THE NATURE OF EXCEPTIONS TO THE PATTERN OF UNIPARENTAL INHERITANCE FOR HIGH LEVEL STREPTOMYCIN RESISTANCE IN *CHLAMYDOMONAS REINHARDI*¹

NICHOLAS W. GILLHAM

*Department of Botany, Josiah Willard Gibbs Research Laboratory,
Yale University, New Haven, Connecticut*

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TWO different classes of mutation to streptomycin resistance have been described in *Chlamydomonas reinhardtii* (SAGER 1954, 1960; GILLHAM and LEVINE 1962). The first, *sr-1*, is resistant to 50 μ g of streptomycin sulfate per ml under the experimental conditions to be reported here. These mutants segregate 1:1 in crosses to wild type (*ss*). The *sr-1* mutants are all closely linked or allelic and lie in the left arm of linkage group IX (SAGER and Tsubo 1961; GILLHAM and LEVINE, 1962). The second class of mutations, *sr-2*, is resistant to 500 μ g of streptomycin sulfate per ml. When *sr-2* cells in the plus mating type (*mt*⁺) are crossed to *ss* cells of the minus mating type (*mt*⁻), almost all tetrads segregate four *sc-2*:zero *ss* progeny. Only one tetrad exceptional to this pattern has been reported (GILLHAM and LEVINE 1962) among several hundred analyzed (SAGER 1954; GILLHAM and LEVINE 1962). In this tetrad the meiotic products gave rise to mixed clones of resistant and sensitive cells. In the reciprocal cross, on the other hand, the large majority of the tetrads segregate zero *sr-2*:four *ss* progeny. The remaining tetrads from this cross, usually less than ten percent, are exceptional; they segregate four *sr-2*:zero *ss* progeny.

The exceptional tetrads from the cross *sr-2 mt*⁻ $\times$ *ss mt*⁺ are paradoxical since the normal pattern of uniparental inheritance of streptomycin sensitivity via the *mt*⁺ parent appears to be reversed. Although the *mt*⁻ parent is resistant to streptomycin in this cross, all four meiotic products produce clones of cells which are resistant rather than sensitive to streptomycin. If the resistant progeny from an exceptional tetrad are crossed to sensitive cells, the usual pattern of uniparental inheritance via the *mt*⁺ parent appears once again (SAGER 1954). Thus, the ability of the *mt*⁻ parent to transmit resistance in a cross appears to be fortuitous and is not inherited by the resistant *mt*⁻ progeny in an exceptional tetrad. Why does an apparent reversal of uniparental inheritance occur in these tetrads? It is the purpose of this paper to show that in the great majority of exceptions a true reversal has not occurred and that the contribution of sensitivity by the *mt*⁺ parent is not lost, but merely masked.

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MATERIALS AND METHODS

Strains: The wild-type strains (137c *mt*⁺ and *mt*⁻) of *C. reinhardi* used in these studies were isolated by the late PROFESSOR G. M. SMITH. Three mutant strains were employed as well, and each was derived from strain 137c. One of these strains is an *sr-2* mutant. The other mutant strains, described in previous publications (LEVINE and EBERSOLD 1960; EBERSOLD, LEVINE, LEVINE and OLMSTED 1962), are the arginine-requiring strain, *arg-2*, located in linkage group I, and a nonmotile strain possessing paralyzed flagella, *pf-15*, located in linkage group III.

Media: The media used in these experiments and their abbreviations are as follows: MA = basal minimal medium after LEVINE and EBERSOLD (1958) plus 2 g of sodium acetate and 15 g of Bacto Agar (Difco) per liter; MS100 = MA medium plus 100 mg streptomycin sulfate (Lilly) per liter. Streptomycin was added after the medium had been autoclaved.

Analysis of dissected tetrads: Tetrad analysis was carried out according to the methods described by EBERSOLD and LEVINE (1959). Two crosses were made between *sr-2 mt*⁻ cells and *ss mt*⁺ cells. In the first cross the marker *pf-15* was segregating and the tetrads were dissected on MA medium. When the meiotic products had given rise to colonies of cells, the colonies were replica-plated to MA and MS100 media. The majority of the tetrads were composed only of sensitive cells, but in the exceptional cases colonies of resistant cells formed on the MS100 plates. The growth of resistant cells on MS100 medium merely indicated that the original colony had contained some resistant cells. It did not eliminate the possibility that this colony was a mixture of resistant and sensitive cells. In order to determine whether such a colony was a mixture of resistant and sensitive cells it was picked from the nonselective MA replica with a wire needle and the component cells were suspended in potassium phosphate buffer (M/150; pH 6.8). An aliquot of this suspension was then plated onto MA medium and the colonies that formed on the MA plate were replica-plated to MS100 medium. If any of these colonies were unable to grow on MS100 medium, it indicated that the colony picked from the MA replica had been a mixture of resistant and sensitive cells.

