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Genetic studies in *Eudorina*

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1. INTRODUCTION

Eudorina is a green, colonial, heterothallic alga belonging to the family of Chlamydomonadaceae. Facts that make this organism attractive as genetic material are a short life-cycle (about 3 weeks), a classical meiosis (Goldstein, 1964), and a growth habit amenable to many of the techniques of bacterial genetics. *Eudorina* grows photosynthetically on a minimal medium at a relatively rapid rate and reaches a stationary phase in about 7 days. Individual cells divide to form a clone of cells which appear as distinct colonies after a week's growth on minimal agar in Petri plates (Plate 1). Such colonies growing on the surface of minimal agar can easily be analysed using the method of replica plating (Lederberg & Lederberg, 1952). Thus it is possible to deal with a very large population of colonies and subject them to selective techniques to permit high resolution genetic analysis. This organism seems to offer substantial promises for studies of problems pertaining to developmental genetics or to genetics of somatic cells (Mishra, 1967). However, the genetics of *Eudorina* is unknown, therefore the present paper reports on the formal genetic analysis of *Eudorina elegans*.

2. MATERIAL AND METHODS

Strains of *Eudorina elegans* used during the present study have been described by Goldstein (1963). These were obtained from the 'Algae Culture Collection', Indiana University, Bloomington, Indiana, U.S.A.

The strains were:

Strain number	*Mating type*
40m, 62m, 738	Female
40f, 62f, 737	Male

The strains are heterothallic and oogamous. Under conditions of gametogenesis cells of the male coenobium form 32-celled sperm packets, whereas the cells in a female coenobium enlarge to form eggs. The life-cycle is represented in Text-fig. 1.

Stock cultures were maintained on the minimal agar medium as described below. All cultures were grown in a growth chamber at 20 ± 1 °C with 16 h of light (400 ft-candles), provided automatically by a clock device from a bank of standard cool

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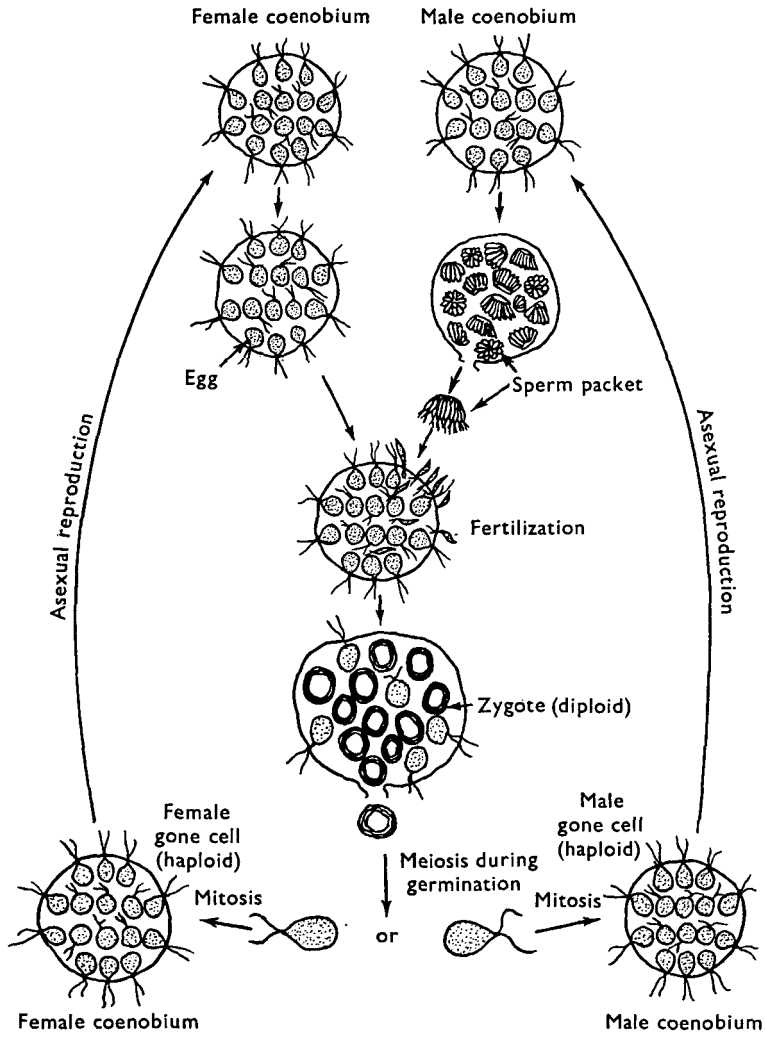
white fluorescent tubes, followed by 8 h of darkness. Cultures, irradiated for isolation and characterization of nutritional mutants, were grown on complete medium and later replicated on to appropriately supplemented agar medium. Cultures treated with antimetabolites and antibiotics were grown either on minimal agar or on the peptone medium described below.



