of different mating types.

TABLE IX. The distribution of mating types at conjugation in crosses involving recently derived pure type VIII caryonides from a variety of sources, including particularly those clones from pairs which showed changes in the previous generation.

Crosses	Mating types of exconjugants: VII VII	VII Self.	VII VIII	VIII Self.	VIII VIII	Total	Changes to VII	Changes to VIII
20	6	8	348	26	21	409	14	47

DISCUSSION

These observations concerning mating type inheritance in the Group B varieties may be understood on the basis of a reasonably simple conceptual scheme. That the macronuclei control the phenotype is shown by the occasional occurrence of caryonidal distribution and more particularly by Sonneborn's(14) demonstration of the correlated distribution of mating types and macronuclei under certain experimental conditions.

The second essential fact in the Group B system is that the cytoplasm directs the course of macronuclear development. This is indicated first by the usual perpetuation of the parental mating types through conjugation—when the genetic constitutions of the pair members are made equivalent—and secondly by the results observed when massive cytoplasmic exchange is induced. When the cytoplasms remain separate, the macronuclei in the paired cells usually develop in *different* ways; when the cytoplasms are fused, the macronuclei in the paired cells usually develop in the *same* way.

The third aspect of the system involves the relationship between the macronucleus and the cytoplasmic conditions. It is conceivable that the cytoplasm is independent of nuclear control and contains a "self-perpetuating" system of some sort. On the other hand, the cytoplasm may be under the direct control of the nucleus. The available evidence leads decisively to this second interpretation. First, when two macronuclei developing in the same cell at the same time are differentiated to control different mating types, the cytoplasmic conditions necessary for the

TABLE X. The distribution of mating types at conjugation within selfing *clones*.

Crosses	Temp.	Mating type of exconjugants: VII VII	VII Self.	VII VIII	Self. Self.	VIII Self.	VIII VIII	Total	Changes to VII	Changes to VIII
4*	12–14°C.	1	1	59	0	5	2	68	2	7
	21°C.	2	0	39	0	2	5	48	2	7
	32°C.	2	0	59	0	6	14	81	2	20
1†	12°C.	8	8	6	0	0	0	22	16	0
	32°C.	1	6	8	1	4	2	22	8	7

* Crosses within clones presumably containing pure caryonides of different types.
† A cross within a clone presumably containing at least one unstable caryonide.

perpetuation of these types at the next reorganization are separated at the same division at which the new macronuclei are separated. Thus, like the mating types themselves, the cytoplasmic conditions are "caryonidal". Since the macronuclei control the mating types, we must conclude that the macronuclei also control the cytoplasmic conditions. This interpretation is further supported by Sonneborn's experiments on mating type segregation mentioned above. When diverse macronuclei are separated—even several fissions after conjugation—not only are the mating types distributed in a correlated manner, but again the cytoplasmic conditions necessary for the perpetuation of those types are segregated. Hence, two activities of the differentiated nuclei may be distinguished—action on the phenotype of the cell and action on the cytoplasm, which determines the macronuclear condition (and phenotype) in the next generation.

The behavior of selfing caryonides demonstrates that a partial separation of these two functions may occur under certain conditions. Selfing caryonides may consist predominantly of mating type VIII cells (99% or more) and yet upon reorganization may yield chiefly type VII progeny (10% or less pure type VIII caryonides). In what way are these observations to be interpreted? One possibility is that two different systems of differentiation are involved—one responsible for mating type control and the other for cytoplasmic control. These different systems would, however, have to be linked in some manner since a complete separation has not been observed. Such a scheme might also lead to the expectation of another type of caryonide—predominantly type VII, but giving rise chiefly to type VIII at reorganization. In Sonneborn's unpublished studies a very few such caryonides were obtained, but we have never encountered them and their status is problematical. Sonneborn's studies utilized autogamy instead of conjugation as the means of reorganization and this difference may have been significant, particularly since certain other differences in mating type determination at the two kinds of reorganization have been noted. Under the circumstances it appears best to ignore these observations until such type VII caryonides are again obtained and characterized.

If only the three types of caryonides documented in this paper are considered, a simpler interpretation is possible. We may conceive of the macronuclei in selfing caryonides as "mixtures" of type VII and type VIII propensities. Without assigning any particular material significance to the terms we may think of a macronucleus which is 30% type VII, 60% type VII, etc. If this VII fraction requires different threshold levels for manifestation of the two kinds of activity previously noted, the activities would be partially dissociated in cells with certain types of macronuclei. Thus a nucleus which is 60% type VII may not produce enough of the VII products to determine the cell as type VII; yet the threshold for the products acting through the cytoplasm may be such that 60% type VII is sufficient to influence the majority of new nuclei to develop as type VII. Thus a cell could manifest mating type VIII, but most of its progeny would be mating type VII.

