

tronquées; deux dépressions buccales opposées l'une à l'autre commençant du milieu du corps et allant jusqu'à l'extrémité postérieure; l'un des bords de chaque dépression buccale est développé en une expansion aliforme; de l'angle antérieur de chaque dépression buccale part un flagelle ayant à peu près la longueur du corps; de chaque côté 3 cils buccaux, insérée plus bas, ne dépassent pas la dépression buccale. Deux noyaux marginaux en forme de croissant, placés tout-à-fait à l'extrémité antérieure du corps et se touchant par leur partie antérieure renflée; de cette façon l'ensemble des deux noyaux figure une sorte de fer à cheval à concavité dirigée en arrière.

A la division tout le matériel chromatique est employé à la formation de chromosomes en forme de grains (16 pour chaque noyau).

Dans le genre *Hexamitus* il y a des espèces libres et des espèces parasites; les genres *Octomitus* et *Giardia* ne renferment que des espèces parasites; les *Trepomonas* sont des Flagellés libres, mais certaines espèces (p. ex. *T. agilis* Duj.) peuvent être parasites facultatifs.

Théodosie (Crimée).

## 2. Copulation in Amoeba.

By Ralph E. Hedges, Instructor in Zoology, Pennsylvania State College, with an appended letter by Maynard M. Metcalf, Oberlin College.

(With 5 figures.)

eingeg. 4. November 1913.

While working in the Zoological laboratory of Oberlin College during the summer of 1912 I found a small species of *Amoeba* in a hay infusion which I was examining. These I took to be *Amoeba limax*. Although I was not working upon the Protozoa at the time, these Amoebae attracted my attention because they were particularly active, and because I several times saw two Amoebae move toward each other, touch and after remaining in contact for a few seconds move apart.

In most cases it was impossible to distinguish a nucleus, for the Amoebae were small and filled with bacteria of which there was an abundant supply in the infusion. There was however very little debris upon the slide, so that the Amoebae could be seen very clearly. The material examined was not surface scum but was drawn up with a pipette from the sides of the receptacle and from the hay beneath the surface.

The slide upon which I first found these Amoebae had not been under observation for more than ten minutes before I saw two Amoebae come into contact, and after remaining quiet in this position for perhaps twenty seconds, the distinct line of contact between the two individuals broke through for a portion of its length, and the protoplasm of one *Amoeba* flowed rapidly into the protoplasm of the other *Amoeba*. The

opening grew larger as the protoplasm flowed through and this flowing did not take more than three or four seconds to be completed. From the behavior of the other *Amoebae* which were on the slide and which as stated above had been touching and then separating I had anticipated some such action as this and was fortunate enough to have sketched the two which I saw unite, as they were moving toward each other, and as they united (fig. 1 *a—e*). These drawings were made by the double vision method under the high power of the microscope (265 diameters), and with respect to shape and relative size are fairly accurate, although I was unable in either *Amoeba* to distinguish the nucleus.

This union I believe to have been Copulation<sup>1</sup>. It was not a temporary union or contact, neither was it an engulfing or devouring of one *Amoeba* by the other. The two individuals were of nearly the same size and the process was a distinct and rapid flowing of the protoplasm of one into the protoplasm of the other, the resulting individual being a slightly larger *Amoeba*. The abundance of food contained in the *Amoebae* made this flowing all the more plainly visible, and enabled me to see clearly the definite currents as indicated by the arrows (fig. 1 *d*). The transfer of protoplasm was plainly seen to be complete, none being cast off in the process, for the *Amoebae* at the time of copulation were in a clear field and there was no chance of confusion arising from the presence of other objects in the field.

The only noticeable differences between the two *Amoebae* that copulated were the differences in shape and the fact that individual *B* (fig. 1) was the more active of the pair while *A* was less active. These factors may be unimportant although there is some relation between form and movement, the form of *B* (fig. 1 *b*) being the form taken by this species of *Amoeba* in moving in a definite direction, while the form of *A* (same figure) is that taken by the *Amoeba* when moving less rapidly and in no definite direction. In this case copulation was accomplished by the protoplasm of the less active flowing into the protoplasm of the more active individual. The afternoon of the following day I observed this copulation process again in two other pairs, and in one of these (fig. 2) the movement of the protoplasm was again from the distinctly less active to the distinctly more active individual. In the third pair observed (fig. 3) both *Amoebae* were more nearly alike in activity and in shape, and, although I could not tell, which was the more active in this case, the process of copulation was the same as in the other two pairs. Whether this flowing from the less to the more active individual

<sup>1</sup> The German usage is here followed, and as the union of the gametes is complete and not partial and temporary, the word copulation instead of conjugation is used.

