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Experimental research on the development of Ceratium hirundinella O.F.M.

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INTRODUCTION

The two authors have come together for the joint work under consideration in the pursuit of their special studies, each hoping to obtain solutions to certain questions in his own field of study, through the observation of the processes of germination in the Ceratium cysts.

Through studying the anomalies of form in Cer. hirundinella G. Huber [1] has raised morphological and biological questions, whose solution could only be expected through observation of the hitherto incompletely known processes of the germination of Ceratium cysts, as well as through experimental studies on the germinating cyst. In wider pursuit of his mud studies in Lake Zürich Nipkow [2] questioned the life and development capabilities of the dormant stages of numerous organisms found in the deep mud-cores and thus chanced upon the Ceratium cysts. It was natural for him, therefore, to wish to cause them to develop.

However, the most important mutual aim of study lay in filling in the great gap in our knowledge of the processes of germination in the Ceratium cyst and the early developmental stages in the standing stock of C. hirundinella.

This aim has now been reached, as will be shown below.

Wesenberg-Lund [1] had already been concerned with the germination of Ceratium cysts in 1912. By exposing mud cores from the bottom of a Danish lake, to the bright daylight in glass

1. Huber, G., Formanomalien bei Ceratium hirundinella O.F.M. Internat. Rev. 1914

2. Nipkow, F., Vorläuf. Mitteilungen üb. Untersuchungen d. Schlammabsatzes im Zürichsee. Zeitschrift. f. Hydrologie. I. Jahrgang. Heft 1 u 2 1920 (Aarau).

1. Wesenberg - Lund, C., Studier over de Danske Soeers Plankton. Kopenhagen 1914

dishes, he succeeded in inducing germination and obtained Ceratium from the cysts. He does not give details of the finer processes of germination and of the juvenile stages, so that we must simply be satisfied with the fact that he cultivated Ceratium from cysts. In connection with the question, is there a normal two-horned transitional stage in C. hirundinella, [2] G. Huber, in 1915 took mud from a pond on the Zürichberg (spring water) and added tap water to it. The material proved to be very poor for cysts. A number of empty cyst cases were to be seen and also the emergence of the contents of the cyst observed, but the development of the youngest stages could not be followed with enough certainty.

On the basis of the Nipkow material from Lake Zürich, which mostly contained rich cysts, we now succeeded extraordinarily well in pursuing the consistent development of Ceratium from the cyst to the completed cell. Moreover, a series of experiments were carried out on the cysts and the juvenile stages of Ceratium, which showed very interesting results.

We shall present in I. a general - descriptive part: the normal processes of germination in Ceratium cysts and the development of the juvenile stages, in order to show in II. an experimental part: the changes in form of C. hirundinella under the influence of temperature, light and varying salinities.

General descriptive part

1. The research material.

We used as starting material the mature cysts of C. hirundinella from the stratified cold-water saprobal activated sludge from Lake Zürich mentioned above.

Nipkow[2] showed that the saprobal activated sludge in Lake Zurich at a depth of 100-140m shows a clear annular stratification. The individual layers consist of a pale grey calcium carbonate-bearing summer layer and on account of ferrous sulphide, a blackened winter layer. (Fig. 1) The cysts normally lie on the lower edge of the black layer. Those cysts which are abundantly produced during and probably also as a result of the autumnal fall in temperature, are deposited in Lake Zürich at the onset of the Autumn circulation, and are then covered by Winter sedimentation and remain ultimately buried in the stratified mud. As the temperature at this depth throughout the year rarely exceeds 4 - 5°C, these low temperatures are not sufficient according to observations in situ and the research undertaken, to cause the cysts to germinate. If the spring circulation extends to a depth of about 100m, it finds the greater part of the cysts already buried

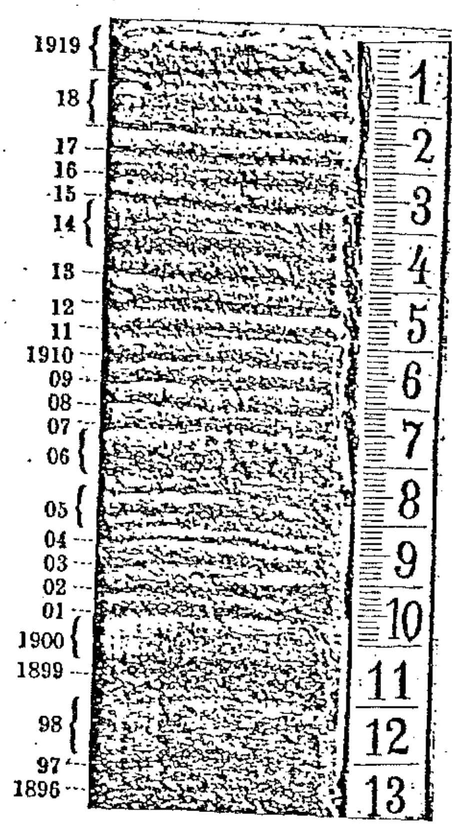

Abb. 1. Geschichteter Kaltwasserfaulschlamm aus dem Zürichsee. Schlammprofil aus 132 m Seetiefe. Schwarze Winterschicht und helle Sommerschicht bilden zusammen eine Jahresschicht. Die Jahrgänge 1918, 1900 und 1898 enthalten Sedimente, die von Uferrutschungen herrühren.

2. loco cit. S. 29 (im Separatdruck).

in the sediment. On the other hand it is obvious, that the cysts from the shallower part of the lake bottom rise once more when in March the full Spring circulation forces higher temperatures down to these depths and allows the cysts to develop on the spot.

The well-established annual stratification allows the age of the cysts to be determined with full certainty. Not all the annular layers contain the *Ceratium* cysts. They were particularly numerously settled in Autumn *1920, 1919*, and 1916, which indicates that both the formation of the cyst and the mass deposition depends upon particularly suitable circumstances. On the other hand one must take into consideration the annual layers in which there are no cysts capable of germination, cysts which are empty or only to be found in very small numbers. One must also consider the possibility that the resting cyst would perhaps be deposited after all but under special circumstances (very strong circulatory currents), but could still have found their way from the benthos into the water body. A proportion of the cysts disappear on sinking.

The assay of the ability of the cysts of particular years to germinate showed a most remarkable fact; that the cysts of *C. hirundinella* from the year 1914 - ∴ 6½ yr old cysts - were still capable *of germination*. Older seasons had lost viability. The duration of ability to germinate of *Ceratium* cysts (for Lake Zürich) can also be set at 6½ years.

Ceratium is surpassed in this respect by *Peridinium cinctum*: the oldest *but still* viable cysts were 16½ years old *(from the year 1904)*. The cold-water saprobal activated sludge in which the cysts lay enveloped for 6½ - 16½ years, shows itself to be a relatively good conserving material. The reason for *Peridinium* cysts remaining viable for considerably longer than the *Ceratium cysts*, may be because, apart from particular properties of the *protoplasts*, the parietal layer of the *Peridinium* cyst is thicker and more resistant than those of *Ceratium*.

Well-formed, viable *Ceratium* cysts occur most frequently in the Autumn stratum of 1919, whi*lst those* in the material of 1914, for example, are seldom encountered. It is, therefore pre-eminently the first-named stratum, which produced the cysts for the study of germination and further experiments. By way of comparison, material from Autumn 1920 and also from other years is used and processed further.

The mud cores themselves were taken from the lake bottom by Nipkow with the help of the Naumann corer which he modified himself, and the mud-core so obtained, further processed by the methods described by him *loc. cit*.

The methods practised by us have the great advantage that the age of the material can be determined exactly, and that an unlimited equivalent material is always available, so that control assays and the continuation of biological experiments at any time is possible.

In the preparation of a culture, several forceps-tips of mud rich in cysts were taken from the desired Autumn stratum, carefully separated and mixed with 50 ccms of fresh spring water (or any particular solution), shaken up in a suitable flask, the flask covered with paper, and then simply left it in diffused daylight. For the control development, at specific intervals, a part of the remaining water was centrifuged and examined under the microscope. Simultaneously the mud sediment was also examined micro scopically through the bottom of the flask. For the purposes of control, as is customary, a similar culture was always set aside.

