Studies on *Lagenophrys tattersalli* (Ciliata Peritricha, Vorticellinae)

Part II. Observations on Bionomics, Conjugation, and Apparent Endomixis

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INTRODUCTION AND PREVIOUS WORK

THE present paper records the continuation of work on *Lagenophrys tattersalli* Willis, a marine, vorticellinid ciliate found epizoic upon the gill-plates of the amphipod crustacean *Gammarus marinus* Leach (Willis, 1942).

The existence of conjugation ('Copulation', Awerinzew, 1936) in *Lagenophrys* has been known almost from the creation of the genus. Nevertheless, previous accounts of the process are very fragmentary, a result of the acknowledged technical difficulties in studying conjugation in an epizoic, loricate protozoan. In general form, the conjugation of *Lagenophrys* resembles that of other Vorticellinae, and is of the dimorphic type, involving the association of micro- and macroconjugants (cf. Text-fig. 3A). The microconjugant was first observed by Stein (1851). Ubisch (1913) has given the fullest account of the formation of the microconjugant (named by her, variously, as a

'Conjugationsschwarmer' or 'Mikrogamet'). According to this description, based mainly on the freshwater species *L. platei*, the microconjugants are formed by the divisions of an organism produced by *budding* from a normal vegetative individual. Unfortunately the term 'Conjugationsschwarmer' is also applied by Ubisch to this bud. Awerinzew (1936) distinguished two types of microconjugant ('grossen' and 'kleinen' Gameten) in an unnamed, freshwater lagenophryid which was found in West Africa on the crustacean *Telphusa*. These microconjugants are described as being formed from two types of organisms (respectively, the 'grossen' and 'kleinen' Gametocyten) produced by the unequal *fission* of a normal vegetative individual. Since the terminology of Ubisch and Awerinzew is both confusing and inexact, the term 'protoconjugant' is applied in the present work to the organism which divides to form the microconjugants (cf. Text-fig. 2A–C). In the study of *L. tattersalli* special attention has been paid to the formation and subsequent division of the protoconjugant, and a comparison has been made between protoconjugant formation and first-type division (as previously described, Willis, 1942).

In previous work on *Lagenophrys*, little attention has been paid to the nuclear phenomena of conjugation. Awerinzew (1936) alone gives a brief description of the formation of the pronuclei and the synkaryon. Similarly, there is no record of the divisions by which the macronuclear Anlagen, formed from the synkaryon, are distributed among the lineal descendants of the synconjugant (exconjugant). For this reason special attention has been paid to these distributive divisions in *L. tattersalli* where they have an added interest at the period of ecdysis, when they conform to the pattern of a special type of obligatory division (second-type division, Willis, 1942).

There is no record of any endomictic process in *Lagenophrys*, although it may be questioned whether some of the stages figured and described by some earlier workers are not, in fact, stages in endomixis. The difficulty in distinguishing between possible endomixis and conjugation consists in (i) the relatively early fusion of the two partners in conjugation, thus obscuring the distinctive form of the process, and (ii) the fact that the organisms cannot be isolated and cultured away from the host. No attempt has been made in previous work to follow the alternative method of study, namely to trace the behaviour of known individuals upon isolated gill-plates subjected to continual irrigation. The latter method has been employed in the present work.

In conclusion attention must be drawn to certain descriptive terms used in the following account. The surface of the organism applied to the gill-plate, and the free surface, are referred to as the lower and upper surfaces respectively. The diameter passing through the middle of the lorica mouth (oral region) is distinguished as the main diameter, with oral and aboral extremities. The main diameter, together with the diameter at right angles to it, divides up the body into quadrants which are referred to as the right and left oral, and the right and left aboral quadrants.

Methods and Material

The occurrence and collection of the host and the method for examining living material for prolonged periods by means of Kitching's (1934) irrigation apparatus have been described previously (Willis, 1942).

The difficulties in studying conjugation in *Lagenophrys* may be overcome, in part, by removing the gill-plates from the host, and setting up preparations for continuous irrigation under the microscope. The outline of the gill-plate and the position of the epizoites may then be recorded by means of a camera lucida. In this way the behaviour of conjugants (and other stages) can be followed for periods ranging from 3 to 6 days, and the early phases of conjugation can be distinguished from endomictic or other processes of nuclear reorganization. The sequence of observations on living organisms is interrupted by the first distributive division of the synconjugant, since, as yet, no means has been devised for following the swarmer after its liberation from the parent lorica.

The sea-water used for culturing the hosts, and for the irrigated cultures, was renewed every third day by samples brought directly from the shore at Swanbridge, on the Bristol Channel, where the material was collected.

