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TRANSMISSION, SEGREGATION AND RECOMBINATION OF NON-MENDELIAN GENETIC MARKERS AND CHLOROPLAST DNAS IN INTERSPECIFIC CROSSES BETWEEN *CHLAMYDOMONAS EUGAMETOS* AND *C. MOEWUSII*

A Dissertation

Presented to

the Faculty of the Graduate Studies

Dalhousie University

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

---

by

Claude Lemieux

November 1981

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ABSTRACT

The density, molecular weight and cellular repetition of DNA molecules associated with the β-DNA satellite of the interfertile algae *Chlamydomonas eugametos* and *C. moewusii* were determined. The similarities between these values and those for the cpDNA in the related alga *Chlamydomonas reinhardtii* indicate that this satellite represents chloroplast DNA (cpDNA).

Differences in the restriction endonuclease fragmentation patterns of cpDNA from *C. eugametos* and *C. moewusii* were employed to follow the transmission of cpDNA in interspecific crosses between these algae and to correlate this transmission with that of non-Mendelian genetic markers in the same crosses. The results provide the first physical evidence for the recombination of cpDNA in any plant system and they strongly indicate that the non-Mendelian genetic markers followed are encoded in this DNA.

Analysis of cpDNAs from ten randomly selected F_1 hybrids, in each case, revealed cpDNAs to be recombinant for parental restriction sites. In backcrosses between an F_1 *mt+* and *C. moewusii mt-* strains, seven randomly selected B_1 hybrids showed cpDNA restriction patterns either identical or very similar to that of the *mt+* parent. It is proposed that cpDNA molecules are transmitted predominantly by the *mt+* parent in both F_1 and B_1 generations but that selection favours survival of F_1 hybrids with recombinant chloroplast genomes, thus avoiding nucleo-chloroplastic incompatibilities.

Correlations between the inheritance of non-Mendelian genetic markers and cpDNA fragmentation patterns were more evident among the B_1 hybrids than the F_1 hybrids. Many of these correlations were noted among the mitotic segregants of meiotic products initially mixed for non-Mendelian genetic markers and cpDNA sequences from both parents. Moreover, despite the fact that most of the 34 hybrids analysed revealed recombinant cpDNAs, the inheritance of one *Ava*I and two *Bst*EII generated cpDNA fragments characteristic of *C. moewusii* was always correlated with the inheritance of the non-Mendelian streptomycin sensitive marker from the *C. moewusii* parent.

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LIST OF ABBREVIATIONS

ρ	buoyant density
APBA	2-amino-3-phenyl butanoic acid
ATCC	American Type Culture Collection
BP	biparental
cpDNA	chloroplast DNA
MMS	methyl methanesulfonate
mt	mating-type
mtDNA	mitochondrial DNA
PAR	photosynthetically active radiation
UP	uniparental
UTEX	University of Texas Culture Collection

To my wife Monique,

whose absolute support made it all possible

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

An important goal for investigators of organelle genetics - chloroplast and mitochondrial - is to explain the inheritance, recombination and vegetative segregation of organelle genetic markers in terms of the behaviour of these organelles and their DNAs. The interfertile unicellular algae *Chlamydomonas eugametos* and *C. moewusii* represent potentially valuable organisms for such studies. Although still designated as distinct species, there is convincing evidence and agreement for their being conspecific (Gowans 1963; Lewin 1974). Nevertheless, the high lethality (over 90%) typically observed among the meiotic products of hybrid zygotes produced in an interspecific *C. eugametos* X *C. moewusii* cross, suggests important differences between them (Cain 1979; Gowans 1963, 1976). This dissertation, therefore, developed from the expectation that the chloroplast DNAs (cpDNAs) of these species might be sufficiently different in nucleotide sequence, as revealed with restriction endonuclease enzymes, to allow the following of their transmission in interspecific crosses. Such transmission patterns might then be correlated with those of non-Mendelian mutations, at least [redacted] of which have been identified in *C. eugametos* (McBride and McBride 1975). The non-Mendelian mutations recovered in the related alga *Chlamydomonas reinhardtii* map in a single linkage group assumed to represent cpDNA (reviewed by Gillham, 1978). This assumption has been strengthened recently by the demonstration of coordinate uniparental transmission of non-Mendelian genetic markers and an altered cpDNA restriction pattern characteristic of the mutant strain (Grant et al. 1980).

Dr. Laurens Mets has independently begun studies of the *C. eugametos-C. moewusii* system for reasons similar to those stated above and there have been two preliminary reports of his progress. In the first (Mets 1977), it was noted that whole cell DNA prepared from *C. eugametos* and *C. moewusii* contains a major satellite DNA component for each species. This component was identified as cpDNA on the basis of similarities to the major satellite DNA of *C. reinhardtii* known to be cpDNA. It was further reported that the cpDNAs of *C. eugametos*, *C. moewusii* and *C. reinhardtii* form distinguishable fragmentation patterns after digestion with the restriction endonucleases *Hae*III, *Hpa*II or *Hha*I. In the second report (Mets 1979), the characteristic *C. eugametos* and *C. moewusii* cpDNA fragmentation patterns were employed to demonstrate the uniparental inheritance of cpDNA in a rare complete tetrad derived from a hybrid zygote in which the inheritance of a non-Mendelian antibiotic resistance marker was also uniparental.

This dissertation has two major objectives. The first (Chapter I) is to provide further evidence that the major satellite DNA of *C. eugametos* and *C. moewusii* is cpDNA and to confirm and extend the results of Mets (1977) showing species-characteristic cpDNA fragmentation patterns after digestion with restriction endonucleases. The second objective (Chapter II) is to follow the transmission of species-characteristic cpDNA restriction sites in interspecific crosses and to correlate these results with the transmission, recombination and vegetative segregation of non-Mendelian genetic markers in the same crosses. This objective, which depends on the success of the first, will also require the isolation of non-Mendelian genetic markers in *C. moewusii* (Appendix). It is hoped that this dissertation will provide evidence for, or against,

the association of non-Mendelian genetic markers with cpDNA and that it will provide some insight into the inheritance and possible recombination of cpDNA in intraspecific crosses.

CHAPTER I

CHARACTERIZATION OF CHLOROPLAST DNA IN *CHLAMYDOMONAS EUGAMETOS* AND *C. MOEWUSII*

INTRODUCTION

As stated previously, the transmission of cpDNA might be followed in interspecific crosses between *C. eugametos* and *C. moewusii*, provided that the cpDNAs of these algae form distinguishable fragmentation patterns after digestion with an appropriate restriction endonuclease. Before testing this possibility, it will be first necessary to devise a methodology for the isolation of cpDNA from *C. eugametos* and *C. moewusii*.

The isolation of DNA from a preparation of chloroplasts is impracticable in *Chlamydomonas* as the single large chloroplast characteristic of this genus is usually damaged with cell breakage. After centrifugation in equilibrium CsCl density gradients, however, it is hoped that the whole cell DNA recovered from *C. eugametos* and *C. moewusii* will display a cpDNA satellite comparable to that observed in *C. reinhardtii*. A similarity in density, molecular weight and cellular repetition between the cpDNA of *C. reinhardtii* and presumptive cpDNA components of *C. eugametos* and *C. moewusii* would provide rather conclusive evidence that such components are cpDNAs. If that approach proves successful, it would then be possible to isolate the cpDNAs of *C. eugametos* and *C. moewusii* from preparative CsCl density gradients without the need for chloroplast isolation. Because the identification and characterization of cpDNA in *C. eugametos* and *C. moewusii* depend heavily, if not exclusively, on the literature about the DNA of *C. reinhardtii* in general and that of its chloroplast in particular, it is appropriate to review this literature in some detail.

A. DNA Species of *C. reinhardtii*

Estimates of the *C. reinhardtii* cellular DNA content, based on the diphenylamine colour reaction, range from 120 to 200 $\mu g/10^9$ cells (Sueoka et al. 1967; Whiteway and Lee 1977). The total cellular DNA can be resolved into four density components after equilibrium density gradient centrifugation in CsCl. The major component which accounts for 85% of the cellular DNA is termed α-DNA and has a density of 1.723 g/ml (Sueoka et al. 1967). This DNA is certainly of nuclear origin since it represents most of the cellular DNA and is greatly enriched in preparations of nuclei (Robreau and LeGal [illegible]5). A minor satellite DNA, termed γ-DNA, with a density of 1.712 g/ml, accounts for about 1% of the cellular DNA and contains the nuclear genes for ribosomal RNA (Bastia et al 1971a; Howell 1972; Marco and Rochaix 1980).

Another minor satellite DNA, termed δ-DNA, with a density of 1.706 g/ml, also accounts for about 1% of the cellular DNA. The δ-DNA is believed to be of mitochondrial origin because it is the only DNA component which was recovered from mitochondrial pellets treated with DNase (Ryan et al. 1978). Electron microscopic examination of δ-DNA preparations revealed mostly linear molecules with only 1% of super-coiled or circular molecules with a contour length of 4.7 μm (Ryan et al. 1978). The linear molecules have unique ends, consequently they cannot result from randomly sheared circular DNA (Grant and Chiang 1980). Molecular weight estimates based on measurement of intact circles, kinetic complexity and restriction fragment analysis are in agreement that the δ-DNA has a molecular weight of 9.5 megadaltons (Ryan et al. 1978; Grant and Chiang 1980). Using this value, it was calculated that

C. reinhardtii contains about 50 copies of δ-DNA per cell (Ryan et al. 1978).

The major satellite DNA, termed β-DNA, with a density of 1.696 g/ml, comprises 10-15% of the cellular DNA and is associated with the single chloroplast of *C. reinhardtii*. The chloroplast occupies about 40% of the cell volume and usually lyses when the cell is broken. Thus, as discussed earlier, intact chloroplast preparations are not easily made. Nevertheless, crude broken chloroplast preparations were found to be enriched for the 1.696 g/ml DNA component, but this component accounted for only about 40% of the DNA extracted from such preparations (Sager and Ishida 1963). The remaining DNA had the density of the α component. Hence, the possibility that part of the cpDNA has the same density as the α-DNA cannot be excluded. Dron et al. (1979), however, using a cell-wall deficient mutant of *C. reinhardtii* and more sophisticated techniques of cell fractionation, were able to obtain preparations of apparently intact chloroplasts. The DNA extracted from these preparations revealed a major density component of 1.696 g/ml and a minor contaminant of 1.723 g/ml which is likely to represent trapped nuclear DNA (α-DNA).

Due to the difficulties encountered with the direct isolation of DNA from chloroplasts, investigators of cpDNA from *C. reinhardtii* were forced to recover this DNA by fractionation from CsCl gradients of total cellular DNA. It was found that such preparations contained circular molecules with a contour length of 62 μm corresponding to a molecular weight of 134 megadaltons (Behn and Herrmann 1977). This value shows good agreement with the molecular weight estimates obtained by summing the molecular weights of *Eco*RI restriction fragments (Howell

et al. 1977; Rochaix 1977). Reassociation kinetic analysis, however, indicated complexities equivalent to 200 megadaltons for the β-DNA fraction (Bastia et al. 1971b; Howell and Walker 1976; Wells and Sager 1971). As pointed out by Kolodner and Tewari (1972), the discrepancy between the molecular weight estimates based on reassociation kinetics and those based on length measurements and restriction fragment analysis might be explained by the use of an inaccurate molecular weight value for the T4 DNA employed as standard for the determinations of kinetic complexity. An estimate of 130 megadaltons for the molecular weight of β-DNA indicates the presence of about 80 copies of this DNA per chloroplast of *C. reinhardtii* (Gillham 1978).

Recent advances in the molecular biology of *C. reinhardtii* have demonstrated that the β-DNA is the chloroplast genome. Restriction endonuclease generated fragments of β-DNA have been ordered into a circular map and it has been shown that chloroplast ribosomal RNAs hybridyze to specific β-DNA fragments (Rochaix 1978). Furthermore, the gene coding for the large subunit of the CO_2-fixing enzyme, ribulose-1, 5-bisphosphate carboxylase, has also been localized on the physical map of *C. reinhardtii* β-DNA (Gelvin et al. 1977; Malnoe et al. 1979).

B. Objectives of this Chapter

The general aim of this chapter is to provide the biochemical foundation for the proposed investigation on the transmission of cpDNA in interspecific crosses between *C. eugametos* and *C. moewusii*. The specific objectives are as follows:

(1) characterize the DNA density components of *C. eugametos* and

C. moewusii and compare these components with those recovered from *C. reinhardtii*, as reported in the literature and confirmed here;

(2) develop a methodology for the isolation of *C. eugametos* and *C. moewusii* cpDNAs;

(3) test if species-characteristic fragmentation patterns are obtained after digestion of *C. eugametos* and *C. moewusii* cpDNAs with restriction endonucleases;

(4) find the restriction endonucleases that maximize the distinguishing features between *C. eugametos* and *C. moewusii* cpDNAs.

MATERIAL AND METHODS

A. Strains and Culture Conditions

The wild-type strains of *C. eugametos* (UTEX 9) and *C. moewusii* (UTEX 97) (Starr 1978) were kindly supplied by Dr. C.S. Gowans of the University of Missouri-Columbia. The sexual equivalency of strains 9 and 97 as "male" or "mating type plus" (*mt+*) has been recently stated by Gowans (1976). The wild-type (*mt+*) strain of *C. reinhardtii* was provided by Drs. N.W. Gillham and J.E. Boynton of Duke University.

One or eight liter synchronous cultures were grown at 25°C under alternating 12 hour light and 12 hour dark periods with 3% CO_2 in air, using 3/10 HS minimal medium (Sueoka et al. 1967) for *C. reinhardtii* and the minimal medium of Gowans (1960) for *C. moewusii* and *C. eugametos*. The smaller cultures were surrounded by cool white fluorescent tubes which provided 1000 $\mu E/m^2s$ photosynthetically active radiation (PAR) at the vessel surface. Fluorescent tubes were placed on opposite sides of the larger cultures and provided 3500 $\mu E/m^2s$ PAR at the vessel surface facing the light. Growth was monitored both by cell counting in a hematocytometer and by turbidity measurements (OD_{750nm}) in a Bausch and Lomb Spectronic 20 spectrophotometer. For long term storage, the strains were maintained at 25°C on slants of liquid minimal medium solidified with 1.5% Difco Bacto agar. These cultures were subjected to alternating 12 hour light (50 $\mu E/m^2s$ PAR) and 12 hour dark periods.

B. Whole Cell DNA Isolation

Cells from one liter synchronous cultures were harvested at the

onset of the light period when the culture density had reached 2-4 x 10^6 cells/ml. Cell number routinely increased about four-fold in the previous light-dark cycle. Aliquots containing 3-6 x 10^8 cells were rapidly cooled on ice and harvested by centrifugation at 6000 x g for 10 minutes at 4°C. Cell pellets were resuspended in 5 ml of cold saline-EDTA (50 mM Tris-HCl, 5 mM EDTA, 50 mM NaCl, pH 8.0) and centrifuged in 13 x 100 mm screw-capped test tubes with teflon lined caps at 1000 x g for 10 minutes at 4°C. The pellets were washed as before in 5 ml of buffer A (10 mM Tris-HCl, 10 mM EDTA, 10 mM NaCl, pH 8.0). The cells were resuspended in 0.2 ml of buffer A and lysed by the rapid addition of 2 ml of a sodium dodecyl sulfate (SDS)-proteinase K solution (100 mg SDS, electrophoresis grade, BioRad, U.S.A. and 5 mg proteinase K, Merck, Germany, in 15 ml of buffer A), and then incubated for 12 hours at 37°C. The lysates were stored at 4°C for at least two days. In order to determine the exact number of cells lysed, the cells lost during the harvesting and washing steps were counted in the pooled supernatants. Prior to DNA extraction, a known amount of an internal DNA standard (*Bacillus subtilis* phage SP8 DNA, 20 µg/10^9 cells) was added to the lysates and the volumes were completed to 3.0 ml with buffer A. The mixtures were stored overnight at room temperature. DNA was then isolated by the method of Gross-Bellard et al. (1973).

C. β-DNA Isolation

Cells from eight liter synchronous cultures were harvested at the onset of the light period when the culture density had reached 2-4 x 10^6 cells/ml. Aliquots containing 10^{10} cells were cooled on ice and harvested with a Sharples continuous flow centrifuge. Cells were washed suc-

cessively in ice-cold saline-EDTA and buffer A, before being resuspended in 50 ml of buffer A containing proteinase K at 200 µg/ml. After incubation for 15 minutes at 37°C, the cell suspension was lysed by the rapid addition of 50 ml of buffer A containing 2.5% SDS and incubated for 4-6 hours at 37°C. The lysate was extracted twice with an equal volume of water saturated distilled phenol neutralized to pH 8.0 with Tris-base. After dialysis of the resulting aqueous solution against 1/5 saline-EDTA, it was adjusted to 1 M NaCl and 10% (w/v) polyethylene glycol-6000 (PEG-6000) by the addition of 5 M NaCl and 50% PEG-6000, and then stored at 4°C for 12 hours. The precipitate was collected by low speed centrifugation (2000 x g, 5 minutes, 4°C) and dissolved in 35 ml 1/5 saline-EDTA. The solution was adjusted to 55.8% (w/v) CsCl and then to 2.5 µg/ml ethidium bromide. Cellulose nitrate tubes previously treated overnight with 10% sarkosyl NL-97 (ICN-K and K laboratories, Plainview, U.S.A.) and each containing 6.5 ml of the above solution, were overlaid with paraffin oil and centrifuged in an IEC A-269 rotor (20° fixed angle) at 41,000 rpm for 48 hours at 18°C. After centrifugation, the α- and β-DNA bands were visualized by fluorescence under long-wave UV light (365 nm). The upper band containing the β-DNA was collected by puncturing the tubes just above this band with a syringe (20-gauge needle). This fraction was extracted twice with two volumes of isoamyl alcohol, dialyzed exhaustively against 1 mM Tris-HCl, 1 mM EDTA, 1mM NaCl, pH 7.5 and lyophilized. The DNA was then redissolved in distilled water at a concentration of 500 µg/ml. The yield obtained was 200-300 µg of β-DNA.

D. Isolation of the 1.707 g/ml Satellite DNA from *C. moewusii*

The fraction between the α- and β-DNA bands was collected with

a syringe from several equilibrium CsCl density gradients of whole cell nucleic acid. The preparation of whole cell nucleic acid, the composition of the CsCl solutions and the conditions of centrifugation were as described in the methods of β-DNA isolation. The pooled DNA fraction was then subjected to a second CsCl density gradient centrifugation in an IEC A-321 rotor (35° fixed angle) at 43,000 rpm for 48 hours at 18°C. The centrifuge tubes previously treated with sarkosyl NL-97 were filled with 8.5 ml of solution containing 10 µg/ml pooled DNA fraction, 55.0% CsCl (w/v), 2.5 µg/ml ethidium bromide and 60 µg/ml netropsin sulfate which was extracted from *Streptomyces netropsis* (ATCC #23940) according to the method of Finlay et al. (1951). After centrifugation, the upper band containing the β-DNA and the middle band containing the 1.707 g/ml DNA were collected from each tube with syringes. Netropsin sulfate and ethidium bromide were removed from each pooled DNA fraction by passage throughout a small column (0.4 x 2.5 cm) of Na^+ Dowex AG 50W-X2 that had been extensively washed with 10 mM EDTA, pH 8.0. The collected DNA solutions were then exhaustively dialyzed against 1/5 saline-EDTA. The yield obtained was 3 µg of the 1.707 g/ml DNA from 2 x 10^9 cells.

E. DNA Density Profiles and Quantification

DNA preparations were analysed in equilibrium CsCl density gradients (Mandel et al. 1968) in a Spinco Model E analytical ultracentrifuge. The UV absorption photographs of the gradients were traced with a Joyce-Loebl scanning microdensitometer and the DNA densities were calculated according to the equations of Schildkraut et al. (1962) using *Bacillus subtilis* phage SP8 DNA (ρ = 1.742 g/ml) as a reference. This reference DNA (kindly supplied by Monique Turmel, Dalhousie University)

shows a sharp band free of any other density components in CsCl gradients. To confirm the DNA nature of the components resolved in the analytical CsCl density gradients of whole cell DNA, this DNA in 10 mM Tris-HCl, 3 mM $MgCl_2$, pH 8.0 was digested with deoxyribonuclease I at 20 µg/ml for 3 hours at 25°C. After dialysis against 1/5 saline-EDTA, the solution was analysed by analytical CsCl density gradient centrifugation.

The total DNA and β-DNA contents of lysates derived from a known cell number were determined from the microdensitometer traces of UV absorption photographs taken after equilibrium CsCl density gradient centrifugation of whole cell DNA. Amounts of total and β-DNAs were estimated by comparing the area under all density components of cellular DNA and that under the β-DNA component, respectively, with the area under the internal standard DNA (phage SP8 DNA) peak. In order to obtain a direct correspondence between film density and DNA concentration, it was necessary to integrate the reference and β-DNA peaks from one centrifuge cell containing a DNA concentration of 5.5 µg/ml and the α-DNA peak from another centrifuge cell containing a four-fold gravimetric dilution of the former solution. For further details see Whiteway and Lee (1977).

F. Endonuclease Digestion and Gel Electrophoresis

β-DNA (2 µg) was digested in 25 µl of incubation mixture with sufficient enzyme to give complete cleavage of the DNA at 37°C for 3 hours, with the exception that digestion with *Bst*EII was done at 60°C. Compositions of the incubation mixtures were the following: *Ava*I (Bethesda Research Labs) - 20 mM Tris-HCl, 30 mM NaCl, 10 mM $MgCl_2$,

100 µg/ml bovine serum albumin, pH 7.4; *Bam*HI (Bethesda Research Labs)- 20 mM Tris-HCl, 100 mM NaCl, 7 mM $MgCl_2$, 2mM 2-mercaptoethanol, pH 7.0; *Bgl*I (Bethesda Research Labs)- 20 mM Tris-HCl, 7 mM $MgCl_2$, 7 mM 2-mercaptoethanol, pH 7.0; *Bgl*II (Bethesda Research Labs)- 20 mM Tris-HCl, 7 mM $MgCl_2$, 7 mM 2-mercaptoethanol, pH 8.0; *Bst*EII (Bethesda Research Labs)- 6 mM Tris-HCl, 50 mM NaCl, 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol, pH 7.9; *Eco*RI (Miles Laboratories)- 100 mM Tris-HCl, 50 mM NaCl, 5 mM $MgCl_2$, 2 mM mercaptoethanol, pH 7.2; *Hind*III (Miles Laboratories)- 20 mM Tris-HCl, 60 mM NaCl, 7 mM $MgCl_2$, pH 7.4; *Hpa*II (Bethesda Research Labs)- 20 mM Tris-HCl, 7 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.4; *Msp*I (New England Biolabs)- 20 mM Tris-HCl, 6 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 µg/ml bovine serum albumin, pH 7.4; *Pst*I (Bethesda Research Labs)- 20 mM Tris-HCl, 50 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 100 µg/ml bovine serum albumin, pH 7.5; *Sal*I (Bethesda Research Labs)- 8mM Tris-HCl, 150 mM NaCl, 6 mM $MgCl_2$, 0.2 mM Na_2EDTA, pH 7.6; *Sma*I (Bethesda Research Labs)- 15 mM Tris-HCl, 15 mM KCl, 6 mM $MgCl_2$, pH 8.0; *Sst*II (Bethesda Research Labs)- 14 mM Tris-Hcl, 90 mM NaCl, 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol, pH 7.5. Each reaction was stopped by the addition of 5 µl 250 mM EDTA, pH 7.0, 5 µl glycerol and 2.5 µl 0.5% bromophenol blue.