The average weight of a "forceps-tip-full" is 4 cmm. The cyst count in this mass of mud is actually always rather variable according to the place it is taken from: in earlier years, isolated examples contained less than a dozen. In the season of 1919, however, the cysts were to be found in large numbers; the count was 500 - 600 cysts per forceps-tip.[1] Before each setting-up of the culture, the mud was first tested for its cyst content, in order to supply the necessary solution for the mass of the cysts and the Ceratium to be expected, as also for the types of cyst and their relative numbers. The material was invariably examined live. The examination under the microscope was, when possible, on the slide without the coverslip.

When it concerned very exact observation of particular details, also measurements, a coverslip was put on. Collective drawings were prepared with drawing apparatus which was often particularly difficult with moving forms. The enlargement is the same for all diagrams (with the exception of a single one (Fig.8). The figures are therefore comparable with each other.

2. The resting cysts. Morphology of the cysts.

In the mud of Lake Zürich, 3 types of cyst can be clearly distinguished:

1. Spherical, 3-horned, small cysts (*c.* 50 - 60μ long) and 40 - 45μ wide), with short, crumpled horns. On each cyst, according to its formation, one can clearly distinguish a front and rear, left and right, dorsal and ventral.

1 In the face of the numbers cited above, a proper notion can scarcely be had as to the extent of the Ceratium lost to the lake, through this burying of the cyst. In late Autumn a veritable rain of cysts which cannot again enter the cycle of the plankton world, must sink to the bottom where the stratification of the sediment starts at a depth of about 100 - 140 m (perhaps from 60 - 80 m upwards).

Those cysts reproduced in Fig. 2. are all so placed, that the horn pointing upwards, which as a rule stands upright, corresponds to the apical horn, and that the two rear horns, which here, as a rule are short and bent inwards, correspond to the so-called rear horns. Certainly the horn of the latter coming from deep within (Fig. 2a,b) corresponds to the ant-apical and the higher inserted horn corresponds to the right rear-horn (cysts in dorsal position). The cyst wall is not remarkably thick, has no particular casing (e.g. jelly) and is (in opposition to Schulling [1]) unstratified. In the centre of the cyst reddish brown pigment is accumulated; the edge-zone of the cyst is coloured pale yellowish brown. The whole cyst is full of fine vesicles, probably mostly fat granules (reserve-matter), which cover the chromatophores as well as the nucleus. There were to be seen in the centre a number of well-defined reddish-brown larger vesicles. In this case the otherwise diffusely distributed reddish-brown pigment is accumulated in this vesicle.

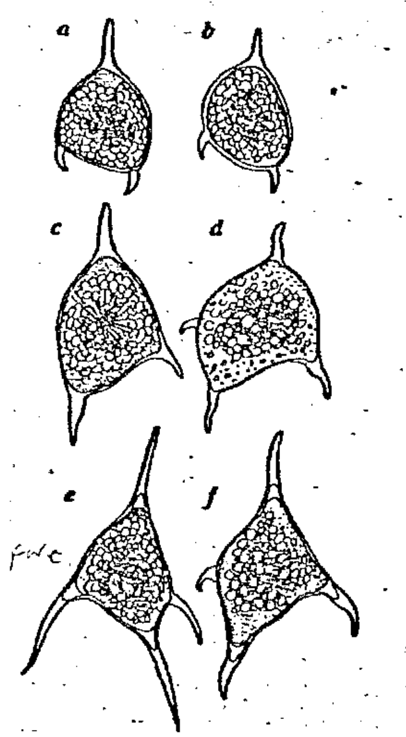

Fig. 2. Resting cysts of Ceratium hirundinella. a and b small, spherical, 3-horned cysts (in dorsal position). c and d large, 3 - and 4 - horned cysts (in ventral position). e and f large, rhomboid, 4-horned cysts (e in dorsal, f in ventral position). The large, fat-grain content can be seen, as well as the central accumulation of pigment and in e - the nucleus.

1. Schulling, J.A. Die Süsswasserperidineen I. -D. Basel 1891.

We could see resulting from these spherical, 3-horned cysts (they are not quite spherical, as the anterio-posterior diameter is rather smaller than the cross-diameter) in our culture, Ceratia, which belong to the group of f.gracile (Bachmann [2]) longer forms, with more or less parallel rear horns, thin habit and relatively narrow width). We describe these cysts as "gracile-cysts".

2. Spherical, 3- and 4- horned, larger cysts 92 - 100μ long (horns included), 50 - 60μ wide. Their form is frequently more attenuated than these mentioned previously; often they are greater in width, particularly in the 4- horned form (Abb. 2 d). Their horns are relatively short, but frequently rather longer, and, particularly the rear horns, less crumpled than in the gracile-cysts. - The centre of the cyst is also coloured diffuse red to reddish-brown; this is followed by a colourless to pale brownish marginal zone. Here and there one can see the rod-shaped chromatophores arranged so that they radiate from the centre (2 c). The contents of the cyst is always strongly granular, and permeated by a considerable number of large and strongly diaptric vesicles (fat-spheres). The nucleus is not, as a rule, visible. The 4-horned cysts (Fig. 2 d in dorsal view) shows as 4 a small horn, which corresponds to the left rear horn (l. postequatorial horn).

Ceratia issue from these larger, spherical 3- to 4- horned cysts, which correspond to the f. austriacum Zederbauer [1] (with wider middle bodies and rather spreading horns).

3. Flat 3-, mostly, however, 4-horned, rhomboid cysts. They are always oblong, angular, the largest of all cysts 100 - 120μ long (+ horns), 80μ wide; to be distinguished from all other cysts by their long, mainly pointed, less crumpled and widely spread horns. The reddish - brown pigment is also again limited to a diffuse colouration at the centre. The margin zone is yellowish-green. Chromatophores are not, as a rule, to be seen. Sometimes the sharpley - defined nucleus is visible (Fig. 2e). That reproduced in Fig. 2 e is the most frequent, that in 2f on the other hand the less frequent form of this type of cysts.

From these cysts issue Ceratia, which suggest, f. piburgense Zederbauer, but are not completely identical with them. (Thinner form, conical middle body and strong spreading of the rear horns. The middle horn does not point in the direction of the fore-horn. These Ceratia are also mostly rather smaller than the two first types of form.) We describe these cysts for the present as "piburgense - cysts".

On the basis of our cultures it is now possible for the first time to identify the cyst with certainty and to follow up their relationship to certain Ceratia-types.

2. Bachmann, H. Das Phytoplankton d. Sussw. Linzern 1911.

1. Zederbauer, C., Ceratium hirundinella. i.d. Österr. Alpenseen. Öster.bot.Zeitschr. Wien. 1904. No.4 u. 5.

In isolated assays so far it has not failed. Thus, Brutschy [1] reproduced cysts from Lake Zuger observed by him to be Ceratium - forms.

The resting cyst possesses a simple, flat membrane of medium strength The reddish-brown to yellowish-brown pigment is lodged in the centre of all the cysts and usually completely covers the nucleus. [2] The colourless, diaptric fat-droplets lie more around the margin. The 3-horned spherical cysts are as a rule coloured throughout darker than the much paler-seeming rhomboid, 4-horned cysts. The whole contents fill the cyst tightly to the base of the horns, vacuoles and their like are not to be seen. It is just as unlikely to see vesicle movement in the resting cyst.

3. The germinating cyst.

Not many hours after the culture is set at 18°C room-temperature, in the centre of the cyst, processes manifest themselves which start the germination. The whole cyst swells up somewhat, obviously the hitherto impermeable cyst wall (as a result of the influence of temperature) becomes permeable to water, and this enters the cell. In 3-horned cysts the horns begin to broaden at the base and to fill up with cyst contents. The swelling of the whole cyst and the basal broadening of the horns are to be attributed to the increased turgor. A slow differentiation of the cyst contents now taken place hand in hand with the swelling of the cyst. The coarser cells are displaced more and more towards the centre of the cyst, while the horns appear pale and a lively movement of the finest granules in the sense of the brownian molecular movement is to be noticed in them. Only after one day has elapsed from the start of the culture (with the onset of the cell-movement in the horns) instead of the mostly only diffuse brownish-red pigment in the centre of the cyst, a number of (up to 12) sharply defined reddish-brown granules become noticeable, and also the rod shaped chromatophores gradually show themselves in their typical form. Now, usually the nucleus becomes clearly prominent, surrounded by the red-brown flecks. At this time in many cysts the nucleus is still not sharply defined (covered by reserve - and colouring matter).

