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THE NATURE AND MODE OF ACTION OF THE MATING TYPE SUBSTANCES¹

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Mating type inheritance in group B, *Paramecium aurelia*, is explained in part by analogy with the gene-kappa-paramycin system. Group B (Sonneborn and Dippell, 1946) animals usually do not change mating type at conjugation. Thus one variety 4 conjugant, produces a type VII clone; its mate produces a type VIII clone. Occasionally, however, both conjugants produce type VII or type VIII clones, indicating in each case that one conjugant changed mating type. Similarly, change of type from VII to VIII or VIII to VII occurs at autogamy in many stocks. Sonneborn (1947) correlates change of mating type at conjugation with delay in separation of the conjugating animals. This delay in separation is associated with exchange of cytoplasm. Cytoplasmic factors for mating type included in the exchanged cytoplasm are believed to effect the change of type. Nothing further is known concerning mating type inheritance in group B. Since no mating type genes have been reported, it is at present necessary to regard cytoplasmic factors alone as the mating type determining agents. This scheme requires at least two cytoplasmic factors in each variety, one for each mating type. Furthermore, when applied to change of type at conjugation or autogamy, the scheme implies competition of these cytoplasmic factors for "effective substrate."

It is apparent from this brief discussion that assumptions concerning fundamental issues arise at once when

¹The studies of Metz and Foley, which form a major part of this report, were aided by a grant from the National Institute of Health, U. S. Public Health Service.

one approaches mating type inheritance. Thus further investigation of mating types and the factors controlling them should add much to our knowledge of the relation between genes, cytoplasmic factors and characters. Although the studies to be reported here concern the physiology of conjugation, it is hoped that the methods, facts and points of view developing from this work on variety 4, *P. aurelia*, may eventually help to unravel the relationship between the mating type characters and the agents controlling them.

THE MATING REACTION AND CONJUGATION

Conjugation in *Paramecium* involves several types of union between the conjugants and a variety of internal changes in these animals. The initial step in conjugation is a superficial contact and adhesion of animals (Sonneborn, 1937). Under appropriate conditions this may take the form of mass agglutination and is referred to as the mating reaction. In fact, occurrence of the mating reaction on mixture of clones from diverse sources serves to distinguish and identify complementary mating types.

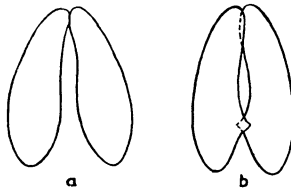


Fig. 1. Types of Union in conjugating, and pseudo-selfing paramecia.
a Holdfast union in early conjugants and pseudo-selfing animals.
b Holdfast and paroral cone union in more advanced conjugants.

Following the initial agglutination, potential conjugants unite in a more intimate but not inseparable fashion at a region near their anterior ends (Figure 1a) known as the *holdfast region* (Metz, 1947). Finally, the *paroral cones* (Diller, 1936), which have formed in the posterior region, unite (Figure 1b). From this time on the conjugants can not be separated.

Subsequent to holdfast union, but prior to paroral cone fusion, the conjugants lose their ability to give the ag-

glutinative mating reaction. This loss of mating activity results in breakdown of the mating reaction agglutinates into conjugating pairs. In *P. aurelia* the first signs of nuclear activity appear at about this time (Diller, 1936; Metz, 1947). These involve enlargement of the micronuclei and formation of the first meiotic prophase. Macronuclear breakdown or fragmentation begins shortly after completion of the second meiotic division. Nuclear behavior subsequent to meiosis will not enter into the discussion to follow.

PHYSICAL BASIS OF THE MATING TYPE DIFFERENCES

In any consideration of conjugation, and particularly in any analysis of the initial agglutinative mating reaction, it is essential to realize that hormones, gamones or sex stuffs are never found in the fluid from Paramecium cultures. Thus serious analogy with *Clamydomonas* (Moewus, 1939) or *Euplotes* (Kimball, 1943) is of little value. Consequently, consideration must be given at once to the surface structure of the Paramecium for an understanding of the mating reaction. More particularly, attention must be directed to the structure of the cilia, since Jennings (1939) has shown that the mating reaction involves these organelles. It seems reasonable to suppose that the agglutinative mating reaction results from interaction of definite complementary mating type substances or at least complementary configurations. This view is supported by several lines of evidence. Thus Sonneborn (1937, 1942) has shown that two paramecia of the *same* mating type can clump together temporarily if one of them has previously been in contact with an animal of opposite mating type. This indicates transfer of mating type substance from one animal to another. Boell and Woodruff (1941) and Metz (1947) have found that dead paramecia of one mating type will clump strongly and specifically with living animals of opposite mating type, again suggesting interaction of moderately stable sub-

