Short Communication

SATYENDRA N. BANERJEE* and ROBERT J. HORSLEY*: Cytological Studies in the Haploid Male and Female Strains of *Oedogonium cardiacum*

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A detailed analysis of the chromosome morphology is lacking in all of the studies on chromosome numbers in *Oedogonium cardiacum* investigated by many authors¹⁻⁴. There is also some controversy regarding the presence or absence of satellite-bearing chromosomes and the number of chromosomes. We have been using *Oedogonium cardiacum* extensively for radiobiological studies^{5,6}, and have studied in detail the chromosome morphology of this species for future work on chromosome aberrations in irradiated cells.

Oedogonium cardiacum male and female strains were obtained from Dr. R. C. Starr (Indiana culture collection). The cells were grown in an inorganic solution in 5 gallon jars under 12 hour photoperiod. For cytological studies 5-6 month old cultures were induced to form zoospores which were collected on slides for a short time interval (1-2 hours). The detailed procedures for zoospore induction, collection and growth in the inorganic solution have been given in our previous communications⁶⁻⁸.

Chromosome analyses were done both in the long chains and in the zoospores. In the first case, collected zoospores were grown for 10 days and then fixed. For chromosome studies in the sporelings, a synchronized population was grown in an inorganic medium containing colcemide (500 $\mu\text{g/ml}$) and samples were fixed regularly at half hourly intervals from 12 hours to 24 hours from the mid-point of collection. Cells treated with colcemide were arrested at mitosis between late prophase and metaphase. The samples were then fixed in Carnoy's solution saturated with ferric-ammonium sulphate, coated with a thin film of collodion and stained by the Feulgen reaction as previously described^{9,10}.

The normal chromosome number of this species as determined from 60 good metaphase plates was found to be $n=19$ (Figs. 1 and 3). Variations in the size of chromosome ranged from 3.5 μ to 8 μ . On the basis of size difference the chromosomes are classified under the following groups: (1) 9 short chromosomes (3.5 μ to 4.1 μ); (2) 8 medium sized chromosomes (5.2 μ to 5.5 μ); and (3) 2 long chromosomes (8 μ). The two long chromosomes are seen to bear satellites or secondary constrictions. Apart from this normal number, cells with 18 chromosomes have also been noted in 2 cases (Fig. 2). In both the plates containing 18 chromosomes, a short chromosome was found to be missing. Detailed study of the chromosome morphology in plates containing 19 chromosomes is presented in Table 1 and Fig.

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Fig. 1. A typical metaphase plate seen in long chains of male or female *Oedogonium cardiacum*. The two SAT chromosomes are marked by arrows. $\times 3200$.

Fig. 2. A metaphase plate in zoospores treated with colcemide (500 $\mu\text{g/ml}$) for 16 hours from the mid point of collection. $\times 1500$.

Fig. 3. Karyotype analyses of the chromosomes as seen in Fig. 1.

3. It is interesting to note that in the F type each of the two long chromosomes has two constrictions, primary and secondary. In one chromosome the primary constriction is in the subterminal position and the secondary constriction is in the terminal position. The other chromosome has a primary constriction in the submedian position and the secondary constriction is in the subterminal position. In both chromosomes, the primary and secondary constrictions are well marked (Fig. 1).

Previous reports on the number of chromosome in *Oedogonium cardiacum* have claimed 19 chromosomes^{2-4,11,12}. Howard and Horsley¹³ have also mentioned 18 chromosomes for the same species. In our studies, however, we have observed 19 chromosomes in all but 2 cases. We thus confirm the previous reports of 19 chromosomes in *Oedogonium cardiacum*. The two cases of 18 chromosomes may have arisen due to non-disjunction of one of the small chromosomes (Type A). However, cells with 20 chromosomes were not observed.