Material was extracted from the cultures at various stages and fixed in Schaudinn, Champy, or mercuric chloride plus acetic acid for later cytological study.

Since the presence of a lorica made it difficult to obtain an even differentiation, regressive stains, like Heidenhain's iron-alum haematoxylin, were not wholly satisfactory. Diluted Delafield's haematoxylin (1 part stain to 5–10 parts of distilled water), used progressively, gave good pictures of the nucleus, especially in metamorphosing forms. In most cases, however, Feulgen's 'Nuclealfärbung' proved to be the most satisfactory process for demonstrating the nuclear material sharply and evenly. Despite the value of the Feulgen process for nuclear studies, an unqualified reliance upon the method in the study of the complex nuclear phenomena of conjugation does not appear justifiable at the present time. As a result, to assess the value of the method, the Feulgen preparations have been compared, at all critical stages, with preparations made with orthodox nuclear stains. From this comparison, it is clear that the chromatin (basichromatin) is stained energetically by the Feulgen method until the final dissolution of the necrochromidium.

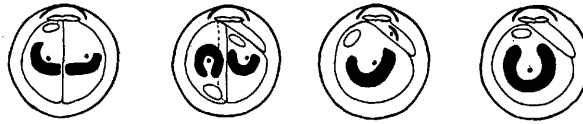
To apply the Feulgen method, the gill-plates were taken, after fixation in mercuric chloride plus acetic acid, and transferred to a little sieve made of cheese-cloth tied around a glass ring (diameter 1 in., depth $\frac{1}{2}$ in.). In this way the preparations can be removed from one fluid to another with the minimum loss of time. This is a great advantage in controlling the critical hydrolysis in N.HCl. Despite the presence of a lorica, the optimum time for the latter is 4 minutes, as in the usual technique for sections of material fixed in mercuric chloride plus acetic acid (Gatenby and Painter, 1937). Some of the preparations were counterstained in light green to give a sharp differentiation of the background.

Estimates of the percentage of the infection transferred from one instar to another of the host were made by taking the moulted cuticles from isolated individuals, and counting the number of empty loricae; the new instar was then killed, after an interval of 3 days, and the new infection counted.

The figures were made with an Abbe camera lucida with a Zeiss oil immersion lens ($\frac{1}{12}$ inch, N.A. 1·30).

Bionomics

Like all species of its genus, *L. tattersalli* is a sessile epizoite. It is restricted to the gill-plates of its host, and occurs most abundantly upon the inner



Text-fig. 1. *Lagenophrys tattersalli.* Diagrams illustrating various modes of the divisions occurring between moults and at ecdysis (based on Willis, 1942).

A. 'First-type' division. This normally occurs in the inter-moult period and produces a swarmer (on the left) and a residual organism. The swarmer escapes while the residual organism remains behind in the lorica (thick black outline), grows, and repeats the 'first-type' division process until the ecdysis of the host. B. Mode *a* of 'second-type' division, involving the rapid succession of 'first-' and 'second-type' divisions. The latter is unequal and produces a small residual organism. Two swarmers are thus produced and both escape. The small residual organism remains in the lorica and degenerates. Occurs at ecdysis. C. Mode *b* of 'second-type' division involving the unequal fission of entire organism (i.e. without an immediately preceding first-type division). The micronucleus divides equally, the macronucleus unequally. Occurs at ecdysis. D. Mode *c* of 'second-type' division. The nuclei do not divide. Occurs at ecdysis.

Macronucleus as a broad band (black), micronucleus as a dot (black), peristome as an ovoid outline in swarmer, lorica as a heavy outline. Ciliation omitted.

surfaces of these structures. The reproductive bodies, or swarmers, are 'hypotrichous' forms, adapted for moving over surfaces. They do not appear to become pelagic at any time. From the surface-dwelling habits of the swarmers and the fact that the entire colony is removed periodically by the ecdysis of the host arise two unusual problems for *L. tattersalli*: (i) the problem of maintaining the infection from instar to instar of the host, and (ii) the problem of securing the initial infection of the host. No attention has been paid to these problems in previous work on the genus.

(i) The onset of ecdysis in the host is correlated with the occurrence of second-type divisions of the protozoon (Willis, 1942). Each of these produces a normal swarmer and a small residual body. The various modes of second-type divisions and their relationships to the first-type divisions normally occurring in the inter-moult period are set out diagrammatically in Text-fig. 1. Since all the organisms composing the infection respond to the onset of ecdysis in this way, the whole colony is mobilized when the old cuticle is shed.

