

STUDIES ON THE NUCLEAR APPARATUS OF PERITRICHOUS CILIATES.

PART II. THE NUCLEAR APPARATUS OF *CARCHESIUM SPECTABILE* EHRLG.

by C. M. S. DASS, *I.C.I. Research Fellow, N.I.S.I., Department of Zoology, Central College, Bangalore.*

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INTRODUCTION

The only work on the cytology of the nuclear apparatus of the genus *Carchesium* is that by Popoff (1908) on *C. polypinum*. Recent studies on allied genera of Peritricha, e.g., *Trichodina* sp. (Diller, 1928), *Zoothamnium arbuscula* (Furssenko, 1929), *Vorticella microstoma* (Finley, 1943), *Urceolaria synaptae* (?) (Colwin, 1944), *Lagenophrys tattersalli* (Willis, 1948), *Vorticella convallaria* (Seshachar and Dass, 1951), *Rhabdostyla vernalis* (Finley, 1952) and *Epistylis articulata* (Dass, 1953) have shown a number of interesting features in the cytology of this group, viz., variation in the number of micronuclei, form and shape of the macronucleus, behaviour of the macronucleus during binary fission, difference in the number of progamic divisions of the micronucleus in the two conjugants and behaviour of the macronucleus during conjugation. The present study is an attempt to interpret the condition in *Carchesium spectabile* in consonance with these recent findings.

MATERIAL AND METHODS

The colonies of *Carchesium spectabile* are found attached to weeds and rocks in running sewage water. Pillai and collaborators (1942) have shown that among the dominant organisms met with when raw sewage is aerated, are peritrichous ciliates of the genera *Vorticella*, *Epistylis*, *Carchesium*, *Zoothamnium* and *Opercularia*. This fact was made use of in obtaining large supplies of material. The material was collected from flowing sewage waters about six miles out of Bangalore and while some of it was fixed on the spot, large quantities were brought to the laboratory for further examination and treatment. The material was fixed in hot Schaudinn's, Carnoy's and San Felice's fluids and stained in Feulgen-light green.

OBSERVATIONS

The vegetative individual.

The nuclear apparatus, as in other euciliates, comprises two entities, the macronucleus and the micronucleus. The macronucleus is a greatly elongated, cylindrical body. It has no definite shape and can best be described as an irregularly bent cylinder. It exhibits a fine granular appearance and gives an intense Feulgen reaction. During the height of trophic activity a number of Feulgen negative areas are seen in the macronucleus (Pl. IV, fig. 1). They are not seen during binary fission nor do they occur in conjugating animals. The micronucleus, on the other hand, is a spherical body averaging about 5μ in diameter. It is faintly stained, compared with the macronucleus. One micronucleus is generally found in each animal. However, instances are abundant where multimicronucleate individuals occur. Animals with two, three and even four micronuclei are found in my material (Text-fig. I, figs. *a*, *b* and *c*; Pl. IV, fig. 4). The future behaviour of such multimicronucleate animals indicates that once the variation is introduced, it becomes perpetuated in a colony for a considerable time, all the micronuclei taking part in division.

Binary fission.

This is the common method of asexual reproduction. The division activity is heralded by changes in the micronucleus. It loses its uniform appearance and thread-like chromosomes are faintly discernible. The nucleus increases in size and later assumes a spindle shape. The chromosomes move to the poles and the daughter nuclei are pushed far apart by the central spindle that develops between the two anaphase chromosome sets. As the two daughter micronuclei separate the cleavage furrow starts from the oral end and the macronucleus stretches across it. Eventually the macronucleus is cut into two almost equal halves (Pl. IV, fig. 2). This sequence of events takes place in quick succession.

In some members of the colony, another type of binary fission, where the macronucleus condenses into a deeply staining polymorphic body prior to its splitting, is also noticed (Pl. IV, fig. 3). Such a phenomenon was found in *Epistylis articulata* (Dass, 1953) but the fine filamentar processes produced by the macronucleus into the cytoplasm in *Epistylis* were not seen in *Carchesium*.

Conjugation.

The process of conjugation can be divided into four phases: (*a*) formation of conjugants, (*b*) fusion of the conjugants and formation of synkaryon, (*c*) formation of nuclear anlagen, and (*d*) reorganization fissions.

(a) Formation of conjugants

As in all Peritrichous ciliates, the two individuals participating in conjugation differ in size. The smaller microconjugant is produced earlier than the macro-

conjugant. At certain periods one notices the production, in very large numbers, of the microconjugants in the colony; it would seem the stimulus for their formation pervades the whole colony. Microconjugants are produced by normal individuals of a colony by a series of fissions. The first division gives rise to two animals which appear to pause for a while until each develops a stalk. Two divisions follow this successively,—each stalk bearing four small animals (Text-fig. I, fig. *d*). All the four microconjugants detach themselves soon and swim about in search of macroconjugants.

The formation of the macroconjugant appears to involve no preparatory division. Any vegetative individual of the colony seems capable of acting as the macroconjugant. However, I have noticed a slight change in the nuclear apparatus of potential macroconjugant. The macronucleus exhibits a tendency to be drawn out into a skein, while the micronucleus increases in size and becomes fainter.

