GENETICS OF *CHLAMYDOMONAS REINHARDTII* DIPLOIDS. II. THE EFFECTS OF DIPLOIDY AND ANEUPLOIDY ON THE TRANSMISSION OF NON-MENDELIAN MARKERS

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ABSTRACT

The transmission of two non-Mendelian drug resistance markers has been studied in crosses of *Chlamydomonas reinhardtii* involving diploids and aneuploids with different mating type genotypes. Under normal laboratory conditions for gametogenesis, mating and zygote maturation, the transmission pattern of the non-Mendelian markers *sr-u-1* (resistance to streptomycin) and *spr-u-1-27-3* (resistance to spectinomycin) is primarily determined by the mating type genotypes of the parental cells. Our results confirm and expand an earlier observation suggesting that an apparent codominant function of the female (mt^+) allele in regulating chloroplast gene transmission in meiosis appears to be distinct and separate from its recessive function in regulating mating behavior. The chloroplast DNA complement (as indexed by the number of extranuclear DNA-containing bodies) may exert a secondary effect on the transmission of these markers. Within a mating type group (mt^+/mt^- or mt^-/mt^-) a cell line with more chloroplast DNA tended to transmit its non-Mendelian markers more frequently than a cell line with less chloroplast DNA.

DATA accumulated over the last several years strongly suggest that the chloroplast DNA (cpDNA) of *Chlamydomonas reinhardtii* carries the well-characterized maternal non-Mendelian genes in this organism (for review see BIRKY 1978). Patterns of mutability (LEE and JONES 1973), the marker phenotypes and demonstrations of altered chloroplast ribosome properties in some of the non-Mendelian mutants (see HARRIS, BOYNTON and GILLHAM 1976) all indicated a chloroplast location for the genome. More recently, reciprocal crosses made between a strain of *C. reinhardtii* with physical deletions in the cpDNA and a strain carrying two non-Mendelian antibiotic resistance markers showed a strict cotransmission of the maternal genetic and physical markers (GRANT, GILLHAM and BOYNTON 1980). Similarly, SPREITZER and METS (1980) showed that a mutant ribulose-1,5-biphosphate carboxylase, the gene for which has been located on the physical map of *C. reinhardtii* cpDNA, is transmitted in the uniparental pattern. These results unequivocally demonstrate that known cpDNA sequences are inherited maternally and are consistent with the assignment of the genetic markers for antibiotic resistance to the cpDNA.

There is also cytological evidence indicating that the paternal cpDNA is lost during zygote maturation (KUROIWA *et al.*, 1982).

Most (>90%) diploid zygotes of *C. reinhardtii* produce haploid meiotic progeny that express the non-Mendelian markers of the mt^+ (maternal) parent only. Based on the studies mentioned earlier, it appears that the mt^+ cpDNA is preferentially preserved through the sexual cycle and is transmitted to the progeny in most of the zygotes. However, a mechanism for the elimination of the mt^- cpDNA or for the preservation and replication of only the mt^+ cpDNA has not been demonstrated. A number of models and hypotheses detailing possible mechanisms have been advanced. In general, these have been suggested by the ways in which the patterns of non-Mendelian transmission can be perturbed. (For a brief review see BIRKY 1978; MATAGNE and HERMESSE 1980, 1981; or VAN WINKLE-SWIFT 1980). None of the current hypotheses explain all of the genetic data, and many of the key elements of the various models remain to be demonstrated.

In this report we describe the patterns of transmission of non-Mendelian markers in crosses involving an apparent spontaneous homozygous mt^- diploid strain (EVES and CHIANG 1982) and compare these patterns with those from crosses involving diploids heterozygous for mating type and aneuploid strains of various mating type compositions. Our intent was to begin to apportion the influences of the factors of (1) mating type composition and (2) amount of cpDNA on the degree of exceptional transmission of non-Mendelian markers.

MATERIALS AND METHODS

Strains: The strains of *C. reinhardtii* used in these studies are listed in Table 1. Several of the hybrid (hd) and aneuploid (an) strains are presented as heterozygotes for particular markers (Table 1, column 2). Heterozygosity at those loci was determined by the fractions of meiotic progeny that expressed the various phenotypes. For a more complete explanation of this analysis, see EVES and CHIANG (1982).

The Mendelian markers employed are *ery-M3a*, resistance to 200 μg/ml of erythromycin; *msr-1*, resistance to 300 μg/ml of L-methionine-D,L-sulfoximine; *ac-17*, requirement for 2 mg/ml of sodium acetate; *pyr*, resistance to 1 μg/ml of pyrithiamine; mt^+ or mt^-, mating type; *nic-7*, requirement for 0.75 μg/ml of nicotinamide, sensitive to 75 μg/ml of 3′-acetylpyridine; *ac-29a*, pale green to yellow on acetate-containing medium; *act*, resistant to 10 μg/ml of actidione (cycloheximide); *can-1a*, resistant to 1 mg/ml of canavanine; *sr-1a*, resistant to 50 μg/ml of streptomycin; *nic-13*, same phenotype as *nic-7* but nonallelic; *pf-2*, paralyzed flagella.