In the second cross the marker *arg-2* was segregating. The procedure used was similar to the one described for the first cross except that all media were supplemented with 10 mg of L-arginine monohydrochloride per liter, and an MA plate was included among the replica plates so that the segregation of *arg-2* from its wild-type allele could be followed.

Analysis of undissected tetrads: Since exceptional tetrads are infrequent and dissection of tetrads is time consuming, an effort was made to supplement the information gained from the analysis of dissected tetrads by a method which circumvented the problem of tetrad dissection. Cells of the genotype *ss arg-2 mt*⁺ were crossed to cells whose genotype was *sr-2 arg-2*⁺ *mt*⁻. The resulting zygotes were handled according to the procedures described by EBERSOLD and LEVINE (1959). After the zygotes had matured, they were scraped off the surface

of the maturation medium with a chisel-edged wire implement and suspended in distilled water. Aliquots (0.2 ml) of the suspension were plated onto MA medium, and the plates were chloroformed for 35 seconds to kill any vegetative cells that might have been plated along with the zygotes. Upon germination the zygotes produced colonies of cells which were derived from the two *arg-2*⁺ meiotic products. The two arginine-requiring meiotic products did not survive, since MA medium does not contain arginine. Although this procedure made it impossible to derive any information concerning segregation of *sr-2* and *ss* from the *arg-2* progeny, it had been found previously that even on arginine-containing media *arg-2* competes very poorly with *arg-2*⁺. Therefore, the simplest procedure was to confine the analysis to the two *arg-2*⁺ meiotic products by eliminating the two *arg-2* meiotic products from consideration.

The colonies that formed on the MA plates were transferred to fresh MA medium (30 colonies per plate) with a wire needle. Several days later they were replica-plated to MA and MS100 media (first replicas). Colonies containing resistant cells, ~~judged from growth~~ on the selective MS100 replica, were removed from the nonselective MA replica with a wire needle and suspended in phosphate buffer. Aliquots of the suspension were plated on MA medium. The cells which were present in the aliquots formed colonies, and these colonies were replica-plated to MA and MS100 media (second replicas). The occurrence of sensitive colonies of cells on the second MS100 replica indicated that the colony which had been tested from the first MA replica was a mixture of resistant and sensitive cells.

To establish whether one of the *arg-2*⁺ meiotic products in a tetrad had given rise to resistant cells, sensitive cells, or a mixture of the two types, mating-type tests were performed on colonies of resistant and sensitive cells isolated from the second MA replica. Since *mt* and *arg-2* are chromosomal loci (LEVINE and EBERSOLD 1960), three types of tetrads arise in crosses of *arg-2 mt*⁺ × *arg-2*⁺ *mt*⁻. About 30 percent of the tetrads are parental ditypes (PD) and nonparental ditypes (NPD), and the ratio of PD to NPD is 1:1. In a PD the *arg-2* progeny are *mt*⁺ and the wild-type progeny are *mt*⁻. In an NPD the reverse is true. The other 70 percent of the tetrads are tetratypes (T) in which the *arg-2* progeny are of opposite mating type as are the *arg-2*⁺ progeny. The PD and NPD tetrads could not be used for analysis since both of the *arg-2*⁺ meiotic products were of the same mating type and hence the progeny of one product could not be distinguished from the progeny of the other. In the T tetrads, on the other hand, the *arg-2*⁺ meiotic products produced clones of opposite mating type, and the mating-type test served to distinguish the progeny of one meiotic product from the other. Thus, in the T tetrads it was possible to determine whether each meiotic product had given rise to resistant cells, sensitive cells, or a mixture of the two types.

Terminology: In order to aid in understanding the results, it is necessary to define certain of the terms that will be used throughout this paper. The term *segregation* is used in two senses. First, it is used in the Mendelian sense to describe the segregation of the chromosomal genes *arg-2*, *pf-15*, and *mt*. Second, it is used to indicate the segregation of *sr-2* and *ss*, and no relationship to the Men-

delian segregation of genes is implied. As will become evident from the results, a given meiotic product can give rise to a clone of sensitive cells, a clone of resistant cells, or to a clone in which both resistant and sensitive cells are present. A clone of the last type will be referred to as a *mixed clone*. Finally, *exceptional tetrads* are defined as tetrads to which the *sr-2* factor has been transmitted by the *mt*⁻ parent. They arise in crosses of the type *sr-2 mt*⁻ × *ss mt*⁺.

