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# On the genus *Ancistruma* Strand (= *Ancistrum* Maupas).

## II. The Conjugation and Nuclear Reorganization of *A. isseli* Kahl.

By

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(With Plate 1.)

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## Introduction.

A discussion of the general morphology, division and habitat of *Ancistruma isseli* is to be found in an earlier paper on this genus (Kidder, 1933 b). The present account deals with the process of con-

jugation and subsequent reorganization with special reference to the nuclear behavior.

This work was carried on at the Marine Biological Laboratory at Woods Hole, Massachusetts and the Zoological Laboratories of Columbia University in New York City. I am indebted to the faculty of Columbia University for providing me with research facilities. I wish to acknowledge the very helpful advice and criticism of the manuscript given by Prof. GARY N. CALKINS. Thanks are also due to RUTH R. KIDDER for valuable technical assistance.

## Methods.

The general methods employed in the first of this series of papers (KIDDER, 1933 b) were used in the present study. A variety of fixatives were used but the ones found to give the most satisfactory results were BOUIN'S, GILSON-CARNOY'S and SCHAUDINN'S fluids and sublimate-acetic in 95 % alcohol. For a study of the micronuclear phenomena during maturation HEIDENHAIN'S haematoxylin was found to be by far the most satisfactory. Stages of exconjugant reorganization were studied after staining in the BORREL mixture and after the FEULGEN thymonucleic acid reaction.

## Macronucleus during conjugation.

Conjugation epidemics of *Ancistruma isseli* have been found to occur rather frequently. I have noted the presence of large numbers of conjugants in material collected at intervals throughout the summer of 1932. The process apparently lasts but two or three days at a time and one to three weeks ensue before another epidemic takes place. In dealing with a commensal of this type it is evident that no exact data as to time relationships are available, and the extent of an epidemic and the frequency of its recurrence are only approximated, based on daily collections of the host, in this case *Modiola modiolus*. The justification of the term "epidemic" is the fact that, at such a time, the majority of ciliates from a collection are either conjugating or in some stage of postconjugant reorganization.

During conjugation two ciliates become attached in a slightly asymmetrical manner. The oral surfaces come together and a bridge of protoplasm is formed between the two. This bridge connects a region about midway in the anterior third of the peristomal groove of one of the conjugants with the extreme anterior tip of the other. Thus one of the pair is slightly in advance of the other (Pl. 1

Figs. 1—5). The ventral surface of one conjugant and the dorsal surface of the other are always presented to the observer. This position is similar to that of *Chilodon* during conjugation.

There appears to be no constant size difference between the members of a conjugating pair.

The macronucleus of *Ancistruma isseli* during conjugation presents a characteristic form. Very shortly after fusion of the conjugants it loses its spherical shape and becomes elongated into a spindle-shaped mass, the long axis of which is parallel to the long axis of the ciliate. It moves from the center of the cell to a position near the left margin of the conjugant well toward the posterior end. So close does it seem to be pushed to this margin that it often conforms to the shape of the left boundary of the ciliate. The two macronuclei of the conjugating pair are seen to be as far away from each other as possible (Pl. 1 Figs. 1—4).

Very little change in the staining capacity of the macronuclear chromatin is noted during the early stages of conjugation. However, during the later stages and the early exconjugant reorganization stages it becomes more basophilic and vacuolated, until ultimately it is broken down and absorbed into the cytoplasm.

## Micronucleus during conjugation.

My observations on the micronuclear phenomena during conjugation are very incomplete, due entirely to the difficulty encountered in staining certain stages. The micronucleus of *Ancistruma isseli* is quite small during vegetative divisions. It enlarges somewhat during conjugation but it also loses most of its capacity to stain. Dozens of preparations were obtained where no trace of the micronuclei could be seen although the macronuclei and other cell structures were beautifully shown. Certain stages, however, could be demonstrated with ease.

### First maturation division.

Shortly after the fusion of the two conjugants the micronucleus of each swells and becomes entirely negative to any basophilic stain. The location may, at times, be noted as a clear vacuole-like space in the anterior cytoplasm. These both move back to the region opposite the bridge. Within this enlarged nucleus the first indication of chromatin to be looked for is a band of tiny granules in the equatorial region. These granules become more and more

basophilic and collect into a definite metaphase plate from which the spindle fibers are seen to radiate. The granules are too small and too compact to permit an accurate count (Pl. 1 Fig. 1). These granules separate into two groups which move to opposite poles of the now definite spindle (Pl. 1 Fig. 1). I have no preparations showing the separation of the daughter nuclei, so I am unable to tell whether or not there is a contraction of the chromatin.