DNA restriction fragments (0.5-1 µg) or undigested DNAs (0.5-10 µg) were resolved by electrophoresis in agarose horizontal slab gels (25 x 15 x 0.3 cm) at 50 volts for 12 hours. The electrophoresis buffer consisted of 40 mM Tris-acetate, 5 mM sodium acetate, 1 mM EDTA, pH 7.8. After electrophoresis, the gels were stained for 1 hour in the above buffer containing 0.5 µg/ml ethidium bromide. Gels were trans-illuminated with 300 nm UV light (Brunk and Simpson 1977) and photo-

graphed through a red filter (Kodak Wratten # 26) on Kodak high contrast copy film 5069.

G. Determinations of Molecular Weight and Fragment Content of DNA Bands in Agarose Gels

Each agarose gel was standardized with DNA fragments of known molecular weight. The reference fragments, with sizes ranging from 0.5 to 24 megadaltons (Figure 1), were from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA (Rochaix 1978). The molecular weight of each DNA band in agarose gels was determined from a calibration curve relating the electrophoretic mobility and molecular weight of the reference fragments (Figure 2).

Microdensitometer traces of UV fluorescence photographs of restriction patterns were used to determine the number of fragments in each band (see Figure 2) (Rochaix 1977). The ratio between the area under each peak and the molecular weight of the corresponding DNA band is a relative measure of the molar amount of fragments present in the peak.

RESULTS

A. DNA Density Profiles and Quantification

As shown in Figure 3, after equilibrium CsCl density gradient centrifugation the whole cell DNA from *C. reinhardtii* displays the expected density species. These include the main band or α component (ρ = 1.723 g/ml), the major satellite or β component (ρ = 1.696 g/ml) and the two minor satellites γ (ρ = 1.712 g/ml) and δ (ρ = 1.706 g/ml). The density profiles of whole cell DNA prepared from *C. moewusii* and *C. eugametos* resemble that of *C. reinhardtii* with respect to having a main DNA component (ρ = 1.718 g/ml) and a major satellite DNA (ρ = 1.700 g/ml). For conformity with *C. reinhardtii*, these DNA components are hereafter designated the α- and β-DNAs. With respect to the minor satellite DNAs, the preparation of *C. moewusii* reveals two with densities of 1.707 and 1.732 g/ml whereas only one minor satellite with a density of 1.710 g/ml is visible in the *C. eugametos* preparation. All density components described above were DNA as revealed by the disappearance of the bands from CsCl gradients after digestion with DNase.

The results of two independent determinations of whole cell DNA content, using cells from synchronously dividing cultures of each of the three species (removed at the dark/light transition point), are shown in Table 1. Comparable values of whole cell DNA content, ranging from 183 to 252 $\mu g/10^9$ cells, were estimated for the three species by direct DNA quantification in the analytical ultracentrifuge. The *C. reinhardtii* values agree with those obtained with the diphenylamine colorimetric assay (Sueoka et al. 1967; Whiteway and Lee 1977). Estimates of the β-

DNA content in the same cell samples (see Table 1) indicate that the β component of all three algae consistently amounts to 11-15% of the cellular DNA. The α component represents most of the remaining proportion as the minor satellite DNAs account for less than 3% of the cellular DNA.

B. Purity of the β-DNA Preparations

Centrifugation analysis of samples enriched for β-DNA from each of the three species reveals only minor contaminations by the α-DNA (Figure 4) while fractionation of these samples by agarose gel electrophoresis (Figure 5) reveals trace amounts of low molecular weight DNAs in the *C. reinhardtii* and *C. moewusii* samples. The *C. moewusii* low molecular weight DNAs are characterized by one prominent band with a molecular weight of 3.9 megadaltons and one fainter band of 4.4 megadaltons. At least two even fainter bands of lower molecular weight can be distinguished when larger amounts of DNA are applied to the gel (data not shown). All of these components account for less than 2% of the DNA in the β-DNA fraction of *C. moewusii*. No similar low molecular weight components are detected in equivalent amounts of the β-DNA fraction from *C. reinhardtii* or in an intentionally overloaded sample from the *C. eugametos* preparation. The *C. reinhardtii* sample, however, reveals a 9.5 megadalton DNA contaminant which has been reported to originate from the 1.706 g/ml satellite DNA (δ-DNA) associated with the mitochondrial fraction (Rochaix 1978; Ryan et al. 1978). The 9.5 megadalton component is clearly absent from the *C. moewusii* sample; its possible detection in the *C. eugametos* sample could have been obscured by the excess of DNA applied to the gel. However, other gels loaded

with half this amount of *C. eugametos* β-DNA preparation clearly indicated the absence of a 9.5 megadalton DNA band (data not shown).

C. Molecular Weight of the *C. moewusii* 1.707 g/ml DNA Component

Unlike the 1.706 g/ml DNA component (δ-DNA) of *C. reinhardtii*, the analogous 1.707 g/ml DNA component of *C. moewusii* does not have a low molecular weight. The fractionation of the 1.707 g/ml DNA component from CsCl gradients of whole cell nucleic acid is hindered by the poor separation between this component and the α- and β-DNA bands (Figure 6, traces A and B). As shown in Figure 7, however, the resolution of the DNA components of *C. moewusii* is greatly enhanced when netropsin sulfate is added to the CsCl gradients. Netropsin sulfate preferentially reduces the density of DNA rich in (dA + dT)-sequences because of its preferential binding affinity for these sequences (Wartell et al. 1974). The increase in resolution so obtained allowed the enrichment up to 80% purity of the 1.707 g/ml DNA component (Figure 6, trace C) and the isolation of a β-DNA fraction free of any contaminating density components (Figure 6, trace D). Analysis of these DNA preparations showed that the DNA in both preparations migrated as one major band of molecular weight clearly higher than 25 megadaltons (Figure 8, samples A and B). When larger amounts of the β-DNA preparation were applied to the gel (Figure 8, sample C), low molecular weight DNA bands were seen in the same proportion as those observed in the β-DNA prepared in absence of netropsin sulfate. Comparable amounts of the 1.707 g/ml DNA preparation were not analysed as an insufficient quantity of this DNA was recovered.

D. Restriction Endonuclease Fragment Analyses of *C. eugametos* and *C. moewusii* β-DNA Preparations

The electrophoretic patterns of *C. eugametos* and *C. moewusii* β-DNAs digested with eleven different restriction endonucleases are compared in Figures 9 to 13. Distinct restriction patterns are observed with every enzyme tested; however, the characteristic patterns of the two species always share bands in common. For each species, the proportion of these common bands depends on the enzyme employed. For example, in the *Eco*RI and *Sma*I restriction patterns of *C. moewusii* β-DNA, the proportions of bands common to both species are respectively as low as 20% and as high as 70%.

The β-DNAs of *C. eugametos* and *C. moewusii* were also digested with the restriction endonucleases *Hpa*II and *Msp*I in order to determine if these DNAs were methylated. Both enzymes have the same recognition sequence (CCGG), but a 5-methyl group at the internal C residue protects this sequence from *Hpa*II cleavage but not from *Msp*I cleavage (Waalwijk and Flavell 1978). As shown in Figure 14, both enzymes produce identical fragmentation patterns with the same β-DNA, but the patterns produced with the β-DNAs from the two species are different. Thus the distinctive restriction fragmentation patterns for *C. eugametos* and *C. moewusii* most probably reflect differences in their β-DNA nucleotide sequences.

E. Molecular Weight and Cellular Repetition of β-DNAs

The sizes of the *C. eugametos* and *C. moewusii* β-DNAs were estimated by summing the molecular weights of all fragments detected in individual *Eco*RI, *Ava*I, *Bst*EII and *Sma*I restriction patterns. As in-

dicated in Tables 2 to 5 and as summarized in Table 6, similar values were calculated for the two species and these are comparable to the molecular weight values of around 130 megadaltons reported for the β-DNA from *C. reinhardtii* (Behn and Herrmann 1977; Howell et al 1977; Rochaix 1977). The estimates for *C. eugametos* range from 142 to 163 megadaltons and those for *C. moewusii* range from 137 to 161 megadaltons. The above-mentioned restriction patterns proved the most suitable for molecular weight determinations with regard to the number, size and fragment content of the bands. The 3.9 megadalton band observed in the *Ava*I and *Sma*I restriction patterns of *C. moewusii* is present in submolar amounts and therefore was not considered in the calculations. This non-stoichiometric band probably represents the 3.9 megadalton DNA contaminating the β-DNA preparation (see preceeding section). The 3.9 megadalton band, however, is not detected in the *Eco*RI and *Bst*EII restriction patterns, thus suggesting that this contaminant of β-DNA is cut by the restriction enzymes.

The β-DNA molecules are present in multiple copies in cells of *C. reinhardtii*, *C. eugametos* and *C. moewusii*. From the estimates of β-DNA molecular weight and content per cell, similar values of repetition (about 100 molecules as indicated in Table 7) were calculated for the β-DNAs of the three species. The copy number per cell reported here for the *C. reinhardtii* β-DNA is significantly higher than that reported by Whiteway and Lee (1977) because the molecular weight of β-DNA (200 megadaltons) used by these workers has proven to be too high. However, when the copy number of *C. reinhardtii* β-DNA is recalculated using the data of Whiteway and Lee and a molecular weight of 130 megadaltons, a value in agreement with that reported here is obtained.

DISCUSSION

A. Characterization and Cellular Location of *C. eugametos* and *C. moewusii* DNA Density Components

Like *C. reinhardtii*, the α- and β-DNA components of *C. eugametos* and *C. moewusii* undoubtedly represent the respective nuclear and cpDNAs of these algae. There are several lines of evidence obtained from comparative studies of DNA from *C. eugametos*, *C. moewusii* and *C. reinhardtii* that support this conclusion. First, the density profiles of whole cell DNA from *C. eugametos* and *C. moewusii* strikingly resemble that observed for *C. reinhardtii* with regard to the densities and proportions of the α- and β-DNAs. Second, the molecular weight values of β-DNA from *C. eugametos* (154 megadaltons) and *C. moewusii* (150 megadaltons) are similar to that reported for the cpDNA of *C. reinhardtii* (130 megadaltons). Third, the β-DNA molecules in cells of *C. eugametos* and *C. moewusii* are present in the same number of copies as the cpDNA of *C. reinhardtii* (about 100 molecules per cell).

The possible functional homology between the minor satellite DNAs of *C. eugametos* (1.710 g/ml) and *C. moewusii* (1.707, 1.732 g/ml), and those of *C. reinhardtii* (1.706, 1.712 g/ml) is not as obvious as with the α- and β-DNAs. A DNA density component that can be convincingly equated with the mitochondrial DNA (*mt*DNA) of *C. eugametos* and *C. moewusii* was not identified. Although the minor 1.707 g/ml satellite DNA of *C. moewusii* is similar in density to the mtDNA of *C. reinhardtii* (ρ = 1.706 g/ml), the molecular weight of this satellite DNA is more than two-fold greater than that of the mtDNA of *C. reinhardtii*. One

normally finds the molecular weight of mtDNA to be more uniform within a single genus (reviewed by Gillham, 1978). The possible association of the *C. moewusii* 1.707 g/ml DNA component, as well of the *C. eugametos* 1.710 g/ml DNA component, with nuclear genes for cytoplasmic ribosomal RNAs seems more likely and must be tested. Such an association has been demonstrated for the 1.712 g/ml satellite DNA of *C. reinhardtii* (Bastia et al. 1971a; Howell 1972).

The low molecular weight DNAs detected in the *C. moewusii* β-DNA preparation are also unlikely candidates for mtDNA because of their apparent absence from *C. eugametos*. Furthermore, it seems impossible to resolve the low molecular weight DNAs and the β-DNAs in CsCl density gradients; a highly purified β-DNA preparation appeared to contain the same proportion of contaminating low molecular weight DNAs as a less purified one. This observation suggests a density for the low molecular weight DNAs lighter than that of the *C. reinhardtii* mtDNA. Similar low molecular weight DNAs of non-mitochondrial origin have been recovered from *Acetabularia* (Green 1976), *Euglena* (Nass and Ben-Shaul 1972) and yeast (Guerineau et al. 1974). Nevertheless, the similarity between the combined molecular weights of *C. moewusii* low molecular weight DNAs and that of *C. reinhardtii* mtDNA makes one hesitant to rule out a mitochondrial origin for these DNAs of *C. moewusii*.

The identical densities of the cpDNAs (β-DNAs) from *C. eugametos* and *C. moewusii* and of their respective nuclear DNAs (α-DNAs), as well as the difference between these values and those obtained for the comparable components of *C. reinhardtii* whole cell DNA, are consistent with the hypothesis that *C. eugametos* and *C. moewusii* are closely related to each other and more distantly related to *C. reinhardtii* (reviewed by

Gowans, 1976). Nevertheless, differences between *C. eugametos* and *C. moewusii* with respect to minor satellite DNAs and, as discussed later, with respect to cpDNA endonuclease fragmentation patterns are also consistent with genetic data suggesting some divergence between these two algae (Cain 1979; Gowans 1963). The variations in nuclear and cpDNA densities observed in this limited sampling from the genus *Chlamydomonas* are consistent with variations in the densities of these DNAs within the Chlorophytan genus *Chlorella* or more broadly throughout the division *Chlorophyta* (see Kirk and Tilney-Bassett 1978). Among higher plants (angiosperms), cpDNA is remarkably constant in density (1.697 ± 0.001 g/ml) while the density of nuclear DNA appears uniform within genera but variable between genera (see Kirk and Tilney-Bassett 1978).

Finally, as in *C. reinhardtii*, it is possible to purify the cpDNAs from *C. eugametos* and *C. moewusii* by collecting the β-DNA fraction from CsCl density gradients of whole cell DNA. Even though there is less separation between the α- and β-DNA components of *C. eugametos* and *C. moewusii* compared to that observed for *C. reinhardtii*, β-DNA fractions of high purity are easily prepared from *C. eugametos* and *C. moewusii*. The utilization of synchronous cultures harvested at the cell-cycle stage where cells are the most easily lysed avoids the need for cell-wall deficient mutants. Such mutants are employed in the standard procedure of cpDNA extraction from *C. reinhardtii* (Rochaix 1980). No similar mutants have been reported for *C. eugametos* and *C. moewusii*. Nevertheless, even if wall-less mutants were available, they probably would have been undesirable in this investigation as their sensitivity to micromanipulation complicates genetic analysis.

B. Detection of *C. eugametos* and *C. moewusii* Specific CpDNA Nucleotide Sequences

The cpDNAs from *C. eugametos* and *C. moewusii* can be distinguished by analysis of their restriction endonuclease fragmentation patterns. Failure to detect methylation of the *C. eugametos* and *C. moewusii* cpDNAs with the restriction endonucleases *Hpa*II and *Msp*I suggests that their characteristic fragmentation patterns reflect differences in nucleotide sequence or rearrangements of common sequences, rather than differential methylation. CpDNA from wild-type vegetative cells of *C. reinhardtii* is also unmethylated, although cpDNA from one mutant (Grant et al. 1979) and from gametes (Burton et al. 1979; Royer and Sager 1979; Sager et al. 1981) show varying degrees of methylation.

Although distinguishable with every restriction enzyme tested, the cpDNAs of *C. eugametos* and *C. moewusii* possibly share extensive homology in nucleotide sequence. The high proportion of fragments showing identical electrophoretic mobility in the compared *C. eugametos* and *C. moewusii* cpDNA restriction patterns is not likely due to non-homologous fragments which by chance migrate at the same rate. This is particularly true in the case of the patterns produced by *Sma*I where as many as 70% of the bands were found to be common to both species. It seems reasonable, therefore, that most pairs of common fragments are homologous in nucleotide sequence. This hypothesis could be tested by hybridizing such common restriction fragments purified from one species to all the restriction fragments from the other species after electrophoresis in agarose gel. The finding that the common restriction fragments hybridize to their counterparts from the other species would support the presence of homologous nucleotide sequences. The same ap-

proach could be used to detect restriction fragments possibly homologous to species-characteristic restriction fragments.

In conclusion, the original expectation concerning the *C. eugametos*-*C. moewusii* system proved to be fully justified. The characteristic restriction patterns of *C. eugametos* and *C. moewusii* cpDNAs can be utilized to follow the transmission of these DNAs in interspecific crosses. Among the restriction enzymes assayed in this study, it was found that *Ava*I, *Bst*EII and *Sma*I produce the most suitable patterns for this purpose. Other restriction enzymes tested revealed more species-characteristic cpDNA fragments, but these fragments were less easily distinguished by electrophoresis. Different restriction sites are recognized by the *Ava*I, *Bst*EII and *Sma*I enzymes and these sites appear to be randomly distributed among the cpDNA molecules as revealed by the good agreement between the calculated number of fragments expected from a random sequence of bases and the observed number of fragments (see Table 8). A total of at least 75 restriction fragments characteristic to *C. eugametos* or *C. moewusii* are generated by *Ava*I, *Bst*EII and *Sma*I. Assuming that cpDNA sequences of *C. eugametos* and *C. moewusii* are homologous except for a single restriction site, one would expect the generation of up to three species-characteristic restriction fragments. Hence, a minimum of 25 *Ava*I, *Bst*EII and *Sma*I species-characteristic restriction sites must be dispersed on the cpDNA molecules of *C. eugametos* and *C. moewusii*.

CHAPTER II

TRANSMISSION, SEGREGATION AND RECOMBINATION OF NON-MENDELIAN GENETIC MARKERS AND CHLOROPLAST DNAS IN INTERSPECIFIC CROSSES BETWEEN *CHLAMYDOMONAS EUGAMETOS* AND *C. MOEWUSII*

INTRODUCTION

Since the discovery of the first non-Mendelian genetic marker in *C. reinhardtii* by Sager (1954) numerous other mutations with similar inheritance patterns have been identified in this alga and over the past few years there has accumulated persuasive but not conclusive evidence that these genetic markers are encoded in cpDNA. Nevertheless, abundant genetic information on the sexual transmission, segregation and recombination of the non-Mendelian genetic markers is not yet fully explained at the chloroplast genome level (reviewed by Gillham, 1978 and by Birky, 1978). The major aim of this chapter, therefore, is to use differences in the distribution of restriction sites in the cpDNA of *C. moewusii* and *C. eugametos* to follow the transmission of cpDNA in crosses between these interfertile algae. It is hoped that this approach together with studies on the behaviour of non-Mendelian genetic markers in the same crosses will help establish firmly a cpDNA location for such markers in the genus *Chlamydomonas* and at the same time provide molecular data which will help explain features of non-Mendelian gene inheritance. Before expressing the objectives of this chapter in more detail, it is necessary to review certain background information.

A. Non-Mendelian Genetics of *Chlamydomonas*

Chlamydomonas reinhardtii. The sexual cycle of *C. reinhardtii* is advantageous for genetic analysis because of its simplicity and its readily controllable stages. Under conditions of nitrogen deprivation, vegetative cells of mating-type plus (*mt+*) and mating-type minus (*mt-*)

can be differentiated into gametes (Sager and Granick 1954). When mixed, the morphologically similar gametes of opposite mating-type first aggregate, then pair and shortly thereafter (within 15 minutes), fuse laterally to form diploid planozygotes. The two gamete nuclei and the two gamete chloroplasts fuse during the first three hours following zygote formation (Cavalier-Smith 1970). After a suitable period of maturation, the zygotes can be induced to undergo meiosis, with each zygote germinating into four haploid meiotic products, two of which belong to each mating-type. The clones produced by each meiotic product can be induced to undergo gametic differentiation by nitrogen deprivation.

Two distinct genetic systems have been identified in *C. reinhardtii*. Mutations belonging to the first system segregate in a classical 2:2 Mendelian fashion in tetrads after meiosis. The system is therefore assumed to be chromosomal and to reside in the nucleus. In contrast, mutations belonging to the second system exhibit non-Mendelian segregations. In crosses involving strains with any of the non-Mendelian mutations, three kinds of zygotes are encountered. The majority of zygotes transmit the non-Mendelian genetic markers from only the *mt*+ parent to the four meiotic products (uniparental or UP+ zygotes); a few zygotes (less than 10%) transmit the non-Mendelian genetic markers from both parents (biparental or BP zygores); and a minority (less than 1%) transmit the non-Mendelian genetic markers from only the *mt*- parent (uniparental or UP- zygotes). The frequencies of BP and UP- zygotes can be greatly enhanced by treating the *mt*+ gametes with UV prior to mating (Sager and Ramanis 1967). Furthermore, the gametogenesis regime of the *mt*- parent (Sears et al. 1980) and the duration of zygote maturation (Sears 1980a, b) have been found to affect slightly the transmission pattern of the non-

Mendelian genes.

The BP zygotes are of critical importance for studies of non-Mendelian gene segregation and recombination. Such zygotes which are heteroplasmic for opposite parental alleles of non-Mendelian genes segregate progeny homoplasmic for one or the other parental allele during meiotic and post-meiotic mitotic divisions (see Sager 1977 and Gillham 1978). The segregation is usually completed within 20 divisions. Unlike Mendelian genes, therefore, non-Mendelian genes continue to segregate after meiosis. During segregation, recombinant non-Mendelian genotypes are produced in addition to parental genotypes (Gillham 1965; Sager and Ramanis 1965). Reciprocal recombinant genotypes are formed but are often not derived from the same zygote (Gillham 1965; Sager and Ramanis 1976b).

The laboratory of Sager and that of Gillham and Boynton have reported contrasting results regarding the allelic ratio of both spontaneous and irradiation-UV induced BP zygotes. The allelic ratio of a BP zygote is defined as the fraction of progeny clones in the total progeny bearing a given allele of a non-Mendelian gene (Gillham et al. 1974). Among the progeny of spontaneous rare BP zygotes, Sager and Ramanis (Sager and Ramanis 1968, 1976a; see also Sager 1972) have observed a 1:1 ratio for cells homoplasmic for a given pair of alleles while Gillham, Boynton and colleagues (Gillham 1963; Gillham et al. 1974; Boynton et al. 1976) have observed marked deviations from this ratio, the non-Mendelian allele carried by the *mt+* parent being nearly always present in excess. Sager and Ramanis (1976a) have detected no effect of UV treatment of *mt+* gametes on the allelic ratio of BP zygote; a 1:1 ratio has been consistently observed for almost all non-Mendelian genes examined regardless of the UV dose. Exceptional deviations from this ratio were found to be associated

with specific gene loci. In contrast, Gillham, Boynton and colleagues (Gillham et al. 1974; Forster et al. 1980) have observed a skew in favour of the allele carried by the *mt+* parent at low UV doses. At higher doses, the average allelic ratio for a population of BP zygotes approached 1:1 although the allelic ratios from individual zygotes were not normally distributed around a mean of 0.5. As will be discussed later, the yet unexplained discrepancy between the two sets of data has led to divergent views about the mechanism of inheritance of the non-Mendelian genes.

Because of the tendancy of the non-Mendelian genes to segregate during mitosis and their ability to undergo recombination, methods for mapping these genes have been devised (reviewed by Adams et al., 1976 and by Sager, 1977). In fact, *C. reinhardtii* is at present the only photosynthetic organism in which one can map non-Mendelian genes by formal genetic methods. Non-Mendelian genetic maps have been constructed by the laboratory of Sager and that of Gillham and Boynton (see Sager 1977 and Gillham 1978). Although there are some discrepancies in map order between the two laboratories, the mapping studies concur in showing that all stable non-Mendelian mutations mapped to date are associated with a single linkage group. The majority of these non-Mendelian mutations confer antibiotic resistance or dependence on chloroplast ribosomes.

Finally, evidence has emerged recently for a second non-Mendelian genetic system in *C. reinhardtii*, which is thought to be of mitochondrial origin (Alexander et al. 1974; Wiseman et al. 1977). As the evidence favouring the existence of this system is involved and in the end somewhat questionable, it will not be discussed further. The interested reader is referred to a thorough review on this subject by Gillham (1978).

Chlamydomonas eugametos and *C. moewusii*. The sexual cycles of *C. eugametos* and *C. moewusii* are almost identical to that of *C. reinhardtii*, the main difference being in the method of zygote formation (Gowans 1976). Fusion of opposite mating-type gametes in *C. eugametos* or *C. moewusii* is completed later than in *C. reinhardtii*, usually four to eight hours after the formation of the mating pairs. Furthermore, this fusion takes place without any of the lateral flexion exhibited by *C. reinhardtii*.