The non-Mendelian markers in these studies are *spr-u-1-27-3*, which confers resistance to 100 μg/ml of spectinomycin, and *sr-u-1*, which confers resistance to 500 μg/ml of streptomycin (CHUDER and CHIANG 1974). In crosses containing both *sr* markers, clones and colonies were scored on both concentrations of the drug. (See footnote to Table 6 in EVES and CHIANG 1982).

Media: Media and the conditions of culture and maintenance have been described previously (EVES and CHIANG 1982).

Streptomycin·SO_4 was obtained from Sigma Chemical Company. Trobicin (spectinomycin·2HCl) was purchased from Upjohn Company, and spectinomycin·SO_4 was obtained courtesy of GEORGE B. WHITFIELD of the Upjohn Company.

Gametogenesis and mating: Cells were scraped from 7- to 10-day-old plates and resuspended at approximately 10^6/ml in nitrogen-free (N-free) medium in 60-mm Petri dishes, which were then placed in the light for gametogenesis. Approximately 12 hr were required for gametogenesis of haploids and up to 18 hr for aneuploids and diploids. Plus and minus mating type gametes were then mixed in approximately equal numbers in Petri dishes, which were returned to the light for

TABLE 1

Strains

Strain	Markers	Source
137c	*mt*$^+$ or *mt*$^-$ wild types	N. W. GILLHAM (GB-124, GB-125)[a]
L_0	*mt*$^+$ or *mt*$^-$, wild types	R. P. LEVINE
89	*mt*$^-$	Indiana University Culture Collection
90	*mt*$^+$	Indiana University Culture Collection
nic-7	*mt*$^+$, *nic-7*	N. W. GILLHAM (GB-85)[a]
ac-29a	*mt*$^-$, *ac-29a*	N. W. GILLHAM (GB-44)[a]
ery-M3a (*ery-2y*)	*mt*$^-$, *ery-M3a*	N. W. GILLHAM (GB-73)[a]
spr-u-1-27-3	*mt*$^+$ or *mt*$^-$, *spr-u-1-27-3*	N. W. GILLHAM (GB-104, GB-103)[a]
LTS (linkage tester strain)	*mt*$^+$ or *mt*$^-$, *ac-17, pyr, act, can-1a, sr-1a, nic-13, pf-2*	R. SMYTH
d *ery-M3a sr-u-1*	*mt*$^-$, *ery-M3a, sr-u-1*	Produced via streptomycin mutagenesis of *ery-2y*, originally a haploid
nic-7 ery-M3a sr-u-1 (clone 3)	*mt*$^+$, *nic-7, ery-M3a sr-u-1*	*nic-7* × *ery-M3a sr-u-1* (haploid)
nic-7 ery-M3a sr-u-1 (clone 16)	*mt*$^+$, *nic-7, ery-M3a, sr-u-1*	*nic-7* × *ery-M3a sr-u-1* (haploid)
sr-u-1 spr-u-1-27-3 (clone 3)	*mt*$^+$, *sr-u-1, spr-u-1-27-3*	*spr-u-1-27-3* × *ery-M3a sr-u-1* (haploid)
nic-7 ery-M3a sr-u-1 spr-u-1-27-3 (clone 4)	*mt*$^+$, *nic-7, ery-M3a, sr-u-1, spr-u-1-27-3*	*nic-7 ery-M3a sr-u-1* (clone 3) × *spr-u-1-27-3*
ery-M3a sr-u-1 spr-u-1-27-3 (clone 14)	*mt*$^-$, *ery-M3a, sr-u-1, spr-u-1-27-3*	*nic-7 ery-M3a sr-u-1* (clone 3) × *spr-u-1-27-3*
ery-M3a sr-u-1 spr-u-1-27-3 (clone 16)	*mt*$^-$, *ery-M3a, sr-u-1, spr-u-1-27-3*	*nic-7 ery-M3a sr-u-1* (clone 3) × *spr-u-1-27-3*
an $18A_2$-1-1	*mt*$^+$/*mt*$^-$, *spr-u-1-27-3*	*spr-u-1-27-3* × d *ery-M3a sr-u-1*
an $18A_2$-1-2	*mt*$^+$, *ery-M3a/ery-M3a, spr-u-1-27-3*	*spr-u-1-27-3* × d *ery-M3a sr-u-1*
an $18A_2$-1-3	*mt*$^-$/*mt*$^-$, *ery-M3a, spr-u-1-27-3*	*spr-u-1-27-3* × d *ery-M3a sr-u-1*
an $18A_2$-1-4	*mt*$^+$/*mt*$^-$, *ery-M3a*/+, *spr-u-1-27-3*	*spr-u-1-27-3* × d *ery-M3a sr-u-1*
hd 2-5-1	*mt*$^+$/*mt*$^-$, *nic-7*/+, *ac-29a*/+	*nic-7/ac-29a*
hd 2-5-9	*mt*$^+$/*mt*$^-$, *nic-7*/+, *ac-29a*/+	*nic-7/ac-29a*
hd 30-1-2	*mt*$^+$/*mt*$^-$, *nic-7*/+, *ac-29a*/+ *ery-M3a*/+, *sr-u-1, spr-u-1-27-3*	*nic-7 ery-M3a sr-u-1 spr-u-1-27-3* (clone 4)/*ac-29a*