RESULTS

Analysis of undissected tetrads: A cross was made between *sr-2 arg-2*⁺ *mt*⁻ cells and cells of the genotype *ss arg-2 mt*⁺. After the zygotes had matured, they were germinated on MA medium and the colonies formed by the *arg-2*⁺ progeny in each tetrad were handled according to the protocol outlined previously. Among the tetrads obtained, 13 percent (161/1228) were exceptional. The progeny cells produced by 90 of the exceptional tetrads were tested to see if sensitive as well as resistant cells were present. In all but two cases (2.2 percent), sensitive cells were found. Mating-type tests were performed on the sensitive and resistant progeny cells derived from a number of these tetrads, and a total of 30 T tetrads were found. In the 30 T tetrads it was possible to determine whether each meiotic product had produced a pure or mixed clone of cells. The 30 T tetrads included the progeny of 60 meiotic products. Among these 60 meiotic products, 37 (61.7 percent) produced mixed clones, while 16 (26.6 percent) gave rise only to sensitive progeny, and 7 (11.7 percent) to resistant progeny.

Analysis of dissected tetrads: Tetrads were dissected in two different crosses (Table 1). In both crosses over 90 percent of the zygotes germinated. A total of 773 complete tetrads was obtained of which 25 were exceptions. The segregation of the marker *pf-15* was followed in the first cross, whereas the segregation of *arg-2* was followed in the second cross. Each of the markers exhibited a 1:1 segregation in all of the tetrads that were examined. This included all of the exceptional tetrads.

TABLE 1

Segregation of resistance (sr-2) and sensitivity (ss) in exceptional tetrads derived from two crosses between sr-2 mt⁻ *and ss mt*⁺. In each cross a chromosomal marker was segregating. M = Mixed clone

Tetrad types	Cross		Total number of tetrads	Frequency of each tetrad type
	<i>sr-2</i> + <i>mt</i> ⁻ × <i>ss arg-2 mt</i> ⁺	<i>sr-2</i> + <i>mt</i> ⁻ × <i>ss pf-15 mt</i> ⁺		
0 <i>sr-2</i> : 3 <i>ss</i> : 1 M	2	5	7	0.28
0 <i>sr-2</i> : 2 <i>ss</i> : 2 M	0	2	2	0.08
0 <i>sr-2</i> : 1 <i>ss</i> : 3 M	0	4	4	0.16
0 <i>sr-2</i> : 0 <i>ss</i> : 4 M	0	2	2	0.08
1 <i>sr-2</i> : 0 <i>ss</i> : 3 M	1	3	4	0.16
2 <i>sr-2</i> : 0 <i>ss</i> : 2 M	0	1	1	0.04
3 <i>sr-2</i> : 0 <i>ss</i> : 1 M	0	0	0	0.00
4 <i>sr-2</i> : 0 <i>ss</i> : 0 M	1	4	5	0.20
Total tetrads	4	21	25	

In comparison to previous studies (SAGER 1954; GILLHAM and LEVINE 1962) a variety of segregations for resistance and sensitivity were found among the exceptional tetrads (Table 1). Although 48 percent (12/25) of the tetrads were scored as segregating only resistant progeny on MS100 medium, analysis of the same tetrads based on the nonselective MA replica showed that in seven of the tetrads one or more of the meiotic products had produced a mixed clone of resistant and sensitive cells. In the five remaining tetrads the presence of sensitive cells could not be demonstrated, but for reasons to be considered in the Discussion, it seems possible that sensitive cells were simply not detected.

In the other 52 percent (13/25) of the exceptional tetrads, between one and three of the meiotic products produced only sensitive progeny; the remaining meiotic products gave rise to either mixed clones or clones of resistant cells. Of all the exceptional tetrad segregations observed, the most frequent type (seven tetrads) was one in which three meiotic products produced only sensitive cells, whereas the fourth gave rise to a mixed clone.

Several hundred incomplete tetrads were obtained in addition to complete tetrads. Most of these segregated only sensitive cells, but nine three-celled exceptions were found. In addition to resistant cells, all nine of these exceptions included meiotic products which segregated sensitive cells either in pure clones or in mixed clones.

