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Patterning of Ciliary Structures in *janus* Mutant of *Tetrahymena* with Mirror-image Cortical Duplications. An Ultrastructural Study

Synopsis. The fine structure of the somatic and oral infraciliature was studied in three lines of *janus* mutant of *Tetrahymena thermophila*. This mutant perpetuates a large-scale reversal in location and orientation of some surface structures, including oral apparatuses and contractile vacuole pores (Jerka-Dziadosz and Frankel 1979).

The somatic kineties in the mutant show normal ultrastructure of the components and normal pattern of proliferation. The ultrastructure of the contractile vacuole pore is described. No difference in fine structure of the primary and secondary CVP sets was found.

The primary oral apparatus shows nearly normal pattern of disposition of basal bodies and their microtubular derivatives. In the kinetosomes of the secondary OA the microtubules show normal orientation, but the arrangement of basal bodies is frequently modified into abnormal pattern.

Development of the primary OA was studied. Kinetosomes produced in the anarchic field assemble into pairs, which next align into promembranelles. The arrangement of basal bodies in adoral membranelles of late dividing cell differs from that in adult cells indicating that the final patterning of oral structures takes place in the terminal stages of morphogenesis.

In the Discussion new features of *Tetrahymena* cortex are outlined and the mode of interaction of simple and complex ciliary structures with the reversed morphogenetic fields are discussed.

The analysis of abnormal cellular patterns produced by chemical operation on the genome seems to be a powerful method in analysing mechanisms of cell differentiation. Recently due to an advance in *Tetrahymena* genetics, many developmental and other mutants have been isolated and analysed (Bruns et al. 1976, Doerder et al. 1975, Frankel et al. 1976, 1977, Kaczanowski 1975, 1976).

The *janus* mutant of *Tetrahymena thermophila* seems to be of particular interest. The analysis of the phenotype and the nature of genic control of the *janus* syndrome has been already published (F r a n k e l and J e n k i n s 1979, J e r k a - D z i a d o s z and F r a n k e l 1979). The cortical features that distinguish the original mutant clone (CU-127) bearing the mutated gene *jan* from all previously examined *T. thermophila* are: (1) stable propagation of an unusually high number of ciliary meridians that appear sporadically in all *janus* clones, (2) capacity to produce a second oral apparatus with characteristic abnormalities at position close to 180° to the primary oral apparatus, (3) frequent possession of two sets of contractile vacuole pores, always located on the cells side, that is to the right of the normal OA and to the left of the second-
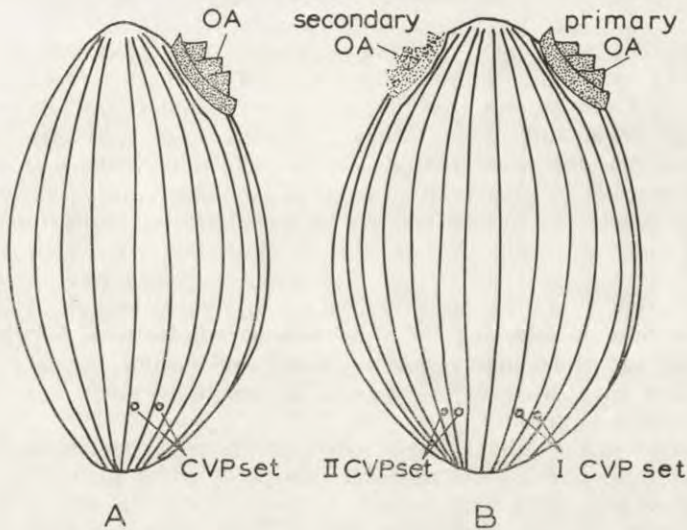
Fig. 1. Schematic diagrams of general feature of wild type (A) and *janus* mutant of *Tetrahymena thermophila* (B). Longitudinal lines represent ciliary meridians. Small circles in the posterior part of the cell indicate the pores of contractile vacuole (CVP). The *janus* phenotype is represented in its highest state of expression i.e., the cell possesses two oral apparatuses (OA) and two sets of CVP's

ary OA (Fig. 1), (J e r k a - D z i a d o s z and F r a n k e l 1979), (4) a phenotypic defect in development of exconjugants when *janus* homozygotes are crossed with each other (F r a n k e l and J e n k i n s 1979).

Dividing cells manifest numerous different combinations of traits in anterior and posterior moieties of the same cell. Oral apparatuses on the primary axis are always formed and appear normal, whereas those

on the "secondary axis" are sporadically formed and always abnormal. The abnormalities consist of reversed adoral membranelles. It seems that the stage most sensitive to the action of the factors responsible for the "handedness" of structures to be patterned is the stage 2–4 of oral development when kinetosomes of the anarchic field become organized into structures specific for the OP — the kinetosomal pairs. In the Discussion new features of cortical ultrastructure of *Tetrahymena* are outlined and the modes of interaction of simple and complex ciliary pattern (J e r k a - D z i a d o s z and G o l i ń s k a 1977) with an reversed morphogenetic field are discussed.

Material and Methods

Three lines of *Tetrahymena thermophila* were used in this study. These were the wild type B strain of *T. thermophila* and three clones bearing the *jan* gene. These were: the original CU-127 line (*jan/jan*) isolated after N-methyl-N-nitroso-guanidine mutagenesis by Dr. P. Bruns, and lines No. 30 and No. 46 of *jan/jan* mutants, which resulted from crosses between different lines. Line No. 30 is a homozygous *jan/jan* clone derived from a cross between *jan/jan+* heterozygote descended from CU-127 and line A* (*jan+/jan+*). A-star is a defective line described by W e i n d r u c h and D o e r d e r (1975). Line No. 46, also *jan/jan* resulted from cross between another *jan/jan+* clone and A+(*jan+/jan+*) (F r a n k e l and J e n k i n s 1979). Cultures were kept in 1% PPY medium in 5 ml tubes at 27° and loop transferred twice a week. To improve the expression of the *janus* condition, two other enriched media were used. These were Thompson's medium containing 0,5% dextrose and Fe^{2+} + EDTA complex in addition to 2% proteose-peptone and 0,2% yeast extract (T h o m p s o n 1967), and Orias enriched medium containing glucose and vitamins (O r i a s and R a s s m u s s e n 1976). *Janus* cells grown in the Thompson medium manifest rather high expression of cells with double oral apparatuses and many cells with double oral primordia (F r a n-k e l and J e n k i n s 1979), they, however, show signs of poor fixation after standard procedures used in electron microscopic preparation of material.

For EM fixation a drop of tube cultures was inoculated into 50 ml of medium in Ehrlenmayer flasks and grown for about 20 h at 27°C. After that, cells were pelleted in a laboratory centrifuge and fixed in 1:1 (vol) mixture of 2% osmic acid and 3% glutaraldehyde dissolved in cacodylate buffer, pH 7.2. After 1 h fixation in refrigerator (+4°C) cells were washed in buffer, collected into agar blocks and dehydrated through alcohols, propylene oxide, and embeded into Epon 812. This fixation preserves rather well the microtubular, microfibrillar and membraneous structures, but causes quite substantial extraction of the cytoplasm. Therefore some samples of *janus* and wild type cells were fixed first in glutaraldehyde and then postfixed with osmic acid following standard methods used by other authors (i.e., A u f d e r h e i d e 1979). Some sections were additionally stained with tannic acid 0.1% before collection into agar blocks. Blocks were sectioned on a LKB ultramicrotome with diamond knife and examined under JEM 100 B transmission electron microscope.

Results

(a) Somatic Cortical Structures

The somatic cortical ultrastructure of *Tetrahymena pyriformis* strain W has been carefully and extensively described by A l l e n (1967, 1969), therefore the findings of this investigation were compared to A l l e n's description.

The pellicular structure of *janus* mutant appears very similar to that of *T. pyriformis* W. It is composed of three membranes — the outer one and two inner ones covering the subpellicular alveoles (Pl.I 6 and Pl.II 11). Under the third membrane a fibrogranular sheet of epiplasm is located. In the peripheral layer of *janus*, like in the WH and GL strains of *T. piriformis*, mucocysts are present (Pl.I 4). Fixation in the mixture of osmic acid and glutaraldehyde frequently causes extrusion of mucocysts. Traces of "empty" mucocysts are sometimes visible on grazing sections through the pellicle (Pl. I 3).

The basal bodies of somatic cilia in *janus* cells all have the same orientation. In cross sections, looking from the exterior of the cell — the microtubular triplets turn to the right of the viewer (Pl. I 1). Each somatic kinetosome (as in Allen's study) possesses 1 fiber and 3 sets of microtubules (Pl. I 1–7, Pl. II 8–10). The kinetodesmal fiber is associated with the proximal end of the basal body at its right anterior quadrant. The kinetodesmal fiber appears striated and extends anteriad into the right of the more anterior basal body in the same kinety and appears to end in close proximity to the longitudinal microtubular band (lm). In cross sections the longitudinal microtubular bands which extend from the anterior apex to the posterior end — appear to be composed of 9–13 microtubules, situated between alveoles and the epiplasm (Pl. I 6). The microtubules on the right side of the band have "free" ends on the anterior side (Pl. I 3). Microtubules on the opposite side have feree ends on their posterior side (compare with N g 1979). Microtubular ends are seen also in the middle of the band (Pl. I 3).

Each somatic basal body possesses two microtubular ribbons, respectively attached to the left and right sides of the basal body. The postciliary ribbons (Pc) arise next to the posterior right of the proximal region of basal body and extend obliquely posterior and to the right of the kinety, as they bend away from the basal body and progress toward the surface (Pl. I 1, 7). The transverse fibers (T) arise on the anterior side of the proximal end of the basal body (Pl. I 6, 7). This set is usually composed of 5–6 microtubules, extends under the surface subjacent to the epiplasm, and crosses transversely toward the longitudinal micro-

tubular band (Pl. I 3, 7). The microtubules are not equally long, the posterior-most being the shortest. The three sets of microtubules: longitudinal, transverse and postciliary appear morphologically similar in their ultrastructural picture in *janus* cells and in wild type tetrahymenas.

The somatic kineties are accompanied by single or double longitudinal basal microtubules running along a ciliary row on its left side at the level of the proximal ends of kinetosomes (Pl. I 1). The anterior ends of somatic kineties show characteristic differentiations. These were first observed in *Tetrahymena* by M c C o y (1974) on protargol preparations and in electron microscope by N g and W i l l i a m s (1977). Many kineties of both *janus* and wild type cells possess a kinetosomal couplet at their most-anterior ends, composed of one ciliated (posterior) and one non-ciliated (anterior) basal body (Pl. II 8, 9). The anterior basal body lacks the postciliary and kinetodesmal fiber. It possesses only the transverse fiber, which however, is oriented differently from other transverse fibers present in front of the other somatic kinetosomes. In cross section the anterior (Pl. II 9), transverse fiber is situated in front of the anterior kinetosome and is oriented perpendicularly to the kinety. Between the two basal bodies a parasomal sac is located in the typical position. The posterior basal body shows a well developed kinetodesmal fiber, postciliary ribbon and transverse fiber in their typical locations. The two basal bodies lie close together and are connected by dense desmoses which sometimes appear striated. The distribution of couplets in wild type cells is on kineties 5 to n-1 (M c C o y 1974) and hence is asymmetrical with respect to the two sides of the cell. In many *janus* cells with one OA this same pattern is seen, however, in cells with two OAs the pattern is reversed around the secondary OA (F r a n k e l personal communication). The anterior kinetosome of the apical couplets in wild type cells and in *janus* tetrahymenas are underlain by a ring made of fine filaments with the thinnest microfilaments having diameter of 4–7 nm. This ring shows a banding pattern with period ranging from 90–200 nm. The structure of this ring is described separately (J e r k a - D z i a d o s z 1981 c). The apical couplets are formed during cell division in the incipient furrow region (N e l s e n et al. 1981). In the same region in *janus* cells a ring made of 4–7 nm microfilaments originate. This ring is involved in division furrow formation and cell constriction (J e r k a - D z i a d o s z 1981 c). A contractile ring was described in *Tetrahymena pyriformis* dividing synchronously by Y a s u d a et al. (1980).

It should be pointed out that all examined somatic basal bodies in *janus* clones showed normal asymmetry with respect to their internal constitution (the orientation of triplets in a kinestosome) as well as the position of the accessory microtubular ribbons. Sections through pro-

liferating kinetosomes revealed that new somatic basal bodies are formed in a classic position (A l l e n 1969) anterior to pre-existing basal bodies (Pl. I 5).

Another cortical structure associated with the surface layer in *Tetrahymena* is the pore of the contractile vacuole (CVP). As has been shown in the analysis of the *janus* phenotype (J e r k a - D z i a d o s z and F r a n k e l 1979) about 60⁰/₀ of the population of *janus* cells possess two sets of CVP's located in the posterior region of the cell, predominatly close to the left of kineties 4.5 and 7.8 (in cells having 23 kineties) — counting from the postoral meridian of the normal oral apparatus (OA) (Fig. 1 B). The pore manifests a distinct and complicated ultrastructure (P a t t e r s o n 1980). The CVP's found on sectioned material from *janus* cells do not differ from those found in wild type cells sectioned as controls and are similar to those already described in the literature (P a t t e r s o n 1980, P e c k 1977). The CVP appears as an indentation in the pellicle lined with outer cell membrane (Pl. II 10, 11). Up to the middle part of the pore the subpellicular alveolar structures are present. The inner part of the CVP is covered by only a single unit membrane underlain on the cytoplasmic side by a narrow sheet of epiplasm. At the bottom of the pore, parallel to the outer membrane, a single membrane of the pulsating vacuole is located. In saggital sections (Pl. II 11) cross-section of about 9–11 microtubules can be clearly observed in the lower part of the pore, under the unit external membrane. These microtubules are surrounded by a dense fibro-granular substance which appears somewhat similar to the epiplasm although it is slightly thicker. Since cross-sections through microtubules are seen in sections perpendicular to the cell surface, cut at different angles with respect of the antero-posterior axis of the body, it appears that the microtubules actually form a ring surrounding the lower part of the pore. To this microtubular ring are attached other microtubules running obliquely inside the cell (Pl. II 10) and attaching to the membrane of the pulsatile vacuole. Since all somatic kineties of *janus* cells have normal orientation, it is expected that all CVP's found in this mutant will show normal ultrastructure. In fact all CVP's sectioned manifest the same ultrastructural characteristics as CVP's found in wild type cells and those described in the literature (P a t t e r s o n 1980, P e c k 1977).

Summarizing this section it can be stated that structures already described in wild type cells of *Tetrahymena* are completely normal, confirming the earlier conclusions derived from studies of protargol preparations (J e r k a - D z i a d o s z and F r a n k e l 1979). Other structures, such as the transverse and longitudinal bundles of microtubules, the anterior ciliary couplets and the CVP's have not been described in

the same detail in wild type cells, yet it can be assumed that the structures seen in *janus* cells are the same as in wild type *Tetrahymena*.

(b) Primary Oral Apparatus

As is known from the previous analysis of *janus* phenotype, in log-phase cultures (clone CU-127) about 15% of the non-dividing cells possess two oral apparatuses, and in dividing cells about 25% of dividers manifest two sets of oral primordia. In protargol stained cells as well as in scanning EM microphotographs, the "primary oral apparatus" always appeared normal (J e r k a - D z i a d o s z and F r a n k e l 1979). Transmission EM examination of ultrathin sections revealed that the composition of the oral structures as well as their pattern of distribution is the same as described previously in other strains of *Tetrahymena* by N i l s s o n and W i l l i a m s (1966), W i l l i a m s and L u f t (1968) N i l s s o n (1976) and E l l i o t and K e n n e d y (1973), S a t t l e r and S t a e h e l i n (1976, 1979).

The oral apparatus consists of four sets of membranelles: three adoral membranelles (AZM): M-1, M-2 and M-3 composed basically of three rows of ciliated basal bodies each (Pl. III 17). These are located on the left side of the oral opening. On the right side of the OA an undulating membrane (UM) is present. The UM contains two rows of basal bodies (Pl. III 12). The outer row is ciliated, the inner one is non-ciliated (Pl. III 14, 15). The three adoral membranelles differ among each other in number of constituting elements, their arrangement and the position of ancillary structures, such as microtubular derivatives and parasomal sacs. The M-1 membranelle is the largest one. In adult oral apparatuses the anterior row of cilia is shorter than the remaining two rows, and possesses "intraciliary bodies" which were first described by W i l l i a m s and L u f t (1968) and later studied in detail by S a t t l e r and S t a e h e l i n (1974) (Pl. III 17).

In newly divided cells the three rows of basal bodies seem to be equally long (Pl. IV 19). This means that some of the cilia, and perhaps basal bodies, of the anterior row on the left side of M-1 membranelle are resorbed during final patterning. The right side of M-1 membranelle shows a characteristic "sculpturing" (Pl. IV 19, IV 26, III 17). Parasomal sacs accompany all basal bodies from the posterior row. In the posterior row the parasomal sacs are located in between the postciliary ribbons. In the anterior row the parasomal sacs are located anteriorly to the basal bodies. Kinetosomes of the middle row lack the parasomal sacs, with a few indicative exceptions, that is one or two parasomal sacs are visible to accompany the first two basal bodies within the characteristic sculpturing of the right side of the membranelle.

The M-2 membranelle is slightly smaller than M-1 membranelle. It is composed generally of three rows of ciliated basal bodies. Again as in M-1 membranelle the M-2 on its right side shows (Pl. III 17), characteristic sculpturing which is similar, though not identical to that described by M c C o y (1974).

M-3 is the smallest membranelle, in young OA's it is formed from three rows of basal bodies of equal length (Pl. V 24). In adult OA's this regular arrangement becomes significantly modified.

In cross-sections through the basal bodies of the oral membranelles characteristic microtubular derivatives can be seen (Pl. III 12, 13, 15, 16, 17). The postciliary fibers of the AZM accompany only the posterior rows of basal bodies in each membranelle and in young OA also the first basal bodies of all membranellar rows from the right side (the UM side) (Pl. III 17, VI 25). In cross sections, the Pc ribbons of the posterior rows of membranelles are directed toward the posterior-right, those on the right side are directed diagonally toward the UM, that is toward the right side of the cell (Pl. VI 25). These last postciliary fibers probably take part in the construction of the cytostomal wall (Pl. III 15). Transverse microtubules were not found in adult oral membranelles. Besides the postciliary microtubules, there are other systems of essentially microtubular structures often accompanied by filaments having the diameter of intermediate filaments (10 nm) (Pl. IV 20). Besides microtubules some microfilamentous material is also found in the oral apparatus of *Tetrahymena* (Pl. III 15). This seems to be connected with the UM and cytostomal region (G a v i n 1977, N i l s s o n and W i l l i a m s 1966, S a t t l e r and S t a e h e l i n 1974, N u m a t a et al. 1980, J e r k a - D z i a d o s z 1981 c).

The undulating membrane surrounds the oral apparatus on the right and posterior sides. The outer row is ciliated, the inner row is non-ciliated and bears the postciliary fiber (Pc). In these oral kinetosomes the Pc is located on the posterior left side of a basal body, rather than posterior right as in the somatic basal bodies (compare Pl. I 1 and Pl. III 12). The postciliary microtubules originate as direct continuation of the posterior left microtubular triplet in the basal body. They run upward to the cell membrane slightly bending posteriorly (Pl. III 13). Therefore on sections through the middle and distal part of kinetosome the Pc microtubules appear to be located in between of the subsequent basal bodies. As already noted by S a t t l e r and S t a e h e l i n (1976) three basal linkers (Pl. III 12 arrowheads) connect a ciliated basal body located in the outer row with the closest posteriorly-located non-ciliated basal body in the inner row. A ribbed wall exists along part of the right posterior surface of the buccal cavity (S a t t l e r and S t a e h e-

lin 1979). Each rib is supported by 2 ribbons of microtubules with the characteristic $2 + 4$ pattern. In tangential sections (Pl. III 15) and in cross sections the fine filamentous reticulum exhibits a distinctive regular pattern of dense bands oriented at right angles to the filaments. The dense bands (see also J e r k a - D z i a d o s z 1981 c) are spaced at intervals 82–105 nm (S a t t l e r and S t a e h e l i n 1979). Apart from that there is also a course filamentous reticulum (W i l l i a m s and L u f t 1968).

From this brief survey it follows that the "primary oral apparatus" in *janus* clones appears very similar to the wild type OA. Again, as in the case of the somatic structures, some aspects have not been studied with the same detail in wild type cells, therefore it is still possible that the primary OA in *janus* cells may not be completely normal.

(c) Formation of the Oral Apparatus

The development of oral primordia in the *janus* mutant of *Tetrahymena thermophila* has been described based on a light microscopical study of protargol-stained and silver-impregnated cells, and on scanning electron-microscopic microphotographs (J e r k a - D z i a d o s z and F r a n k e l 1979). It was concluded that the oral primordium formed on the primary axis appears the same as in wild type tetrahymenas (F r a n k e l and W i l l i a m s 1973, M c C o y 1974). The secondary oral primordia showed profound abnormalities, many were resorbed before full maturation, and some showed mirror-image reversal of global asymmetry.

In this study developing primordia of only *janus* cells were examined, therefore it is not known whether the ultrastructural details in oral primordia of *janus* cells differ from those of wild type cells or not. In only few cases was it possible to state whether the sectioned oral primordium is the primary or the secondary one. This is possible only when both primordia are visible on the same section, or on serial sections from the same cell. In such cases, however, the primordia are usually cut sagitally (Pl. IV 22), that is kinetosomes are sectioned longitudinally, and therefore an analysis of the arrangement of kinetosomes and their microtubular derivatives is practically impossible. A tangential section through the oral primordium allows this kind of analysis, but discrimination between primary and secondary primordia is very difficult.

Plate V shows pictures of oral primordia which have been interpreted as the "primary oral primordia". The oral primordium shown on Pl. V 21 is in stage 3 of development (staging after F r a n k e l and W i l l i a m s 1973). Many of the visible kinetosomes are arranged into kine-

tosomal pairs (Pl. V 21 a). A pair is composed of two kinetosomes accompanied by specific microtubular derivatives. The posterior kinetosome bears a well developed postciliary ribbon showing a clear convergent configuration, and a transverse ribbon (Tp) which is located at the right anterior side of this kinetosome. The anterior basal body of pair has only one transverse microtubule (Ta) located in front of the pair. This microtubule is very short and not always visible. The microtubule marks the anterior side of the pair and the Pc the posterior side of the pair. In stage 3 of development kinetosomal pairs line up with a homogeneous orientation, in such a way that all Pc fibers are directed toward the posterior-right of the cell. In this way an obliquely oriented promembranelle composed of two rows of kinetosomes originate. The posterior kinetosomal row of the promembranelle bears the postciliary ribbons. After alignment the promembranelles separate somewhat and a second round of kinetosome proliferation occurs anteriorly in each promembranelle. In this way the third anterior row of basal bodies in each membranelle is formed (Pl. V 23).

The origin of the UM has not been studied in detail, at the ultrastructural level. Especially, the origin of the paired row visible in the second half of morphogenesis is unclear. In stage 5 the UM is visible more or less in the form resembling adult UM's (Pl. V 24, VI 25). It is composed of two rows of basal bodies, one ciliated (exterior) one nonciliated (interior) with postciliary microtubules directed toward the AZM membranelles. Plate V 24 shows an OA where signs of UM patterning can be observed. The old exterior row (ex) is in the process of desintegration, whereas new row is being formed (arrowheads) adjacent to old interior row (see also N e l s e n 1981).

In comparable stage of development, proliferating basal bodies are also present in the anterior part of the M-2 membranelle (Pl. VI 25). These new basal bodies are probably involved in the formation of the "sculpturing" of the right side of the M-2 membranelle (M u s z y ń s k a et al. 1981).

(c) Secondary Oral Apparatus

Since a rather small fraction of the population of *janus* cells, expresses the secondary oral apparatus, finding and recognizing the abnormal OA in a fixed random sample appears very difficult. It several dozen sectioned oral apparatuses no trace of abnormal kinetosomes were found. In all cases, the microtubular triplets in cross-sectioned basal bodies from the oral apparatuses showed the same direction of turning as found in their neighbouring somatic kinetosomes. Therefore, it is concluded that the abnormality of the secondary OA does not comprise the mirror-ima-

ge reversal of the internal organization of microtubules within the basal body.

The oral apparatus shown on Pl. VI 27 possesses three AZM membranelles (M-1, M-2 and M-3) and an undulating membrane with the inner non-ciliated row of basal bodies and the ribbed wall that is quite disorganized. The orientation of triplet microtubules in the basal bodies of the adoral membranelles is clock-wise, as can vaguely be noticed on the few basal bodies from the M-2, seen on the right side of the photograph. A second proof for the normal orientation of microtubules can be found on cross-sections through cilia where the arms of the A microtubule point counter-clockwise (compare Pl. VI 27 with Pl. III 18).

Looking into the characteristic structures normally found on the right side of membranelles, although it is difficult to interpret the arrangement of infraciliature, based on cross-sections through cilia, it can be stated that normal sculpturing of the right side of membranelles (the left side of the photo) is not completely ruled out. The left sides of the first two membranelles are, however, modified and their pattern deviate from the normal membranelles.

In M-1 membranelle on Pl. VI 27 (compare with Pl. III 17 and Pl. IV 19, 20) all three rows of basal bodies are ciliated. It should be noted, however, that the anterior-left ciliary shafts show signs of either growth or resorption. If resorption of cilia is taking place, then this would indicate that final patterning of the left side of the M-1 membranelle occurs in such a way as to produce the normal left side pattern of M-1 membranelle, with the anterior row of cilia shorter.

If, however, growing of cilia occurs, then some sort of right side sculpturing may be expected at the level of infraciliature.

Between M-1 and M-2 on the cell's left side a group of basal bodies is seen, which again are difficult to interpret. The first possibility is, that this group of kinetosome represent a "right" margin sculpturing of the M-2 membranelle arranged abnormally in a modified mirror-image fasion. This is lightly supported by the position of the postciliary ribbon in the left-most kinetosomes, and by the position of the parasomal sac. The second possibility is that the group of basal bodies represent an abortive membranellar fragment not related either to M-1 or M-2 membranelle.

From the above analysis of the abnormal OA, it can be concluded that the secondary oral apparatus in this cell of the *janus* mutant is formed from normal kinetosomes, but the pattern of arrangement of basal bodies may be modified. The above conclusions have been confirmed by observations of several sectioned doublet cells where both primary and secondary oral apparatuses can be seen at the same section (or series

of sections from the same cell). Based on comparison of the organization of kinetosomal microtubules, and the axonemal microtubules of cilia of the AZM, UM and the somatic cilia located nearby, it is concluded that the *janus* mutation does not alter the internal microtubular organization of a ciliary unit, somatic or oral (J e r k a - D z i a d o s z 1980).

The development of the secondary oral primordia have not been studied on sectioned material.

Discussion

Ultrastructure of Surface Organelles in *Tetrahymena thermophila*

The ultrastructure of cortical layers of *Tetrahymena* has been studied extensively over the last decades and is rather well known. Therefore only the details which have not been described so far will be commented upon briefly.

(1) The apical crown of paired kinetosomes. This structure was described by M c C o y (1974), based on light optic observation of protargol stained cells. In the present study the details of ultrastructure of the pair were given. The apical pair in *Tetrahymena* appears morphologically similiar to couplets occurring in the anterior termini of kineties in *Dexiotricha* described by P e c k (1977). The anterior basal bodies are underlain by filamentous band, composed of 4–7 nm microfilaments. This ring appears similar in some respects to a contractile ring of filaments found in the division furrow region of *janus* cells (J e r-k a - D z i a d o s z 1979, 1981) and in other strains of *Tetrahymena* (N u m a t a et al. 1980, Y a s u d a et al. 1980).

(2) The kinetosomal pairs produced in the anarchic fields, as an intermediate step in the formation of oral membranelles.

Involvement of paired kinetosomes in stomatogenesis have been described to occur in representatives of *Polyhymenophora* (P a u l i n and B u s s e y 1971, B o h a t i e r 1979, D u b u c h e t et al. 1979, G r i-m e s 1972, R u f f o l o 1976). The spatio-temporal aspects of pair formation and their role in patterning of polymembranelles of *Paraurostyla weissei* have recently been analysed (J e r k a - D z i a d o s z 1981 a, b) M c C o y (1974) using protargol stained cells of *Tetrahymena*, noticed kinetosomal pairs in the oral primordium. The present study gives some details of organization of microtubules in the pairs. The oral pair of *Tetrahymena* differs from oral pairs known in *Polyhymenophora* in that a conspicious transverse microtubular ribbon accompanies the po-

sterior kinetosome of the pair. The oral pair in *Tetrahymena* differs significantly from somatic apical pair: (a) in the position of accessory microtubules, (b) in the lack of a kinetodesmal fiber in the oral pair, (c) in the different types of desmoses between the kinetosomes of a pair, and (d) in a difference in origin of the pair: the kinetosome of the somatic pair are related generatively — the anterior one assembles with a connection to the posterior one and remains associated with it. The oral pair may originate by random association of kinetosomes produced earlier in the anarchic field, and therefore the kinetosomes in an oral pair are not necessarily related generatively.

One qualification should be made: suitable cross-sections through the oral anlagen in the stage of pair formation were found only in one *janus* cell from line No. 46 grown in Thompson medium and fixed in the mixture of glutaraldehyde and osmic acid. Therefore there are two open questions: first, whether the details found in *janus* cells can be generalized for wild type *Tetrahymena* as well, and second, whether insufficiently good fixation might have obscured fine details of the ultrastructure of the oral pair.

Sections through later stages of developing oral primordia showed, that in the patterning of the oral ciliature, two rounds of kinetosome proliferation are involved. The first one is the random proliferation occurring in the early anarchic field stage of oral differentiation. The second one occurs in already organized adoral promembranelles and aligned UM parts of the oral primordium. Basing on the studies performed by N e l s e n (1981), on reorganization of the anterior oral apparatus occurring during division, it is tempting to suggest that the two rounds of kinetosome proliferation may be separable, that is in the anterior OA only the second round kinetosome proliferation or part of it takes place.

(3) Ultrastructure of the contractile vacuole pore. The description given in this paper provides more details of the structure of the CVP than known so far (E l l i o t and B a k 1964). The organization of microtubules in the CVP of *Tetrahymena* appears identical to that described in some scuticociliates (P e c k 1977, P a t t e r s o n 1980).

(4) Ultrastructure of the longitudinal microtubular (lm) bands. Grazing sections of lm showed that the pattern of arrangement of microtubules in the longitudinal band is similar to that described in *Glaucoma* and *Calpidium* by P i t e l k a (1961). N g (1979 a, b) in considerations concerning the propagation of the longitudinal band postulated that microtubules "extended until ultimately reaching or defining the right edge of the band". Plate I 3 shows a microtubule whose elongation has apparently stopped or slowed down as compared to neighbouring micro-

tubules, before reaching the right margin of the band. This may indicate that sliding of microtubules against each other is taking place.

Pattern Reversal in *Tetrahymena*

The study presented in this paper confirmed and extended the conclusions derived from the light-optic and scanning electron microscopic study on the *janus* mutant of *Tetrahymena thermophila* (J e r k a - D z i a d o s z and F r a n k e l 1979). The previous study showed that the somatic ciliature in the *janus* mutant does not differ from that in wild type cells. This includes the presence and orientation of basal bodies, postciliary, transverse and longitudinal ribbons which can be seen on suitable protargol preparations.

Ultrastructural studies showed that in addition to this, the configuration of microtubules in basal bodies, ciliary axonemes and accessory microtubular ribbons (Pc, T and lm) also do not differ in wild type and *janus* cells. Other pellicular differentations of the somatic cortex such as the subpellicular alveoli, parasomal sacs, as well as cellular organelles located in the perpheral layer of the cell (mucocysts and mitochondria) appear normal.

It is interesting to note that in mirror-image doublets of a hypotrich ciliate *Pleurotricha* the orientation of somatic cirri in the symmetry-inverted half is the same as in the normal half (G r i m e s et al 1980), whereas the global pattern of oral structures is mirror-image inverted. Similarly in *Blepharisma*, K u m a z a w a (1979) surgically produced double cells which occasionally formed a third adoral band with a mirror-image orientation. He noted: "that the reversed peristome forms in spite of the intrinsic polarity of the kinetosomes, although the latter is expressed as deviations from exact mirror relation" (K u m a z a w a 1979, p. 11).