The swarmers may then be observed moving freely over the surface of the host. Access to the surface of the underlying new cuticle is provided by splits which appear in the old cuticle before the latter is finally shed. The transference of the infection is not complete, but appears to be about 50–70 per cent. effective. The influence of the obligatory, second-type divisions upon the form of the distributive divisions of the synconjugant is discussed later (*see* Conjugation).

(ii) The initial infection of the host by *L. tattersalli* takes place during the breeding periods of the former. During these periods some of the swarmers emerging from loricae on the gill-plates of female hosts appear to migrate into the brood-pouch which is developed by the latter during breeding periods. If hatching of the eggs contained in the pouch has already occurred, some of the migrating swarmers settle down on the gill-plates of the young gammarids. The brood-pouch is formed by the overlapping oostegites occurring on the anterior thoracic appendages of the female host, and since these oostegites lie close to the heavily infected inner surfaces of the anterior gill-plates of the parent amphipod, a direct pathway is provided for the migration of the swarmers into the brood-pouch. The actual penetration of the swarmers into the pouch has not been observed, but inspection of the gill-plates of young gammarids extracted from the pouch shows that a high proportion are always infected. This initial infection is always low and rarely consists of more than 4–6 specimens of *L. tattersalli* upon a single young gammarid. It follows from this observation, that the colonies of *L. tattersalli* on the gill-plates of a single host are likely to be highly clonic, i.e. to consist of the descendants of the relatively few swarmers which transmit the infection from host to host.

Conjugation

Formation and Division of the Protoconjugant

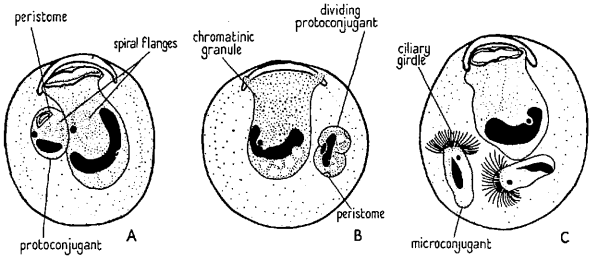
Conjugation in *L. tattersalli* resembles that of other Vorticellinae and involves the association of two dissimilar conjugants—a larger, non-motile macroconjugant and a smaller, motile microconjugant (Text-fig. 3A). Before the attachment of the microconjugant the macroconjugant resembles a normal vegetative organism in its general morphology, and in the apparent characters of the nucleus. On the other hand, the microconjugant is a specialized organism formed by the binary fission of a protoconjugant (Text-fig. 2A–C).

The protoconjugant is formed by the unequal fission of a vegetative organism (Text-fig. 2A). The plane of cleavage passes through the left oral and aboral quadrants, parallel to the main diameter. In first-type division, cleavage is along the plane of the main diameter.

The first stage in division is the movement of the micronucleus along the anterior border of the macronucleus to a point about two-thirds to three-quarters of the length of the macronucleus from its right extremity. This point marks the position of the future cleavage plane. In first-type division

the micronucleus moves in a similar direction to a point midway along the anterior border of the macronucleus.

The adoral spiral of the parent becomes contracted and the cilia and undulating membranes disappear. Before cleavage is completed structures which resemble the peristome, vestibular cavity, and spiral flange of the early swarmer (Willis, 1942) appear in the protoconjugant (Text-fig. 2A). In the swarmer these structures are formed by the division of the parental peristome, vestibular cavity, and adoral spiral, respectively. There is no con-



TEXT-FIG. 2. Stages in microconjugant formation. (×480.) A. Late stage in protoconjugant formation. B. Division of the protoconjugant. From the lower surface. C. Two mature microconjugants within the lorica. Ciliation not visible in the parent organism, and may be absent at this stage. (Feulgen preparations.)

clusive evidence to show whether the peristome, vestibular cavity, and spiral flange of the protoconjugant arise *de novo*, or by division, from the parent.

There is no evidence, in *L. tattersalli*, for the formation of the protoconjugant by budding, as described by Ubisch (1913) for *L. platei*. In the unnamed species described by Awerinzew (1936) the large protoconjugants are formed by a cleavage resembling that which invariably occurs in *L. tattersalli*. On the other hand, the small protoconjugants are said to be formed by a cleavage which runs obliquely across the left aboral quadrant. There is no indication of this type of cleavage in the formation of the protoconjugant in *L. tattersalli*, nor of any dimorphism in size among the protoconjugants. Neither Ubisch (1913) nor Awerinzew (1936) appears to have observed any cytoplasmic differentiation of the protoconjugant which may be compared with that described above for *L. tattersalli*.