(b) *Fusion of conjugants and formation of synkaryon*

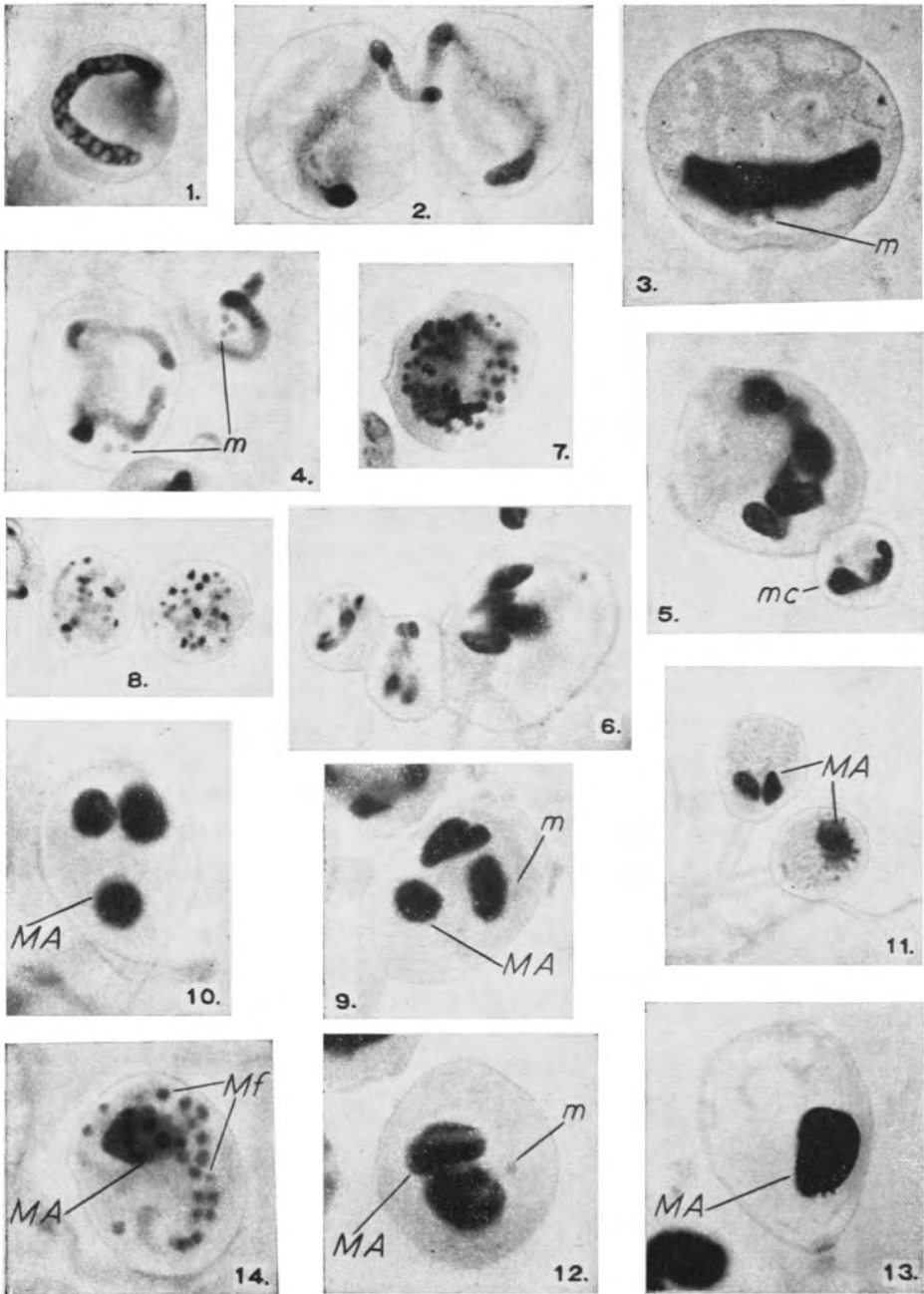
When the microconjugant comes in contact with the macroconjugant, it spins on its surface for a while and attaches firmly. The position on the body of the macroconjugant where it attaches is highly variable and perhaps the entire surface of the body is receptive to it. But the most common sites of fusion are at the base, on either side of the stalk (Pl. IV, fig. 5), and on the sides of the body. More than one microconjugant is often seen in association with a macroconjugant. It is also seen, in some instances, that one microconjugant is attached to another which is in association with a macroconjugant (Pl. IV, fig. 6).

The nuclear changes in the two conjugants can be treated under two heads: (a) the micronucleus and (b) the macronucleus.

(a) *The micronucleus.*—The beginnings of micronuclear activity in the microconjugant are seen even before its attachment with the macroconjugant. The micronucleus starts the first division, and if the time spent by the microconjugant in search of a macroconjugant is long enough, this division is completed. As a consequence, at the time of contact, the microconjugant has two micronuclei, while the macroconjugant has only one (Text-fig. I, fig. *e*). In those instances where the microconjugant comes in contact with the macroconjugant immediately after the former's release, it is seen that the micronucleus of the macroconjugant waits for the completion of the first division of the micronucleus of the microconjugant. The first and second divisions of the micronucleus of the macroconjugant are completed along with the second and third divisions of the micronucleus of the microconjugant (Text-fig. I, figs. *f* and *g*). Consequently, eight progamic nuclei are found in the microconjugant while only four are seen in the macroconjugant. One progamic nucleus of each of the conjugants becomes the pronucleus; the rest are absorbed in the cytoplasm.