If the three-celled tetrads are added to the complete tetrads, the total number of exceptional tetrads in the two crosses amounts to 34. These 34 tetrads produced 127 meiotic products of which 28 (22.0 percent) segregated only resistant cells, 39 (30.8 percent) segregated sensitive cells, and 60 (47.2 percent) produced mixed clones. By adding these figures to the totals obtained in the analysis of undissected tetrads, we have a population of 187 meiotic products, 35 (18.7 percent) of which produced only resistant progeny, 55 (29.4 percent) of which gave rise only to sensitive cells, and 97 (51.9 percent) of which produced mixed clones. It is evident from these data that approximately half of the meiotic products give rise to mixed clones while the other half segregate pure clones of resistant or sensitive cells. It is also notable that when a meiotic product produces a pure clone of cells it is more often sensitive than resistant.

Frequency of resistant and sensitive cells in mixed clones derived from exceptional tetrads: When mixed clones were tested for the presence of resistant and sensitive cells, striking departures from a 1:1 ratio of resistant to sensitive cells were noted (Table 2). Of the clones that were tested, 77 percent (23/30) possessed a much higher proportion of sensitive than resistant cells. Only 13.3 percent (4/30) had proportions of resistant cells between 20 and 80 percent, while the remaining ten percent (3/30) of the clones were composed almost entirely of resistant cells. Thus, mixed clones tend to contain a high proportion of sensitive cells.

Several interpretations may be offered for the high frequency of sensitive cells in mixed clones. First, it is possible that sensitive cells replica plate more efficiently than resistant cells. Second, sensitive cells may have a selective advantage over resistant cells when the two types are growing in competition in a mixed clone.

TABLE 2

Frequency of resistant cells in mixed clones

Frequency of resistant cells in mixed clone	Number of clones	Percent of mixed clones analyzed
0.001-0.01	13	43.3
0.01-0.10	6	20.0
0.10-0.20	4	13.4
0.20-0.30	1	3.3
0.30-0.40	1	3.3
0.40-0.50	0	0.0
0.50-0.60	0	0.0
0.60-0.70	1	3.3
0.70-0.80	1	3.3
0.80-0.90	0	0.0
0.90-1.00	3	10.0
Total	30	

Third, during growth a mixed clone may segregate sensitive cells more frequently than resistant cells.

A reconstruction experiment was performed in order to ascertain the most probable explanation of the high frequency of sensitive cells in mixed clones. Two crosses were made. The first cross was between *ss* cells of opposite mating type. This cross produced only sensitive progeny. The second cross, between *ss mt⁻* and *sr-2 mt⁺*, produced only resistant progeny. Zygotes from the two crosses were germinated together on the same plate of MA medium. A single sensitive meiotic product from the cross between *ss* cells was then placed next to a single resistant meiotic product from the cross *ss mt⁻ × sr-2 mt⁺*. The two meiotic products were teased into position with a glass needle and this procedure was repeated until 31 separate pairs of meiotic products had been placed next to each other. The paired meiotic products formed colonies which were indistinguishable from the colonies formed by single cells. After three or four days of growth, the colonies were transferred to fresh MA medium with a wire needle. Growth was allowed to continue for several days before the colonies were replica-plated to MA medium. After colonies had appeared on the MA replica, they were analyzed for the presence of resistant and sensitive cells in the manner already described for the exceptional tetrads.

The results of the reconstruction experiment are presented in Table 3. Four colonies are omitted from the table since they contained only resistant or sensitive cells. It is assumed in each case that one of the two original cells had died. As expected, resistant and sensitive cells were found in the other 27 colonies. It is evident from the table that there is no marked tendency towards a high proportion of sensitive cells in these contrived colonies. These results appear to rule out the possibility that sensitive cells are replica-plated more efficiently than resistant cells and the possibility that they are at a selective advantage to resistant cells in a mixed colony. Therefore, the simplest interpretation seems to be that when a meiotic product produces a mixed clone this clone segregates sensitive cells more

TABLE 3

*Frequency of resistant cells in contrived "mixed clones" in the reconstruction experiment.
See text for experimental details*

Frequency of resistant cells in "mixed clones"	Number of clones	Percent of "mixed clones" analyzed
0.001-0.01	1	3.7
0.01-0.10	2	7.4
0.10-0.20	2	7.4
0.20-0.30	3	11.2
0.30-0.40	3	11.2
0.40-0.50	2	7.4
0.50-0.60	2	7.4
0.60-0.70	3	11.2
0.70-0.80	3	11.2
0.80-0.90	0	0.0
0.90-1.00	6	22.2
Total	27	

frequently than resistant cells. This interpretation would be in agreement with the observation made previously, that when a meiotic product in an exceptional tetrad gives rise to a pure clone of cells, it is more often sensitive than resistant. Further supporting evidence for this point of view has been obtained from a study of the cells in mixed clones which were produced during the first three or four postmeiotic mitotic divisions (GILLHAM, unpublished). These observations indicate that a meiotic product which gives rise to a mixed clone segregates more sensitive than resistant cells during the early divisions following meiosis.