At the end of cleavage the protoconjugant separates from the parent organism. At a comparable stage in the development of the swarmer free cilia are formed upon the spiral flange; this never occurs in the protoconjugant. The latter also differs from the mature swarmer in the absence of a sucker cavity and ciliary girdle on the lower surface.

After a short period of free existence the protoconjugant divides within the lorica (Text-fig. 2B) to produce two organisms which transform directly into the functional microconjugants. Before this division the micronucleus takes

up a position midway along the peristomal border of the macronucleus. The cleavage plane passes through this point and the middle of the peristome, and thus, in its general orientation, resembles the cleavage plane of first-type division. In each young microconjugant, a peristome and a small spiral flange are present. Similar structures were not observed by Ubisch (1913) nor by Awerinzew (1936) in the microconjugants of the species studied by them. It is likely that the peristome and the spiral flange are formed by the division of the comparable structures in the protoconjugant, although the evidence for this is not complete.

After the separation of the two young microconjugants, the peristome and the spiral flange disappear in each, and both organisms become elongated (Text-fig. 2c). A ciliary girdle develops around the apical extremity of each microconjugant, in contrast to the swarmer, where the ciliary girdle develops on the lower surface.

It may be concluded from the above account that in *L. tattersalli* the microconjugants are formed by two successive divisions, each of which is comparable, at least in the orientation of the cleavage plane, to first-type division.

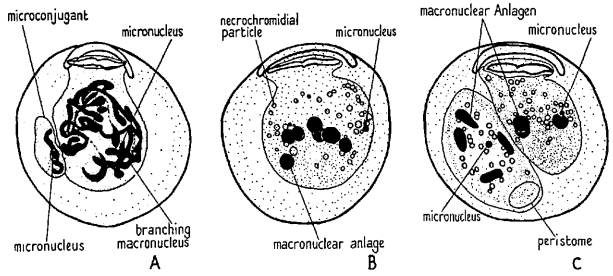
Behaviour of the Microconjugant

After the formation of the ciliary girdle the microconjugants remain within the lorica of the parent for varying periods (in one case for 2 days) before finally escaping. They usually show slow rotating movements. In those cases in which the formation of the microconjugants was traced in life from the protoconjugant, the sequence of observations ended with the escape of the microconjugants through the mouth of the lorica. In many cases, however, the organisms died before escaping. As a result the possibility that some of the microconjugants may fail to escape, and may conjugate with the parental organism, cannot be excluded. In view of the observed escape of some of the microconjugants this conclusion may appear rather fine-drawn. Nevertheless, it is enhanced by the following facts and considerations: (i) The microconjugants are pelagic organisms and are not adapted for moving over surfaces. It is thus difficult to see how they can maintain themselves on the gill-plates while exposed to the respiratory current of the host. It can hardly be supposed that any chemical attraction is exerted upon them by the macroconjugant, since any substance diffusing from the latter would not remain localized. (ii) In a high proportion (about 50 per cent.) of all preparations of early conjugating stages the functional microconjugant is accompanied, in the lorica, by a degenerating body which is clearly comparable, in many cases, to a microconjugant. In view of the above consideration (i), it seems probable that, in such cases as these, the two microconjugants formed from a protoconjugant have been retained within the lorica, and that one has become a functional microconjugant while the other degenerates. (iii) In *L. platei* Ubisch (1913) states that the microconjugant enters the macroconjugant by boring through the lorica. No evidence for this mode of entry has been found

in any living or fixed specimen of *L. tattersalli*. From (i), (ii), and (iii) above it seems reasonable to conclude that the possibility of automictic (paedogamic) conjugation is supported by circumstantial evidence. Among the peritrichous ciliates, paedogamic conjugation has been observed by Enriquez (1907) in *Opercularia*.

Nuclear Phenomena in the Micro- and Macroconjugants

In living material the first sign of impending conjugation is the rapid darting movement of the microconjugant towards the surface of the left



TEXT-FIG. 3. Stages in conjugation up to the first distributive division. Ciliation omitted. (×480.)

A. Early stage in the association of the conjugants. The macronucleus of the macroconjugant has the branching form which is developed shortly before its fragmentation. B. Mature synconjugant. The micro- and macroconjugants have fused. C. Appearance of the first distributive division in the inter-moult period. (Feulgen preparations.)

aboral quadrant of the macroconjugant. Although there is no visible differentiation of the macroconjugant in this area, the fact that the movements of the microconjugant are always directed towards it leads to the supposition that it must exert some attraction. It is physically possible for the microconjugant to attack any point on the surface of the macroconjugant. The movements of the microconjugant are brought about by the concerted, backward sweep of its cilia.