Nomenclature: d, homozygous diploid; hd, hybrid diploid; an, aneuploid; those lacking specific designation are haploid (h).

[a] Gillham-Boynton strain number, Duke University.

a 30- to 60-min mating period. The time required for mating was cross dependent; diploids, aneuploids and paralyzed flagella strains required more time to mate than did wild-type haploids. Aliquots of the mating mixture (0.5–0.75 ml) were transferred to maturation plates when numerous fused gamete pairs could be observed but before zygote aggregates could form.

Zygote maturation and germination: Two different protocols were employed for zygote maturation. Mating mixtures were plated either onto HSM plates and incubated for 2 days in the light (100 footcandles, 1075 lux) and 5 days in the dark (protocol A, based on SUEOKA, CHIANG and KATES 1967) or onto N-free medium and incubated in the light (200 footcandles, 2150 lux) for 3 days (protocol B from VAN WINKLE-SWIFT 1977).

After maturation, zygotes were scraped from the maturation plates, deposited on fresh solid medium (supplemented as required for any input markers) and positioned with a glass loop for analysis. Prior to germination in the light, these plates were inverted over chloroform for 35 sec to kill any vegetative cells. Diploid zygotes matured by the A protocol germinate in 12–16 hr. The B maturation protocol increased the germination time by 10–100%, depending on the cross.

Zygote colony isolation, tetrad dissection, replica plating and scoring: The germinated zygotes were allowed to form colonies approximately 1–2 mm in diameter before they were transferred to master plates. On tetrad dissection plates the germination products (usually four or eight) were separated within areas defined by circles drawn on the bottoms of the plates. Zoospore clones were allowed to grow up for several days or until a diameter of 1–2 mm was attained. Then all viable clones were transferred to fresh plates. For pedigree analysis, the four (or rarely eight) zoospores of a zygote were widely separated to lie in sectors drawn on a plate. When the zoospores released the first set of daughter cells, these were separated within the sector; time of this "first division" and the number of daughters were noted. In some crosses the zoospores of a single zygote divided at different times. Thus, the number of division products varied from two to 16. The clones were allowed to grow to 1–2 mm and were then transferred to master plates.

Zygote colonies, progeny clones and streaks of the parental strains were allowed to grow up on master plates until they attained a size sufficient for replica plating. The master plates were inverted onto a sheet of Whatman no. 1 sterile filter paper that had been fastened over a replica plating block. Replicas were made on the desired media, and the replica plates were placed in the light.

The growth interval required between replicating and scoring depended on the strains and markers involved. Products from haploid × haploid crosses could usually be scored at 7–10 days. Haploid × diploid and haploid × aneuploid cross products required up to 20 days due to the slow growth of some aneuploids.

Nucleoid counts: 4-6-Diamidino-2-phenylindole dihydrochloride (DAPI) is an AT-specific, DNA-binding fluorescent stain that has proven useful for the detection of small amounts of DNA (WILLIAMSON and FENNELL 1975, 1978). The DAPI used here was obtained through D. H. WILLIAMSON, National Institute of Medical Research, Mill Hill, Great Britain and B. J. STEVENS, University of Chicago. Cells were fixed and slides were prepared as described previously (EVES and CHIANG 1982). A solution of 0.2 μg/ml of DAPI in distilled water (WILLIAMSON and FENNELL 1978) was pipetted onto a slide, and staining was allowed to proceed for 10 min at room temperature. The slides were then rinsed thoroughly with distilled water and air dried. DAPI-stained cells were observed and photographed using a Leitz Orthoplan microscope equipped for fluorescence photomicrography. Nucleoids were counted from the negatives using a low-power stereoscopic microscope.

Terms: The transmission of non-Mendelian markers from the mt^- (paternal) parent is termed "exceptional." Exceptional clones result from single meiotic products (zoospores) or can be produced by gene segregation in postmeiotic, mitotic cell divisions. If the paternal allele is recessive, the latter type can be reliably distinguished only in pedigree analysis.