Second maturation division.

All the products of the first maturation division enter into and complete the second division. The chromatin is now drawn out into ribbon-like chromosomes on the metaphase plate (Pl. 1 Fig. 2). These number about ten. (I have attempted to make accurate counts on many favorable preparations but I find it impossible to do more than approximate this. The number given, ten, is the most frequent one encountered.) These long chromosomes segregate or divide into two groups which move toward opposite poles (Pl. 1 Fig. 2). The nuclei then divide and each conjugant possesses four products. Three daughter nuclei of the second maturation division now move toward the posterior part of the cell where they become compact, deeply-staining spheres. These are destined to disintegrate, while the one daughter left in the region of the bridge of each individual will divide once more.

Third maturation division.

The two nuclei in the regions of the bridge form characteristic, sharply pointed spindles. The chromatin condenses into deeply staining chromosomes on the equatorial plate (Pl. 1 Fig. 3). These chromosomes, though quite even, are nevertheless very close together and hard to count. I am quite sure there are fewer than in the preceding division, the number appearing to be around five. This is what one would expect if the reduction during the second maturation division, found to take place in the majority of ciliates, is realized.

The danger of confusing this stage with the preceding one is slight, when we consider that the degenerating products of the second maturation division are clearly visible, and may be counted, until the first amphinuclear division.

The division of the nuclei results in the formation of the pronuclei. The telophase of this division is quite characteristic and resembles that described for *Boveria subcylindrica* by STEVENS (1910).

The chromatin is very densely staining and is drawn out into a long strand between the two polar masses (Pl. 1 Fig. 4).

I have never obtained preparations showing the migration or fusion of the pronuclei. These stages must take place very rapidly as I have examined hundreds of conjugants without once observing either stage.

## The exconjugant.

The amphinuclear divisions.

The first amphinuclear division takes place before the two conjugants have completely separated. The amphinuclei are found in the same location as the maturation nuclei, near the former bridge. The spindle and chromosomes closely resemble those of the second maturation nuclei. There are about ten slightly ragged chromosomes on the metaphase plate (Pl. 1 Fig. 5). A number of points, however, enable the observer to distinguish one from the other. In the second maturation division there are always two spindles in each cell, while there is only one spindle per cell during the first amphinuclear division. There are no disintegrating nuclei at the time of the second maturation division while these degenerating masses of chromatin are quite evident during the first division of the amphinucleus. The appearance of the macronucleus also aids in the identification of the stage. It is compact and somewhat regular during the early maturation divisions but becomes ragged and roughly granular by the time the amphinucleus divides for the first time (Pl. 1 Fig. 5).

Shortly after or during the first amphinuclear division the conjugants separate. The ensuing steps in the reorganization of the cell must be followed in single exconjugants.

The two products of the amphinucleus divide by typical mitoses. These spindles are slightly smaller than those of the preceding divisions but are larger than the normal vegetative spindle (Pl. 1 Fig. 6). From this division four apparently equal products are formed. During this period the old macronucleus is becoming more ragged and reduced in size. The exact state of its disintegration varies somewhat in different organisms (Pl. 1 Fig. 7).

The third and last amphinuclear division resembles the second except that four spindles form instead of two. These spindles are usually located in the mid-region of the cell (Pl. 1 Fig. 8) but I have found them widely separated. This division results in eight small, compact spheres (Pl. 1 Fig. 9). These are deeply staining

and have every appearance of the vegetative micronucleus. I have been unable to detect any visible difference between these spheres.

Differentiation of macronuclear anlagen.

The eight products of the third amphinuclear division become separated and move into the anterior part of the cell. One of the eight remains compact and becomes the functional micronucleus, while the other seven lose their staining capacity and swell into finely granular spheres, the macronuclear anlagen (Pl. 1 Fig. 10). This phenomena almost exactly parallels the differentiation of macronuclear anlagen in *Conchophthirius mytili* (KIDDER, 1933 a). These anlagen are stained very faintly after the FEULGEN reaction and appear green with tiny points of red after the BORREL stain.

During this time the old macronucleus has become fragmented and the remnants, still very deeply staining, are being absorbed into the cytoplasm. At the time of the differentiation of the macronuclear anlagen only small masses of the old macronucleus are seen and these very shortly disappear.