Assembly of basal bodies in *Tetrahymena* and patterning of other cortical structures apparently related to them, such as positioning of cortically located mitochondria (A u f d e r h e i d e 1979, 1980) and fine positioning of CVP (N g 1980), appears normal in cell regions where the postulated morphogenetic field with reversed symmetry is operating.

A way of visualizing of how can basal bodies in general remain normal, when being confronted with factors responsible for dextro-sinistral inversion of the morphogenetic field, is to postulate that they are somehow insensitive, or "immune" to this factor. This can be related to aspects of the relative independence of somatic kinety propagation, such as well documented cases of transmission of inverted kineties (B e i s s o n and S o n n e b o r n 1964, N g and F r a n k e l 1977),

and phenomena defined collectively as "cortical inertia" (N a n n e y 1980). A mechanistic basis for explaining these phenomena is the assumption that basal bodies form through self-assembly, where each preceding step limits the succeeding one (A n d e r s o n 1977) and where patterning occurs together with assembly (A u f d e r h e i d e et al. 1980).

Development of the oral ciliature in *Tetrahymena* requires more steps than does the production of a somatic cilium. Production of poly-kineties (oral membranelles) requires several rounds of proliferation. The question arises, which step in the production of the secondary oral apparatus is the most sensitive to the action of the postulated reversed morphogenetic field. From the analysis of protargol stained cells it can be concluded that defects in oral development can be observed starting from the earliest stages. The secondary anarchic fields are amaller and frequently do not develop further and later become resorbed. In the early anarchic field single kinetosomes with or without postciliary microtubules associate into pairs. Microtubules associated with basal bodies are not assembled at that time, but filamentous structures used for connecting the two kinetosomes are produced. A kinetosomal pair differentiates further — this becomes expressed a little later when pairs start to align from random into homogeneous orientation. Apparently only parallell lining up of pairs can be stabilized, probably because the microtubular triplets in kinetosomes are no longer equivalent, and the asymmetry of the pair is expressed in specific proteins associated with microtubules on both sides of paired kinetosomes. How, if at all, the inverted morphogenetic field influences the morphology of a pair is unknown.

It can only be hypothetized that the orientation of kinetosomal pairs in the secondary oral primordia in *Tetrahymena* is affected in such a way that it frequently results in rotations of about 90° of each super-ficially normal appearing kinetosomal pair. This then could give rise to a "mirror-image" configuration of adoral promembranelles such as seen on Fig. 18 b of J e r k a - D z i a d o s z and F r a n k e l (1979), p. 190 and on Pl. VI 27 of this paper.

Some details of components of mirror-image inverted oral apparat-uses have been described in *Tetrahymena* (J e r k a - D z i a d o s z and F r a n k e l 1979 and this paper) and in hypotrich ciliate *Pleurotricha* (G r i m e s et al. 1980). In *Tetrahymena* the adoral membranelles of the perfect mirror-image oral apparatus have the antero-posterior axis unchanged: kinetosomes with postcilliary microtubules are located at the posterior margin of oral membranelles (Pl. III 17, Pl. V 21, Pl. VI 27). In *Pleurotricha* as can be judged from the protargol stained cells (G r i-m e s et al. 1980) and the mode of formation of polymembranelles in

hypotrich ciliates (J e r k a - D z i a d o s z 1981 a) each membranelle is rotated upside-down and therefore, the postciliary microtubules should accompany the (cell's) anterior kinetosomal rows in each membranelle. Whether the difference in orientation of membranelles in *Tetrahymena* and *Pleurotricha* is related to the fact that in the former, the defect is caused by single recessive gene mutation, and in the latter, by surgical manipulation, remains to be resolved. It seems that in both cases, however, the final mirror-image orientation of the zone of membranelles results from modified alignment of kinetosomal pairs in the anarchic field. The fact that single somatic kinetosomes remain normal in the territory of the inverted morphogenetic field supports again the conclusion that they are under strictly local determination and that the spatial orientation of membranelles is independent of the kineties.

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EXPLANATION OF PLATES I–VI

Pl. I. Somatic cortical structures of *Tetrahymena thermophila*. Cells shown on phot. 1 and 4 were fixed in glutaraldehyde and postfixed in osmic acid. The remainder photographs show pictures of cells fixed in direct mixture of glutaraldehyde and osmic acid. All stocks were grown in PPY medium

1: Basal bodies in a kinety. Pc-postciliary microtubules, kd — kinetodesmal fiber, bmt — basal microtubules running along the proximal region of kinetosomes in a kinety. *Janus* line No. CU-127. (30 000 ×)

2: Grazing section through the pellicle of the wild type cell (B). The left side of the longitudinal microtubular band (lm) is seen. Arrows point to the ends of microtubules. (13 000 ×)

3: Grazing section through the pellicle of the wild type cell (B). Longitudinal microtubular band seen on its right side. Arrow point to ends of microtubules. T — transverse microtubular ribbon, Mu — "empty" sac after extruded mucocyst. (15 000 ×)

4: Mucocysts in *janus* CU-127 cells fixed with glutaraldehyde and postfixed with osmic acid. (30 000 ×)

5: Proliferation of new basal bodies near an equatorial region of *janus* CU-127 cells. (35 000 ×)

6: Longitudinal section through a somatic kinetosome and cilium from the anterior region of *janus* line No. 46 cell. The transverse fiber (T) is terminating under the longitudinal microtubular band (lm). Three pellicular membranes are seen: oM — outer cellular membrane and aM — alveolar membranes. Kinetodesmal (kd) and postciliary (Pc) ribbons are cross sectioned (33 000 ×)

7: Grazing section through the pellicle of *janus* line No. 30. The transverse microtubules (T) are terminating non-evenly under the adjacent kinety (14 000 ×)

Pl. II. The apical crown and the contractile vacuole pore. Photographs. No. 8, 9 and 11 show cells fixed in mixture of glutaraldehyde and osmic acid. Phot. 10 shows a cell fixed in glutaraldehyde and postfixed in osmic acid. All stock were grown in PPY.

8: Tangential section through the apical region of somatic kineties in a cell from *janus* mutant line No. 46. The filamentous ring (AR) is seen underlying the anterior kinetosomes of the ciliary couplets (15 000 ×)

9: Cross section through two apical couplets of a cell from *janus* line No. 30. Note the desmoses (c) between kinetosomes and the positions of postciliary (Pc) and transverse (T) microtubules and the kinetodesmal fiber (kd). Contrasted with tannic acid. (35 000 ×)

10: Cross section through the opening of the contractile vacuole (CVP) in a *janus* cell CU-127. oM — the outer cellular membrane, mtR — circular microtubules surrounding the pore, mt — microtubules running radially toward the vacuole bb — basal body situated close and anteriad from the CVP. (35 000 ×)

11: Longitudinal section through the contractile vacuole pore of a cell from *janus* line No. 46. oM — outer cellular membrane, aM — alveolar membrane, ivM — inner vacuolar membrane Mi — mitochondria, ep — epiplasm, mtR — cross sections through the microtubular ring surrounding the base of the pore, mt — microtubules radiating from the ring toward the vacuole. (37 000 ×)

Pl. III. Oral organelles. All sections are from cells fixed with mixture of glutaraldehyde and osmic acid.

12: Cross section through the kinetosomes of the undulating membrane (*janus*

line No. 46). Note that kinetosomes are connected by desmoses (arrowheads) into oblique pairs. The posterior kinetosomes of pair is non-ciliated and bear post-ciliary microtubules (Pc) (34 000 ×)

13: Longitudinal section through the inner row of non-ciliated basal bodies of UM (*janus* line CU-127). Note the course of postciliary microtubules and the underlying filamentous network (34 000 ×)

14: Longitudinal section through kinetosomes (*janus* line No. 46) of the UM (34 000 ×)

15: Cross section through a posterior portion of the oral apparatus (*janus* CU-127). UM and ribbed wall are visible. Note the microtubules of oral ribs and the fibro-reticular mesh (FR) underlying the oral ribs (OR), (18 000 ×)

16: Cross section through the oral ribs equipped with two sets of microtubules (*janus* CU-127). Between the ribs alveole (A) are present (90 000 ×)

17: Cross section through adoral membranelles (*janus* line No. 46). Note the infraciliary differentiations (arrows) in the anterior row of M-1 membranelle. Postciliary ribbons are constituted of about 10 microtubules. Note the position of parasomal sacs in the M-2 membranelle (16 000 ×)

18: Higher magnification of a cross section through a cilium of M-1. The dynein arms of the A microtubules (arrow) point counter-clockwise (85 000 ×)

Pl. IV. Adoral membranelles from the primary OA. Cells fixed in mixture of glutaraldehyde and osmic acid.

19: M-1 and M-2 membranelle. Note the right side sculpturing of the M-1 membranelle, the position of postciliary microtubules (Pc) and parasomal sacs (Ps). *Janus* mutant, line No. 30, grown in Thompson's medium (25 000 ×)

20: Cross section through the adoral membranelles of line CU-127 from PPY. The sculpturing of the right side of the M-2 membranelle is clearly visible. Note the position of parasomal sacs. Filaments interconnecting the membranelles are well visible (23 000 ×)

Pl. V. Developing oral structures. Cell fixed in mixture of glutaraldehyde and osmic asid.

21: Primary oral primordium. Note that kinetosomes are arranged into pairs. The pairs are oriented diagonally with Pc microtubules directed toward the right side of the cell. *Janus* line No. 30 grown in Thompson's medium (20 000 ×)

21 a: Higher magnification of a kinetosomal pair from the oral primordium. Ta — anterior transverse microtubule, Tp — posterior transverse microtubules. Pc — postciliary microtubules (75 000 ×)

22: Sagittal section through an oral primordium, nk — proliferation of kinetosomes. *Janus* line No. 46 (20 000 ×)

23: Section through adoral membranelles. Proliferation of kinetosomes in the anterior rows of M-1 and M-2 is visible. (Line No. CU-127) (20 000 ×)

24: Section through oral apparatus of *janus* CU-127 cell. Note the organization of UM. Arrow point to young kinetosome oriented perpendicular to the neighboring interior one. gp — gap in the exterior (ex) row of ciliated basal bodies (20 000 ×)

Pl. VI. Oral organelles in the primary and secondary oral apparatus. All sections are from cells fixed with mixture of glutaraldehyde and osmic acid. Cells were grown in PPY.

25: Section through differentiating oral apparatus of *janus* cell from line *janus* CU-127. Note the position of the postciliary microtubules (Pc), nk — new kinetosomed added in front right part of the M-2 promembranelles (13 000 ×)

25 a: Higher magnification of UM pairs. Note the ancilliary microtubular structures (73 000 ×)

26: Section through the anterior-right part of M-1 membranelle from the primary oral apparatus of *janus* cells No. 30. Note the position of parasomal sacs (Ps) (18 000 ×)

27: Section through adoral membranelles of a secondary oral apparatus of *janus* cell from line CU-127. Short cilia (nc) are seen in the anterior-left part of M-1 membranelle. Note the positions and orientation of postciliary ribbons (Pc) in the M-2 membranelle and the positions of parasomal sacs (Ps) (23 000 ×)

27 a: Higher magnification of a cilium from M-1 membranelle. The dynein arms of the A-microtubules point counter-clockwise (compare with phot. 18 on Pl. III) (50 000 ×)
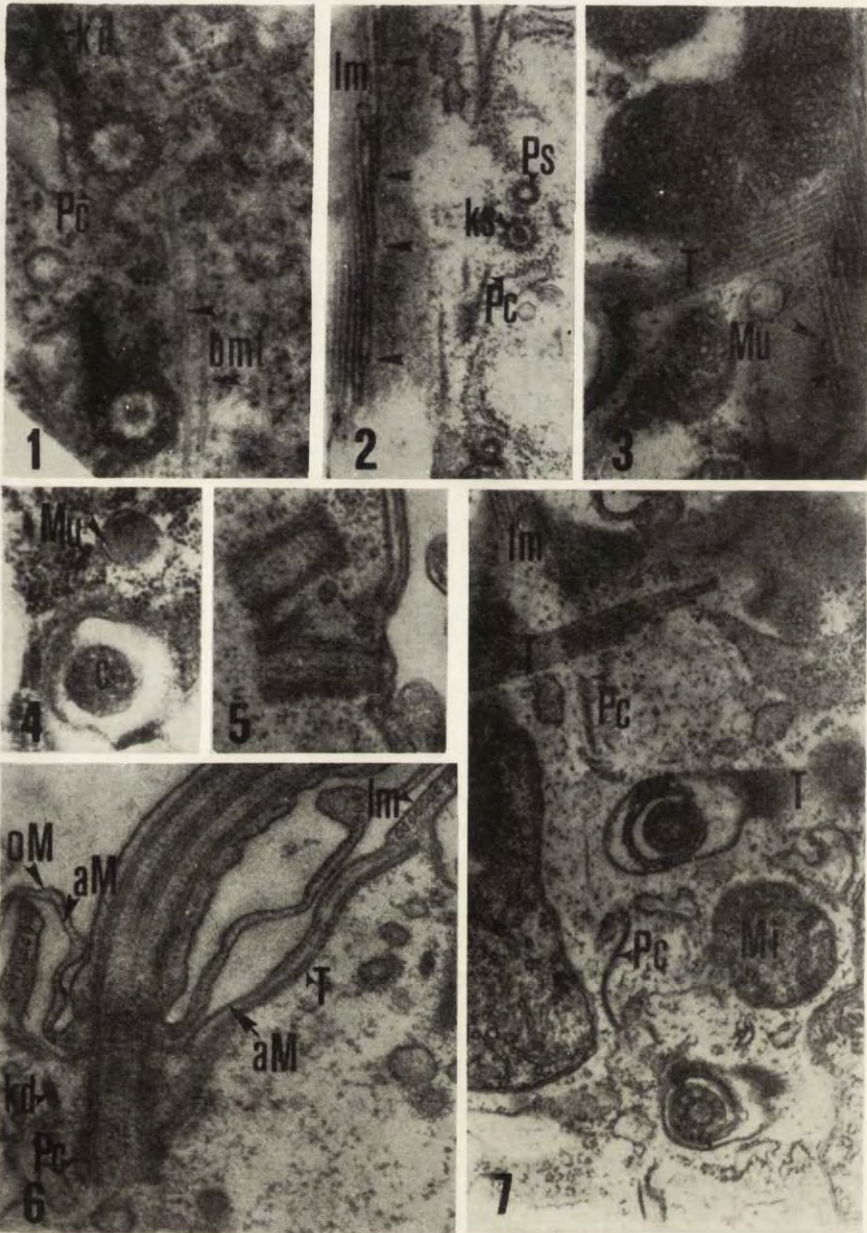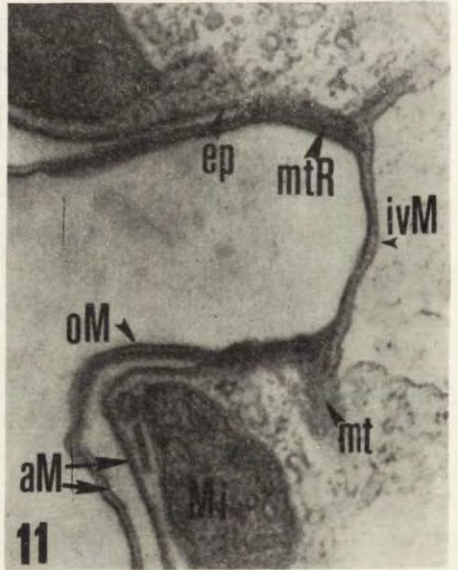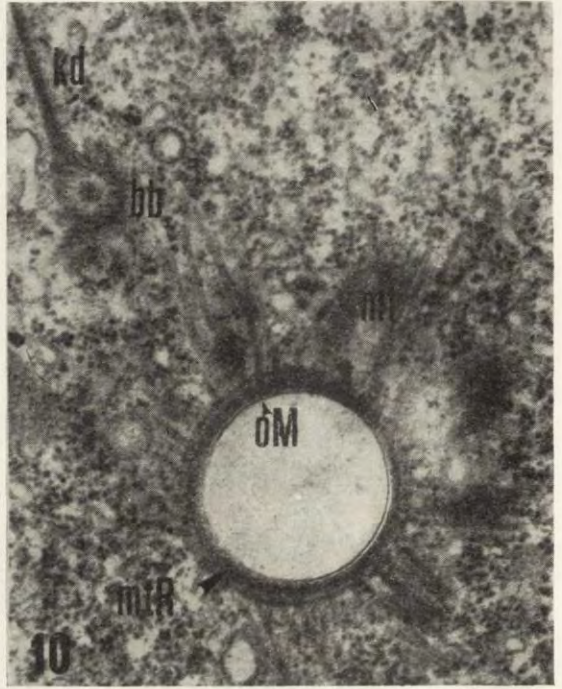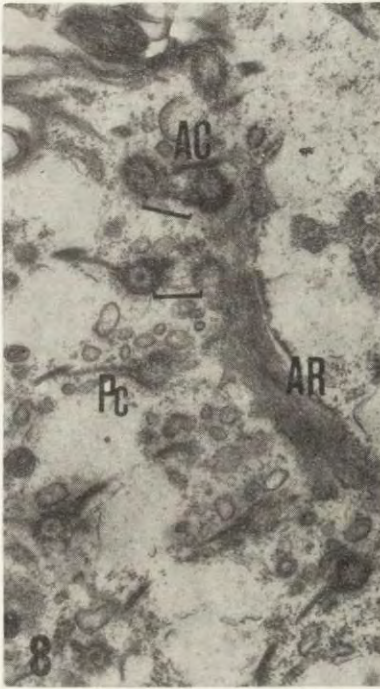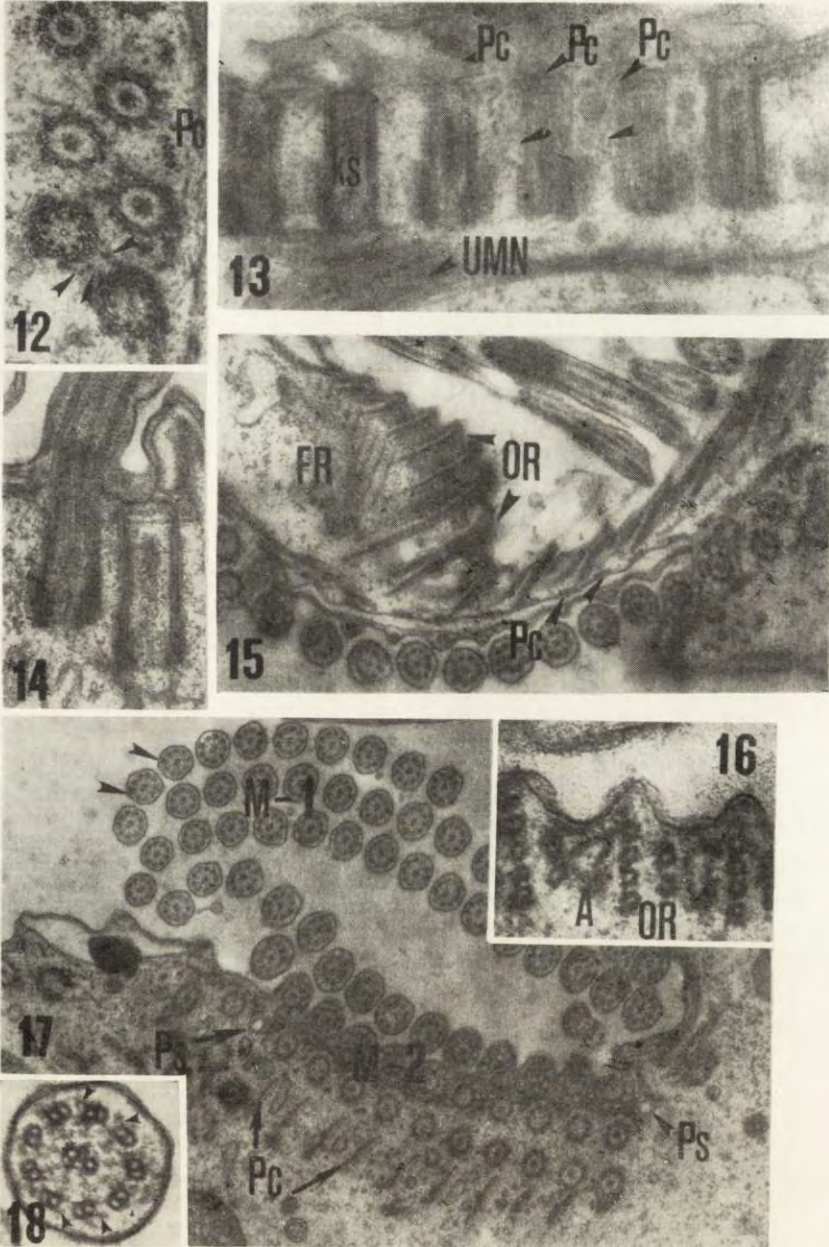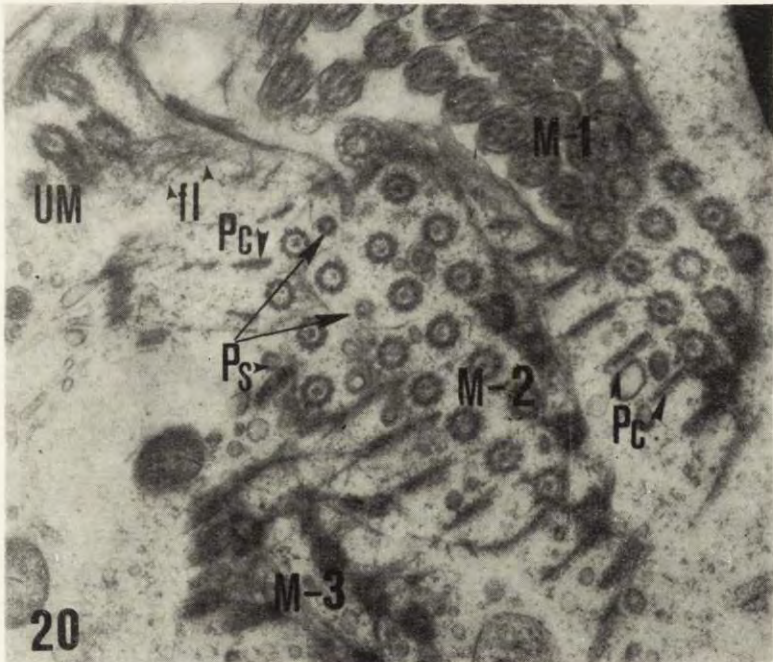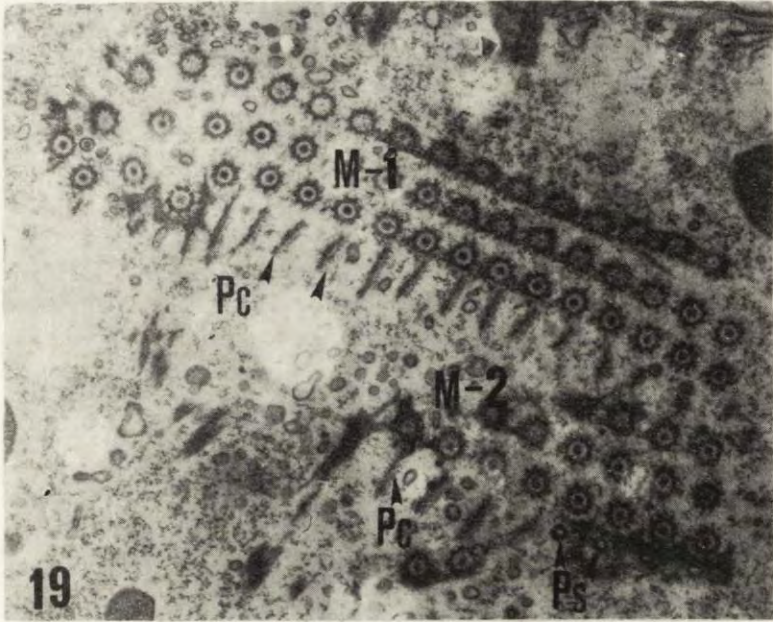
PLATE I

PLATE II

M. Jerka - Dziadosz

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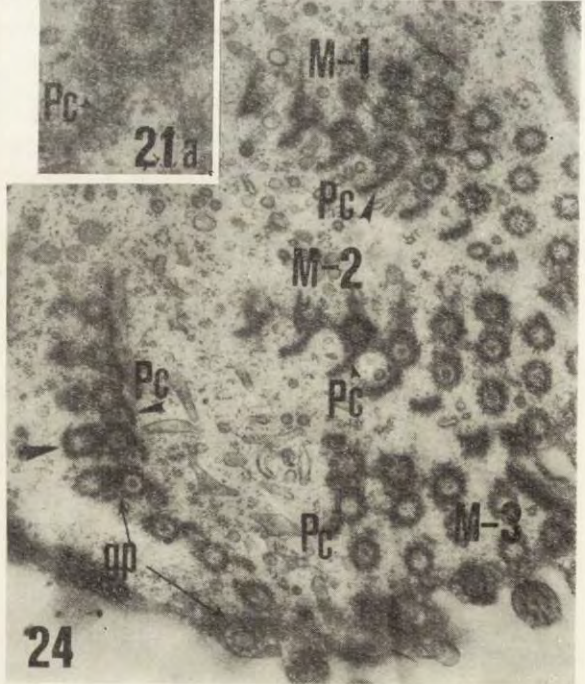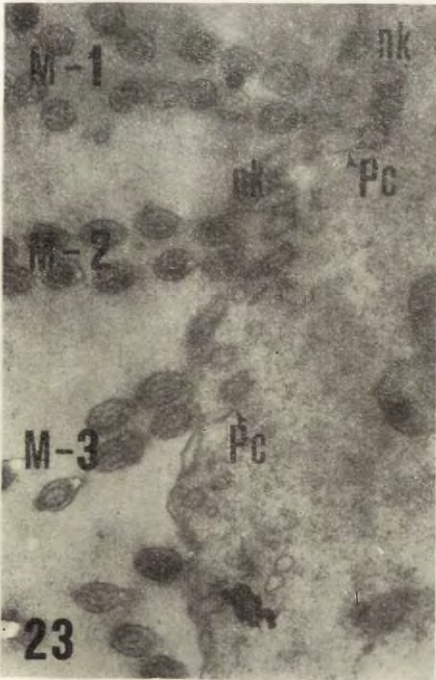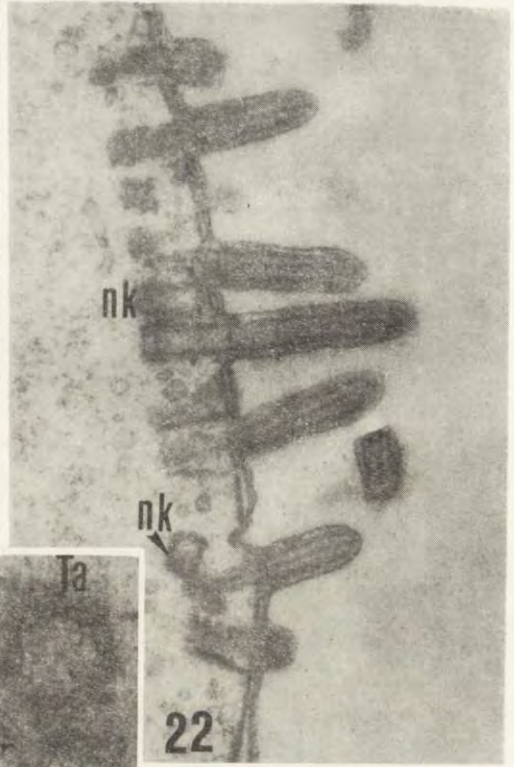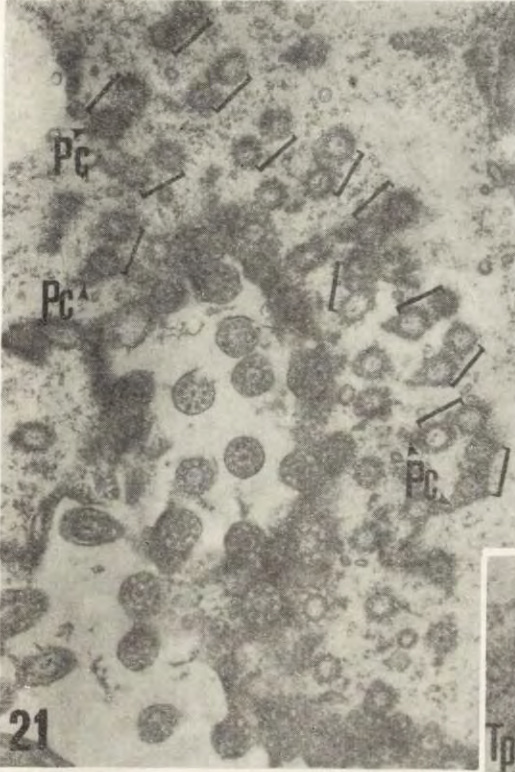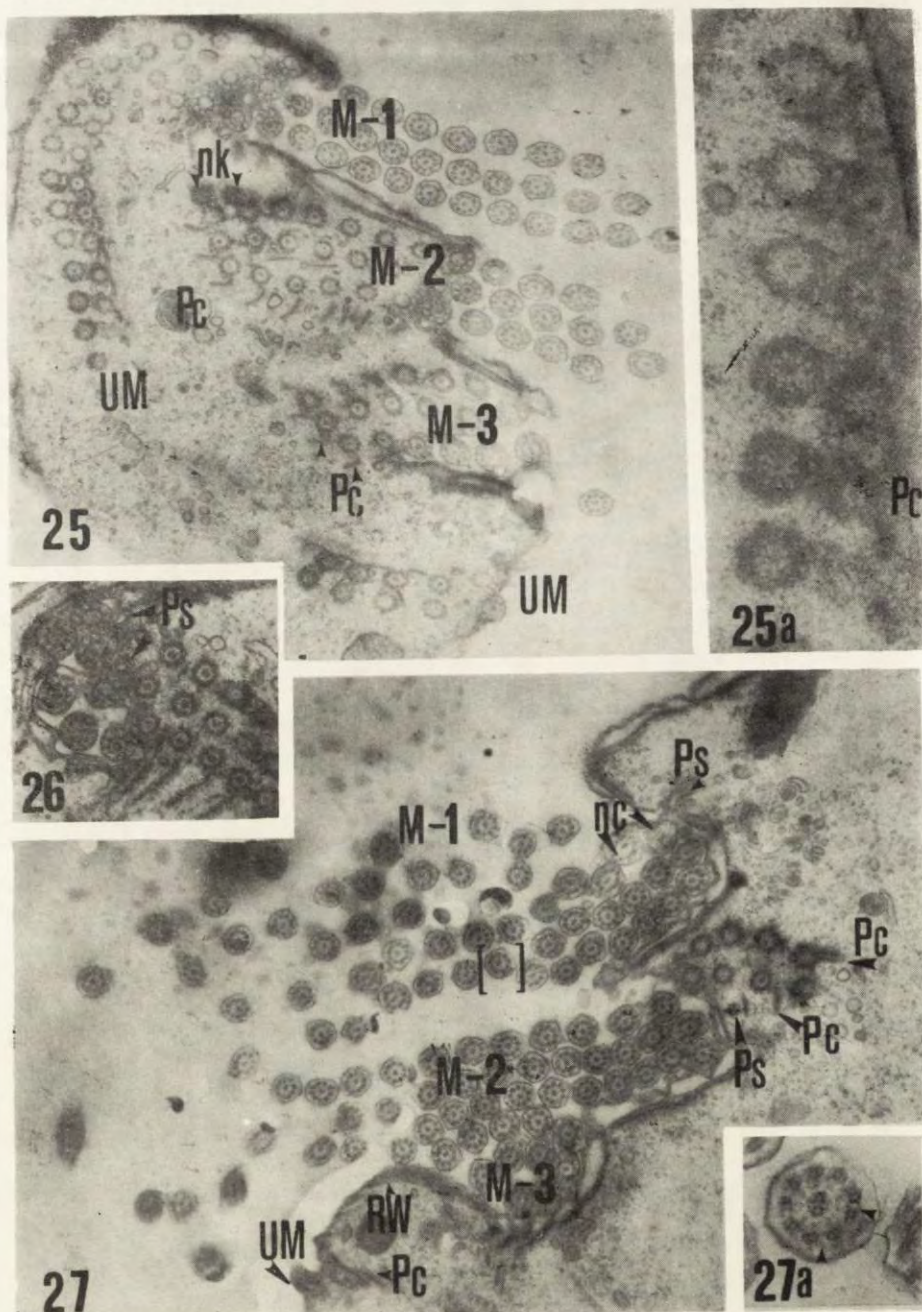M. Jerka - Dziadosz   auctor phot

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Maria WOLSKA

Studies on the Genus *Triadinium* Fior. (*Ciliata, Entodiniomorphida*) Comparison of *Triadinium galea* Gass. and *Triadinium caudatum* Fior.