The term "exceptional zygote" is used in this report to designate a zygote that produced one or more exceptional clones or one that was not dissected following meiosis but which, as a colony, expressed a paternal marker. Exceptional zygotes and clones are of two types; those expressing non-Mendelian markers from both parents are termed "biparental" (BP), whereas those expressing only mt^- markers are "paternal" (P).

RESULTS

Transmission genetics: The crosses performed for this study and their results are summarized in Table 2. The non-Mendelian *sr-u-1* and *spr-u-1-27-3* mark-

ers undergo exceptional transmission in about 2% of the zygotes in 12 haploid × haploid crosses (no. 1–12, Table 2). This frequency is higher than that usually observed by SAGER and RAMANIS (1974, 1976) but well within the range of frequencies cited by GILLHAM (1969). In crosses involving hybrid diploids (no. 18, 19 and 20), the frequencies of exceptional transmission for these markers through meiosis increase to about 45%. Again, this is in good agreement with previous reports (GILLHAM 1969; VAN WINKLE-SWIFT 1976). However, in crosses involving our spontaneous mt^-/mt^- homozygous diploid, (d mt^- *ery*-M3a *sr-u-1*) (EVES and CHIANG 1982), the frequency of exceptional zygotes decreases to about 10% for the *sr-u* marker and to slightly less than that for the wild-type (sensitive) *spr-u* allele carried by the diploid (no. 13–17, Table 2). These results indicate that prior to chloroplast fusion the presence of an mt^+ allele in the diploids exerts a marked beneficial effect on the preservation of the diploid chloroplast genome. This pattern appears among crosses involving aneuploids as well (no. 21, 22, 27 and 28 *vs.* 25 and 26, Table 2). In each cross, the frequency of exceptional clones derived from meiotic products was lower than the frequency of exceptional zygotes. This was expected since usually the majority of the meiotic products from an exceptional zygote express the maternal non-Mendelian markers (M clones), whereas a minority express either the paternal non-Mendelian markers (P clones) or markers from both parents (BP clones).

Exceptional transmission in crosses involving the aneuploid progeny from triploid meioses was highly variable (no. 21–26 Table 2); the overall frequencies for mt^+/mt^- and mt^- aneuploids were similar to their diploid counterparts.

Marker segregation: The means by which paternal non-Mendelian markers are occasionally preserved throughout the sexual cycle are important to the formulation of models for the normal functioning of the non-Mendelian mechanism. To determine whether the higher frequencies of exceptional transmission that occur in the crosses involving diploids and aneuploids (Table 2) might be attributable to an increase in marker rescue via recombination, we have examined the phenotypes of the exceptional clones in crosses in which the dominant alleles of the two non-Mendelian markers entered in coupling. These data are presented in Table 3. For haploid × haploid crosses the numbers of spontaneous exceptional zygotes and hence of P and BP clones were quite small. In all types of crosses shown in Table 3 the P clones outnumber the BP clones. If paternal non-Mendelian alleles were being rescued by random recombination or conversion, BP clones expressing only one of the paternal markers would be expected to outnumber the P clones unless *sr-u-1* and *spr-u-1-27-3* are rather tightly linked. Such tight linkage seems unlikely since the two markers segregate from each other rapidly during the early postmeiotic divisions (data not shown). Such rapid postmeiotic segregation of non-Mendelian markers has been reported previously (GILLHAM 1969; SAGER 1972; SAGER and RAMANIS 1976; FORSTER *et al.* 1980). The decrease in the P/BP clone ratios in crosses involving diploids is not readily explicable. It may be that in triploid zygotes the increase in the number of copies of cpDNA promotes increased recombination either before or after meiosis.