(b) *The macronucleus.*—The meeting of the conjugants brings attendant changes in their macronuclei also. The macronucleus of the microconjugant splits into three or four bodies which later break up into 15–20 rounded bodies. The behaviour of the macronucleus of the macroconjugant, however, is interesting. It is thrown out into a skein, as in other peritrichous forms. A number of small deeply stained bodies are extruded from it and these are absorbed in the cytoplasm (Text-fig. I, fig. *e*). A little later, the macronucleus breaks up into a number of elongate bodies, which gradually fragment into spherical bodies numbering about 150–200. The fragments average 2–3 μ in diameter.

The fusion of the pronuclei takes place almost immediately after the boundary between the two conjugants breaks down and in the earliest synconjugant, the pale large and spherical synkaryon can be easily differentiated from the many macronuclear fragments derived from both conjugants that literally fill the cytoplasm.



(c) Formation of the nuclear anlagen

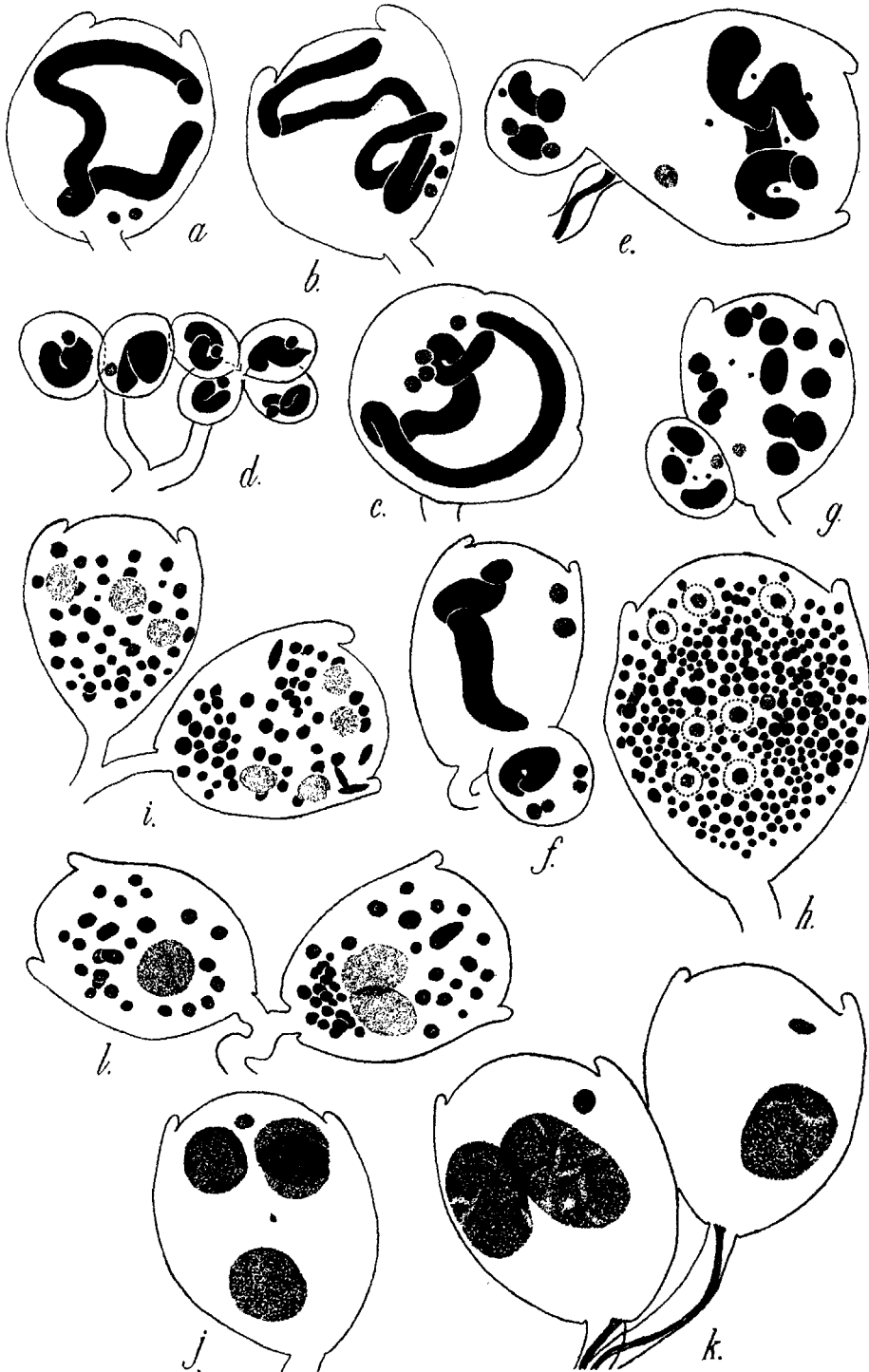
Immediately after its formation, the synkaryon divides successively thrice giving rise to eight nuclei; each is about $5\ \mu$ in diameter and a faint indication of a granular network-like structure can be seen in it. Soon, seven of them enlarge in size to about $15\ \mu$ in diameter while the other shrinks in size to about $3\ \mu$ (Text-fig. I, fig. *h*). The seven enlarged bodies are the macronuclear anlagen while the eighth is the micronuclear anlage. The macronuclear anlagen can be distinguished from the micronuclear anlage not only from their larger size but also from the fact that while in the former, the network-like appearance is seen clearly, the latter presents a homogeneous appearance characteristic of the definitive micronucleus.