The presence of satellite-bearing chromosomes in *Oedogonium* has been a matter of controversy. Hastischka-Jenschke²⁾ reported three distinct SAT-chromosomes for *Oedogonium cardiacum*. Hoffman³⁾ suggested the possibility of 2 SAT-chromosomes. In our studies we have seen two chromosomes with distinct secondary constrictions. The two chromosomes are easily distinguishable since the primary and the secondary constrictions are at different positions.

Hoffman¹²⁾ did not find any variation of chromosome morphology in young and old cultures as observed by Tschermak¹³⁾. Our observations in this regard support these findings of Hoffman. According to Hoffman³⁾ chromosome number and morphology of the male and female strains is similar. We also have not observed any difference in chromosome morphology between the male and female strain of *Oedogonium cardiacum* and thus, confirm the findings of Hoffman.

We are tempted to report here one interesting observation regarding the effects of colcemide treatment on *Oedogonium cardiacum* cells. Although colcemide treated cells were arrested at metaphase, this did not prevent stretching of the cell walls of the newly formed daughter cells as shown in Fig. 2. This sign of initiation of cell division in *Oedogonium* is first seen after completion of telophase in untreated control cells. These abnormal divisions suggest that the cell division protein in *Oedogonium* is different from the protein which constitutes the spindle fibres. We have reported before that colcemide interferes with SS-linked fibrous protein and inhibits spindle development¹⁰⁾. The initiation of cytokinesis before the completion of karyokinesis in colcemide treated cells lend us support to postulate that the cell division protein may not contain disulphide bonds as in the case of spindle fibres. It may also imply that in *Oedogonium*, completion of karyokinesis is not a prerequisite for cytokinesis.

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Table 1. Measurement of mitotic chromosomes of *Oedogonium cardiacum*.

Chromosome types	A	B	C	D	E	F SAT.
No. Chromosomes for each type	7	2	4	2	2	2
Length in μ	1.2+2.3 =3.5	1.1+3 =4.1	1.2+4.3 =5.5	2.2+3.3 =5.5	2.6+2.6 =5.2	1.5+5+1.5SAT=8 (F ₁) 1.5SAT+1.5+5=8 (F ₂)
Relative length	44	51	69	69	65	100
Form %	34%	27%	22%	40%	50%	25% (F ₁) 38% (F ₂)
Centromere	sm	st	st	sm	m	st (F ₁) sm (F ₂)

Relative length: length of each chromosome/length of longest chromosome. Form %: (length of short arm/total length of chromosome) $\times 100$. m, median; SAT, satellite; sm, submedian; st, subterminal.

References

- 1) Howard, A. and Horsley, R. J., Intern. J. Rad. Biol. 2: 319 (1960).
- 2) Hastischka-Jenschke, G., Osterr. Bot. Z. 107: 194 (1960).
- 3) Hoffman, L. R., Ph. D. Diss., Univ. Texas, Austin (1961).
- 4) Sinha, J. P., Cytologia 28: 194 (1963).
- 5) Banerjee, M., Banerjee, S. N. and Horsley, R. J., Intern. J. Rad. Biol. 11: 395 (1966).
- 6) Horsley, R. J., Fucikovsky, L. A. and Banerjee, S. N., Rad. Bot. 7: 241 (1967).
- 7) Horsley, R. J., Banerjee, S. N. and Banerjee, M., Rad. Bot. 7: 465 (1967).
- 8) Banerjee, S. N. and Horsley, R. J., Amer. J. Bot. 55: 514 (1968).
- 9) ——— and ———, Exptl. Cell Res. 53: 549 (1968).
- 10) Banerjee, S. N., Horsley, R. J. and Pujara, C. M., Phyton 26: 213 (1969).
- 11) Howard, A. and Horsley, R. J. (Abstract), Radiat. Res. 9: 131 (1958).
- 12) Hoffman, L. R., Amer. J. Bot. 54: 271 (1967).
- 13) Tschermak, E., Chromosome 2: 493 (1948).