TABLE 2

Summary of exceptional transmission for two non-Mendelian markers

Type of cross[a]	Cross no.	$mt^+ \times mt^-$	Exceptional[b] zygote[c] frequency			Exceptional[b] clone frequency		
			sr-u	*spr-u*	N	*sr-u*	*spr-u*	N
h $mt^+ \times$ h mt^-	1	*nic-7 ery-M3a sr-u-1* (clone 16) × 89	0.020		48	0		84
	2	LTS × *spr-u-1-27-3*		0.011	374		0	72
	3	*nic-7 ery-M3a sr-u-1* (clone 3) × *spr-u-1-27-3*	0	0	15	0	0	60
	4	*nic-7 ery-M3a sr-u-1* (clone 16) × *spr-u-1-27-3*	0	0.046	43	0	0.016	64
	5	*nic-7 ery-M3a sr-u-1-27-3* (clone 4) × L_0	0.009	0.009	226	0	0	104
	6	*nic-7 ery-M3a sr-u-1 spr-u-1-27-3* (clone 4) × 89	0.018	0.018	220	0.048	0.038	104
	7	*sr-u-1 spr-u-1-27-3* (clone 3) × *ery-M3a*	0.020	0.020	51	0.013	0.013	76
	8	*nic-7* × *ery-M3a sr-u-1 spr-u-1-27-3* (clone 14)	0	0	9	0	0	34
	9	90 × *ery-M3a sr-u-1 spr-u-1-27-3* (clone 14)	0.100	0.100	50	0.052	0.052	193
	10	137c × *ery-M3a sr-u-1 spr-u-1-27-3* (clone 14)	0.023	0.023	43	0.012	0.012	172
	11	90 × *ery-M3a sr-u-1 spr-u-1 spr-u-1-27-3* (clone 16)	0	0	7	0	0	28
	12	L_0 × *ery-M3a sr-u-1 spr-u-1-27-3* (clone 16)	0.095	0.095	21	0.060	0.060	84
		All haploid × haploid	0.022[d]	0.020[d]	1107	0.023[d]	0.022[d]	1075
h $mt^+ \times$ d mt^-/mt^-	13	90 × d *ery-M3a sr-u-1*	0.130		23			
	14	L_0 × d *ery-M3a sr-u-1*	0.078		51	0.071		28
	15	137c × d *ery-M3a sr-u-1*	0.060		50	0.037		27
	16	*spr-u-1-27-3* × d *ery-M3a sr-u-1*	0.082	0.066	61	0.035	0.018	57
	17	LTS × d *ery-M3a sr-u-1*	0.125		136	0.089		246
		All haploid × diploid	0.100[d]	0.066[d]	321	0.075[d]	0.018[d]	358
h $mt^+ \times$ hd mt^+/mt^-	18	*nic-7 ery-M3a sr-u-1 spr-u-1-27-3* (clone 4) × hd 2-5-1	0.402	0.318	107	0.392	0.329	158
	19	*nic-7 ery-M3a sr-u-1 spr-u-1-27-3* (clone 4) × hd 2-5-9	0.283	0.250	60	0.281	0.250	128
	20	*nic-7* × hd 30-1-2 (*sr-u-1 spr-u-1-27-3*)	0.712	0.770	139	0.360	0.460	50
		All haploid × hybrid diploid	0.520[d]	0.510[d]	306	0.345[d]	0.318[d]	336

h mt^+ × an mt^+/mt^-	21	90 × an 18A$_2$-1-1(mt^+/mt^-)	0.400	15	0.435	23
	22	LTS × an 18A$_2$-1-1(mt^+/mt^-)	0.279	43	0.311	61
	27	137c × an 18A$_2$-1-4(mt^+/mt^-)	0.341	44	0.316	57
	28	LTS × an 18A$_2$-1-4(mt^+/mt^-)	0.373	59	0.314	105
		All haploid × aneuploid (+/−)	0.342[d]	161	0.325[d]	246
an mt^+ × h mt^-	23	an 18A$_2$-1-2(mt^+) × 137c	0.091	11	0.023	44
	24	an 18A$_2$-1-2(mt^+) × LTS	0.205	73	0.202	109
		Aneuploid (+) × haploid	0.190[d]	84	0.144[d]	153
h mt^+ × an mt^-/mt^-	25	137c × an 18A$_2$-1-3(mt^-/mt^-)	0.333	9	0.154	39
	26	LTS × an 18A$_2$-1-3(mt^-/mt^-)	0.012	58	0.010	98
		Haploid × aneuploid (−/−)	0.060[d]	67	0.051[d]	137

[a] Abbreviations used are: h, haploid; d, homozygous diploid; hd, hybrid diploid; an, aneuploid.
[b] Exceptional zygotes or clones are those that express an *sr-7* or *spr-u* phenotype different from that of the mt^+ parent.
[c] Zygotes were scored as described in MATERIALS AND METHODS. Those scored as undissected zygote colonies will lead to an underestimate of exceptional transmission in crosses in which the paternal allele is recessive.
[d] Overall frequency, not a mean.