(d) Reorganization fissions

The seven macronuclear anlagen continue to increase in size, and reach a diameter of $20\text{--}25\ \mu$. During this later period of growth the fine network-like appearance is lost and the anlagen present a faint homogeneous and granular structure. At this stage the reorganization fissions are initiated. During the first fission the macronuclear fragments (derived from both the conjugants) become somewhat localized at the base of the synconjugant while the macronuclear anlagen lie free in the cytoplasm towards the oral end of the animal. The micronucleus divides by mitosis and the two daughters separate. With the movement and separation of the micronuclei, the macronuclear anlagen also segregate into two groups of three and four (Text-fig. I, fig. *i*). The division of the cytosome also ensures an approximately equal distribution of macronuclear fragments between the two daughter individuals. There is a considerable lapse of time between the first reorganization fission and the second. During this period the macronuclear anlagen increase in size to about $45\text{--}50\ \mu$ and also become progressively deeply stained. The second reorganization fission segregates the macronuclear anlagen among the daughters so that they get 2, 2, 2, and 1 (Text-fig. I, fig. *k*; Pl. IV, fig. 11). There is a considerable time interval between the second and third reorganization fissions. The cell grows in size during this period, as also the macronuclear anlagen, which at this stage show a number of poorly stained furrows. The macronuclear anlagen has reached a size of $120\text{--}150\ \mu$ in diameter by the time the third reorganization fission starts. At the end of the third reorganization fission, each individual has one macronucleus and a micronucleus (Pl. IV, fig. 13). Maximum nucleination of the anlagen is noticed at this period. The macronuclear anlagen retain their more or less spherical shape during reorganization fissions.

An interesting feature in regard to the macronuclear anlagen refers to their rate of nucleination. It was observed that in a number of instances there was no uniformity in this matter in the anlagen derived from the same synkaryon—while in one daughter cell, the nucleination of the anlagen had progressed considerably, and they appeared in Feulgen preparations, brilliantly stained, in the sister individual, nucleination had not advanced to the same extent and the anlagen were relatively faint (Text-fig. I, fig. *l*). It was even noticed that the anlagen in the same organism sometimes differed in their nucleination as judged by their reaction to Feulgen. In an individual with two anlagen, of about the same size, one was decidedly more positive and brilliant than the other. In all such cases where nucleination was poor, the assumption of the final cylindrical form of the macronucleus was delayed until the anlagen had acquired the requisite amount of nucleic acid, the anlagen remaining as polymorphic bodies during the process.

There appears to be an interesting correlation between the rate of nucleination of the macronuclear anlagen and the rate of absorption of the macronuclear fragments. In most cases, with every reorganization fission the macronuclear fragments get distributed between the daughter individuals almost equally, but most of them are absorbed by the second reorganization fission. In a few cases, however, they



TEXT-FIG. I. (For Explanation see foot of page 179.)

persist till after the second fission and are sometimes seen even in the completely reorganized individuals (Pl. IV, fig. 14). In cases where the fragments are absorbed early, the macronuclear anlagen are conspicuous by their deep Feulgen reaction (Pl. IV, figs. 9, 10 and 12), while in instances where the fragments persist long, the anlagen are faint and only slowly and gradually acquire their final intensity (Pl. IV, figs. 11 and 14). This appears to offer evidence of some type of a relationship between the dissolution of the macronuclear fragments and the nucleination of the anlagen.

DISCUSSION

Nuclear apparatus.

The nuclear apparatus of *Carchesium spectabile* resembles that of other Peritrichous ciliates. The great length and tortuous nature of the macronucleus, however, are very striking. The appearance of Feulgen negative areas in the macronucleus during certain phases of life-history of these ciliates has been recorded in other Peritricha, e.g., *Zoothamnium alternans* (Fauré-Fremiet, 1930), *Vorticella microstoma* (Finley, 1943), *Urceolaria synaptae* (?) (Colwin, 1944) and *Epistylis articulata* (Dass, 1953). Colwin (1944) regards these areas as pits caused by the throwing off of chromatin balls into the cytoplasm and compares the process with the phenomenon of 'hemixis' described by Diller (1936) in *Paramecium aurelia*. I have not observed in *C. spectabile* any chromatin elimination in the vegetative animal. In *E. articulata* (Dass, 1953) also these areas were noticed in the macronucleus and the application of Unna's methyl green-pyronin mixture showed them to be stained deeply with pyronin. A similar condition obtains in *C. spectabile* also and it is possible that these areas represent pockets of ribonucleic acid. It is interesting to see that such areas are present during the height of trophic activity and do not occur during the division phase. In this, as well as in its nucleic acid content, they resemble nucleoli of Metazoa.

Binary fission.

Of the two types of binary fission that have been recorded, it is the second, where the macronucleus becomes a polymorphic body, that is more interesting. A similar type of division was also observed in *E. articulata* (Dass, 1953). The

EXPLANATION OF TEXT-FIG. I (see page 178).