Text-fig. 1. Life-cycle of *Eudorina elegans* (strains 62m and 62f).

The minimal medium was made up from the ingredients of Bristol medium (Starr, 1960). It consisted of 10 ml of each ingredient, 1 ml of trace-element solution (Wilbois, 1958) and glass-distilled water up to 1 l.

Soil water extract (Starr, 1960) and modified Bristol agar medium (Goldstein, 1963) were used for making crosses and zygote germination. Minimal medium containing peptone 5 mg/ml, sodium acetate (1 mg/ml) and sodium glutamate

(1 mg/ml) is called peptone medium in this paper. The complete medium consisted of 2·5 g casein hydrolysate, 1 g yeast extract, 2·5 ml nucleic acid hydrolysate, 5 ml vitamins solution dissolved in 1 l. of minimal medium. The nucleic acid hydrolysate and vitamins solution were made up as described elsewhere (Gowans, 1956). Media were solidified whenever needed, by adding 1·5 % bactoagar.

Crosses were made in liquid soil-extract medium following Starr's method (Starr, 1960). After 7–9 days of maturation in light, the zygotes were harvested and washed carefully in distilled water. The zygotes were then spread on modified Bristol agar medium in Petri plates and exposed to chloroform vapour for 30 s to kill the vegetative clones. The Petri plates containing the zygotes were incubated at 37 °C for about 70 h before returning to the growth chamber. Zygotes were found to germinate during the following 4–5 days.

(i) *Mating type test*

The individual gone colonies were isolated singly into test-tubes containing minimal agar. After growth for about 7–10 days, the individual isolates were back-crossed to two known mating type strains using the method described earlier (Starr, 1960).

(ii) *Method of irradiation*

For ultraviolet (u.v.) irradiation, cells from the log. phase culture of strain 62m and 62f were collected photoactically. An aliquot of 5 ml (cell density 1×10^6/ml) was exposed to u.v. light at a distance of 10 cm from a Hanova u.v. lamp (CH 1/988) for a period of 5–25 min. Samples (1 ml) of the irradiated cells were transferred to small Erlenmeyer flasks, each containing 4 ml of complete medium. They were kept in the dark for about 6 h to prevent photoreactivation. The flasks and their contents were then returned to the growth chamber for further growth.

Samples (5 ml) of actively growing cultures of strain 62f (cell density 1×10^6/ml) were exposed to gamma-rays from a cobalt-60 source housed at the McMaster University Nuclear Reactor. The dose of gamma-radiation was measured by a Phillips Universal Ionization Chamber giving 100 rads/min at a distance of about 1·5 ft. These samples were exposed to doses of gamma-radiation varying from 9000 rads to 40000 rads. One ml aliquots of the irradiated cultures were transferred to Erlenmeyer flasks containing 4 ml of complete medium. These flasks were then returned to the growth chamber for further growth.