TABLE 3

Yields of paternal and biparental clones in the various types of crosses

Type of cross	Cross	Exceptional clones			Sample size[a]
		P	BP	P/BP	
Haploid × haploid	*mt*$^+$ *sr-u-1 spr-u-1-27-3* (clone 3) × *mt*$^-$ *ery-M3a*	5	1	5:1	3
	mt$^+$ *nic-7 ery-M3a sr-u-1 spr-u-1-27-3* (clone 4) × *mt*$^-$ 89				
Haploid × haploid	*mt*$^+$ *90* × *mt*$^-$ *ery-M3a sr-u-1 spr-u-1-27-3* (clone 14)				
	mt$^+$ 137c × *mt*$^-$ *ery-M3a sr-u-1 spr-u-1-27-3* (clone 14)	14	3	4.7:1	8
	mt$^+$ L_0 × *mt*$^-$ *ery-M3a sr-u-1 spr-u-1-27-3* (clone 16)				
Haploid × diploid (*mt*$^+$/*mt*$^-$)	*mt*$^+$ *nic-7 ery-M3a sr-u-1 spr-u-1-27-3* (clone 4) × hd 2-5-1				
	mt$^+$ *nic-7 ery-M3a sr-u-1 spr-u-1-27-3* (clone 4) × hd 2-5-9	67	43	1.6:1	79
	mt$^+$ *nic-7* × hd 30-1-2	15	10	1.5:1	18

[a] Number of zygotes in which at least one exceptional clone was scored.

Nucleoids: Nucleoids or extranuclear aggregates of DNA are usually located in the chloroplast region of the cell in Chlamydomonas and are generally assumed to be cpDNA (RIS and PLAUT 1962; VAN WINKLE-SWIFT 1980; CHIANG *et al.* 1981). These nucleoids are readily visualized when fixed cells are stained with the fluorescent dye DAPI (see Figure 1 and COLEMAN 1978; MATAGNE and HERMESSEE 1981; MATAGNE 1981; KUROIWA *et al.* 1982). We have counted the nucleoids in vegetative cells and gametes of several of our strains to ascertain (1) whether the mean number of nucleoids per cell varies with ploidy and (2) whether the number of nucleoids in a paternal strain can be correlated with the level of transmission of its non-Mendelian markers. The mean number of nucleoids per cell has been regarded as an index of the cpDNA content of a strain, and the predictive value of this index for non-Mendelian transmission has been assessed.

The results of the nucleoid counts are presented in Table 4. The number of nucleoids observed per cell varies a great deal. MATAGNE and HERMESSE (1981) report one to seven nucleoids per haploid cell; KURIOWA *et al.* (1982) report eight to ten nucleoids per cell. We have seen two to eight nucleoids per vegetative cell in haploid strains, three to ten nucleoids per vegetative cell in hybrid diploid strains and broad ranges in our aneuploid strains. In most of the strains the mode for nucleoid number coincided with, or was very close to, the mean.

Nucleoids vary in intensity as well as number. Since we have not determined the extent to which intensity on the negative may be influenced by the focal plane of the field, *i.e.*, the amount of overlying cellular material, it is uncertain whether fluorescence intensity is directly related to DNA content. In assembling the data presented in Table 4, all visible nucleoids are included without

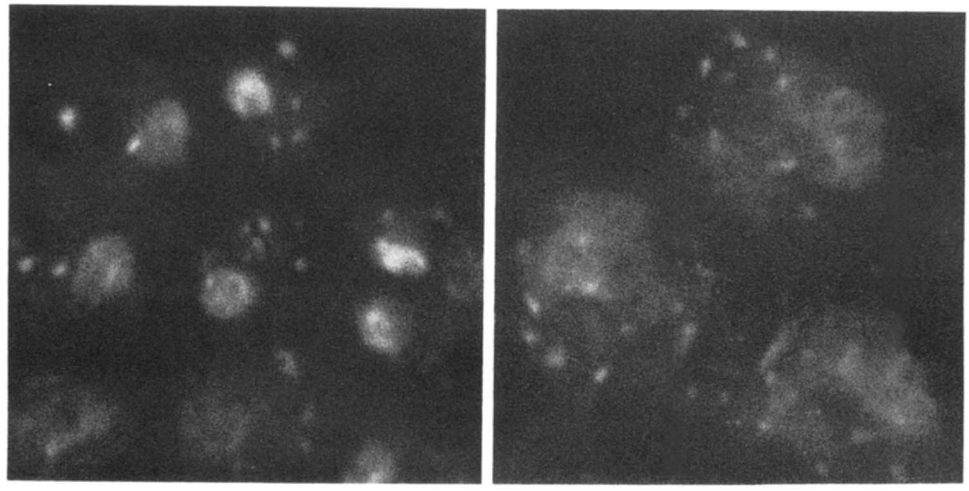

FIGURE 1.—Cells fixed and then stained with DAPI (see MATERIALS AND METHODS): Left panel, *mt⁻ ery-M3a* cells; right panel, d *mt⁻ ery-M3a sr-u-1* cells.