- FIGS. a, b, c.—Bi-, tri- and tetramicronucleate individuals. $\times 700$. Feulgen-Light green.
- FIG. d. Microconjugant formation. On one stalk bunch of four microconjugants seen, while on the other they are still in the process of formation. $\times 700$. Feulgen-Light green.
- FIG. e. Conjugation. In the microconjugant first progamic division is over while in the macroconjugant the micronucleus is in prophase. $\times 700$. Feulgen-Light green.
- FIG. f. Conjugation. The second progamic division of the micronucleus of microconjugant and first progamic division of that in macroconjugant are over. $\times 700$. Feulgen-Light green.
- FIG. g. Conjugation. Pronucleus in each conjugant ready. The residual progamic nuclei are seen as small dark bodies. Already a number of them have been absorbed in the cytoplasm. $\times 700$. Feulgen-Light green.
- FIG. h. Synconjugant. The cytosome filled with macronuclear fragments. Macronuclear anlagen differentiated. Faint network-like structure still seen in the anlagen. Micronucleus small and faint. $\times 700$. Feulgen-Light green.
- FIG. i. F_1 individuals. The macronuclear fragments greatly reduced in number. $\times 700$. Feulgen-Light green.
- FIG. j. F_1 individual. All fragments absorbed in the cytoplasm. $\times 700$. Feulgen-Light green.
- FIG. k. F_2 individuals. The macronuclear anlagen have increased in size. All the macronuclear fragments are absorbed. $\times 700$. Feulgen-Light green.
- FIG. l. F_2 individuals. The rate of nucleination of the anlagen is not the same in sister individuals. The macronuclear fragments still persistent. $\times 700$. Feulgen-Light green.

condition seen in *C. spectabile*, however, differs from that in *E. articulata* in that fine filamentar processes given off from the macronucleus in the latter species are not seen. The significance of the process is not clear.

Micronuclear variation.

Generally there is a single micronucleus in *C. spectabile*, but occasionally individuals with two, three or four micronuclei are met with. It would appear, among Peritricha only *Epistylis* shares this feature with *Carchesium*, though in other euciliates it is a common enough phenomenon (*Paremecium bursaria*, Hamburger, 1904; Woodruff, 1931; *P. trichium*, Wenrich, 1926; *P. caudatum*, Wichterman, 1946). Whatever the agency responsible for the introduction of multimicronuclearity in other ciliates, in *C. spectabile* it would seem to be due to a mis-step in binary fission, the cytosome and the macronucleus not following micronuclear division. It was observed that binary fission is initiated by the micronucleus, and only long after the separation of the two daughter micronuclei do the macronucleus and cytosome divide. A failure of the cell to divide after the division of the micronucleus would result in bimicronuclearity, and a repetition of the same phenomenon would lead to a further multiplicity of micronuclei. It was noticed that once this condition was introduced it was perpetuated over a number of generations, until perhaps at conjugation, it was rectified and normalcy restored.

Conjugation.

The phenomenon of conjugation can be broadly divided into four stages: (a) formation of conjugants, (b) fusion of conjugants, (c) formation of nuclear anlagen and (d) reorganization fissions.

(a) *Formation of conjugants*

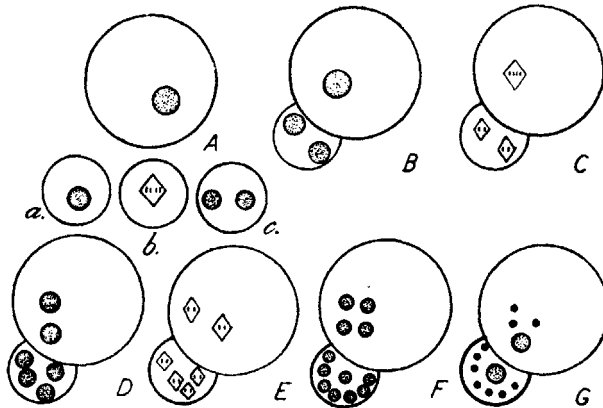
The Peritricha as a rule are characterised by dissimilar conjugants. The macroconjugant is generally a vegetative individual, sometimes with a definite position in the colony as in *Zoothamnium arbuscula* (Furssenko, 1929), *Z. alternans* (Summers, 1938) or placed at random as in *Carchesium polypinum* (Popoff, 1908) and *Ophrydium versatile* (Kaltenbach, 1916) and *E. articulata* (Dass, 1953). It is produced by a special 'sex-differentiating' division of a vegetative individual in *Opercularia coarctata* (Enriques, 1907) and preconjugation division in *Vorticella microstoma* (Finley, 1943) and *Lagenophrys* sp., (Awerinzew, 1912). In *C. spectabile* any vegetative individual of the colony seems capable of being transformed into a macroconjugant. In such individuals the macronucleus shows an incipient skein and the micronucleus, an initiation of the division process.

The microconjugant, on the other hand, is very much smaller. It is produced by at least four different methods in Peritricha: (1) 'Rosette formation', where a vegetative individual of the colony undergoes two or three successive divisions, viz., *Vorticella monilata*, *V. nebulifera* and *V. putrina* (Maupas, 1888), *Carchesium polypinum* (Popoff, 1908), *Zoothamnium (arbuscula) alternans* (Stein, 1867), *Z. geniculatum* (Wesenberg-Lund, 1925), *Epistylis plicatilis* (Claparède and Lachmann, 1858-1861), *E. simulans* (Plate, 1888), *E. articulata* (Dass, 1953), *Trichodina* sp., (Padnos, 1939), *Glossatella tintinnabulum* (Penard, 1922), *Opercularia infusionum* (Stein, 1867) and *Ophrydium versatile* (Kaltenbach, 1916); (2) Unequal division of the vegetative individual, smaller of the two products becoming the microconjugant, viz., *Vorticella convallaria* and *V. campanula* (Engelmann, 1876), *V. microstoma* (Finley, 1943), *Vaginicola (Cothurnia) crystallina* (Penard, 1922), *Cothurniopsis* sp., (Penard, 1922), *Lagenophrys labiata* (Awerinzew, 1912; Penard, 1922), *Pyxidium inclinans* and *P. curvicaule* (Penard, 1922), *Opercularia coarctata* (Enriques, 1907), *Opisthonecta henneguyi* (Rosenberg, 1940); (3) Metamorphosis of special cells—the microzooids—