Fig. 1.



is at all constant I am unable to say from these limited observations, but record it only as observed.

I was not able satisfactorily to distinguish the nucleus in any of

the individuals of the three pairs in which the union was plainly seen to occur. However the water and food vacuoles were seen to be carried across intact especially in the pair shown in fig. 2, in which case each *Amoeba* contained two large water (?) vacuoles. The resulting individual contained the four.

Regarding the phenomena following the union of the two individuals I can give only enough data to show that there are in all probability resulting phenomena of interest. Immediately after copulation in each case, the resulting *Amoeba* remained comparatively quiet for a period of several minutes and then, in the case of the last two pairs of individuals observed (fig. 2 and 3), the resulting Amoebae crawled into a mass of debris and were lost. The individual resulting from the first pair observed to copulate (fig. 1) was followed for a period of four hours. This copula after remaining quiet for a few mi-

Fig. 2.



Fig. 3.



minutes, having the shape shown in *e*, then began sending out pseudopodia as in *f* and crawled into a mass of bacteria where it remained for about ten minutes assuming shapes similar to *g* and apparently feeding. It then took the shape shown in *h*, and moved rapidly across the field in a comparatively straight line and toward a line of bacteria near the edge of the slide. There were many other Amoebae on the slide and almost all had also taken the shape shown in *h*, and were moving in the same direction as the one under observation. In the posterior and pointed end of the copula as many as three vacuoles would appear and would then burst into one which would then contract. The "club shape" of the *Amoeba* is the typical *Amoeba limax* shape and the fact that it was the predominant shape of all the individuals observed, is one reason for believing this to have been *Amoeba limax*.



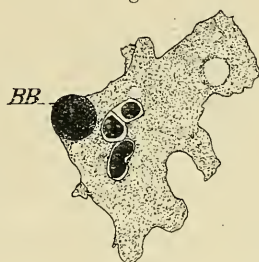
As it came nearer to the line of bacteria the copula rounded up (*i*) and crawled slowly among the bacteria. This was about a half hour after copulation and the *Amoeba* stayed here during the remainder of the time that it was under observation, as did the other *Amoebae* which had been moving toward the bacteria.

About an hour after copulation I noticed a large clear space formed in the copula, which had much the appearance of a large vacuole as shown in *j*. The *Amoeba* now became more or less "knotted" in appearance and the wall of the clear space which had been taken for a vacuole became thicker and brownish in color, with a small semi-clear space near the center (*k*). Two hours after copulation and an hour after its first appearance, this structure had become very distinct and seemed near to the outside of the body and at one time appeared to be almost

Fig. 4.



Fig. 5.



cut off. During the remaining two hours during which the *Amoeba* was observed it remained in much the shape shown in *l* and finally crawled out of sight and in amongst some other *Amoebae* and was lost. Many other *Amoebae* on the slide showed this same "brown body" and other similar bodies were seen lying loose upon the slide, but this was the only case in which what appeared to be the development of one of them was observed.

During the period between the arrival of the zygote *Amoeba*, above described at the line of bacteria and the end of the observation, the *Amoeba* approached or was approached by one near-by individual five separate times, but in each instance, after touching, the two separated. These may have been merely collisions. Similar collisions of individuals in a condition prepared for copulation might lead to fusion. This individual, being itself a zygote, perhaps repulsed instead of attracting the others which it touched.

My work was unavoidably interrupted for a day and not being able to find any more of the *Amoebae* copulating after that, I mounted some of the material. Among those successfully stained with Delafield's Haematoxylin and mounted, only two individuals show more than one

nucleus. These are shown in figs. 4 and 5. In fig. 4 there are two nuclei side by side, while there is a darker portion of the protoplasm which has somewhat the appearance of one of the "brown bodies" (*BB*). In the other individual (fig. 5) there are two nuclei side by side as in fig. 4 but there is also an additional, elongate nucleus in this case. The brown body is much more clearly defined in this individual, is much darker and has more of the appearance of the body as seen in the living specimens. It has taken the stain rather deeply.

