Cytological Studies on *Oedogonium cardiacum* Wittrock and One Another *Oedogonium* sp.¹

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Material and methods

Much has been said about the correlation of the nuclear division and the formation of cell plate (Kraskovits 1905 and Wisselingh 1908). The present communication deals with the cytological observations which have been made only on mitosis and morphology of the chromosomes of *two Oedogonium* spp. that were investigated.

The species of *Oedogonium cardiacum* Wittrock was obtained identified from Cambridge Culture Collection of Algae and Protozoa through the Curator Mr. E. A. George in 1956. Another *Oedogonium* species was collected in a vegetative condition from Wise Ean Tarn, near the laboratory of the Fresh-water Biological Station in the Lake District Britain. The width of the filaments of this sp. varies from 55 to 65 μ and the cells are shorter than broad or as long as broad. In culture, formation of sex organs did not take place. So it was not identified.

Culture was maintained in the biphasic media (Pringsheim 1946), Godward's solution (Godward 1942) and soil extracts, culture media. The soil extracts were prepared by adding three times distilled water to the volume of John Innes Compost, in a 2000 cc. conical flask of pyrex glass and the flask was autoclaved at 20 atmosphere pressure for 30 minutes. After cooling and filtered, solution was again autoclaved under the same conditions. The extract was then ready for use. Godward's medium was diluted to half with the extract and the resulting mixture was then used as a culture solution. The cultures were maintained throughout at a temperature between 20°—24°C. Sterilised wide-mouthed milk bottles, pyrex culture-tubes and petri dishes were used as culture vessels. Stock cultures were maintained in 12 hours light and 12 hours darkness, and artificial light proved successful for maintaining cultures. The materials flourished well and the growth was found to be satisfactory.

The filaments of *Oedogonium*, after inoculation in fresh biphasic culture solutions or in inorganic solution mixed with soil extract produced zoospores within 4 days which after a swarming period settled down at the surface of the culture solution. The male and female filaments of *Oedogonium* were

¹ Part of Ph.D. work.

inoculated singly in culture media and zoospores were soon to be produced from them.

In old cultures generally after three months, antheridia and oogonia were formed. The sex-organs were formed more profusely and more rapidly (within a week) when they were grown in an atmosphere of carbon dioxide (Starr 1955, pp. 261 and 265). This was done by placing the *Oedogonium* in watch glasses enclosed in petri-dishes containing 5% sodium bi-carbonate.

Cytological technique. Materials of cytological study were kept in petri-dishes in order to get uniform light. The freshly prepared fixing fluid in the proportion of 3:1 Glacial acetic acid and absolute alcohol was used and the materials were left about two hours till they were completely decolourised.

Fixation was made within 24 to 48 hours after the materials were transferred from stock culture to fresh culture medium.

The method of staining by acetocarmine after mordanting with 4% aqueous iron alum was used throughout the investigation as employed by Dr. Godward (1948). The slides with materials mounted in acetocarmine under a cover slip were heated very slowly over the naked flame of a spirit lamp, taking all precautions to prevent drying of the preparations. The slow gradual heating was found to dissolve the starch grains and give better staining of the chromosomes with a clear back ground.

In general, mitotic divisions were found in the morning, when the material was kept after 24 hours in a temperature 2°C higher (24°C) than that at which it has previously been kept (22°C). When the material was kept in continuous light, the divisions were not concentrated at any particular hour.

Figs. 1-3. $\times 2400$. Mid prophase and late prophase in *O. cardiacum* Wittrock. 1, mid prophase with long intertwined chromosomes showing spiral structure. 2-3, late prophase showing intertwined arrangement of chromosomes.

Cytological observations

Oedogonium cardiacum Wittrock

Mitosis The resting nucleus is circular or slightly oblong, surrounded by a delicate nuclear membrane and containing either one large nucleolus or two smaller nucleoli. The diameter of the nucleus measures nearly 7μ . Two dot shaped prominent bodies are frequently seen within one nucleolus, which presumably represent the regions of *N. O. Chromosomes*; the details of which have not been made out at this stage. In early prophase, the nucleus enlarges considerably to about 18 to 20μ in diameter. The outline of the nuclear membrane becomes irregular. In very early prophase, minute chromatin granules appear within whole nuclear cavity which seem to be arranged in

Figs. 4-6. $\times 2400$. Anaphase in *O. cardiacum* Wittrock. Microphotograph of an anaphase group. At this focal level 3 chromatids are out of focus. Localised centromeres are clearly seen at points of bendings. 5, diagrammatic representation of Fig. 4 ($n=19$). The chromatids show median, submedian and subterminal centromeres. 6, the chromatids of Fig. 5 are arranged in three groups, to show the positions of the centromeres. A, with median centromeres. B, with submedian centromeres. C, with subterminal centromeres

linear order as observed by Kretschmer (1930) in *Oedogonium pachyandrium*. Presumably the discontinuous staining of the early prophase chromosomes may indicate alternation of euchromatin and heterochromatin regions. At about mid-prophase, long chromosome threads are visible which are intertwined together very much. Sometimes, these are seen to be orientated lengthwise (Fig. 1), as pointed out by Kretschmer (1930) who used the term "Polfeldlage". The spiral structure of the chromosomes is frequently seen (Fig. 1). They are of various sizes as reported by Ohasi (1930) in *Oedogonium grande*. The

