Pl. V. in the next number.

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On the Behaviour of the Nuclei
during
the Conjugation of *Zygnema*.

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With Plate V.

The fusion of nuclei of two cells during the conjugation-process of several species of *Zygnema* has been established by Dr. H. Klebahn.⁽¹⁾ It is a well-known fact that we distinguish two modes of conjugation in *Zygnema*: firstly, that in which zygote is formed within one of the two conjugating cells, and secondly,

⁽¹⁾ Klebahn, Ueber die Zygosporen einiger Conjugaten. Ber. d. deuts. bot. Ges. Bd. VI, 1888.

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that in which it is formed, not within any of these cells, but within the common canal formed between them. **Klebahn's** observations refer exclusively to *Zygnema* belonging to the first class. It is the purpose of this paper to establish the fusion of two nuclei in one species belonging to the second class and to describe their somewhat peculiar behaviour during the process.

Now firstly as to the mode of preparation. My observations have almost exclusively been executed on materials which were hardened, stained, and imbedded in Canada-balsam, because fresh materials give but an imperfect image. Algal filaments are hardened and stained in either of the two ways mentioned below: firstly, they are hardened and stained simultaneously by means of **Pfitzer's Picro-nigrosin**⁽¹⁾, in which they lie at least for one night and are then washed with very dilute glycerin; secondly, they are hardened by 1 % chromic acid, washed during several hours with running water, stained with **Delafield's Haematoxylin**, in which they lie at least for one night, washed with 2 % alum solution, and then washed repeatedly with distilled water. Algae hardened and stained in either of these two ways are then transferred to 20-50 % alcohol, which is then made to concentrate gradually almost to 100 % by diffusion method⁽²⁾, then again transferred to 10 % solution of turpentine in absolute alcohol; **Overton's** arrangement⁽³⁾ is then made to make

(1) **Pfitzer**, Ueber ein Härtung und Färbung vereinigendes Verfahren für die Untersuchung des plasmatischen Zelleibes. *Ber. d. deuts. bot. Ges.* Bd. I, 1883.

(2) **Strasburger**, *Botanisches Practicum*, 2. Aufl., 1887, p. 515.

(3) **Overton**, Mikrotechnische Mittheilungen etc. *Zeits. f. wiss. Mikroskopie.* Bd. VII. Heft 1.

this solution gradually change into almost pure turpentine, after which algae can safely be imbedded in Canada-balsam in turpentine (not too much concentrated) with no contraction at all. Thus I have obtained beautiful specimens of *Zygnema*, in which only pyrenoids, nuclei, and nucleoli are stained and other parts are almost colourless.

The specific name of *Zygnema* in question could not be determined. For its structure I refer the readers to the middle cell (on the left side and not concerned in conjugation) in *Fig. 4 a*.

The first step of the conjugation is the formation of two processes, one from each cell (*Fig. 1*), which then meet and fuse with each other (*Fig. 2*). Then follows the disappearance of the common wall which separates the two processes (*Fig. 3*) Up to this stage we see no change at all in both nuclei. The next stage is represented by the upper pair of cells in *Fig. 4*, where the nuclei of the two cells are already concerned in the process of fusion. At this stage, it seems as if both nuclei are connected by a very fine thread (*t*), but this so-called "thread" part seems to be nothing more than a part of the nuclei specially thinned during the conjugation. I was not fortunate enough to be able to follow the development of this "thread," but its formation is not difficult to be comprehended and proceeds probably as follows: Each nucleus produces a thin process tapering inwards and these two processes meet to form the "thread." This view is made highly probable by the fact that each nucleus tapers inwards very gradually and the "thread" is therefore finest at its middle part, which indicates the meeting point of the two processes of nuclei. In the next

stage (*Fig. 5*), the "thread" becomes broader, its width being almost equal to that of the body of the nucleus; in this stage, the two nucleoli do not yet come into fusion. Then the "thread," which may now rather be called a "band," becomes shorter and the two nucleoli approach each other (*Fig. 6*). When this stage is over, two chromatophores pass from each cell into the common canal, which widens and becomes bladderly; now, the real fusion of the two nuclei takes place, for both nucleoli become fused into one, and the resulting nucleus takes the normal shape (*Fig. 7*). For the formation of zygote, it remains then nothing but to form a thick coat around the whole apparatus of these chromatophores and nuclei.

In short, during the conjugation of this species of *Zygnema*, after the fusion of the two processes of cells, one of the nuclei produces a thin slender process which advances inwards and meets with that produced similarly from another and thus the so-called "thread" is formed. The latter then becomes wider, the two nucleoli approach each other and fuse together, and the nucleus takes the normal shape. This is the whole process relating to the fusion of the two nuclei.

It may here be remarked that chromatophores (pyrenoids as well as starch-granules) seem to grow in those cells concerned in conjugation. For this I refer the readers to the *Fig. 4a* in which cells concerned and those not concerned are found both in one and the same filament.

In conclusion, I have to add that I could follow also almost all successive stages of the behaviour of nuclei during the conjugation of a species of *Zygnema* belonging to the first class above mentioned. I ap-

pend here figures (*Fig. 8, a-g*) explaining some of these stages, of which *c*, *e*, and *f* correspond respectively to *Figs. 11, 12*, and *13* of Klebahn⁽¹⁾⁽²⁾. These figures explain themselves, but I refer those readers who want further information to the Explanation of Plate affixed below.

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⁽¹⁾ Klebahn, l. c.

⁽²⁾ Of course, various stages represented in my figures are drawn in fresh state in common systematic books, such as Cooke's *British Fresh-water Algae* (e. g. Pl. 31, fig. 2 b) or Wolle's *Fresh-water Algae of the United States* (e. g. Pl. CXLIII, fig. 5), but in these works cell-contents are coloured entirely green, so that I can distinguish neither chromatophores nor nuclei.

Explanation of Plate V.

All figures drawn from *Picro-nigrosin* preparations (except Fig. 8, which is drawn from *Chromic-Haematoxylin* preparations) with Abbe's camera lucida under the magnification by Zeiss, Homog. Immers. $\frac{1}{12}$ + Ocular 2.

Fig. 1. Cells preparing to conjugate; two processes produced from them about to meet each other. p signifies *pyrenoid*; c, *chromatophore*; n, *nucleus*; k, *nucleolus*.

Fig. 2. Two processes fused, and common wall yet present.

Fig. 3. Common wall already disappearing.

Fig. 4. Nuclei connected by the so-called "thread" (processes of two nuclei) (t).

Fig. 4 a. In the uppermost and the lowest pair of cells, which are concerned in conjugation, chromatophores are large, whereas in the middle cell not concerned in conjugation they are small.

Fig. 5. Thread now becoming a band; two nucleoli yet distinct.

Fig. 6. Two nucleoli approaching each other.

Fig. 7. Chromatophores passing into the common bladdery canal; two nuclei, together with their respective nucleoli, fusing.

Fig. 8. Represents various stages of conjugation in a species of *Zygnema* belonging to the first class:—

- a. Cell not concerned in conjugation;
- b. Contents of one cell on the way of passing towards the other cell; two nuclei distinct;
- c. Contents of two cells found together in one of them; both nuclei yet distinct;
- d, d'. Nuclei coming into contact with each other;
- e. Nuclei fusing together, both nucleoli yet distinct;
- f. Nuclei and nucleoli fusing;
- g. Abnormal form, in which both nuclei and nucleoli fuse together already before the contents of one cell have not yet completely passed away into the other cell.