The microconjugant eventually becomes attached to the macroconjugant by its apex, and not by its side. This is in contrast to the behaviour of the smaller organism produced in apparent endomixis (*see* Observations on Apparent Endomixis). The cilia of the microconjugant later disappear.

The cytological study of specimens at this early stage shows that there are no preparatory nuclear changes in either conjugant. This observation is of some importance, since Ubisch (1913) concluded that in *L. platei* there were preparatory nuclear changes in the macroconjugant, before the union of the latter with the microconjugant. The evidence for this conclusion is criticized later (*see* Metamorphosis and the Later Distributive Divisions).

After the disappearance of the cilia from the microconjugant the protoplasm of both conjugants becomes confluent at the point of attachment. The

micronucleus of each conjugant moves towards the point of confluence and undergoes a series of divisions. The divisions are synchronous in both conjugants, and are exactly comparable to those observed by Awerinzew (1936) in *Lagenophrys* sp. and to the maturation divisions of other Vorticellinae. In *L. tattersalli*, during the maturation divisions, the chromatin appears finely dispersed through the substance of the nuclei and no chromosome-like structures are visible. After the exchange of the migratory pronuclei, a synkaryon is formed in the macroconjugant. No synkaryon was observed in the microconjugant. The nuclear behaviour of the two conjugants ceases to be synchronous after the exchange of the pronuclei. In the microconjugant the latter quickly degenerate and disappear. This degeneration is accompanied by the absorption of the microconjugant by the macroconjugant. In view of this it seems preferable to describe the macroconjugant, subsequently, as a syn- rather than ex-conjugant. Further, in view of the fusion of the conjugants and the formation of only one functional synkaryon there is some justification for describing the sexual process of *Lagenophrys* as copulation (as defined by Doflein, 1929), rather than as conjugation.

After the attachment of the conjugants the macronucleus of the macroconjugant undergoes a remarkable transformation which has not been observed in any other species of *Lagenophrys*, and which has no exact counterpart in other ciliates (Text-fig. 3A). The macronucleus throws out branching processes which appear to become drawn around in the cytoplasm. In this process parts of the branches become attenuated and finally break, so that large separated fragments of the macronucleus become dispersed throughout the cytoplasm. At this stage there occurs a further fragmentation of the macronuclear substance and, as in conjugation and endomixis in other ciliates, an extensive necrochromidium is formed. This consists of a large number of small vesicles, each with a cortex of chromatin which surrounds a clear space.

The Maturation and the First Distributive Division of the Synconjugant

After syngamy the synkaryon moves into a central position and undergoes three successive divisions to form eight nuclei (as in *Vorticella*, Maupas, 1888, and *Carchesium*, Popoff, 1908). Seven of these become macronuclear Anlagen, one forms a micronuclear Anlage. Before dividing the synkaryon increases in size and becomes a somewhat ovoid vesicle. In Feulgen preparations, this is seen to consist of a pale-staining matrix of finely dispersed chromatin containing larger granules which stain intensely.

In most cases the divisions of the synkaryon occur in rapid succession and are synchronous. Therefore, in later stages of maturation, when the chromatin content of the Anlagen increases, the latter differentiate uniformly. The micronuclear Anlage later decreases in size to the dimensions of a normal micronucleus and its chromatin becomes condensed, the entire Anlage appearing progressively more homogeneous and deeply staining after Feulgen. In the mature macronuclear Anlagen the chromatin appears coarsely granular

and is arranged in a network. The general appearance of the mature synconjugant is shown by Text-fig. 3B. The macronuclear Anlagen and the micronucleus usually take up a position which roughly corresponds to the positions of a macro- and micronucleus in a normal, vegetative organism.

In certain cases the successive divisions of the synkaryon appear to occur less rapidly and without synchronization. This condition is indicated by the small number of maturing synconjugants in which the members of each set of macronuclear Anlagen show variation in size, and in chromatin concentration.

Between moults the first distributive division of the mature synconjugant is always a first-type division which is normal in all but the condition of the macronuclear material. The micronucleus moves into a central position on the main diameter and determines the position of the future cleavage. The seven macronuclear Anlagen become segregated into groups of three and four. Usually the group of four Anlagen passes to the swarmer (Text-fig. 3C), the remainder being retained in the residual organism. The lipoid and fatty reserve materials are either divided equally or segregated in the swarmer, as in normal, non-distributive first-type divisions (Willis, 1942). The food vacuoles are retained in the residual organism while the necrochromidium is divided equally between the swarmer and the residual organism. Since all these inclusions (i.e. fat, lipoid, food vacuoles, and necrochromidium) are uniformly dispersed throughout the cytoplasm before division, it is clear that simple cleavage cannot account for the independent segregations which have been observed. As yet no explanation can be given of this feature of the division process. At the end of cleavage the macronuclear Anlagen of the swarmer become elongated. This change of form seems to be comparable to the elongation and attenuation of the macronucleus in the non-distributive swarmer of asexual reproduction (Willis, ibid.).