Synopsis. The somatic and the buccal ciliature as well as the structure of the cortex have been studied in *Triadinium galea*. Some new information are also added to the knowledge of the buccal ciliature in *T. caudatum*. Important differences have been found between both species concerning their buccal ciliature and the structure of the cortex as well.

Taxonomy of ciliates of the order *Entodiniomorphida,* occurring in the horse intestine, is differently approached by various authors (S t r e l- k o v 1939, L a t t e u r and D u f e y 1967, C o r l i s s 1979) showing on the necessity of further studies on these ciliates. The genus *Triadinium* has been already the object of my investigation (W o l s k a 1969, 1971, 1978 b). As a result, the knowledge of morphology of *T. caudatum* Fiorentini, 1890 has been enlarged and the species *T. minimum* Gassovsky, 1919 removed from the genus *Triadinium* Fiorentini, 1890. Then, my attention has been turned to the next species of the genus, namely to *T. galea* Gassovsky, 1919 and again to the buccal ciliature of *T. caudatum*.

The aim of the present paper was to examine the somatic and the buccal ciliature and the structure of the cortex in *T. galea* and to compare this species with *T. caudatum* taking into account the former as well as the present investigations.

Material and Methods

Samples of horse intestine content were taken just after killing of animals in Rawicz slaughtery and transported in 1 l vacuum flasks to the laboratory in Łódź. From a part of the material fixed in 10% formaldehyde, were made the

preparations stained with Dobell's haematoxyline (M a c k i n n o n and H a w e s 1961) or with silver solution after Bielshowsky. For electron microscopical studies the ciliates *T. galea* were fixed in buffered osmium tetroxide. The procedure of silver impregnation of preparations as well as preparing of ultra thin and semi thin sections has been published earlier (W o l s k a 1980 a, b).

Results

I. *Triadinium galea*

Light Microscope Investigation

The ciliate body is rounded in outline and flattened laterally. The vestibulum opens at the posterior end of the body, near to the ventral edge. Such orientation of the body is assumed due to vital observation of the ciliate moving with the pole opposite to the buccal opening directed forward. This observation is concordant with S t r e l k o v's (1939) but inconsistent with H s i u n g's (1930) views. The posterior end of the body, at the right side of the dorsal edge, is provided with a process usually bent ventrally; at the left side the body extends into a triangular lobe (Fig. 1, Pl. I 1 a). This lobe frequently has a concave
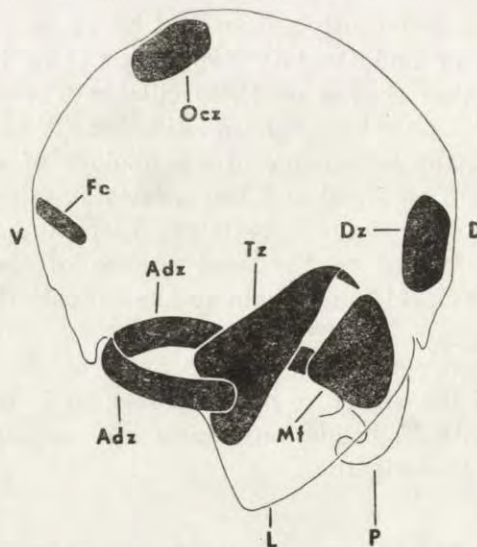

Fig. 1. *Triadinium galea*. Scheme of ciliary pattern; left side view. The ciliate drawned as a transparent one. Ventral edge (V), dorsal edge (D), process (P), lobe (L), occipital zone (Ocz), dorsal zone (Dz), adoral zone (Adz), mussel-like fragment (Mf), triangular zone (Tz), free cilia (Fc)

ventral margin being thus similar to a hood. Elongate macronucleus lays along the body axis, being slightly nearer to the right side. Its ends are bent towards the dorsal side, slightly deviating to the left (Pl. I 1, 2). Small spherical micronucleus lays near the anterior and of the macronucleus (Pl. I 2). The contractile vacuole is situated dorsally, at the posterior bend of the macronucleus. Sometimes another smaller vacuole may be seen at the anterior end of the macronucleus. Cytopyge is situated dorsally, in the posterior part of the body. B o d y   d i m e n-s i o n s: length 44–63 μm (process and lobe not included), width 45–55 μm.

Ciliature is limited to only some groups of cilia. In non-silvered ciliate the following ciliary zones may be discerned: (1) dorsal zone near the posterior end of the body, at right side, (2) anterior zone, named occipital zone by S t r e l' k o v (1939), shifted almost completely to the left side, and (3) circumoral or adoral zone (Pl. I 1). Particulars of the structure of these zones, as well as other parts of ciliature, may be seen only in silvered protozoans.

I n f r a c i l i a t u r e. When silvered both somatic zones, the dorsal and the occipital one, are in shape of short bands with irregularly rounded ends (Fig. 1, Pl. I 3). Each of these zones is composed of den-sely arranged rows of kinetosomes, slant to the longer axis of the band. Distances between particular rows are the same in the whole zone. The kinetosomes in particular rows are very densely arranged. Along the zone, somewhat obliquely, runs, a series of deeply impregnated large granules forming a prominent dark strand (Pl. I 3). Sometimes another such strand is visible at the anterior margin of the zone. Moreover, loosely dispersed single granules may be seen in various parts of the zone. The circumoral zone is in form of a long, narrow ribbon beginning at the left side, near the triangular lobe. It passes through the ventral edge to the right side and, at the base of the dorsal process, sharply bends into the vestibulum extending there in a characteristic way. This wider part of the zone, lying on the right side of the vestibulum, will be named further a mussel-like fragment (Fig. 1, Pl. I 3). When bending over the margin of the vestibulum cavity the adoral zone seems to be slightly teared so the mussel-like fragment may be seen as a separate element. However, it is joined with the zone through a narrow isthmus difficult to see. That the narrow zone surrounding the opening of the vestibulum and the mussel-like fragment form a unity, proves a pri-mordium of this part of ciliature (Pl. III 9, IV 11). It clearly shows that the distinct mussel-like fragment is an integral part of the adoral zone. At the posterior and the anterior margins of the adoral zone the rows of dark granules similarly as in the somatic zones may be observed,

being more pronounced at the posterior margin. Moreover, silver impregnation reveals that a large ciliary zone forming an irregular triangle (Fig. 1, Pl. II 5), is situated on the left wall of the vestibulum. The peak of this triangle is strongly extended; it passes to the right wall of the vestibulum and approaches to the mussel-like fragment (Pl. II 8, I 4). The base of this triangle — a distal margin of the triangular zone, is cut out (Pl. II 5, IV 11, 12). The triangular zone is formed of parallel rows of very densely arranged kinetosomes situated approximately in line with the main axis of the body (Pl. II 6). The triangle has no junction with the circumoral zone. In dividing specimens a separate primordium of this zone may be seen (Pl. IV 12, 11).

At the ventral edge, fairly far from the adoral zone, a small group of kinetosomes is revealed due to silver impregnation (Pl. II 7). These kinetosomes, as it will be shown below in electron microscope investigation, give rise to so called "free cilia" known in other *Entodiniomorphida* (W o l s k a 1978 a, b, 1980 a, b). The primordium of this group of kinetosomes is visible in close proximity of the adoral zone primordium but at another level (Pl. III 10).

Electron Microscope Investigation

The body of ciliate is covered by cell membrane sometimes folded and detached here and there from a thin layer of epiplasma lying beneath it (Pl. V 13, 14). Somewhat deeper longitudinally arranged microtubules, in groups of 2 or 3, are situated (Pl. V 13, 14). Under them there are bars of dense substance (Pl. V 13, 14, 16) oriented in the same direction as the microtubules. At the level of microtubules, and partly at the level of bars, numerous vesicles with granular or homogenous electron dense content are distributed among groups of microtubules. Sometimes, these vesicles are partly discharged of the substance or completely empty (Pl. V 13, 14, 16). Longitudinal bars are connected in regular intervals by transverse strands of dense substance (Pl. V 14, 16). Directly under the above described net of dense substance a rather fine laver of microfibrils is situated with single barren kinetosomes dispersed in it (Pl. V 16). In the most part of the left body side the cortex passes directly into the endoplast. At the right side of the body the ectoplast, containing the nuclear apparatus and the contractile vacuoles, is separated from the endoplast by a fibrillar border EE (Pl. VII 20, 21). This border is formed of double layer of microfibrils (Pl. VII 20, 21). The border layer gives numerous branchings to the surface of the cell (Pl. V 15) and other ones twining around the nucleus. This fairly loose net work with strands running to the surface (Pl. VIII 24) forms the structure similar to the karyophore. The layer

of microfibrils attains greater thickness and compactness in the places where the macronucleus comes close to the cytopharyns (Pl. VIII 25).

The kinetosomes of ciliary zones are provided with associated fibres. In my opinion these fibres are poorly developed kinetodesmal and transverse ones (Pl. VI 17). The bases of kinetosomes are closed and surrounded by strongly developed dense substance. Sheats of dense substance of particular kinetosomes of one row are conterminous forming a common baguet lining under each row of kinetosomes (Pl. VI 17, 23), however, the borders of dense substance pertaining to each kinetosome are marked (Pl. VII 23). Particular baguet push out transverse stripes to neighbouring ones. These connections are well visible at some level (Pl. VI 19). In particular baguet thickenings may be observed getting to inside the cell (Pl. VII 22). Probably, these thickenings are mentioned in the description of ciliary zones as dark granules revealed in silver impregnated preparations. More or less developed nematodesmata, composed of small or large number of microtubules, take their origin from the dense substance at the bases of kinetosomes. Most of them are small or medium size nematodesmata. The large ones are distributed at the borders of zones or in their median part, concomittantly with thickenings of dense substance in baguet. At their beginning the nematodesmata spread under the zone of cilia, parallelly to the surface (Pl. VI 18, VII 22). Then, they may change the direction, running far from the place of their origin, cutting the ectoplast also in places deprived of the ciliature. The kinetosomes are provided usually with axial granules (Pl. VII 22).

The kinetosomes of the groups of cilia called "free cilia" do not show any anomalies in structure, while the cilia are deformed. In transverse sections (Pl. IX 30, 31) these cilia show an irregular shape, being flattened or dilated. Also the arrangement and the number of peripheral doublets of microtubules are disturbed (usually less than 9 doublets occur) and the central pair is lacking. It seems that the kinetosomes of "free cilia" have the same associated fibres as the kinetosomes of ciliary zones described above. However, the material in my disposal was not sufficient to set forth such statement.

The complex cytostome-cytopharynx has a typical structure. The bottom of the vestibulum is provided with characteristically arranged microtubules (rideaux de tubules or ribbons of microtubules). Supposedly, these ribbons of microtubules are formed by transverse fibres derived from kinetosomes of the triangular zone and of the mussel-like fragment. The layer of large and small nematodesmata and of individual microtubules, as well as the outer layer of microfibrils, form a timbering of the cytopharynx (Pl. VIII 26, 27, IX 28). The cytopharynx, widely

opened at first, quickly narrows running along the nucleus. In transverse sections through the cytopharynx, near its end, the number of microtubular ribbons is 12 (Pl. IX 29).

II. *Triadinium caudatum*

The adoral zone of *T. caudatum* (Pl. X 32) has been described earlier (W o l s k a 1969, 1971) as a zone the dorsal part of which (or a ribbon of short kineties) was completely reduced. Careful reexamination of silver impregnated preparations has brought some new data concerning the structure of this zone. The dorsal part of the adoral zone of *T. caudatum* is strongly reduced indeed but exists in a vestigial form. It is composed of a few very short rows of kinetosomes. This short ribbon joins the ends of strongly developed ventral part invaginated into the vestibulum. Due to bending of the adoral zone in the shape of eight observations of particular parts, lying in various planes and shadowing themselves, is very difficult. Only accidentally an atypical position of the ciliate in the preparations may favour observation of the dorsal part, usually weakly impregnating with silver nitrate. A set of several good photographs gave me the possibility to demonstrate this part (Pl. X 33, 34, 35). In the light of these new data it must be conceded that the adoral zone of *T. caudatum* does not differ in its general architectonic from the adoral zone of other *Entodiniomorphida* from horses, such as *Cycloposthium* (F e r n a n d e z - G a l i a n o 1958), *Tripalmaria* and *Tetratoxum* (W o l s k a 1978 a, 1980 b).

Discussion

It is worth to remind here that the dorsal process in *T. galea* is deprived of cilia in contrast to *T. caudatum*. The cilia, in living specimens of *T. galea* (in these parts of the ciliature which may be observed in such conditions), are grouped is small brushes — syncilia. In this respect *T. galea* does not differ from *T. caudatum* as well as from other *Entodiniomorphida*. The presence of syncilia is not a matter for discussion here, as their occurrence in *Entodiniomorphida* is commonly known (for the definition vide C o r l i s s 1979). The ciliature of the vestibulum in *T. galea* differs clearly from that in *T. caudatum*. In *T. galea* the band of syncilia surrounding the opening of vestibulum bends inward the vestibulum at its right side in more pronounced way and attains a characteristic appearance. This difference becomes much more pronounced due to the presence of the triangular zone directed

to the dorsal side. If we assume that the triangular zone is homologous to the ribbon of short kineties in *T. caudatum,* then, we must admit great difference in the degree of development of this zone in *T. galea* and *T. caudatum.* In *T. caudatum* it is a very small vestigial element while in *T. galea* this is a well developed structure characteristic of the species. Thus, *T. galea* resembles to some degree those species of *Ophryoscolecidae* which have the dorsal part only slightly less developed than the ventral part (N o i r o t - T i m o t h e e 1960).

The character and the position of the group of cilia called "free cilia" in *T. galea* is the same as in *T. caudatum* (W o l s k a 1969). They are situated at the ventral edge of the body being shifted to the right side at a fairly great distance from the adoral zone and differ in this respect from other *Entodiniomorphida* having "free cilia" near the adoral zone.

The cortex of *T. galea* shows the same structure as of *T. caudatum* but in the latter the microtubules are abundant while in *T. galea* they are not numerous. The occurrence of barren kinetosomes in the microfibrillar layer is a common feature of both species, but it is known also in other representatives of *Ditoxidae* Strel'kov, e.g., in *Cochliatoxum* (S e n a u d and G r a i n 1972) and *Tetratoxum* (W o l s k a 1980 b). According to my observations the kinetosomes in ciliary zones of *T. galea* are closed while in *T. caudatum* they are open (W o l s k a 1978 b). Appearance of dense substance underlying the rows of cilia in *T. galea* is very similar to that described in *Ophryoscolecidae* (N o i r o t - T i m o t h e e 1960). The cytopharynx in *T. galea* is more simple than in *T. caudatum,* the layer of nematodesmata is less developed and less compact.

The above comparison shows that *T. galea* markedly differs from *T. caudatum* for which the genus *Triadinium* Fiorentini has been created. Thus, I suppose that *T. galea* ought to be excluded from the genus *Triadinium.* However, the final decision whether to create a new genus for it, or to place it in any other genus, ought to be suspended up to obtaining new data, especially about the adoral zone, in the representatives of the genus *Ditoxum* Gassovsky of the same family (*Ditoxidae*) and *Cochliatoxum* Gassovsky, after all recently excluded from *Ditoxidae* (C o r l i s s 1979).

Information about division morphogenesis is only fragmentary. Observation of dividing specimens of *T. galea* shows that this is a complicated process in which the ciliary primordia undergo rotation and considerable dislocation. However, the right sequence of these phenomena has not been established because of scarcity of dividing specimens in examined samples. Early stages of division never has been observed. Electron microscope observations show that the primordia of ciliary zones in *T. galea* develop in vacuoles. Such mode of ciliary zone formation has

been also observed in *T. caudatum*, as well as in other *Entodiniomorphida*. The above data exhaust the knowledge of division morphogenesis in the genus *Triadinium*. Further studies are necessary; they should concern also other species of the genus, namely: *T. magnum* Hsiung and *T. elongatum* Strel'kov from the intestine of donkey and zebra. The data presented in this paper ought to be regarded as a contribution to the knowledge of the genus *Triadinium* Fiorentini.

EXPLANATION OF PLATES I–X

Triadinium galea 1–31, *Triadinium caudatum* 32–35. Light micrographs 1–12 and 32–35 (4 — semi-thin section, 1, 1 a, 2, and 32 — haematoxyline stained preparation, the remaining ones silver impregnated).
1: General view from the left side. Ventral edge (V), dorsal edge (D), occipital zone (Ocz), dorsal zone (Dz), adoral zone (Adz). (1050 ×)
1 a: General view from the right side. Dorsal edge (D), ventral edge (V), process (P), outline of lobe (L), adoral zone (Adz). (1050 ×)
2: Left side view. Micronucleus (arrow). (1050 ×)
3: Posterior part of the body. Dorsal zone (Dz), mussel-like fragment (Mf). (1050 ×)
4: Semi-thin section. Longitudinal section near the right body side. Rapprochement of the proximal end of the triangular zone (Tz) and the mussel-like fragment (Mf). Cytopyge (Cp). (1500 ×)
5: General view of position of the triangular zone in relation to the adoral zone and the mussel-like fragment (1700 ×)
6: Triangular zone. Orientation of kinetosome rows is visible. Anterior (A) and posterior (P) ends. (1700 ×)
7: Ventral part of a ciliate. Kinetosomes of "free cilia" (Kfc). (1700 ×)
8: Mussel-like fragment of the adoral zone with approaching apex of the triangular zone (arrow). (1700 ×)
9: Dividing specimen. An outline of the adoral zone primordium with the mussel-like fragment (arrow) is visible. (1700 ×)
10: Dividing specimen. Primordium of a group of kinetosomes of "free cilia" (Kfc) is visible against the adoral zone primordium (arrow) in the background. (1700 ×)
11: Dividing specimen. Primordium of the adoral zone with the mussel-like fragment (Mf). Parental triangular zone (Tz). (1700 ×)
12: Dividing specimen. Parental triangular zone (Tz) and the primordium of a new triangular zone (arrow). (1700 ×)
13: Section oblique to the cortex. Cell membrane (M). Microtubules (T), longitudinal bars of dense substance (Lb), microfibrils (Mf), vesicles (V). (35 000 ×)
14: Slightly oblique section through the cortex. Epiplasma (Ep), vesicles (V), microtubules (T), longitudinal bars (Lb), transverse strands (Ts). (35 000 ×)
15: Section tangential to the surface. A strand of microfibrils (Mf). (19 200 ×)
16: Section tangential to the surface. Longitudinal bars of dense substance (Lb), transverse strands of dense substance (Ts), layer of microfibrils (Mf), kinetosome (arrow). (22 000 ×)
17: Oblique section through a zone of somatic cilia. Kinetodesmal fibres (Kd), transverse fibres (Tr), baguet underlying rows of cilia (B). (31 200 ×)
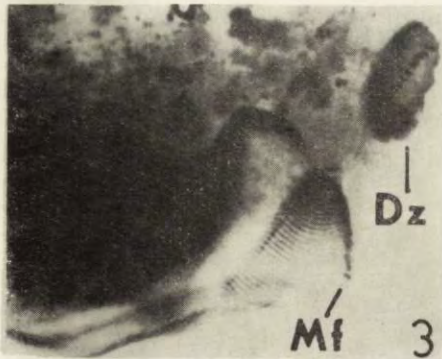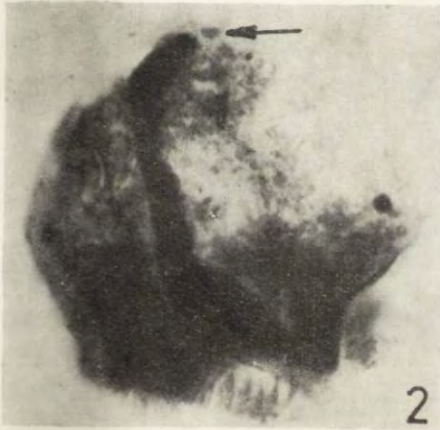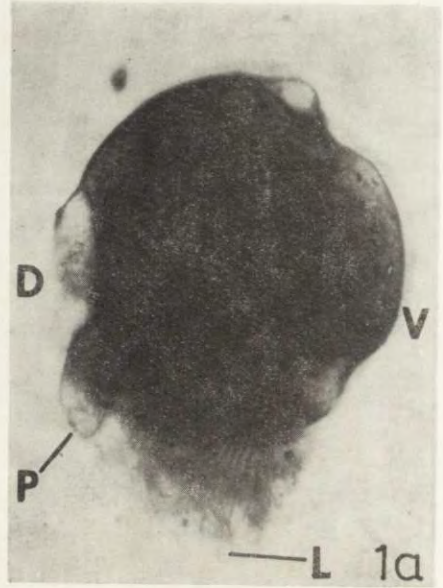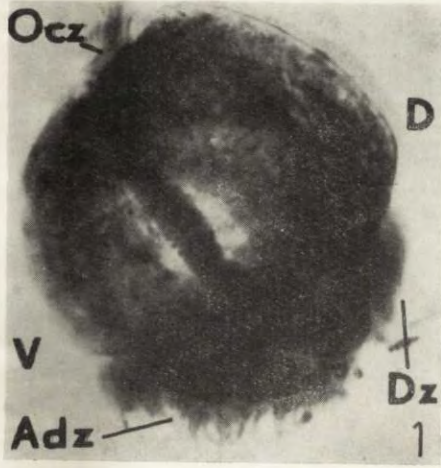18: Section through a ciliary zone with an initial fragment of the nematodesma (arrow). (20 000 ×)
19: Section through the mussel-like fragment of the adoral zone, Transverse junctions between baguet are visible. (12 400 ×)
20: Section through the dorsal zone at the right body side. EE border composed of microfibrils (arrow). (16 000 ×)
21: EE border in the anterior body part (arrow). Macronucleus (Ma), micronucleus (Mi). (10 000 ×)
22: Section perpendicular to the surface through a ciliary zone. Baguet underlying
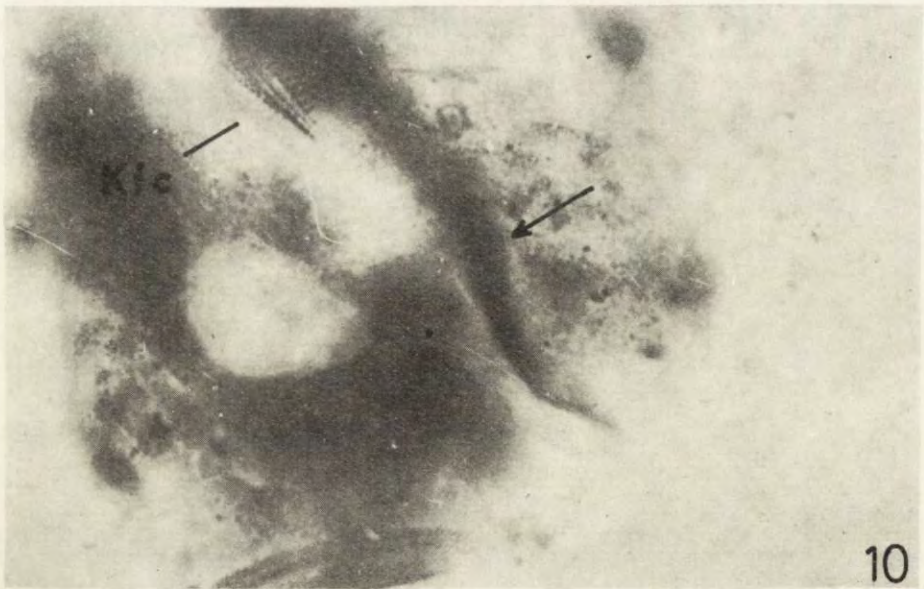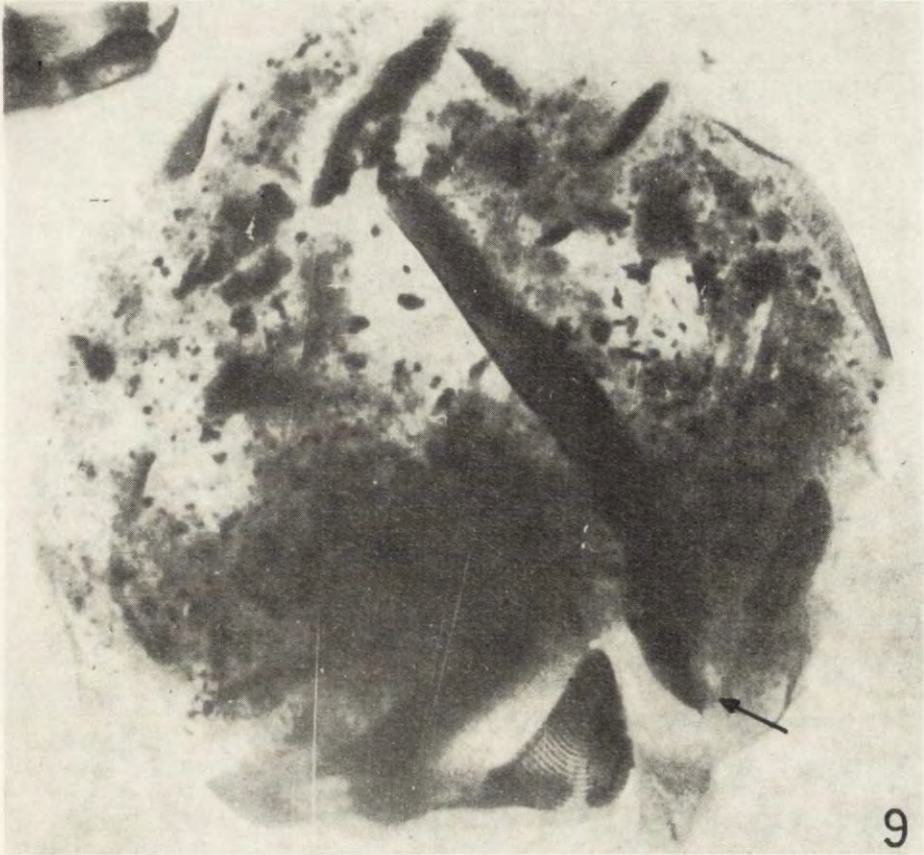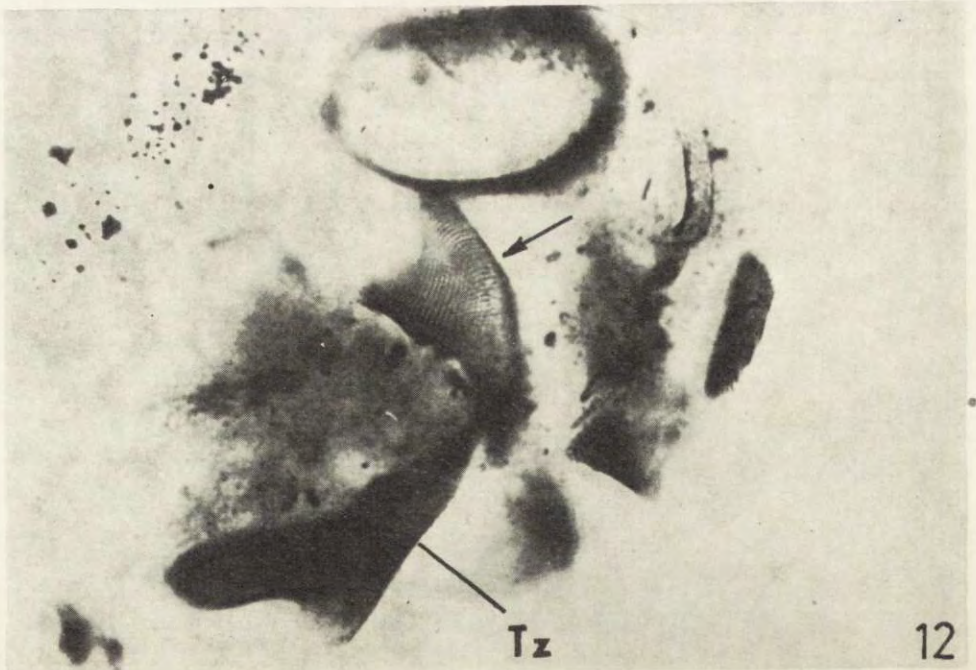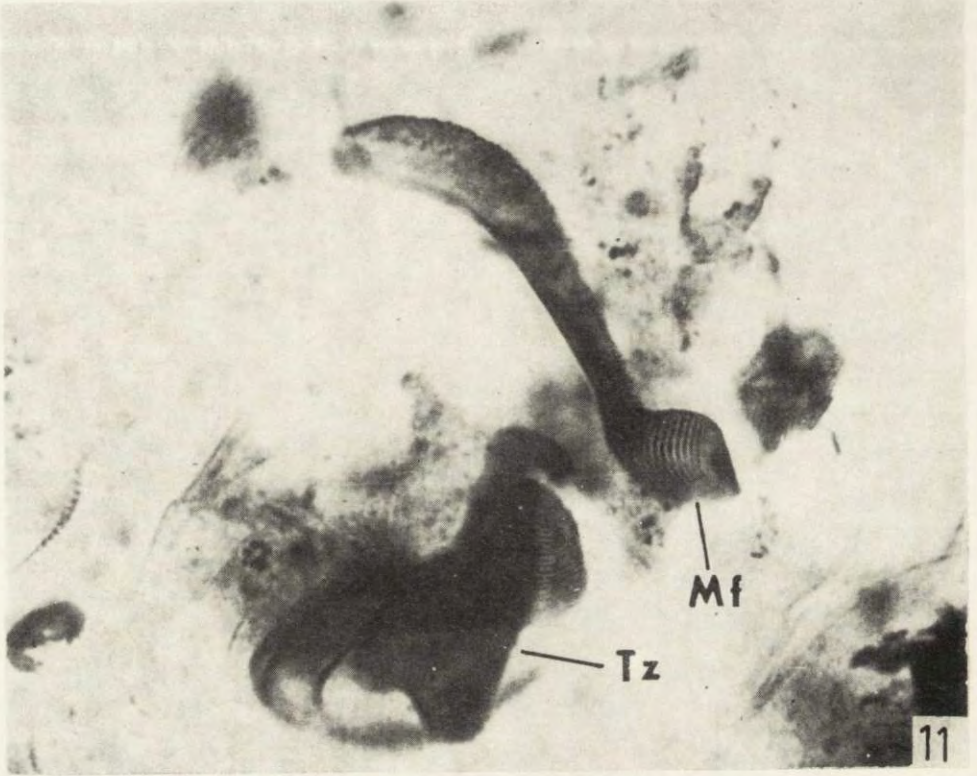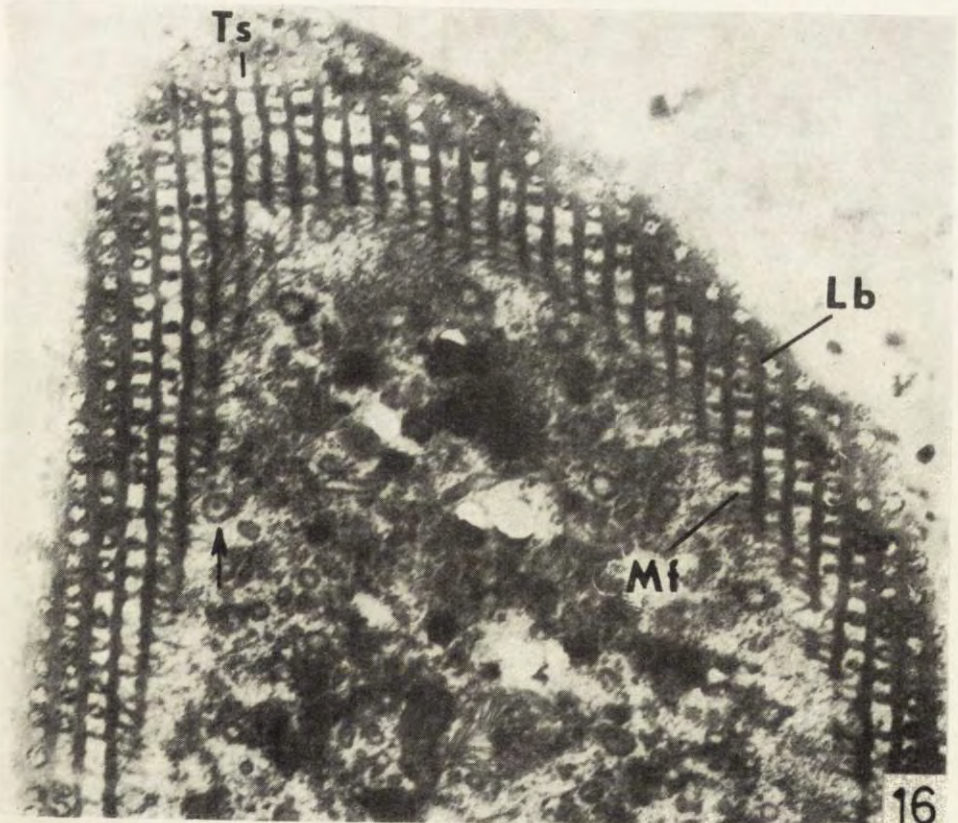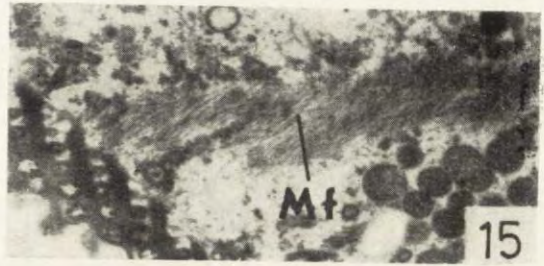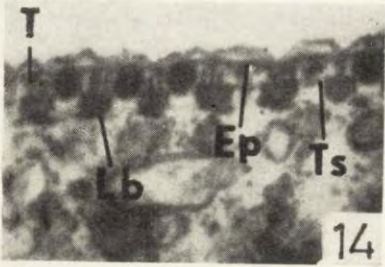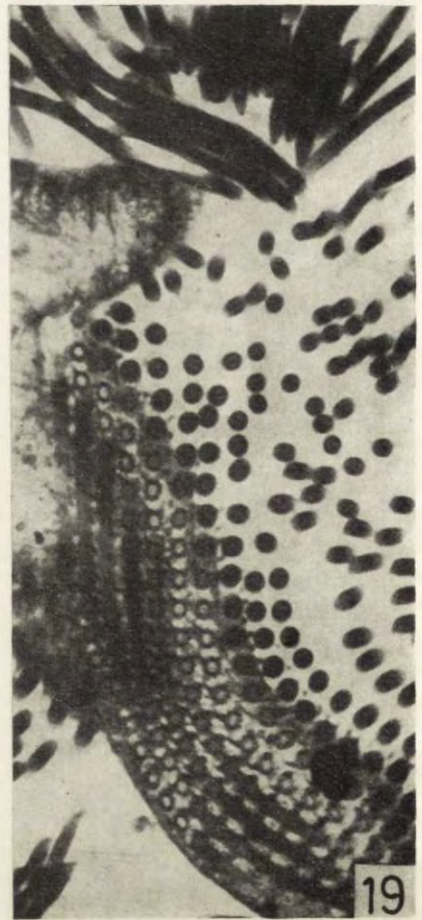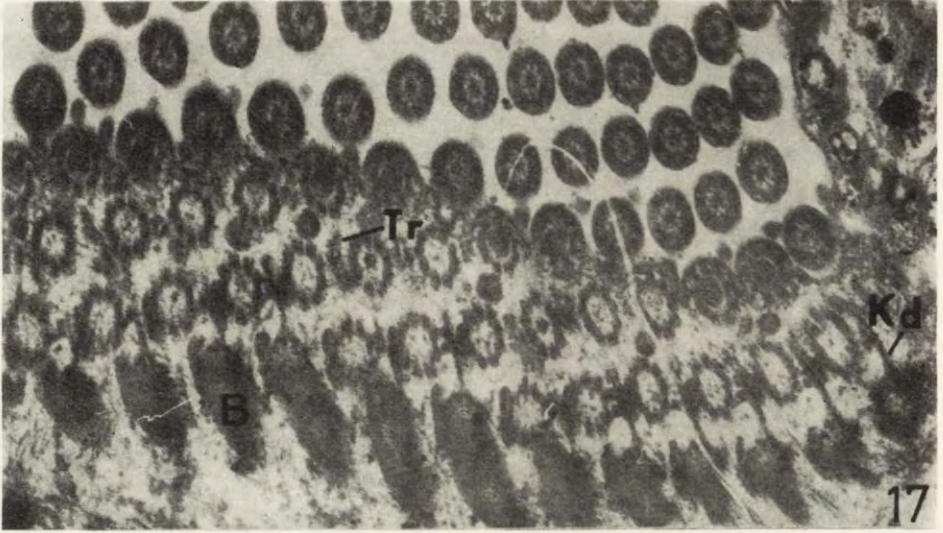
PLATE I