of the colony, viz., *Zoothamnium alternans* (Summers, 1938a); and (4) Sex-differentiating division, producing a macroconjugant and a protoconjugant the latter dividing again once or twice to give rise to microconjugants, viz., *Lagenophrys tattersalli* (Willis, 1948) and *Rhabdostyla vernalis* (Finley, 1952). The microconjugant formation in *C. spectabile* is of the first type, but there is seen a time lag between the first and the succeeding two divisions, thus the microconjugants are found in two bunches of four each borne on different stalks.

(b) Fusion of conjugants

Conjugation involves the meeting of the two conjugants, attendant changes in the nuclear apparatus and the final fusion and merging of the microconjugant into the body of the macroconjugant to produce a synconjugant. The point of interest during conjugation in Peritricha centres round the micronuclear divisions in the two conjugants. Popoff (1908) observed that in *C. polypinum* the micronucleus of the microconjugant undergoes three divisions. He described the first division as 'Preliminary division', which takes a long time to complete while the subsequent two divisions occur in quick succession. Of the eight nuclei so produced one persists and the rest degenerate. This persistent nucleus divides again and two nuclei are produced. One of these degenerates and the other becomes the



TEXT-FIG. II. Diagrammatic representation of the sequence of micronuclear divisions in micro- and macroconjugants. Only the micronucleus and its products are shown.

FIG. A. Macroconjugant ready for conjugation. The micronucleus has entered the prophase of first proгамic division.

FIG. a. Free microconjugant just released from the parent stalk. The micronucleus in resting condition.

FIG. b. Free microconjugant. Metaphase of first proгамic division in progress.

FIG. c. Free microconjugant. First proгамic division is complete.

FIG. B. Conjugation. Microconjugant just come in contact with the macroconjugant. The micronucleus of macroconjugant is still in prophase of first proгамic division, while that in microconjugant has completed the first division.

FIG. C. Conjugation. First proгамic division in macroconjugant and second proгамic division in microconjugant in metaphase.

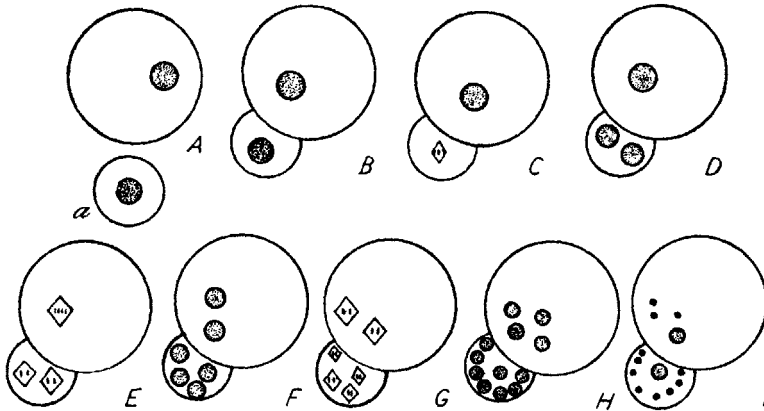
FIG. D. Conjugation. First proгамic division of micronucleus in macroconjugant and second proгамic division in microconjugant are over.

FIG. E. Conjugation. Second proгамic division metaphase in macroconjugant and third proгамic division metaphase in microconjugant.

FIG. F. Conjugation. Second proгамic division in macroconjugant and third proгамic division in microconjugant are over.

FIG. G. Conjugation. The micronucleus in each conjugant is ready. The dark bodies are the residual proгамic nuclei.

pronucleus. Thus the pronucleus of the microconjugant, according to Popoff, is a product of the fourth division of the micronucleus. In the macroconjugant also, according to him, a similar process takes place in the production of the pronucleus. However, recent work on other Peritricha (Finley, 1943 and 1952; Dass, 1953) has revealed that the number of divisions of the micronucleus is not the same in both conjugants—the micronucleus of the microconjugant undergoes three divisions, while that of the macroconjugant, two. Generally the first division of the micronucleus of the microconjugant is considered as a 'Preliminary division'. Finley (1952) considers that the first division of the micronucleus of the microconjugant in the members of the order Peritricha 'is a vestigial process reminiscent of a phylogenetically earlier stage when the smaller conjugant was formed by repeated equal divisions'. He has also suggested that 'this extra division has arisen *de novo* in the microconjugant line and has no homologue in the macroconjugant or in the conjugation of other ciliates'. In this context the condition obtaining in *C. spectabile* is illustrative. It may be recalled that in most cases at the time of meeting of the macroconjugant the micronucleus of the microconjugant has undergone the first progamic division, a condition identical with that seen in *Vorticella microstoma* (Finley, 1943) and *Rhabdostyla vernalis* (Finley, 1952). The micronucleus of the macroconjugant



TEXT-FIG. III. Diagrammatic representation of the sequence of micronuclear division in micro- and macro-conjugants when conjugation occurs precociously. Only the micronucleus and its products are shown.

FIG. A. Macroconjugant ready for conjugation. The micronucleus has entered prophase of first progamic division.