nucleoli gradually disappear during prophase. Kretschmer (1930) has also reported dissolution of the nucleolus during prophase. In late prophase, the

chromosomes become distinct but the long chromosomes appear somewhat intertwined (Figs. 2 and 3). At metaphase they usually arrange themselves at equator. The chromosomes at metaphase appear short or long and rod-shaped. Chromatids are easily recognisable at pre-metaphase. However, the chromosomes are not seen at one level in metaphase, so it was not possible to estimate their correct numbers at this stage; neither was it possible at late prophase. The number of chromosomes therefore, has been estimated from the counts of chromatids at anaphase, the number found being 19 (Figs. 4 and 5). In the photograph (Fig. 4), three chromatids are out of focus. Fig. 5, in which all 19 chromatids are shown is a diagrammatic sketch made from the preparation from which the above photograph was taken at one focal level. At this stage, the positions of centromeres are easily recognised as nonstaining regions, but even more readily at points of bending. The position of centromeres in all chromatids has been observed carefully and is represented in Fig. 6, the chromatids being arranged in three groups

Figs. 7-8. $\times 2400$. Anaphase and telophase in *O. cardiacum* Wittrock. 7, late anaphase. 8, telophase. Spiral structure of the chromosomes in the daughter nuclei is clearly seen.

Figs. 9. $\times 1400$. A squashed pre-metaphase stage in an *Oedogonium* sp. from which an approximate count of 41 chromosomes is made. Localised centromeres are clearly seen in some chromosomes.

as follows :—

- A. 5 chromatids showing median centromeres.
- B. 6 chromatids showing sub-median centromeres.
- C. 8 chromatids showing subterminal centromeres.

At anaphase, the chromatids are separated in a normal way and they are more closely crowded at both ends of poles (Fig. 7). Spindle fibres appear to be delicate. Ohasi (1930) has reported fine spindle fibres. The spiral structure of chromosome is easily made out at telophase (Fig. 8).

The process of nuclear division and the structure of chromosomes of *Oedogonium* appears similar to that of the higher plants.

Oedogonium sp: A brief account of mitosis of another *Oedogonium* species is as follows. The resting nucleus is spherical or oblong, measuring nearly 9 to 10 μ in diameter containing one prominent nucleolus in which sometimes a dot shaped thick body is often recognisable, presumably a variety of heterochromatic chromosome. The enlarged nucleus, in prophase measures nearly 18 to 22 μ in diameter and becomes irregular in shape. The nucleolus seems to be gradually dissolved. Early prophase chromosomes show discontinuous staining and appear as strings of fine dots. At late prophase short and long chromosomes are distinguishable. The number of the chromosomes was estimated as approximately 41. In Fig. 9, though heavily squashed, some chromosomes are not separated from each other. The sizes of the chromosomes vary considerably. Localised centromeres in most chromosomes are easily recognisable.

Anaphase is normal. In telophase, spiral structure of the chromosomes is often recognisable.

Summary

1. Cytological investigations as regards the morphology of the chromosomes during mitosis have been made out in two *Oedogonium* spp.

2. The process of nuclear division during mitosis follows a normal course and the number of chromosomes has been estimated 19 in *Oedogonium cardiacum* from the counts of chromatids at anaphase and in another *Oedogonium* sp., the number of chromosomes has been estimated as approximately 41 during late prophase.

3. The morphology of the chromosomes in regard to the position of the centromeres has been studied. Localised centromeres are easily recognisable as non staining regions at points of bending of chromatids during anaphase. As regards the positions of centromeres, the chromatids are found to be of three types, with median centromeres, sub-median centromeres and sub-terminal centromeres.

4. The spiral structure of the chromosomes of various sizes is easily recognisable in mid-prophase and at telophase. Ohasi (1930) has reported

about the spiral structure of the chromosomes in *Oedogonium grande*. Delicate spindle fibres appear to be present as pointed out by Ohasi (1930).

5. The nucleus enlarges considerably during early prophase, the nuclear membrane becomes irregular and minute chromatin granules arranged in linear order, appear within whole nuclear cavity as observed by Kretschmer (1930) in *Oedogonium pachyandrium*. At about mid-prophase, long intertwined chromosome threads are visible, some of which are seen orientated lengthwise as pointed out by Kretschmer (1930).

6. Chromatids are easily recognisable at pre-metaphase and at metaphase, the chromosomes appear short or long rod-shaped. Anaphase follows a normal course of division and the chromatids are seen more closely crowded at both ends of poles.

7. The process of nuclear division and the structure of chromosomes of both spp. of *Oedogonium* appears similar to that of the higher plants.

8. The method of staining by acetocarmine has been used throughout the investigation for cytological study.

9. The sex organs were formed generally after three months in old cultures. But they were formed profusely and more rapidly within a week when they were grown in an atmosphere of carbon dioxide by placing the filaments in watch glasses enclosed in petri-dishes containing 5% sodium bicarbonate as reported by Starr (1955).

10. After inoculation in fresh culture solutions, zoospores were produced within 4 days. The male and female filaments of *Oedogonium cardiacum* were inoculated singly in culture media and zoospores were seen to be produced from them.

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