As in *C. reinhardtii*, Mendelian and non-Mendelian genetic systems have been identified in *C. eugametos* and *C. moewusii*. The non-Mendelian genes of *C. eugametos*, like those of *C. reinhardtii*, are transmitted predominantly by the *mt+* parent. The first genetic studies by McBride and McBride (1975) indicated that both a mutation conferring resistance to streptomycin (*sr-2*) and a mutation conferring dependence on neamine (*nd*) are transmitted to the meiotic progeny exclusively by the *mt+* parent; no exceptional zygotes (BP or UP-) were detected. This strictly uniparental transmission pattern of non-Mendelian genes can be altered by employing different conditions for crosses. Indeed, during the course of this investigation, crosses were made that involved the same *sr-2* mutant isolated by McBride and McBride but under conditions slightly different from those they employed; it was observed that 80% of the zygotes recovered were UP+ while the remaining were BP or UP- (Appendix).

The inheritance pattern of non-Mendelian genes in *C. moewusii* is remarkably different from that in *C. reinhardtii* and *C. eugametos*. This finding was also made during the course of the present investigation and involved the first non-Mendelian mutants recovered from *C. moewusii*: a mutant resistant to streptomycin (*sr-nM1*) and a mutant resistant to erythromycin (*er-nM1*). Zygotes from reciprocal crosses between the mu-

tant and wild-type strains, in almost all instances, transmitted non-Mendelian genes from both parents (Appendix). These BP zygotes yielded tetrads containing a majority of heteroplasmic meiotic products which, during the following post-meiotic divisions, segregated progeny homoplasmic for one or the other parental allele of a given non-Mendelian gene. Furthermore, as reported by Gillham, Boynton and colleagues for *C. reinhardtii*, there was an excess of the *mt+* parental allele among the progeny of BP zygotes.

Although *C. eugametos* and *C. moewusii* are still designated as distinct species, there is convincing evidence and agreement for their being conspecific (Gowans 1963; Lewin 1974). Cytological studies have failed to detect morphological and chromosomal differences between the two algae (Gowans 1963). The gametes of *C. eugametos* and *C. moewusii* pair then fuse readily and the resulting zygotes germinate with a high frequency. Germination of the zygotes typically releases four meiotic products, however, there is considerable subsequent lethality and rarely does more than one product per F_1 tetrad survive (Gowans 1963). This low survival must be due to hybridity as Cain (1979) found progeny survival to be high after the intraspecific crosses with *C. eugametos* and with *C. moewusii*. Cain (1979) also reported the frequency of progeny survival to be higher in crosses having *C. eugametos* as the *mt+* parent (8% survival) than in the reciprocal crosses (2% survival). Finally, no detailed study on the transmission of non-Mendelian genes in interspecific crosses between *C. eugametos* and *C. moewusii* has been reported to date.

B. Cellular Location of Non-Mendelian Genes

Chlamydomonas reinhardtii. Since their discovery, the non-Men-

delian genes of *C. reinhardtii* have been assumed by most to be located on cpDNA (reviewed by Gillham, 1978). To demonstrate this assumption, several experimental approaches have been exploited more or less fruitfully.

Chiang (1968, 1970, 1976) and Sager and her colleagues (Sager 1972; Sager and Lane 1972; Sager and Schlanger 1976) labelled differentially the *mt+* and *mt-* parental cpDNAs to study the fate of these DNAs in populations of zygotes. In *Chlamydomonas*, both *mt+* and *mt-* gametes contribute equal amounts of cpDNA to the zygote. As mentioned earlier, the majority of zygotes transmit to their meiotic progeny the non-Mendelian genes from only the *mt+* parent (UP+ zygotes). If the non-Mendelian genes are located on cpDNA, then the behaviour of cpDNA in zygotes should reflect that of the non-Mendelian genes. One would therefore expect that most of the cpDNA from the *mt-* parent would disappear in zygotes prior to meiosis. The experiments of Chiang and Sager and her colleagues, which are discussed in detail by Gillham (1978), yielded unfortunately contrasting results. The results of Chiang showed the conservation of cpDNA from both parents while those of Sager and her colleagues showed the conservation of cpDNA from only the *mt+* parent. These different findings might be reconciled if pools of DNA precursors and DNA turnover were associated with the experiments of Chiang. An alternate experimental approach which eliminates pool problems was therefore required to demonstrate clearly the parallel behaviour of cpDNA and non-Mendelian genes in sexual crosses.

Grant et al. (1980) employed altered sequences of cpDNA as physical markers for correlating the inheritance of cpDNA and non-Mendelian genetic markers in tetrads recovered from UP+ zygotes. After a systematic screening of various *C. reinhardtii* mutant strains, they discovered one in which cpDNA exhibited two 100 base-pair deletions. In

reciprocal crosses between strains with normal and deleted cpDNAs; cpDNA physical markers and non-Mendelian genetic markers were found to be cotransmitted strictly from the *mt+* parent to all meiotic progeny. Since the two deletions were separated by about one-half of the chloroplast genome, the results suggested that a large part, if not all of the chloroplast genome, is uniparentally transmitted by the UP+ zygotes. Although the results were consistent with the chloroplast location of the non-Mendelian genes, the possibility that these genes are located on other uniparentally inherited cytoplasmic DNAs (e.g. mtDNA) cannot be excluded.

Another piece of evidence for the location of the *C. reinhardtii* non-Mendelian genes on cpDNA was recently provided by Spreitzer and Mets (1980) who identified the inheritance pattern of a mutation affecting the activity and structure of a gene product known to be encoded by cpDNA. This gene product is the large subunit (LS) of the CO_2-fixing enzyme, ribulose-1, 5-bisphosphate carboxylase. Spreitzer and Mets found that the LS mutation was transmitted in a uniparental non-Mendelian fashion from the *mt+* parent in sexual crosses. The LS mutation, however, has not been genetically mapped. If it is found to be linked to the other non-Mendelian mutations so far mapped, then the association of the non-Mendelian genetic linkage group with cpDNA will be definitively proven.

Chlamydomonas eugametos and *C. moewusii*. Mets employed differences in the distribution of restriction endonuclease cleavage sites in the cpDNA of *C. eugametos* and *C. moewusii* as physical markers to study the transmission of cpDNA in interspecific crosses between these algae and to correlate this transmission with that of a non-Mendelian streptomycin resistant marker (*sr*-2, McBride and McBride 1975) associated with

C. eugametos. In a preliminary report (1979), he communicated the first successful recovery of a rare F_1 hybrid zygote (0.01% of the zygotes examined) in which all four products survived and another equally rare zygote from the reciprocal cross in which three of the four germination products survived. In both instances, the *Hae*III cpDNA restriction sites of only the *mt+* parent were inherited and this was correlated with the uniparental inheritance of the non-Mendelian streptomycin resistant or sensitive marker associated with the *mt+* parent. In the same interspecific crosses, however, Mets also found the strict transmission from the *mt+* parent of low molecular weight DNA species present only in *C. moewusii*. These low molecular weight DNAs are the same as those detected in *C. moewusii* during the present investigation (see Chapter I). In summary, the results of Mets supported the hypothesis that the *sr-2* gene is encoded in cpDNA, but as was concluded in similar experiments with *C. reinhardtii* (Grant et al. 1980), the possible association of this non-Mendelian gene with other uniparentally inherited DNAs was not excluded.

C. Alternate Models of Chloroplast Genome Segregation

In *C. reinhardtii*, non-Mendelian genes hereafter designated chloroplast genes segregate during vegetative asexual division. Such a vegetative segregation of organelle genes in other organelle systems is commonly interpreted in terms of a random physical movement and sorting-out of the multiple organelles containing these genes during cell division (reviewed by Birky, 1978). *Chlamydomonas*, however, has a single large chloroplast which divides as the cell divides (Sager and Palade 1954). Thus, the vegetative segregation of chloroplast genes in this

genus must be attributed to the physical segregation of the chloroplast genomes.

The molecular mechanism governing the segregation of chloroplast genes has not yet been elucidated. Many tentative models have been formulated to explain the segregation mechanism of chloroplast genomes in *C. reinhardtii* but, to date, none of them has been adequately tested by molecular or cytological studies. These models often antagonize each other primarily because they are based on genetic data that are unfortunately conflicting between laboratories. In particular, the persisting controversy between the laboratory of Sager and that of Gillham and Boynton concerning the allelic segregation ratio for chloroplast genes among the mitotic progeny of BP zygotes has led to different estimates of the copy number of the chloroplast genome. A detailed description of all existing models is inappropriate here; thorough reviews on this subject have been published (Sager 1977; Birky 1978; Gillham 1978; Van Winkle-Swift 1980). It is relevant, however, to present briefly the model of Sager and Ramanis (1976a, 1977) and that of VanWinkle-Swift (1980) which offer the extreme views about the segregation mechanism of chloroplast genomes.

According to the model of Sager and Ramanis, segregation of parental chloroplast alleles into opposite daughter cells requires recombination between parental chloroplast genomes. Vegetative cells are assumed to contain two functional copies of the circular chloroplast genome. The model further postulates the existence of a centromere-like membrane attachment point directing the movement of chloroplast genomes. During the vegetative division of an heteroplasmic chloroplast, the movement of the attachment point is directed in such a way that one

replica of each parental genome enters each daughter chloroplast. In the absence of recombinational events, heteroplasmic daughter chloroplasts are therefore produced. Homoplasmic chloroplasts are segregated only if there is recombination (gene conversion or crossing-over) between opposite parental genomes prior to their segregation. This process is analogous to the production of homozygous daughter cells by mitotic crossing-over in the nucleus.

In striking contrast, the model of VanWinkle-Swift assumes that most segregation events occur without recombination of chloroplast genomes. The model proposes that the 50 to 100 copies of cpDNA molecules observed per chloroplast are equally competent genetically. Furthermore it is assumed that genomes clustered into a small number of discrete areas, rather than individual cpDNA molecules, are the segregation units. Such DNA clusters, termed nucleoids, have been cytologically observed in *C. reinhardtii* (Coleman 1978; Goodenough 1970). According to her model, the distribution of nucleoids to daughter cells is not actively directed; although non-random, it is dictated solely by the spatial arrangement of parental nucleoids with respect to the plane of chloroplast division. Because the model postulates that the nucleoids found in heteroplasmic chloroplasts are, in most cases, individually homoplasmic (i.e. composed of genomes of one parental origin) and that interactions between nucleoids are relatively rare, recombination between opposite parental chloroplast genomes should occur infrequently.

Studies on the inheritance of cpDNA sequences in hybrid progeny of *C. eugametos* and *C. moewusii* may offer direct evidence in support of, or against, alternative models proposed to explain the vegetative segregation of chloroplast genes. Indeed, the differences in the distribution

of restriction endonuclease cleavage sites in the parental cpDNAs may allow one to trace the inheritance of cpDNA among the homoplasmic mitotic progeny segregated by meiotic products heteroplasmic for chloroplast genetic markers. The finding of cpDNAs recombinant for restriction sites in most mitotic segregants would support segregation mechanisms such as those proposed by Sager and Ramanis (1976a), Gillham et al. (1974) and Birky (1978), which require the recombination of chloroplast genomes. On the other hand, the frequent observation of non-recombinant cpDNAs among mitotic segregants would be consistent with the segregation mechanism postulated by VanWinkle-Swift (1980), provided that recombination between *C. eugametos* and *C. moewusii* cpDNA sequences is detectable and that non-homologies between these sequences do not alter the normal course and pattern of recombination.

D. Objectives of this Chapter

The principal goal of this chapter is to investigate the transmission of cpDNA in interspecific crosses between *C. eugametos* and *C. moewusii*. This approach is possible because particular parental cpDNA sequences can be conveniently distinguished by restriction endonuclease analysis. Every restriction site characteristic of one or the other parent can be effectively employed as a physical marker to score the inheritance of parental cpDNA sequences in hybrid progeny. As discussed in Chapter I, the enzymes *Ava*I, *Bst*EII and *Sma*I together generate a minimum of 25 species-characteristic restriction sites. Assuming that these sites are distributed randomly on the cpDNA molecules, one should be able to detect the occurrence of most recombination events.

The inheritance of non-Mendelian genetic markers of *C. eugametos*

(*sr*-2) and *C. moewusii* (*er-nM*1) will be followed in hybrid progeny concurrently with the inheritance of parental cpDNA sequences. Progeny derived from typical hybrid zygotes, i.e. those yielding only one viable product per tetrad, will be selected randomly for cpDNA analysis before scoring the inheritance of the non-Mendelian genetic markers. If meiotic products heteroplasmic for non-Mendelian genetic markers are detected, then the inheritance of cpDNA sequences will be examined in various homoplasmic mitotic segregants derived from individual heteroplasmic products. These segregants will be selected to represent each of the available non-Mendelian phenotypes (opposite parental and possibly reciprocal non-parental phenotypes). Any possible correlation between the inheritance of cpDNA sequences and the inheritance of non-Mendelian genetic markers in the overall hybrid progeny will be sought.

The specific objectives of this chapter are as follows:

(1) establish the typical inheritance patterns of cpDNA sequences and non-Mendelian genetic markers in hybrid progeny of *C. eugametos* and *C. moewusii*;

(2) test for linkage between the *C. eugametos sr*-2 and the *C. moewusii er-nM*1 genetic markers by formal genetic analysis;

(3) confirm the preliminary results of Mets showing the probable location of the *sr*-2 gene on cpDNA and extend this cpDNA location to the *er-nM*1 gene;

(4) determine whether or not recombinant cpDNA molecules are found in the homoplasmic mitotic progeny derived from hybrid meiotic products heteroplasmic for non-Mendelian genes.

As discussed earlier, the last objective may provide direct evidence in support of, or against, tentative models proposed to explain

the vegetative segregation of chloroplast genes in *C. reinhardtii*. Of course, the results will be conclusive if the non-Mendelian genes of *C. eugametos* and *C. moewusii* are located on cpDNA and if these genes within the progeny of heteroplasmic meiotic products behave similarly to the chloroplast genes of *C. reinhardtii*.

MATERIALS AND METHODS

A. Strains and Culture Conditions

The *mt+* and *mt-* wild-type strains of *C. moewusii* (UTEX 97 and 96) (Starr 1978), the male (*mt+*) and female (*mt-*) wild-type strains of *C. eugametos* (UTEX 9 and 10) and the *mt+* strain of *C. eugametos* carrying the non-Mendelian streptomycin resistance marker *sr-2* (McBride and McBride 1975) were provided by Dr. C.S. Gowans of the University of Missouri-Columbia. The mutant strain of *C. moewusii* requiring 1 µg/ml nicotinamide (*nic-1*) and the other requiring 1 µg/ml p-aminobenzoic acid (*pab-1*) were isolated from the *mt-* wild-type strain after UV mutagenesis. The *nic-1* and *pab-1* mutations are inherited in a Mendelian fashion and are unlinked to the *mt* locus. The *C. moewusii* non-Mendelian mutant *er-mM1*, resistant to 400 µg/ml erythromycin, was isolated from the *mt+* wild-type strain after methyl methanesulfonate mutagenesis (Appendix). *C. eugametos*, *C. moewusii* and *C. eugametos-C. moewusii* hybrid strains were maintained under the conditions described in the Materials and Methods of Chapter I with the exceptions that the minimal medium was supplemented with 4 g/l Difco Bacto yeast extract for the growth of strains with *nic-1* and with 1 mg/l p-aminobenzoic acid for the growth of strains with *pab-1*.

For cpDNA isolation, eight liter synchronous cultures were grown in the minimal medium of Gowans (1960) under the conditions described in the Materials and Methods of Chapter I. For the determination of mutant phenotypes, cultures were grown on solid minimal medium at 22°C in continuous cool white fluorescent light (400 µE/m^{2}s PAR). Solid minimal medium was prepared by adding 15 g Difco Bacto agar per liter of liquid minimal

medium. The medium was supplemented with 75 µg/ml streptomycin (from streptomycin sulfate, Pfizer) for the detection of *sr-2* and with 100 µg/ml erythromycin (from erythromycin lactobionate, Abbott) for the detection of *cr-nM1*. Sterile streptomycin and erythromycin solutions were added to molten agar medium after autoclaving. All liquid and solid media were supplemented with appropriate growth factors when necessary and again, sterile solutions of growth factors were added to the medium after autoclaving.

B. Procedure for Crosses

All crosses employed in this study are listed in Table 9. The opposite mating-type strains to be crossed were first grown separately at 18°C on solid minimal medium supplemented with 4 g/l Difco Bacto yeast extract under alternating 12 hour light and 12 hour dark periods. The illumination associated with this and all subsequent steps described below was provided at 400 µE/m^2s PAR from cool white fluorescent tubes. After 5-6 days of growth under alternating light-dark conditions, a heavy inoculum of each strain was transferred to solid minimal medium containing 1/10 of the normal NH_4NO_3 concentration and cultured under the above conditions for 5-6 days. At the completion of the last dark period, cells from each strain were suspended separately in 0.5 ml sterile distilled water saturated with $CaCO_3$ and densities were adjusted to approximately 10^6-10^7 cells/ml. Gametogenesis was induced by incubating the cell suspensions at 18°C in light for 3-6 hours. The suspensions of gametes of opposite mating-type were then mixed and allowed to mate for 1-2 hours under the above conditions of gametogenesis. After this time, 0.5 ml of each mating pair suspension was plated on minimal medium and incubated

at 18°C under continuous light for 48 hours. The plates were then wrapped in aluminium foil and incubated at 18°C for 6 days. At the end of this period, mature zygotes were scraped from the plates, transferred to fresh agar plates and individual zygotes were isolated as described by Ebersold and Levine (1959). The plates were illuminated continuously at 22°C and germination of the zygotes occurred within the following 24 hours. The meiotic products were separated according to the method described by Ebersold and Levine (1959). As recommended personally by Dr. J.R. Cain, a fine tungsten wire (0.127 mm diameter, Ventron, Alfa Division) was used to manipulate the zygotes and meiotic products. Sex and growth requirements of meiotic products were determined as described by Gowans (1960) except that gamete suspensions were prepared by the method described above.

C. Non-Mendelian Genetic Analyses

Inheritance of non-Mendelian genetic markers in meiotic products. In crosses where the *sr*-2 and *er-nM*1 markers are in repulsion (crosses 2, 4 and 5 of Table 9), the inheritance of these non-Mendelian genetic markers was scored after meiotic products had undergone about 20 mitotic divisions (primary clone). About 10^4-10^5 cells from each primary clone were plated on streptomycin and on erythromycin agar medium for the detection of resistant cells. The meiotic products were classified as UP+ when growth was noted only on the antibiotic to which the *mt*+ parent was resistant. The meiotic products were classified as UP- when they grew only on the antibiotic to which the *mt*- parent was resistant. When growth was noted on both antibiotic media, meiotic products were classified as BP.

Vegetative segregation of non-Mendelian genetic markers. To determine whether a meiotic product was homoplasmic or heteroplasmic for non-Mendelian genetic markers, 100 subclones were first recovered as colonies on non-selective agar medium after streaking a liquid suspension of the primary clone of this product. About 10^4-10^5 cells from each subclone were then plated on antibiotic containing agar medium for the detection of resistant cells. A meiotic product was considered homoplasmic for the resistance or the sensitivity to a given antibiotic when all 100 subclones were resistant or sensitive to this antibiotic. Alternatively, a meiotic product was considered heteroplasmic for the resistance and sensitivity to a given antitiotic when some subclones were resistant and others sensitive to this antibiotic.

The same procedure was utilized to prove the homoplasmic state of mitotic segregants subcloned from meiotic products heteroplasmic for the resistance and sensitivity to streptomycin and for the resistance and sensitivity to erythromycin. About 10^4-10^5 cells from each of 100 subclones recovered from individual mitotic segregants (about 20 generations after their initial subcloning) were plated on streptomycin, on erythromycin and on streptomycin-erythromycin containing agar medium to determine the non-Mendelian phenotype of these subclones. A mitotic segregant was considered homoplasmic for a given parental or non-parental non-Mendelian phenotype when all 100 subclones derived from this segregant displayed that phenotype.

D. CpDNA Analyses

CpDNA (β-DNA) preparations, endonuclease digestions and electrophoreses in 0.75% agarose gel were performed as described in the Materials

and Methods of Chapter I except that cpDNA was extracted from 2 x 10^9 cells and all isolation procedures were scaled down five-fold. The molecular weight and fragment content of bands from restriction patterns were also determined as described in the Materials and Methods of Chapter I.

The electrophoretic restriction patterns of cpDNA from the hybrid progeny were compared to those of the parents. Progeny and parental bands with identical electrophoretic mobility were designated as "characteristic" bands when they were specific to one or the other parental patterns and as "common" bands when they were present in both parental patterns. Progeny bands with electrophoretic mobility not corresponding to the mobility of any parental bands were named "new" bands. When characteristic or common progeny bands differed in multiplicity from the parental ones, the fragments in these progeny bands were classified according to Figure 15.

CpDNA sizes were determined from *Sma*I restriction patterns by summing the molecular weights of all fragments. Because *Ava*I and *Bst*EII restriction fragments of low molecular weight were difficult to detect under the conditions of electrophoresis employed, cpDNA sizes were estimated from *Ava*I and *Bst*EII patterns by summing the molecular weights of fragments higher than 1.2 megadaltons. Such estimates are satisfactory for the comparison of cpDNA sizes in hybrid progeny as, for *C. eugametos* and *C. moewusii*, the summations of molecular weights lower than 1.2 megadaltons yielded similar values and these values represented less than 7% of the cpDNA size (*Ava*I: 10.0 megadaltons for *C. eugametos* and 7.6 megadaltons for *C. moewusii*; *Bst*EII: 5.3 megadaltons for *C. eugametos* and 8.1 megadaltons for *C. moewusii*). The non-stoichiometric 3.9 mega-

dalton band sometimes seen in *Ava*I and *Sma*I patterns was not considered for the calculations of cpDNA size.

RESULTS

A. Survival of F_1, B_1 and B_2 Hybrid Meiotic Products

Germination of most hybrid zygotes from interspecific crosses and from first and second generation backcrosses yielded four meiotic products, however, as shown in Table 10 overall survival of these products was low. In general agreement with Cain (1979) and Gowans (1963) only 9% of the F_1 products survived; although the majority of the zygotes produced no viable products, about one-third of the population provided one surviving product and a few produced two. Overall survival and the average number of viable products per tetrad increased with successive backcrossing. Nevertheless, even after the second backcross only one of 25 tetrads yielded four viable products. Within this complete tetrad, a 2:2 segregation of the *nic*-1 alleles was observed and the surviving progeny of incomplete tetrads were frequently non-parental with respect to the unlinked *pab*-1, *nic*-1 and *mt* Mendelian loci.

B. Inheritance of the *sr*-2 and *er*-*nM*1 Non-Mendelian Markers in F_1, B_1 and B_2 Hybrid Meiotic Products

Table 10 also shows that in the F_1 generation the great majority of the viable products inherited non-Mendelian genetic markers uniparentally from the *mt*+ parent (UP+ products); in first and second generation backcrosses, however, the frequency of products showing transmission of non-Mendelian genetic markers from the *mt*- parent increased. Most of this increased transmission from the *mt*- parent is reflected in meiotic products inheriting non-Mendelian resistance markers from both parents

or biparentally (BP products). The proportion of these BP meiotic products in the successive hybrid crosses increased from 2% to 17%.

C. Segregation of the *sr*-2 and *er*-*nM*1 Non-Mendelian Markers Among the Mitotic Progeny of F_1 and B_1 Biparental Hybrid Meiotic Products

The non-Mendelian phenotypes of subclones derived from the F_1 BP product (F-1-k) and from four of the B_1 BP products (B-1-i, B-1-j, B-1-k, B-1-l) of Table 10 were examined. The results presented in Table 11 show that after about 20 generations of mitotic growth, all F_1 and B_1 BP products segregated subclones with parental and non-parental non-Mendelian phenotypes. Although both parental phenotypes were detected among the segregants of most BP products, segregants with one or the other parental phenotype were usually in great excess. Finally, the two reciprocal non-parental phenotypes were found among the segregants from F-1-k, B-1-i, B-1-j while only one of the non-parental phenotypes was detected among the segregants from B-1-k and B-1-l.