1. Brutschy, A., Monograph. Studien a. Zugersee - Archiv. f. Hydrobiol.u.Hydrographie. E. Schweizerbarth, Stuttgart. 1912.

2. Bütschli - Bronns Klass u.Ord.d.Tierreichs, Tat: 53, Fig. 9c, reproducing a 4-horned cyst with a very clear nucleus, which is obviously too schematically portrayed. The same applies to sev. drawings of Steins. Lieberkühn shows (acc. to Bütschli) a clear covering similar to the usual shell, on the casing of the cyst; we have never seen this.

Not until shortly before the emergence of the contents of the cyst is it quite distinct. (Fig. 3a - c.)

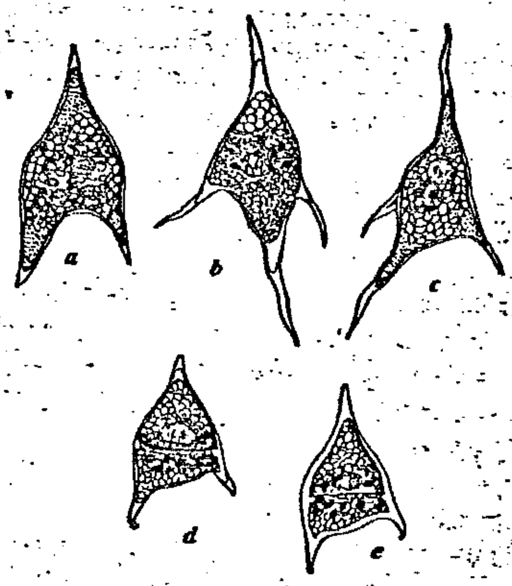

Abb. 3. Keimende Cysten.

Fig. 3. Germinating cysts.

a) Large, 3-horned cysts, 44 hours after the start of the culture. Length 100, width 48μ - whole cyst rather swollen; horns broadened at the base, with clear movement of the cells. The filaceous nucleus is visible; moreover, a number of dark, (reddish brown) cells in its vicinity. Differentiation of the cyst content in an apical and an antapical part, a paler zone between: onset of formation of transverse grooves b, c) 4-horned, rhomboid cysts. Nucleus clear and surrounded by reddish-brown cells. In C the cyst contents are forced into the horns, with clear movement of cells.

d, e) Small, 3-horned cysts. The contents show a clear transverse groove, as well as the reserve fat and the nucleus. The reddish-brown cells are displaced to the rear part of the cell. The contents surround themselves with their own fine membrane and retreat from the cyst wall.

The contents now differentiate themselves more and more clearly in two halves, one towards the apical horn of the cyst, and one towards the antapical side of the same half. The two halves are divided by a pale-seeming zone. After about 36 -40 hours, i.e. a few (perhaps 3 - 4) hours before the emergence, a slight indentation is to be seen under the cyst wall, in the middle zone of the cyst (i.e. approx. in the middle between the fore- and rear-horns), which becomes clearer and clearer and finally appears as a transverse groove. In the meantime the cyst contents under the cell wall have also formed their own very thin membrane. The transverse groove becomes more and more clearly defined. The whole cyst contents becomes rounder, in that it retreats from the horns a little and moves away from the cyst wall. This retraction is quite characteristic. The cell movement becomes slower and shortly before the emergence is no longer visible (Fig. 3d, e.).

At the end of this germination process, the following picture presents itself. The outer form of the cyst is unchanged in its basic features, only a more or less visible swelling is to be seen, whereby the horns are often distended at their base. - A swelling of the cyst membrane does not occurr. Inside, a spherical body has formed, which draws back more and more from the cyst wall. The horns are free from the cyst contents. The so changed contents of the cyst shows a clear, more or less steep diagonal groove twisted to the left, which runs continuously over the dorsal side to spread flat over the ventral side. In the vicinity of the transverse groove, as a rule pushed nearer to the apical part, the nucleus is located (the'red granules' are distributed). In its vicinity, mostly towards the antapical part, The rod-shaped brown chromatophores have arranged themselves along the transverse groove at the periphery of the cell in the direction of the longitudinal axis of the same. Apart from this the cell is still filled with round, more plate-like, mostly only blurred chromatophores, as well as fat granules. The longitudinal groove is still not clearly seen inside the cyst.

The cyst looks like this ca. 40 - 48 hours after the culture is set up. It can stay in this state for about 2 - 3 hours.

If one can watch continuously a transformed cyst like this, it is possible to observe beautifully the emergence of the cyst contents. The emergence takes place at an almost constant place, namely, between apical- and right rear-horn (nearer the apical horn). The apical part of the germ goes first, in the emergence. The attached pictures show it clearly. The resulting split in the cyst wall apparently runs mostly longitudinally. The opening is mostly rather narrow, so that during the whole process of emergence, the cell body shows a covering form. The more the cyst empties itself, the more clearly does the well-developed long flagellum move towards the (opening) being the last organ to leave the cyst along with the rear end of the self-freeing germ. The confirmation of the existence of a transverse cilium is only possible on rare occasions. (Fig. 4.)

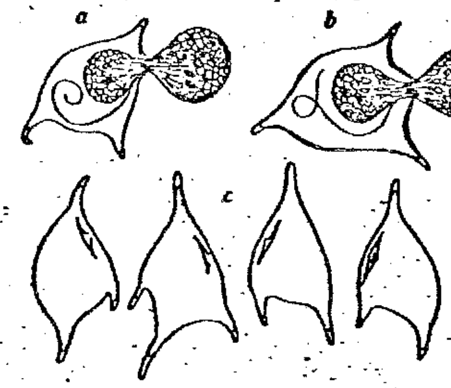

Abb. 4. Keimende Cysten.

Fig. 4. Germinating cysts.

a and b) side sac form of the emerging germ. The germ is near the front (apical) end. Very clear longitudinal flagellum. In the middle of the germ the rod-shaped chromatophores. Cyst b drawn fairly vertically foreshortened. c) Several cysts (ventral and dorsal view), which show clearly the tear between the front and right rear-horn.

The duration of the emergence amounts to 1 - 2 minutes. From the moment of the setting up of the culture to the emergence of the young germ takes at 18°C ca. twice 24 hours.

It is now extraordinarily interesting to observe, how low temperatures slow down the metamorphosis in the cyst and the emergence. At 10 - 11°C it takes about 6 days; at 7 - 9°C the emergence takes place on the 7th day at the earliest and at 4 - 7°C not until 4 - 5 weeks have elapsed. At higher temperatures (20 - 26°) the germination is considerably accelerated, so that is has finished after only 36 hours. A clear difference in the germination time of 3- and 4-horned cysts of different Ceratium types has not at this point been noticeable.

Immediately upon leaving the cyst, the young germ takes on a thoroughly characteristic form: it shows the complete characteristics of a Gymnodinium. We have, therefore, described this stage of development of Ceratium as the Gymnodinium stage, and the Ceratia in this stage as Gymnoceratia.

4. The Gymnodinium stage

The germ which has emerged from the cyst, the Gymnoceratium, has not yet great similarities with a Ceratium; it shows, as already mentioned, much more of the behaviour of a Gymnodinium cell. An apical part is very easily distinguished from an antapical part, by a usually, steep transverse groove winding to the left.

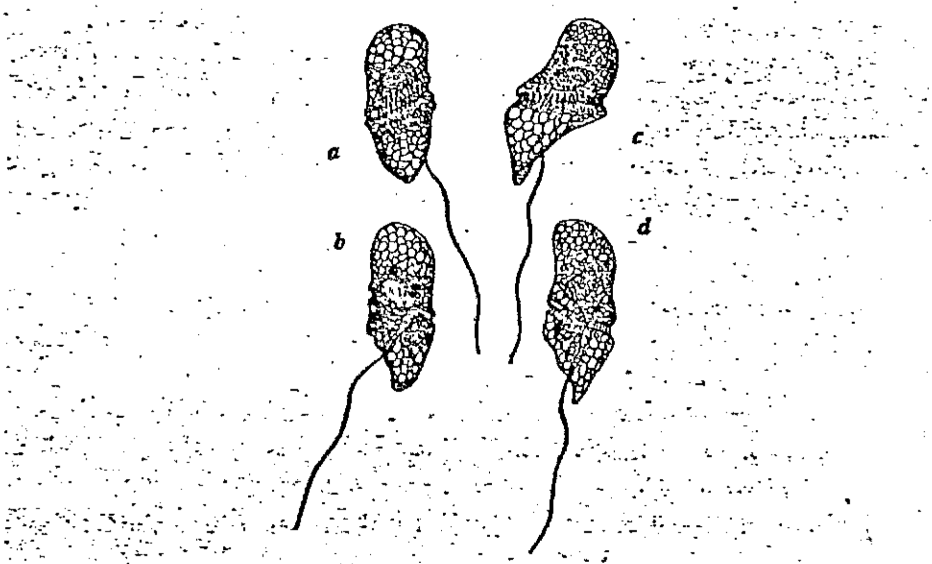

Abb. 5. Ceratiumkeime im Gymnodiniumstadium (Gymnoceratien) etwa 10 Minuten nach dem Ausschlüpfen.