The seven macronuclear anlagen increase in size and staining capacity until they come to occupy most of the volume of the anterior part of the cell (Pl. 1 Fig. 11). At this stage they certainly might be interpretated as seven fragments of an original macronucleus. This is the assumption that ISSEL (1903) made, and many others have fallen into the same error. This point will be discussed more fully later in this paper.

## Reorganization divisions of the exconjugant.

The micronucleus of the exconjugant which has undergone three amphinuclear divisions and has completely differentiated its macronuclear anlagen, now forms a typical vegetative spindle and divides. As this process is going on a peculiar thing happens in every one of the macronuclear anlagen. Many granules within the sphere move to the periphery of the anlagen. These granules become packed into balls of chromatin and, by the time the division of the cell is well under way, are pushed out as homogeneous spheres (Pl. 1 Fig. 12). These extrusion masses are similar to those of *Conchophthirius mytili* (KIDDER, 1933 a) but are proportionately larger. They show their connection with the anlagen for a short time and then break down and disappear in the cytoplasm.

The first division of the cell body results in a segregation of nuclei, four macronuclear anlagen and one daughter micronucleus

passing to one daughter ciliate while the remaining three macronuclear anlagen and daughter micronucleus go to the other. The resulting ciliates are of two types, as shown by Pl. 1 Figs. 13 and 14.

The second exconjugant division is characterized, as is the first, by an early micronuclear division. Within the anlagen a second set of granules collects and moves to the periphery (Pl. 1 Figs. 13 and 14). The daughter micronuclei move apart and the ciliates divide, further segregating the anlagen. As plasmotomy begins the masses of chromatin are cast out of each anlage (Pl. 1 Figs. 15 and 16). Immediate disintegration of these extrusion masses begins as soon as their connections with the anlagen are severed. Examples of this are seen in the rough outline of the masses in Pl. 1 Fig. 15.

The ciliates resulting from the second exconjugant division are of two types, those possessing two macronuclear anlagen and those possessing one. The latter has the normal nuclear number. Its anlage metamorphoses by growth and increase of staining capacity into the large, spherical macronucleus of the vegetative form. The ciliate possessing two macronuclear anlagen (Pl. 1 Fig. 17) must undergo a third segregating division. The details of this are a duplication of the preceding ones. The extrusion chromatin again collects (Pl. 1 Fig. 18) and is cast out during plasmotomy (Pl. 1 Fig. 19).

I have never seen any irregularities in the segregation of macronuclear anlagen in *Ancistruma isseli*, such as were occasionally noted for *Conchophthirius mytili* (Kidder, 1933 a). The types found were those with seven, four, three and two anlagen. In most of these the micronucleus was in the resting stage indicating that this stage is of long duration. Less frequently were found the early stages of micronuclear activity as in Pl. 1 Figs. 13, 14 and 18. The later stages of division were easily recognized under the low powers of the dissecting binoculars, picked out with a fine pipette, and fixed.

## Discussion.

Many accounts and figures of "macronuclear fragmentation" are found in the literature dealing with the Boveridae, the Ancistrumidae and the Conchophthiriidae. In his monograph on the Ancistridae of the Gulf of Naples, Issel (1903) describes this fragmentation in *Ancistruma* (*Ancistrum*) *cyclidioides*, *A. compressum*, *A. tellinae* and *A. isseli* (his *A. mytili*). He is very definite about the source of the

nuclear spheres or fragments and says they are the result of a breaking up of the macronucleus and not the result of conjugation. This point he reiterates in a later paper (ISSEL, 1926).

STEVENS (1910) briefly describes and figures two to three spherical macronuclei in *Boveria subcylindrica*. The normal form has only one large macronucleus. She expresses the opinion that these multi-macronucleate forms were in reality exconjugants because she found them only at times when conjugation was taking place. She believed the "fragments" originated from the old macronucleus, however, and not from products of amphinuclear divisions.

CHEISSIN (1931) in his paper dealing with certain members of the families Ancistrumidae and Boveridae, describes the fragmentation of the macronucleus into seven spheres in both *Tiarella baicalensis* and *Ancistrella choanomphali*. He figures, in consecutive order, organisms having one, two, three and so forth up to seven spheres. He also describes, within these "fragments", large masses of chromatin that stain after the FEULGEN reaction. These he refers to as "nucleoli".

In the light of the investigation on *Ancistruma isseli* above described and the recent work on *Conchophthirius mytili* (KIDDER, 1933 a) I believe it highly probable that the "fragments" described by CHEISSIN will be found to be macronuclear anlagen and the "nucleoli" to be extrusion chromatin. The intermediate stages must be found in order to establish or invalidate this contention. As to the assertions of ISSEL (1903) (1926) concerning macronuclear fragmentation in *Ancistruma isseli* it is quite apparent from the above investigation that the spheres are derived from the products of amphinuclear divisions and not from the fragmentation of the old macronucleus. I believe the spheres described in *A. cyclidioides*, *A. compressum* and *A. tellinae* will also fall into this category.