stances. Furthermore, Metz (unpublished) has found that treatment of dead animals with antiserum inhibits the mating reaction between dead and living animals. Although this action of antiserum was not mating type specific, the results nevertheless indicate blocking of surface groups.

Direct examination of cilia has so far given no clue to the nature of the reactive surfaces. Thus Jakus and Hall (1946) observed bundles of fibrils in cilia by use of the electron microscope, but they were unable to determine what held these fibrils together or to detect a limiting sheath.

All attempts to extract mating substances from *P. aurelia* have so far failed (Metz, 1946a). Heating, grinding, freeze-thawing or extraction with acid, alkali, salt solution, urea, or organic solvents produced no fraction containing mating substance activity (specific action on animals of opposite mating type; inhibition of the mating reaction). Although large fragments of dead *P. aurelia* clumped with living animals, just as do fragments of living *P. bursaria* (Tartar and Chen, 1941), all mating activity disappeared when the animals were completely broken up by either physical or chemical means.

In a study of the effect of various agents on the mating type substances, Metz (1946a) found that *P. aurelia* could be killed by appropriate treatment with a rather wide variety of physical and chemical agents without destruction of mating activity. Such dead animals clumped strongly and specifically with living animals of opposite type. Unfortunately these results give little indication of the nature of the mating type substances. However, pursuit of this line of investigation should prove fruitful.

INDUCTION OF AUTOGAMY AND PSEUDO-SELFING PAIR FORMATION IN *P. aurelia*

As might be expected, the question eventually arose: does specific clumping with dead animals of opposite type

induce nuclear reorganization or any other conjugation effects in living paramecia? The answer obtained to this question was quite strikingly positive (Metz, 1946b, 1947). While clumped to formalin-killed animals of one mating type, living animals of the opposite mating type unite to form pseudo-selfing pairs. The pseudo-selfing animals subsequently lose their ability to give the agglutinative mating reaction, they separate from the dead animals, and finally swim freely in the medium. These free swimming pairs can remain united for at least five hours. Union involves only the anterior or holdfast regions of the pair members (Figure 1a). Paroral cones form but these structures do not fuse. The two selfing pair members come from a single clone of a single mating type; furthermore both pair members give rise to clones of this same original mating type. Thus pseudo-selfing pair formation involves union of paramecia of the same mating type and is not regularly associated with permanent change of mating type.

Cytological examination shows that the pseudo-selfing pair members undergo meiosis and macronuclear breakdown (Metz, 1947) and that these nuclear changes are morphologically and temporally identical with corresponding changes in conjugating animals. Preliminary genetic studies (Jacobson, unpublished) indicate that the pseudo-selfers undergo autogamy.

Induced macronuclear breakdown and meiosis are not confined to the pseudo-selfers, for these changes can be induced independently of pseudo-selfing pair formation in single isolated living animals by formalin killed animals of opposite type. From this result it may be concluded with a reasonable degree of assurance that clumping with dead animals will induce autogamy directly and specifically in living animals of opposite mating type.

These effects of dead animals upon living animals are not causally related to the natural autogamy that occurs periodically in *P. aurelia*. In fact, they are not peculiar

to *P. aurelia*. Thus specific clumping between formalin-killed and living animals followed by meiosis, macronuclear breakdown and pseudo-selfing pair formation has been obtained in the Yale stocks of *Paramecium calkinsi* (Metz and Foley, unpublished). This study is of particular interest because spontaneous nuclear reorganization (endomixis, natural autogamy) has not been reported in *P. calkinsi* (Woodruff, 1921; Spencer, 1924) and could not be found in the stocks used in this study. Actually these changes have been induced in living Type II *P. calkinsi* only. Positive results have not been obtained in the reverse combination (dead type II plus living type I).