The available data permit a more precise estimation of the fractions necessary to get a particular effect. We know something of the effect (on the cytoplasm but not on the phenotype) of equal quantities of type VII and type VIII nuclei; antiserum-induced double animals contain one type VII and one type VIII macronucleus and the large majority of new nuclei developing in such an environment are determined as type VIII. Since most of the progeny of the predominantly type VIII selfers become type VII, the VII fraction in the macronuclei of the selfers must be appreciably larger than 50%. Then, as indicated above, we must assume that the threshold for determining the VII phenotype is higher than the threshold for determining the characteristic VII cytoplasm. Hence, the fraction of macronuclear VII necessary to obtain the VII phenotype is not only greater than 50%, but probably very much greater and perhaps close to 100%. The unstable caryonides studied, therefore, have a very large fraction of type VII and the macronuclear composition is intermediate between the two threshold levels.

This interpretation involves the manipulation of "quantities" of abstract type VII and VIII determiners, but it is not possible at the present time to give any precise meaning to these quantities. A prior problem is the nature of the VII and VIII distinctions in *pure* macronuclei. Are these differences "qualitative" or are they in some meaningful sense "quantitative"? Either type of difference could yield quantitative differences in selfing clones if macronuclei are constructed of different relative "amounts" of the two types. Certain evidence now available, however, suggest *quantitative* distinctions even between pure VII and pure VIII cells. Chao(1) found a striking relationship in stock 51 between the mating type of a cell and the amount of kappa (killer cytoplasmic particles) in the cytoplasm. A given genotype showed twice as much kappa in cells of type VII as in cells of type VIII. Chao also found that cells of the same mating type contained twice as much kappa when they had the genotype KK as when they had the genotype Kk. Speculation on these facts gave rise to a gene-dosage hypothesis of mating type determination(6), according to which one mating type (VII) contained twice as many K (and perhaps other) genes as its

alternative. Attempts to verify this hypothesis have been inconclusive, but disappointing. An explanation based on simple polyploid differences is no longer tenable. The DNA quantities expected to be associated with such differences were not found(3); deliberate alterations in micronuclear (and hence macronuclear) ploidy failed to yield the expected mating types and indeed revealed another and previously unsuspected factor in mating type differentiation(14). Finally, studies on *Tetrahymena*(8) reveal a very similar system of caryonidal determination which operates in a multiple mating system with many alternative nuclear conditions; no simple polyploid series is conceivable here. From this it must not be concluded that the gene-dosage hypothesis is disproved, but only that one formulation of the hypothesis—based upon a doubling of *all* chromosomes in one mating type—is not acceptable. Interpretations involving quantitative variations in either the amount or activity of single chromosomes or parts of chromosomes are still possible. Regardless of the resolution of this problem, the basic observations remain as provocative indications of the nature of mating type differences and provide, moreover, an independent test of the speculations set forth in the preceding paragraph. We concluded there that selfing caryonides certainly contain more than 50% type VII in the macronucleus and possibly much more. We would expect, therefore, that the number of kappa particles in such cells should be much greater than half-way between the normal VII and normal VIII kappa levels. Upon examination Chao(2) found that the unstable caryonides possessed approximately as much kappa as normal type VII cells. Hence, the analysis receives significant support from this direction.

The hypothesis of macronuclear heterogeneity in selfing caryonides may be viewed in one of two general ways. The simplest interpretation would hold that the macronuclei are *structurally* impure and contain some parts (subnuclei perhaps) which are type VII and some which are type VIII. The assortment of these parts at fission would result in macronuclei with different proportions of the two types of material, controlling different mating types and different cytoplasmic conditions. The chief reason for rejecting this interpretation comes not from *Paramecium* but from *Tetrahymena*(9). Unstable nuclei occur here also, but stabilization can be achieved by starvation. Unless an extreme intranuclear selection occurs during starvation, this hypothesis would demand that the sub-nuclear elements—though differentiated—retain some plasticity. If the parts of the macronucleus are to be considered plastic, it is simpler to assume that plasticity is characteristic of the entire macronucleus and to dispense with the additional complication of structural diversity, since it becomes no longer useful.

If the nuclear instability is not to be explained in structural terms, some explanation in physiological terms is in order. A model of some utility is provided by a concept of a nuclear flux equilibrium which has —in addition to two normal steady states—one or more metastable intermediate conditions, represented by the selfing caryonides. The chief disadvantage in being driven to such a formulation lies in the difficulty of designing critical tests for the hypothesis. Many problems remain to be solved before a completely satisfactory explanation for nuclear differentiation is available; both the stable and the unstable nuclear conditions must be considered in the final resolution.

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