KAHL (1931) suggests that the spheres described in various species of *Ancistruma* may be the result of endomixis. If one were to interpret what I have called the second and third amphinuclear divisions as second and third micronuclear divisions of a single form then this suggestion would seem to be well founded. I do not believe this to be the case, however, as the appearance of these amphinuclear divisions was noted only during epidemics of conjugation. The old macronucleus was seen to have the same approximate shape and position as the macronuclei in the members of the conjugating pair.

A few cases of multiple vegetative macronuclei are recorded among the ciliate commensals of molluscs. One definite case is that described by Rossolimo and Frau Jakimowitsch (1929) for *Conchophthirius steenstrupii*. That this case is different from that of the reorganization stages is evidenced by the fact that no segregation of the macronuclear spheres occurs. They each undergo fission at cell division with the result that each daughter receives seven macronuclei and one micronucleus.

Maupas (1883) described *Cryptochilum echini* as having one to three macronuclei, but it is clear from the recent work of Daïn (1930) that the individuals so described were exconjugants. *Cryptochilum echini* is very similar to *Ancistruma isseli* in its reorganization. Macronuclear differentiation takes place after the second amphinuclear division, however, instead of after the third, and there does not appear to be any chromatin elimination. Three macronuclear anlagen and one micronucleus are formed from the four products of the second amphinuclear division. The macronuclear anlagen are segregated out during subsequent cell divisions while the micronucleus undergoes mitosis.

It is quite apparent that the conjugation and reorganization of *Ancistruma isseli* follows the type shown by *Conchophthirius mytili* (Kidder, 1933 a). In the latter form differentiation of the macronuclear anlagen takes place after the fourth amphinuclear division while in *Ancistruma isseli* the anlagen are differentiated after the third. The extrusion chromatin forms in a much more regular fashion in *Conchophthirius* than in *Ancistruma* but the method of extrusion is the same.

The ciliates fall into definite groups as regards the time after conjugation of macronuclear differentiation. Summaries of these groups are to be found in the paper on *Uroleptus mobilis* by Calkins (1919) and in the work of Dogiel (1925). To bring the list up to date I shall review the observations of numerous investigators on this point.

Group A. Macronuclear anlagen differentiated after first amphinuclear division.

To this group belong *Chilodon uncinatus* (Enriques, 1908) (Mac Dougall, 1925), *Opisthotrichum janus* and *Cycloposthium bipalmatum* (Dogiel, 1925), *Prorodon griseus* (Tannreuther, 1926), *Balantidium* (Scott, 1927), *Dileptus gigas* (Visscher, 1927) and *Metopus sigmoides* (Noland, 1927).

In *Balantidium* (SCOTT, 1927) states that occasionally two divisions of the amphinucleus, instead of the normal one, take place before differentiation. Only one of these four products becomes a micronucleus while the other three enlarge and become macronuclear anlagen. These anlagen presumably segregate at subsequent divisions.

In *Metopus sigmoides* only one exconjugant is viable, according to NOLAND (1927). This is the one that receives all the pronuclear material and some of the cytoplasm of its partner. As a usual thing only one pair of pronuclei fuse, the others disintegrating. Occasionally, however, four products are seen. These, Noland thinks, are the result of the formation of two amphinuclei, each of which has divided once.

Group B. Macronuclear anlagen differentiated after second amphinuclear division.

To this group belong *Didinium nasutum* (PRANDTL, 1906), *Glaucoma scintillans*, *Leucophrys patula*, *Spirostomum teres*, *Euplotes charon* and *Onychodromus grandis* (MAUPAS, 1888), *Lionotus fasciola* (PROWAZEK, 1899), *Stylonychia pustulata* (PROWAZEK, 1909), *Paramaecium bursaria* (HAMBURGER, 1904), *Blepharisma undulans* (CALKINS, 1912), *Stentor coeruleus* (MULSOW, 1913), *Cryptochilum echini* (DAÏN, 1930), *Oxytricha fallax* (GREGORY, 1923), *Collinia* (*Anoplophrya*) *branchiarum* (COLLIN, 1909), *Collinia* (*Anoplophrya*) *circulans* (BRUMPT, 1913), *Dogielella sphaerii* (POLJANSKY, 1926), *Pleurotricha lanceolata* (MANWELL, 1928), *Uroleptus mobilis* (CALKINS, 1919), *Dallasia frontata* (CALKINS and BOWLING, 1929), *Colpidium colpoda* (HOYER, 1899) and *Euplotes patella* (TURNER, 1930).