(iii) *Method of screening biochemical mutants*

The irradiated cultures, after 56 h of incubation in the growth chamber, were diluted ten times; about 0·1 ml aliquots were spread on complete agar medium in Petri plates. The plates were incubated in the growth chamber for 6–7 days. Colonies, growing on these plates, were replicated with the aid of a filter paper (Whatman no. 1) onto Petri plates containing (i) minimal, (ii) minimal + amino acids (a.a.) + nucleic acids (n.a.), (iii) minimal + a.a. + vitamins, (iv) minimal + n.a. + vitamins. Colonies growing on the supplemental medium but not on the minimal alone were isolated for further analysis of specific growth requirements.

(iv) *Method of screening drug-resistant mutants*

About 0·1 ml aliquots of strain 62f or 62m from cultures in the log. phase of growth were spread over minimal agar, in Petri plates, containing the particular drug in various concentrations and then returned to the growth chamber. After 10 days growth, the surviving colonies were isolated as presumptive drug-resistant mutants for further analysis. These were then grown on minimal agar in the absence of the drug for several generations.

(v) *Method of testing drug-resistant isolates*

The presumptive isolates were tested for drug-resistance in the following way: colonies from individual isolates were suspended in liquid minimal medium; a drop of this suspension was plated on minimal agar with and without the drug. Under the test conditions, the wild-type isolates were sensitive to the drug and died on the minimal agar containing the drug. However, the mutant isolates were resistant to the drug and could grow equally well on minimal agar with or without the drug.

The following drugs were used: DL-methionine-DL-sulfoximine, streptomycin-sulphate, dihydrostreptomycin-sulphate, neomycin sulphate, actidione and erythromycin. All were obtained from the Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A., except erythromycin (erythrocin lactobionte) which was kindly donated by the Abbott Laboratories, Montreal.

3. RESULTS

(i) *Effects of irradiation*

The u.v. irradiated cells in the absence of photoreactivation, yielded a standard kill curve (Text-fig. 2A). Under the present experimental conditions an exposure to u.v. light for 25 min was found to kill 99·9 % of the cells. The survival curve following gamma-radiation is non-linear (Text-fig. 2B). A dose of 9000 rads was found to kill only about 30 % of the cells, but no cells survived an exposure to a dose of 40000 rads.

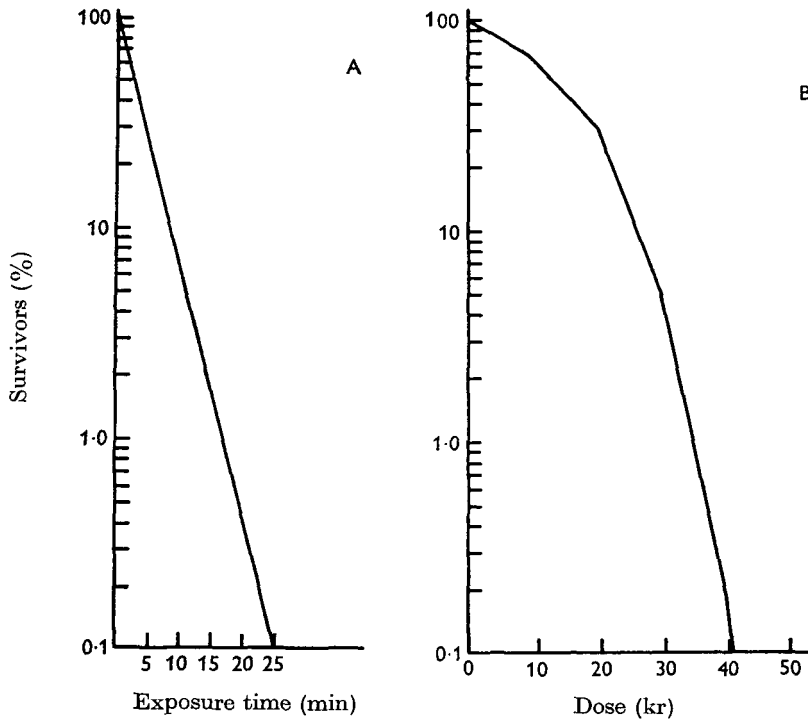
A few presumptive mutant colonies were isolated following u.v. or gamma-radiation. On subsequent examination, these were found to have no specific requirements and to show wild-type growth on minimal agar. To date, no mutant with a specific growth requirement or morphological change has been isolated.