All the BP meiotic products analysed above were initially heteroplasmic for resistance and sensitivity to streptomycin and for resistance and sensitivity to erythromycin. It is likely, however, that most segregants recovered from these BP meiotic products were homoplasmic because all 17 of a sample of segregants selected for cpDNA analysis were found to be homoplasmic for their non-Mendelian phenotype. Nevertheless, even after the 20 generations of growth provided, a few subclones derived from B-1-i and B-1-j were still heteroplasmic (H). Such subclones contained streptomycin resistant cells, erythromycin resistant cells but no cells resistant to both antibiotics (Table 11).

D. Inheritance of CpDNA Restriction Fragments in Randomly Selected F_1 and B_1 Hybrid Meiotic Products

Ten F_1 and seven B_1 hybrid progeny were randomly selected from single surviving meiotic products recovered from interspecific crosses (crosses 1 and 2 of Table 9) and from first generation backcrosses (crosses 3 and 4 of Table 9). Subsequent subclone analysis proved all of these products to be homoplasmic for the non-Mendelian phenotype of the *mt+* parent.

F_1 hybrids. CpDNA isolated from a subclone of each of the ten F_1 hybrid products (F-1-a through -j) displayed *Ava*I (Figure 16), *Bst*EII (Figure 17) and *Sma*I (Figure 18) restriction patterns of cpDNA distinct from that of either parent. A mixture of bands unique to one or the other parent was found in all F_1 progeny, with some of the bands characteristic of *C. eugametos* (the *mt+* parent) or *C. moewusii* (the *mt-* parent) being uniformly present among all ten hybrids. One *Ava*I band, one *Sma*I band and several *Bst*EII bands not associated with either parent were detected in various F_1 hybrids. Finally, the 13 *Ava*I, the nine *Bst*EII and the eight *Sma*I bands common to both parents were detected in all F_1 progeny except in F-1-i which showed 12 *Ava*I bands common to both parents. Tables 12 and 14 summarize further features of the inheritance of *Ava*I, *Bst*EII, and *Sma*I bands and fragments. For the *Ava*I and *Bst*EII patterns, fragment number in agreement with band number usually showed that significantly less than half of those unique to *C. moewusii* were detected in all F_1 hybrids. In contrast, the *Sma*I restriction patterns displayed most of the bands characteristic of the *C. moewusii* parent and only a few bands characteristic of the *C. eugametos* parent. Several *Ava*I and *Bst*EII

restriction fragments were not found in new bands but were detected as changes in the multiplicity of common or characteristic bands (see Figure 15). Finally, Tables 12 to 14 show that cpDNA sizes, as estimated by summing the molecular weights of all fragments higher than 1.2 megadaltons, were comparable (±2% for *Ava*I; ±7% for *Bst*EII; ±8% for *Sma*I) to the parental values.

In summary, the results reveal that the cpDNAs from all ten F_1 hybrids are recombinant for restriction sites. The presence of fragments characteristic of both parents, the absence of many species-characteristic parental fragments and the similarities between the molecular weight estimates of parental and hybrid cpDNAs clearly indicate that the cpDNA restriction patterns observed for the F_1 hybrids did not result from a simple mixture of parental patterns. This biparental inheritance of cpDNA restriction sites in all F_1 hybrids is in contrast to the uniparental inheritance of non-Mendelian genetic markers in the same hybrids.

B_1 hybrids. The electrophoretic patterns of *Ava*I (Figure 19) and *Bst*EII (Figure 20) digests of cpDNA from subclones of seven B_1 hybrid products (B-1-a through -g) were found identical or highly similar to that of the F_1 *mt*+ parent. Unlike F_1 hybrids, therefore, the uniparental inheritance of cpDNA restriction sites in some B_1 hybrids was correlated with the uniparental inheritance of non-Mendelian genetic markers from the same *mt*+ parent. The cpDNA results, as summarized in Tables 15 and 16, show B-1-a, -b and -c to have fragment patterns indistinguishable from that of the F_1 *mt*+ parent, while the patterns of B-1-d, -e, -f and -g showed a maximum of two fragment differences from this parent. B-1-d and -g displayed fragments characteristic of only the F_1 *mt*+ parent. A band characteristic of the *C. moewusii mt*- parent, however,

was detected in the *Ava*I patterns of B-1-e and -f, thus indicating the cpDNAs of these hybrids to be recombinant. The inheritance of parental *Sma*I restriction fragments in the B_1 progeny could not be adequately determined because only one band distinguished the *Sma*I cpDNA restriction patterns of the parents (see Figure 18 and Table 14).

In contrast to the F_1 hybrids which all showed recombinant cpDNAs, only two of the seven B_1 hybrids inherited cpDNAs recombinant for parental *Ava*I and/or *Bst*EII restriction sites. The presence of restriction fragments characteristic of both parents is, in the view of this investigator, the only valid criterion for the identification of recombinant cpDNA. CpDNA molecules not meeting this criterion but displaying patterns slightly different from that of the *mt*+ parent (e.g. cpDNA from B-1-d and B-1-g) could be attributed to either recombinantion or to deletion of cpDNA sequences.

E. Inheritance of CpDNA Restriction Fragments in Mitotic Segregants Derived from F_1 and B_1 Biparental Hybrid Meiotic Products

Although relatively rare, F_1 and B_1 hybrid meiotic products which inherited non-Mendelian genetic markers from both parents were recovered, and it was of interest to score the inheritance of parental cpDNA restriction fragments in the genetically homoplasmic segregants of these products. To this end, segregants derived from one F_1 BP product (F-1-k) and from four B_1 BP products (B-1-i through -l) were selected to represent each of the available non-Mendelian phenotypic classes (S, E, + ES) shown in Table 11. Growth rate variation in liquid medium (non-selective) was noted among the segregants derived from the same meiotic products, but no attempts were made to quantify this variation. It was clear,

nevertheless, that unusual growth rates were not associated with any specific non-Mendelian phenotypes.

F_1 segregants. Despite the genetic differences among all four F-1-k segregants (S, E, + ES), the cpDNAs of these segregants displayed almost identical patterns after digestion with *Ava*I (Figure 21) and after digestion with *Bst*EII (Figure 22). As shown in Table 17, the majority of the *Ava*I and *Bst*EII bands characteristic of the *C. moewusii* parent were found in the cpDNA restriction patterns of all four segregants. No bands characteristic of the *C. eugametos* parent were detected in any of the *Bst*EII patterns; however, a few bands characteristic of this parent were detected in all *Ava*I patterns. This latter observation indicates that the cpDNAs from all four segregants were recombinant for restriction sites.

B_1 segregants. Unlike F-1-k, each of the four B_1 BP meiotic products B-1-i, -j, -k, and -l gave rise to at least two segregants displaying strikingly dissimilar *Ava*I (Figures 23 and 24) or *Bst*EII (Figures 25 and 26) restriction patterns of cpDNA. Among the seven segregants with one or the other parental non-Mendelian phenotype (E or S), six showed *Ava*I and *Bst*EII patterns quite similar, but never identical to that of the parent having the same non-Mendelian phenotype. Of these, the cpDNA patterns from B-1-i(E), -j(S) and -j(E) appeared to be non-recombinant as no bands characteristic of the parent with the opposite non-Mendelian phenotype were detected, while the patterns from B-1-k(S), -k(E) and -l(E) revealed cpDNAs recombinant for restriction sites (Tables 18 and 19). The *Ava*I and *Bst*EII patterns from the seventh segregant B-1-i(S) also revealed cpDNA recombinant for restriction sites. In contrast to the patterns from B-1-k(S), -k(E) and -l(E), however,

most of the species-characteristic bands in the pattern from B-1-i(S) were from the parent having the opposite non-Mendelian phenotype. Finally, all six segregants with a non-parental non-Mendelian phenotype (+ or ES) displayed recombinant cpDNAs.

In summary, the F_1 and the four B_1 meiotic products heteroplasmic for resistance and sensitivity to streptomycin and for resistance and sensitivity to erythromycin were mixed for at least certain cpDNA sequences. The segregation of these sequences during mitotic division apparently occurred concurrently with the segregation of non-Mendelian phenotypes as all 17 analysed mitotic segregants were found homoplasmic for their non-Mendelian phenotype and homogeneous for their cpDNA. The cpDNAs of the segregants were homogeneous in composition because no heterogeneity could be detected in the cpDNA restriction patterns and because the estimates of cpDNA size were comparable to the parental values. Finally, restriction analysis of cpDNA revealed a much wider range of cpDNA sequences for the B_1 mitotic segregants than for the F_1 mitotic segregants. Indeed, both recombinant and non-recombinant cpDNA restriction patterns were observed among the B_1 segregants whereas only a few specific recombinant cpDNA restriction patterns were detected among the F_1 segregants.

F. Correlation Between the Inheritance of Non-Mendelian Genetic Markers and the Inheritance of Species-Characteristic CpDNA Restriction Fragments in Hybrid Progeny

To determine if the non-Mendelian genetic markers of *C. eugametos* and *C. moewusii* are located on cpDNA, a search was made for some correlation between the inheritance of these markers and the inheritance of

cpDNA in the overall hybrid progeny. A parallel inheritance of non-Mendelian genetic markers and characteristic cpDNA restriction fragments from one or the other parent was the kind of correlation being sought. Only eight of the 34 hybrids examined, however, showed both *Ava*I and *Bst*EII cpDNA restriction patterns with fragments characteristic of only one parent (i.e. non-recombinant cpDNA patterns): three of these hybrids, recovered from genetically homoplasmic products (B-1-a, -b, -c), revealed intact parental cpDNA restriction patterns, while the others recovered from genetically homoplasmic products (B-1-d, -g) and from homoplasmic segregants of heteroplasmic products [B-1-i(E), -j(S), -j(E)] revealed patterns slightly different from that of either parent. The characteristic cpDNA restriction fragments and non-Mendelian genetic markers inherited in each of these eight hybrids were of the same parental origin.

Even though the cpDNAs from most of the 34 hybrid progeny examined were recombinant for restriction sites, a strict correlation was found between the inheritance of the streptomycin sensitive marker of the *C. moewusii* parent and the inheritance of an *Ava*I band and a *Bst*EII band characteristic of this parent; the presence of these bands was always associated with the streptomycin sensitivity while their absence was always associated with streptomycin resistance. According to the system of band classification employed in Chapter I, the *C. moewusii* characteristic bands correlated with the streptomycin sensitivity are *Ava*I band number 9 (Figure 10) and *Bst*EII band number 17 (Figure 11). No correlation was noted between the inheritance of species-characteristic bands and the inheritance of streptomycin resistance, erythromycin resistance or erythromycin sensitivity.

DISCUSSION

A. CpDNA Location of *C. eugametos* and *C. moewusii* Non-Mendelian Genetic Markers

The results presented in this study are entirely consistent with the hypothesis that the non-Mendelian genetic markers of *C. eugametos* (*sr*-2) and *C. moewusii* (*er-nM*1) are located on cpDNA. There are three lines of evidence which support this hypothesis. First, in each case where non-recombinant cpDNA restriction patterns were observed in the hybrid progeny, the characteristic cpDNA restriction fragments and the non-Mendelian genetic markers were inherited from the same parent. Second, meiotic products which inherited non-Mendelian genetic markers from both parents were always mixed for certain parental cpDNA sequences; moreover, these genetic markers and cpDNA sequences segregated concomitantly during the mitotic growth of biparental products. Third, and perhaps most importantly, the inheritance of particular *Ava*I and *Bst*II restriction fragments characteristic of the *C. moewusii* parent was always associated with the inheritance of the streptomycin sensitive marker from that parent.

It should be pointed out that the non-Mendelian genetic markers conferring resistance and sensitivity to a given antibiotic in the *C. eugametos* and *C. moewusii* parents of interspecific crosses are *a priori* not necessarily allelic. This is, however, hardly credible as the chloroplast ribosomal proteins which are thought to be affected by non-Mendelian antibiotic resistance mutations (Gillham et al. 1976) are probably conserved in the closely related algae *C. eugametos* and *C. moewusii*.

The failure to establish a correlation between the inheritance of erythromycin resistance or sensitivity and the inheritance of a parental cpDNA restriction fragment may be due to the location of these non-Mendelian genetic markers on fragments common to *C. eugametos* and *C. moewusii*. Alternatively, correlations between the inheritance of these genetic markers and that of species-characteristic cpDNA fragments may have been disrupted by recombination. Nevertheless, there is still little doubt about the cpDNA location of these markers as that for erythromycin resistance (*er-nM1*) appeared to be genetically linked to that for streptomycin resistance (*sr*-2) in crosses that involved these markers in repulsion. Among the progeny of biparental hybrid meiotic products, the parental non-Mendelian phenotypes were observed much more frequently than the non-parental non-Mendelian phenotypes. Similar observations in intraspecific crosses with *C. reinhardtii* that involved the non-Mendelian streptomycin resistant and neamine resistant markers in repulsion represented the first evidence for the linkage of non-Mendelian genes in this alga (Gillham 1965).

Hereafter it will be assumed that the non-Mendelian genetic markers of *C. eugametos* and *C. moewusii* are encoded in cpDNA, even if a mtDNA location of these markers has not been completely ruled out. Such a mtDNA location is unlikely in view of the fact that all 34 hybrids examined showed *C. moewusii* characteristic cpDNA restriction fragments associated with the *C. moewusii* streptomycin sensitive marker. It seems improbable that all these hybrids, many of which revealed extensive cpDNA recombination, would have maintained parental combinations of certain cpDNA and mtDNA sequences. Nevertheless, data of the kind reported here cannot be conclusive unless the parental mtDNAs can be

identified, distinguished and shown to be transmitted independently of the cpDNA.

It seems evident that the *C. eugametos*-*C.moewusii* hybrid system will be useful in locating chloroplast genetic markers on *C. eugametos* and *C. moewusii* cpDNA restriction maps and thus in comparing the relative positions of chloroplast genetic markers on both physical and genetic maps. As shown here for the *C. moewusii* streptomycin sensitive marker, other chloroplast genetic markers can be associated with species-characteristic restriction fragments if they are not localized on fragments common to both parents. The construction of *C. eugametos* and *C. moewusii* cpDNA restriction maps will demand much effort as there are many fragments generated by the restriction enzymes which reveal the most distinguishing features between the cpDNAs of these algae. Nevertheless, once the restriction fragments are ordered in one species, it is likely that the construction of the other species restriction map will be facilitated by identification of the homologous cpDNA restriction fragments in both species.

Finally, it should be noted that because *C. eugametos* and *C. moewusii* are obligate phototrophs (Lewin 1950; Wetherell 1958), the isolation of chloroplast mutations in these species is unfortunately limited to those not permanently impaired in photosynthetic activity (e.g. conditional non-photosynthetic and drug resistance mutations). *C. reinhardtii*, in contrast, is capable of heterotrophic growth in the dark with acetate as the carbon source and thus non-photosynthetic chloroplast mutations can be readily recovered in this species (see Gillham 1978).

B. Selection of Specific Recombinant CpDNA Sequences for Nucleo-Chloroplastic Compatibilities

CpDNAs from several of the *C. eugametos-C. moewusii* hybrids proved to be recombinant for restriction sites. This physical evidence for cpDNA recombination is not surprising in light of genetic evidence indicating the recombination of chloroplast markers in crosses; however, the high proportion of F_1 hybrid progeny relative to the B_1 hybrid progeny showing recombinant cpDNAs was unexpected. All randomly selected F_1 hybrids revealed recombinant cpDNA restriction patterns, whereas the majority of randomly selected B_1 hybrids revealed cpDNA restriction patterns identical or highly similar to that of the *mt+* parent, (i.e. non-recombinant cpDNA patterns). Furthermore, the F_1 progeny compared to the B_1 progeny with recombinant cpDNAs, on the average, inherited a much higher proportion of fragments characteristic of the *mt-* parent.

Assuming that cpDNA molecules are transmitted primarily by the *mt+* parent in both F_1 and B_1 generations, the exclusive recovery of F_1 progeny with recombinant cpDNAs suggests that incompatibilities between parental chloroplast genomes and hybrid nuclei can be reduced or eliminated in F_1 progeny with specific recombinant cpDNAs. Perhaps once a recombinant chloroplast genome compatible with a combination of *C. eugametos* and *C. moewusii* nuclear genes has been established, further modifications are less critical to survival in the subsequent backcross generations. The three- to four-fold difference in survival noted for reciprocal *C. eugametos* X *C. moewusii* crosses (Cain 1979) supports the hypothesis that nucleo-chloroplastic incompatibilities are an important source of lethality in these crosses.

The inheritance of recombinant cpDNA molecules in randomly se-

lected F_1 hybrids may appear inconsistent with the simultaneous uniparental inheritance of one or two chloroplast genetic markers from the *mt+* parent. It is possible, however, that these markers are by chance localized on *mt+* parental cpDNA sequences which are present in all recombinant cpDNA molecules. This is consistent with the fact that several cpDNA restriction fragments characteristic of the *mt+* parent are common to the restriction patterns of all F_1 hybrid cpDNAs examined, while many other fragments are common to both parents. The inheritance pattern of the common fragments cannot be determined by restriction analysis; thus, the possibility exists that at least some of these fragments are also transmitted uniparentally. The uniparental inheritance of particular *mt+* parental cpDNA sequences may result from selection against progeny with the homologous *mt-* cpDNA sequences. The disadvantage conferred to the progeny inheriting these *C. moewusii* sequences may decrease in backcrosses to *C. moewusii* where the proportion of *C. moewusii* nuclear genes increases in the progeny. Thus, an increased inheritance of the streptomycin sensitive and erythromycin resistant markers from the *mt-* parent, as was observed, may be expected.

The cpDNA restriction patterns observed for the homoplasmic mitotic segregants derived from F_1 and B_1 biparental hybrid products are consistent with the idea that specific recombinant cpDNA sequences are required in F_1 progeny for nucleo-chloroplastic compatibilities but that recombinant cpDNA sequences are less critical for survival of the B_1 progeny. The four F_1 segregants with parental or non-parental non-Mendelian phenotypes revealed recombinant cpDNAs sharing the same basic fragmentation pattern, while the 13 B_1 segregants analysed showed a wider range of cpDNA sequences, including cpDNA sequences highly similar

to the parental ones (i.e. non-recombinant cpDNAs). Nevertheless, the differences observed in the growth rate of B_1 segregants derived from a given biparental product and containing different proportions of *C. eugametos* and *C. moewusii* cpDNA sequences suggest that nucleo-chloroplastic incompatibilities are not completely eliminated in the B_1 progeny.

The *C. eugametos*-*C. moewusii* hybrid system appears to offer a unique opportunity for the study of nucleo-chloroplastic interactions since one can readily obtain progeny which are recombinant for both chloroplast and nuclear genes of two species. It would be interesting, for example, to examine macromolecules with chloroplast and nuclear coded subunits (e.g. ribulose-1,5-bisphosphate carboxylase and chloroplast ribosomes) to see if certain subunit combinations in hybrid progeny are never observed and hence a possible source of lethality.

C. Inconclusive Results with Regard to the Segregation Mechanism of the Chloroplast Genomes

The restriction analysis of cpDNA from homoplasmic mitotic segregants derived from biparental hybrid meiotic products did not provide evidence for, or against, any of the proposed mechanisms for the segregation of chloroplast genomes in *Chlamydomonas*. Because nucleo-chloroplastic incompatibilities and selection of recombinant cpDNAs appear to prevail in interspecific crosses, the frequent observation of recombinant cpDNAs in the hybrid progeny does not necessarily indicate that the parental chloroplast genomes recombine extensively in the zygotes and/or their meiotic progeny. Hence, the detection of a high proportion of mitotic segregants with recombinant cpDNAs provides apparent but unconvincing evidence for segregation mechanisms such as those proposed by Sager and

Ramanis (1976a), Gillham et al. (1974) and Birky (1978), which require the recombination of chloroplast genomes. Despite possible nucleo-chloroplastic incompatibilities, the finding of cpDNAs highly similar to those of both parents (i.e. non-recombinant cpDNAs) in some B_1 segregants may be consistent with the segregation mechanism of chloroplast genomes proposed by VanWinkle-Swift (1980).

Nevertheless, the *C. eugametos-C. moewusii* hybrid system could be useful for investigating the segregation mechanism of chloroplast genomes during vegetative cell division. The observation of an increasing survival of the progeny with successive hybrid backcrosses to *C. moewusii* seems to indicate increasing nucleo-chloroplastic compatibilities. Hence, it is conceivable that there are hybrid backcrosses in which the selection of specific cpDNA sequences may not be necessary for survival of the progeny. Hopefully, the parents involved in such backcrosses would retain enough differences in their cpDNA fragmentation patterns to enable one to determine if recombination between parental cpDNA sequences is required for the production of homoplasmic progeny during vegetative division of heteroplasmic meiotic products.

D. Comparison of this Study with Other Studies on the Inheritance of Organelle DNA in Interspecific Hybrids

The results reported here provide the first physical evidence for recombination of cpDNA. Similar evidence for mtDNA recombination in interspecific sexual crosses with *Saccharomyces* (Fonty et al. 1978) and in interspecific parasexual crosses with *Nicotiana* (Belliard et al. 1979) has been reported and, as observed for cpDNA in this study, progeny with recombinant mtDNAs were recovered very frequently. The results presented

here also provide the first evidence for cpDNA segregation among the mitotic progeny of individual cells initially mixed for certain parental cpDNA sequences. The segregation of mtDNA sequences has been similarly demonstrated in *Saccharomyces* hybrids (Fonty et al. 1978). Belliard et al. (1978) have shown that parental cpDNAs were recovered from different cell lineages of *Nicotiana* parasexual hybrids initially mixed for physically distinguishable parental cpDNA sequences, however, they did not demonstrate the segregation of opposite parental cpDNA sequences from the same cell lineage.

The observation of recombinant cpDNA in the F_1 progeny examined here is in contrast to the uniparental transmission of *Hae*III cpDNA restriction sites from the *mt+* parent in reciprocal *C. eugametos* X *C. moewusii* crosses as reported by Mets (1979). These differences might be explained by the fact that the *Hae*III endonuclease reveals fewer distinguishing features between the two parental cpDNAs than is the case for the *Ava*I and *Bst*EII enzymes employed here. Even enzymes producing similar numbers of distinguishing features can differ in their ability to detect changes. As shown here, *Bst*EII has proven more sensitive than *Ava*I in the detection of recombinant cpDNA patterns. Hence, existing recombination may have been missed in the study by Mets (1979). Alternatively, the nuclear make-up of the hybrid meiotic products which Mets recovered from rare complete or nearly complete tetrads may have been atypical in a way which reduced the selection pressures proposed to explain the recovery of recombinant cpDNAs detected in progeny of the more common hybrid zygotes, i.e. those yielding only one viable product.

To date the inheritance of chloroplast genetic markers and cpDNA

restriction sites has not been examined extensively in interspecific crosses when *C. moewusii* is the *mt*+ parent. However, it is known that the inheritance patterns of chloroplast genetic markers differ dramatically for the two intraspecific crosses, being predominantly uniparental through the *mt*+ parent in crosses with *C. eugametos* (McBride and McBride 1975; and Appendix) and predominantly biparental in crosses with *C. moewusii* (Appendix). Thus, a continuation of the types of analyses reported here, to include the reciprocal *C. moewusii mt*+ X *C. eugametos mt*- cross, will be necessary for a fuller understanding of the factors favouring the recovery of recombinant cpDNA molecules from interspecific hybrid progeny.