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PLATE II

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PLATE III

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PLATE IV

PLATE VI

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PLATE VII

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PLATE IX

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PLATE X
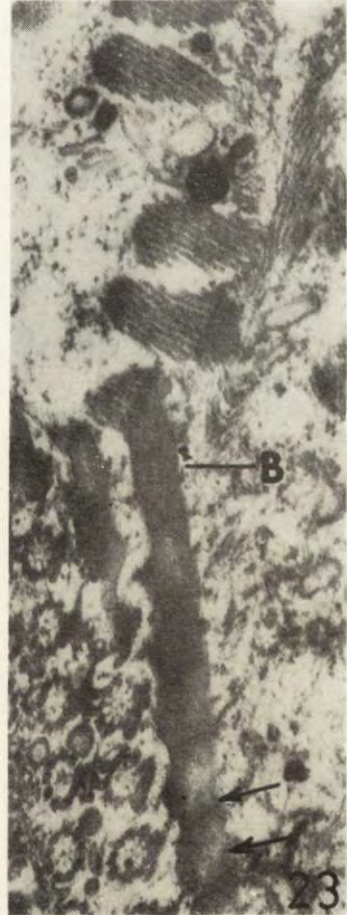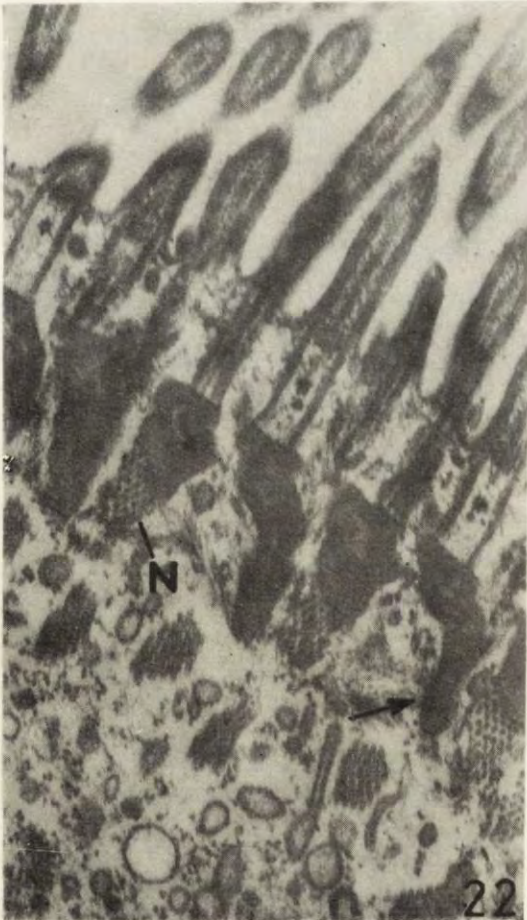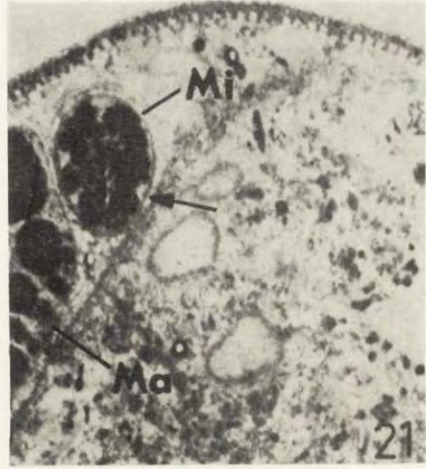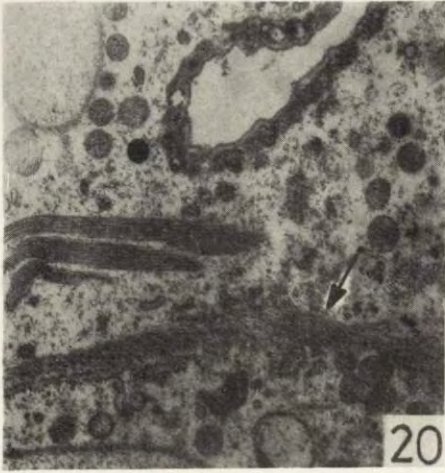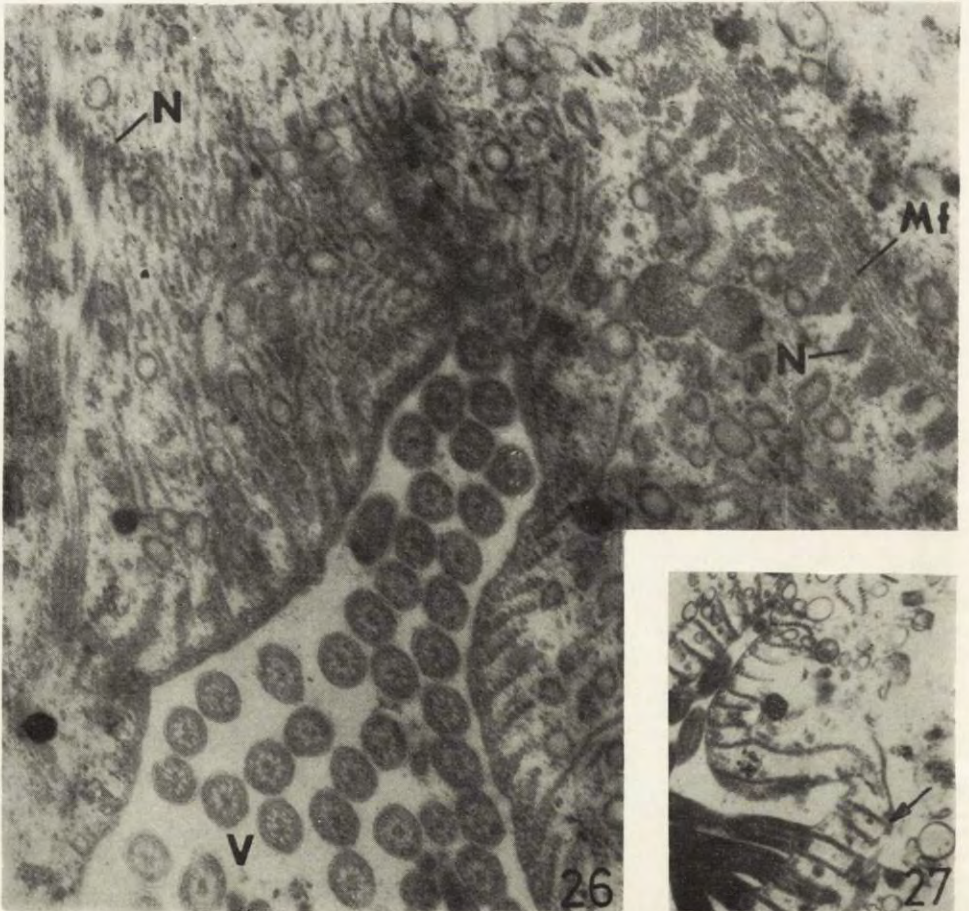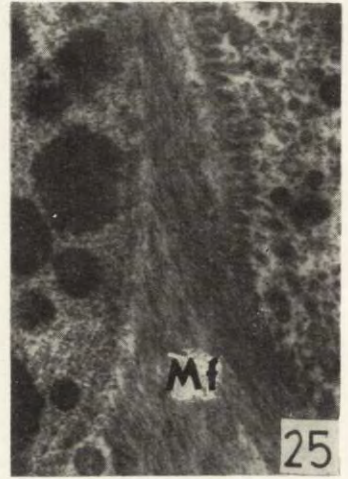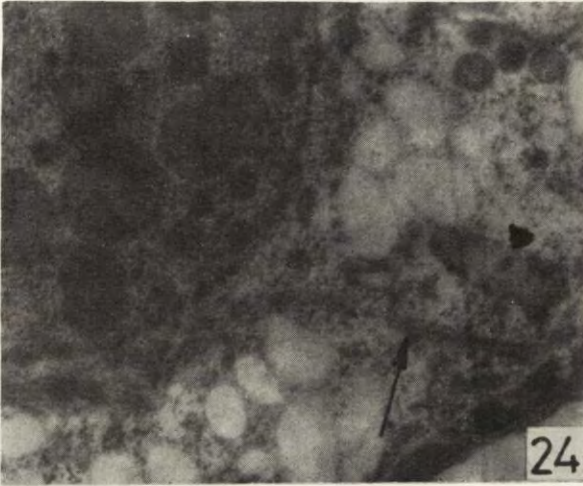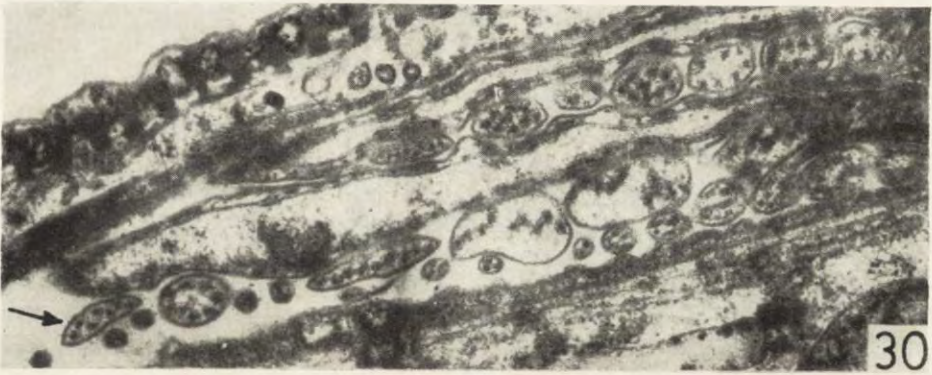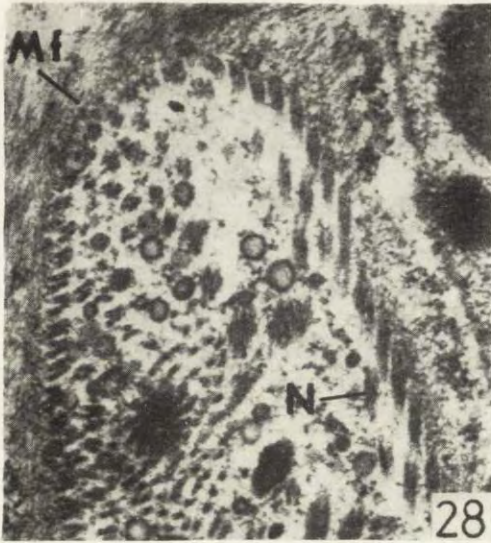
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rows of cilia are transversely cut, some of them show pronounced growth (arrow). Nematodesma (N). (33 000 ×)

23: Obligue section through a ciliary zone. Baguet (B), borders of dense substance pertaining to particular kinetosomes (arrow). (31 200 ×)

24: Section through the macronucleus. Fibrillar net surrounding the nucleus with branchings getting off towards the surface (arrow). (18 400 ×)

25: Section through the nucleus near cytopharynx. Microfibrils (Mf). (18 400 ×)

26: Section through the vestibulum (V) and the beginning of cytopharynx. Nematodesmata forming a timbering of the cytopharynx (N). Layer of microfibrils (Mf). (32 000 ×)

27: Section through the vestibulum near cytopharynx showing on the origin of microtubules forming the "rideau de tubules". The kinetosome from which the microtubules begin (arrow). (16 800 ×)

28: Section through the cytopharynx (arrow) and its timbering. Nematodesmata (N), microfibrils (Mf). (32 000 ×)

29: The same as above. (36 000 ×)

30: Transverse section through "free cilia" situated in furrows. Strongly flattened cilium with markedly disturbed pattern of microtubules (arrow). (32 000 ×)

31: The same as above. (40 000 ×)

32: *T. caudatum*. General view from the right side. (800 ×)

33: *T. caudatum*. The adoral zone. A ribbon of short kineties joining the ends of the band of syncilia (arrow). (1500 ×)

34: The same as above. (1650 ×)

35: The same as above. (1500 ×)

Anna CZAPIK

La morphogenèse chez le cilié *Diophrys oligothrix* Borror

Synopsis. L'auteur a étudié la stomatogenèse chez le cilié hypotriche *Diophrys oligothrix* en s'appuyant sur les préparations imprégnées de protéinate d'argent. Le debut de la division est signalé par l'apparition de la bande de reorganisation sur le macronoyau. Le primordium oral est formé au-dessus du premier cirre transversal. Les champs ciliaires donnent naissance à deux complets de cirres nouveaux.

Ce cilié a été trouvé parmi les autres espèces psammophiles dans le fond sablonneux d'un lac saûmatre au bord de la mer Baltique. Les ciliés transportés dans le laboratoire étaient cultivés dans l'eau saûmatre et nourris d'une façon très simple: on jetait dans les boites de Petri les enchytrés coupés en morceaux sur lesquels se développaient les bactéries. Le cilié se nourissait de bactéries ainsi que de petits ciliés (*Cyclidium*) qui apparaissaient souvent dans les cultures.

La morphologie et les processus morphogénétiques ont été étudiés sur les préparations imprégnées à protéinate d'argent selon la méthode de T u f f r a u (1967).

Resultats

Le corps du cilié mesuré sur les individus fixés a 57–80 µm de long et 34–46 de large. Le péristome est entouré de 30–35 membranelles. La parorale est double. Les cinq cirres frontales sont disposés en deux groupes: 2 et 3. Plus bas on voit deux cirres ventraux et sur le côté gauche au-dessous du péristome deux cirres postoraux. Cinq cirres transversaux très puissants forment une rangée au-dessous du péristome. Trois, rarement quatre cirres caudaux sont situés près du bout du corps sur le côté droite. Sur la surface dorsale du cilié il y a quatre

rangées de cils. Le protéinate d'argent revèle la présence de deux fibres qui sortent de deux premiers cirres transversaux et aboutissent sur le dernier cirre frontal. (Pl. I 1).

Le macronoyau est composé de deux fragments en forme de boudin. Chaque fragment est accompagné par un micronoyau. L'ensemble des caractères cités laisse supposer qu'on avait à faire avec l'espèce *Diophrys oligothrix* décrite par B o r r o r (1965). Néanmoins il faut noter, que la taille de notre cilié était plus petite et les membranelles moins nombreuses.

H i l l (1978) a publié une courte déscription de la morphogenèse corticale chez *Diophrys scutum* sans s'occuper du macronoyau et de cirres caudaux. L'étude présente ajoute certains détails.

Le debut de la division est signalé par les changements dans le macronoyau. Sur tous les deux macronoyaux la bande de reorganisation apparait. Le bout de chaque fragment prend la forme d'un capuchon. A cette stade le macronoyau ressemble à deux grégarines (Fig. 1 A,
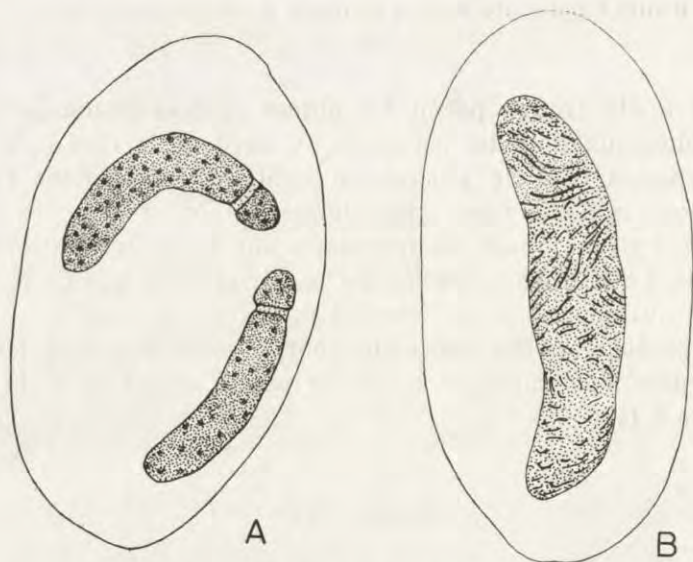
Fig. 1. *Diophrys oligothrix* Borror. Changements dans le macronoyau. A — apparition de la bande de reorganisation dans le macronoyau, B — integration du macronoyau

Pl. I 2). Au fur et à mesure que le processur morphogénétique avance, toutes les deux parties du macronoyau s'approchent et finalement se touchent. Ensuite les bandes de réorganisation disparaissent et la structure interne change: au lieu des granulations on voit une masse filamenteuse. Maintenant tous les deux fragments s'unissent en un macro-
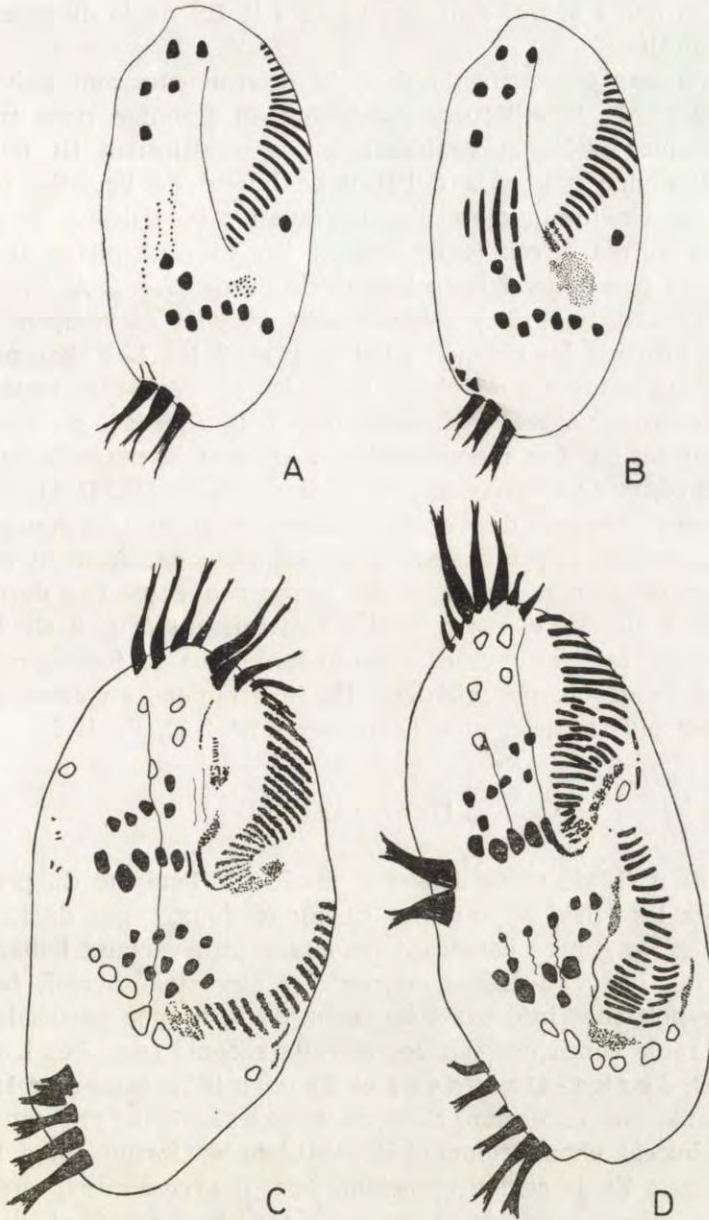
Fig. 2. *Diophrys oligothrix* Borror. Morphogénése. A — apparition du primordium buccal et des cinétosomes responsables des champs ciliaires, B — champs ciliaires. Developpment de nouveaux cirres caudaux chez l'opisthe, C — desagregation de l'AZM chez le proter. Formation de AZM chez l'opisthe, D — deux nouveaux complets de cirres ventraux. Formation des cirres caudaux chez le proter

noyau épais (Pl. I 3) qui s'allonge et vers la fin de la division se romp en deux moitiés.

Les premiers changements dans le macronoyau sont suivis par la reorganisation de la ciliature. Au-dessus du premier cirre transversal les cinétosomes isolés apparaissent; en se multipliant ils forment un champ qui donne naissance à l'ébauche buccal de l'opisthe (Fig. 2 A, Pl. I 4). Les cinétosomes apparaissent aussi à côté droite du corps au-dessus des autres cirres transversaux. En se multipliant ils forment trois rangées parallèles à l'axe longue du corps (Fig. 2 A, Pl. II 5). Ces rangées, d'abord très fines s'épaississent et puis se rompent en fragments, en formant les champs ciliaires (Fig. 2 B). Les champs ciliaires forment deux complets de cirres frontales et ventraux; cependant les vieux cirres commencent à disparaitre (Pl. II 7). AZM du proter subit une reorganisation. Les membranelles ainsi que la parorale se desagrègent et au cours de la division ils se reconstituent (Pl. II 6).

Les cirres caudaux de l'opisthe subissent aussi une réorganisation. Les cinétosomes qui apparaissent dans leur voisinage forment trois stries épaisses qui deviennent les bases des cirres nouveaux. Ces derniers sont formés avant que les cirres primitifs disparaissent (Fig. 2 C). De même chez le proter les cinétosomes apparaissent dans la future region caudale au stade de champs ciliaires. Ils se groupent en courtes rangées pour former plus tard les cirres caudaux (Fig. 2 D, Pl. II 8).

Discussion

Tuffrau (1969, 1970) dans son étude sur l'origine du primordium buccal chez les ciliés hypotriches distingue deux types de la stomato-genèse: chez les genres possedant les cirres transversaux l'ébauche buccal se forme juste au-dessus du premier cirre transversal, tandis que chez les hypotriches inferieurs à gauche de la rangée postorale des cir-res. Cette régle confirmée par les travaux recents (p.e. Jerka-Dzia-dosz 1972, Jerka-Dziadosz et Janus 1972, Borror 1979) n'est pas pourtant sans exception. Sapra et Dass (1970) ont constaté que l'ébauche buccal chez *Stylonychia pustulata* se forme entre les cirres postoraux près de la region cytostomienne. Il arrive aussi, que chez les espèces du même genre la stomatogenèse peut procéder des façons differentes: par exemple chez *Oxytricha platystoma* Grolière (1969) a observé la formation du primordium buccal à partir du premier cirre transversal tandis que chez *Oxytricha agilis* (Buitkamp 1975) ce pro-cessus ressemble celui chez *Stylonychia pustulata*. Un cas singulier re-présente la stomatogenèse chez *Onychodromus acuminatus* (Jareño

et T u f f r a u 1979) où le macronoyau participe physiquement à la neo-formation du primordium. Chez *Diophrys oligothrix* la stomatogenèse procède conformément à la régle générale du T u f f r a u.

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EXPLICATIONS DES PLANCHES I–II

1: *Diophrys oligothrix* Borror — morphologie générale
2: changements dans le macronoyau: la bande de reorganisation
3: integration du macronoyau
4: apparition du primordium buccal
5: apparition des cinétosomes responables des champs ciliaires
6: stade de champs ciliaires. Desagrégation des structures buccales chez le proter
7: deux complets des cirres nouveaux
8: reconstitution des structures buccales chez tous les deux individus
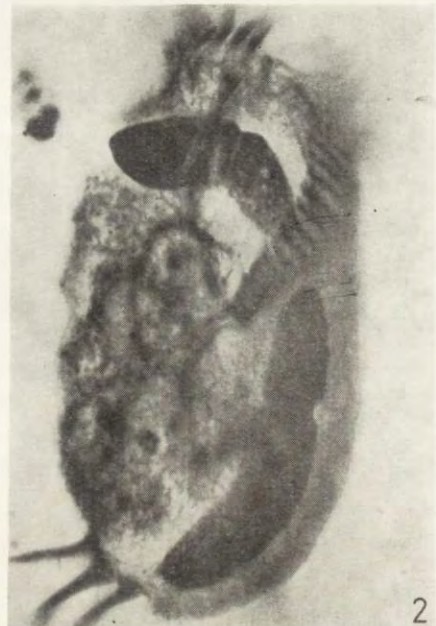
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Régéneration chez *Gastrostyla steinii*

Synopsis. Les événements corticaux qui se produissent au cours de la morphogenèse de régénération de *Gastrostyla steinii* sont décrits à l'aide du proteinate d'argent. Les phénomènes nucléaires qui accompagnent la régénération sont:
(a) Synthèse d'ADN et division micronucléaire.
(b) Fragmentation des macronoyaux sans préalable synthèse d'ADN.
Le processus de régénération est indépendant de la synthèse d'ADN.

Les travaux sur la régénération chez les ciliés Hypotrichous ont été effectués essentiellement sur l'Urostylides (Jerka-Dziadosz 1963, 1965, 1974, 1976, 1977, Jerka-Dziadosz et Frankel 1969, 1970). Aussi la régénération d'*Oxytricha* (Hashimoto 1961), *Stylonychia* (Grimes et Adler 1978, Grimes et L'Hernault 1979), *Laurentiella* (Martin et al. 1977, Torres et al. 1980) et *Euplotes* (Yow 1958, 1961) ont été etudiés.

Chez *Urostyla* (Jerka-Dziadosz et Frankel 1970) et *Laurentiella* (Torres et al. 1980) les événements corticaux de régénération s'accompagnent de fragmentation macronucléaire et division micronucléaire et la régénération est indépendante d'une synthèse d'ADN. Chez *Euplotes,* Yow (1961) a observé des bandes de réorganisation macronucléaires pendant la régénération, mais le processus n'est pas bloqué par le chloramphénicol à dose qui arrête la synthèse d'ADN.

Dans ce travail nous avons etudié les événements corticaux et nucléaires qui se produissent au cours de la régénération chez *Gastrostyla steinii*.

Matériel et méthodes

Les cultures de *Gastrostyla steinii* sont maintenues au laboratoire sur milieu de Pringsheim à 20°C, avec *Chlorogonium* sp.

L'appareil nucléaire est teinté par la coloration de Feulgen, et le dosage de

l'ADN est effectué par cytophotométrie selon les techniques déjà décrites (T o r r e s et al. 1979).

La morphologie et morphogenèse corticale ont été étudiées par l'emploie de l'imprégnation au protargol, décrite par T u f f r a u (1967).

La régénération est provoqué par traitement avec l'urée (concentration finale 3%) pendant 30 s. Après le dommage corticale les ciliés sont placés rapidement dans le milieu de Pringsheim frais pour éliminer les traces d'urée.

L'hydroxiurée a été utilisée comme inhibiteur de la synthèse d'ADN.

R e s u l t a t s

M o r p h o l o g i e   g é n é r a l e — Les ciliés de cette espèce présentent un corps rigide de forme ovale. La taille oscille entre 200–250 µm de longeur et 50–70 µm de largeur. Dans la face ventral (Pl. I 1) on peut distinguer la ZAM formée par 30–40 membranelles composées de 4 rangées de cinétosomes, au côté gauche de la zone adorale il y a deux membranes ondulantes. Le reste de la ciliature ventrale est constitué de cirres entre lesquelles on peut distinguer les groupes suivants: Frontoventraux (CFV), un groupe (8) qui se situe à la partie antérieure de l'animal et une rangée oblique qui part du versant peristomatique et arrive jusqu'aux cirres transversaux, Transversaux (CT) en numéro de 5 localisés à la partie postérieure de l'individu et qui forment un groupement pareil à une "J", Marginaux, qui entourent l'individu de gauche et de droite et confluent à la partie postérieure.

M o r p h o g é n è s e   c o r t i c a l e   d e   r é g é n é r a t i o n — Les cellules traitées avec l'urée á 3%, dans la forme que nous avons décrite dans les méthodes, perdent la partie antérieure de la ZAM, la trame antérieure des membranes ondulantes et quelques cirres frontaux (Pl. I 2).

Le dommage cortical déclenche un processus morphogénétique qui conduit en dernier ressort à la formation d'une nouvelle dotation complete d'organelles corticaux qui remplacent les préexistants.