Fig. 5. Ceratium germ in the Gymnodinium stage (Gymnoceratia) about 10 minutes after emerging. a and b) the same Gymnoceratium (a) dorsal and (b) ventral view.
c and d) another example in the same layer. Apical and antapical separated by the steeply left-winding transverse groove (with transverse flagellum). Longitudinal groove with longitudinal flagellum (b and d). The large reserve fat granules clearly seen in the front and rear part. The large filamentous nucleus lying transversely in the apical part. Red-brown drops of colour, particularly in the rear part. The rod-shaped chromatophores arranged longitudinally are only to be seen clearly dorsally.

The apical part is bulky, rounded and has rather more mass than the antapical part, lightly drawn back from the transverse groove here and there. The antapical part is thinner and runs away more or less to a point. At the place where the rear horn later forms, a "corner" can frequently be seen; however, horns or any other striking features are not present. Also, the antapical pole is blunt; however, the antapical end is always clearly distinguishable from the apical end. The transverse groove runs continuously along the dorsal side, ending on the ventral side at various heights. In the left ventral part of the antapical half a longitudinal groove is clearly visible. A long flagellum arises from it. The transverse flagellum which runs in the ring groove is already existing, it is just difficult to see. The bulky apical part has a wide-eliptical transverse cut; the antapical part conversely, is flattened by the transverse groove dorsoventally and shows a light bowl-shaped hollow, with its concavity directed to the ventral side. Simultaneously the antapical part opposite the apical part is rather twisted to the left. The large oval nucleus lies in the apical part of the cell with the longitudinal axis lying transversley. In the upper part of the apical half of the cell the fat is clearly laid in droplet form. In the antapical part are equal numbers of smaller and larger fat granules; besides this the large reddish-brown granules are arranged. The dark yellowish-brown chromatophores are not dispersed evenly over the whole cell; they are most abundant in the middle zone and in the antapical part. The membrane is very delicate and smooth, it shows neither plate markings nor granul(is)ation. A metabolism is likewise not evident. The length is 60μ, the width 36μ. (Fig. 5)

Immediately after the emergence from the cyst the young germ moves with the help of the increasingly strongly beating longitudinal flagellum or with the apical part pointing straight ahead, or it rotates on its longitudinal axis, thus showing the transverse flagellum very clearly.

The Ceratium remains in this Gymnodinium stage for about two hours.

The Gymnoceratia, which swim about freely after 10 minutes and those, which are approx. 1 - 1½ hours old differ clearly in their outward appearance. This can readily be seen from our figures. The Gymnoceratia which belong to the f. austriacum, can also mostly be distinguished from other more delicate Gymnoceratium forms by their powerful form. (Fig. 6,d, e).

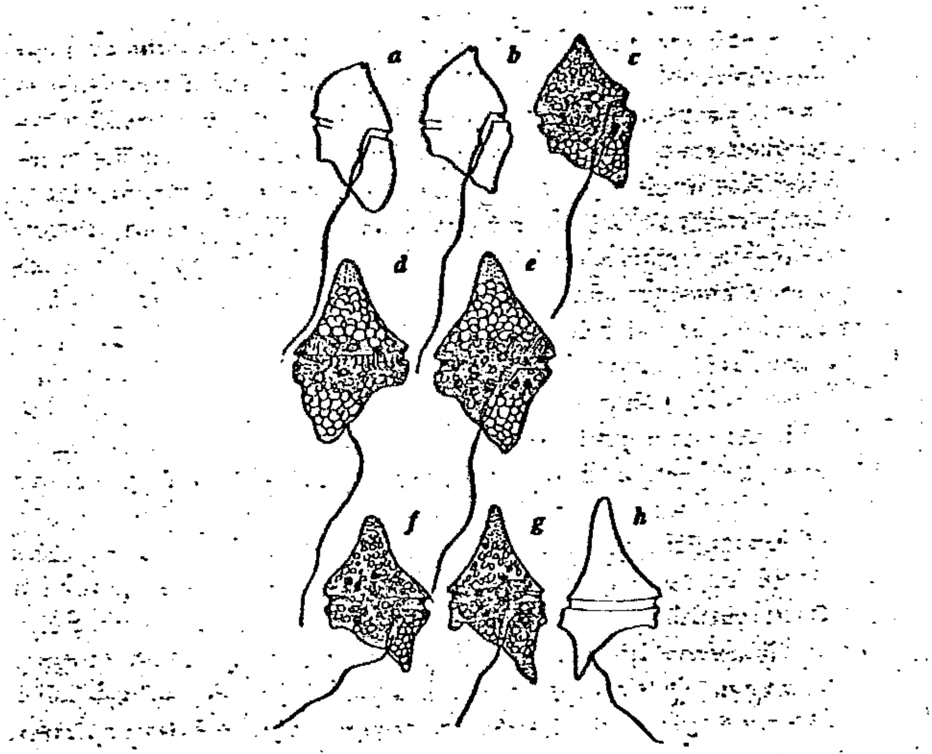

Abb. 6. Gymnoceratien gegen den Schluß des Gymnodiniumstadiums.

Fig. 6. Gymnoceratia towards the end of the Gymnodinium stage.
a) 1 hour after the emergence, 60μ long, 32μ wide.
b) 1¼ hours after the emergence, 60μ long, 37μ wide.
c) 1¾ hours after the emergence, 60μ long, 40μ wide.
The same individual was, after 2½ hours 70μ long, 50μ wide. Noteworthy is the behaviour of the nitches on the polar horns from a - c.
d,e) Gymnoceratia of the large Austriacum form ventral (d) and dorsal view (e), 76μ long, 52μ wide. Nucleus, chromatophores (d), reddish brown granules, fat granules, flagella. The site of the apical horns moves forward.
f, g, h) the polar horns become more and more distinct; the right rear horn is only weakly indicated. - Smaller forms. f: 56μ long, 44μ wide, g: 60μ long, 40μ wide. All individuals are still without shells, the site of the horns f - h gradual transfer to pre-Ceratium stage.

One hour later it can be seen that certain changes take place on the Gymnoceratium body: the apical area loses its typical rotundity and slowly takes on a wide conical form; moreover the outermost zone of this area becomes more and more hyalin. A totally striking phenomenon follows: On the tip of the lightly drawn out apical area (becoming hyalin) a clear sunken area can be observed, so that two very short, blunt horns come into view on the tip of the apical pole. Folgner [1] has already made a similar observation on the Gymnoceratium germ of Ceratium cornutum. Of course in the above cases and our own, it is not a matter of individuals, which afterwards produce a cleft apical horn (we did not find any such forms in the culture under discussion). It must then be a case of racial history. We did not see, as mentioned, the shelled phenomenon in all Gymnoceratia, and we observed a large number. We established the existence of a similar fine indentation on the antapical pole.

As the apical horn developes in length, in these Gymnoceratia, the clearer the depression becomes. The relationship of this with the apical horns could not be followed with enough certainty.

Such changes do not take place exclusively in the apical area, but also in the antapical area. Periodically, of course development takes a leap forward. There are also stray protrusions on the antapical part of the cell body, which start the shape of the right rear horn.

These changes form the transfer to a new phase, which the Ceratium germ enters, a phase during which further changes take place in the young Ceratium cells, which gradually lead into the definitive form. This new phase, which lasts several hours and is characterised by important metamorphism of the cell body, has been labelled the pre-Ceratium stage, whereby it is implied that it is this phase of development, which immediately precedes the typical "Ceratium state", and leads to it.