APPENDIX

BIPARENTAL TRANSMISSION OF NON-MENDELIAN GENES IN INTRASPECIFIC CROSSES WITH *CHLAMYDOMONAS MOEWUSII*

When this investigation was undertaken, non-Mendelian genetic markers were available in *C. eugametos* (McBride and McBride 1975) but not in *C. moewusii*. In order to follow the transmission of such markers as well as that of cpDNAs in interspecific crosses between *C. eugametos* and *C. moewusii*, it was necessary to recover non-Mendelian mutants in *C. moewusii*. As reported here, two non-Mendelian mutants were identified in *C. moewusii*: one resistant to streptomycin (*sr-nM1*) and the other resistant to erythromycin (*er-nM1*). In intraspecific crosses involving each of these mutants and the wild-type strain, the great majority of the zygotes transmitted both the mutant and the wild-type alleles to the meiotic progeny. This biparental transmission pattern of non-Mendelian genes contrasts with the uniparental transmission pattern reported for the non-Mendelian genes of *C. reinhardtii* (see Gillham 1978) and *C. eugametos* (McBride and McBride 1975).

MATERIALS AND METHODS

A. Strains and Culture Conditions

The strains and culture conditions employed in this study are described in the Materials and Methods of Chapter II.

B. Recovery of Antibiotic Resistant Mutants

For the recovery of streptomycin resistant mutants, *mt+* wild-type cells of *C. moewusii* were mutagenized by growth in liquid minimal medium containing 10 µg/ml 2-amino-3-phenyl butanoic acid (APBA), as described by McBride and McBride (1975). Aliquots of 10^6 cells were then plated on minimal agar medium supplemented with 12.5 µg/ml strep-

tomycin (from streptomycin sulfate, Pfizer) and incubated at 20°C under 400 μE/m^{2}s PAR from cool white fluorescent tubes.

For the recovery of erythromycin resistant mutants, *mt+* wild-type cells of *C. moewusii* were mutagenized with methyl methanesulfonate (MMS) by a modification of the method described by Hawks and Lee (1976). Aliquots of cells were removed from synchronous cultures at the onset of the light period, harvested by centrifugation at 1000 x g for 10 minutes at 25°C and resuspended in 30 mM phosphate buffer pH 6.8 at a final concentration of 10^6 cells per ml. The cell suspension was made 30 mM for MMS and then incubated for 30 minutes at 25°C in darkness. Under these conditions, about 50% of the cells survived. Mutagenized cells were washed once with minimal medium, resuspended in this medium to a final concentration of approximately 2 x 10^5 cells per ml and 1.0-ml aliquots were delivered to each of several tubes. The tubes were kept at 25°C in an incubator flushed with 3% CO_2 in air and illuminated continuously under 1000 μE/m^{2}s PAR from cool white fluorescent tubes. After 48 hour incubation during which cell number increased about 40-fold, the content of each tube was plated on minimal agar medium containing 25 μg/ml erythromycin (from erythromycin lactobionate, Abbott) and incubated at 20°C under 400 μE/m^{2}s PAR from cool white fluorescent tubes.

Streptomycin resistant and erythromycin resistant colonies were recovered after two weeks of culture on the respective antibiotic media. The resistance level of the mutant strains so recovered was determined on minimal agar media containing increasing concentrations of streptomycin or erythromycin.

C. Procedure for Crosses

The procedure for intraspecific crosses with *C. moewusii* was the same as that employed for interspecific crosses between *C. eugametos* and *C. moewusii* and is described in the Materials and Methods of Chapter II. Sex of meiotic products was also determined as described in the Materials and Methods of Chapter II.

D. Genetic Analysis

To establish whether the antibiotic resistance mutations are inherited in a Mendelian or a non-Mendelian fashion in tetrads, crosses were made between *mt*+ streptomycin or erythromycin resistant mutants and the *mt*- wild-type strain. The inheritance of each resistance allele was scored after meiotic products had undergone about 20 mitotic divisions on non-selective medium (primary clone). An aliquot of each primary clone containing 10^4-10^5 cells was plated on streptomycin or on erythromycin agar medium for the detection of resistant cells. Mendelian inheritance was revealed by 2:2 segregation of resistance and sensitivity in each tetrad and non-Mendelian inheritance by a consistent departure from this pattern of segregation.

In crosses involving a non-Mendelian antibiotic resistant mutant and a wild-type strain, meiotic products that give rise to resistant mitotic progeny may also segregate sensitive progeny but these are rarely identified with the technique described above. Detection of a mixture of resistant and sensitive cells usually requires the testing of subclones for antibiotic resistance. This method was employed to analyse meiotic products from crosses between the *mt*+ wild-type strain and *mt*- strains with non-Mendelian streptomycin or erythromycin resistance mutations.

After meiotic products had undergone about 20 mitotic divisions on non-selective medium (primary clone), 50 subclones from each primary clone containing resistant cells were tested for growth on streptomycin or erythromycin agar medium. A meiotic product was considered homoplasmic for the resistance allele when all subclones grew on the antibiotic medium. Alternatively, a meiotic product was considered heteroplasmic for the resistance and sensitivity alleles when some subclones grew and some did not.

RESULTS AND DISCUSSION

As shown in Table 20, the resistance levels of the streptomycin and the erythromycin resistant mutants isolated during this study are significantly higher than the wild-type resistance level of 5 µg/ml for both streptomycin and erythromycin. The streptomycin resistant mutants were recovered with an incidence of $1/10^5$ viable cells plated on agar medium after mutagenesis with APBA. The majority of these mutants (90%) showed a resistance level of 50 µg/ml and rare mutants (10%) showed a resistance level of 250 µg/ml. A mutant representing each of the two resistance classes was crossed to the *mt-* wild-type strain and the inheritance of the resistance mutations was scored in tetrads. Table 20 shows that the mutation conferring low level resistance (*sr*-50) was inherited in a Mendelian fashion while the mutation conferring high level resistance (*sr-nM*1) was inherited in a non-Mendelian fashion. On the basis of this limited analysis, it appears, as reported for non-mutagenized *C. reinhardtii* (Sager 1954) and APBA mutagenized *C. eugametos* (McBride and McBride 1975), that non-Mendelian streptomycin resistant mutants are recovered with a

lower incidence and display a higher resistance level than Mendelian mutants.

Attempts to isolate erythromycin resistant mutants after APBA mutagenesis were unsuccessful. After mutagenesis with MMS, however, four independent mutants were recovered from 7×10^6 mutagenized cells. As shown in Table 20, only the *er-nM1* mutant proved non-Mendelian. Thus, as observed for the streptomycin resistant mutants, the incidence of Mendelian mutants was higher than that of non-Mendelian mutants and like the non-Mendelian erythromycin resistant mutants of *C. reinhardtii* (Davidson et al. 1978), those of *C. moewusii* could not be distinguished from the Mendelian mutants by their resistance level alone.

When the *mt+ sr-nM1* and *er-nM1* mutants were crossed to a *mt-* wild-type strain, the respective resistance alleles were transmitted to all four meiotic products in each tetrad analysed; however, some products were obviously heteroplasmic for the resistance and sensitivity alleles. Such heteroplasmic products were revealed by the appearance of resistant cell clusters within a background of dead cells upon transfer of the primary clones from non-selective medium to antibiotic medium.

The segregation pattern of the non-Mendelian resistance and sensitivity alleles was further examined in tetrads obtained from the reciprocal crosses in which the *sr-nM1* and the *er-nM1* mutations were associated with the *mt-* parents. Table 21 shows that all 30 zygotes recovered from the two crosses, with one exception, were biparental as both resistance and sensitivity alleles were inherited among the meiotic progeny. Most meiotic products (99/120) were themselves biparental, i.e. heteroplasmic for the parental resistance and sensitivity alleles. Indeed, the most frequent tetrad class was one in which the four meiotic

products were heteroplasmic. Among the homoplasmic products (21/120) that were detected, all but one expressed the sensitivity allele of the *mt+* parent. As shown in Table 22, an analysis of the frequency of resistant cells in the primary clones derived from heteroplasmic meiotic products also revealed a biased output in favour of the sensitivity allele of the *mt+* parent.

The predominantly biparental transmission of non-Mendelian genes observed here in crosses with *C. moewusii* contrasts with the transmission pattern of similar genes in crosses with *C. reinhardtii*. In this species, the *mt+* parent transmits its non-Mendelian genes to all four of the meiotic products in 90% or more of all zygotes; non-Mendelian genes are transmitted from the *mt-* parent in up to 10% of the zygotes (see Gillham 1978). Two kinds of the latter zygotes have been found: those that transmit non-Mendelian genes from both parents (biparental zygotes) and the rarer zygotes that transmit non-Mendelian genes only from the *mt-* parent. It is interesting to note that the sole non-biparental zygote observed here in crosses with *C. moewusii* transmitted the non-Mendelian genetic marker only from the *mt+* parent. If a larger sample of zygotes had been analysed, it is probable that zygotes transmitting the non-Mendelian genetic marker only from the *mt-* parent also would have been detected.

There is a discrepancy between the laboratory of Sager and that of Gillham and Boynton concerning the frequency of non-Mendelian gene segregation during the meiotic division of biparental zygotes in *C. reinhardtii*. Gillham (1963) has observed a high frequency of non-Mendelian gene segregation during meiosis, whereas Sager and Ramanis (1963) found none. In this study with *C. moewusii*, the segregation of non-Mendelian alleles during meiosis was observed, but less frequently

than reported by Gillham.

A second discrepancy between these two laboratories concerns the ratio of cells homoplasmic for a given pair of alleles among the post-meiotic mitotic progeny of biparental zygotes (allelic ratio). Sager and Ramanis (1968, 1976a; see also Sager 1972) have consistently reported an allelic ratio of 1:1 for individual non-Mendelian genes, whereas Gillham, Boynton and colleagues (Gillham 1963; Gillham et al. 1974) have observed marked deviations from this ratio. As reported here in *C. moewusii*, Gillham, Boynton and colleagues found that biparental zygotes segregated progeny expressing mainly the alleles derived from the *mt+* parent.

The predominantly biparental transmission of non-Mendelian genes in crosses with *C. moewusii* also contrasts sharply with the exclusively uniparental transmission of similar genes in crosses with the closely related alga *C. eugametos*. McBride and McBride (1975) have shown that a non-Mendelian mutation conferring resistance to streptomycin (*sr-2*) and another conferring dependence on neamine (*nd*) were transmitted to all four meiotic products by the *mt+* parent. In a sample of 3000 zygotes, none was found to transmit the non-Mendelian mutations from the *mt-* parent.

Variations in the conditions used for crosses have been shown to affect the non-Mendelian gene transmission pattern of *C. reinhardtii* (Sears 1980a, b; Sears et al. 1980). For this reason, differences in the procedures employed for intraspecific crosses with *C. reinhardtii*, *C. moewusii* and *C. eugametos* may, at least partially, explain the distinctive non-Mendelian gene transmission patterns revealed by these species. Indeed, during this study, crosses were performed with *C. eugametos* using the procedure employed for crosses with *C. moewusii*

and it was found that non-Mendelian genes were not transmitted exclusively by the *mt+* parent as reported by McBride and McBride. In crosses between the *mt+* wild-type strain of *C. eugametos* and a *mt-* strain with the same *sr-2* mutation used by McBride and McBride, 20% of the zygotes recovered (56/287) transmitted the *sr-2* allele to one or more meiotic products. This proves that genetic differences must also contribute to the distinctive *C. eugametos* and *C. moewusii* non-Mendelian gene transmission patterns as intraspecific crosses with both species were preformed under the same conditions.

TABLE 1. Total DNA and β-DNA contents of cell lysates prepared from *C. reinhardtii*, *C. eugametos* and *C. moewusii*. Two independent determinations of these values were made for each species. The proportion of total DNA in each β-fraction is also given.

	Total DNA ($\mu g/10^9$ cells)	β-DNA ($\mu g/10^9$ cells)	% β-DNA
C. reinhardtii	197	26	13.1
	183	20	10.9
C. eugametos	218	29	13.2
	252	38	15.3
C. moewusii	195	23	11.8
	234	30	12.9

TABLE 2. Molecular weight of fragments detected after 0.75% agarose gel electrophoresis of *Eco*RI digests of β-DNA from *C. eugametos* and *C. moewusii*. When two or more comigrating fragments are found in a band, the fragment number is indicated in parentheses. Estimates of β-DNA size, based on the summation of molecular weights of all fragments, are also given. Fragments of identical mobility in the electrophoretic patterns of *C. eugametos* and *C. moewusii* are indicated by +.

Band No.	Molecular Weight (megadaltons)					
	C. eugametos			*C. moewusii*		
1	12.0	(2)	+	12.0		+
2	9.1	(2)		9.0	(3)	
3	7.5	(2)		7.9		
4	6.7	(2)		7.4		
5	6.3			6.9	(2)	
6	5.9			6.0	(2)	
7	5.3		+	5.4		
8	4.8			5.3		+
9	4.7	(2)		5.0		
10	4.5			4.7	(3)	
11	4.0	(2)		4.5		
12	3.6		+	4.2		
13	3.4			3.8		
14	2.7	(2)		3.7		
15	2.4	(2)		3.6		+
16	2.1			3.3	(2)	
17	1.8			2.6		
18	1.6			2.4		
19	1.5			2.1		
20	1.4			1.85		
21	1.35	(2)		1.75		
22	1.25			1.50		
23	1.20			1.45	(2)	
24	1.05	(2)	+	1.40		
25	1.00			1.30		
26	0.93	(2)	+	1.20		
27	0.88			1.05	(2)	+
28	0.80	(2)		0.97		
29	0.66		+	0.93		+
30	0.50			0.87		
31				0.66		+
32				0.49		
β-DNA Size (megadaltons)	154.15			161.32		

TABLE 3. Molecular weight of fragments detected after 0.75% agarose gel electrophoresis of *Ava*I digests of β-DNA from *C. eugametos* and *C. moewusii*. When two or more comigrating fragments are found in a band, the fragment number is indicated in parentheses. Estimates of β-DNA size, based on the summation of molecular weights of all fragments, are also given. Fragments of identical mobility in the electrophoretic patterns of *C. eugametos* and *C. moewusii* are indicated by +.

Band No.	Molecular Weight (megadaltons)				
	C. eugametos			*C. moewusii*	
1	20.0		+	20.0	+
2	9.6	(2)	+	9.6	(2) +
3	7.4		+	8.3	
4	7.2		+	7.4	+
5	7.0			7.2	+
6	5.9			5.25	(2) +
7	5.35			4.80	(2)
8	5.25			4.30	(2)
9	4.90			4.10	
10	4.20			3.70	+
11	3.80			3.65	
12	3.70	(2)	+	3.60	+
13	3.60		+	3.15	(2)
14	3.10			2.80	
15	2.95			2.75	
16	2.70			2.40	+
17	2.60			2.25	+
18	2.40		+	2.15	(2)
19	2.25		+	1.90	(2)
20	2.20	(2)		1.80	
21	2.05	(2)		1.65	+
22	1.95	(2)		1.55	(2)
23	1.85			1.45	+
24	1.75	(3)		1.25	+
25	1.65		+	1.20	(3) +
26	1.45		+	1.10	+
27	1.37			1.07	+
28	1.33			0.83	(3)
29	1.25		+	0.68	(2) +
30	1.20	(3)	+	0.60	(2) +
31	1.15	(2)		0.40	+
32	1.10		+		
33	1.07		+		
34	1.00				
35	0.86	(2)			
36	0.68	(2)	+		
37	0.60		+		
38	0.45				
39	0.40		+		
β-DNA Size (megadaltons)	157.35			150.92	

TABLE 4. Molecular weight of fragments detected after 0.75% agarose gel electrophoresis of *Bst*EII digests of β-DNA from *C. eugametos* and *C. moewusii*. When two or more comigrating fragments are found in a band, the fragment number is indicated in parentheses. Estimates of β-DNA size, based on the summation of molecular weights of all fragments, are also given. Fragments of identical mobility in the electrophoretic patterns of *C. eugametos* and *C. moewusii* are indicated by +.

Band No.	Molecular Weight (megadaltons)					
	C. eugametos			*C. moewusii*		
1	17.0		+	17.0		+
2	14.0			13.0		
3	9.3	(2)	+	9.3		+
4	8.2		+	8.2		+
5	7.9			7.6		
6	7.3	(2)	+	7.3	(2)	+
7	5.2	(2)		5.8		
8	4.4			5.6		
9	3.5		+	4.1	(2)	
10	3.1	(2)		3.5		+
11	2.75	(2)		3.4		
12	2.70	(2)		3.3		
13	2.35		+	3.2		
14	2.13			2.73	(2)	
15	2.05			2.65		
16	1.95	(2)	+	2.35		+
17	1.85	(2)		2.25	(2)	
18	1.65			2.10	(3)	
19	1.40		+	1.95	(2)	+
20	1.35	(2)	+	1.80	(3)	
21	1.28			1.75		
22	1.13			1.55	(2)	
23	1.04			1.40		+
24	0.95			1.35		+
25	0.75		+	1.25		
26	0.72		+	1.20		
27	0.67			0.98	(2)	
28				0.91	(2)	
29				0.87	(2)	
30				0.75		+
31				0.72		+
32				0.59		
33				0.55		
β-DNA Size (megadaltons)	142.12			151.44		

TABLE 5. Molecular weight of fragments detected after 0.75% agarose gel electrophoresis of *Sma*I digests of β-DNA from *C. eugametos* and *C. moewusii*. When two or more comigrating fragments are found in a band, the fragment number is indicated in parentheses. Estimates of β-DNA size, based on the summation of molecular weights of all fragments, are also given. Fragments of identical mobility in the electrophoretic patterns of *C. eugametos* and *C. moewusii* are indicated by +.

Band No.	Molecular Weight (megadaltons)					
	C. eugametos			*C. moewusii*		
1	24.0		+	24.0		+
2	19.0		+	19.0		+
3	15.9		+	15.9		+
4	14.5	(2)	+	14.5	(2)	+
5	9.5			9.2		+
6	9.2		+	8.8		
7	7.5	(2)		7.3	(2)	
8	6.9	(2)		4.7		
9	5.1	(2)		4.2	(2)	+
10	4.2		+	2.40		+
11	4.0			1.00		+
12	2.70	(2)				
13	2.40		+			
14	1.00		+			
β-DNA Size (megadaltons)	162.6			137.0		

TABLE 6. Summary of the *C. eugametos* and *C. moewusii* β-DNA sizes (from Tables 2 to 5). The size values were estimated from the *Eco*RI, *Ava*I, *Bst*EII and *Sma*I β-DNA restriction patterns by summing the molecular weight of all fragments present in each of these patterns. An average value of β-DNA size is given for each species.

Endonuclease	β-DNA Size (megadaltons)	
	C. eugametos	*C. moewusii*
*Eco*RI	154	161
*Ava*I	157	151
*Bst*EII	142	151
*Sma*I	163	137
Average Value	154	150

TABLE 7. Molecular weight, cellular content and copy number per cell of β-DNA from *C. reinhardtii*, *C. eugametos* and *C. moewusii*. The molecular weight of *C. reinhardtii* β-DNA is based on the contour length of circular molecules as reported by Behn and Herrmann (1977) while those of *C. eugametos* and *C. moewusii* β-DNAs each represent an average value of the molecular weights estimated from the *Eco*RI, *Ava*I, *Bst*EII and *Sma*I β-DNA restriction patterns (see Table 6). The values of β-DNA cellular content were determined by averaging the data from Table 1.

	Molecular Weight (megadaltons)	Content per Cell (grams)	Copies per Cell
C. reinhardtii	134	23×10^{-15}	103
C. eugametos	154	34×10^{-15}	133
C. moewusii	150	26×10^{-15}	92

TABLE 8. Expected and observed number of fragments obtained after digestion of the *C. eugametos* and *C. moewusii* cpDNAs with various restriction endonucleases. The expected number of fragments was calculated by assuming a random sequence of bases for the *C. eugametos* and *C. moewusii* cpDNAs, each having 230 x 10^3 base-pairs and a G+C content of 41%. The observed number of fragments was taken from Tables 2 to 5.

Endonuclease	Recognition Sequence	Number Expected	Number Observed	
			C. eugametos	*C. moewusii*
*Ava*I	CPyCGPuG	102	51	45
*Bst*EII	GGTNACC	35	36	46
*Sma*I	CCCGGG	17	19	14

TABLE 9. List of crosses performed in this study. Hybrid F-1-a was recovered from cross 1 while hybrid B-1-b was recovered from cross 3.

Cross No.	*mt*+ Parent		*mt*- Parent
	Interspecific Crosses		
1	*C. eugametos* (*sr*-2)	X	*C. moewusii* (*pab*-1)
2	*C. eugametos* (*sr*-2)	X	*C. moewusii* (*er-nM*1)
	First Generation Hybrid Backcrosses		
3	F-1-a (*sr*-2 *pab*-1)	X	*C. moewusii*
4	F-1-a (*sr*-2 *pab*-1)	X	*C. moewusii* (*er-nM*1)
	Second Generation Hybrid Backcross		
5	B-1-b (*sr*-2)	X	*C. moewusii* (*er-nM*1 *nic*-1)

TABLE 10. Viability and inheritance of non-Mendelian genetic markers (*sr*-2 and *er-nM*1) among meiotic products recovered from an interspecific cross and from first and second generation backcrosses. The number of surviving products for each class of non-Mendelian inheritance pattern is given in parentheses. *Pab*-1, *nic*-1 and *mt* are Mendelian genetic markers. Abbreviations: UP+ = uniparental for the non-Mendelian erythromycin sensitive marker of the *mt*+ parent; UP- = uniparental for the non-Mendelian streptomycin sensitive marker of the *mt*- parent; BP = biparental for the non-Mendelian resistance markers of the *mt*+ and *mt*- parents.

Cross	Viable Products per Tetrad (No. of Tetrads)					Surviving Products	Non-Mendelian Inheritance Patterns (Frequency)		
	4	3	2	1	0		UP+	BP	UP-
A. Interspecific Cross.									
C. eugametos (*sr*-2 *mt*+) X *C. moewusii* (*er-nM*1 *mt*-)	0	0	3	38	81	9%	0.96(42)	0.02(1)	0.02(1)
B. First Generation Hybrid Backcross.									
F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*er-nM*1 *mt*-)	0	2	19	92	65	19%	0.93(127)	0.06(8)	0.01(1)
C. Second Generation Hybrid Backcross									
B-1-b (*sr*-2 *mt*+) X *C. moewusii* (*er-nM*1 *nic*-1 *mt*-)	1	6	13	5	0	53%	0.83(44)	0.17(9)	0

TABLE 11. Non-Mendelian phenotypes (parental and non-parental) of subclones derived from F_1 and B_1 biparental hybrid meiotic products. F-1-k was recovered from the cross *C. eugametos* (*sr*-2 *mt*+) X *C. moewusii* (*cr*-*nM*1 *mt*-) while B-1(-i through -l) were recovered from the cross F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*cr*-*nM*1 *mt*-). One hundred subclones derived from each biparental product were analysed except for F-1-k where 200 subclones were tested. Abbreviations: S = resistant to streptomycin and sensitive to erythromycin; E = resistant to erythromycin and sensitive to streptomycin; + = sensitive to streptomycin and to erythromycin; ES = resistant to streptomycin, to erythromycin and to a mixture of streptomycin and erythromycin; H = resistant to streptomycin and to erythromycin but sensitive to a mixture of streptomycin and erythromycin.