In some of the above cases all four of the products of the second amphinuclear division remain functional, while in others one or two degenerate. Those showing no degeneration are *Didinium*, *Glaucoma*, *Leucophrys*, *Spirostomum*, *Paramaecium bursaria*, *Stentor coeruleus*, *Cryptochilum echini*, *Blepharisma*, *Oxytricha* and *Collinia branchiarum*. Those having one product degenerate are *Lionotus*, *Stylonychia*, *Colpidium*, *Dogielella*, *Pleurotricha* and *Uroleptus*. Those having two products degenerate are *Euplotes patella*, *Euplotes charon* and *Collinia circulans*.

It should be noted that the first amphinuclear division is said to differentiate the macronuclear anlage in *Euplotes patella* (TURNER, 1930) but both the macro- and micronuclear anlagen proceed to a second division, one product of each degenerating.

Group C. Macronuclear anlagen differentiated after third amphinuclear division.

To this group belong *Cryptochilum nigricans*, *Vorticella nebulifera* and *Vorticella monilata* (MAUPAS, 1888), *Opercularia coarctata* (ENRIQUES, 1907), *Carchesium polypinum* (POPOFF, 1908), *Ophrydium versatile* (KALTENBACH, 1915), *Stentor polymorpha* (MULSOW, 1913), *Paramaecium caudatum* (CALKINS and CULL, 1907), *Ancistruma isseli* (KIDDER, described above), *Loxocephalus* (BEHREND, 1916), *Bursaria truncatella* (POLJANSKY, 1928) and *Paramaecium putrinum* (DOFLEIN, 1911).

*Cryptochilum*, *Vorticella*, *Opercularia*, *Carchesium*, *Ophridium* and *Ancistruma* form seven macronuclear anlagen and one micronucleus and there is no degeneration. In *Cryptochilum* the seven macronuclear anlagen are said to fuse to form the new macronucleus, but in the remaining species the seven anlagen are segregated during subsequent cell divisions, the micronucleus dividing each time.

In *Paramaecium* four of the eight amphinuclear products become macronuclear anlagen while four become micronuclear anlagen. All products are functional according to CALKINS and CULL (1907), while MAUPAS (1888) and DOFLEIN (1911) believe that three of the micronuclear anlagen degenerate.

In *Loxocephalus* BEHREND (1916) described seven macronuclear anlagen (placenta) being formed but varying numbers of these are said to be resorbed. He described "pseudonucleoli" being given off into the cytoplasm from each anlage. These quite certainly correspond to the extrusion chromatin of *Ancistruma isseli* and *Conchophthirius mytili* (KIDDER, 1933 a).

Group D. Macronuclear anlagen differentiated after fourth amphinuclear division.

To this group belong *Paramaecium multimicronucleata* (LANDIS, 1925) and *Conchophthirius mytili* (KIDDER, 1933 a). According to PROWAZEK (1899) *Bursaria truncatella* should be included in this group, but the later work of POLJANSKY (1928) on this species does not confirm PROWAZEK's results (see above).

*Paramaecium multimicronucleata* is peculiar in that three of the products of the second amphinuclear division degenerate. The remaining one divides into two equal parts and these two divide again. Of these four two enlarge and become macronuclear anlagen, while the remaining two form the micronuclear anlagen. The macronuclear anlagen divide once more, forming four, while the micronuclear anlagen divide two more times to form eight products. The first cell division segregates two macro- and four micronuclear anlagen into each daughter. At the next cell division the macro-

nuclear anlagen are segregated again, while each micronuclear anlage divides. Thus the resulting daughters are reorganized, each having one macronucleus and four micronuclei.

The fourth division of the amphinucleus in *Conchophthirius mytili* results in sixteen apparently equal products. There is no degeneration, but twelve to fifteen enlarge and form macronuclear anlagen which segregate at the subsequent cell divisions. The remaining four to one contract and become the micronuclei, which undergo mitosis at each subsequent cell division.

So far no ciliate has been described in which the differentiation of macro- and micronuclear anlagen is delayed beyond the fourth amphinuclear division.