5. The Pre-Ceratium Stage.

When Ceratium has remained for about two hours in the condition described as the Gymnoceratium stage and has, towards the end of this phase lost the original rotund form, especially in the area of the apex and the middle zone, now the generally still thin form of the cell begin to extend in width in the middle part which is occupied by the transverse groove. Many individuals show an increase in width from 36μ (in the Gymnodinium stage) to 56μ (in the pre-Ceratium stage). Hand in hand with this goes a stray dorsoventral flattening, particularly of the apical part. In the same way as the middle zone of the cell widens, narrows simultaneously with the lengthening of the apical part, so that this takes on an increasigly clear cone shape. On the antapical part too, which from the beginning was more pointed than its antipode, changes are taking place simultaneously: the area which is becoming the antapical horn becomes more and more sharply pronounced, it also lengthens a little, thereby causing the end to be more pointed. Nothing is yet to be seen of the right postequatorial horn (so called right rear horn) at this stage, or,

1. Folgner, V., Beitr.z.Kenntnis d. Entw.Süsswasserperideen Oster.bot.Zeidschr. 1899. Jahrg. 49.

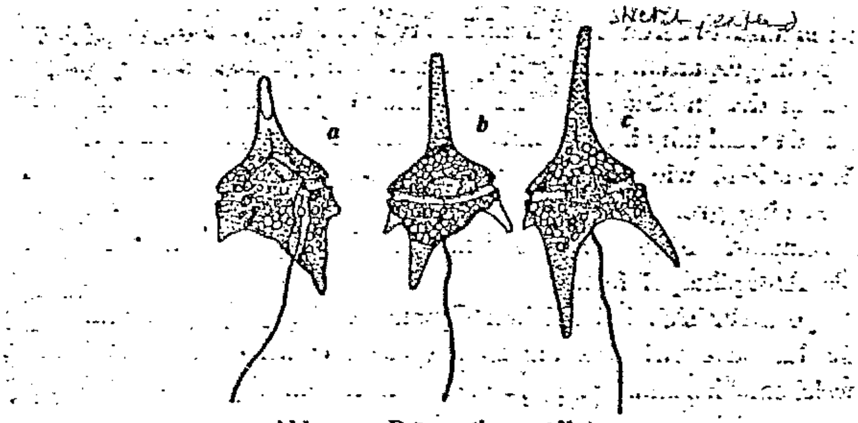

Abb. 7. Präceratiumstadium.

Fig.7. Pre-Ceratium stage.
a) Ca 4 hour-old pre-Ceratium (from a blue-glass culture, which inhibits development). Plate-division and granulation already present, but extremely fine. Length 100 μ, width 56 μ. Appearance of post-equatorial horns already clear.
b) 4-horned. individual, 3½ hours old from regular daylight culture. A scarcely projecting plate is present at this stage. Hyaline horns still without shell.
c) Approx. 4hours old, pre-Ceratium place pattern still delicate as is the granulation. Suggestion of plate formation on apical horn.

one sees a broad, shrimp-shaped indication. Here and there it keeps pace in its correlative formation with both pole horns.

In the previous section we have emphasized that in various Gymnoceratia, whose development we particularly followed, we observed a fine indentation at the apical pole. During further development this protrusion bearing the indentation becomes pushed forward by the apical horn stretching along its length and finally disappears. For a time one has the impression, that a canal leads funnel-like from this softening indentation, to the main body; possibly this indentation is the first manifestation of the apical pore resp. apical canal. [1] In any case, the indentation is only a quickly passing phenomenon on the Ceratium body.

The pre-Ceratium stage lasts approx. 6 hours.

Towards the end of this period, as a rule the right postequatorial horn becomes increasingly clearly manifested; even the left postequatorial horn (so called 4-horn) is sometimes already indicated as a tiny protruberance, from which can be deduced that the Ceratia generation leaving the cyst can be 4 - horned.

1. It is to be noted similarly as after Schilling a similar exists with three notched lobes as found in Cystodinium which itself possesses no apical pore.

There are forms in this pre-*Ceratium* stage, in which the 3 rear horns are already clearly recognizable (4-horned forms). However, it is not uncommon to find forms where there is still very little to be seen of the antapical-horn of the right rear horn, so that these forms give the total impression of 2-horned Ceratia. So, if Hüber surmises that a 2-horned transitsional stage exists in *C. hirundinella*, then for a least part of the Ceratia in the culture of 16 - 18°C, this is in fact the case. (The 3 horn is then subsequently formed.) However, we have established cultures of 7 - 9°C, where the 2-horned Ceratium forms appeared in over 80%, and not just as transitional forms, but as persistent cold forms.

If the transformed stages in the middle zone of the cell are in substance discharged, and the slow development of the horns established - *Ceratium* swims around in the water during this time, then the first marks of shell development become apparent on the main body (about 4 - 5 hours after the emergence from the cyst): the separation into plates appears initially very faintly; also, a very fine net-like pattern granulation is indicated. There is therefore already in existence a delicate shell, on which first of all hyaline unprotected horns are to be seen. There is a small granule movement to be seen on the horn tips, which was already visible before the clear occurrence of the shell. The growth of the horns and the shell seems to last longest there.

The last period of this pre-*Ceratium* phase shows two phenomena above all: first, a strong growth in length, originating in the development of the apical and antapical horns (less striking, is the development of the right post equatorial horn), then the increasingly clear projection of the shell structure, which makes itself known through the greater prominence of the plate (cement edge) and the granulation. If the length of the cell at the end of the Gymnodinium stage is still 56 - 60µ, now it reaches values of 100 - 200µ or even higher.

Very soon growth to the diameter comes to an end; if it showed values of, say, 36µ in the Gymnodinium stage, now it finally has values in the pre-*Ceratium* stage of 56 - 60µ according to the form of the *Ceratium*.

The rear horns on the tip of the exoskeleton (with the delicate hyaline end cone) reach the end of their growth through the hardening of the shell on the tips of these horns. However, it is not improbable, that on the basis of the same, over a certain period of time, an inter-kalar growth zone exists. The front horn generally remains open; it bears the apical pore. At the end of the pre-*Ceratium* stage lengths of 180µ and 220 - 256µ can be reached. (Fig. 8).

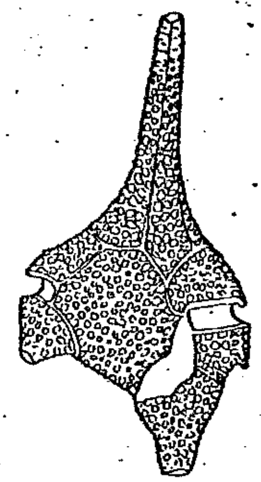

Abb. 8. Panzer gegen

Fig. 8. Shell towards the end of the pre-Ceratium stage. The division into areas and the granulation of the shell can be seen clearly. The rear horns are still not closed (in the live individual hyaline end cores project from them). Shells found in such a condition can imitate an [Autotomie] (Kofoid). The apical horn remains open. (Apical pore).

We can see, therefore, which intensive and striking changes C. hirundinella undergoes during the pre-Ceratium stage, changes in the whole architecture of the cell: formation of horns, the laying down of an exoskeleton. It is also understandable, that this phase takes about 3 times as long as the initial phase, the Gymnodinium phase, in which the "bare" outline of the horns is first faintly discernible.

We think we are fully justified, then in characterising this important and well-defined phase in the stages of development of C. hirundinella, in that we describe it as the pre-Ceratium stage. For the next stage in Ceratium, which gives this dinoflagellate its "definitive" form, brings something completely new and characteristic, the ability of the cell to divide.

6. The full-grown stage of *Ceratium hirundinella* : *Ceratium* stage.

We have followed the hitherto unknown immature stage through to the final fully-developed stage of *Ceratium*. On this itself we can be brief, in that in both its external and internal construction many single observations and collective descriptions exist. We shall only refer to one:

We have also seen in our cultures according to the type of cyst, the variety of forms of *Ceratium* arising. From the small, rotund, predominantly 3-(seldom 4-) horned cysts, a large, slender *Ceratia* - form developed, which is described as *f. gracile*; from the rather larger, likewise rotund 3-4 horned cyst with the rather longer horns, we saw the strong, robust *f. austriacum* issuing and from the rhomboid 4-horned pale cyst we cultivated a yet more slender form with greater spreading of the antapical horns: *f. prope piburgense*. The individual *Ceratium* types show an absolutely consistent course of development.