MECHANICS OF FERTILIZATION IN PARAMECIUM

It is now appropriate to attempt an interpretation of the facts outlined above. In conjugation, natural autogamy, pseudo-selfing and probably nuclear reorganization induced in single isolated animals, essentially the same series of events is observed, namely:

- (1) Loss of mating activity
- (2) Paroral cone formation
- (3) Meiosis
- (4) Macronuclear breakdown

The essentially identical nature of the series in the several types of behavior suggests a similar origin for these events in conjugation, natural autogamy, pseudo-selfing and reorganization induced in single animals. This similar origin is best visualized as a predetermined chain of reactions following from a common initiating mechanism. As stated elsewhere (Metz, 1947), the nature of the reaction between living and dead paramecia suggests that the common initiating mechanism may be an interaction of mating type substances. (It will be seen presently that natural autogamy is initiated through a separate mechanism). According to this view, interaction of mating type substances at the animal's surface would "acti-

vate" the Paramecium in the same sense that the spermatozoan activates the metazoan egg. In other words mating type substance interaction would perform a function in Paramecium analogous to that postulated by Lillie (1919) for the interaction of fertilizin and sperm receptor (Tyler's, 1942, sperm antifertilizin) in metazoan fertilization.

Support for this view was sought in a study of a non-conjugating race of *Paramecium aurelia* (Metz and Foley, 1947; unpublished). This study also suggests an interesting explanation of pseudo-selfing pair formation. The non-conjugating, "can't mate" or CM¹ animals give the initial mating or clumping reaction with normal animals. However, they do not form more permanent union with normal animals or undergo nuclear reorganization through association with normal animals. In other words, the CM animals can not be activated by animals of opposite mating type. The results of the investigation are summarized in Table I.

Table I Induction of pseudo-selfing and macronuclear breakdown in living normal and CM *P. aurelia* of one mating type by formalin-killed normal and CM animals of opposite type

Living	Normal	Normal	CM	CM
Dead	Normal	CM	Normal	CM
Initial clumping	+	+	+	+
Pseudo-selfing	+	+	-	-
Macronuclear breakdown	+	+	-	-

It was found that dead CM animals could induce pseudo-selfing in normal stocks (Table I). Indeed, even *living* CM animals induced pseudo-selfing pair formation in living normal animals. Thus the ability to induce pseudo-selfing is not peculiar to dead animals.

It was further found that the living CM animals never formed pseudo-selfing pairs when treated with dead normal animals (Table I). The mechanism previously sug-

¹ The writer is most grateful to Professor T. M. Sonneborn for supplying the CM stocks.

gested (Metz, 1947) to account for pseudo-selfing pair formation offers no ready explanation for this failure of the CM animals to self. Thus pseudo-selfing pair formation can not result directly from transfer of mating substances from dead to living animals unless the CM animals are endowed with very special properties.¹ It seems more reasonable, therefore, to attribute holdfast union, the union of pseudo-selfing, to interaction of separate substances which are not the mating type substances. It is not unreasonable to assume that these holdfast substances appear as a result of activation and are to be classed with loss of mating activity, paroral cone formation, meiosis and macronuclear breakdown.

As previously stated, dead normal (non-CM) animals activate single isolated normal animals. Likewise formalin-killed CM animals induce macronuclear breakdown and meiosis in single isolated normal animals. This demonstrates beyond question that the CM animals possess the activation initiating mechanism. However, the CM animals can not be activated by either living or dead normal animals of opposite mating type. Thus it appears that some block, *the CM block*, prevents activation from proceeding much beyond the initial step in CM animals. Depending upon the nature of the activation-initiating mechanism, at least two possibilities exist for the position of this CM block. If activation is initiated simultaneously in conjugants by interaction of a single system consisting of two complementary substances (Figure 2a) then the CM block must be placed in a position "internal" to the intact initiating system. This follows from the fact that CM animals can activate normal animals.

Conceivably activation of the two conjugants could involve interaction of two separate systems each consisting of two complementary substances, an "inducer" (I) and a "reactor" (R), such that each conjugant possessed the

¹ The possible nature of such special properties will be discussed in a later publication.