The differentiation of micronucleus and macronuclear anlagen from the eight products of the third amphinuclear division in *Ancistruma isseli* proceeds rapidly. There is a swelling of seven of the products and simultaneously a decrease in nucleic acid, as evidenced by the FEULGEN thymonucleic acid reaction. There appears to be no way of knowing beforehand just which of the eight products will form macronuclear anlagen and which will become the micronucleus. It is conceivable that chance position in relation to some region within the cell may influence development toward a micronucleus (germinal) or toward a macronucleus (trophic). This was suggested by DILLER (1928) as a cause for micronuclear determination in *Trichodina*. If this is so then the determining influence must differ in position with different individuals, as there seems to be no localized general region where the micronucleus becomes differentiated. I have found the micronucleus in a great variety of positions in relation to the macronuclear anlagen and other cell regions.

Too little is known about the chemical and genetic make-up of the protozoan nucleus to allow for any exact interpretation of the meaning of the regular extrusion of chromatin from the macronuclear anlagen. It is perhaps logical to suppose that this chromatin represents the germinal substance and is given off in the purification process of the trophic nucleus. This has been suggested in the case of *Trichodina* (DILLER, 1928) and *Conchophthirius mytili* (KIDDER, 1933 a). It is necessary to assume, therefore, that the amphinucleus contains both germinal and trophic substances and that the new micronucleus and the micronuclei of the generations that follow are also composed of both substances or at least the potentiality of forming them. Some evidence for this assumption is found in the

fact that during endomixis the micronuclei may give rise to macronuclei (WOODRUFF and ERDMANN, 1914; DILLER, 1928) but in amicronucleate races (DAWSON, 1919; WOODRUFF, 1921) the macronucleus is incapable of forming the germinal nucleus. It is possible, therefore, to compare the separation of germinal chromatin from the trophic chromatin as it occurs in the different types of ciliates. In *Ancistruma isseli*, *Conchophthirius mytili* (KIDDER, 1935 a) and *Loxocephalus* (BEHREND, 1916) this separation takes place by a casting out of chromatin. This extrusion chromatin I believe to be comparable to the degenerating amphinuclear products of many forms (*Uroleptus mobilis* CALKINS, 1919; *Pleurotricha lanceolata* MANWELL, 1928; etc.). In the forms where no degeneration occurs (*Chilodon uncinatus* MACDOUGALL, 1925; *Prorodon griseus* TANNREUTHER, 1926; etc.) one must suppose that the separation of germinal from trophic materials takes place during an amphinuclear division and the division is, at least qualitatively, heteropolar.

If the above speculation is correct it may be possible to compare the extrusion of chromatin in *Ancistruma isseli* to the chromatin diminution that occurs in some metazoa. If the extrusion chromatin is germinal and the nuclei sloughing it off are no longer capable of sexual activity then *Ancistruma* might be considered to follow the *Ascaris* type (BOVERI, 1899). In *Ascaris* the nucleus of one of the blastomeres in the two cell stage undergoes diminution whereby a large amount of chromatin is cast into the cytoplasm to disintegrate. The products of this blastomere always form soma. The blastomere which does not undergo diminution is capable of forming germ cells. In the fly, *Miastor* KAHLE (1908) and HEGNER (1912) have described a similar case of chromatin diminution, but somewhat more delayed than in *Ascaris*. Here diminution takes place in the third and fourth cleavages. Only the primordial germ-cell and its descendants contain the whole compliment of chromatin, while the cells casting out chromatin are no longer capable of developing into germ cells. In other cases this process of diminution is delayed still farther until in the beetle *Dytiscus* (GIARDINA, 1901) it occurs in the last four divisions of the germ line, while in some of the Lepidoptera (*Phragmatobia*, *Orgyia* and *Limantria*) SEILER (1914) has described diminution as occurring in the first polocyte division during the maturation of the egg. In this case it appears to be the "trophochromatin" that is eliminated.

It should be pointed out that there exist many differences of opinion as to the theoretical interpretations of diminution in the

metazoa. FOGG (1930), in discussing chromatin diminution in *Ephestia kühniella* says that "diminution plays no primary or essential part in differentiating the germ line from the somatic. It is rather a by-product of conditions existing in the cytoplasm which may vary widely in different species in respect to the time of its occurrence, its modus operandi, and its physiological significance".

It is obvious that no definite conclusions can be drawn as regards the comparison of chromatin extrusion in protozoa with chromatin diminution in the metazoa, at least until more data concerning its frequency, time of occurrence, and chemical significance in both groups are obtained.

## Summary.

1. The conjugation and reorganization of *Ancistruma isseli*, a ciliate commensal in the horse mussel, *Modiola modiolus*, is described.