Pour décrire la morphogenèse de régénération nous avons établis les suivants stades:

S t a d e 0. L'état dans lequel se trouvent les individus immediatement après le dommage cortical provoqué avec l'urée. La Pl. I 2 montre les caractéristiques morphologiques des individus qui se trouvent dans cette phase.

S t a d e I. Correspond à le début de la morphogenèse de régénération. Les premiers cinétosomes néoformés apparaissent en étroite relation avec les cirres transversaux I/II et I/III (Pl. I 3 et 4). Les cinétosomes ne procèdent pas des préexistants dans les anciennes plaques, sinon qu'elles se forment de "novo" dans les proximités de ces plaques sans que celle-ci démonstrent une désintégration préalable.

S t a d e II. Prolifération des cinétosomes et migration du primordium oral (PO) vers l'avant, acquérant une morphologie triangulaire (Pl. II 5). Pendant la croissance du PO ont peut observer la contribution de cinétosomes procédent de quelques plaques de la rangée ventral.

S t a d e III. Une fois que le PO a atteint les immédiations du versant péristomatique, il se détache de sa zone antérieure une rangée de cinétosomes qui se place parallelement aux membranes ondulantes et qui formera plus tard les nouvelles membranes ondulantes. D'autre part la désorganisation des cirres frontaux détermine l'apparition d'un champ de cinétosomes non ordonné (Pl. II 6 et 7).

S t a d e IV. Se caractérise par la formation de cinq rangées de cinétosomes qui proviennent de l'ordre des précédents des cirres frontaux et qui vont former le futur système frontoventrotransversal (FVT). A ce stade le PO est ordonné en membranelles dans sa partie antérieure, cet ordre a lieu de droite à gauche et depuis la partie antérieure jusqu'à la postérieure (Pl. II 8).

S t a d e V. Fragmentation transversale des cinq hilaires de primordies cirraux (PCs) donnant lieu à la formation de plaques basales des cirres ventraux, à l'éxception du cirrus buccal qui a son origine à partir de l'éxtremité antérieure d'une des membranes ondulantes (Pl. III 9). À ce stade se produit la fusion du PO au reste de l'ancienne ZAM et se forment les primordies marginaux.

S t a d e VI. Pendant ce stade a lieu l'émigration des plaques basales individualisées vers sa position définitive, en même temps que se produit la réabsorption des anciens cirres (Pl. III 10).

S t a d e VII. Fin du processus de régénération.

Pour dèterminer l'évolution des différents stades de la morphogenèse de régénération corticale nous avons effectué des préparations 0.5, 1, 1.5, 2, 2.5 et 3 h après le traitement avec l'urée. Dans les différentes préparations on a déterminé le pourcentage d'individus qui se trouvaient dans les différents stades, les Fig. 1 A–F montrent les résultats de cette étude. Après une heure il apparaît un 33⁰/₀ de cellules dans le stade I et à la troisième heure toute la population (98⁰/₀) se trouve dans le stade VII.

P h é n o m è n e s n u c l é a i r e s p e n d a n t l a r é g é n é r a t i o n. Accompagnant la morphogenèse corticale de régénération, des changements se produisent dans l'appareil nucléaire tant au nivau des macronoyaux comme dans les micronoyaux. Les phénomènes nucléaires furent suivis utilisant la tinction de Feulgen.

M a c r o n o y a u x. La Fig. 2 montre l'évolution du nombre de fragments macronucléaires 1, 1.5, 2, 2.5, 3, 3.5 et 4 h après le traitement avec l'urée, chaque point de la figure se détermine effectuant la moitié
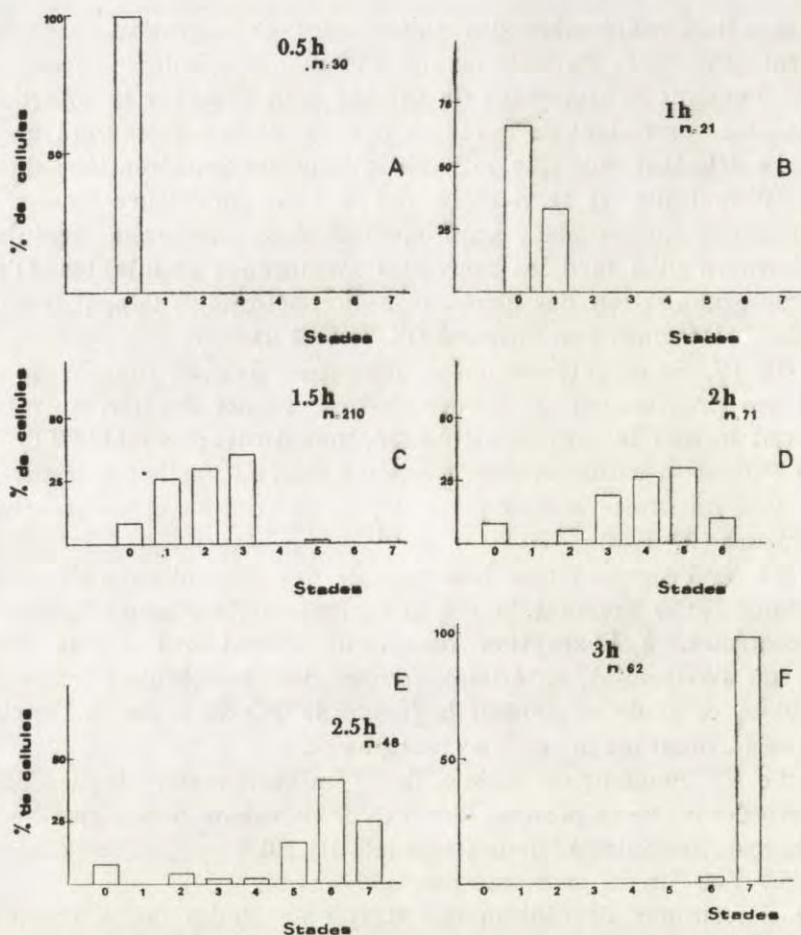
Fig. 1. Pourcentage de cellules qui se trouvent dans les différents stades de la morphogenèse de régénération 0.5 h (A), 1 h (B), 1.5 h (C), 2 h (D), 2.5 h (E) et 3 h (F) après du traitement avec l'urée (3%, 30 s)

du nombre de fragments macronucléaires présents dans 70–100 cellules.

Comme on peut l'apprécier, durant la régénération a lieu une fragmentation macronucléaire, étant donné que les cellules présentent une heure après le traitement un nombre moyen de fragments macronucléaires égal à 4.38 et après 3 h la valeur moyenne est de 7.09. Cette fragmentation macronucléaire se produit entre deux et trois heures.

M i c r o n o y a u x. Dans les préparations teintées suivant le méthode de Feulgen on peut observer que la division mitotique des micronoyaux commence approximativement deux heures après le traitement avec l'urée. Normalement la division micronucléaire est antérieure à la fragmentation macronucléaire quoique dans certaines occasions la dernière

étape de la mitose micronucléaire peut coïncider avec la fragmentation des macronoyaux.

Afin de dèterminer si la division micronucléaire est précédée de synthèse d'ADN nous avons effectué des mesures cytophotométriques sur des micronoyaux de cellules en régénération à 1, 2 et 3 h après le traitement avec l'urée.
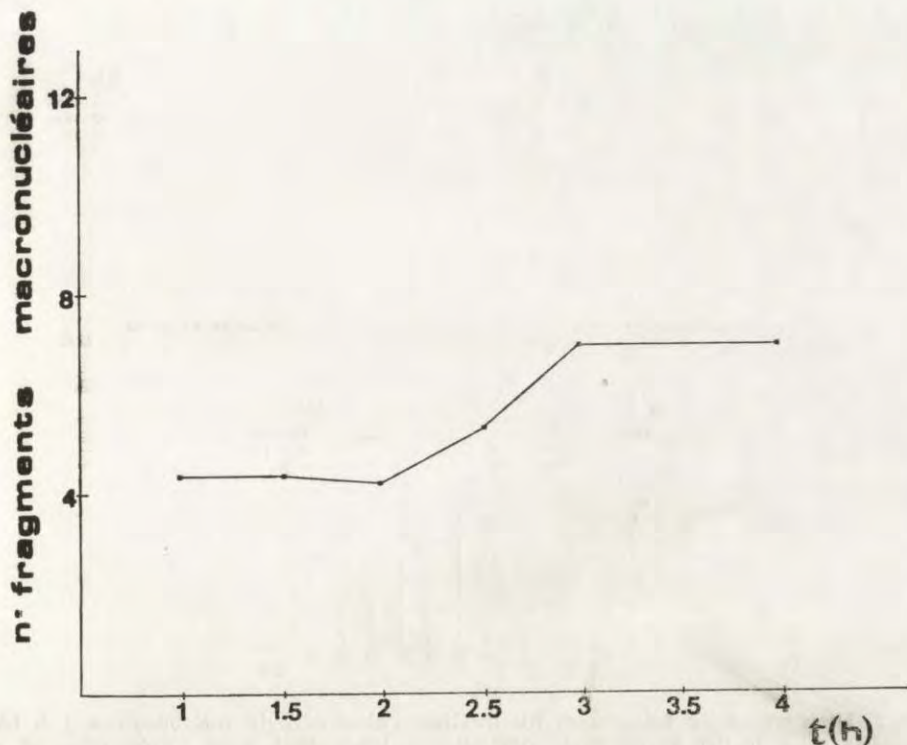
Fig. 2. Variation face au temps du nombre des fragments macronucléaires après le traitement avec l'urée. Chaque point de la figure s'est déterminé effectuant la moitié du nombre de fragments macronucléaires présents en 70–100 cellules. Observez la fragmentation macronucléaire entre 2–3 h

La Fig. 3 A montre le contenu d'ADN de 19 micronoyaux une heure après le dommage cortical. Dans la Fig. 3 B on expose la quantité d'ADN de 20 micronoyaux deux heures après le traitement avec l'urée. La Fig. 3 C montre la même étude sur 30 micronoyaux trois heures après du lésion cortical.

Nous devons faire observer qu'arrivés à trois heures les micronoyaux mesurés se trouvent en télophase ou viennent de terminer leur division,

donc la quantité d'ADN micronucléaire en ce moment peut être considérée comme 2 n.

D'autre part l'étude montre qu'entre une et deux heures s'effectue une synthèse d'ADN micronucléaire.

Inhibition de la synthèse d'ADN pendant la régénération. Pour préciser si le processus de régénération dépend ou non de la synthèse d'ADN on a effectué une étude avec l'hydroxiurée.
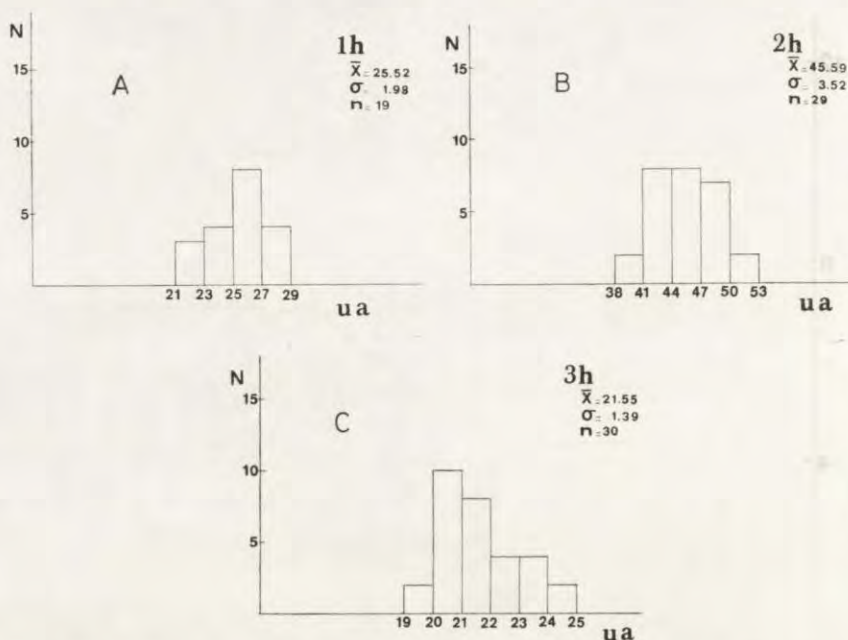
Fig. 3. Histograme de fréquences du moitiés d'absortion de micronoyaux 1 h (A), 2 h (B) et 3 h (C) après avoir effectué le traitement avec l'urée (3%, 30 s). *Abscisse*: Quantité d'ADN exprésée en unités arbitraires (ua). *Ordonnée*: Nombre de micronoyaux

Pour déterminer la concentration de l'inhibiteur à utiliser on a selectionné des cellules qui se trouvaient en G_1 et on les transféra aux cultures avec différentes concentrations d'inhibiteur (1, 5, 10, 15 et 20 mg/ml). Dans chaque concentration on a étudié l'évolution de la culture. La Fig. 4 montre les résultats de cette étude. Nous avons sélectionné la concentration de 5 mg/ml comme la plus appropiée entendu qu'elle bloque la division cellulaire et permet aux cellules de survivre pendant 56 h.

Afin d'étudier l'effet de l'HU (5 mg/ml) sur la régénération, les cellules, une fois traitées avec l'urée, on les mit en présence de l'inhibiteur

pendant tout le processus de régénération. Pour réaliser cette étude on a déterminé par la moyen de la technique du protargol le pourcentage de cellules qui se trouvent dans les différents stades de la régénération 1, 2 et 3 h après le dommage cortical et en présence du HU (5 mg/ml). Les Figs. 5 A–C montrent les résultats. Veuillez observer qu'il n'éxiste aucune différence entre les stades de la morphogenèse corticale des cellules en présence ou en absence de l'inhibiteur, pourtant l'inhibiteur n'affecte pas à la régénération corticale.
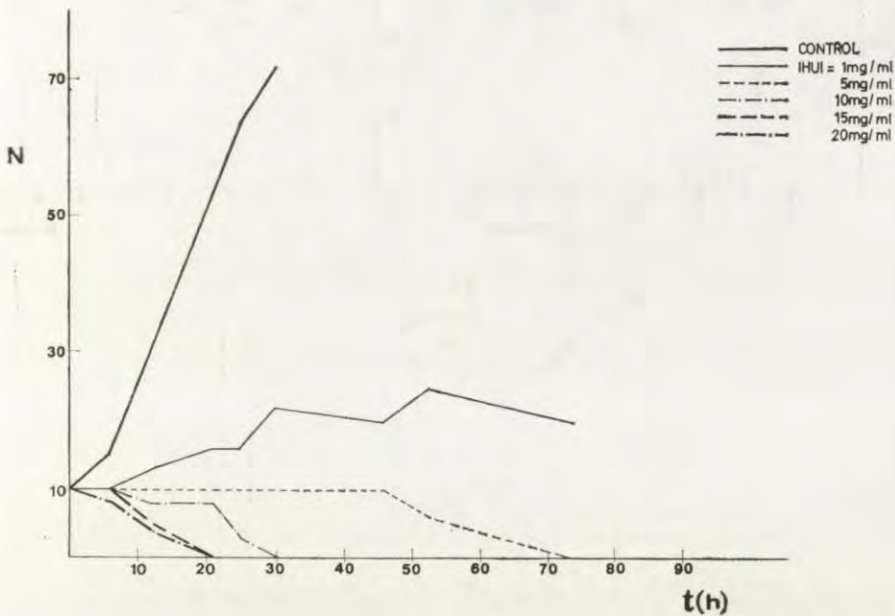
Fig. 4. Effet de la HU aux différentes concentrations (1–5, 10–15, 20 mg/ml) sur la division cellulaire. *Abscisse*: Temps écoulé après le traitement. *Ordonnée*: Nombre d'individus

Pour déterminer l'effet de l'HU sur la synthèse d'ADN micronucléaire, des préparations faites 1, 2 et 3 h après le dommage cortical et en présence d'HU furent soumises à la tinction nucléaire de Feulgen. Les Figs. 6 A–C montrent les contenus d'ADN micronucléaire 1, 2 et 3 h après le traitement respectivement.

On peut observer comme la quantité d'ADN reste pratiquement constante tout le long du temps du à l'effet inhibiteur de l'hydroxiurée qui bloque la synthèse d'ADN et la suivante mitose micronucléaire.

Ayant pour objet de vérifier si la fragmentation macronucléaire qui se fait pendant la régénération dépend ou non de la synthèse d'ADN

on a déterminé le nombre de fragments macronucléaires 1 et 3 h après le dommage cortical en présence de HU (5 mg/ml).

Les Figs. 7 A et 7 B montrent les résultats de cette étude. Il faut noter que le nombre de fragments macronucléaires arrive à être de 4.74 une heure après le traitement jusqu'à 8.23 a trois heures, d'où nous pouvons déduire que la fragmentation macronucléaire est indépendante de la synthèse d'ADN.
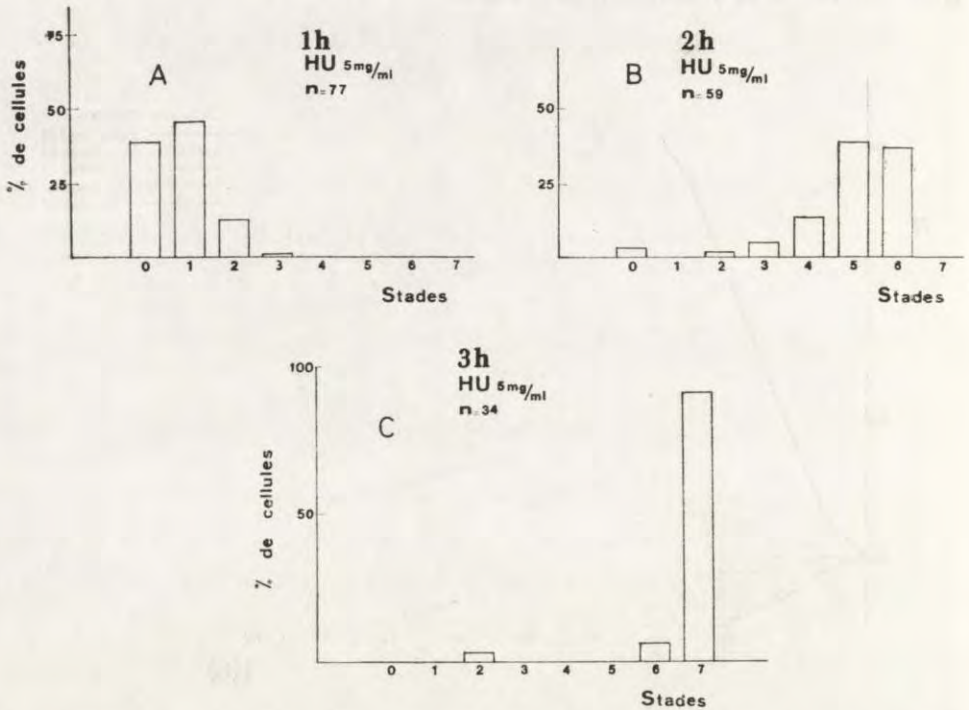
Fig. 5. Pourcentage de cellules qui se trouvent dans les différents stades de la morphogenèse de régénération 1 h (A), 2 h (B) et 3 h (C) après du traitement avec l'urée (3%, 30 s) et en présence du HU (5 mg/ml) comme inhibiteur de la synthèse d'ADN

Discussion

Les études de régénération chez les ciliés ont l'avantage de déclencher experimentalement un processus morphogénétique de reconstruction de l'appareil bucal similaire à celui qui se produit pendant la division cellulaire.

Selon De Terra (1974, 1975), dans le cas de la régénération le stimulus inducteur de la formation du primordium oral est une modifica-

tion artificielle du rapport entre la dimension du cortex bucal et du cortex somatique provoqué par l'élimination des structures orales.

Dans la morphogenèse corticale de régénération de *Gastrostyla steinii* le primordium oral se développe en relation avec les cirres transversaux I/II et I/III intervenant aussi les cinétosomes de plaques basa-
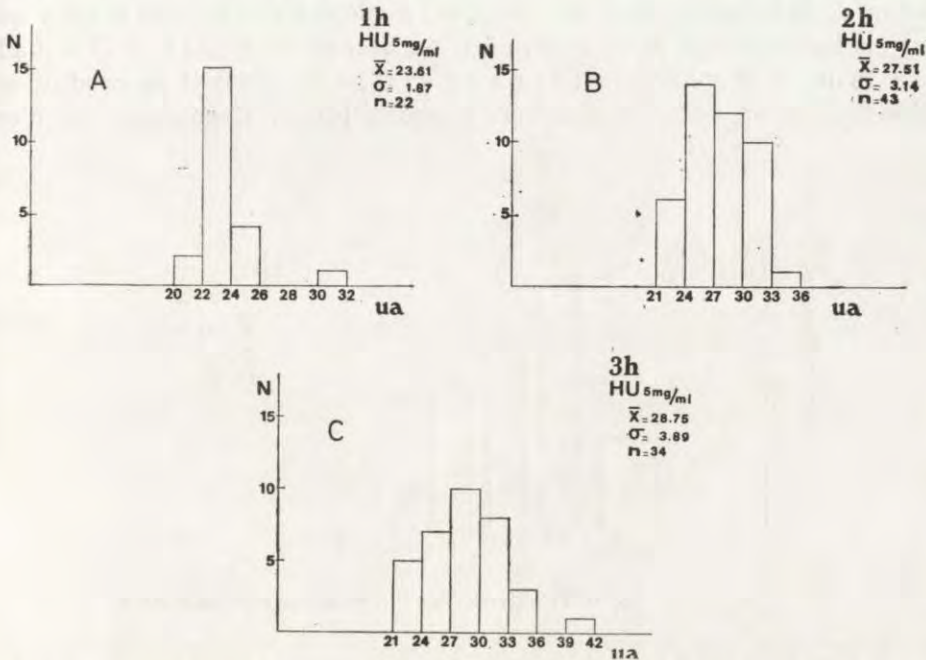
Fig. 6. Histograme de fréquences de moitiés d'absortion de micronoyaux 1 h (A) 2 h (B) et 3 h (C) après avoir effectué le traitement avec l'urée (3%, 30 s) et en présence du HU (5 mg/ml) — comme inhibiteur de la synthèse d'ADN

les de l'hilaire ventral. L'origine du système ventral est mixte, entendant que dans sa formation interviennent autant les plaques basales de l'ancienne ciliature que les cinétosomes dérivés du primordium oral.

Le cirrus bucal a son origine partant d'une des membranes ondulantes.

Dans le processus morphogénétique, qui dure trois heures nous avons distingué sept étapes sequencielles, et on a determiné tout le long du temps le pourcentage des cellules qui se trouvent dans chaque état. Les stades I (apparition des premiers cinétosomes) et VI (migration des plaques basales du système frontoventrotransversal) apparaissent comme les états de plus longue durée.

Pendant la régénération de *G. steinii* il se produit deux phénomènes nucléaires associés aux événement corticaux:

(1) Synthèse d'ADN micronucléaire et division des micronoyaux.

(2) Fragmentation macronucléaire.

Ces phénomènes nucléaires ont été aussi observés pendant la régénération des ciliés Hypotriches *Laurentiella acuminata* (T o r r e s et al. 1980) et *Urostyla weissei* (J e r k a - D z i a d o s z et F r a n k e l 1970). Dans certaines ciliés Hétèrotriches on a aussi observé pendant la régénération des changements dans l'appareil nucléaire semblables à ceux qui ont eu lieu pendant la division, ainsi en *Stentor* (P e l v a t et D e H a l - l e r 1979) et *Blepharisma* (P a r k e r et G i e s e 1966) il se produit un allongement et une rénodulation macronucléaire. Cependant, ce n'est

Fig. 7. Pourcentage de cellules avec 2, 3, 4, ... fragments macronucléaires, 1 h (A) et 3 h (B) après du traitement avec l'urée et en présence du HU (5 mg/ml) comme inhibiteur de la synthèse d'ADN

pas une situation géneral étant donné que dans *Condylostoma magnus* (B o h a t i e r 1978) il existe un comportement différent suivant l'origine de l'espéce: dans certaines souches les macronoyaux ne passent pas par de changements morphologiques, cependant que dans d'autres souches il se produit un cycle typique de condensation et d'allongement.
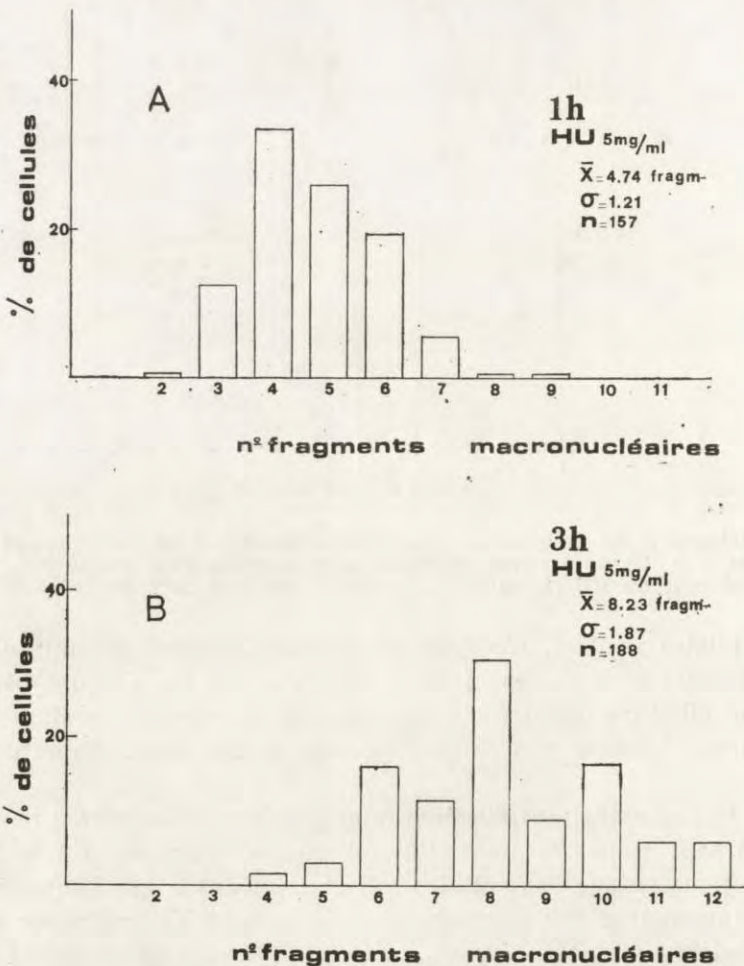
Dans le groupe des Hypotriches la synthèse d'ADN micronucléaire et la division de ces noyaux sont associées à la morphogenèse corticale, tant dans la division que dans dékystement (G u t i e r r e z et al. 1981), la régénération (J e r k a - D z i a d o s z et F r a n k e l 1970, T o r r e s et al. 1980) et la conjugaison (J e r k a - D z i a d o s z et J a n u s 1975). Ces résultats confirment l'idée (J e r k a - D z i a d o s z et F r a n k e l 1970) de ce que la morphogenèse corticale et la mitose micronucléaire constituent un bloc de phénomènes lancés par un même signe.

La fragmentation macronucléaire qui a lieu pendant la régénération survient sans duplication de l'ADN puisque les macronoyaux ne sont pas parcouris par des bandes de répliquation et experimentent une division en présence de l'inhibiteur. Par conséquent la synthèse d'ADN et la division macronucléaire sont deux processus régularisées par des systèmes de contrôle differents comme a déjà été indiqué par J e r k a - D z i a d o s z et F r a n k e l (1970).

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EXPLICATIONS DES PLANCHES I–III

1: Vue ventral de *Gastrostyla steinii* après la technique au proteinate d'argent. (270 ×)

2: *G. steinii*: Stade 0. Individu après être soumis à un traitement avec l'urée. 280 ×

3: *G. steinii:* Stade I. On aperçoit un nombre très réduit de cinétosomes (flèche) près du cirre transversal I/II. (800 ×)

4: *G. steinii*: Stade I. On aperçoit des cinétosomes relationées avec le cirre transversal I/III (flèche), pendant que le primordium oral (PO) ascend vers la part près du cirre transversal I/II. (800 ×)

5: *G. steinii*: Stade II. PO avec la morphologie triangulaire. On peut observer la incorporation au PO de cinétosomes procédents de la désintégration d'un cirre ventral (flèche) (550 ×)

6: *G. steinii:* Stade III. Une rangée de cinétosomes se détache du primordium oral (double flèche) pendant que les plaques basales des proximités se désintégrent. (300 ×)

7: *G. steinii*: Stade III. On aperçoit les primordies cirraux (PCs) qui se développent mais ne forment pas des rangées ordonnées. Le PO commence sa ordre en membranelles. (310 ×)

8: *G. steinii*: Stade IV. On aperçoit cinq rangées de primordies cirraux. On peut observer (flèche) une plaque basale ventral qui contribuit au primordium oral. (380 ×)

9: *G. steinii*: Stade V. Les premiers états de la fragmentation des primordies cirraux en plaques basales.
Les primordies marginaux (PMs) se forment et le primordium oral (flèche) se joint au reste de la zone adorale de membranelles préexistentes. 360 ×

10: *G. steinii*. Stade VI. Migration des nouvelles structures à une position définitive. (300 ×)

PLATE I

J.. J. Nieto et al.

PLATE II

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PLATE III
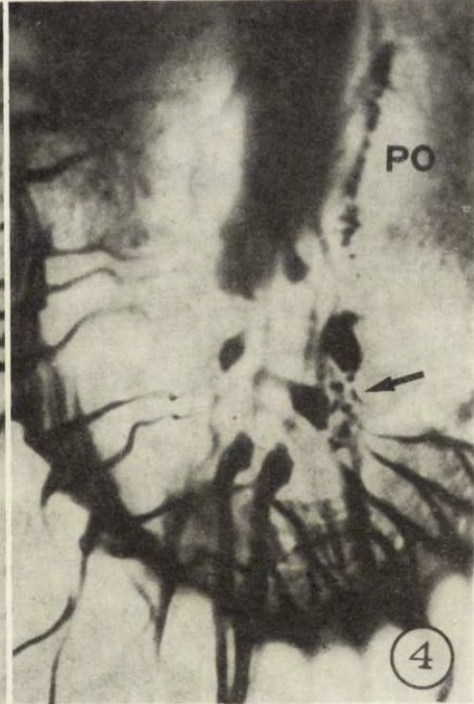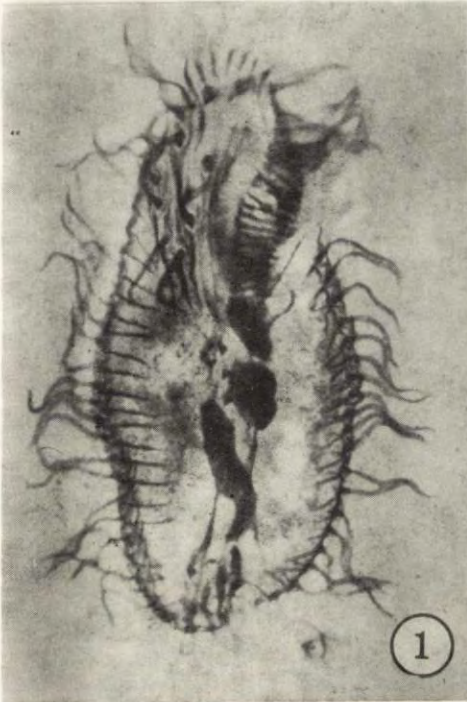
J. J. Nieto et al.

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Stanisław L. KAZUBSKI

Further Investigation on Morphological Variability of
Semitrichodina sphaeronuclea f. *macrodentata* (Lom)
(*Ciliata, Peritrichida*), a Parasite of Land Snails

Synopsis. Morphological variation and its sources have been studied in
a ciliate *Semitrichodina sphaeronuclea* f. *macrodentata* (Lom) parasit-
izing slugs of the family *Limacidae*. Statistically significant variation,
connected with host species, geographical origin and belonging of cil-
iates to particular local populations have been observed as well as the
variation among subpopulations. The last one appeared to be dominant.
It has been found that in comparison with other hitherto examined
trichodinas the variation in *S. s.* f. *macrodentata* is more compound
and diverse, being conditioned by genetic as well as by ecological
factors.