We succeeded, therefore, in a simple fashion in cultivating *Ceratium* cysts through all the stages to the ability of the individual to divide. The whole duration of the development - from the beginning of the culture through to the completed formation of individuals capable of division - lasts at 18°C a minimum of about 50 - 60 hours, i.e. 48 hours to the emergence from the cyst, 2 hours for the Gymnodinium -, and about 6 hours for the pre-*Ceratium* stage. Many individuals need 70 - 80 hours and more for their complete development.

Several hours after the first well-formed 3- or 4-horned *Ceratia* appear in the culture, isolated individuals can already be observed, in the process of dividing, particularly in the early hours of the morning, an established fact which is completely identical with the observation in nature. (As is known, the divisions in *C. hirundinella* take place almost exclusively in the hours after midnight into the earliest hours of the morning, so that in the course of the morning, one comes across dividing stages as well as *Ceratia* already divided).

We can omit the more precise processes of division, here, as these do not differ from these facts already known. Only two pictures have been reproduced here, to show two stages in the division of a *Ceratium* raised in a culture.

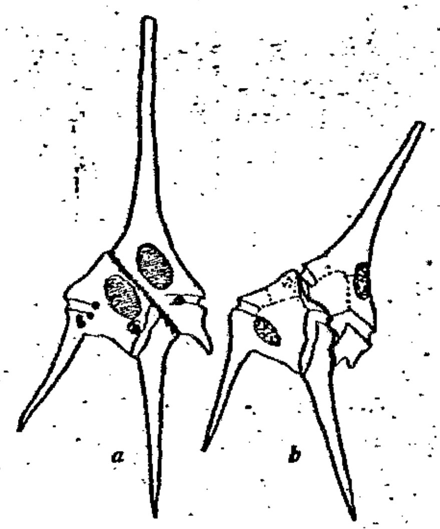

Abb. 9. Zwei Teilungsstadien von Ceratium hirundinella. Aus einer Kultur (vom 19. III. 21 morgens) am 24. III. 21, 8h 30 vormittags. Beide Individuen mit Zeichnungsapparat gezeichnet; die meisten Einzelheiten weggelassen. a) Länge 220 μ. Breite 60 μ.

Fig. 9. Two stages of division of Ceratium hirundinella. From a culture (from 19. III 21 in the morning) to 24.III 21, 8.30 a.m. Both individuals drawn with drawing apparatus; most details are left out.
a) length 220 μ. Width 60 μ.

Figure 9a, from 8.30 a.m. Shows an individual, in which the division of the nucleus has already taken place (imitating the cell division as always) and the steep transverse wall has already formed. As a rule, in the individual which is lying on its back, this runs from the space between the antapical horn and the left post equational horn (so called 4 horn) steeply up to the right and finishes, cutting the transverse groove at the base of the apical horn on its right side. The two nuclei are still very close to the transverse wall.

In Fig. 9b we see the process of cell division further advanced: the cell nuclei, which can be seen to have contracted after the division, are widely separated from each other. The original apical part of the cell sits up sharply from the antapical part, and seems to be pushed away by the developing protruberances of the lower half of the cell becoming the apical horn as well as by the opposite growth force. Within, the complete separation of the daughter individuals will soon have taken place.

During the latter part of the morning one finds in the cultures of 16 - 18° fairly numerous forms with very short apical horns and proportionately long rear horns and some with still undeveloped short hind horns and long front horns. The regeneration of the cell parts lost by division takes only a few hours (about 6).

In order to deal with the Ceratia types themselves rather more precisely, we shall reproduce a few figures of the forms obtained as demonstration; for such examples from the culture no longer exist (s. Fig. 10,11,12). We shall not neglect this opportunity to emphasize now that in our experience from the temperature experiments as well as the plankton catches the classification of types existing at the time (Selijo[1], Bachmann, [2] Schröder [3]) are not satisfactory and it will not do, to depend on the body length, the direction of the pole horns and the spread of the rear horns as a principle of classification. Such a system is not tenable or at any rate only within restricted boundaries. We have reproduced in our figures, these which can be interpreted as f. gracile, austriacum and "piburgense", based on the hitherto current nomenclature (Bachmann). The latter form is fairly distinguishable from the Zederbauer form, as our form has the stronger divergence of rear horns with moderate total length, but with a rather conical rather than cap-shaped apical part [1]

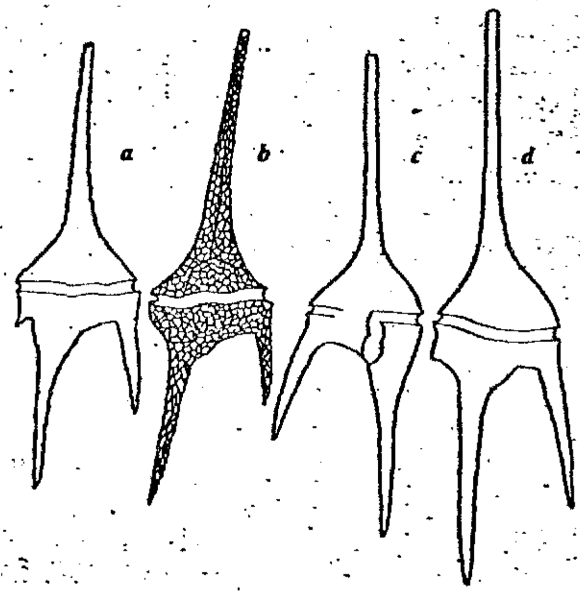

Abb. 10. Aus Cysten gezogene Ceratien: gracile-Formen. Lange.

Fig. 10. Ceratia cultivated from cysts: gracile forms long, thin body, rear horns almost paralle, axes of the pole horns accordant directed, 4th horn missing or only suggested as a "corner". Cultivated from the sediment of 1919 in large numbers.

a) 16 hours old.
b) length 220 μ, width 55 μ. 24 hours old.
c) length 216 μ, width 52 μ.
d) length 256 μ, width 56 μ.

1), 2) See Schulling, A.J. in Paschers Süsswasserflora.
3) Heft "Dinoflagellata". 1913

3) Schröder, Br.,Die neun wesentlichen Formentypen von C. hirund. Arch.f.Naturgesch. 1981 8.H.

1. Bally, W. (Upper Lake Zürich Arch.f.Hydrob.u.Hydrogr., Stuttgart, 1907) gives two figures corresponding with this form. (S. 139, 10 and 11).

We would like to retain the name description f. piburgense for the present and only through the addition of accedeus (acc. or prope) suggest that it is not identical with the "original form". (More exact expositions on this in the experimental part.)

For comparison, the following can serve to show if differences in form exist between those cultivated by us and those observed in nature: after we had established a predominantly austriacum count on the basis of the cysts in the mud (sediment) of the 1920 season, and had also seen a corresponding majority of austriacum forms developing in our cultures, we could expect to find the same behaviour in nature. Plankton catches of May; 1921 confirmed our expectations: the large majority of Ceratia belong in fact to the austriacum type. There exists, however, through exact observations, a certain distinction in the form of those we cultivated and those Ceratia which developed spontaneously: the width of the latter is on average greater than those examples germinated from cysts; the cross-section of the middle body is more strongly bent into a kidney shape, the dorso-ventral flattening more pronounced and the shell pattern even stronger. [1]

This comparison is not unimportant; it shows, that it is still different for an individual has at its disposal 50 cm^3 water as its undisturbed environment, or a large lake, where there is always a greater or lesser wave-movement. Ceratium seems to be adjusted to a mass action.