I hesitate to draw any conclusions regarding the nuclear phenomena feeling that the stages fig. 4 and 5 represent only two phases of what is probably a relatively long series of changes. All of the observations are preliminary and may perhaps be continued if it is possible again to get material in this stage of development.

State College, Pennsylvania, May 25st 1913.

My dear Hedges:

I have read your paper and studied your slides with much interest. Two points among your observations seem to me of especial interest — first, the great rapidity of the copulation process, and second, the fact that the gametes are of considerable size and do not, so far as I can determine from your slides, contain caryosomes in their nuclei.

Copulation has been observed by a number of students in about a dozen different species of *Amoeba*. These are, however, all small forms with nuclei containing caryosomes. The large vegetative forms of *Amoeba*, like the common *Amoeba proteus*, have nuclei without caryosomes, but these have never been found in copulation. The minute gametes of *Amoeba proteus*, whose copulation I have observed, have nuclei containing caryosomes. If you really have here gametes with nuclei which contain no caryosomes, and this appears to be the case, it is of much interest, for caryosome nuclei are almost unquestionably the more primitive, and gamete nuclei, not only in *Amoeba proteus*, but in general, show a somewhat primitive condition. If in your species even the gametes show the modified type of nucleus, the species is, in this important feature, the most aberrant *Amoeba* known.

From your slides and description I cannot identify the species upon which you worked, nor is it likely that I could have identified the living individuals. Identification of species in this "genus" is often very difficult. Different forms taken by individuals of the same species in corresponding and different stages of the life history have been given invalid specific names. Probably several species assume the *limax* form, and it is doubtful if there is a distinct species which can properly be called *A. limax*. The same is true of *A. radiosa*, and several other forms.

Specific descriptions of Amoebae should include the complete life history, as do for example some of Schaudinn's and Nägler's descriptions. Except in the case of unusually distinct forms, confidence in identification is not possible without a knowledge of the full life history.

Faithfully,

Maynard M. Metcalf.

Oberlin, Ohio, June 10, 1913.

### 3. Über die Hancocksche Drüse von *Oecanthus pellucens* Scop.

Von Dr. V. v. Engelhardt, Moskau.

(Mit 4 Figuren.)

eingeg. 8. Februar 1914.

In seiner Mitteilung »Über die Begattung und die Spermatophoren bei einigen Locustodea und Gryllodea«<sup>1</sup> beschreibt Boldyrev ziemlich eingehend das Liebeswerben von *Oecanthus pellucens* Scop. Bei dieser Art findet der obenerwähnte Forscher ein eigentümliches Organ auf dem Metanotum des Männchens, das im Liebesleben dieser Art von großer Bedeutung zu sein scheint. Es handelt sich um eine besondere Drüse, oder, besser gesagt, um einen Drüsenkomplex, der auf dem Hinterrücken des Männchens ausmündet. Boldyrev hat beobachtet, daß das Weibchen während, und besonders nach vollzogener Copulation, diese Drüse fortwährend ableckt, was oft eine halbe Stunde in Anspruch nehmen kann.

Nach Ablauf dieser Zeit geht das Pärchen auseinander, und das Weibchen verzehrt fast augenblicklich die schon vollkommen entleerte Spermatophore. Auf Grund dieser Beobachtung macht Boldyrev den durchaus richtigen Schluß, daß, obwohl diese Drüse vom *Oecanthus*-Männchen einen Reiz auf das Weibchen ausübt, so hat doch dieselbe eine wichtigere Bedeutung für die Erhaltung der Art. Seiner Ansicht nach ist diese Drüse weniger ein »Lockapparat«, — vielmehr aber ein spermaschützendes Organ, das das Weibchen von der vorzeitigen Vernichtung der Spermatophore abhält, da in der Zeit, während das Weibchen fleißig das Drüsensecret ableckt, der ganze Sameninhalt der Spermatophore in das Receptaculum einwandert, so daß schließlich ein leerer Sack vom Weibchen verzehrt wird. Die Bedeutung der Hinterrückendrüse wurde von Boldyrev durch folgendes Experiment bewiesen: ohne das Weibchen zu stören, entfernte er ganz vorsichtig während der Copulation das Männchen, — und als Folge davon konnte man sehen, daß das Weibchen fast sofort die vom Sperma gefüllte

<sup>1</sup> B. Th. Boldyrev, »Über die Begattung und die Spermatophoren bei einigen Locustodea und Gryllodea«. Rev. Russe d'Entomologie. T. 13. 1913.

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