“inducer” of one system and the “reactor” of the other system. Interaction of I and R in one system would activate one conjugant, and interaction of R' and I' of

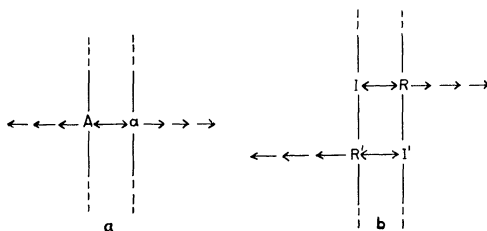


Fig. 2. Two possible activation-initiating mechanisms. Each series of arrows represents the main activation chain in one conjugant. (a) Simultaneous activation of conjugants by interaction of a single pair of surface substances. (b) Simultaneous activation of conjugants by interaction of two pairs of surface substances.

the other system would activate its mate (Figure 2b). In such a scheme the CM block could lie in the initiating mechanism rather than “internally,” if the CM animals lacked reactor (R).

The existence of the CM block, whatever its nature, is of considerable interest, and especially so since the CM animals regularly undergo natural autogamy. Because the CM animals can undergo natural autogamy, but can not be activated by animals of opposite mating type, it follows that different activation initiating reactions operate in sexually induced activation (conjugation, cytogamy and their experimental variants) and natural autogamy. Not only must different mechanisms initiate activation in conjugation and natural autogamy, but in natural autogamy the CM block is ineffectual. It appears, then, that in natural autogamy the main reaction chain is activated beyond the CM block, in other words between the CM block and the first bifurcation of the reaction chain. This will assume added interest if the CM block should prove to be independent of the activation initiating mechanism of conjugation. These relations are summarized in Figure 3.

In normal conjugation interaction of surface substances (position a, Figure 3) sets in motion a chain of reactions that ultimately branches into side reactions¹ (position d) leading to the several end effects of activation (position e), namely: holdfast substance formation (tentative),

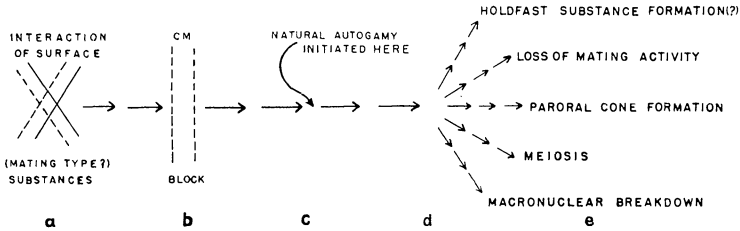


Figure 3. Scheme for activation in *Paramecium*. (a) Initiating reaction (mating type substance interaction?) in sexually induced activation. (b) CM block, here assumed to lie "internal" to the initiating reaction, (a). (c) Position where main chain is activated in natural autogamy. (d) Break-up of main activation chain into side reactions leading to (e) the various end effects of activation.

loss of mating activity, paroral cone formation, meiosis and macronuclear breakdown. In the CM animal the main chain is blocked at or near the initiating reaction by the CM block (position a or b, but assumed at b). Natural autogamy is not initiated through the same initial reaction but at some point in the main chain (possibly via a side reaction) beyond, or "internal" to the CM block (position e).

POSSIBLE RELATION OF MATING TYPE SUBSTANCES TO ACTIVATION

As previously suggested, interaction of mating type substances may initiate activation in *Paramecium*. The CM study supports this view. As shown above and also by Sonneborn (1942), holdfast union very probably does

¹There is no evidence to indicate dependence of one side reaction upon another. However, it is possible, in fact, likely, that all side chains do not arise simultaneously and independently from the main chain. Indeed, the holdfast-substance side chain may arise between (b) and (c). This follows from the fact that holdfast union has never been observed in naturally autogamous animals. This may indicate that holdfast substances are not formed in natural autogamy or that holdfast substances are formed but that union requires the intimate contact found only in clumps or agglutinates.

not involve mating type substances. Since the CM animals can not be activated and can not form holdfast attachments, but can nevertheless activate single isolated normal animals, it follows that holdfast attachment or interaction of holdfast substances, is not essential for activation. The only other known substances that could be involved in activation, then, are the mating type substances (as defined by Metz, 1947). Since nothing is known of the number of these substances, this view is consistent with either of the activating mechanisms suggested in Figure 2. For the present it is either necessary to postulate that interaction of completely unknown and unsuspected substances initiate activation or to assume that mating type substance interaction initiates activation. The latter possibility is tentatively accepted in the scheme presented (Figure 3 position a).

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