A Note on Conjugation in *Zygnema*.

BY

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With Plate XII and two Figures in the Text.

MATERIAL. Certain preserved material of *Zygnema*, which was known to have been in the possession of the Department for a number of years, although it was unfortunately impossible to discover its source, was found on examination to show abundance of zygospores. As conjugation in *Zygnema* is rare in nature, and has not hitherto been induced experimentally, it was felt that a preliminary account of the stages shown in this material would be of interest. In order to confirm the observations on the structure of the chloroplast in the fixed material, fresh material of another species (showing only the vegetative stage) was also studied. The resemblance in general character of the chloroplasts in the two species was very striking.

Treatment. The preserved material was in 70 per cent. alcohol, but as an additional precaution was fixed for 24 hours in dilute chromacetic acid. It was then placed in 5 per cent. glycerine, which was concentrated until of the thickness of pure glycerine. The material was then stained in safranin and aniline blue, and mounted in Venetian turpentine. Material thus treated showed the nucleus stained pink, the chloroplasts light blue, while the pyrenoid took a very dark blue. The cytoplasm remained practically colourless. Paraffin sections $6\ \mu$ thick were also cut, and stained with Heidenhain's iron-alum haematoxylin, but owing to the small size of the nucleus and the originally imperfect fixation, no details of nuclear structure were made out.

The fresh material was studied in water and iodine mounts: the latter reagent brought out very clearly the structure of the chloroplast and the pyrenoid.

Description. By comparison with herbarium material at the Natural History Museum, the conjugating material was identified as *Zygnema stellinum*, (Vauch.) Ag. The fresh material corresponded to *Z. cruciatum*.

Zygnema stellinum: cells of filament sub-cylindrical $30-35$ (33) μ in diameter, $70-80$ μ in length. The cell-wall is thin cellulose, with no signs of lamellation even when treated with caustic potash. The cytoplasm is somewhat vacuolated, with an apparently denser median strand (zugon), in which the nucleus is suspended. The nucleus is small (3 μ), sub-spherical, with well-marked membrane and nucleolus. No details of nuclear division were observed. In the vegetative cell there are two elaborate chloroplasts, suspended in a small amount of cytoplasm in the centre of the cell. Each plastid contains a single large (3 μ) pyrenoid, which is surrounded by a more or less diffuse starch zone. The exact method of starch-formation was not determined. The plastid is never apparently seen side-on, as it is in *Mougeotia*, so that it would seem to be a radially organized body. It appears (in the fresh material) as a complex mass of much-dissected fronds, radiating from and incurved over the central pyrenoid (see Plate XII, Fig. 1).

TEXT-FIG. 1. *Zygnema stellinum*, showing scalariform type of conjugation. ($\times 500$ times.)

In the fixed material the dissection was not quite so marked, due possibly to shrinkage during manipulation.

Conjugation. The material at hand showed abundance of zygospores, but no earlier stages in the process of conjugation. Conjugation was by the scalariform method, the zygospore being lodged in one or other of the conjugating cells, which is not inflated or distorted in any way (Text-fig. 1). 'Cross'-conjugation was not observed, but there were numerous cases of differentiation of sex in a single filament, as has been frequently reported in *Spirogyra* (Cunningham (1)).

The young zygospore is $30-33$ μ in diameter, and approximately spherical. The wall is thin ($1.5-2$ μ), and not yet cuticularized: usually there are four inwardly-directed points on the inner surface. The four chloroplasts derived from the two conjugants remain quite distinct, and retain their characteristic complex shape as seen in the vegetative cell. They are commonly arranged in a tetrahedral fashion (see Plate XII, Fig. 2).

The nucleus is approximately double the size of the vegetative nucleus ($7-8\ \mu$), and frequently appears constricted. Whether this appearance is due to incomplete fusion or incipient division was not determined.

The cytoplasm is much vacuolated, with many highly refractive droplets which dissolve completely in absolute alcohol or ether, and so would seem to be of an oily nature (Text-fig. 2, *a*).

Occasional azygospores are found, which are more ovoid, and contain

TEXT-FIG. 2. *a*. Young zygospore of *Z. stellinum*, showing four chloroplasts and fusion nucleus in centre of spore. *b*. Young azygospore, ovoid, with only two chloroplasts. *c*. Mature zygospore, showing sculpturing of the mesosporium: the four chloroplasts remaining distinct. (All $\times 1,300$.)

only two plastids. The nucleus also is no larger than the vegetative nucleus (Text-fig. 2, *b*).

The mature zygospore (Text-fig. 2, *c*) shows sculpturing of the mesosporium into blunt bosses, which are yellow-brown and cuticularized. The four chloroplasts retain their individuality: even at this stage there is no sign of any fusion or division of the plastids. The four tetrahedrally arranged ingrowths on the wall may indicate a subsequent tetrad division, similar to that described in *Spirogyra* (Tröndle (2)), for the first division of the zygote, but this point must be left for further observation to decide.

SUMMARY.

Zygnema stellinum shows scalariform conjugation, with the zygospore lodged in one or other of the conjugating cells.

The vegetative cell contains two complex chloroplasts, consisting of much-dissected fronds, incurved over a central pyrenoid. The nucleus is suspended in a strand of denser cytoplasm in the centre of the cell.

The young zygospore is spherical, with a thin wall and four chloroplasts. The fusion nucleus is in the centre of the spore, and is twice the size of the vegetative nucleus and often constricted.

Occasional azygospores are found, which are more ovoid and have only two chloroplasts.

The mature zygospore shows sculpturing of the mesosporium, which is yellow-brown and cuticularized. The four chloroplasts remain distinct even at this stage.

In conclusion, I wish to express my thanks to Professor R. R. Gates, at whose instigation the work was undertaken, for his constant advice and criticism during the course of the investigation; also to Mr. Gepp, of the Natural History Museum, for his kind assistance in identifying the species.

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EXPLANATION OF PLATE XII.

Fig. 1. Vegetative cell of *Zygnema cruciatum*, showing two complex chloroplasts, each with one pyrenoid, nucleus suspended in centre of cell in a strand of denser cytoplasm.

Fig. 2. Young zygospore of *Z. stellinum*, showing four chloroplasts, less dissected than those of the vegetative cell. (Both figures $\times 2,300$.)

1.

2.

Huth lith et imp.

SMITH — ZYGNEMA.

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