FIG. a. Free microconjugant. The micronucleus in resting stage.

FIG. B. Conjugation. The microconjugant just come in contact with macroconjugant. The micronucleus of macroconjugant still in prophase of first progamic division. The micronucleus of microconjugant in resting condition. Compare this with Fig. B of text-fig. II.

FIG. C. Conjugation. The micronucleus of macroconjugant in prophase of first progamic division. The micronucleus of the microconjugant in metaphase of first progamic division.

FIG. D. Conjugation. The micronucleus of macroconjugant in prophase of first progamic division. The micronucleus of the microconjugant has completed the first progamic division. Condition similar to Fig. B of text-fig. II.

FIG. E. Conjugation. First progamic division metaphase in macroconjugant and second progamic division metaphase in microconjugant.

FIG. F. Conjugation. First progamic division in macroconjugant and second progamic division in microconjugant are over.

FIG. G. Conjugation. Second progamic division metaphase in macroconjugant and third progamic division metaphase in microconjugant.

FIG. H. Conjugation. Second progamic division in macroconjugant and third progamic division in microconjugant are over.

FIG. I. Conjugation. The pronucleus in each conjugant is ready. The dark bodies are the residual progamic nuclei.

which has already entered prophase in preparation for conjugation now proceeds further to reach first progamic metaphase. Simultaneously the second progamic metaphase is reached in the microconjugant also. The second progamic division in the macroconjugant and third progamic division in the microconjugant take place synchronously (Text-fig. II, figs. A-G). While this is the normal picture, in some instances, the meeting of the microconjugant and the macroconjugant takes place early, before the first progamic division of the micronucleus of the macroconjugant has taken place. In such instances, though the micronucleus of the macroconjugant has already entered the prophase of first progamic division it stays at the stage till the first progamic division is completed in the microconjugant. It is only after this, the micronucleus of the macroconjugant enters first progamic metaphase and simultaneously the micronucleus in the microconjugant also enters second progamic metaphase. The third division in the microconjugant and second division in the macroconjugant occur simultaneously (Text-fig. III, figs. A-I). The latter condition indicates that the difference in the number of progamic divisions of the micronucleus in the two conjugants may be due to the behaviour of the micronucleus of the macroconjugant. At any rate this seems more plausible than considering the first progamic division in the microconjugant as having arisen *de novo*. The time taken for completion of the first progamic division in the macroconjugant is equivalent to the time taken for the two divisions of the counterpart in the microconjugant. The macroconjugant behaves as a specialized cell which requires a stimulus for the first progamic division to progress and this seems to be provided when the first progamic division is completed in the microconjugant. A similar, but slightly different, condition has been recorded in *Epistylis articulata* (Dass, 1953), where the first division of the micronucleus in both conjugants start simultaneously and proceed up to metaphase. Thereafter, the division process is arrested in the macroconjugant and is resumed only with the second progamic division metaphase in the microconjugant. In this difference in time factor during progamic divisions, as also the size and future behaviour, the macroconjugant of *Peritricha* resembles the ovum of metazoa.

(c) Formation of nuclear anlagen

Formation of the sinkaryon and nuclear anlagen follows the complete fusion of the microconjugant with the macroconjugant. In no instance was a residual microconjugant observed, though Popoff (1908) found it in *Carchesium polypinum*.

Generally, soon after the fusion of the pronuclei the unused progamic nuclei are absorbed in the cytoplasm. In a few cases, viz., *Vorticella microstoma* (Finley, 1943), *Rhabdostyla vernalis* (Finley, 1952), *Zoothamnium arbuscula* (Furssenکو, 1929) and *Epistylis articulata* (Dass, 1953) they persist for some time, and can be recognized long after the sinkaryon has started its division. In *C. spectabile*, however, all progamic nuclei except one are absorbed in their respective conjugants long before their fusion.

The sinkaryon is formed only in the macroconjugant and this is the condition observed in most peritrichous ciliates. But a reciprocal exchange of pronuclei with the formation of a sinkaryon in each conjugant has been reported in *Zoothamnium arbuscula* (Furssenکو, 1929) and *Urceolaria synaptae* (?) (Colwin, 1944). The sinkaryon divides thrice successively to give rise to eight bodies, a feature common to all peritrichous ciliates so far studied. They are all alike and a fine network of thread-like structures is seen in all. Later, however, in one of the eight bodies the network-like structure gives place to a uniform homogeneous appearance. It also undergoes a reduction in size. This is the micronucleus. The other seven are the macronuclear anlagen.

A variation in the number of macronuclear anlagen has been recorded by Colwin (1944) in *Urceolaria synaptae* (?) but Finley (1952) has pointed out that

the author may have confused a macronuclear fragment for a macronuclear anlage. Willis (1948) observed in *Lagenophrys tattersalli* a number of instances where 'the morphologically single macronucleus shows a gross, moniliform character, with two, three, four or seven lobes, i.e., with the number of lobes corresponding to the number of discrete anlagen found in normal distributive stages. This may indicate that in certain cases the anlagen become fused together'. Outside this observation of Willis, in no other Peritricha has such a fusion of macronuclear anlagen been reported. Only in one instance, a departure from the conventional condition has been recorded; in case of *Vorticella convallaria* (Seshachar and Dass, 1951) where instead of seven of the eight bodies becoming transformed into macronuclear anlagen, only six do so and the remaining two become micronuclear anlagen.

