tropic effects on various other characters the distribution of these genes will be at random relative to their action on bristle number.

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Transmission and Segregation of a Non-chromosomal Factor controlling Streptomycin Resistance in Diploid *Chlamydomonas*

Chromosomal and non-chromosomal mutations to streptomycin resistance have been described in *Chlamydomonas reinhardi*[1–3]. The chromosomal mutants, *sr*-1, are resistant to low levels of streptomycin (50–100 μg/ml.), possess a Mendelian pattern of inheritance, are closely linked or allelic, and lie in the left arm of linkage group IX. The non-chromosomal mutants, *sr*-2, are resistant to high levels of streptomycin (500 μg/ml.) and exhibit a uniparental pattern of inheritance. When a mating is made between wild-type cells (*ss*) and cells of an *sr*-2 mutant, all the progeny in each tetrad are, with rare exceptions[1,4], endowed with the resistance-level of the mating type plus (mt^+) parent. The purpose of the present communication is to show that this pattern breaks down when diploids are made between *sr*-2 and *ss* cells and that a large proportion of the diploid clones segregate both resistant and sensitive cells during vegetative growth.

The method for obtaining diploid cells in *C. reinhardi* was recently devised by Ebersold[5], and only a brief description of the procedure will be presented here since it will be described in detail elsewhere[5,6]. In the present experiments, reciprocal matings were made between two non-allelic arginine-requiring mutants according to the method of Ebersold and Levine[7]. The two mutants, *arg*-1 and *arg*-2, complement in diploids[5,6] and are located six map units apart in linkage group I (ref. 7). The *arg*-2 parent always carried the *sr*-2 mutation while the *arg*-1 parent was always *ss*. In each experiment the cells were mated for 1 h and then the mating mixture was plated directly on minimal medium supplemented with sodium acetate[3]. The plates were incubated in the light at 27° C for several days after which visible colonies had appeared on them.

Since the original mating mixture had contained normal zygotes as well as diploid cells, the colonies included prototrophic haploid recombinants produced by germinated zygotes in addition to diploid cells. Usually, the diploid colonies were larger than the colonies formed by the wild-type recombinants and they could be selected on this basis for further testing. The most likely explanation of this phenomenon seems to be that the diploids begin to divide soon after being plated while the zygotes giving rise to wild-type recombinants probably do not begin to germinate for at least 36 h. After a number of presumed diploid colonies had been isolated, they were tested for mating type and streptomycin resistance. The *mt* gene lies in linkage group VI (ref. 8) and assorts independently of the *arg* mutants so that one half of the wild-type recombinants are mt^+ and the other half are mt^-. Diploids, on the other hand, are always mt^- in phenotype probably because mt^- is dominant to mt^+ (refs. 5 and 6). Therefore, the mating type test could be used to estimate the proportion of diploids and wild-type recombinants isolated in a given experiment. Fortunately, all colonies tested proved to be mt^- in the experiments to be discussed. In a number of cases presumptive diploids were tested further by crossing them to haploid wild-type mt^+ cells and screening the resulting tetrads for arginine-requiring progeny. The two arginine-requiring mutants can be distinguished by testing them on ornithine-containing medium since *arg*-1 will grow on either ornithine, citrulline, or arginine whereas *arg*-2 will only utilize arginine[7]. These crosses showed that all the presumptive diploids carried both the markers.

Table 1. Transmission and Segregation of Streptomycin Resistance and Sensitivity in Diploids derived from Reciprocal Crosses between *sr*-2 and *ss* Cells

Cross	*sr*-2 mt^+ × *ss* mt^-			*ss* mt^+ × *sr*-2 mt^-		
Experiment	1	2	3	1	2	3
Percentage of *ss* cells in diploid clone						
100	5·55 (3/54)	1·24 (1/82)	1·85 (3/179)	24·50 (22/90)	44·50 (12/27)	6·70 (6/90)
100–80	0·00 (0/37)	1·86 (1/53)	0·00 (0/28)	0·00 (0/12)	15·10 (3/11)	4·91 (3/57)
80–60	5·10 (2/37)	3·72 (2/53)	3·50 (1/28)	12·50 (2/12)	15·10 (3/11)	8·25 (5/57)
60–40	7·65 (3/37)	13·00 (7/53)	7·02 (2/28)	12·50 (2/12)	15·10 (3/11)	16·30 (10/57)
40–20	15·30 (6/37)	7·45 (4/53)	10·50 (3/28)	12·50 (2/12)	10·00 (2/11)	34·40 (21/57)
20–0	20·40 (8/37)	22·30 (12/53)	38·60 (11/28)	31·90 (5/12)	0·00 (0/11)	26·20 (16/57)
0	45·80 (18/37)	50·40 (27/53)	38·60 (11/28)	6·25 (1/12)	0·00 (0/11)	3·26 (2/57)

Figures not in parentheses indicate the percentage diploids in a given cross belonging to each of the categories listed. Figures in parentheses show the actual number of diploids in a given category and the total number of diploids examined. In all crosses the *sr*-2 parent carried the *arg*-2 marker and the *ss* parent carried the *arg*-1 marker.

Two important points emerge in the experimental results (Table 1). First, 50–> 90 per cent of the diploids arising from reciprocal crosses between *sr*-2 and *ss* cells produce mixed clones containing both resistant and sensitive cells. This phenomenon cannot be explained by the assumption that the diploid nucleus is unstable since it has been found that three unlinked chromosomal markers do not segregate in a similar manner when the diploids are grown under non-selective conditions[8]. Secondly, although 50 per cent or more of the diploid clones are mixtures of *sr*-2 and *ss* cells, a certain amount of the polarity characteristic of an ordinary cross is retained. Thus, in a cross of *sr*-2 mt^- × *ss* mt^+ up to 50 per cent of the diploid clones may be composed entirely of *ss* cells while less than 10 per cent are composed only of *sr*-2 cells. In the reciprocal cross the reverse is true.

If it is assumed that *sr*-2 and *ss* represent alternate mutational states of the same factor, then in a normal cross the transmission of the factor present in the mt^- parent is usually blocked while the transmission of the factor present in the mt^+ parent is never blocked. Diploids probably arise because some function necessary for normal zygote formation is never realized. Whatever this function may be, the function which results in the uniparental transmission of the *sr*-2 factor is also impaired or missing in most newly formed diploid cells. As a result, the majority of diploid cells contain both *ss* and *sr*-2 determinants and these determinants segregate during subsequent mitotic divisions.

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