The variation in *Semitrichodina sphaeronuclea* (Lom) parasitizing the
mantle cavity of land snails has been already the object of some in-
vestigations. Great differences were noted between the ciliates occurring
in *Oxychilus (Cellarius) orientalis* (Cless.) of the family *Zonitidae* and
those parasitizing slugs, *Bielzia coerulans* (Bielz), *Limax maximus* L.
and *L. cinereoniger* Wolf of the family *Limacidae*. This was the ground
for discrimination of two forms: *Semitrichodina sphaeronuclea* f. *micro-
dentata* and *S. s.* f. *macrodentata* occurring respectively in the represen-
tatives of *Zonitidae* and *Limacidae* (K a z u b s k i 1971). The variation
of *S. s.* f. *macrodentata* from *Bielzia coerulans* in connection with the
altitude of the host habitat above the sea level has been already describ-
ed. The study, made on the material collected on slopes of the Babia
Góra Mt., has shown an increase of the body dimensions — of the ad-
hesive disc and of the number of denticles, with growing altitude above
the sea level (K a z u b s k i 1976).

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The up to date investigations made on summarized material, however, did not take into consideration the variation within and among populations. Moreover, some new material has been collected by the author from a new host, *Lehmannia marginata* Müll. from Poland and France. Renewed analysis of the former and of the new data on *S. s.* f. *macrodentata,* using the analysis of variance with respect to inter- and intrapopulational variation, has shown interesting possibilities of interpretation as well as more accurate recognition of the sources of variation in these ciliates. The analysis has been restricted only to *S. s.* f. *macrodentata.* The other form, *S. s.* f. *microdentata* parasitizing land snails of the family *Zonitidae,* due to small dimensions of the host snails is not abundant making difficult collection of representative samples of particular subpopulations from single host individuals.

Material and Methods

In this study subpopulations of *S. sphaeronuclea* f. *macrodentata* (Lom), used in former investigations (K a z u b s k i 1971, 1976) and those from *Bielzia coerulans* from the Bieszczady Mts, Beskid Śląski Mts. and Babia Góra Mt. were used as well as the new material collected from *Lehmannia marginata* from the slopes of the Babia Góra Mt. in Poland and from Besse-en-Chandesse and Les Moulins near Le Mont Doré (dep. Puy de Dome) in France (Table 1). The term "subpopulation" is applied to a group of ciliates from a single host specimen similarly as in former papers (K a z u b s k i 1979, 1980, K a z u b s k i and P i l e c k a - R a p a c z 1981).

The snails were dissected or examined alive. In the last case smears of the mucus from the mantle cavity were made or impressions of the folded out mantle on slides. This method allows to remain alive snails and to take the ciliates several times from the same host specimen.

Smears were dried and silver impregnated after the method of Klein. For the present investigation only these subpopulations of *S. s.* f. *macrodentata* were used which comprised at least 30 ciliates (with only a few exceptions) good enough to be measured. The measurements were made according to principles described in previous papers (K a z u b s k i 1979, 1980, K a z u b s k i and P i l e c k a - R a - p a c z 1981). The following features were measured: (1) body diameter, (2) diameter of the adhesive disc with the border membrane, (3) diameter of the adhesive disc without the border membrane, (4) diameter of the denticulate ring, (5) number of the denticles, and (6) length of the denticles. Moreover, for each subpopulation mean length of an arch of the denticulate ring corresponding to one denticle was counted according to the formula:

$$\frac{\text{denticulate ring diameter}}{\text{mean number of denticles}} \times \pi.$$

The variation in trichodinas was studied with the aid of statistical methods. Mainly the analysis of variance was made using two and three level nested ANOVA with unequal sample sizes (S o k a l and R h o l f 1969, Box 10.4 and 10.5),

Table 1

Mean values (M) and standard deviation (SD) of main characters in samples of 24 subpopulations and examined host, geographical and local groups of *Semitrichodina sphaeronuclea* f. *macrodentata* (*n* — sample numerosity)

Host	Locality	Altitude above sea level (m)	Date	No. of sub-population	Diameter (µm) body M	body SD	body n	adhesive disc with border membrane M	SD	n	adhesive disc M	SD	n	denticulate ring M	SD	n	No. of denticles M	SD	n	Length of denticle (µm) M	SD	n	Width of denticle (µm)
Lehmannia marginata	Babia Góra	1125	24.07.1974	8264	57.09	4.38	33	42.24	2.44	34	37.86	2.61	35	19.35	1.54	35	26.06	0.86	33	9.57	0.81	14	2.33
		1050	7.09.1979	10245	58.06	7.26	18	43.06	3.31	17	38.79	3.07	17	20.86	1.81	18	25.50	1.45	14	10.56	0.62	8	2.57
				10249	57.45	5.44	38	42.78	3.16	32	38.40	3.26	31	20.62	1.73	30	26.37	0.96	30	10.08	0.71	19	2.46
	Besse-en-Chandesse	1000	19.07.1976	8631	59.08	5.92	33	41.47	2.71	32	34.23	3.43	33	19.53	1.87	33	26.61	1.12	33	10.75	0.76	32	2.31
			27.07.1976	8733	57.89	6.03	28	41.63	3.12	28	36.34	3.16	28	19.13	1.92	28	27.46	1.35	28	10.36	0.56	28	2.19
			28.08.1976	9153	57.40	4.56	30	40.97	2.38	30	35.20	2.22	30	18.53	1.49	30	27.47	1.00	30	10.07	0.67	27	2.12
				9154	56.46	8.47	28	40.87	5.24	24	35.61	4.70	28	19.02	2.45	28	26.39	1.17	28	9.87	0.72	15	2.26
			31.08.1976	9256	55.93	5.60	29	41.26	4.17	27	36.16	3.46	29	18.98	1.73	29	26.45	0.78	29	10.11	0.72	19	2.25
				9257	57.47	5.40	30	41.20	3.16	30	35.97	2.91	30	18.42	1.83	30	26.57	1.16	30	10.34	0.57	29	2.18
	Les Moulins	1070	5.08.1976	8823	58.83	5.42	30	42.20	3.44	30	36.30	3.20	30	19.20	1.78	30	25.83	0.87	30	10.80	0.55	30	2.34
	Bieszczady	1080	28.07.1958	2757	59.36	9.89	11	46.13	3.97	15	41.54	3.36	14	23.02	3.19	22	28.94	1.31	18	10.76	1.64	19	2.50
Bielzia coerulans	Beskid Śląski	650	13.05.1960	4026	58.04	5.32	28	46.57	2.84	21	39.05	3.38	20	22.19	1.89	21	27.86	1.28	21	10.69	1.05	21	2.50
	Babia Góra	760	23.07.1974	8246	59.47	5.52	34	45.22	3.37	41	36.85	6.24	41	22.34	1.83	41	28.93	1.19	41	10.43	0.72	41	2.42
				8249	62.37	6.85	35	45.16	3.52	40	38.45	2.99	40	21.60	1.69	40	27.97	1.02	40	10.64	0.91	40	2.43
				8250	59.59	5.96	37	45.03	3.29	32	39.50	3.63	38	22.39	2.07	43	27.52	1.21	42	10.59	0.93	28	2.56
			28.07.1974	8291	69.09	6.99	34	50.09	5.39	21	44.61	4.61	36	25.24	2.66	39	29.41	1.33	39	11.00	0.71	13	2.70
				8292	61.65	6.66	40	45.26	3.48	43	39.65	2.77	43	22.04	1.71	43	28.04	1.05	46	10.83	0.63	38	2.47
		1050—1125	24.07.1974	8256	66.11	6.23	38	47.04	2.58	27	42.37	3.77	35	24.05	2.07	39	28.95	1.12	41	10.97	0.94	17	2.61
				8257	62.33	6.12	42	45.86	3.55	44	41.43	3.06	45	22.78	2.02	45	28.55	1.16	47	11.25	0.81	44	2.51
				8258	65.57	6.68	37	46.14	3.66	35	42.31	4.49	39	23.88	2.09	39	29.07	1.05	40	11.10	1.00	29	2.58
				8260	64.49	6.01	37	46.96	3.87	28	41.84	4.52	32	23.50	2.15	40	28.81	1.21	41	10.92	1.07	26	2.56
		1225—1300	27.07.1974	8278	67.46	6.29	35	50.24	4.32	37	45.45	4.11	40	25.84	2.64	42	30.41	1.52	44	11.06	0.62	25	2.67
				8279	68.29	6.55	44	49.77	5.32	26	44.39	2.95	44	25.67	2.20	47	30.30	1.35	47	10.85	0.85	17	2.66
				8280	64.41	7.74	39	48.05	4.26	44	43.48	3.76	44	24.32	2.37	44	30.20	1.53	46	11.11	0.91	44	2.53
Lehmannia marginata	Babia Góra				57.44	5.45	89	42.61	2.90	83	38.25	2.95	83	20.18	1.79	92	26.08	1.06	77	10.00	0.80	41	2.43
	Besse-en-Chandesse				57.41	6.08	178	41.24	3.46	171	35.55	3.41	178	18.94	1.91	178	26.82	1.18	178	10.31	0.71	150	2.22
	France				57.62	6.00	208	41.39	3.47	201	35.66	3.38	208	18.98	1.89	208	26.68	1.19	208	10.39	0.71	180	2.23
Bielzia coerulans	Babia Góra	760			62.53	5.60	180	45.76	4.04	177	39.70	4.90	198	22.70	2.37	206	28.36	1.34	208	10.65	0.80	160	2.51
		1050—1125			64.56	6.37	154	46.40	3.48	134	41.96	3.93	151	23.52	2.12	163	28.83	1.14	169	11.10	0.94	116	2.56
		1225—1300			66.76	7.04	118	49.22	4.62	107	44.41	3.68	128	25.28	2.48	133	30.30	1.46	137	11.05	0.79	86	2.62
	Babia Góra				64.26	7.11	452	46.85	4.26	418	41.68	4.70	477	23.65	2.54	502	29.03	1.53	514	10.89	0.87	362	2.56
Lehmannia marginata — summarized data					57.56	5.83	297	41.74	3.35	284	36.40	3.47	291	19.35	1.94	300	26.52	1.19	285	10.32	0.74	221	2.29
Bielzia coerulans — summarized data					63.79	7.25	491	46.82	4.20	454	41.57	4.64	511	23.57	2.56	545	28.98	1.53	553	10.87	0.93	402	2.56
Summarized data					61.45	7.39	788	44.86	4.61	738	39.70	4.92	280	22.07	3.10	845	28.14	1.84	838	10.67	0.90	623	2.46

with computation of expected mean squares and degrees of freedom. Probability of the error within the range 1 to 5% was interpolated in diagrams of tabular values of the function F at defined degrees of freedom. The analysis was made for three features: (1) diameter of the adhesive disc without the border membrane, (2) diameter of the denticulate ring, and (3) the number of denticles. Choice of these characters regarded as representative for trichodinas was argued by K a-z u b s k i (1979).

The variation between groups of trichodinas from both host species was studied as well as the variation among local groups and particular subpopulations within the whole material. Additionally, the trichodinas parasitizing *Lehmannia marginata* in Poland and France were compared. Moreover, the influence of the altitude above the sea level on the adhesive disc dimensions, denticulate ring diameter and the number of denticles in *S. s. f. macrodentata* was analysed again.

Results

The values of metric and meristic characters of the examined sub-populations of *S. s. f. macrodentata* are given in Table 1. The mean values of particular groups of trichodinas (host and local groups) are also given in this Table. All the values presented in this Table are highly differentiated. Especially great differences in body dimensions and in the number of denticles occur in trichodinas from different hosts. However, these trichodinas do not differ in general appearance and can

Table 2

Three-level nested ANOVA table for three examined characters of *Semitrichodina sphaeronuclea* f. *macrodentata* from different hosts and localites

Source of variation	Degree of freedom	F_0-value			Critical value		
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{0.025}$	$F_{0.01}$
Among groups from various hosts	1	8.41 s	20.59 s	11.21 s	6.61	10.01	16.26
Among local groups	5[a]	4.77 s	4.12 s	8.14 s	2.81	3.44	4.34
Among particular subpopulations	17[a]	6.69 s	7.28 s	6.54 s	1.64		2.00
Within subpopulations	n[b]−24	—	—	—			

[a] Degree of freedom computed after Sokal and Rholf, [b] diameter of adhesive disc $n = 802$, diameter of denticulate ring $n = 845$, and number of denticles $n = 836$

not be regarded as distinct species on the ground of commonly accepted criteria (see Pl. I). Some small differences occur also between the trichodinas parasitizing *Lehmannia marginata* from Poland and France and between those parasitizing *Bielzia coerulans* from habitats situated on slopes of the Babia Góra Mt. at various altitudes above the sea level.

The analysis of variance for all 24 subpopulations (Table 2) has shown highly significant differences among groups of trichodinas from various hosts. In the case of the denticulate ring diameter the value of F_0 coefficient largely overpassed the critical value at 1% risk of error, while the risk of error of rejection of the null hypothesis is 2% in the case of the number of denticles and approximate to 3.5% in the case of the adhesive disc diameter. Also the differences among local groups are high. The significance of differences between the means of the adhesive disc diameter may be concluded with the risk of error less than 1%, and between the means of the denticulate ring diameter with similar but slightly more than 1%. The differences in the number of denticles are more pronounced and the value of F_0 coefficient is greatly higher than the critical value of F at 1% risk of error. The highest differences occur, however, among particular subpopulations of examined trichodinas. In this case the value of F_0 coefficient is several times higher than the critical value of F at 1% risk of error.

In order to elucidate the influence of the geographic factor the analysis of variance was made for 10 subpopulations of ciliates from *Lehmannia marginata* originating from Poland and France. This analysis (Table 3) has shown that the means of the adhesive disc in both groups

Table 3

Two-level nested ANOVA table for three examined characters of *Semitrichodina sphaeronuclea* f. *macrodentata* from *Lehmannia marginata* from Poland and France

Source of variation	Degree of freedom	F_0-value			Critical value		
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{0.025}$	$F_{0.01}$
Among groups from various countries	1	26.33 s	10.27 s	2.31 ns	5.32	7.57	11.26
Among particular subpopulations	8[a]	1.43 ns	2.56 s	7.59 s	1.97		2.57
Within subpopulations	$n^b - 10$	—	—	—			

[a] Degree of freedom computed after Sokal and Rholf, [b] diameter of adhesive disc, $n = 291$, diameter of denticulate ring $n = 300$, and number of denticles $n = 285$

are significantly different and the value of F_0 coefficient is more than twice greater than the critical value of F at 1% risk of error. The adhesive disc diameters are also different. In this case the null hypothesis may be rejected with the risk of error only slightly more than 1%. As the number of denticles is concerned no important difference has been found between both compared groups. But, when particular subpopulations are considered, the means of the adhesive disc diameter do not show any significant difference, the differences in the denticulate ring diameter are significant at 1% risk of error, and the differences in the number of denticles are highly significant. In the last case the value of F_0 is almost three times higher than the critical value at 1% risk of error.

The analysis of variance was made also in order to verify the former hypothesis on the influence of the altitude of host habitats above the sea level on the morphology of *S. s.* f. *macrodentata* (K a z u b s k i 1976). Detailed data on the ciliates and their hosts have been given earlier (K a z u b s k i op. cit.), in the present study also the interpopulational variation is taken into account. The results (Table 4) ascertained

Table 4

Two-level nested ANOVA table for three examined characters of *Semitrichodina sphaeronuclea* f. *macrodentata* from *Bielzia coerulans* from different altitude of its habitats above see level

Source of variation	Degree of freedom	F_0-value			Critical value		
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{0.025}$	$F_{0.01}$
Among groups from various altitudes	2	5.70 s	5.60 s	13.74 s	4.26	5.71	8.02
Among particular subpopulations	9[a]	9.57 s	10.39 s	7.46 s	1.91		2.45
Within subpopulations	$n^b - 12$	—	—	—			

[a]   Degree of freedom computed after Sokal and Rholf, [b]   diameter of adhesive disc $n = 477$, diameter of denticulate ring $n = 502$, and number of denticles $n = 514$

the influence of the altitude on all the three examined parameters in *S. s. f. macrodentata*. The differences in the adhesive disc and in the denticulate ring diameters appeared to be significant at about 2.5% risk of error, while those concerning the number of denticles at the risk of error less than 1%. Simultaneously, fairly high variation among particular subpopulations were observed in the examined material. In all the three parameters the values F_0 several times overpassed the critical value at 1% risk of error.

Conclusions

The material presented in this paper shows that the sources of va-
riation in *Semitrichodina sphaeronuclea* f. *macrodentata* are diverse and
numerous. They may be enumerated as follows:

(1) Host species. The trichodinas occurring in two species of hosts,
namely in *Bielzia coerulans* and *Lehmannia marginata*, were significantly
different although they were collected in the same area, e.g., in the
wood covering the slopes of the Babia Góra Mt. near Zawoja.

(2) Geographical distribution. Some differences were noted resulting
from different geographical origin of the ciliates. The populations from
Poland and those from France, harboured by the same host species –
Lehmannia marginata, significantly differed by the adhesive disc and
the denticulate ring dimensions (greater mean dimensions were noted
in trichodinas from Poland), while the number of denticles did not show
any difference. In both cases the material was collected in mountains,
at approximatively the same altitude above the sea level and in similar
climatic conditions.

(3) Belonging to defined groups of local populations. This factor plays
an important role in the variation of *S. s.* f. *macrodentata* and the obser-
ved differences are highly significant. In this type of variation the tem-
perature characterizing the habitat in which a group of local populations
occurs is the most important factor. The variation connected with the
altitude above the sea level in *S. s.* f. *macrodentata* parasitizing *Bielzia
coerulans* on the slopes of the Babia Góra Mt. may serve as an example.
This problem has been investigated earlier (K a z u b s k i 1976) being
now reexamined with attention paid to interpopulational variability. It
has been ascertained that, independently of other factors, the body di-
mensions — the adhesive disc diameter, the denticulate ring diameter
and the number of denticles, in *S. s.* f. *macrodentata* increase with inc-
reasing altitude of the habitat above the sea level and with related
decrease of temperature.

(4) Interpopulational variation. This type of variation in *S. s.* f. *ma-
crodentata* has the greatest importance. In all examined cases the diffe-
rences were significant and the values of F_0 several times overpassed
the critical value F at 1% risk of error. This type of variation seems to
be connected with genetic differences between particular subpopulations
and the degree of isolation among them (K a z u b s k i 1979, 1980).

Interesting results gives also the comparison of variation in *S. s.* f.
macrodentata with the variation in other species of trichodinas exami-
ned in the same manner. Such studies have been hitherto made on *Tri-
chodina vesicularum* Fauré-Fremiet, *T. faurefremieti* Kazubski and

T. ranae de Cunha parasitizing the urinary vesicle of amphibians (K a-z u b s k i 1979, 1980) and on *T. nigra* Lom from the gills of *Lucioperca lucioperca* (L). (K a z u b s k i and P i l e c k a - R a p a c z 1981). It has been found that the interpopulational variation is dominant in the three species from amphibians while in the last one this kind of varia-tion, although statistically significant, gives way to other types of varia-tion. The variation in *S. s. f. macrodentata* as described in the present paper, has rather indirect and more compound character.

The interpopulational variation, being of the genetic origin, is domi-nant in this species of trichodina. It is however, less pronounced than in the species parasitizing the urinary vesicle of amphibians but slightly more than in *T. nigra* from fish gills. From the other hand, high varia-tion connected with other factors, such as host species, geographical distribution and other local conditions, has been also observed. At the ground of this variation lays probably isolation of particular groups of subpopulations, resulting from their occurrence in distant territories, or inhabiting hosts living in various habitats, as well as from other local conditions of outer environment, such as temperature of the habitat.

The character of variation in *S. s. f. macrodentata* seems to be con-nected also with the mode of life of these ciliates inhabiting the mantle cavity of slugs of the family *Limacidae*. They form fairly small subpopu-lations, up to some hundreds of ciliates. A part of them occurs at the margin of the mantle so their exchange during the contacts of host snails (during copulation or in other circumstances) seems to be not so difficult. Such contacts may be fairly frequent as the slugs occur numerously in their proper habitats. From the other hand their biotopes are restricted to small territories and the mobility of slugs is rather low. These are the reasons for some morphological uniformity in populations occurring in delimited areas and, simultaneously, for the maintenance of differen-ces among particular groups of local populations, exposed to the action of various factors, among others the factors of the outer environment. Thus, in variation of *S. s. f. macrodentata*, beside the genetic ones, the ecological conditions play an important role.

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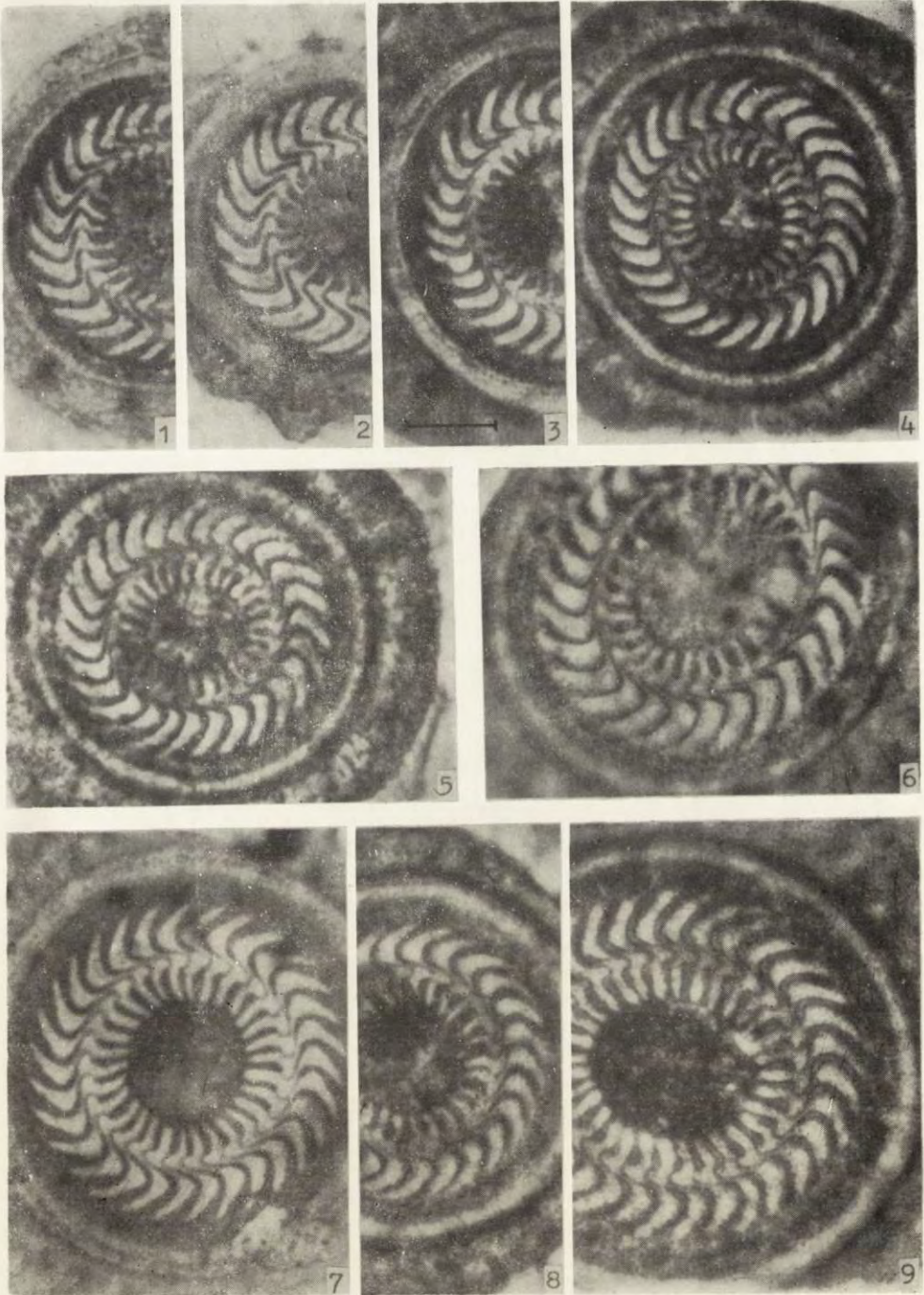
EXPLANATION OF PLATE I

Semitrichodina sphaeronuclea f. *macrodentata*

1—6 specimens from *Lehmannia marginata*, 1—2 from Babia Góra Mt., Poland, and 3–6 from Besse-en-Chandesse, France

7–9 specimens from *Bielzia coerulans* from Babia Góra Mt.

Note the specimen in phot. 9 with great number of denticles from the highest locality on Babia Góra Mt.

Scale-bar in phot. 3 is 10 μm long

S. L. Kazubski   auctor phot.

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The Ethogram of *Oxytricha bifaria* Stokes (*Ciliata, Hypotrichida*)
I. The Motile Behaviour [1]

Synopsis. The motile behaviour of single and paired *Oxytricha bifaria* was studied by the time-exposure-dark-field-technique (D r y l 1958) and by the video-tape recording. (1) Single animals. (a) Creeping. The oxytrichas move along an irregular series of geometric arcs ($\chi^2 < 10^{-3}$, radius $= 800 \pm 450$ μm), bent to the left, at a mean speed of 500 ± 290 μm/s. Radiuses and speeds are correlated positively ($n = 25$, $r = 0.87$). (b). Side Stepping Reaction (SSR). Four stages can be recognized: sudden stop, backward motion (100 ± 45 μm), clockwise rotation ($63 \pm 12°$), forward motion. (c) Swimming. The swimming pathway, lefthand and helicoidal (pitch $= 220 \pm 75$ μm, radius $= 40 \pm 20$ μm) results into a generally straight trajectory, the speed is of 750 ± 300 μm/s. (d) Stop and Reorientation Reaction (SSR). After a short stop, the oxytrichas resume swimming in a new direction, invariably forming obtuse angles of $120 \pm 20°$, with the previous direction. (2) Paired animals. (a) Creeping. Radiuses of arcs (300 ± 180 μm) and speeds (820 ± 570 μm/s) are not correlated. (b) Side Stepping Reaction (SSR). It is quite the same as for the single animals. (c) Swimming. The trajectory is actually straight and the pair keeps turning to the left, around its own longitudinal axis (pitch $= 470 \pm 70$ μm, radius $= 0$ μm), at a mean speed of 760 ± 100 μm/s. (d) Stop and Reorientation Reaction (SRR). It is quite the same as for single animals

The studies previously conducted on the reproduction biology of *Oxytricha bifaria* Stokes led us to consider the mating behaviour of this ciliate several times (R i c c i et al. 1975 a, b, E s p o s i t o et al. 1976, R i c c i et al. 1980 a). To gain new insight into this aspect of the *Oxytricha*'s biology, it was planned an exhaustive study of its entire behaviour, in the line of the "action systems" suggested by J e n n i n g s

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(1906). A precise catalogue of all the behavioural patterns of *O. bifaria*, namely its "ethogram", according to the definition of E i b l - E i b e s- f e l d t (1970), resulted from these investigations and it constitutes the basis necessary for any further study of the behaviour of this hypotrich (R i c c i 1981 b).

Our goal was not only to recognize and to describe as many beha- vioural patterns as possible, but also to conduct the quantitative analysis of the patterns themselves, in an attempt of describing them by simple quantitative parameters. Although this kind of abstraction could intro- duce some inexactnesses in the description of a certain behaviour (cfr. H i n d e 1966), three major advantages substantiated our attempt: (a) the possibility of quantitative comparisons between ethograms of different species and/or strains; (b) the possibility of choosing the most appro- priate behavioural parameter to judge the effect (s) of possible experi- mental treatments; (c) the possibility of appreciating quantitatively the effectiveness of experimental treatments. Finally, our attempt of quan- tifying the behaviour of *O. bifaria* was somehow suggested and some- what justified by the observation that no ethogram had been ever drawn for any ciliate, although the behaviour of Protozoa had been already studied very extensively, from the most different points of view (for comprehensive reviews, see J a h n and B o v e e (1967), various Au- thors at the "Symposium on Motile Systems of Cells" (1971). G r e l l (1973), K u n g (1973), D r y l (1973, 1974), M a c h e m e r (1974), S l e i g h (1974), K u n g (1976), various Authors at the Symposium on "Cell Motility" (1978)).

Material and Methods

Oxytricha bifaria was cultured according to the techniques recently standardized (cfr. R i c c i et al. 1981 b), and a particular attention was paid to the temperature, which always ranged around $22° \pm 1°C$.

To record the tracks of cells it was used the D r y l 's (1958) time exposure dark field microphotographic technique, slightly modified to match our purposes and experimental tools. A Leitz Orthoplan photomicroscope was routinely used, together with its dark field condenser. The times of exposure ranged from 5–7 to 20–30 s. Animals were put between a slide and a coverslip, by a micropipette: it was observed that their number per surface unit was very important to avoid not only rare observations, but also intense cell interactions and track over- lapping: under our experimental conditions, the most appropriate cell density ranged around 500 cells/cm². Once the rolls were processed and printed, the photographs were carefully numbered, and the rolls themselves as well. Each particular point or behavioural pattern is identified by four figures: the first

individuates the roll and the second the single frame, while the third refers to the track and the fourth to the single point progressively noticed along it.

To study tridimentional patterns, the swimming, for instance, it was used the same Leitz Orthoplan photomicroscope, coupled with a T. V. camera Philips LDH 4310/00, with a phototube Vidicon, connected to a videotape recorder Philips FI-3402A/00, kindly lent by the Institute for the Elaboration of Information of C.N.R. (Pisa). The cells were put either between a slide and a coverslip, or simply into a depression of a three spots depression slide, kept in a Petri dish, as a moist chamber, to avoid any evaporation of the culture fluid. To analyze the single tracks, a triacetate sheet (Schwan-Stabilo, 7208) was put onto the T. V. monitor and the tape was scored frame by frame. The positions of a cell were registered every five frames on the sheet, to have a good estimate of the variations of the instantaneous velocities. The times requested to move along a certain track were measured three times and the mean value was used to calculate the velocity of that animal. If it is self-evident that the study of tracks by the video-tape recordings gives a very exact idea of a certain behaviour and that it let us measure with a great accuracy the various parameters, it is also true that the number of tracks scored by this technique is always far smaller than by the analysis of microphotographs.

Statistical analyses of different data were made by a Hewelett Packard HP 27 and by a Hewelett Packard HP 42: the latter, in particular, was programmed to analyze the fitting of the tracks into geometric arcs of circumferences, by means of the χ^2 test.

Results

1. Single Animals.   a. The Creeping

This aspect of the ethogram is treated the first, because *O. bifaria,* a thigmotactic ciliate as all the Hypotrichs are, far prefers to move on the substrate than to swim around through the medium. Photographs with the tracks of a population show a very intriguing pattern (Fig. 1), could it be possible to recognize and to describe different and simple behavioural patterns, which, once freely and randomly combined with each other, could account for what, at a first glance, cannot but puzzle the investigator?

As clearly shown in Fig. 2, the animals always creep along curves, which actually are geometric, perfect arcs of circles. Only very rarely, roughly less than 0.1% of observations, and very briefly, about 500 µm, they move forward straightly. Moreover it was shown that the animals creep unfailingly leftwards, or, in other words, that the centres of the arcs always lie on the left of the track. The mathematical analysis of 12 points lying along the outer border of a track and of 12 more points, lying along the inner border of the same track, demonstrated that (a) the trajectories are perfect arcs ($\chi^2 < 10^{-3}$; $p < 10^{-3}$), (b) the experiment-

al error in evaluating the radius of a certain arc by compasses is less than $\pm 1\%$ of the actual value, (c) the lengths of radiuses are normally distributed around $\bar{x} = 800$ µm ± 450 µm, $n = 220$. It was also found that, if it is true that a certain cell can move along arcs with all the
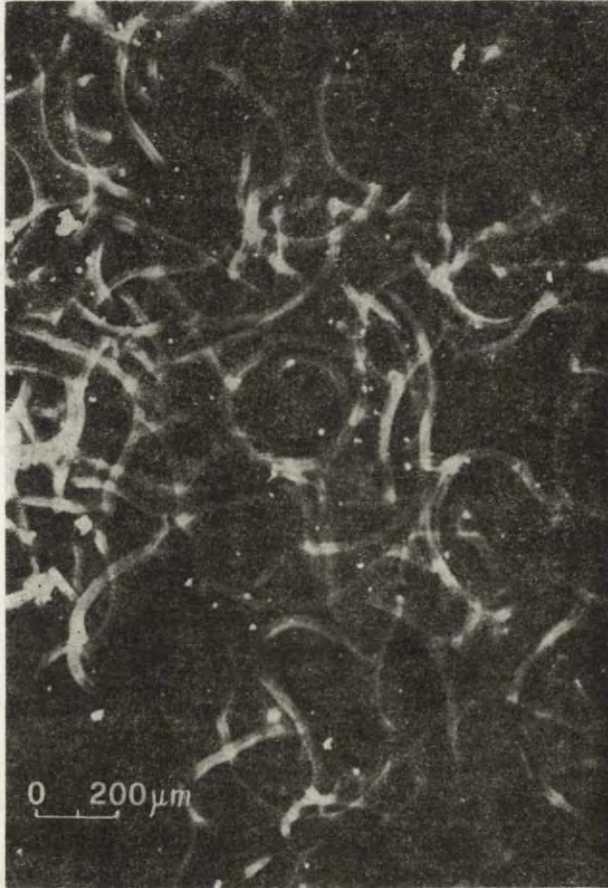

Fig. 1. The intriguing pattern of tracks of creeping *Oxytricha bifaria*. The absence of any straight path is apparent. The length of 200 µm is indicated in the lower left corner

possible radiuses according to the environmental conditions, it is also true that the same animal "prefers" for its trajectories a certain range of radiuses, usually rather limited. No correlation has been found between the radiuses used by a cell and its own length. Such a finding seems to exclude that the degree of curvature of a trajectory might be determined by the dimensions of the animal moving along it.