DISCUSSION

The experiments reported here were undertaken to determine why uniparental inheritance of the *sr-2* factor via the *mt*⁺ parent appears to be reversed in a small proportion of exceptional tetrads derived from every cross between *sr-2 mt*⁻ and *ss mt*⁺. It has been shown that a true reversal does not occur, but that the barrier to transmission of the *sr-2* factor by the *mt*⁻ parent is in some manner overcome. If the pattern of uniparental inheritance were totally reversed, one would expect that the exceptional tetrads would segregate only resistant cells. However, in the overwhelming majority of the cases these exceptions segregate sensitive as well as resistant cells. This observation shows that the *mt*⁺ parent has contributed sensitivity to the cross as usual, but that the *mt*⁻ parent has also contributed resistance to the cross, which is unusual. The following discussion will consider the nature of exceptional tetrads, the few unresolved exceptional tetrads, and the relationship of the findings presented here to the hypothesis that the *sr-2* mutation occurs in a nonchromosomal genetic determinant.

A. The nature of the exceptional tetrad. In almost all of the exceptional tetrads studied, one or more of the meiotic products segregates sensitive cells. Among the 90 undissected exceptional tetrads analyzed, 88 (97.8 percent) produced

sensitive as well as resistant cells. In the analysis of dissected tetrads, 34 exceptions were obtained of which 25 were complete tetrads and nine were three-celled tetrads. All but five of these (85 percent) segregated sensitive and resistant cells. Thus, 94 percent (117/124) of all exceptional tetrads segregated sensitive cells, and the most frequently encountered type of exceptional tetrad can be characterized as one which arises from a zygote to which sensitivity has been transmitted by the mt^+ parent and resistance by the mt^- parent. However, it is also evident that segregation of resistance and sensitivity does not follow a chromosomal pattern of inheritance in exceptional tetrads. A variety of different segregations are found, and approximately half of the meiotic products give rise to mixed clones of cells in which segregation of resistance and sensitivity is postmeiotic.

B. Unresolved exceptions: Five complete tetrads were obtained in which it appeared that all four meiotic products segregated only resistant cells. If it is assumed that one or more of the meiotic products in these tetrads segregated sensitive cells in very low frequency, then these tetrads would differ from the other exceptions only in degree and not in fact. In support of this notion is the observation (Table 2) that mixed clones occasionally do contain a very small proportion of sensitive cells. Although the presence of rare resistant cells in mixed clones is easily detected by replica-plating to MS100 medium, no such selective method is available for rare sensitive cells. For this reason, sensitive cells may simply not have been detected in these five cases.

C. The relationship of the findings to the hypothesis that $sr-2$ is a mutation in a nonchromosomal genetic determinant: SAGER (1960) reviewed the experimental observations which have been made on the inheritance of streptomycin resistance in *C. reinhardtii*, and concluded that the evidence was in best agreement with the hypothesis that $sr-2$ and ss represent alternatives or "alleles" of a single nonchromosomal determinant. If this interpretation is correct, the transmission of the $sr-2$ determinants by the mt^- parent is not blocked or is only partially blocked in zygotes which produce exceptional tetrads. Thus, these determinants are transmitted to the meiotic products together with the ss determinants derived from the mt^+ parent. The fact that in exceptional tetrads over 50 percent of the meiotic products produce mixed clones shows that there is often more than one replicate of the determinant per cell. However, it is not known whether this situation persists indefinitely during the postmeiotic mitotic divisions. The observation that the meiotic products comprising exceptional tetrads produce pure clones of sensitive cells more frequently than pure clones of resistant cells would suggest that the zygote generally contains fewer copies of the $sr-2$ determinant than the ss determinant.

SUMMARY

Mutants resistant to high levels of streptomycin ($sr-2$) exhibit a uniparental pattern of inheritance via the mt^+ parent. However, a small percentage of exceptional tetrads is always encountered in crosses between resistant mating-type minus-cells and sensitive mating-type plus-cells. In these exceptions the

pattern of uniparental inheritance appears to be reversed so that the mating-type-minus parent contributes resistance to all four products of meiosis.

An analysis of many exceptional tetrads reveals that uniparental inheritance is not actually reversed, but that both mating types contribute to the cross, and that the barrier to transmission of *sr-2* by the mating-type-minus parent is merely overcome.

The results are discussed in relation to the hypothesis that resistance and sensitivity represent mutational alternatives of a nonchromosomal genetic determinant.

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