(ii) *Effect of drugs*

The drugs differed in the concentration at which they were lethal to the organism (Table 1). A lethal dose of the drug caused bleaching of cells which was followed by their death. Addition of peptone, acetate or glutamate had no effect on the action of the drug. To date, no colourless surviving colony has been isolated.

When cells from an actively growing culture were plated on minimal agar containing a particular drug, colonies at a very low frequency were found to survive

and grow on such medium. These colonies were isolated as presumptive drug-resistant mutants (i.e. having the ability to grow in the presence of the drug at the concentration tested) for further analysis.



Text-fig. 2. Survival curve of *Eudorina elegans* (strain 62f) following irradiation with u.v. light (A) and gamma rays (B).

Table 1. *Effect of different drugs on the growth of Eudorina elegans strain 62f*

Drug	The minimum concentration (μg/ml) of drug which resulted in 100% kill of the organism, when incorporated in minimal agar
DL-methionine-DL-sulfoximine	50·0
Streptomycin sulphate	1·0
Dihydrostreptomycin sulphate	1·0
Neomycin sulphate	100·0
Erythromycin	1·0
Actidione	0·1

(iii) *Sulfoximine-resistant mutants*

These mutants were selected on minimal agar containing 50 μg/ml of DL-methionine-DL-sulfoximine. The mutant colonies were able to grow on the medium containing the drug on which they first appeared.

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(iv) *Streptomycin-resistant mutants*

These mutants were selected on minimal agar containing either 100 μg/ml of streptomycin or 100 μg/ml of dihydrostreptomycin. When they first appeared on the medium containing the drug, they were able to multiply for a few days. However the growth was considerably enhanced when the mutants were sub-cultured for a few generations on minimal agar; and then returned to the medium containing streptomycin.

(v) *Erythromycin-resistant mutants*

These mutants were selected on minimal medium containing either 100 μg/ml or 10 μg/ml of erythromycin. Most cells plated on 100 μg/ml of the drug died rapidly, and the surviving colonies were unable to grow on the medium on which they first appeared. However, they were found to grow again on minimal agar plus 100 μg/ml of erythromycin upon transfer after a few generations of growth on minimal agar without erythromycin.

However, cells (1×10^5 cells per plate) when plated on 10 μg/ml survived at a greater frequency (10–15 colonies per plate) and were able to multiply very slowly on the medium containing the drugs. All these, except one, E 527, reverted to wild type after two or three weekly transfers on minimal agar.

All mutant strains resistant to the drugs described earlier still maintain their drug-resistant properties after 3 years of growth on minimal agar.

When cells from actively growing cultures were plated on minimal agar containing 1 μg/ml or 10 μg/ml of actidione, bleaching occurred more rapidly than in the case of the other drugs used. To date no actidione-resistant mutant has been isolated.

(vi) *Designation of strains*

The wild-type strains, sensitive to the various drugs, were designated as *mss-500*, *ss-100* or *DHss-100* or *ery-s-100*, indicating sensitivity to DL-methionine-DL-sulfoximine, streptomycin or dihydrostreptomycin and erythromycin respectively.