TABLE 4

Nucleoid counts and exceptional transmission

Strain	Mating type	Nucleoids in vegetative cells	N^a	Nucleoids in gametes	N^a	Exceptional transmission[b]	
						Zygotes	Clones
ery-M3a	mt^-	4.1 ± 0.9	42	2.5 ± 0.9	30	0.020	0.013
spr-u-1-27-3	mt^+	4.1 ± 1.5	24	3.3 ± 1.5	13		
d *ery-M3a sr-u-1*	mt^-/mt^-	7.7 ± 2.3	21	5.1 ± 2.2	26	0.100	0.075
hd 2-5-1, hd 2-5-9	mt^+/mt^-	4.3 ± 1.0	15	4.4 ± 1.3	32	0.401	0.409
an $18A_2$-1-1	mt^+/mt^-	7.4 ± 2.0	11	3.1 ± 1.2	20	0.310	0.345
an $18A_2$-1-2	mt^+	4.3 ± 1.0	15	4.3 ± 1.8	18		
an $18A_2$-1-3	mt^-/mt^-	7.4 ± 1.4	11	3.7 ± 0.8	11	0.060	0.051
an $18A_2$-1-4	mt^+/mt^-	6.3 ± 1.5	10	3.6 ± 1.0	19	0.356	0.310

[a] Sample size.

[b] mt^-, mt^-/mt^- or mt^+/mt^- strains only. These are overall frequencies for crosses involving these strains.

reference to relative intensity. Two general trends are evident in Table 4. The number of nucleoids per cell decreases following gametogenesis in all strains except the hybrid diploids and one aneuploid. MATAGNE (1981) noted a similar decrease. The cpDNA content per cell has been found to decrease during gametogenesis (CHIANG and SUEOKA 1967; SEARS, BOYNTON and GILLHAM 1980), and this correspondence with the decrease in nucleoid number lends increased validity to the use of nucleoid number as a functional index of cpDNA content (also see CHIANG *et al.* 1981). The second trend evident in Table 4 is the correspondence between nuclear ploidy and nucleoid number. The significant exception to this correlation is the small number of nucleoids in the hybrid diploids. The implications of this will be discussed.

Finally, the nucleoid counts reveal that the presence of an mt^+ gene in a diploid or aneuploid strain is more effective in enhancing exceptional transmission than are increased numbers of nucleoids *i.e.*, increased units of cpDNA. For example, the hybrid mt^+/mt^- diploids have, on the average, fewer nucleoids than the homozygous mt^-/mt^- diploid, yet the hybrids transmit their non-Mendelian alleles to five times as many meiotic progeny as the homozygous diploid.

DISCUSSION

The capacity of a male (mt^- or mt^--acting) strain of *C. reinhardtii* to transmit its non-Mendelian markers to meiotic progeny may depend on several factors. Factors that have been exmained to some degree include mating type, the relative complements of non-Mendelian cpDNA in the parental cell lines and the physiological states of the parental cell lines. In matings between wild-type haploid gametes, non-Mendelian markers from the mt^+ parent are usually preserved and those from the mt^- parent are usually lost, implicating mating type or factors under mating type control as the primary determinants of non-Mendelian transmission. This general conclusion was strengthened by the discovery of *mt*-linked mutations which altered non-Mendelian transmission (SAGER and RAMANIS 1973, 1974).

The cpDNA complements of the parental strains were first implicated as influential factors in non-Mendelian transmission when GILLHAM (1963) reported that mt^+/mt^- diploids, which behaved as mt^- cells in mating, transmitted their non-Mendelian markers to a large proportion of their progeny following mating with mt^+ haploids. Presumed damage to the mt^+ cpDNA by UV irradiation also perturbs the non-Mendelian mechanism (SAGER and RAMANIS 1967) as does the reduction of cpDNA quantity which results from exposure of the mt^+ cells to 5′-fluorodeoxyuridine (FUdR) (WURTZ, BOYNTON and GILLHAM 1977). MATAGNE and HERMESSE (1980, 1981) and MATAGNE (1981) have used polyethylene glycol (PEG) to induce asexual fusion of haploid *C. reinhardtii* cells and have examined the non-Mendelian marker phenotypes of the diploid progeny. They found that in these induced fusions disparity between the cpDNA complements of the parents, *e.g.*, vegetative cells fused with non-flagellated gametes (MATAGNE 1981) or FUdR-treated cells fused with untreated cells (MATAGNE and HERMESSE 1981), biased non-Mendelian marker transmission in favor of the parent with the greater complement of cpDNA.

Attempts to dissect the influence of a cell's physiological state on its ability to transmit its non-Mendelian markers have focused almost entirely on aspects of the vegetative cell to gamete to zygote transition, although VAN WINKLE-SWIFT (1978) and SEARS (1980) found that biparental transmission decreases and zygotes of both parental types increase with increasing time between zygote formation and meiosis.

Thus, mating type, cpDNA complement and cellular physiology have all been implicated in the non-Mendelian mechanism. The isolation of a spontaneous homozygous *mt* diploid (EVES and CHIANG 1982) presented an oppor-

tunity to evaluate the relative effects of some of these factors on the transmission of non-Mendelian markers to meiotic progeny via normal gametogenesis and fusion.