2. Conjugation occurs frequently and in epidemics. The conjugants are of similar size.

3. The maturation divisions seem to follow the usual ciliate type, reduction taking place in the second division. Two pronuclei are formed in each cell.

4. The first amphinuclear division proceeds before the conjugants separate.

5. Three divisions of the amphinucleus take place rapidly, giving rise to eight apparently equal products. Of these one becomes the micronucleus and the other seven swell and become the macronuclear anlagen.

6. The macronuclear anlagen are segregated out during subsequent divisions of the exconjugant, the micronucleus undergoing mitoses.

7. Spheres of chromatin, interpreted as germinal chromatin, are extruded from the macronuclear anlagen at each cell division during reorganization. These disintegrate rapidly and are absorbed into the cytoplasm.

8. The types of macronuclear differentiation and the possible theoretical interpretations of the chromatin elimination process are discussed.

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## Explanation of Plates.

### Plate 1.

All figures are of *Ancistruma isseli,* made with the aid of camera lucida. Magnification $\times$ 753. Abbreviations for methods of fixation: B. Bouin's; G. C. Gilson-Carnoy's; Sch. Schaudinn's; S. A. Alc. sublimate-acetic in 95 per cent alcohol. Abbreviations for stains employed: Bor. Borrel; Feul. Feulgen thymonucleic acid reaction; Heid. Heidenhain's haematoxylin.

Fig. 1. First maturation division. One micronucleus in metaphase and one in telophase. The macronuclei are in the characteristic form. B. Heid.

Fig. 2. Second maturation division. Spindles are somewhat smaller than in the first division and the chromosomes are elongate. B. Heid.

Fig. 3. Third maturation division. Spindles are pointed. Only one spindle in each conjugant. The other products of the perceding division can be seen lying in the posterior cytoplasm. B. Heid.

Fig. 4. Late telophase of the third division during the formation of the pronuclei. B. Heid.

Fig. 5. First amphinuclear division. The macronucleus is quite ragged and is becoming clumped. Various disintegrating masses of chromatin are found in the cytoplasm. S. A. Alc. Feul. (counterstained in Borrel II).

Fig. 6. An exconjugant. Second division of the amphinucleus. Sch. Feul.

Fig. 7. An exconjugant. Later than Fig. 6. Four equal products of the amphinucleus. Sch. Feul.

Fig. 8. Exconjugant. Telophase of third amphinuclear division. Sch. Bor.

Fig. 9. Exconjugant. Eight equal products of the third amphinuclear division. Sch. Feul.

Fig. 10. Exconjugant. Differentiation of the seven macronuclear anlagen and the micronucleus. The old macronucleus has nearly disappeared. G. C. Feul.

Fig. 11. Exconjugant. The seven macronuclear anlagen have enlarged and there has been an increase in nucleic acid. G. C. Feul.

Fig. 12. First division of the exconjugant. The micronuclei have divided but the macronuclear anlagen are segregating. Large spheres of chromatin are being extruded from each anlage into the cytoplasm. Sch. Feul.

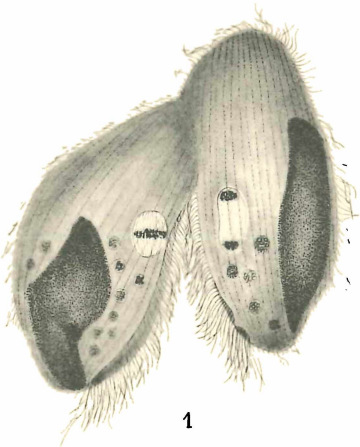
Fig. 13—14. Products of the first exconjugant division. One has four macronuclear anlagen and one has three. The micronucleus of each is preparing to divide. There is a migration of chromatin granules within each anlage toward the periphery. Sch. Bor.

Fig. 15—16. Second exconjugant division. In these divisions chromatin spheres are extruded from each macronuclear anlage. S. A. Alc. Feul.

Fig. 17. The result of the second exconjugant division. The position of the macronuclear anlagen suggests fragmentation of an original macronucleus. B. Heid.

Fig. 18. Preparation for the third exconjugant division. The micronucleus is undergoing mitosis while chromatin granules are collecting near the peripheries of the macronuclear anlagen. S. A. Alc. Bor.

Fig. 19. Late third division stage. Only small bits of chromatin are extruded from the macronuclear anlagen at this division. The anlagen have increased greatly in staining capacity. G. C. Feul.