At ecdysis the obligatory second-type division cuts across the normal progress of maturation, and may considerably modify the character of the first distributive division. In consequence conditions are often observed which appear to have no parallel among the free-living ciliates. A comparison with the behaviour of other ciliates which are epizoic upon arthropods would be of interest, but this is impracticable owing to the lack of previous work on the responses of the epizoites to the ecdyses of their hosts.

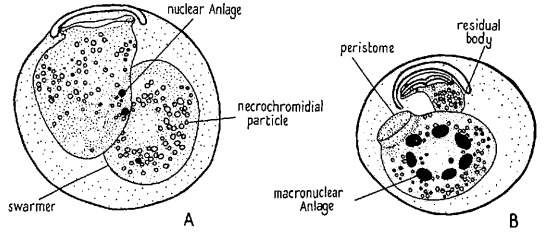
The behaviour of the synconjugant at ecdysis may be considered in two categories, (i) when the synconjugant is mature at ecdysis, and (ii) when it is immature at ecdysis.

(i) The mature synconjugant has been found to respond to ecdysis by modes *a* and *c* of second-type division.

In mode *a* responses (i.e. when there is a rapid succession of first- and second-type divisions) the first-type division is usually comparable to that which normally occurs in the inter-moult period. The following second-type division then produces a swarmer with three Anlagen, and a small residual organism.

In mode *c* responses (Text-fig. 4B) the seven macronuclear Anlagen become grouped in a circle which occupies a position similar to that occupied by the horseshoe-shaped macronucleus of an asexual swarmer. The micronucleus lies centrally, within the macronuclear Anlagen. The latter are ovoid in the distributive swarmer. The small residual organism contains a small portion of the necrochromidium which is cut off from the main mass by the cleavage plane.

(ii) In cases where the synconjugant is immature at ecdysis the second-type divisions may again be by modes *a* and *c*. The first-type division of an



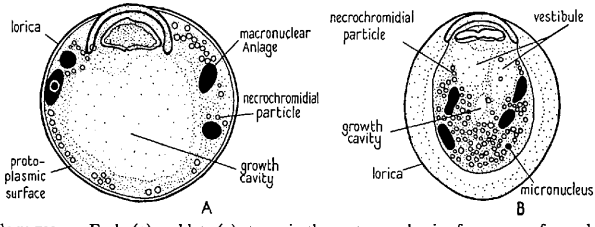
TEXT-FIG. 4. Stages in second-type division at ecdysis, showing modifications of the first distributive division. Ciliation omitted. (×480.)
A. First-type division of a mode *a* response in which one of the divisions of the synkaryon appears to have been cytoclastic. From the lower surface. B. Mode *c* response. (Feulgen preparations.)

incompletely developed mode *a* response is shown by Text-fig. 4A. The swarmer possesses one condensed nuclear Anlage, while two similar bodies are present in the residual organism. These nuclear structures are smaller than the macronuclear Anlagen of a maturing synconjugant. They resemble normal micronuclei in size, but contain less chromatin. In this specimen it seems clear that one of the divisions of the synkaryon has become *cytoclastic*, and has determined the equal, binary fission of the synconjugant. Unfortunately it has not been possible to trace the later behaviour of the swarmers produced by this unusual type of distributive division. It seems reasonable to suppose that the nuclei which are present in such swarmers (and those of the residual organism as well) later divide further, as if they were part of the normal series of nuclei produced by the synkaryon during the intermoult period.

Metamorphosis and the Later Distributive Divisions

Owing to the impossibility of following the path of the swarmer, the lineal descendants of the marked forms kept under observation up to the period of the first distributive division could not be traced. The following account is therefore based upon the seriation of various fixed stages.

The metamorphosis of forms with four Anlagen has been observed frequently (Text-fig. 5A, B). The figured specimens show the metamorphosis of swarmers produced by the first distributive division. The reorganization of the nuclear material produced by conjugation is without effect on the complex process of metamorphosis (as previously described, Willis, 1942), except that (i) the micronucleus may take up various positions, in contrast to normal metamorphosis, when it lies embedded in the right limb of the macronucleus, and (ii) the greatly distended growth cavity appears to exert less pressure upon the scattered macronuclear Anlagen than upon the normal macronucleus, which becomes greatly attenuated during metamorphosis (Willis, 1942).