Our data indicate that the mechanism(s) for the preservation of non-Mendelian markers through the sexual cycle of *C. reinhardtii* must have both specificity and flexibility. Clearly, the presence of an mt^+ gene specifies the preservation of that gamete's non-Mendelian markers, an mt^+ or an mt^+/mt^- line is far more likely to transmit its non-Mendelian markers than is an mt^- or an mt^-/mt^- line. However, there is enough flexibility in the system to allow markers from the paternal parent to be conserved when the maternal parent has been UV irradiated (SAGER and RAMANIS 1967) or when the amount of mt^+ cpDNA is reduced by FUdR prior to mating (WURTZ, BOYNTON and GILLHAM 1977) or, as in our present study, the relative numbers of nucleoids contributed by the parents are altered from the normal state.

With respect to the chloroplast DNA complement size, the anomalies of the vegetative or hybrid diploids require some discussion. Presumably, a hybrid diploid is formed when some mechanism that would lead to zygote development fails. WHITEWAY and LEE (1977) reported that hybrid diploids contain twice as much cpDNA as haploids. In contrast, our hybrid diploids, which were obtained exclusively from sexual crosses, have a mean nucleoid number similar to that of a haploid vegetative cell or that of two haploid gametes. It is not known whether these nucleoids contain more cpDNA than do the nucleoids of haploid cells or the homozygous diploid. The data do suggest that PEG-induced fusion diploids cannot be unreservedly regarded as doubled haploids.

Basically, two distinct models have been proposed for *C. reinhardtii* non-Mendelian gene transmission. One is a system of directed preservation based on a methylation-restricted mechanism, as supported by a number of observations (BURTON, GRABOWY and SAGER 1979; ROYER and SAGER 1979; SAGER, GRABOWY and SANO 1981; SANO, GRABOWY and SAGER 1981). However, extensive methylation of the mt^+ cpDNA as the key to protection proposed originally by SAGER and coworkers seems unlikely in view of the results of BOLEN *et al.* (1982) and FENG and CHIANG (1984). The possibility that a small amount of highly specific cpDNA methylation might be responsible for the non-Mendelian gene transmission (SAGER and GRABOWY 1983) remains open. The second model involves mt^+-biased competition, perhaps for membrane attachment sites (GILLHAM, BOYNTON and LEE 1974; ADAMS *et al.* 1976; BOYNTON *et al.* 1976; BIRKY 1978). Although evidence for this model is still sketchy, the fact that a cell's cpDNA complement influences non-Mendelian transmission does suggest that certain competitive element(s) may exist in such a transmission mechanism.

Although our results do not present incontrovertible evidence for either model currently advanced to explain the mechanism of non-Mendelian transmission, they do support the observation by GILLHAM (1969) that the mt^+ allele or locus exerts a positive effect on the survival of the non-Mendelian genes carried by the cell in which it resides, whether or not the cell carries an mt^- allele as well. Furthermore, our data indicate that this effect is produced

whether the mt^+ is present with an mt^- allele introduced via abortive mating (*i.e.*, a hybrid diploid) or via meiosis (*i.e.*, aneuploid products of triploid meiosis). Another source of *mt* gene expression alteration could be dosage imbalances of other nuclear genes. Although we have not studied this question systematically, no such effect is evident in crosses involving aneuploids (Table 2).

In all types of mt^+/mt^- cells, the mt^- allele or locus controls the mating behavior of the cells so that they mate only with mt^+ cells. The fact that the mating types are apparently domiant in one function and recessive in another suggests that the loci may be (1) regulatory and allelic, (2) each passive with regard to specific functions or (3) complex loci composed of several genes that are resistant to separation by recombination. This last hypothesis may be supported by the linkage map of *C. reinhardtii*; genes linked to the mating types (*e.g., nic-7 ac-29, thi-10*) seem to be inseparably linked, whereas all other syntenic markers (except *fla ts-6*) are recombinatorially remote from the mating types.

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ADDENDUM

Following submission of this study for publication, MATAGNE and MATHIEU (1983, Proc. Natl. Acad. Sci. **80:** 4780–4783) reported similar results using PEG to create diploids homozygous and heterozygous for mating type. Our data are not strictly comparable with theirs since our mt^-/mt^- line arose spontaneously and all of our crosses involved sexual mating, whereas some of theirs were done by PEG fusion. We also looked at aneuploids in which possible imbalances between nuclear and chloroplast gene complements might alter non-Mendelian transmission patterns. Nevertheless, their results and ours are in basic agreement regarding the mt^+ gene as the primary determinant of non-Mendelian gene transmission with the chloroplast DNA content exerting a secondary effect.