Biparental Product	Frequency of Subclone Phenotypes				
	Parental		Non-Parental		
	S	E	+	ES	H
F-1-k	0.945	0.010	0.040	0.005	0
B-1-i	0.76	0.01	0.20	0.02	0.01
B-1-j	0.30	0.58	0.01	0.02	0.09
B-1-k	0.91	0.01	0.08	0	0
B-1-l	0	0.99	0.01	0	0

TABLE 12. Classification of bands and fragments detected after agarose gel electrophoresis of *Ava*I digests of cpDNA from subclones of ten F_1 hybrid meiotic products (F-1-a through -j) and from the *C. eugametos* (C_e) and *C. moewusii* (C_m) parental strains. Estimates of cpDNA size are also given. The genetic backgrounds of the F_1 hybrid subclones are indicated in the legend to Figure 16. Abbreviations: C_e and C_m bands or fragments = characteristic of the *C. eugametos* and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
	C_e	C_m	Com.	New	Total	C_e	C_m	Com.	New	Total	
C_e	17	0	13	0	30	22	0	17	0	39	147
F-1-a	11	2	13	0	26	15	3	18	1	37	142
F-1-b	11	2	13	0	26	15	3	18	1	37	142
F-1-c	12	2	13	0	27	16	3	18	0	37	140
F-1-d	10	3	13	0	26	14	4	18	1	37	145
F-1-e	8	4	13	0	25	12	6	19	0	37	145
F-1-f	8	4	13	1	26	12	5	19	1	37	143
F-1-g	12	2	13	0	27	16	2	18	1	37	143
F-1-h	12	2	13	0	27	16	2	18	1	37	143
F-1-i	7	6	12	0	25	11	8	17	0	36	144
F-1-j	9	4	13	0	26	13	5	19	0	37	145
C_m	0	12	13	0	25	0	18	17	0	35	144

TABLE 13. Classification of bands and fragments detected after agarose gel electrophoresis of *Bst*EII digests of cpDNA from subclones of ten F_1 hybrid meiotic products (F-1-a through -j) and from the *C. eugametos* (C_e) and *C. moewusii* (C_m) parental strains. Estimates of cpDNA size are also given. The genetic backbrounds of the F_1 hybrid subclones are indicated in the legend of Figure 17. Abbreviations: C_e and C_m bands or fragments = characteristic of the *C. eugametos* and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
	C_e	C_m	Com.	New	Total	C_e	C_m	Com.	New	Total	
C_e	12	0	9	0	21	17	0	13	0	30	136
F-1-a	8	4	9	3	24	12	7	13	5	37	150
F-1-b	9	2	9	3	23	13	4	13	6	36	146
F-1-c	9	1	9	1	20	13	2	13	2	30	136
F-1-d	9	4	9	2	24	13	6	13	5	37	147
F-1-e	8	3	9	2	22	12	5	13	5	35	142
F-1-f	7	3	9	0	19	11	4	13	1	29	130
F-1-g	8	4	9	2	23	12	6	13	4	35	143
F-1-h	9	3	9	1	22	13	6	13	2	34	143
F-1-i	7	8	9	1	25	10	10	13	4	37	140
F-1-j	7	5	9	0	21	10	8	14	2	34	140
C_m	0	17	9	0	26	0	25	11	0	36	143

TABLE 14. Classification of bands and fragments detected after agarose gel electrophoresis of *Sma*I digests of cpDNA from subclones of five F_1 hybrid meiotic products (F-1-a, F-1-c, F-1-d, F-1-e, F-1-f) and from the *C. eugametos* (C_e) and *C. moewusii* (C_m) parental strains. Estimates of cpDNA size are also given. The genetic backgrounds of the F_1 hybrid subclones are indicated in the legend of Figure 18. Abbreviations: C_e and C_m bands or fragments = characteristic of the *C. eugametos* and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
	C_e	C_m	Com.	New	Total	C_e	C_m	Com.	New	Total	
C_e	6	0	8	0	14	10	0	9	0	19	163
F-1-a	1	3	8	0	12	1	4	9	0	14	138
F-1-c	3	2	8	0	13	4	2	9	0	15	145
F-1-d	1	3	8	0	12	1	4	10	0	15	142
F-1-e	1	3	8	0	12	1	4	9	0	14	138
F-1-f	2	2	8	1	13	3	2	9	1	15	145
C_m	0	3	8	0	11	0	4	10	0	14	137

TABLE 15. Classification of bands and fragments detected after agarose gel electrophoresis of *Ava*I digests of cpDNA from subclones of seven B_1 hybrid meiotic products (B-1-a through -g) and from the F_1 hybrid (F-1-a) and *C. moewusii* (C_m) parental strains. Estimates of cpDNA size are also given. The genetic backgrounds of the B_1 hybrid subclones are indicated in the legend of Figure 19. Abbreviations: F-1-a and C_m bands or fragments = characteristic of the F_1 hybrid and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
	F-1-a	C_m	Com.	New	Total	F-1-a	C_m	Com.	New	Total	
F-1-a	11	0	15	0	26	16	0	21	0	37	142
B-1-a	11	0	15	0	26	16	0	21	0	37	142
B-1-b	11	0	15	0	26	16	0	21	0	37	142
B-1-c	11	0	15	0	26	16	0	21	0	37	142
B-1-d	11	0	15	0	26	16	0	21	0	37	142
B-1-e	11	1	15	0	27	15	1	21	0	37	142
B-1-f	11	1	15	0	27	15	1	21	0	37	142
B-1-g	11	0	15	0	26	16	0	21	0	37	142
C_m	0	10	15	0	25	0	14	21	0	35	144

TABLE 16. Classification of bands and fragments detected after agarose gel electrophoresis of *BstEII* digests of cpDNA from subclones of seven B_1 hybrid meiotic products (B-1-a through -g) and from the F_1 hybrid (F-1-a) and *C. moewusii* (C_m) parental strains. Estimates of cpDNA size are also given. The genetic backgrounds of the B_1 hybrid subclones are indicated in the legend of Figure 20. Abbreviations: F-1-a and C_m bands or fragments = characteristic of the F_1 hybrid and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
	F-1-a	C_m	Com.	New	Total	F-1-a	C_m	Com.	New	Total	
F-1-a	11	0	13	0	24	16	0	21	0	37	150
B-1-a	11	0	13	0	24	16	0	21	0	37	150
B-1-b	11	0	13	0	24	16	0	21	0	37	150
B-1-c	11	0	13	0	24	16	0	21	0	37	150
B-1-d	10	0	13	1	24	14	0	20	1	35	147
B-1-e	10	0	13	0	23	14	0	21	0	35	147
B-1-f	10	0	13	1	24	14	0	20	1	35	147
B-1-g	9	0	13	1	23	13	0	21	1	35	146
C_m	0	13	13	0	26	0	16	20	0	36	143

TABLE 17. Classification of bands and fragments detected after agarose gel electrophoresis of *Ava*I and *Bst*EII digests of cpDNA from four homoplasmic mitotic segregants derived from an F_1 biparental hybrid meiotic product (F-1-k). The classification of bands and fragments obtained for the *Ava*I and *Bst*EII digests of cpDNA from the *C. eugametos* C_e(S) and *C. moewusii* C_m(E) parental strains are also shown for comparison with the progeny. Estimates of cpDNA size are given. The genetic backgrounds of the F_1 segregants are indicated in the legend of Figure 21. Abbreviations: C_e and C_m bands or fragments = characteristic of the *C. eugametos* and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Enzyme	Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
		C_e	C_m	Com.	New	Total	C_e	C_m	Com.	New	Total	
*Ava*I	C_e(S)	17	0	13	0	30	22	0	17	0	39	147
	F-1-k(S)	3	8	13	0	24	3	12	17	2	34	141
	F-1-k(E)	2	10	13	0	25	2	14	17	0	33	138
	F-1-k(+)	2	10	13	0	25	2	14	17	0	33	138
	F-1-k(ES)	3	9	13	0	25	3	13	17	0	33	138
	C_m(E)	0	12	13	0	25	0	18	17	0	35	144
*Bst*EII	C_e(S)	12	0	9	0	21	17	0	13	0	30	136
	F-1-k(S)	0	15	8	0	23	0	20	12	1	33	132
	F-1-k(E)	0	16	9	0	25	0	23	13	0	36	140
	F-1-k(+)	0	16	8	0	24	0	23	11	1	35	137
	F-1-k(ES)	0	15	9	0	24	0	20	14	0	34	136
	C_m(E)	0	17	9	0	26	0	25	11	0	36	143

TABLE 18. Classification of bands and fragments detected after agarose gel electrophoresis of *Ava*I digests of cpDNA from homoplasmic mitotic segregants derived from four B_1 biparental hybrid meiotic products (B-1-i through -l). The classification of bands and fragments obtained for the *Ava*I digests of cpDNA from the F_1 hybrid [F-1-a(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. Estimates of cpDNA size are given. The genetic backgrounds of the B_1 segregants are indicated in the legends of Figures 23 and 24. Abbreviations: F-1-a and C_m bands or fragments = characteristic of the F_1 hybrid and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
	F-1-a	C_m	Com.	New	Total	F-1-a	C_m	Com.	New	Total	
F-1-a(S)	11	0	15	0	26	16	0	21	0	37	142
B-1-i(S)	3	7	15	0	25	6	9	21	0	36	143
B-1-i(E)	0	10	15	0	25	0	13	21	0	34	142
B-1-i(+)	4	6	15	0	25	8	7	23	0	38	151
B-1-i(ES)	7	2	14	1	24	9	3	22	1	35	140
B-1-j(S)	11	0	15	0	26	16	0	21	0	37	142
B-1-j(E)	0	10	15	0	25	0	13	21	0	34	142
B-1-j(+)	4	7	15	0	26	8	8	21	0	37	147
B-1-j(ES)	1	8	14	0	24	1	11	22	0	34	141
B-1-k(S)	11	1	15	0	27	15	1	22	0	38	146
B-1-k(E)	3	7	15	0	25	4	9	23	1	37	149
B-1-k(+)	3	7	15	0	25	6	9	23	0	38	151
B-1-l(E)	1	8	15	1	25	1	10	22	1	34	142
B-1-l(+)	4	6	15	0	25	7	8	21	0	36	143
C_m (E)	0	10	15	0	25	0	14	21	0	35	144

TABLE 19. Classification of bands and fragments detected after agarose gel electrophoresis of *Bst*EII digests of cpDNA from homoplasmic mitotic segregants derived from four B_1 biparental hybrid meiotic products (B-1-i through -l). The classification of bands and fragments obtained for the *Bst*EII digests of cpDNA from the F_1 hybrid [F-1-a(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. Estimates of cpDNA size are given. The genetic backgrounds of the B_1 segregants are indicated in the legends of Figures 25 and 26. Abbreviations: F-1-a and C_m bands or fragments = characteristic of the F_1 hybrid and *C. moewusii* parental patterns respectively; Com. = common to both parental patterns; New = absent from both parental patterns.

Strain	No. of Bands					No. of Fragments					CpDNA Size Estimate (megadaltons)
	F-1-a	C_m	Com.	New	Total	F-1-a	C_m	Com.	New	Total	
F-1-a(S)	11	0	13	0	24	17	0	20	0	37	150
B-1-i(S)	1	10	13	0	24	2	11	20	1	34	145
B-1-i(E)	0	12	13	1	26	0	14	20	2	36	145
B-1-i(+)	0	12	13	1	26	0	14	19	2	35	145
B-1-i(ES)	6	6	13	1	26	8	7	19	2	36	144
B-1-j(S)	9	0	13	0	22	13	0	21	0	34	141
B-1-j(E)	0	11	12	3	26	0	13	17	5	35	141
B-1-j(+)	1	12	12	2	27	2	14	18	5	39	155
B-1-j(ES)	3	6	13	1	23	4	7	20	2	33	141
B-1-k(S)	10	0	13	0	23	15	0	21	0	36	149
B-1-k(E)	3	8	13	1	25	3	10	22	3	38	155
B-1-k(+)	3	8	13	1	25	4	10	22	2	38	155
B-1-l(E)	3	9	13	1	26	3	11	20	2	36	147
B-1-l(+)	1	11	13	0	25	2	13	20	1	36	150
C_m(E)	0	13	13	0	26	0	16	20	0	36	143

TABLE 20. Inheritance pattern of the antibiotic resistance mutations recovered in *C. moewusii*. Crosses were made between each antibiotic resistant *mt+* strain and the wild-type *mt-* strain. Mendelian inheritance was revealed by 2:2 segregation of resistance and sensitivity in tetrads while non-Mendelian inheritance was revealed by all four meiotic products displaying resistance. All tetrads showed 2:2 segregation of the Mendelian alleles at the *mt* locus.

Mutant Strain	Resistance Phenotype	Inheritance Pattern	Number of Tetrads
sr-50	streptomycin (50 µg/ml)	Mendelian	5
*sr-nM*1	streptomycin (250 µg/ml)	Non-Mendelian	15
er-100a	erythromycin (100 µg/ml)	Mendelian	5
er-100b	erythromycin (100 µg/ml)	Mendelian	5
er-400	erythromycin (400 µg/ml)	Mendelian	4
*er-nM*1	erythromycin (400 µg/ml)	Non-Mendelian	7

TABLE 21. Segregation of antibiotic resistance and sensitivity alleles of non-Mendelian genes in tetrads recovered from two *C. moewusii* crosses. The number of tetrads displaying each type of segregation is given in parentheses. All tetrads showed 2:2 segregation of the Mendelian alleles at the *mt* locus. Abbreviations: S = meiotic product homoplasmic for the sensitive allele derived from the *mt+* parent; R = meiotic product homoplasmic for the resistant allele derived from the *mt-* parent; H = meiotic product heteroplasmic for the resistance and sensitivity alleles.

Tetrad Types	Frequency of Tetrads	
	er-nM1^{+} *mt+* X *er-nM1* *mt-*	*sr-nM1*$^{+}$ *mt+* X *sr-nM1* *mt-*
0S:0R:4H	0.58 (8)	0.69 (11)
1S:0R:3H	0.07 (1)	0.19 (3)
2S:0R:2H	0.14 (2)	0.06 (1)
3S:0R:1H	0.14 (2)	0
4S:0R:0H	0	0.06 (1)
0S:1R:3H	0.07 (1)	0

TABLE 22. Frequency of antibiotic resistant cells in primary clones derived from heteroplasmic meiotic products which were recovered from two *C. moewusii* crosses.

Frequency of Resistant Cells in Primary Clones	Number of Primary Clones	
	er-nM1^{+} *mt+* X *er-nM1 mt-*	*sr-nM1*$^{+}$ *mt+* X *sr-nM1 mt-*
<0.02	12	19
0.02-0.09	7	9
0.10-0.19	6	7
0.20-0.29	1	8
0.30-0.39	4	3
0.40-0.49	2	4
0.50-0.59	4	0
0.60-0.69	3	1
0.70-0.79	1	2
0.80-0.89	2	0
0.90-0.99	2	2
Total:	44	55

FIGURE 1: Electrophoretic patterns in 0.75% agarose gel of *Eco*RI and *Bam*HI digests of β-DNA (cpDNA) from *C. reinhardtii*. On the right of the UV fluorescence photographs are shown the microdensitometer traces of these photographs. The bands are numbered according to the classification of Rochaix (1978). A scale of absolute values of fragment mobility is given for these electrophoretic separations.

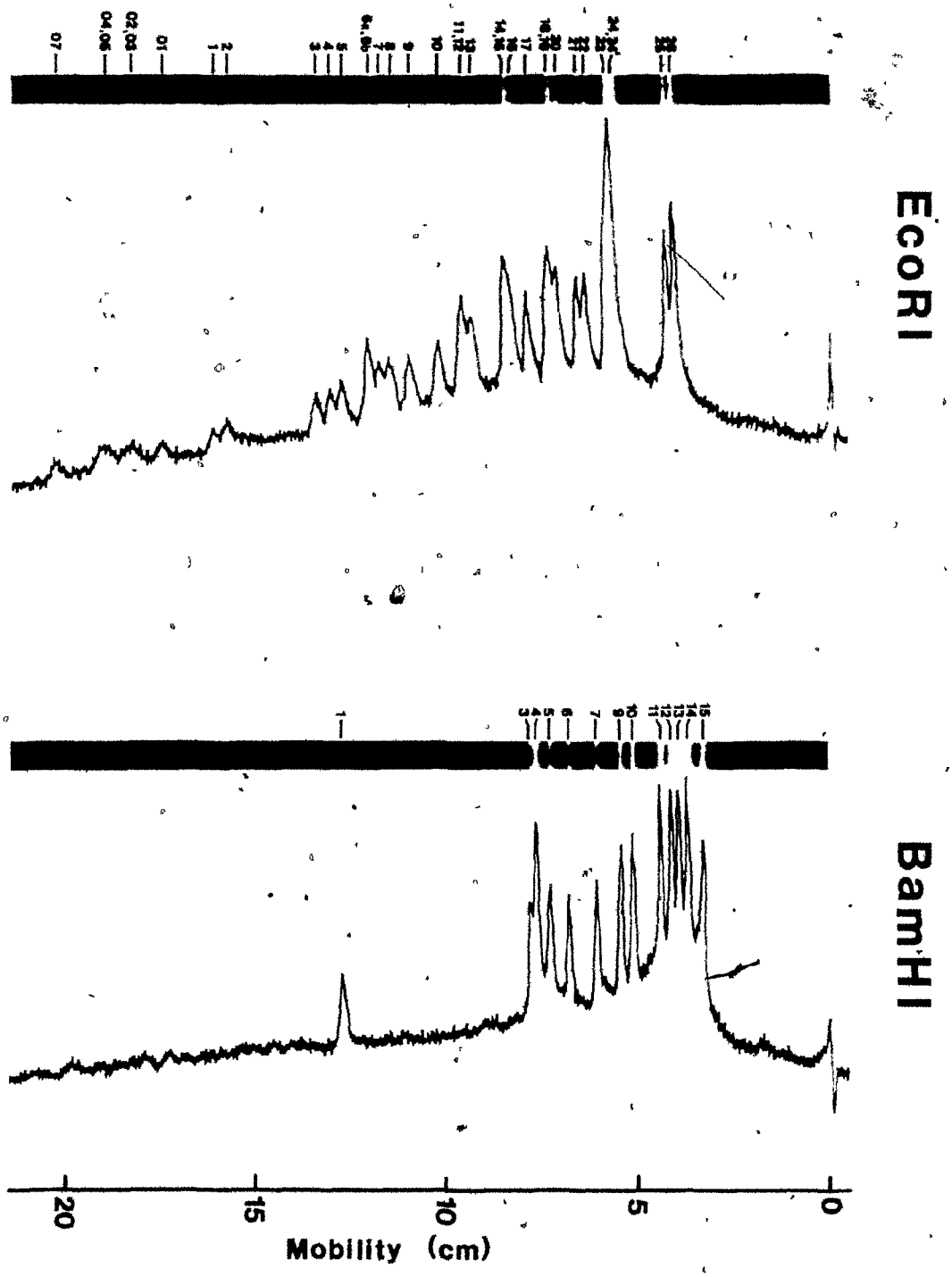

EcoRI
BamHI
20
15
10
5
0
Mobility (cm)

FIGURE 2: Typical calibration curve relating the molecular weight and the electrophoretic mobility in 0.75% agarose gel of *Bam*HI (o) and *Eco*RI (●) restriction fragments of β-DNA (cpDNA) from *C. reinhardtii*. The molecular weight values of the fragments are those reported by Rochaix (1978).

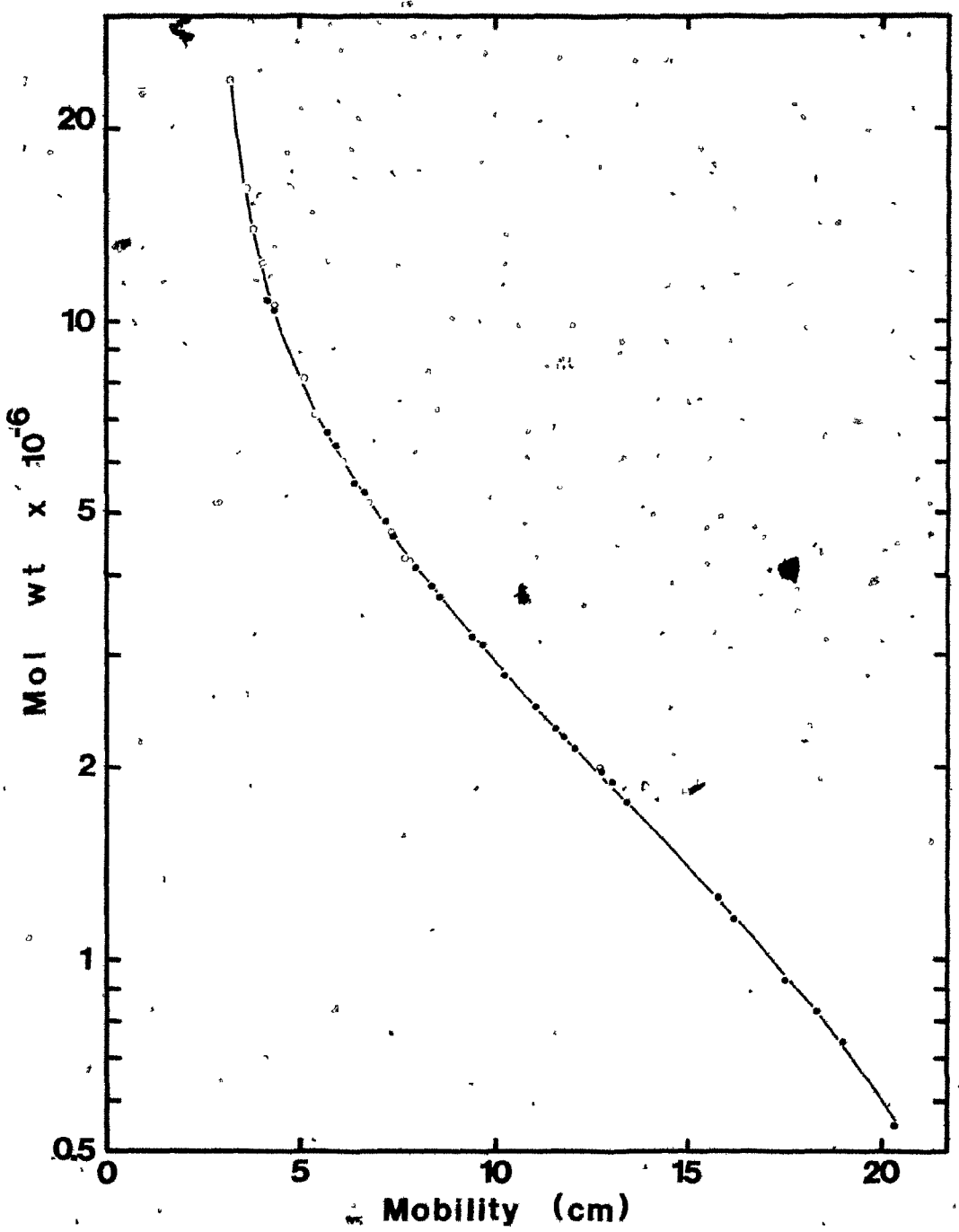

Mol wt x 10^{-6}
20
10
5
2
1
0.5
0
5
10
15
20
Mobility (cm)

FIGURE 3: Microdensitometer traces of UV absorption photographs taken after equilibrium CsCl density gradient centrifugation of whole cell DNA from *C. reinhardtii* (C_r), *C. moewusii* (C_m) and *C. eugametos* (C_e). The density of the DNA components is based on their position relative to phage SP8 marker DNA (ρ = 1.742 g/ml).