TEXT-FIG. 5. Early (A) and late (B) stages in the metamorphosis of swarmers formed by the first distributive division. Ciliation omitted from B. (×480.) (Delafield's Haematoxylin, after Schaudinn fixation.)

In vorticellinids generally, three generations are required to distribute the macronuclear Anlagen of the synconjugant. As a result eight organisms are produced, each with a single macro- and micronucleus. In a number of cases, however, forms are found in which the morphologically single macronucleus shows a gross, moniliform character, with two, three, four, or even seven lobes, i.e. with the number of lobes corresponding to the numbers of discrete Anlagen found in normal distributive stages. This may indicate that in certain cases the Anlagen become fused together. If this is so, then certain apparently normal asexual divisions may in fact be distributive.

The necrochromidium persists throughout the series of distributive divisions and, in some cases, even later. The constituent particles retain their vesicular character.

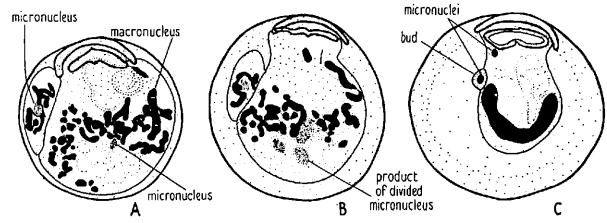
In *L. platei* Ubisch (1913, Text-fig. 44) describes and figures certain specimens which bear a close resemblance to some of the distributive generations of *L. tattersalli*. Ubisch, however, did not consider the distributive divisions, and interpreted these specimens as stages in the maturation of the macroconjugant in readiness for conjugation (Conjugationserwartung). In view of the absence, in *L. tattersalli*, of nuclear preparation in the macroconjugant before its union with the microconjugant, it seems highly probable that the 'Conjugationserwartung' of Ubisch is based upon specimens produced by the distributive divisions of a synconjugant.

Observations on Apparent Endomixis

The Normal Process

The analysis of colonies showing 'epidemic' conjugation always reveals a small number of forms which, to a superficial view, appear to be undergoing conjugation (Text-fig. 6A, B), but which, on closer inspection, show certain differences from the normal mode of that process.

These forms appear to consist of two unequal organisms which never separate. The smaller organism shows some resemblance to a protoconjugant which has failed to separate from its parent. It differs from a protoconjugant in the following characters: (i) the cleavage plane, by which it is formed, runs



Text-fig. 6. A. Early stage in apparent endomixis, showing the formation of the larger and smaller organisms (the smaller organism is on the left-hand side). B. Later stage in apparent endomixis. The micronucleus of the smaller organism is undivided; the micronucleus of the larger organism has undergone two successive divisions. C. Late stage in a process of anomalous micronuclear division. Ciliation omitted. All figures ×480. (Feulgen preparations.)

obliquely across the left oral quadrant of the parent, and (ii) the peristome, vestibular cavity and dexiotropic spiral flange of the typical protoconjugant are absent. The smaller organism differs from a microconjugant in that (i) it is not formed by the binary fission of a protoconjugant, and (ii) it is attached along the whole of its side to the left oral quadrant of the larger organism, and not by its apex to the left aboral quadrant, as in the case of the attachment of the micro- to the macroconjugant in conjugation.

Morphologically, the smaller organism may be regarded as a protoconjugant which has failed to separate from the parent, and which has undergone no further differentiation.

Differences from the process of conjugation are also shown by the behaviour of the nucleus in the larger and smaller organisms. In the first place, the micronucleus of the smaller organism, although it becomes enlarged and vesicular as in the first stages of the maturation of the micronucleus of the microconjugant, does not develop further, and appears to degenerate without division. In the larger organism, the micronucleus takes up a central position. In a macroconjugant, on the other hand, the micronucleus becomes eccentric and moves towards the area of confluence with the microconjugant. Later, the micronucleus of the larger organism becomes enlarged and vesicular, and

divides twice to form four daughter nuclei (Text-fig. 6B). There is no evidence that a third division occurs, as in the case of the dividing synkaryon formed in conjugation. Further differences from the nuclear phenomena of conjugation are also shown in the formation of the necrochromidium. Thus, the filaments produced from the macronucleus are always short and lie, like the vesicles to which they eventually give rise, along the original traverse of the unfragmented macronucleus. The behaviour of the larger organism was not traced beyond the stage represented by Text-fig. 6B. This circumstance may, of course, be owing to a lack of material. On the other hand, it is quite possible that in the later stages the distinguishing morphological feature of the process is obscured by the fusion of the larger and smaller organisms. On the assumption that three of the four nuclei which are formed by the division of the micronucleus in the larger organism become macronuclear Anlagen, the product of the fusion of the larger and smaller organisms would then be indistinguishable from a post-conjugation distributive form with three macronuclear Anlagen.