Then, the creeping velocity was studied both by microphotographs and by T.V. recordings. In the first case the velocity was calculated with
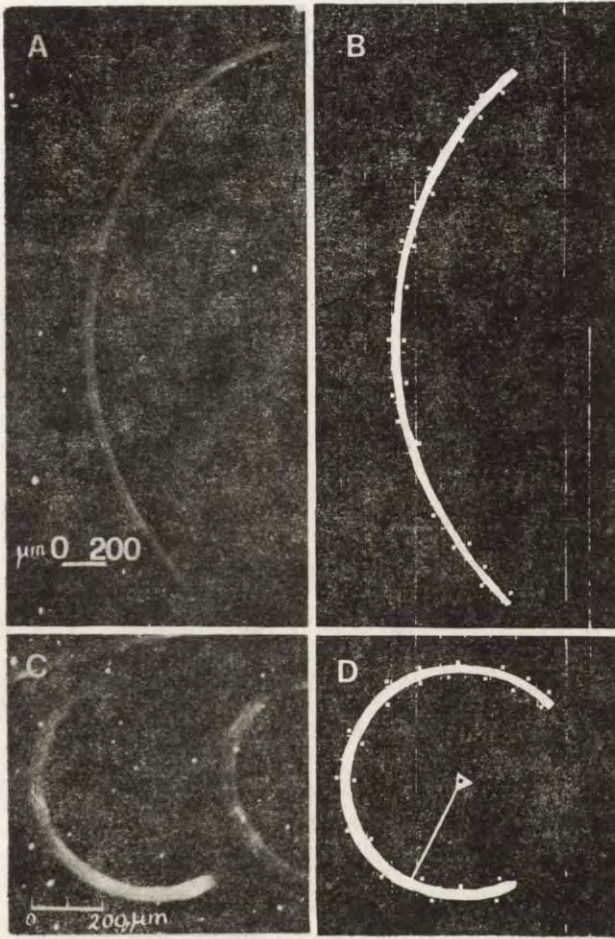
Fig. 2. *O. bifaria* creeps along geometric arcs, two examples of undisturbed tracks. In (A) a long arc, about 2500 μm, with a large radius, about 1500 μm, is shown and in (B) its schematic representation is given, together with the points by which the geometric exactness of the curve was studied. (C) represents an arc about 1600 μm long with a rather small radius, about 300 μm, the centre and the radius of this track are shown in (D), as well as the points used to study the arc. In (A) and in (C) the length of 200 μm is given

the length of the tracks and the relative times of exposure. By T.V. recordings, the velocity was calculated similarly with the length of a certain track, measured on the T.V. monitor, and the average time necessary to move along it. The two pools of data, similar to each other, were normally distributed, with a mean value of 500 ±290 μm/s, $n = 250$. By recording the variations of the instantaneous velocities in time, it was shown that the oxytrichas usually move at a certain speed, roughly constant for a reasonable time lag: however, they can change

dramatically their velocity, almost instantaneously, thus revealing a capability of expressing enormous accelerations, either positive or negative.

In some cases it has been possible to measure both the radius of a curve and the speed of the cell moving along it. It was thus demonstrated that the radiuses and the relative velocities are correlated posi-
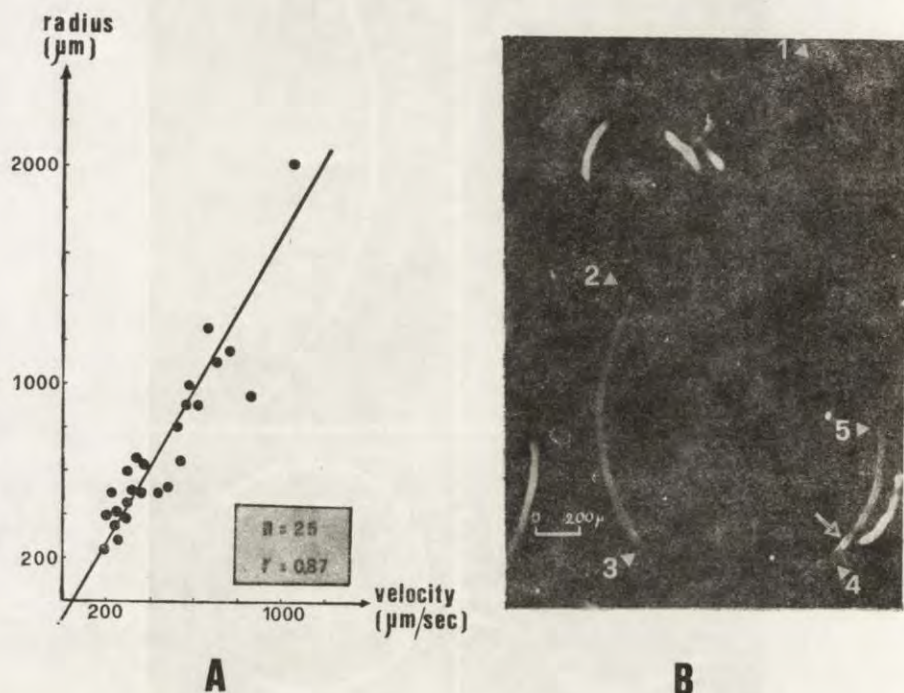

Fig. 3. (A) The graph shows the linear correlation ($p < 10^{-2}$) between the velocities (μm/s) of animals creeping along arcs and the radiuses (μm) of the arcs themselves. (B) Microphotograph of a cell slowing down, the intervals between two successive numbers, along the trajectory, show the motion of the cell in two seconds; this kind of exposure was obtained reducing the intensity of light every two seconds, for two seconds. It is evident that the shorter the arcs, the smaller the radiuses. The arrow indicates a slight Smooth Trajectory Change (cfr. Fig. 6 B)

tively, as shown in Fig. 3. The microphotograph on the right of the Figure, almost unique, represents the track of an oxytricha slowing down almost continuously, the smaller the velocity, the shorter the radius.

Side-stepping Reaction (SSR)

The animals creeping on the substrate periodically show spontaneous motile reactions, which result into changes of the direction and/or avoidances of possible obstacles. Although this kind of reaction in Parame-

cium had been celled "Avoiding Reaction" by J e n n i n g s (1906), we shall refer to it as the "Side-Stepping Reaction", SSR: it is evident beyond any doubt, infact, that only very rarely the SSR is performed by *O. bifaria* to avoid machanical and/or chemical stimuli, while, on the contrary, it is performed spontaneously and very frequently. The SSR, bidimensional and perfectly stereotyped, easily can be studied by mi-
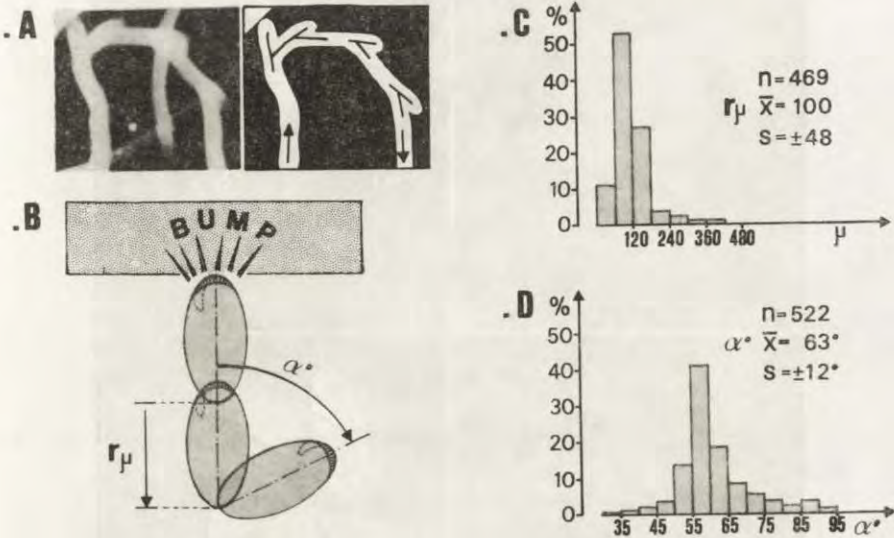

Fig. 4. The Side-Stepping Reaction (SSR). (A) This example shows that *O. bifaria* can avoid possible obstacles (upper, left corner) by a series of several SSRs. (B) Schematic drawing of the SSR. Although, for the sake of evidence, the bumping of an animal against an obstacle has been represented, it must be clear that the SSR periodically and spontaneously occurs in time, even under perfectly constant conditions. The normal distribution of the values of the lengths of backward motions (C) and of the angles of the clockwise rotation (D) are also given, together with the most significant statistical parameters

crophotography, as shown in Fig. 4 A. As indicated in Fig 4 B, an animal, while creeping, suddenly stops and moves backwards for 100 μm (Fig. 4 C), then it rotates clockwise for about 63° (Fig. 4 D) and finally it resumes creeping forward in the new direction. By the way, one can notice that J e n n i n g s, in Fig. 84 of his masterpiece of 1906, describes a series of SSR for *Oxytricha* sp. the mean value for the backward motion is of 220 μm and for the clockwise rotation is of 65°.

The same cell can perform successive SSRs undefinitely in the time, either spontaneously or induced by repeated stimuli, it was shown that neither the length of the backward motion nor the angle of the clockwise rotation are affected in any way. When many SSRs are performed

in a relatively short time lag, two different patterns can be recognized: in the "Radial Reaction" (Fig. 5, upper part) the animal keeps jerking around the same point, while in the "Tangential Reaction" (Fig. 5, lower part) the centre of rotation moves forward of about 40 μm after each jerk, along the perimeter of a sub-exagonal polygon.
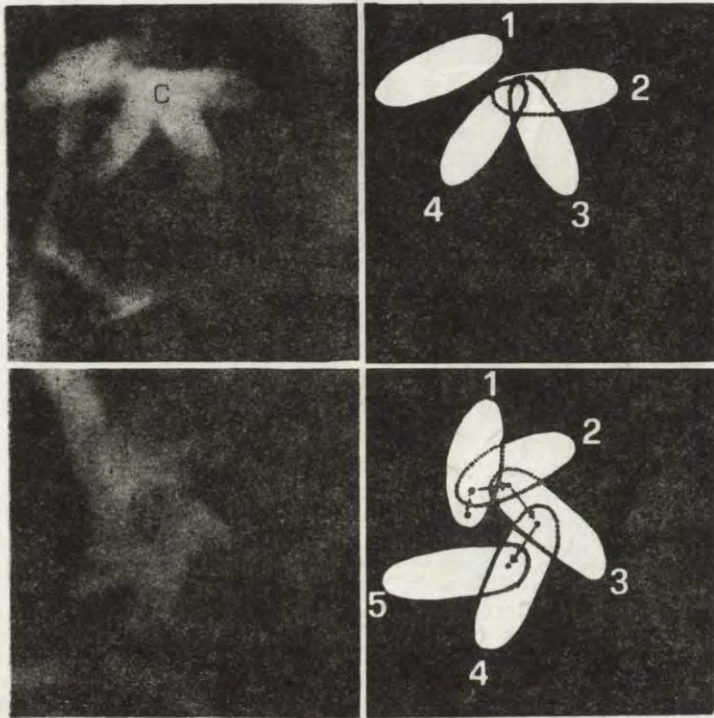

Fig. 5. Upper part. The "Radial Reaction": an *Oxytricha* jerks to several positions keeping constant in the space of the centre, C, of its rotation; on the right the interpretation of the photograph is shown. Lower part. The "Tangential Reaction": *Oxytricha* jerks to new positions, moving forward the centre of rotation of a short space, about 40 μm, as it is more clearly indicated in the interpretation on the right

The careful study of hundreds of tracks, led us to conclude that *O. bifaria* changes its creeping trajectory by means of four major reactions, shown in Fig. 6. (1). Continuous Trajectory Change (CTC), the radius and the centre of the curve, as well as the speed, undergo only little and progressive changes, so that the trajectory itself is entirely bent to the left (Fig. 6A). (2). Smooth Trajectory Change (STC). This kind of correction is obtained by an appreciable and short-lasting reduction of speed, resulting into a significant change of the direction, corresponding to the'

part of the trajectory bent to the right (Fig. 6 B). (3). Rough Trajectory Change (RTC), the animal stops creeping for a moment, during which it changes the direction of the movement: at this level the track shows a corner between two successive arcs, clearly lying in different directions (Fig. 6 C). (4). Wild Trajectory Change (WTC) two successive
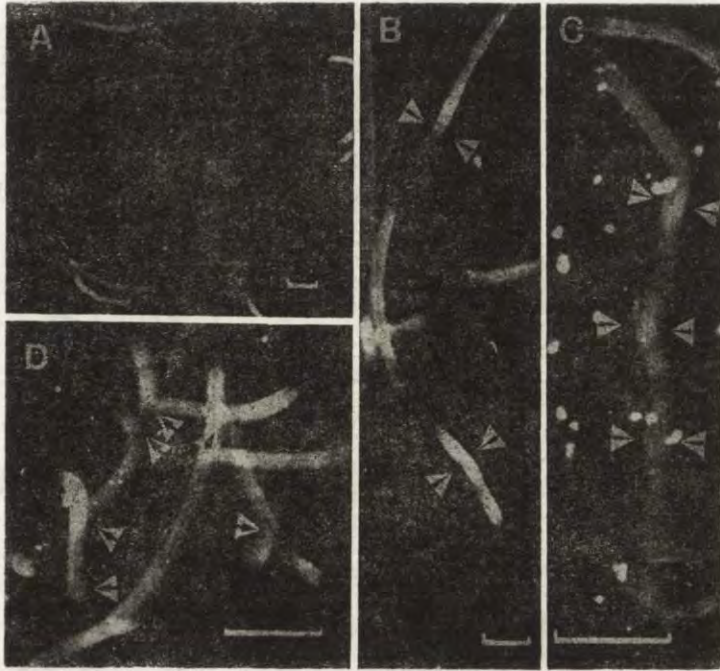

Fig. 6. The four major kinds of trajectory modulation. (A) Continuous Trajectory Change (CTC). (B) Two Smooth Trajectory Changes (STC) are indicated by arrows. (C) Three Rough Trajectory Changes (RTC) are shown by arrows. (D) Wild Trajectory Change (WTC). This example lends itself to show the efficacy of the SSR to change the general direction of an animal, while creeping. In each photograph, the length of 200 μm is indicated

arcs are separated by a SSR, so that each change of direction is of about 63° on the right and the animal can invert the general direction of its movement simply by three WTCs.

Looking again at Fig. 1, it is now possible to read and to interpret the single creeping patterns and a conclusion can be drawn, that an *O. bifaria* moves on the substrate changing continuously not only the radius and the centre of its pathway, but also the general direction of its movement, combining freely and randomly the four mechanisms above decribed.

1. Single Animals.   b. The Swimming

This sort of behaviour is somewhat disregarded by *O. bifaria,* being shown by less than 20% of the cells studied in cultures and, very likely, by a far smaller percentage of animals in their natural environment.

The study of the quantitative parameters of the swimming was to some extent more difficult than for the creeping, mainly due to its tridimensional pattern. Careful and repeated scoring of T.V. recordings showed that the swimming pattern of *O. bifaria* can be generally described as helicoidal. More precisely, the animals swim forward turning to their left, around a straight axis, with their ventral surfaces at a certain distance from it and keeping the anterior end a little outer than the posterior (Fig. 7 A, B, C). The leftward swimming can be easily recog-
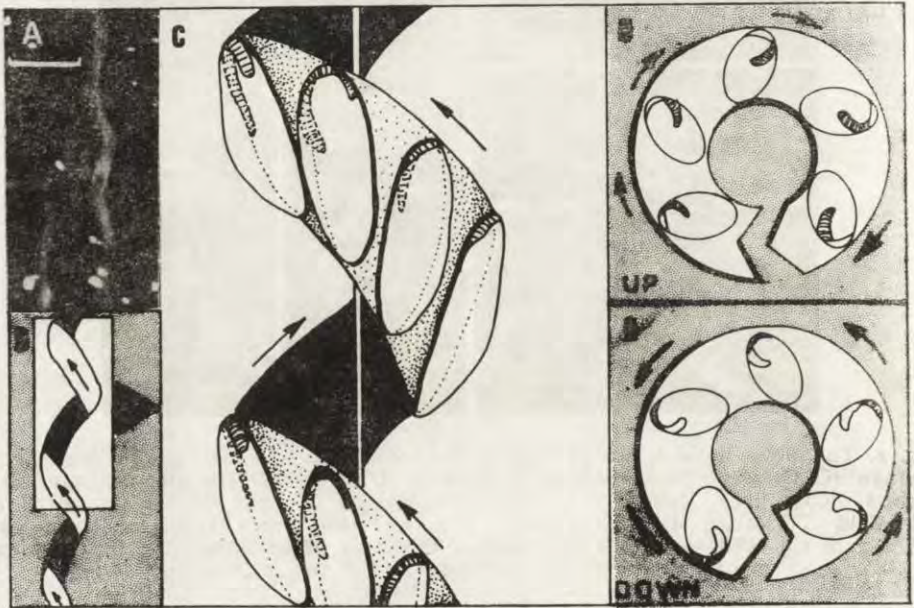

Fig. 7. The swimming of *O. bifaria.* (A). A microphotograph of a swimming animal (200 μm have been indicated) and (B) its schematic explanation. (C) The drawing shows how animals keep turning around a straight axis, with their anterior ends a little outer than the posterior. Diagrams of the upward (D) and of the downward (E) swimming of an oxytricha, as seen from above, are also shown

nized also observing living specimens at a low magnification, 10–20 x, an animal swimming upwards appears as turning clockwise, while an animal swimming downward apparently turns counterclockwise (Fig. 7 D, E). The average distance between two corresponding points of the trajectory, namely the 'pitch' of the movement, is of about 220 ± 75 μm,

and the mean distance of the ventral surface of an animal from the central axis, namely the radius of the movement, is of 40 ± 20 µm. Moreover, we can say that the larger is the radius, the lower is the velocity of that oxytricha. The velocity of *O. bifaria* tends to be more constant in swimming than in creeping, probably due to lack of any influence of the substrate: the velocity is of about 750 ± 300 µm, but it must be recalled that the actual speed along the helicoid is far higher than that.

The swimming trajectory is periodically interrupted by spontaneous Stop-and-Reorientation Reactions (SRR), shown in Fig. 8 A. Scoring T.V. records at low speed, it was found that the animals stop swimming for a while and that, when they resume swimming, they move in a new direction, unfailingly lying at about 120° from the previous one, or, in other words, that the swimming direction can be changed of 60° degrees in any possible direction.

Finally it is interesting to point out that both creeping and swimming are leftward movements and that such a homogeneity is probably
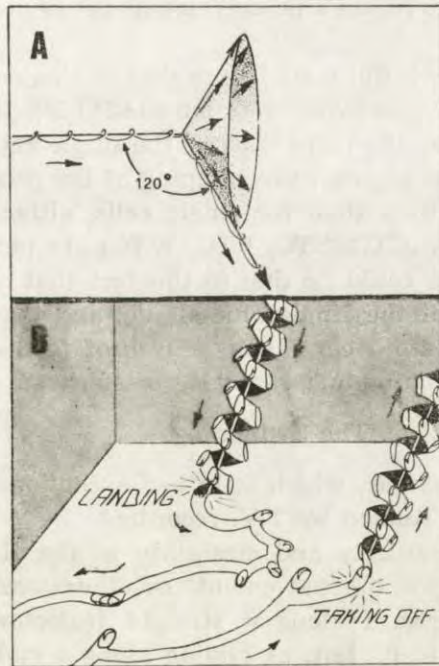

Fig. 8. (A) The "Stop-and Reorientation Reaction" (SRR). The point where the animal stops swimming represents the vertex of a cone, whose lateral surface contains all the possible new directions, lying at about 120° from the previous one. The figure mirrors also the behaviour of a pair. (B) Creeping and swimming are leftward movements: the animals pass from either condition to the other smoothly and without any discontinuity

due to the same mechanism, namely to an expectable unbalance of the "cellular engines".

Although this aspect of the motile behavior deserves a deeper investigation, it is, however, selfevident that one of the advantages of such a situation is that the oxytrichas are always ready "to take off" while creeping, as well as "to land" while swimming (Fig. 8 B), no appreciable discontinuity in the velocity being observable even scoring the T.V. records at very low speed.

2. Paired Animals   a.The Creeping

For paired animals the creeping is even more frequent than for single animals. Soon after pair formation, the locomotory organelles of the partners are immediately ready to work as belonging to a unique cell entity, so that the pair can move as easily and quickly as the single animals do.

Paired animals creep forward along leftward, geometric arcs. The average radius of these arcs is of 300 ± 180 µm and the mean velocity of a pair is of 820 ± 570 µm/s no correlation between these two parameters could be found.

The SSR of a pair is quite similar to that of single animals (backward motion $= 110 \pm 55$µm; clockwise rotation $= 62° + 9°$), although it is performed more rarely by the pairs than by the single animals.

In general one can say that the creeping of the pairs is far more regular and constant in time than for single cells, although the four types of trajectory changes (CTC, STC, RTC, WTC) are performed also by the pairs. This difference could be due to the fact that a pair doesn't feed, differently from what the single animals do, and that, therefore, a pair is not so interested into a continuous search of food or, in other words, into a continuous scanning and exploring of substrate.

2. Paired Animals   b. The Swimming

This kind of behaviour, which is rather exceptional for a pair, as already said, could be studied by T.V. recording.

The pairs spin regularly and straightly in the fluid, actually lying directly on the axis of the movement: in other words, the barycentre of a pair moves forward along a straight trajectory, while the pair itself keeps turning to its left, as gliding along a ribbon, lightly wound (Fig. 9). The pitch of this s. 1. helicoidal movement is of 470 ± 70 µm and the radius of 0 µm. The swimming velocity of a pair is of 760 ± 100 µm: it is very constant not only for the same pair, but also for the population, as shown by the low's'.

When a pair, by a SRR, quits swimming for a moment, it resumes

dorsal   right   ventral   left   dorsal
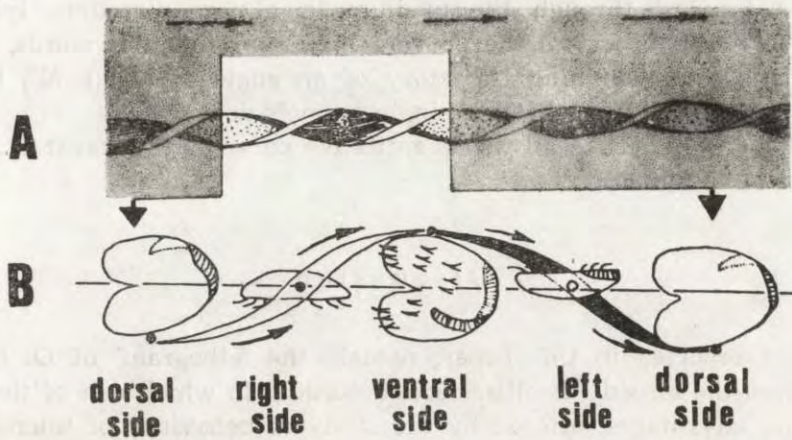**side   side   side   side   side**

Fig. 9. Schematic drawing of the swimming of a pair, turning to the left while moving forward straightly. In (A), the clear surface refers to the dorsal side of the pair and the dark surface to the ventral one. In (B), the movement of one single point, lying on the right side of the pair, is shown, the white strip indicates the part of trajectory which is above the plain of the drawing, while the black strip refers to the part below such a plain

Table 1

Synopsis of the parameters quantified for the motor behaviour of single (on the left) and paired (on the right) *Oxytricha bifaria*

Motor Behaviour	Parameters Considered	Single Animals			Paired Animals		
		$\bar{x}$	s	n	$\bar{x}$	s	n
Forward Creeping	Radius of Arcs (μm	800	450	220	300	180	120
	Velocity (μm/s)	500	290	250	820	570	180
Side Stepping Reaction	Backward Motion (μm)	100	45	460	110	55	150
	Angle (degrees)	63	12	520	62	9	85
Swimming	Pitch of Helicoid (μm)	220	75	50	470	70	50
	Radius of Helicoid (μm)	40	20	50	0	—	39
	Velocity (μm/s)	750	300	150	760	100	110
Stop and Reorientation Reaction	Angle (degrees)	120	20	40	110	20	35

moving forward through the medium in a new direction, lying at
$110° \pm 20°$ with respect to the previous direction, in other words, a pair
can change its swimming trajectory of an angle of about 70°, in any
direction, similarly to what the single animals do.

Table 1 summarizes all the quantitative parameters measured in this
round of experiments.

Discussion

What reported in this paper, namely the 'ethogram' of *O. bifaria*,
reinforces the already familiar idea, according to which one of the most
stricking advantages, offered by the study of behaviour of microorgan-
isms, is the relative simplicity of the systems. *O. bifaria* does not con-
stitute an exception from this point of view, infact we can say that
more than 90% of all the motor patterns possibly performed by *O. bi-
faria* can be described exhaustively. Moreover, this species has been
studied also from several other points of view (cfr R i c c i 1981 a) and
this offers a further chance to gain insight into its more general bio-
logical strategies, comparing and integrating with each other the results
so far obtained. Although already present in literature (D r y l and
G r ę b e c k i 1966, for *Paramecium*), the tendency to describe and
possibly quantify behavioural patterns of ciliates is rather new. Also
for hypotrichs, extensively studied by many authors (J e n n i n g s
1906, M a c h e m e r 1965 a, b, S l e i g h 1968, H i l d e b r a n d 1972, 1975),
no attempt has been made so far to draw exhaustive ethograms of the
different species, in spite of the fact that such an effort cannot but
offer powerful tools to investigate the most different aspects of the
biology of these organisms.

As to the ethogram, one must be aware that environmental condi-
tions, such as temperature, light, pH, gases solved, ionic composition
of medium etc., play key-roles in determining the behaviour of micro-
organisms, as already suggested by D r y l (1974), who stated that per-
fectly controlled experimental conditions constitute the prerequisite to
get reproducible results.

To discuss the geometric exactness of the leftward arcs along which
the oxytrichas creep, it can be said that the results indicate that the
animals are pushed forward by a complex of 'cyto-engines', constantly
more powerful on the right side. Harder task will be to recognize the
different roles singly played by the ciliary organelles, namely the ado-
ral zone membranelles, the undulating membranelles and the ventral
and the marginal cirri, and to quantify their contributes to the move-

ment in its entirety. This particular problem has been investigated in *Stylonychia* by M a c h e m e r (1965 a, b) and by S l e i g h (1966, 1968) and the results they obtained, seem to suit the *Oxytricha's* case too. On the other hand, the problem arises whether, and to what extent it is possible to use what known for *Stylonychia* to interpretate also the *O. bifaria's* behaviour. For the latter, however, a new clue is offered by the comparison of the behaviour of single and paired animals, which mainly differ from each other mainly for their width and cortical array. A pair, infact, although as long as a single animal, is twice as much in width; a pair has an adoral zone membranellar complex almost identical to the single cells, while its ventral surface bears a number of cirri almost twice that of a single animal. The latter trait of the pair's morphology could account for the average radius of their creeping trajectories, which is almost one third of that of single animals (cfr. Table 1); in other words, we can put forward the hypothesis that the differences between the right and the left components of the 'cyto-engines' in the pairs are wider than in single animals and that, in turn, such a larger unbalance results into a reduction of the average radius of the creeping trajectories. But, beyond creeping forward along trajectories far more bent to the left than those of single animals, the pairs can also do that at a very high speed: their wider ventral surfaces, letting the pairs adhere more strongly to the substrate, may account for the observed lack of any correlation between the speeds and the radiuses of the tracks. Moreover, if this is true, we could also conclude that, rather than adoral zone membranelles, which are in the pairs almost the same as in the single animals, the cirri are the ciliary organelles possibly playing the major role in pushing, or pulling, forward the oxytrichas.

Whatever the nature of the 'cyto-engines', *O. bifaria* proved to be capable of modulating continuously and sophisticatedly the ciliary beat, as it is shown by the description of CTC, STC, RTC, WTC, so that the animals can manouver very finely and exactly in response to environmental stimuli and/or internal conditions. We have here to recall that for the microorganisms the movement is determined by the viscoelastic, rather by the inertial forces and that, therefore, the stopping and the reversing of the ciliary beat affects the animal's movement directly and immediately, inducing an instantaneous stop or backward motion, respectively.

As to the Side-Stepping-Reaction (SSR), it has been called that way not only because it is usually performed by the oxytrichas to scan and to explore the substrate, rather than to avoid possible obstacles, but also to recall that such a reaction is shown by *O. bifaria* only while

creeping. The "trial and errors" theory of J e n n i n g s (1906), already criticized by A l v e r d e z (1922) and by P a r d u c z (1956 a, b and 1959) for the *Paramecium's* behaviour, does not fit into what is known today not only for *O. bifaria,* but also for the hypotrichs more in general (S l e i g h 1966, 1973). Comparing the SSR with the SRR of the swimming, it must be recalled that, although similar for their periodic and spontaneous occurrence, they differ from each other because no backward motion was ever observed in the SRR. This trait, moreover, seems to differentiate the behaviour of *O. bifaria* from that of *Stylonychia,* which has been reported to perform some backward swimming (Machemer 1974).

A final remark must be made about the stricking similarity of the quantitative parameters characterizing the SSR and the SRR of both single and paired animals, in spite of their significant morphological differences. Such a similarity seems to suggest that these two behavioural patterns somehow constitute adaptive bottlenecks of the general *O. bifaria's* behaviour, which cannot be altered in any way without affecting seriously their own efficacy.

Thus, although exhaustive from the description point of view, the results here reported about the motor behaviour of single and paired oxytrichas, with their stricking differences and unexpected similarities, showed that further studies on the behaviour not only of *O. bifaria,* but also of as many related species as possible, are requested to gain new insight into the most meaningful traits of this aspect of their biology.

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Stenoductus chondromorphi sp. n. (*Sporozoa: Cephalina*) from the Polydesmoid Millipede, *Chondromorpha kelaarti* (Humbert)

Synopsis. Stenoductus chondromorphi sp. n. from the intestine of the
millipede, *Chondromorpha kelaarti* (Humbert), collected from the Can-
nanore Sree Narayana College Campus, is described in detail with
special reference tomits morphology, life-history and systematic position.

The genus *Stenoductus* Ramachandran, 1976 (Family — *Monoducti-
dae* Ray and Chakravarty 1933) is characterized by (1) sporonts
without epimerites, (2) nucleus spherical, oval or boat-shaped, not teth-
ered by myonemes, (3) gametocyst dehiscence through single sporo-
duct, elongated and tubular or naked and cord-like, and (4) spores
ellipsoid or ovoid, always with hyaline epispore, thick at poles or also
along the equatorial line (Janardanan and Ramachandran
1979). *Stenoductus penneri* Ramachandran (1976 a), *S. gordanus*
Ramachandran (1976 b), *S. carlogoni* Janardanan and Rama-
chandran (1979), and *S. organognathi* Janardanan and Rama-
chandran (1980) are the only four species known under this genus.

The present paper deals with the morphology, life-history and syste-
matic position of a new species of cephaline gregarine from the milli-
pede, *Chondromorpha kelaarti,* collected from the Sree Narayana Col-
lege Campus, Cannanore, Kerala.