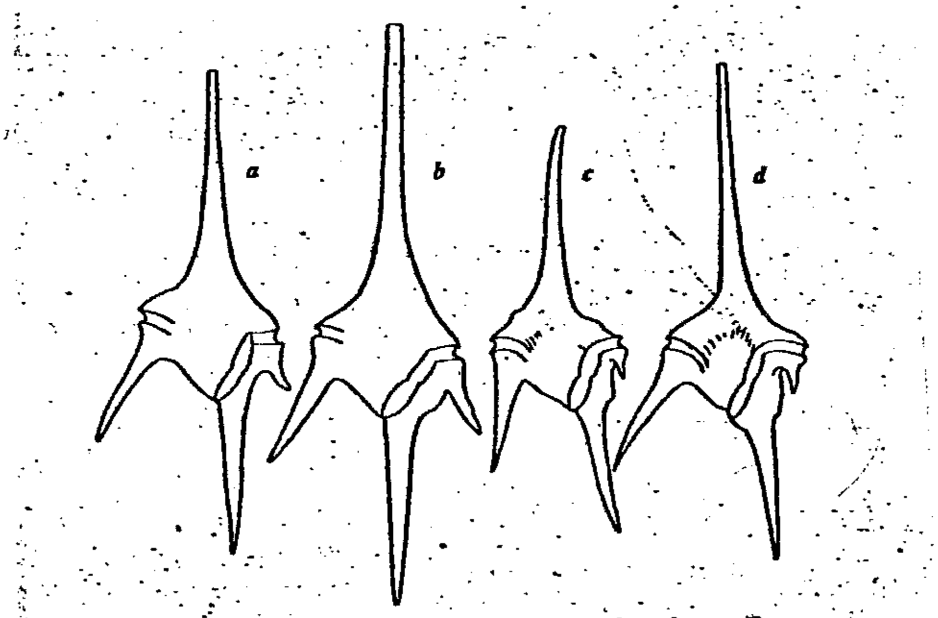

Abb. 11. a und b) Aus Cysten gezogene Austriacum-Formen.

Fig. 11 a and b) austriacum-forms cultivated from cysts.
a) 208: 60μ, b) 232: 60μ
c) and d) from the Lake Zürich plankton, of 24.V.21. (frequent form.)
c) 176: 60μ, d) 220: 72μ.
These forms are mostly 4-horned from the start and rather bulkier than the former.

1. The width amounts to 60 - 72μ, the length 160 - 240μ (majority 160 - 184). In the examples from the culture 60μ was the extreme width. A fourth horn is gently spreading.

We were able to make the following morphological observations on the numerous completely formed individuals of the first generation to have issued from the cysts, established in the cultures of 18°C.
1. The great majority of the Ceratia are well-formed individuals, which exhibit no deviations from the "normal form" of one of the 3 types.
2. Beside, these, are forms, which in a quite normally formed apical part show an inordinately wide spreading of the rear horns, which often are noticeable on the right postequatorial horns. In summer such forms are frequently to be found in the plankton. The 4th horn can also often be noticeably spread away from the cell (Fig. 12.) 3. A particularly prominent phenomenon that amongst the individuals of this Ist generation, there are always a few, which exhibit a cleft right rear horn or, more seldom, a forked antapical horn. Such forms struck us, even as early as the pre-Ceratium stage. As the phenomenon of forked rear horns is much rarer in individuals arising from division, it is obvious that certain

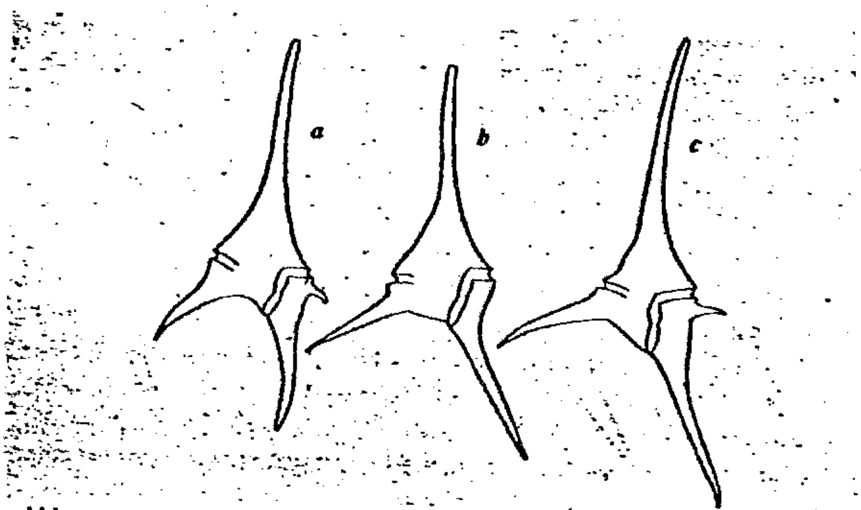

Abb. 12. Aus Cysten gezogene Ceratien: f. piburgense acced. Eher

Fig. 12. Ceratia cultivated from cysts: f. piburgense acced. Rather small form (c 188: 44μ), 3-4 horned, thin, wide spreading of the rear horns, which is almost abnormal with b and c, also the 4th horn stands up in C abnormally. Apical part is increasingly conical: apical horn relatively thin, often gently bowed. Direction of axes of the pole horns mostly very different. Not identical with the Zederbauer f. piburgense, but approaching it by virtue of its widely spread rear horns; therefore described on this basis as f. piburgense accedens. The colouring is (analog. with the cyst) paler and yellowish-green (instead of yellowish-brown of the other forms).

injuries to the protoplasm during the exit from the cyst can happen, which are manifested in the forking of a rear horn. As to why the apical horn is almost never forked (however the apical part of the cell is surely just as exposed to fine lesions as the rather more complicated structure of the antapical part), shows, that the question is not so easily answered.

Observations on a few cell inclusions: On the nucleus itself there is nothing particular to say: it shows a filamentous structure, the threads are just as often fine and long as they are short and thick, with knotty thickenings, in which case the nucleus appears lineated or speckled. As a rule the threads run parallel with the transverse axis.

Chromatophores seem to be present in two forms. In Gymnoceratium and pre-Ceratium we have seen rod-shaped brownish-yellow chromatophores, which are arranged along the length, following the transverse groove, and particularly clearly to be seen, when the individual shows its dorsal side they seem,then, to lie thickly under the surface. At this stage the yellowish-brown or yellowish-green/brown pigment seems mostly diffused through the cell, so that we almost never saw others e.g. lens-shaped chromatophores. Not until the Ceratium stage did we find the rotund lens-shaped chromatophoreplates (more or less) clearly. However, at this stage we no longer found the rod-shaped chromatophores (perhaps overlooked). We know from other Ceratia (C. tripos [Müller] Nitsch), that they possess two sorts of chromatophores, rod- and plate-shaped. We could, therefore demonstrate by C. hirundinella two kinds of chromatophores; they seem to be clearer in one form or the other according to the phase of development: in the immature stage the rod-shaped chromatophores are very clear, the round ones, conversely, indistinct; the reverse true in the mature stage. For a short time the brownish-red colour granules can sometimes be found, on whose nature and physiological significance nothing certain is known. We could follow them from the germinating cyst right through to the Ceratium stage. Numerous were 5 - 8, here and there even 10 - 12 of these colour granules, which were arranged either singly or in small groups of 2 or 3 self-propelling granules. Further, it can be determined, that their number decreases with every division (in the mature state). (See Fig. 9a the upper individual here receives one of these granules, whereby the number of these same is decreased by one in the sister individual.) Hitherto there has been no agreement prevailing as to what this system is. They are regarded by many, at least in the mature Ceratium (the immature stages were unknown hitherto) as eye-spots (stigma) regardless of whether they occur singly or in numbers. We ourselves have not made any further observations on these reddish-brown colour granules.

It is not without interest to draw a comparison between our observations on C. hirundinella and those of Folgner (et al) on C. cornutum. Folgner gives the sequence of the processes in those Ceratium observed by him in the following way: emergence from shell and cyst; appearance of the groove; formation of the two end horn, becoming visible by the sculpturing of the membrane; the side horns arise.

Folgner mentions the "appearance of the groove" after the emergence from the c st. That could well be the case. He did not observe, as he expressly emphazises the emergence of the contents of the cyst, only the freshly emerged germ in the vicinity of a cyst. So it is to be explained, that he supposed the very young 'germ' had formed its transverse groove outside the cyst. We can, however, state with certainity, that C. cornutum does not make an exception here; for Schilling[1] draws (Table II, Fig. 23) a transverse groove inside the Cornutum cyst when he states in his description exactly the reverse of the drawing: ("meanwhile the body rids itself of the rest of its casing and enters upon the formation of the grooves and the lateral horns.")