It is clear from the above that the process under consideration in this section must be sharply distinguished from normal conjugation. From the information available it may be described as an unusual, endomictic reorganization of the nuclear apparatus.

An Anomalous Process of Micronuclear Reduction

At various periods specimens of *L. tattersalli* are found with minute buds attached to the surface of the left oral quadrant, opposite to the left extremity of the macronucleus (Text-fig. 6C). These buds consist of a small mass of protoplasm surrounding a rounded inclusion which resembles a normal micronucleus in size, form, and reaction (to Feulgen and other nuclear stains). This inclusion is undoubtedly a nucleus which is produced by the fission of the micronucleus of the larger organism, to which the bud is attached. This conclusion is based on the discovery of the simple intermediary stages in bud formation. In the first place the parental micronucleus leaves its bay on the right-hand side of the macronucleus, moves along the anterior border of the latter, and eventually reaches a position below the protoplasmic surface in the area where the bud is later formed. The micronucleus divides into two at this point. One of the daughter nuclei, with a small quantity of undifferentiated protoplasm, forms the bud. Whether the latter is formed by true budding—i.e. by an outflow of the protoplasmic surface—or by fission has not been ascertained. The macronucleus remains unmodified throughout the process.

Buds of a similar type, and up to seven in number in a single lorica, were observed by Ubisch (1913) in *L. platei*. Ubisch regarded bud-formation as a process of micronuclear reduction which preceded conjugation. The evidence for this conclusion seems negligible, and to be based on specimens in which the buds occur, in the same lorica, together with a larger vegetative organism which possesses a fragmented macronucleus. In the larger organism,

however, the figures (ibid.) show clearly that the pattern of the nuclear material resembles that of a distributive generation in *L. tattersalli*. It seems likely, therefore, that Ubisch was led into error through failing to recognize the distributive divisions of the synconjugant.

In *L. tattersalli* the buds are never found in association with conjugants or in any constant relationship to a distinctive phase of the life-history. At present the point of importance appears to be that these buds are actually formed by the organism to which they are attached, and that they are not foreign bodies or degenerated microconjugants. The recognition of this fact has not previously been possible owing to the lack of observations on the intermediary stages of bud-formation.

Summary

1. A study of the bionomics of *L. tattersalli* shows that the initial infection of the host is by the passage of swarmers from the gill-plates of the female host to those of the embryo in the brood-pouch. The transmission of the infection from instar to instar is about 50–70 per cent. effective. It is brought about by (i) the mobilization of the entire colony at ecdysis by an obligatory type of division (second-type division), and (ii) by the penetration of the swarmers to the surface of the new instar through splits which appear in the old cuticle as it is shed.

2. The term 'protoconjugant' is applied to the organism from which the two microconjugants are formed by binary fission. The protoconjugant is formed by the unequal fission of the parent, and not by budding as in *L. platei* (Ubisch, 1913). The divisions which produce the proto- and micro-conjugants are compared with the normal asexual reproductive process (first-type division, Willis, 1942).

3. The conjugation process is described. In view of the early fusion of both conjugants, the term 'synconjugant' is proposed for the macroconjugant after its fusion with the microconjugant. There are no visible nuclear preparations for conjugation in either conjugant before their attachment. The evidence for a state of 'Conjugationserwartung' (Ubisch, 1913) is criticized and shown to be based, in all probability, on specimens produced by the distributive divisions of the synconjugant.

4. The term 'necrochromidium' is proposed for the mass of vesicles formed by the degeneration of the macronucleus in conjugation and endomixis. Before the formation of the necrochromidium the macronucleus of the macro-conjugant becomes elaborately branched.

5. The distributive divisions of the synconjugant are described for the first time. Resemblance to other vorticellinids is shown during the inter-moult period of the host. At ecdysis, the distribution of the nuclear Anlagen is adapted to the obligatory second-type divisions which are undergone by *L. tattersalli* at this period. In some cases one of the divisions of the synkaryon is cytoclastic.

6. A process of apparent endomixis is described. In this process unequal fission produces a small organism, resembling a protoconjugant which fails to separate, and a larger organism. The micronucleus of the smaller organism remains undivided and soon degenerates. The micronucleus of the larger organism undergoes two successive divisions to form four nuclear Anlagen.

7. An anomalous process of micronuclear division and reduction is described. One of the micronuclei produced by the division passes into a small bud of protoplasm. Later the latter falls off and degenerates.

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