Table 2. *Strains of Eudorina elegans characterized during the present investigation*

Isolation number	Strain designation	Mating type	Response to drug in minimal agar
1	*msr-500*	Female	Resistant to 500 μg/ml of DL-methionine-DL-sulfoximine
98	*msr-500*	Male	
S 737-1, S-1, S-2, S-3	*sr-100*	Female	
S 737-2, 36, 42, 177	*sr-100*	Male	Resistant to 100 μg/ml of streptomycin or 100 μg/ml of dihydrostreptomycin or 200 μg/ml of neomycin
D-1, D-2	*DHsr-100*	Female	
D-25	*DHsr-100*	Male	
E 1, E 527	*ery-r-100*	Female	Resistant to 100 μg/ml of erythromycin

The mutant strains resistant to the drugs were designated *msr-500*, *sr-100*, *DHsr-100* and *ery-r-100*. The streptomycin resistant mutants (*sr-100*) having the prefix DH- were originally isolated on minimal agar containing dihydrostreptomycin (100 μg/ml). The male and female mating types have been designated as *m* and *f* respectively. The strains are listed in Table 2.

(vii) *Mutation frequency*

Aliquots of actively growing cultures (1×10^5 cells/ml) were plated on minimal agar containing the particular drug. The plates were incubated in the growth chamber for a week and the mutant colonies were then scored. The frequency at which the mutants appeared was found to vary depending on the drug used (Table 3).

Table 3. *Mutation frequency of drug-resistant mutants selected on minimal agar containing the particular drug*

Mutant	Approximate frequency
msr-500	1 in 10^5
sr-500	1 in 10^6–10^7
DHsr-500	3–5 in 10^6–10^7
ery-r-100	1 in 10^6

(viii) *Cross-resistance of the mutants*

Cells from actively growing cultures of different drug-resistant strains were spot-tested on minimal agar containing a particular drug. The *sr-100* mutants were found to grow equally well on minimal agar containing either 100 μg/ml of streptomycin (dihydrostreptomycin) or 200 μg/ml of neomycin sulphate.

The *DHsr-100* mutant strains were also found to have cross-resistance similar to that of the *sr-100* strains. Also, the growth of these mutants was better on minimal agar + dihydrostreptomycin than on minimal agar containing streptomycin or neomycin. However, these mutants were unable to grow on minimal agar containing DL-methionine-DL-sulfoximine, erythromycin or actidione.

The *msr 500* mutants were unable to grow on minimal agar containing any drugs other than DL-methionine-DL-sulfoximine. Similarly *ery-r-100* mutants were unable to grow on minimal agar containing any drugs other than erythromycin.

(ix) *Inheritance of mating type*

Male and female colonies were found to occur in a ratio of 1:1 among the progeny of the crosses 62m × 62f and 40m × 40f (Table 4).

(x) *Inheritance of zygote pattern*

Strains of *Eudorina elegans* produce two morphologically distinguishable forms of zygotes (Goldstein, 1963). They are the closed type and the scattered type. In the closed type the individual zygotes remain clumped together within a coenobium whereas in the scattered type the coenobial wall breaks after fertilization,

releasing the zygotes. During the present investigation the cross 737 × 738 yielded a closed type of zygote. The crosses 62m × 62f and 40m × 40f produced a scattered type of zygote. But the cross 737 × 62m showed a closed type of zygote. The zygotes from this cross, however, had difficulty in germinating and could not be further analysed.

Table 4. *Inheritance of mating type in different crosses*

Cross	Parental type	Number of gone colonies scored			Remarks
		Male	Female	Total	
40m + 40f	Male × female	46	51	97	
62m × 62f	Male × female	39	36	75	
S-1 × 62m	*fsr-100* × *mss-100*	43	49	92	All isolates were drug-resistant
D-2 × 62m	*fDHsr-100* × *mDHss-100*	53	51	104	
62f × 177*	*fss-100* × *msr-100*	22	28	50	All isolates were drug-sensitive
62f × D-25	*fDHss-100* × *mDHsr-100*	25	23	48	

*A few isolates from this cross were streptomycin-resistant, at least two of these on mating type were found to belong to female mating type.