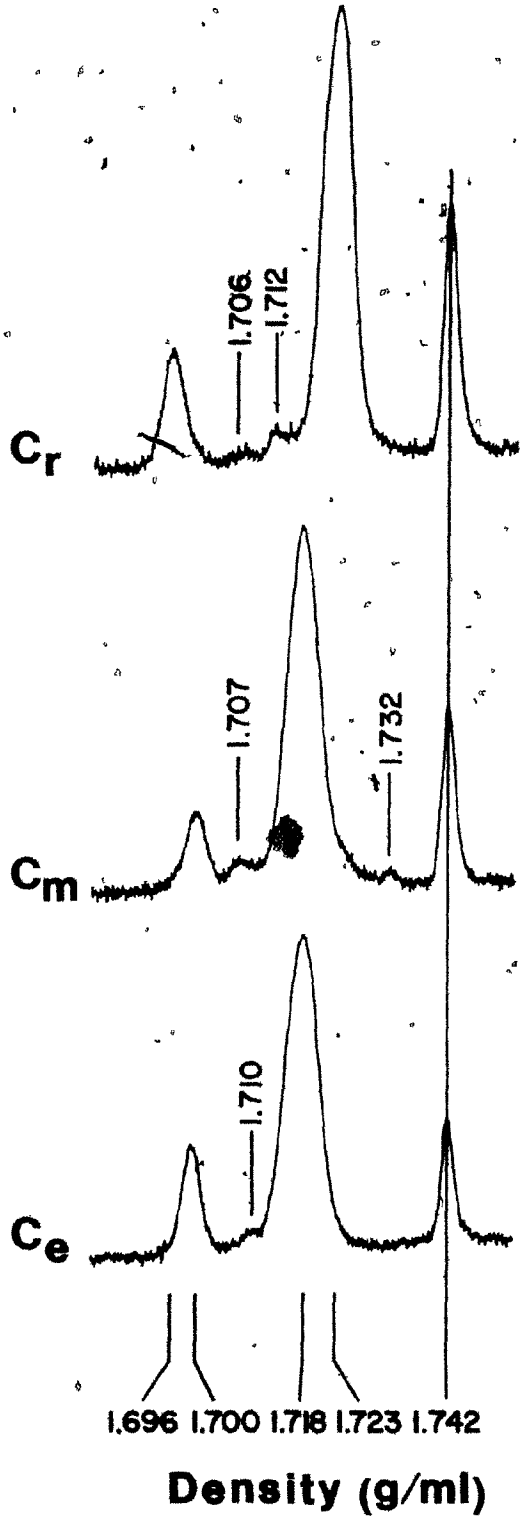

1.706
1.712
C_r
1.707
1.732
C_m
1.710
C_e
1.696 1.700 1.718 1.723 1.742
Density (g/ml)

FIGURE 4: Microdensitometer traces of UV absorption photographs taken after equilibrium CsCl density gradient centrifugation of β-DNA preparations from *C. reinhardtii* (C_r), *C. moewusii* (C_m) and *C. eugametos* (C_e). Each of the DNA samples was obtained by collecting the β-DNA band from preparative equilibrium CsCl density gradients of whole cell nucleic acid. The density of the β-DNAs and the α-DNA contaminants is based on their position relative to phage SP8 marker DNA (ρ = 1.742 g/ml).

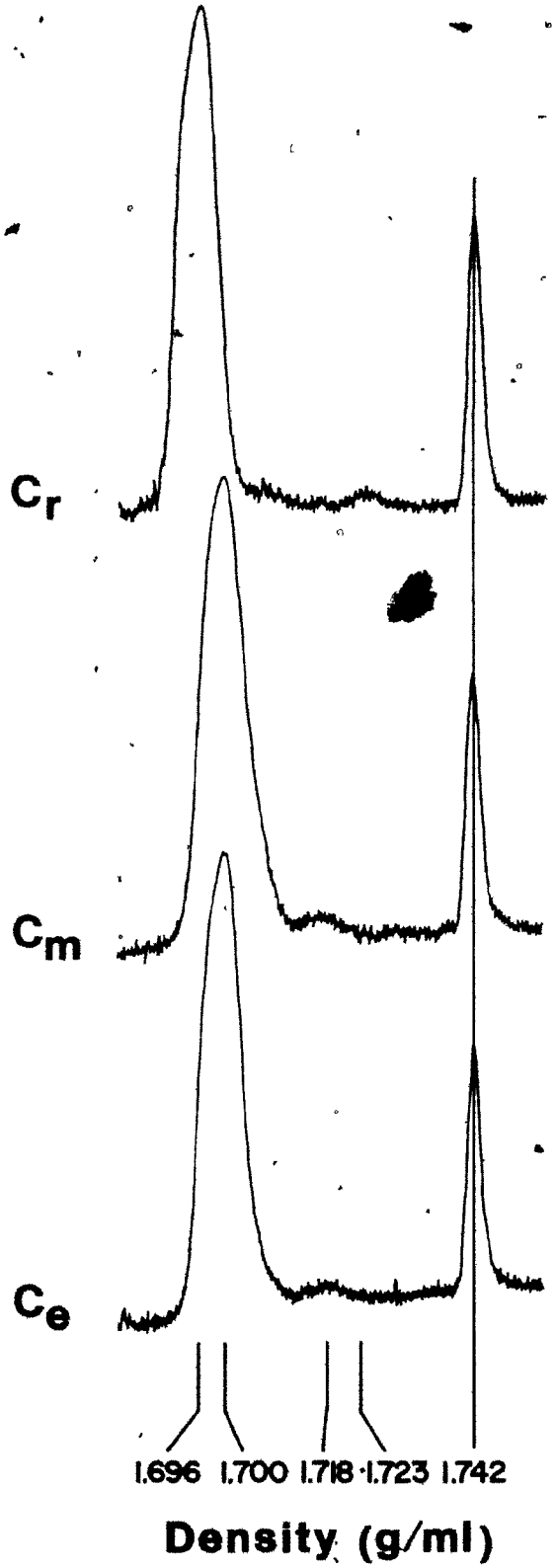

C_r
C_m
C_e
1.696
1.700
1.718
1.723
1.742
Density (g/ml)

FIGURE 5: Electrophoretic patterns in 0.75% agarose gel of β-DNA preparations from *C. reinhardtii* (C_r), *C. eugametos* (C_e) and *C. moewusii* (C_m). Each of these DNA samples was obtained by collecting the β-DNA band from preparative equilibrium CsCl density gradients of whole cell nucleic acid. The amounts of DNA applied to the gel were 5, 10 and 5 µg for the C_r, C_e and C_m samples, respectively. The molecular weight of DNA in the faint bands is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* β-DNA. The average molecular weight of the main DNA components (larger than 25 megadaltons) proved to be too high for a reasonable estimate of size.

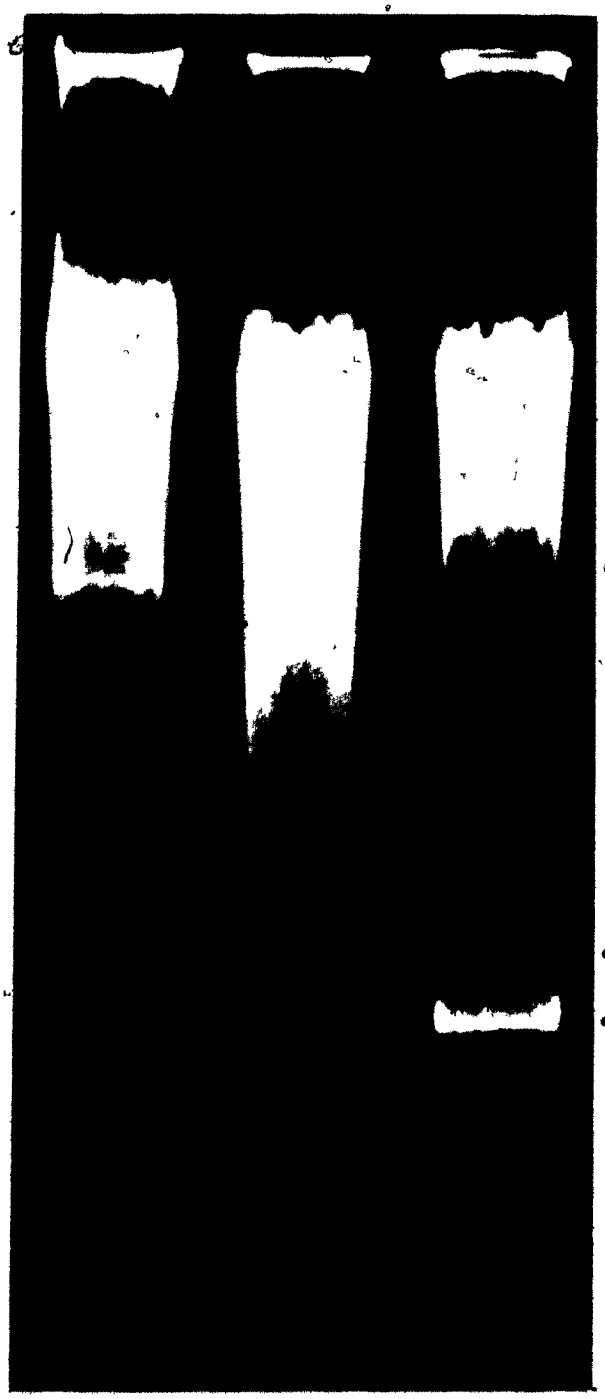

C_r
C_e
C_m
9.5
4.4
3.9
Mol wt x 10^{-6}

FIGURE 6: Microdensitometer traces of UV absorption photographs taken after equilibrium CsCl density gradient centrifugation of the following *C. moewusii* DNA samples: (A) whole cell DNA; (B) DNA enriched for the 1.707 g/ml component by fractionation of preparative CsCl gradients containing whole cell nucleic acid; (C) DNA further enriched for the 1.707 g/ml component by fractionation of preparative CsCl gradients containing netropsin sulfate and the (B) DNA sample; (D) 1.700 g/ml DNA (β-DNA) fraction removed from preparative CsCl gradients containing netropsin sulfate and the (B) DNA sample. The density of the DNA components is based on their position relative to phage SP8 marker DNA (ρ = 1.742 g/ml).

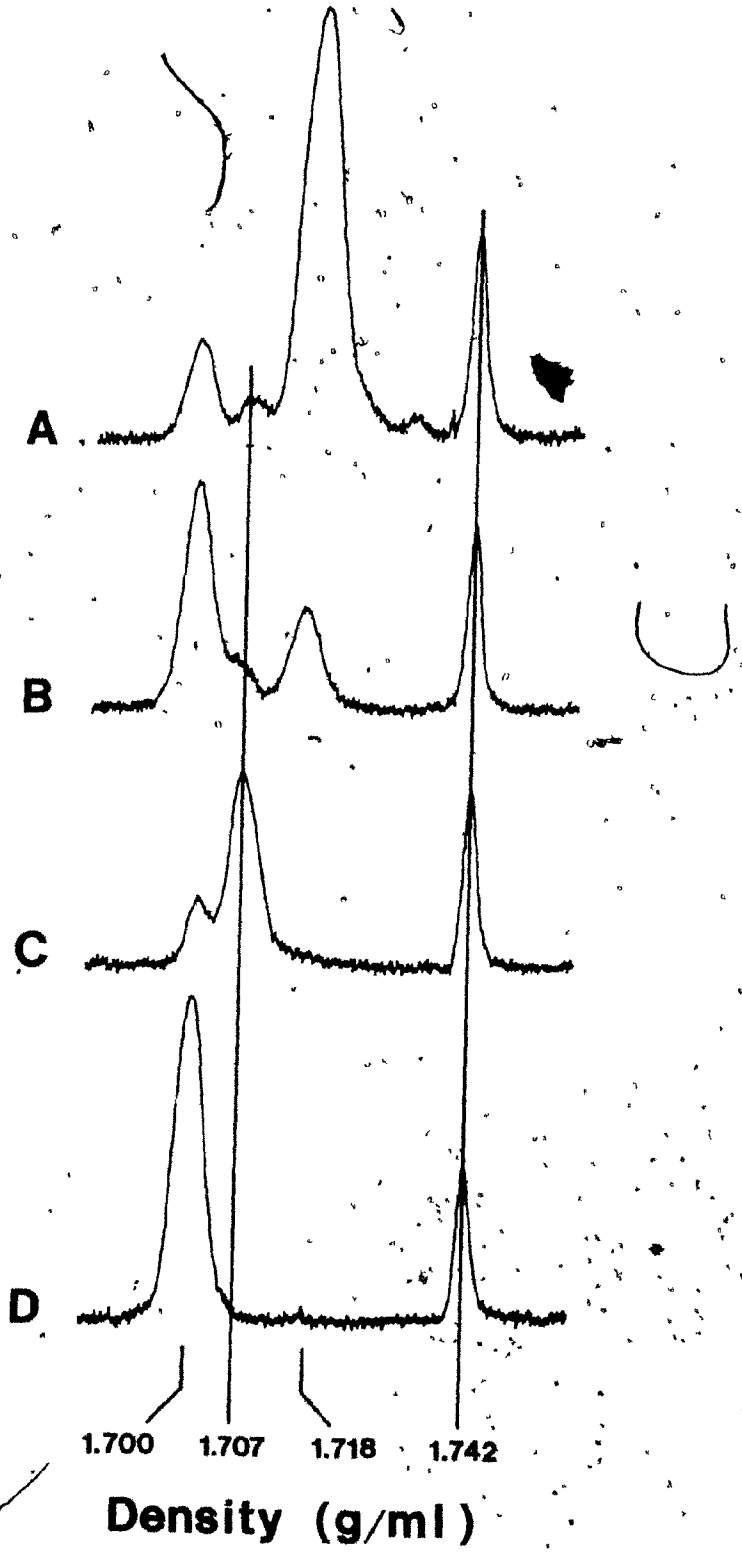

A
B
C
D
1.700
1.707
1.718
1.742
Density (g/ml)

FIGURE 7: UV fluorescent bands of *C. moewusii* DNA components (1.700, 1.707 and 1.718 g/ml) after preparative equilibrium CsCl density gradient centrifugation in the presence of 2.5 µg/ml ethidium bromide. Tube (A) contains whole cell nucleic acid while tube (B) contains netropsin sulfate (6 µg/µg DNA) and the DNA collected between the 1.700 and 1.718 DNA bands of replica (A) tubes. Both tubes were centrifuged under the same conditions (IEC A-321 rotor, 43,000 rpm, 48 hours, 18°C). The fluorescence seen at the bottom of tube (A) is that of pelleted ethidium bromide-RNA complexes.

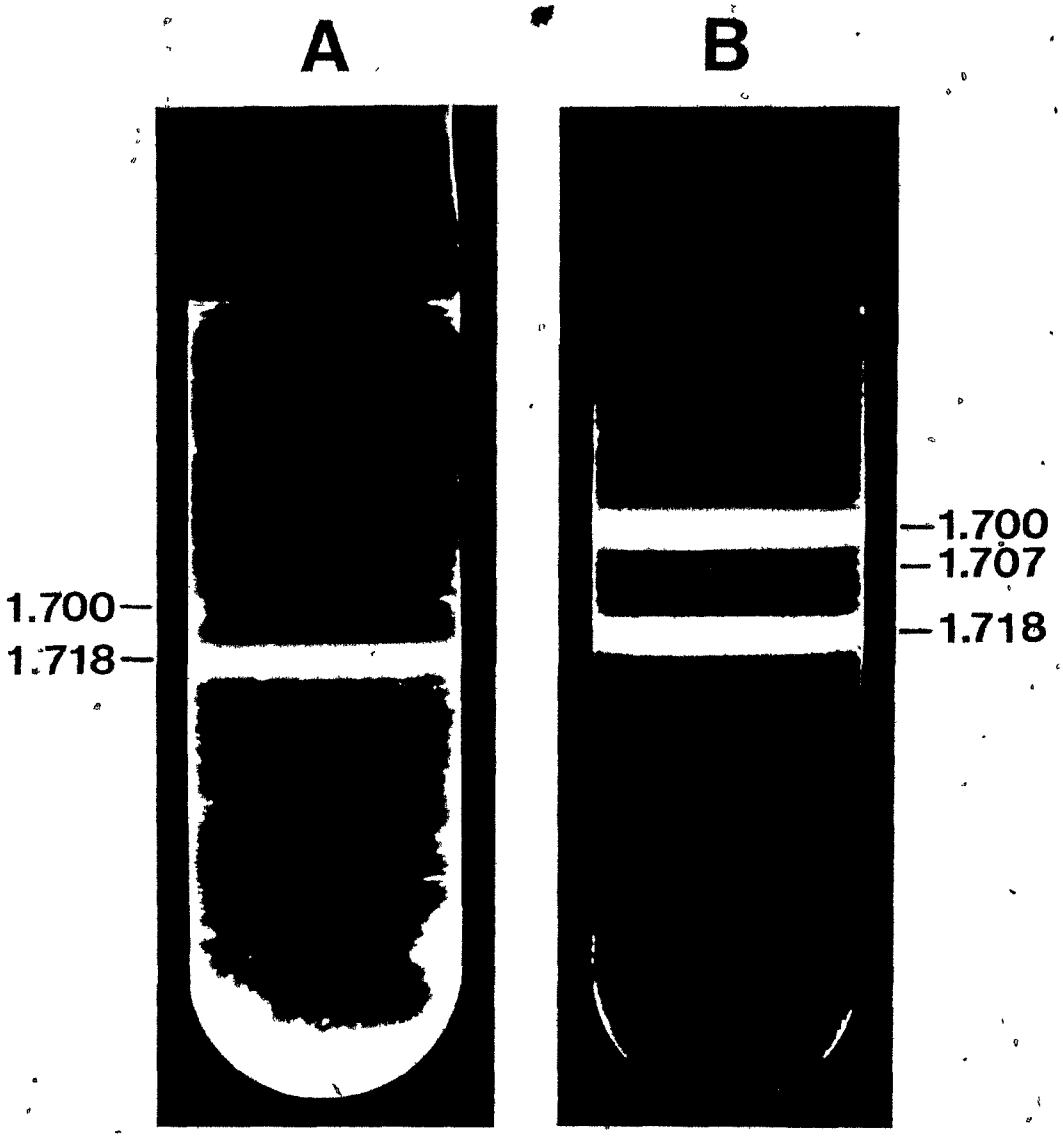

A
B
1.700
1.718
1.700
1.707
1.718

FIGURE 8: Electrophoretic patterns in 0.75% agrose gel of the following *C. moewusii* DNA samples: (A) DNA fraction enriched for the 1.707 g/ml component; (B) and (C) 1.700 g/ml DNA (β-DNA) fraction. These two DNA fractions were recovered from preparative CsCl gradients containing netropsin sulfate (see Figure 7, samples C and D). The amounts of DNA applied to the gel were 0.5, 0.5 and 5.0 µg for the (A), (B) and (C) samples respectively. The molecular weight of DNA in the faint bands which are visible in sample (C) is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* β-DNA. The molecular weight of the main DNA component (larger than 25 megadaltons) of both fractions proved to be too high for a reasonable estimate of size.

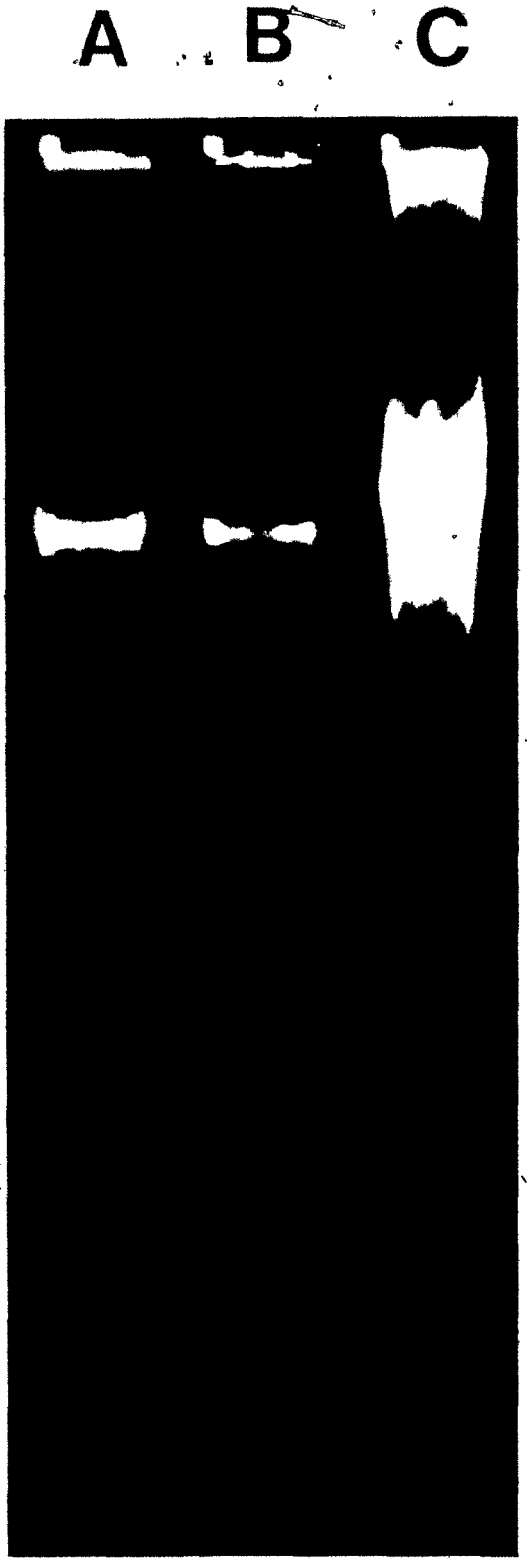

A
B
C
25
4.4
3.9
Mol wt x 10^{-6}

FIGURE 9: Electrophoretic patterns in 0.75% agarose gel of *Eco*RI digests of β-DNA from *C. eugametos* (C_e) and *C. moewusii* (C_m). The bands of each pattern are numbered sequentially in decreasing order of molecular weight. Increasing line thicknesses in the schematic diagrams represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* β-DNA.

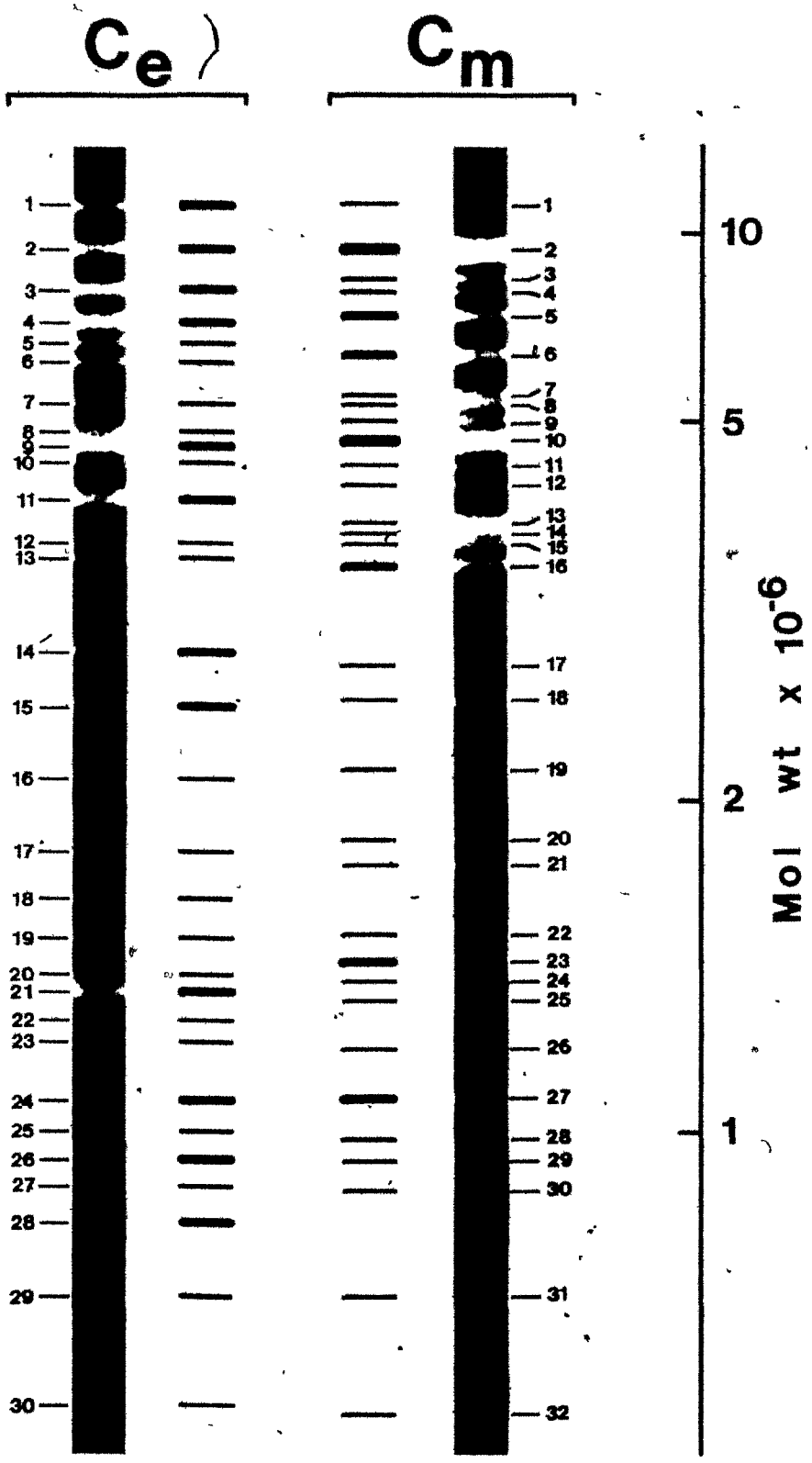

Ce
Cm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
10
5
2
1
Mol wt x 10-6

FIGURE 10: Electrophoretic patterns in 0.75% agarose gel of *Ava*I digests of β-DNA from *C. eugametos* (C_e) and *C. moewusii* (C_m). The bands of each pattern are numbered sequentially in decreasing order of molecular weight. Increasing line thicknesses in the schematic diagrams represent bands containing one, two or three fragments. The dashed line represents the band of the 3.9 megadalton DNA contaminating the β-DNA preparation of *C. moewusii*. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* β-DNA.