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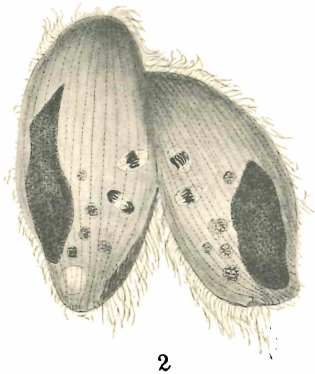

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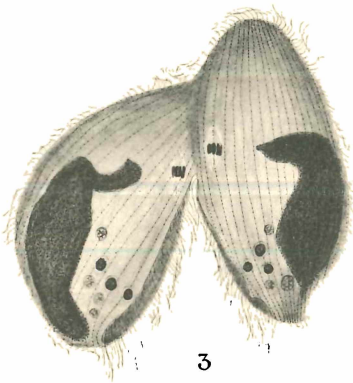

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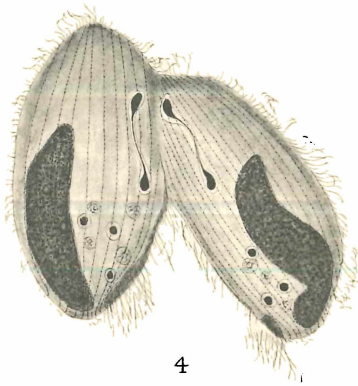

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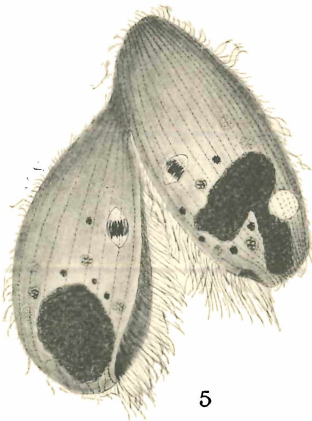

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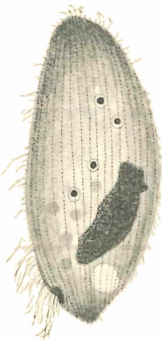

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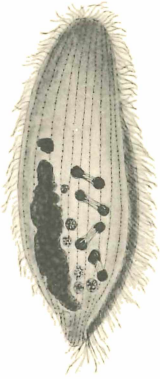

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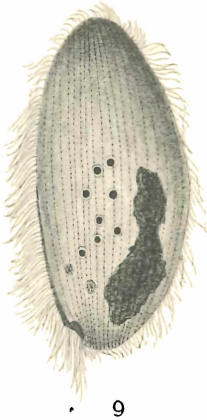

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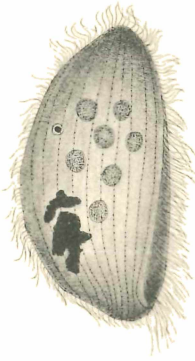

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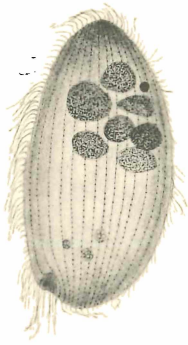

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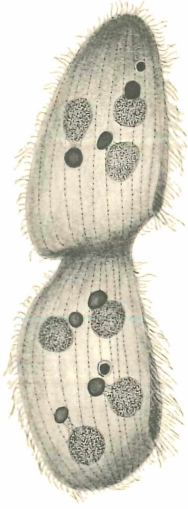

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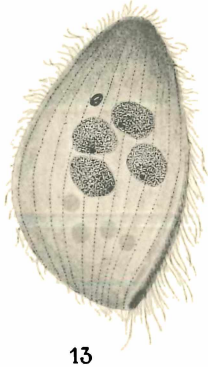

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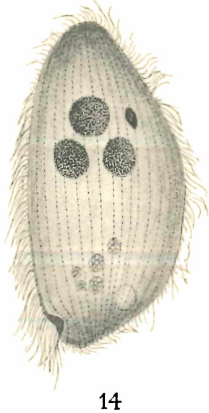

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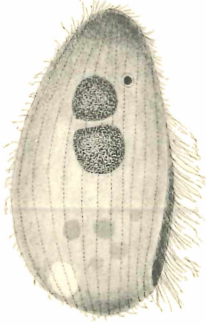

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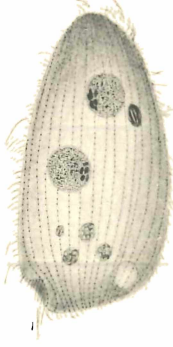

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Fischer in Jena

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