Materials and Methods

The millipedes were collected from the campus during the monsoon months
of 1974 and 1975. The trophozoites and sporonts were studied from stained smears
of the millipede-midgut prepared according to Ramachandran (1976 a). Ga-
metocysts were collected from the hindgut or from moistened fecal pellets and

maintained in moist chamber for further development. Sporozoites were released by exposing mature spores to the millipede's midgut fluid and studied under a phase-contrast microscope. Various development stages of live gregarines were studied by staining them supravitally with crystal violet, neutral red or toluidine blue in dilute concentrations. Random samples of 200 sporonts were studied in stained midgut smears to determine the percentage of incidence of sporonts.

The gregarine and its several stages were sketched with the aid of a camera lucida. The descriptions are based on measurements of a minimum of 20 sporonts.

Results

Stenoductus chondromorphi sp. n.

Description

Sporonts in two morphological forms — designated here typical and atypical. Typical sporonts dominant in a population, with an incidence of 65%.

Typical sporonts (Fig. 1 *1*) elongated, tadpole-like, laterally curved toward the round caudal end, protomerite rectangular, wider than long, deutomerite widest behind the anterior third, becoming uniformly cylindrical at posterior half. Constriction between protomerite and deutomerite inconspicuous. Apical papilla and apical pore absent, epicyte at anterior end thin, epicyte covering of protomerite striated, striations continuous with deutomerite striations. Protomerite formed mostly of ectoplasm, endoplasmic mass in protomerite granular, granules larger than those of deutomerite and staining metachromatically with toluidine blue. Septum circular, flat, its average thickness 3 μm.

Deutomerite elongated, narrow behind septum, maximally dilated behind the anterior third, then narrows to become uniformly cylindrical at the laterally curved, round-ended posterior half, epicyte hyaline, 2 to 3 μm thick, longitudinally striated, with occasional interconnections through their wavy undulations, ectoplasm clear, endoplasm uniformly granular, granules smaller than those in the protomerite.

Nucleus ovoid, nearly visible in fresh sporonts, position of nucleus in deutomerite variable, generally behind the deutomerite dilation, with the long axis of nucleus oriented along the length of deutomerite. Endosome large, round, variable in position, deep-staining with toluidine blue, extraendosome region clear, size of nucleus in a sporont of 429 by 89 μm was 24 by 16 μm, its round, central endosome measured 7 μm.

Atypical sporonts (Fig. 1 *2*) elongated, worm-like, rather laterally curved caudad, protomerite rectangular, wider than long, deutomerite

uniformly cylindrical, ends in round caudal end, constriction between protomerite and deutomerite not conspicuous, protomerite papilla absent, apical pore absent, epicyte at apical end thin, protomerite content mostly of ectoplasm, the endoplasm granular, forming a smaller central mass, endoplasmic granules measured 2 µm to 6 µm, larger than deutomerite granules, the granules stain metachromatically with toluidine
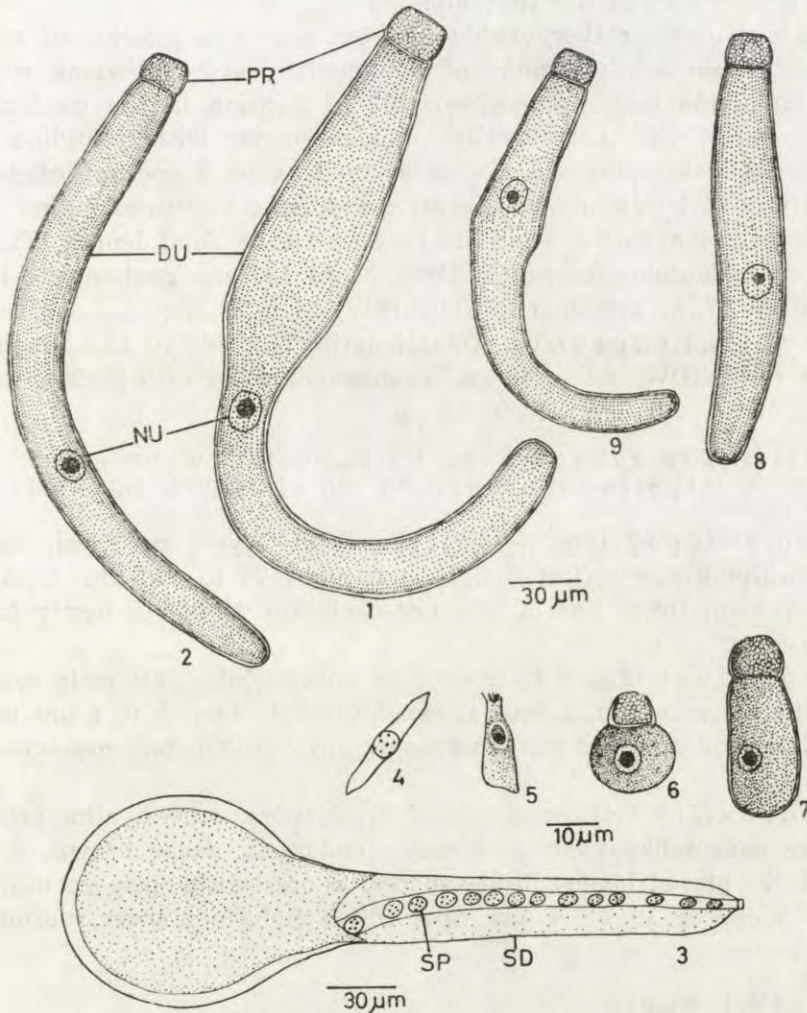

Fig. 1. 1–9 *Stenoductus chondromorphi* sp. n. 1 — Typical sporont, 2 — Atypical sporont, 3 — Gametocyst forming sporoduct and extruding spores, 4 — Spore, 5 — Intracellular aseptate trophozoite, 6–7 — Lumen trophozoites, 8–9 — Young sporonts

DU — deutomerite, NU — nucleus, PR — protomerite, SD — sporoduct, SP — spore

blue. Epicyte covering of protomerite striated, striations continuous with longitudinal deutomerite striations. Septum between protomerite and deutomerite circular, flat, its average thickness 3 μm.

Deutomerite elongated, uniformly cylindrical, ending in a round caudal end, posterior part of deutomerite laterally curved. Epicyte hyaline, 2 to 3 μm thick, forming longitudinal striations, occasionally in touch at points of wavy undulations. Ectoplasm clear, endoplasm granular, light-brown in live sporonts.

Nucleus ovoid, rather visible in fresh sporonts, position of nucleus variable, often at the center of deutomerite, oriented along its long axis. Endosome large, round, variable in position in the nucleus and deep-staining with hematoxylin, metachromatic with toluidine blue, extraendosomal region clear, size of nucleus in a sporont of 480 by 32 μm was 24 by 16 μm, its eccentric endosome measured 7 μm.

M e a s u r e m e n t s: T y p i c a l s p o r o n t s: Total length (TL), 346 to 530 μm, deutomerite width (DW), 51 to 136 μm, protomerite length (PL), 15 to 28 μm, protomerite width (PW), 21 to 36 μm.

A t y p i c a l s p o r o n t s: Total length (TL), 260 to 540 μm, deutomerite width (DW), 32 to 48 μm, protomerite length (PL), 12 to 24 μm, protomerite width (PW), 18 to 28 μm.

R a t i o s: T y p i c a l s p o r o n t s. PL : TL = 1 : 19.7, PW : DW = 1 : 2.8.
A t y p i c a l s p o r o n t s: PL : TL = 1 : 19.3, PW : DW = 1 : 1.6.

G a m e t o c y s t (Fig. 1 3): Fresh gametocysts spherical, opaque, milky-white under reflected light, measured 76 to 120 μm. Cyst wall hyaline, 8 μm thick, line of association clearly visible in newly formed gametocysts.

S p o r o d u c t (Fig. 1 3): Sporoduct milky-white, uniformly cylindrical with a conical tip, a central canal through duct, 5 to 8 μm in diameter. Sporoduct of a gametocyst, 86 μm in diameter, measured 201 by 28 μm.

S p o r e s (Fig. 1 4): Spores ovoid, fresh spores with hyaline epispore, forming dagger-like polar extensions, endospore dense brown, 8 dark, irregularly placed bodies inside spores. Spores with epispore measured 18 by 5 μm or 16 by 4 μm. Sporozoites eight per spore, vermiform, 15 by 2 μm.

Life-Cycle Stages

Gametocysts maintained in moist chamber, at room temperature developed spores in 3 days. The spores arranged one behind the other in a line along the central canal of the sporoduct came out through its tip one by one. Fresh spores placed in midgut fluid of the millipede

host released vermiform sporozoites in 2 min. The eight sporozoites in a spore came out through a pole of the spore one by one or in groups of 2 to 4. The sporozoites showed active, serpentine movements in the medium.

Millipedes seem to pick up infection by ingesting viable spores which release their sporozoites in the midgut on exposure to the midgut fluid. Sporozoites penetrate into midgut epithelial cells, where they develop to trophozoites.

The earliest trophozoite stage observed in the midgut epithelial cells of the millipede was round, undivided, measuring 1.2 µm in diameter, stainable with basic dyes, but for a halo of unstained host-cell region around. The smallest septate trophozoite observed was 4.2 by 3 µm with equally long protomerite and deutomerite. The nucleus was round, uniformly deep-staining with hematoxylin and probably representing the endosome. Trophozoite of 5.6 by 4 µm had a hemispherical protomerite, 2.2 µm long, and an oval deutomerite with a spherical nucleus. The larger trophozoites become detached from the host cells, lie free in the lumen and grow into sporonts. The smallest trophozoite, from the lumen was 28 by 18 µm with a 12 µm long protomerite. The nucleus was spherical with a deep-staining round endosome and an unstained extraendosome region. Epimerite was never observed in the trophozoites. Growth of the lumen trophozoite was rapid, contributed mainly by the faster growth rate of the deutomerite. A trophozoite 112 by 24 µm showed the morphology of the adult sporont. The trophozoites, with further growth and an accompanying accumulation of storage granules, became the full grown sporonts.

Taxonomic Summary

Di a g n o s i s: Sporonts typical and atypical, with laterally curved caudad, typical sporonts tadpole-like, atypical sporonts worm-like, protomerite rectangular, spores extruded through the central canal of a sporoduct, 201 by 28 µm, in a row to pass out at the tip of the duct one behind the other, spores with hyaline epispore, forming dagger-like extensions at poles, the spores measured 18 by 5 µm or 16 by 4 µm.

M e a s u r e m e n t s: Ty p i c a l s p o r o n t s: Total length (TL), 346 to 530 µm, deutomerite width (DW), 51 to 136 µm, protomerite length (PL), 15 to 28 µm, protomerite width (PW), 21 to 36 µm.

A t y p i c a l s p o r o n t s: Total length (TL), 260 to 540 µm, deutomerite width (DW), 32 to 48 µm, protomerite length (PL), 12 to 24 µm, protomerite width (PW), 18 to 28 µm.

Table 1

Comparative characters of species of *Stenoductus*

Comparative characters	S. penneri Ramachandran	S. gordanus Ramachandran	S. carlogoni Janardanan and Ramachandran	S. organognathi Janardanan and Ramachandran	S. chondromorphi sp. n.
Body shape and size	Tadpole-like 1063.2×411.4 μm	Maximally wide at anterior third 1224×244.4 μm	Elongate cylindrical 1064×237 μm	Elongate cylindrical 1238.4×148.5 μm	Two types: typical and atypical with laterally curved caudad. (1) Typical 530×99 μm (2) Atypical 540×44 μm
Epimerite	Absent	Absent	Present on trophozoites	Absent	Absent
Protomerite	Dome-shaped with apical papilla and apical pore	Dome-shaped with apical papilla and apical pore	Dome-like with apical papilla and apical pore	Dome-like with apical papilla and apical pore	Rectangular without apical papilla and apical pore
Deutomerite	Maximally dilated at anterior third	Maximally dilated at anterior third	Cylindrical, rarely dilated at anterior third	Cylindrical	(1) Maximally wide behind anterior third (2) Worm-like
Nucleus	Round or slightly ovoid	Spherical	Boat-shaped with a round endosome and two extraendosome bodies	Boat-shaped with an oval endosome	Ovoid

	Floridobolus penneri	Narceus gordanus	Carlogonus palmatus	Organognathus janardhanani	Chondromorpha
PL : TL	1 : 23.1	1 : 29.6	1 : 15.5	1 : 13.9	(1) 1 : 19.7 (2) 1 : 19.3
PW : DW	1 : 2.8	1 : 2.8	1 : 2.5	1 : 1.7	(1) 1 : 2.8 (2) 1 : 1.6
Gametocysts	Round or slightly ovoid	Spherical	Spherical 135 to 255 μm	Spherical 158.4 to 259.2 μm	Ovoid 76 to 120 μm
Sporocysts	Ellipsoidal, with hyaline epispore with round polar projections	Ellipsoidal with hyaline epispore drawn out at poles to horny projections	Ovoid, with hyaline epispore thick at poles, having equatorial ridge 12×8 μm	Ovoid, with hyaline epispore thick at poles, having equatorial ridge 5.6×4.2 μm	Ovoid, with hyaline epispore forming dagger-shaped polar extensions 18× ×5 μm or 16×4 μm
Host(s)	*Floridobolus penneri* Causey	*Narceus gordanus* (Chamberlin)	*Carlogonus palmatus* Demange	*Organognathus janardhanani* Demange and *Fageostreptus hyatti* Demange	*Chondromorpha Kelaarti* (Humbert)
Locality	Highlands county, Florida (U.S.A.)	Highlands county Florida (U.S.A.)	Chorakkulam, Thottada, and Panoor, Kerala (India)	Pathanamthitta, Varkala, Chirayinkil and Ponmudi Kerala (India)	Sree Narayana College Campus, Cannanore, Kerala (India)

Ratios: Typical sporonts: PL : TL = 1 : 19.7, PW : DW = 1 : 2.8.
          Atypical sporonts   PL : TL = 1 : 19.3, PW : DW = 1 : 1.6.
Host: *Chondromorpha kelaarti* (Humbert)
Location in host: Intestine
Type locality: Cannanore, Kerala, India
Date of collection: June to September of 1974 and 1975
Holotype: To be deposited in the Museum of the Zoological Survey of India,
          34, Chittaranjan Avenue, Calcutta.

Discussion

The sporonts of this gregarine show morphological resemblance to *Stenophora nematoides* Leger and Duboscq, *S. vermiformes* Geus and *Cnemidospora lutea* Schneider. The gregarine, however, differs from them in its measurements and ratios, shape of the protomerite and absence of epimerite. Besides the known gametocysts in these species dehisce by simple rupture.

The present gregarine extrudes its spores through a well-formed single sporoduct and for this reason the species cannot be included in the families *Cnemidosporidae* or *Stenophoridae* where the gametocysts dehisce by simple rupture (G r a s s e 1953, K a r a n d i k a r and R o d g i 1955, G a n a p a t i and N a r a s i m h a m u r t i 1956 and C h a k r a - v a r t y 1959). The mode of dehiscence of the gametocyst through a sporoduct justifies its assignment to the family *Monoductidae* with its genera, *Monoductus* and *Stenoductus*.

The three known species of *Monoductus, M. lunatus* Ray and Cha-kravarty, *M. kelaarti* Karandikar and Rodgi, and *M. tubulosus* Karandikar and Rodgi have prong-bearing epimerites, nuclei with myoneme attachment and spores which are truncated, compressed, spindle-shaped and with longitudinal dorsal ridges. The ovoid spores with hyaline epi-spore, thick at poles, and sporonts without epimerites suggest of its relation to *Stenoductus*, under which it is reported.

The gregarine from *Chondromorpha kelaarti* (Humbert) differs markedly from the four known species of *Stenoductus* in the following points:

(1) Two types of sporonts: typical and atypical
(2) Rectangular protomerite without apical papilla and apical pore
(3) Ratios and measurements
(4) Ovoid spores with hyaline epispore produced into dagger-shaped extensions at poles.

Comparative characters in Table 1 clearly show that the species under investigation differs radically from all the known species of *Ste-*

noductus. It is therefore reported here as *Stenoductus chondromorphi* sp. n. after the generic name of its host, *Chondromorpha kelaarti* (Humbert).

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Rup LAL B. V. P. Reddy and D. M. SAXENA

Effect of DDT on Cell Division and DNA, RNA and Protein Synthesis in a Ciliate Protozoan, *Blepharisma intermedium*

Synopsis: Effects of DDT on cell division and DNA, RNA and protein synthesis of *Blepharisma intermedium* were studied. 100 ppm DDT inhibited cell division in G_1 and S phase whereas cell division was delayed in ciliates treated during G_2 and D phases. DDT treatment also inhibited DNA, RNA and protein synthesis. The degree of inhibition was dependent on the age of the ciliates at the time of treatment.

Dichlorodiphenyltrichlorethane (DDT) and other chlorinated hydrocarbon insecticides are known to influence population of microorganisms (Butler 1977). DDT is also known to bind to cell membrane and bring about changes in its ultrastructure (Hicks and Corner 1973, French and Roberts 1976). However, the precise mode of action of DDT at cellular level in microorganisms is not clearly understood (Rup Lal and Saxena 1980 a). With this in view, a ciliate protozoan, *Blepharisma intermedium* was used to study the effects of DDT on cell division and synthesis of DNA, RNA and proteins.

Material and Methods

Cultures of *Blepharisma intermedium* were maintained in sterilized hay infusion inoculated with bacteria, *Klebsiella aerogenes* at $24 \pm 1°C$. Horlick's malted milk was added periodically to facilitate the growth of bacteria. The old cultures were subcultured once in a week.

Experiments on cell division and the synthesis of DNA, RNA and proteins in *B. intermedium* were carried out in Chalkley's medium. Stock solutions of

DDT were prepared in acetone and added directly to Chalkley's medium containing the organisms. The concentration of acetone was kept 0.5% because this concentration did not have any detrimental effect on the growth of the ciliate (R u p L a l and S a x e n a 1980 b). As the lower concentrations of DDT were not effective a dose of 100 ppm DDT was selected to study the effects on cell division and synthesis of DNA, RNA and proteins.

Effect of DDT on cell division was studied on ciliates of different age groups (0, 2, 6, 12, 20 h old). Twenty-five animals of the same age group were used each time. The ciliates were obtained by isolating the cytokinetic forms from log phase cultures which were allowed to complete division in Chalkley's medium and the time for division was noted in each case. The resulting daughter cells were then treated with 100 ppm DDT for 6 h. Equal number of cells of the same age group in Chalkley's medium having 0.5% acetone served as control. The effect of DDT on the shape, size, pigmentation was also studied by observing the ciliates under a binocular microscope.

In recovery experiments, ciliates (25) of known age group (ranging from 0–20 h) after treatment with 100 ppm DDT for 6 h were washed repeatedly with toxicant free medium and transferred to fresh Chalkley's medium. The time taken by treated ciliates to divide was noted and compared with the corresponding controls.

Radioactive precursors, ^{3}H-thymidine (Sp. Act. 1960 mCi/mM) and ^{3}H-uridine (Sp. Act. 3600 mCi/mM) obtained from BARC, Bombay (India) were used to study the synthesis of DNA, RNA and proteins respectively. *Blepharisma intermedium* does not take up ^{3}H-thymidine and ^{3}H-uridine directly from the medium. These radioactive precursors, therefore, were supplied through prelabelled *Tetrahymena*.

For the study of effects of DDT on DNA and RNA synthesis, 25 ciliates (of the same age group) were isolated, transferred to Chalkley's medium and treated with 100 ppm DDT for 6 h. Appropriate controls were simultaneously kept. Both treated and control cultures were provided *Tetrahymena* (prelabelled with ^{3}H-thymidine/uridine). The ciliates were fixed and processed for autoradiography after 6 h.

Blepharisma intermedium readily incorporates ^{3}H-lysine directly from the medium. In order to study the effect of DDT on protein synthesis, ciliates (25) of the same age group were isolated and treated with 100 ppm DDT for 4 h in Chalkley's medium. Subsequently ^{3}H-lysine (5 uCi/ml) was provided for 2 h. Appropriate controls were maintained simultaneously. After 6 h both control and treated ciliates were fixed and processed for autoradiography. Prior to coating the slides with NTB-3 emulsion, the slides were treated with 5% cold trichloroacetic acid to remove any unicorporated free isotope. The slides were exposed for 3 to 4 weeks and developed in Kodak D-19 developer.

For quantitative estimation by scintillation counting of protein synthesis filter paper disc method (FPM) as described by S h i v a j i et al. (1977) was used. Twenty five ciliates of the same age group were taken and treated with 100 ppm DDT. Appropriate controls were kept simultaneously. After 4 h of treatment both treated and control ciliates were provided with ^{3}H-lysine directly for the next 2 h. The ciliates were then washed with Chalkley's medium repeatedly and transferred to Whatman No. 1 filter paper discs (five ciliate in each disc) and processed for scintillation counts in Tricrab liquid scintillation spectrometer. The experiments were repeated with ciliates of different age group.

Results

Generation time of *Blepharisma intermedium* is 22 ± 1 h. DDT at 100 ppm completely inhibited cell division in the ciliates of 1–20 h of age (Table 1). Twenty one and 22 h old ciliates treated with 100 ppm DDT, divided in the presence of DDT but the division was delayed as compared to the corresponding controls.

Table 1

Effect of 6 h lasting treatment with 100 ppm DDT on cell division in *Blepharisma intermedium*

Age of the animal at the time of treatment (in hours)	Percentage of animals dividing during treatment		Average time taken by animals to divide (h)	
	Control*	Treated*	Control*	Treated*
0	—	—	—	—
4	—	—	—	—
12	—	—	—	—
16	100	—	6	—
18	100	—	4	—
21	100	100	1.5	4.5
22	100	100	1	2.00

* Calculation based on observations of 25 ciliates in each case.

Ciliates belonging to different age groups when treated with 100 ppm DDT for 6 h and then transferred to toxicant free medium recovered from the inhibitory effect of DDT and divided but the recovery time was dependent on the age of the ciliate at the time of treatment (Table 2). Ciliates in G_1 phase (1–11 h old) required less time to recover

Table 2

Recovery time of *Blepharisma intermedium* treated with 100 ppm DDT

Age of the animals at the time of treatment (h)	Average time for completion of division during recovery (h) A*	Average time taken by the control animals to divide (h) B*	Recovery time in h (A − B)*
0	26	21	5
4	27	22	5
8	30	22	8
12	33	22	11
16	42	21.5	20.5

* Calculation based on observations of 25 ciliates in each case.

as compared to the ciliates in S phase (12 to 20 h old). Ciliates in late S phase (16 h old) took maximum time (20.5 h) to recover.

The autoradiographs of *Blepharisma intermedium* of age 1 to 11 h did not show any incorporation of ^{3}H-thymidine in the macronucleus. However, precursor was incorporated into the macronucleus of 12 h old ciliates (Pl. I 1). The incorporation of ^{3}H-thymidine subsequently increased and the macronucleus of 18 h old *Blepharisma intermedium* was densely labelled (Pl. I 2). Ciliates of 21–22 h of age were also provided with prelabelled *Tetrahymena* separately and autoradiographed. Such ciliates did not show any incorporation of ^{3}H-thymidine in the macronucleus. These results show that in *Blepharisma intermedium* G_1 phase lasts for 0–12 h, followed by S phase which lasts for 8 h. The G_2 and D phases together last for 2–3 h.

Blepharisma intermedium treated with 100 ppm DDT in G_1 phase (6 h old) for 6 h did not show any incorporation of ^{3}H-thymidine in the macronuclei. However, the corresponding control ciliates had incorporated ^{3}H-thymidine in the macronuclei (Pl. I 1). Ciliates treated in S phase (12 h old) showed negligible incorporation of ^{3}H-thymidine (Pl. I 3) whereas the corresponding controls were densely labelled (Pl. I 2).

Synthesis of RNA was also reduced significantly in *Blepharisma* treated with 100 ppm DDT. When the ciliates were treated in G_1 phase very little incorporation of ^{3}H-uridine was noticed (Pl. I 4) as compared to the partner control (Pl. II 5). Similarly ciliates treated in S and G_2 phases also showed reduction in the incorporation of uridine.

Protein synthesis was also affected by DDT treatment in *Blepharisma intermedium*. Ciliates in G_1 phase when treated with DDT showed reduction in the incorporation of ^{3}H-lysine (Pl. II 6) as compared to the corresponding controls. DDT treatment also reduced the incorporation of ^{3}H-lysine in S and G_2 phase ciliates. The counts/minute obtained for the treated and control ciliates by the FPM showed maximum incorporation of ^{3}H-lysine in 12 h old ciliates and the incorporation was reduced by 60–80% in G_1 and S phase ciliates treated with 100 ppm DDT (Fig. 1).

Discussion

Exposure of *Blepharisma intermedium* to 100 ppm DDT inhibited cell division. DDT and other chlorinated hydrocarbons are known to reduce cell population growth in other protozoans such as *Euplotes* (P e r s o n n e and U y t t e r s p r o t 1975), *Crithidia fasciculata* (F r e n c h and R o b e r t s 1976), *Stylonychia notophora* (R u p L a l

and S a x e n a 1980 b) and *Tetrahymena pyriformis* (R u p L a l and S a x e n a 1979).

Ciliates treated with 100 ppm DDT in G_1 and S phases of cell cycle failed to divide. However, ciliates treated in G_2 phase divided but the division was delayed. Thus in *Blepharisma intermedium* G_2 phase is
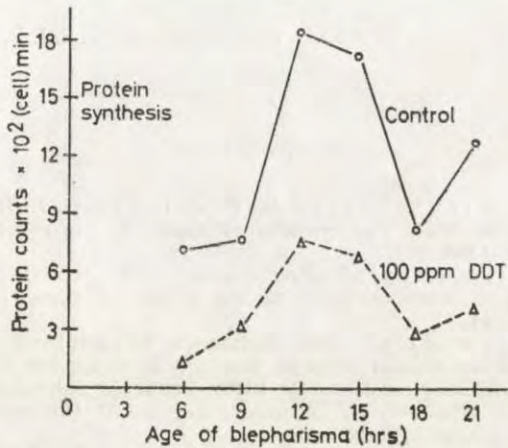

Fig. 1. Effect of 100 ppm DDT on the incorporation of ^{3}H-lysine (CPM) in *B. intermedium* during cell cycle

the transition point from DDT-sensitive to DDT-resistant stage. This indicates that programming of biochemical events necessary for the ensuing cell division is completed before the transition point. *Blepharisma* treated with DDT after this stage i.e., G_2 completed cell division, though it was delayed. It is known that in many eukaryotic systems the G_2 phase of cell cycle stands out as the transition point for the effect of many metabolic inhibitors (F r a n k e l 1962, 1967, P u c k e 1972, K a c z a n o w s k a et al. 1976).

Synthesis of DNA, RNA and proteins was inhibited in *Blepharisma* by DDT treatment. Many other chlorinated hydrocarbon insecticides such as lindane, dieldrin, aldrin, methoxychlor and heptachlor are known to inhibit the synthesis of DNA, RNA and proteins in many organisms such as slime mold, *Dictyostelium discoideum* (B u s h w a y and H a n k s 1976), sea urchin embryo (B r e s h and A r e n d t 1977) many plants (A n d e r e g g et al. 1977), and animals (A n i n a 1975). The exact mechanisms of interaction of DDT and other chlorinated hydrocarbon insecticides with macromolecular synthesis are not known. It is however, known that DDT interferes primarily with Na^+, K^+ and Mg^{++} ATPase system (J o w e t t et al. 1978) and also alters the fine structure

of plasma membrane (F r e n c h and R o b e r t s 1976), which may adversely affect the various biochemical processes including those involved in the synthesis of DNA, RNA and protein.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES I–II

1: Autoradiograph of 12 h old (untreated control) *Blepharisma intermedium* showing the incorporation of ³H-thymidine in the macronucleus. (450 ×)
2: Autoradiograph 18 h old *B. intermedium* (untreated control) showing dense incorporation of ³H-thymidine in the macronucleus. (450 ×)
3: Autoradiograph of *B. intermedium* treated with 100 ppm DDT during S phase. No incorporation of ³H-thymidine in the macronucleus is seen. Ingested pre-labelled *Tetrahymena* is seen (arrow). (450 ×)
4: Autoradiograph of *B. intermedium* treated with 100 ppm DDT during S phase showing decrease in incorporation of ³H-uridine. The arrow indicates the ingested prelabelled *Tetrahymena*. (450 ×)
5: Autoradiograph of *B. intermedium* in S phase showing dense incorporation of ³H-uridine. (450 ×)
6: Autoradiograph of *B. intermedium* treated with 100 ppm DDT in G_1 phase showing a decrease in incorporation of ³H-lysine. (450 ×)
7: Autoradiograph of S phase *B. intermedium* (untreated control) showing dense incorporation of ³H-lysine. (450 ×)

EXPLANATION OF PLATES 1-1

1. Adpressed node of *R. tuberculatum* (natur.). Enclosing internodium showing the incorporation of germinule in an anticlinically 300x1

2. Autoradiograph of an old *R. tuberculatum* tuberated cortical showing plasma incorporation of *H*-thymidine in late prophase xxx. 160

3. Autoradiogram of *R. tuberculatum* treated with *H*-leum 1 *T*-dottum *R* showing the incorporation of *H*-thymidine in the nucleoneus is seen. Labelled into implied *R*-thymidine is seen in xxx. 180

4. Autoradiograph of *R. tuberculatum* treated with the prior CO₂ during *S*-phase showing the role of incorporated of *T*-matter the arrow indicates those implied prelabelled *R*-thymidine. 160x1

5. Autoradiograph of *R* determination in *S*-phase showing during incorporation of *H*-uridine 180x1

6. Autoradiograph of *R* tuberculatum treated with the prior 160x to *C*-phase showing a decrease in incorporation of thymine 180x1

7. Autoradiograph of *R* older *R* tuberculatum incorporation of prior showing a incorporation of thymine 160x1

PLATE I

auctores phot.

Rup Lal et al.

auctores phot.

In preparation:

G. Fernandez-Laborans: Nouvelles observations de deux cilies Oligotriches, *Halteria grandinella* (la morphologie et la morphogenèse) et *Strombilidium gyrans* (la morphologie) — S. L. Kazubski: Morphological Variability of *Trichodina reticulata* Hirschmann et Partsch, 1955 (*Ciliata, Peritrichida*), a Parasite of *Carassius* (L.) from Small Pond in Kortowo (Olsztyn) — S. F. Ng and A. Newman: Isolation of Amicronucleates in *Paramecium tetraurelia* — N. Ricci: The Ethogram of *Oxytricha bifaria* (Stokes) *Ciliata, Hypotrichida*. II. The Mating Behaviour — O. N. Borchsenius and S. I. Fokin: Ultrastructural Changes in the Nuclear Apparatus of *Paramecium bursaria* after Local UV Irradiation of the Generative Nucleus — J. Kołodziejczyk and A. Grębecki: Further Studies on the Relation between Contraction and Streaming Oscillations in the Plasmodial Veins of *Physarum polycephalum* — S. Dryl, C. Demar-Gervais and A. Kubalski: Contribution to Studies on the Role of External Cations in Excitability of Marine Ciliate *Fabrea salina* — H. Szydłowska: Modification of Motile Behaviour in *Paramecium octaurelia* by Cholesterol — P. Kovacs and G. Csaba: Studies on the Lectin Binding Capacity of the *Tetrahymena* — P. Kovacs, G. Csaba and Z. Darvas: Effect of Lasting Treatment with Histamine Antagonists on Lectin Binding of *Tetrahymena* — Cz. Balcerzak and R. Ciepielewski: The Radioprotective Effect of Some Scavengers of the OH Radical on the Radiation — Induced Changes in the Sensitivity of the *Protozoa* Membranes to Sodium Chloride Concentration — J. C. Goutierrez, A. Serrano and F. Parra: Spectrophotometric Identification of a Carotenoid Pigment in the Resting Cysts of a Hypotrichous Ciliate, *Laurentiella acuminata* — Э. И. Успенская и В. А. Иванов: Сравнительное исследование антиленного состава инфузории *Colpoda maupasi* — E. Hadaś: The Lytic Enzymes of Pathogenic and Non-pathogenic Strains of *Acanthamoeba castelanii* and *Naegleria fowleri* — J. J. Lipa: *Nosema euzeti* sp. n. and *Gregarina euzeti* sp. n. Two New Protozoan Parasites of a Mite *Euzetes seminulum* (O. F. Müller) (*Acarina, Oribatei*).

CONTENTS