1 Schilling. I.-O. 1891

Folgner's observations on the "formation of the two end-horn buds" in C.cornutum which he deals with next, concurs with ours on C. hirundinella, insofar as according to Folgner these buds already, in the shelled individual take the form of a translucent bump, as in our so called Gymonodinium stage. In C. cornutum according to v. Stein [1] Schilling and Folgner the buds of the end- or pole-horns follow next. (Not until later, does the formation of the 3rd horn follow, even if the individual has had a shell for a while. The majority of spring forms of C. cornutum, however, have two horns.) In C. hirundinella also, the formation of the pole horns frequently ensues frequently rather quicker than that of the right rear horn, so that one often comes across a number of two-horned hirundinella forms, particularly at lower temperatures. Mostly, however, and especially at optimal temperatures (15 - 26o the right rear-horn keeps pace with the formation of the end horns; even the left rear horn (accessory so-called 4th horn) can develop along with the other horns, almost simultaneously, only slightly delayed.

The appearance of an indentation on the apical horn was first seen by Stein; then Folgner speaks of this observation. We also saw an indentation on the first antapical horn bud; but not just on this one, but also on the antapical-horn. The significance of these indentations has remained obscure to us; according to Folgner's observations it is also a case of phenomena which appear and disappear quickly, which we have not seen in all Gymnoceratia. Furthermore, according to Folgner, the horns of C. cornutum ought to have attained their full length and form by the Gymnodinium stage; and not until this point do they develop the shell. With C. hirundinella this is not the case; here the growth of the horns takes place quite intensively during the pre-Ceratium stage. The formation of the still smooth (naked) horns is, according to Folgner, complete about 2 hours (exactly 1¾ hours) after the start of observations. Then begins the laying on of the shell and so a new stage. However, Folgner now states, that the outermost end of the horns are, for the time being, still transparent, unshelled. That, however, means that the horns, in C. cornutum too, grow still more; they have not, therefore, attained their "full length" in the Gymnodinium stage, and that could rather concur with our observations on C. hirundinella. Folgner mentions further, that he had observed in all those unprotected Ceratium embryo observed by him - our Gymnoceratia - two longitudinal flagella. Surprisingly, we have met this phenomenon not infrequently in otherwise normal Gymnoceratia embryos of C. hirundinella [1]. Folgner did not observe a transverse flagellum, but excluded the presence of such a thing from the rotating motion of the embryo. We firmly established the existence of a transverse flagellum in Gymnoceratia. We must mention still one phenomenon. Folgner regularly determined (as had V. Stein) the presence of several "large red oil droplets". They lay, as in the movable summer form, almost exclusively in the rear, pointed half of the body, whereas normally the fore-parts contain rarely more than two and sometimes none. We made exactly the same observations with C. hirundinella. We observed up to 12 of these large drops. - the duration of the Gymnodinium stage, according to Folgner, is about 2 hours, exactly as with C. hirundinella.

The next stage in the development of C. cornutum is now, according to Folgner the "sculpturing of the membrane becoming visible" and the definitive shape. We have described this phase as the pre-ceratium stage. Folgner's observations here are reasonably in agreement with ours.

1. v. Stein. Org. d. Infus

1. Doyble longitudinal flagella have also been observed in marine Ceratia.

The duration of the pre-Ceratium stage of C. cornutum seems to be about 4 hours. From the emergence of the embryo to the completed form of C. cornutum took about 6 hours according to Folgner (of which c. 2 hours were taken up in the Gymnodinium stage). The duration of the pre-Ceratium stage of C. hirundinella (16 - 18°C) took c- 6 hours, at a higher temperature 4 - 5 hours; therefore a good agreement here is also unmistakable. We did not decide to use the much-used name "[Schwärmer] swarm-spore" here, as usually a fixed stage follows, which is qot the case here. The embryo in Ceratium develops simply in steps, retains its flagella etc. The name Gymnodinium stage (or - phase) means more besides: it is not only a morphological conception, but also opens perspectives on the embryology.

The sharp nomenclature of the two concepts "Gymnodinium- and pre-Ceratium stage" resp. "Gymnoceratia and pre-Ceratia" served well for the experimental studies, as we became acquainted with influences, which, if inserted either in the first or the second stage, had the effect of shape-forming in a different way (mostly in the sense of deformation).

The formation of the Cyst

We have reason to suppose, that it is above all the Autumn fall in temperature, perhaps together with changes in the nutrient medium and the age of the strain, which brings about the encysting in the Ceratia. We do not know if all Ceratia are capable of forming cysts. The formation of cysts is preceded by an (unknown) number of divisions and an accumulation of reserve material.

We have now tried to subject to cooling a "normal" Ceratia population brought to development (18°C, spring water, daylight), by gradually cooling the culture vessel, to about 6°C after about 4 - 5 days and keeping the same at this temperature for about 10 days: Hitherto we have had no success in the formation of cysts through simple cooling. The failure could be attributed to the fact that the Ceratia raised in the culture (through lack of time and perhaps also through insufficient nutrient) could not in the end accumulate enough reserve-nutrient. For, until this point they had lived at the expense of the earlier reserves. Besides this, the cooling could also have been carried out rather too quickly, and finally, the composition of the culture liquid must be given greater attention. In any case we have seen that the low-temperature stimulus alone is not sufficient as an impetus to cyst formation. Other conditions must be fulfilled here. Certainly a special "inner preparation" of the cell, a specific age etc. is also necessary.

These questions are going to be studied. Should we succeed in bringing about cyst formation, the whole cycle of development of Ceratium hirundinella would be experimentally closed. Hitherto, however, the last link in the experimental productive chain of the developmental processes of C. hirundinella is missing.

Summary

1. The stratified cold-water activated sludge of Lake Zürich contains large numbers of Ceratium cysts, especially in individual upper layers. The stratification allows a precise determination of age of the same. We succeeded in causing such cysts to develop into Ceratium capable of division and in following the individual phases.

2. The ability of the Ceratia cysts to germinate lasts about 6 to 7 years.

3. Three types of cyst can be identified: spherical, 3-horned, small cysts, sphèrical, 3 to 4-horned, large cysts and flat, 3-mostly 4-horned rhomboid cysts, which correspond to quite precise Ceratia-types. They are described accordingly as gracile -, austriacum and "piburgense" - cysts.

4. The processes of the germinating cysts consist of a slight swelling up of the whole cyst, spreading of the horns at their base and filling into cyst contents, movement of granules inside, differentiation of the cyst contents into an apical and an antapical part, which results in the formation of the transverse groove (36 - 40 hours after the onset of germination and c. 4 hours before the emergence.) Formation of a very delicate membrane inside the cyst wall and retraction of the so-formed embryo from the cyst wall. Emergence of the embryo at a fairly precise part of the cyst, duration 1 - 2 minutes.

5. The nascent moving embryo (longitudinal and transverse flagellum) has the Gymnodinium characteristics. On this account we describe the phase which the embryo is now passing through as the Gymnodinium stage and the embryo itself as Gymnoceratium. Duration of this stage (at 18°C) about 2 hours. Towards the end of this phase there appear apical- and antapical horn buds, also the bud of the right rear horn and very rarely also that of the left lateral horn - temporary appearance of a sinking in of the pole-horn buds.

6. This phase passes gradually into the pre-Ceratium stage. Characteristic of this is: broadening of the body, greater dorso-ventral flattening, formation of the horns and the exoskeleton, which first appears in the main body in the region of the transverse groove, then becomes visible at the base of the horns, while the distal parts of the horns for the time being remain transparent (places of principal growth). This phase is ended with the complete and clear formation of the shell on the main body as well as the horns. Duration c. 6 hours.

7. The Ceratium stage is characterized first by the ability to divide, which soon establishes the definitive form; then also through the ability to form cysts.

8. We did not succeed in experimentally inducing the formation of cysts. Cooling of freshly cultivated populations is obviously not enough to induce cyst formation.

9. We never observed a copulation.

10. C. cornutum shows a Gymnodinium stage too, whose development was for the greater part observed and described by Folgner. This development shows a Gymnodinium stage which lasts about 2 hours and a pre-Ceratium stage which last 4 hours, both phases agreeing with those stages described by us, even in inner processes.

We are no doubt dealing with, in those processes in the development of C. hirundinella described by us, a general nature which could be common to a universal group of Ceratia (perhaps the whole sub-species of Biceratium [Vanhöffen] Gran).

Notice