Table 5. *Results of crosses to determine the inheritance of resistance and sensitivity to* DL-*methionine-*DL-*sulfoximine* (msr-500/mss-500)*; to erythromycin* (ery-r-100/ery-s-100) *and to streptomycin* (sr-100 *or* DHsr-100/ss-100 *or* DHss-100) *in Eudorina elegans*

Cross	Parental types	Number of gone colonies scored in each class		
		Resistant	Sensitive	Total
1 × 62m	*fmsr-500* × *mmss-500*	69	63	132
E 1 × 62m	*fery-r-100* × *mery-s-100*	24	31	55
S-1 × 62m	*fsr-100* × *mss-100*	92	0	92
D 1 × 62m	*fDHsr-100* × *mDHss-100*	104	0	104
62f × 177	*fss-100* × *msr-100*	7	93	100
62f × D 25	*fDHss-100* × *mDHsr-100*	0	100	100

Table 6. *Results of crosses to determine the segregation of mating type alleles with* msr-500/mss-500 *and* ery-r-100/ery-s-100

Cross	Parental type	Number of male and female gone colonies scored in mutant and wild-type classes			
		Mutant		Wild type	
		Male	Female	Male	Female
1 × 62m	*fmsr-500* × *mmss-500*	16	15	19	22
E 1 × 62m	*fery-r-100* × *mery-s-100*	15	14	11	13

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(xi) *Inheritance of resistance to* DL*-methionine-*DL*-sulfoximine*

The resistant and sensitive colonies were found (Table 5) to occur in 1:1 ratio in the progeny of the cross *fmsr-500* × 62m (wild type). Among both mutant and wild type progeny from the cross *fmsr-500* × 62m the two mating types occurred in a 1:1 ratio (Table 6).

(xii) *Inheritance of resistance to erythromycin*

The resistant and sensitive colonies were found to occur in 1:1 ratio among the progeny of the cross *fery-r-100* × 62m. Among the mutant and wild type progeny of this cross the two mating types occurred in a 1:1 ratio (Tables 5, 6).

(xiii) *Inheritance of resistance to streptomycin*

All the progeny of the crosses *fsr-100* × *mss-100* and *fDHsr-100* × *mDHss-100* were resistant to streptomycin. The reciprocal crosses *fss-100* × *msr-100* and *fDHss-100* × *mDHsr-100* produced progeny all of which, with rare exceptions as noted below, were sensitive to the drug. These data are presented in Tables 4 and 5.

The cross *fss-100* × *msr-100* yielded seven streptomycin-resistant progeny, out of 100 examined. The mating-type test showed two of the seven to be the female mating type and thus their genotypes were *fsr-100*.

(xiv) *Crosses between erythromycin-resistant and sulfoximine resistant mutant strains*

This cross (*mmsr-100 ery-s-100* × *fmss-100 ery-r-100*) was very generally infertile. A few gone colonies, obtained after zygote germination, were tested for their ability to grow on medium supplemented with these drugs. The numbers of colonies scored in each class were: 5 sulfoximine-resistant, 7 erythromycin-resistant, 3 resistant to both drugs and 7 wild type.

4. DISCUSSION

Present results confirm earlier findings (Goldstein, 1963, 1964) of the inheritance of mating types in *Eudorina elegans* due to a single gene difference. Also that the cross 737 × 62f did not yield any germinable zygote suggests that these strains belong to sexually incompatible groups (Goldstein, 1963). The fact that the cross 62f × 62m produced scattered zygotes whereas the crosses 737 (female) × 738 (male) and 737 × 62m resulted in closed zygotes suggest that this character (i.e. closed or scattered zygote types) is inherited through the female parent.

Failure to obtain nutritional mutants in *Eudorina* is comparable to findings in *Chlamydomonas* where only a few markers are known (Levine & Ebersold, 1961). Such lack of nutritional mutants in this organism may be explained on the basis already invoked for *Chlamydomonas* (Ebersold, 1962). The non-linear curve following gamma irradiation of *Eudorina* compares with that of a diploid strain of *Chlamydomonas* (Wetherall & Krauss, 1957). This suggests some duplication of genetic material in *Eudorina*.