(d) Reorganization fissions

Once the macronuclear anlagen are differentiated, the subsequent behaviour is easily followed. The faint network-like structure is soon lost and the anlagen appear homogeneous. This initial differentiation is followed by an increase in size and a progressive nucleination. The first reorganization fission takes place at this stage. The interval between the second and the third reorganization fissions is fairly long and during this period the growth and nucleination of macronuclear anlagen take place. An interesting feature recorded in this form is the differential rate of nucleination of the sister anlagen. Such a condition has not been observed in any other peritrichous ciliate.

The fate of the old macronucleus of the conjugants is interesting. The macronucleus of both conjugants breaks up into a number of fragments which fill the cytosome of the synconjugant. A gradual reduction in their numbers, by absorption, takes place now. Reduction at a more rapid rate takes place later during differentiation of the macro- and micronuclear anlagen. Most fragments are absorbed in the cytoplasm by the third reorganization fission. In some instances, the absorption is so rapid that no fragments are found even in the F_1 individuals. The significance of the rate of absorption of the fragments becomes apparent in instances where there is differential nucleination of the macronuclear anlagen. In some instances the macronuclear fragments are seen to persist up to the end of reorganization fissions and are found even in the fully reorganized individuals. In them, the nucleination of the anlagen is slow and gradual while in cases where the absorption of the fragments takes place early, the nucleination of the anlagen is also early and complete. This confirms the view that the absorption of the macronuclear fragments is perhaps not just 'degeneration' or 'elimination' of the old macronuclear material, but is a reconstitution of the old macronuclear material on a new framework (Seshachar, 1947).

SUMMARY

The nuclear apparatus of *Carchesium spectabile* consists of an irregularly drawn out cylindrical macronucleus and a small spherical micronucleus.

During trophic activity a number of Feulgen negative spaces appear in the macronucleus.

In addition to unimicronucleate forms, in some colonies, individuals with two, three and four micronuclei are also seen.

Two types of binary fission are observed occurring in members of the colony, the criterion of difference being the behaviour of the macronucleus.

A vegetative individual undergoes a preliminary division and both the daughter cells pass through two further successive divisions to produce four microconjugants each. Hence there are eight microconjugants derived from a vegetative individual.

The macroconjugant is produced by differentiation of a vegetative animal.

The microconjugant is liberated as a free-swimming body and during this free-swimming search period its micronucleus undergoes a division. In those instances where the search period is short, the micronuclear division is not complete at the time of attachment to the macroconjugant.

More than one microconjugant may attach itself to a macroconjugant. All of them undergo nuclear changes.

The micronucleus of microconjugant undergoes three divisions while that of macroconjugant two. One of the progamic nuclei in each conjugant becomes a pronucleus and the rest are absorbed in the cytoplasm. The macronucleus of both conjugants fragment.

The microconjugant merges with the macroconjugant *in toto* to form a synconjugant. Two pronuclei fuse to form a synkaryon. The synkaryon divides thrice successively to form eight metagamic nuclei of which seven become the macronuclear anlagen while the eighth is the micronuclear anlage.

The macronuclear anlagen enlarge and progressively become nucleinated. The nucleination is often not uniform among sister anlagen.

The macronuclear fragments are at first absorbed gradually, but later, during the differentiation and growth of the macronuclear anlagen, the absorption is more rapid. In those instances where the nucleination of macronuclear anlagen takes place slowly, it is noticed that the rate of absorption of the fragments is also slow.

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EXPLANATION OF PHOTOMICROGRAPHS

Key to lettering: M—Macronucleus; m—micronucleus; M.C.—Macroconjugant; m.c.—Microconjugant; M.A.—Macronuclear anlagen; M.f.—Macronuclear fragments.

PLATE IV

- FIG. 1. Vegetative individual showing Feulgen negative spaces in the macronucleus. $\times 720$. Feulgen-Light green.
- FIG. 2. Binary fission Type I. $\times 360$. Feulgen-Light green.
- FIG. 3. Binary fission Type II. $\times 360$. Feulgen-Light green.
- FIG. 4. Bi- and tetra-micronucleate individuals. $\times 360$. Feulgen-Light green.
- FIG. 5. Conjugation. Microconjugant just attached to macroconjugant. $\times 720$. Feulgen-Light green.
- FIG. 6. Conjugation. Macroconjugant with two microconjugants. One of them not in direct contact with the macroconjugant. $\times 720$. Feulgen-Light green.
- FIG. 7. Synconjugant. The cytosome is filled with macronuclear fragments. $\times 720$. Feulgen-Light green.
- FIG. 8. F₁ individuals. The number of macronuclear fragments greatly reduced. $\times 360$. Feulgen-Light green.
- FIGS. 9 and 10. F₁ individuals. All the macronuclear fragments absorbed. The macronuclear anlagen showing maximum nucleination. $\times 720$. Feulgen-Light green.
- FIG. 11. F₂ individuals. In one of the individuals the fragments are still persistent while they are absent in the other. The macronuclear anlagen of sister individuals show different rates of nucleination. $\times 180$. Feulgen-Light green.
- FIG. 12. F₂ individual. Prior to third reorganization fission. No macronuclear fragments in the cytosome. $\times 720$. Feulgen-Light green.
- FIG. 13. F₃ individual. No macronuclear fragments in the cytosome. $\times 720$. Feulgen-Light green.
- FIG. 14. F₃ individual. Macronuclear fragments still persistent. $\times 720$. Feulgen-Light green.

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