(i) *Inheritance of drug resistance*

The present findings regarding drug resistance in *Eudorina* suggest the presence of two genetic systems comparable to those in *Chlamydomonas*. In *Eudorina*, DL-methionine-DL-sulfoximine-resistance is inherited in a Mendelian way, while inheritance of streptomycin-resistance has characteristics of non-chromosomal heredity. Inheritance of erythromycin resistance in *Eudorina* is chromosomal. This marker however is not yet known in *Chlamydomonas*. The present data also suggest that none of the following pairs of alleles, *msr-500*/*mss500*, *sr-100*/*ss-100*, *DHsr-100*/*DHss-100* and *ery-r-100*/*ery-s-100*, is linked to the mating type locus in *E. elegans*. It also appears that the loci *msr-100* and *ery-r-100* are unlinked to each other.

(ii) *Non-chromosomal genes in Eudorina*

The particulate nature of non-chromosomal genes (NC genes) has been demonstrated in *Chlamydomonas* (Sager & Ramanis, 1963). The present data in *Eudorina* are comparable to the findings in *Chlamydomonas*. In *Eudorina* streptomycin-resistance is always transmitted through the female parent, i.e. the cross *fsr-100* × *mss-100* transmits streptomycin resistance to all progeny. However, in a few cases streptomycin-resistant progeny were obtained from the cross *msr-100* × *fss-100* in which the streptomycin resistant factor was carried by the male parent.

Two of these streptomycin resistant isolates, on subsequent analysis of mating types, were found to be of the female mating type (i.e. these were recombinant for the chromosomal gene (f) and the non-chromosomal factor (*sr-100*)). The purpose of the mating type test was to show with absolute certainty that these streptomycin-resistant isolates were derived from the zygote and were not merely a result of contamination by the parental type. It appears that these isolates were derived from exceptional zygotes carrying both *ss-100* and *sr-100* factors. However, further analysis is required to determine whether these factors (*ss-100* and *sr-100*) segregate prior to or subsequent to gone formation in *Eudorina*. On the basis of these data, it may be inferred that in *E. elegans* the genetic determinants controlling the streptomycin sensitivity in wild-type strains and streptomycin resistance in mutant strains are, in fact, particulate in nature and may be designated as non-chromosomal genes (NC genes) as in *Chlamydomonas*.

SUMMARY

A formal genetic analysis of the heterothallic, colonial green alga *Eudorina elegans* has been described. Wild-type strains were found to be sensitive to different drugs when grown on minimal agar containing very low concentrations of these drugs. Mutant strains resistant to high concentrations of drugs have been isolated. These are *msr-500* (resistant to 500 μg/ml of DL-methionine-DL-sulfoximine), *ery-r-100* (resistant to 100 μg/ml of erythromycin) and *sr-100* (resistant to 100 μg/ml of streptomycin). The wild-type phenotypes sensitive to these drugs have been designated as *mss-500*, *ery-s-100* and *ss-100* respectively. The *sr-100* also showed cross-resistance to other antibiotics belonging to the streptomycin group.

On genetic analysis, the *msr-500* and *ery-s-100* were found to be inherited in a Mendelian way. These alleles are not linked to each other or to the mating type locus. The inheritance of mating type was found to be due to a single gene difference.

The inheritance of *ss-100/sr-100* was found to be non-chromosomal and was characteristically uniparental, always transmitted through the female parent. The evidence for the non-chromosomal gene (NC genes) controlling *sr-100/ss-100* phenotypes in this organism has been derived from the exceptional zygotes in which the male parent apparently transmits streptomycin resistance to the progeny. Although ultraviolet or gamma-radiation resulted in normal survival curves of the exposed cells, no mutant deficient in any nutritional requirement was isolated.

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