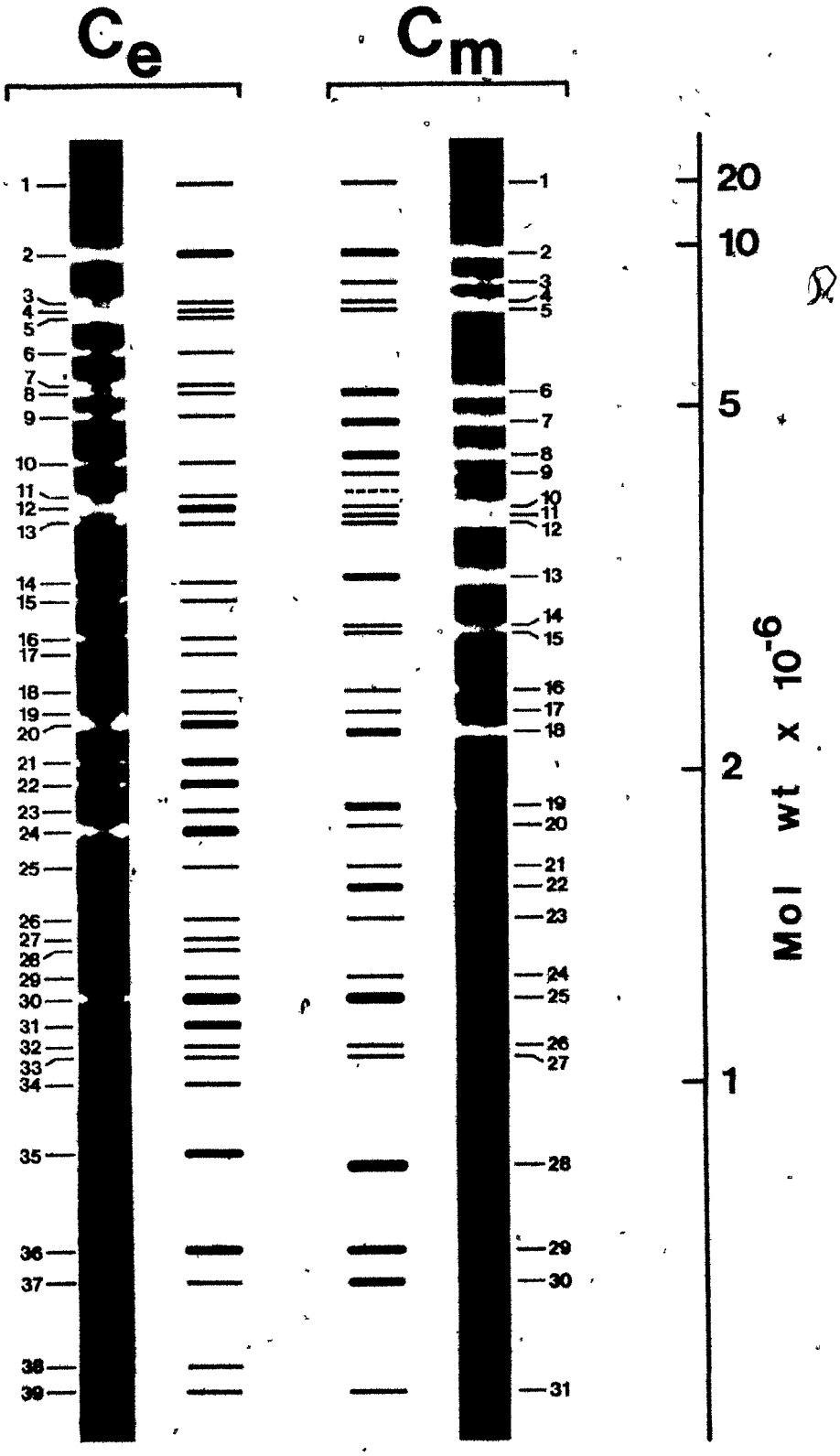

Ce
Cm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
20
10
5
2
1
Mol wt x 10-6

FIGURE 11: Electrophoretic patterns in 0.75% agarose gel of *Bst*EII digests of β-DNA from *C. eugametos* (C_e) and *C. moewusii* (C_m). The bands of each pattern are numbered sequentially in decreasing order of molecular weight. Increasing line thicknesses in the schematic diagrams represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* β-DNA.

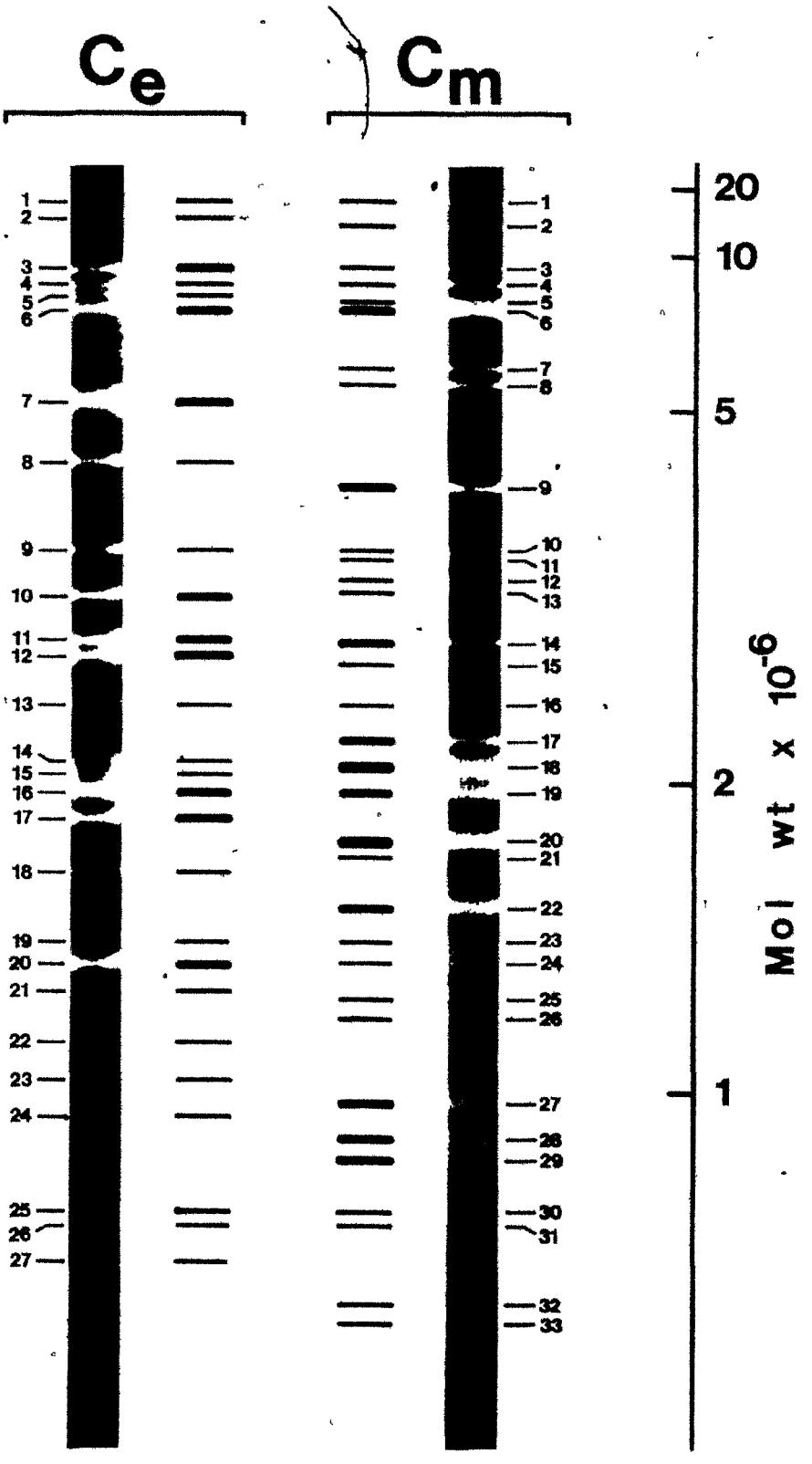

Ce
Cm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
20
10
5
2
1
Mol wt x 10-6

FIGURE 12: Electrophoretic patterns in 0.75% agarose gel of *Sma*I digests of β-DNA from *C. eugametos* (C_e) and *C. moewusii* (C_m). The bands of each pattern are numbered sequentially in decreasing order of molecular weight. Increasing line thicknesses in the schematic diagrams represent bands containing one, two or three fragments. The dashed line represents the band of the 3.9 megadalton DNA contaminating the β-DNA preparation of *C. moewusii*. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* β-DNA.

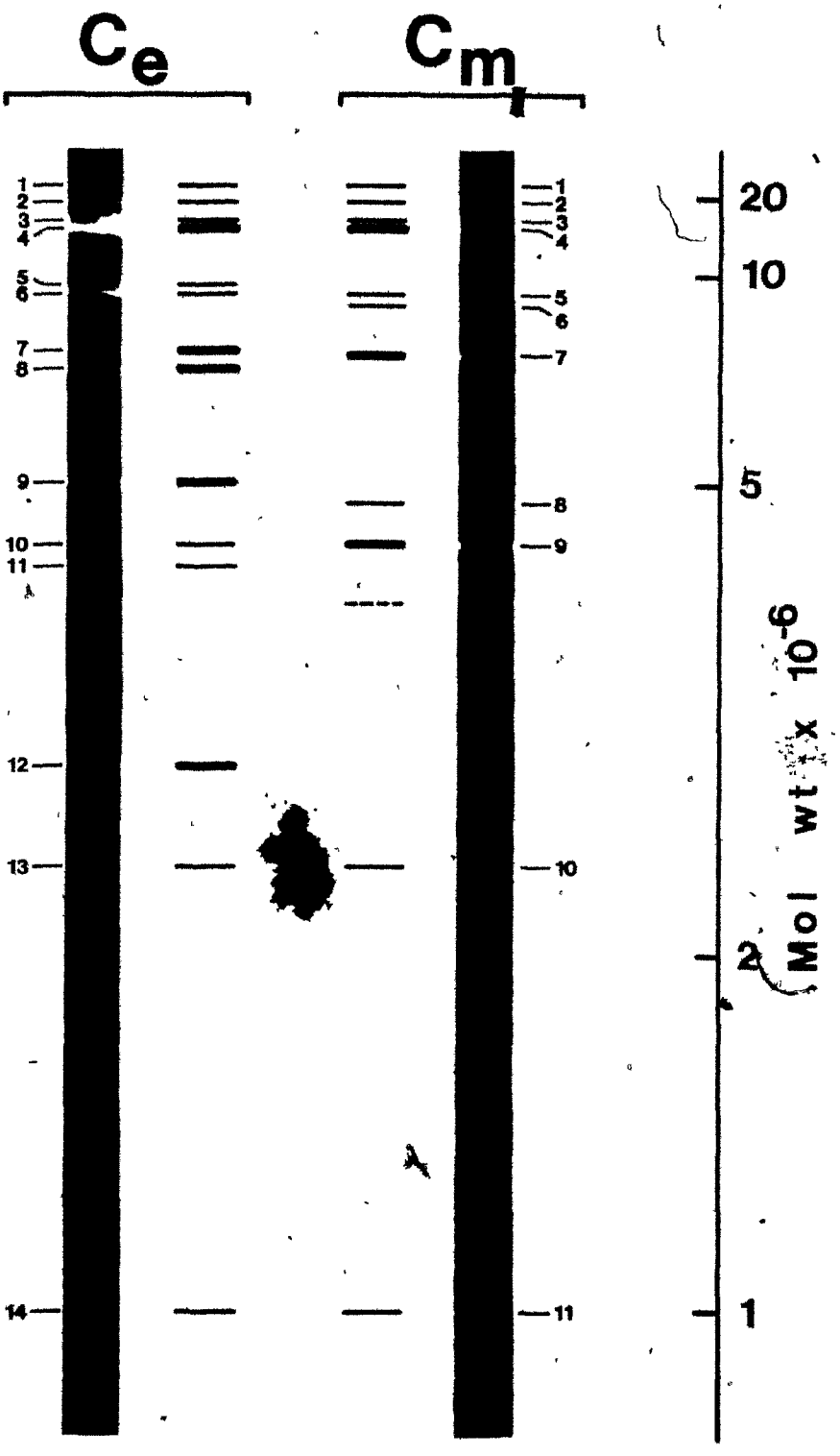

Ce
Cm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
20
10
5
2
1
Mol wt x 10-6

FIGURE 13: Electrophoretic patterns in 0.75% agarose gel of the β-DNA from *C. eugametos* (C_e) and *C. moewusii* (C_m) after digestion with various restriction endonucleases (*Bam*HI, *Bgl*I, *Pst*I, *Bgl*II, *Sal*I, *Sst*II and *Hind*III). The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and *Eco*RI digests of *C. reinhardtii* β-DNA.

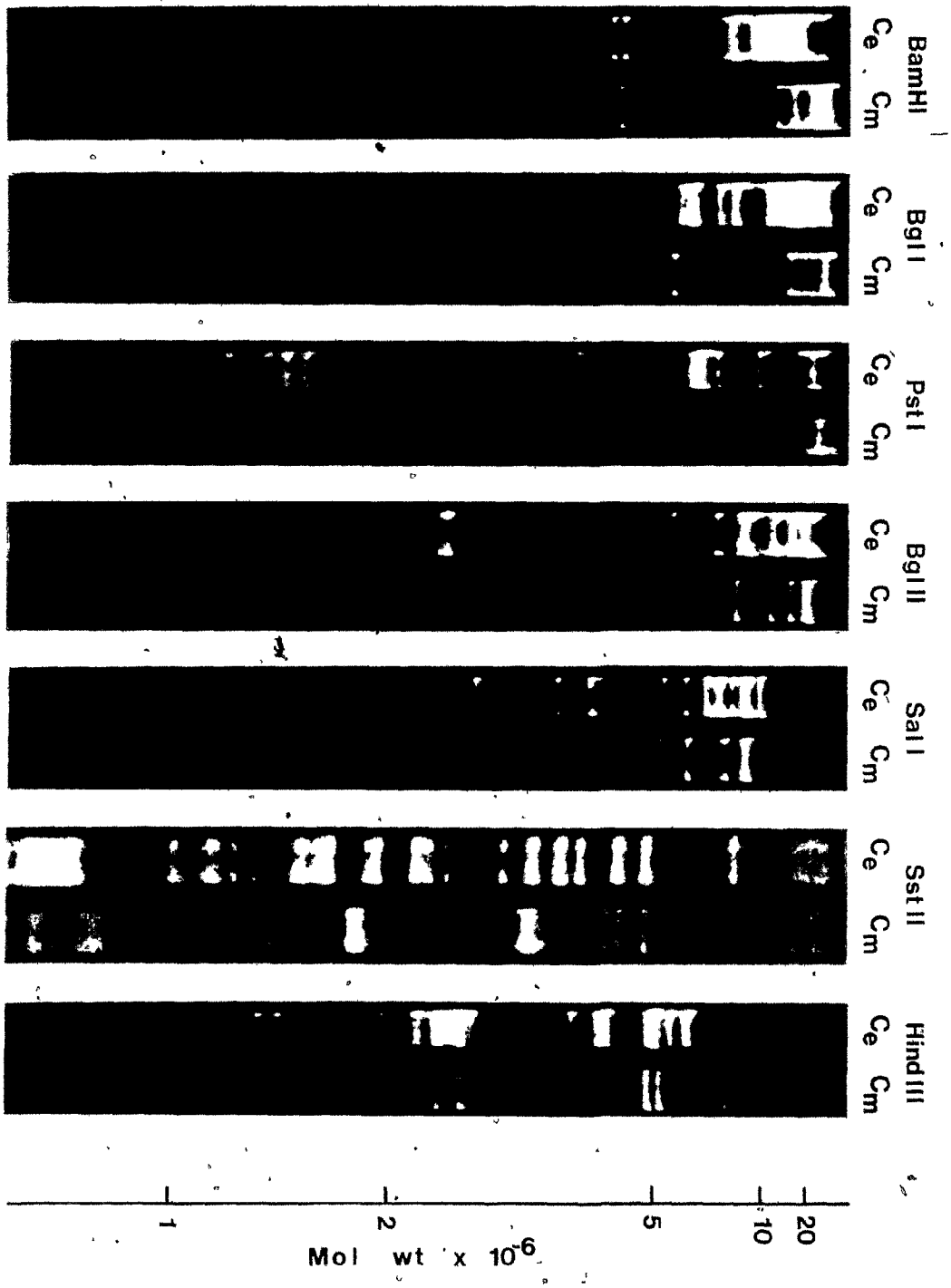

BamHI
Ce Cm
BglI
Ce Cm
PstI
Ce Cm
BglII
Ce Cm
SalI
Ce Cm
SstII
Ce Cm
HindIII
Ce Cm
1
2
5
10
20
Mol wt x 10^-6

FIGURE 14: Electrophoretic patterns in 1.5% agarose gel of *Hpa*II and *Msp*I digests of β-DNA from *C. eugametos* (C_e) and *C. moewusii* (C_m). The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* β-DNA.

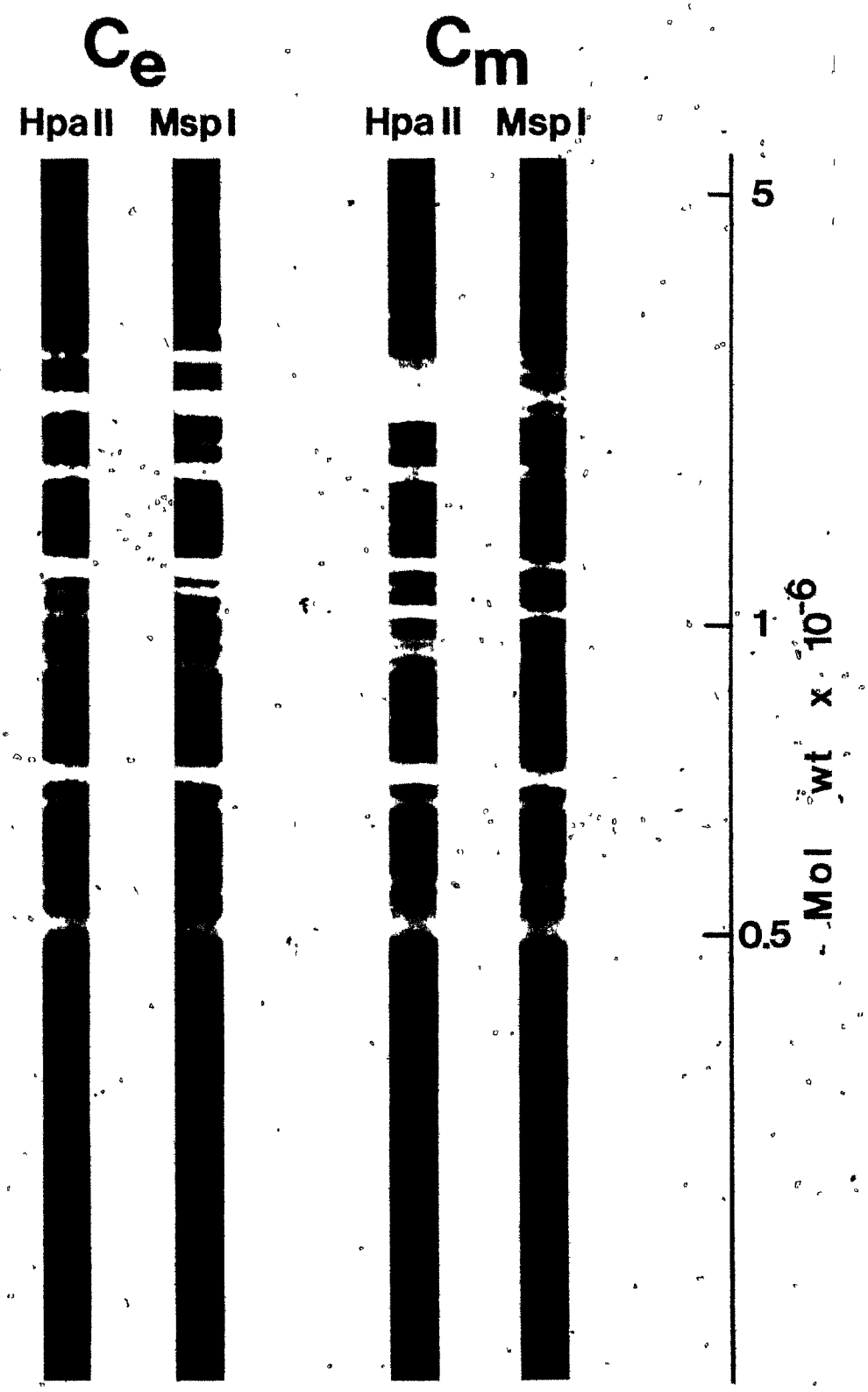

C_e
C_m
HpaII
MspI
HpaII
MspI
5
1
0.5
Mol wt x 10^{-6}

FIGURE 15. Schematic representation of various ways the multiplicity of a progeny band can differ from that of the parent(s). The fragments contained in progeny bands were classified as indicated. Increasing line thicknesses represent bands containing one, two or three fragments.

Parents		Progeny	Classification
mt+	mt−		
▬		—	mt+ characteristic fragment
	—	▬	mt− characteristic fragment + new fragment
—	—	▬	2 common fragments
—	—	▬	2 common fragments + new fragment

FIGURE 16. Electrophoretic patterns of *Ava*I digests of cpDNA from subclones of ten F_1 hybrid meiotic products (F-1-a through -j) and from the *C. eugametos* (C_e) and *C. moewusii* (C_m) parental strains. F-1 (-a through -f) were recovered from the cross *C. eugametos* (*sr*-2 *mt*+) X *C. moewusii* (*pab*-1 *mt*-) while F-1 (-g through -j) were recovered from the cross *C. eugametos* (*sr*-2 *mt*+) X *C. moewusii* (*cr-nM*1 *mt*-). The *Ava*I cpDNA restriction pattern of *C. moewusii* (*pab*-1 *mt*-) was indistinguishable from that of *C. moewusii* (*cr-nM*1 *mt*-). All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. The 3.9 megadalton DNA band visible in the *C. moewusii* digestion pattern (see arrow) is not represented in the schematic diagrams because this DNA is not considered to be a digestion product of cpDNA (see Chapter I). Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA.

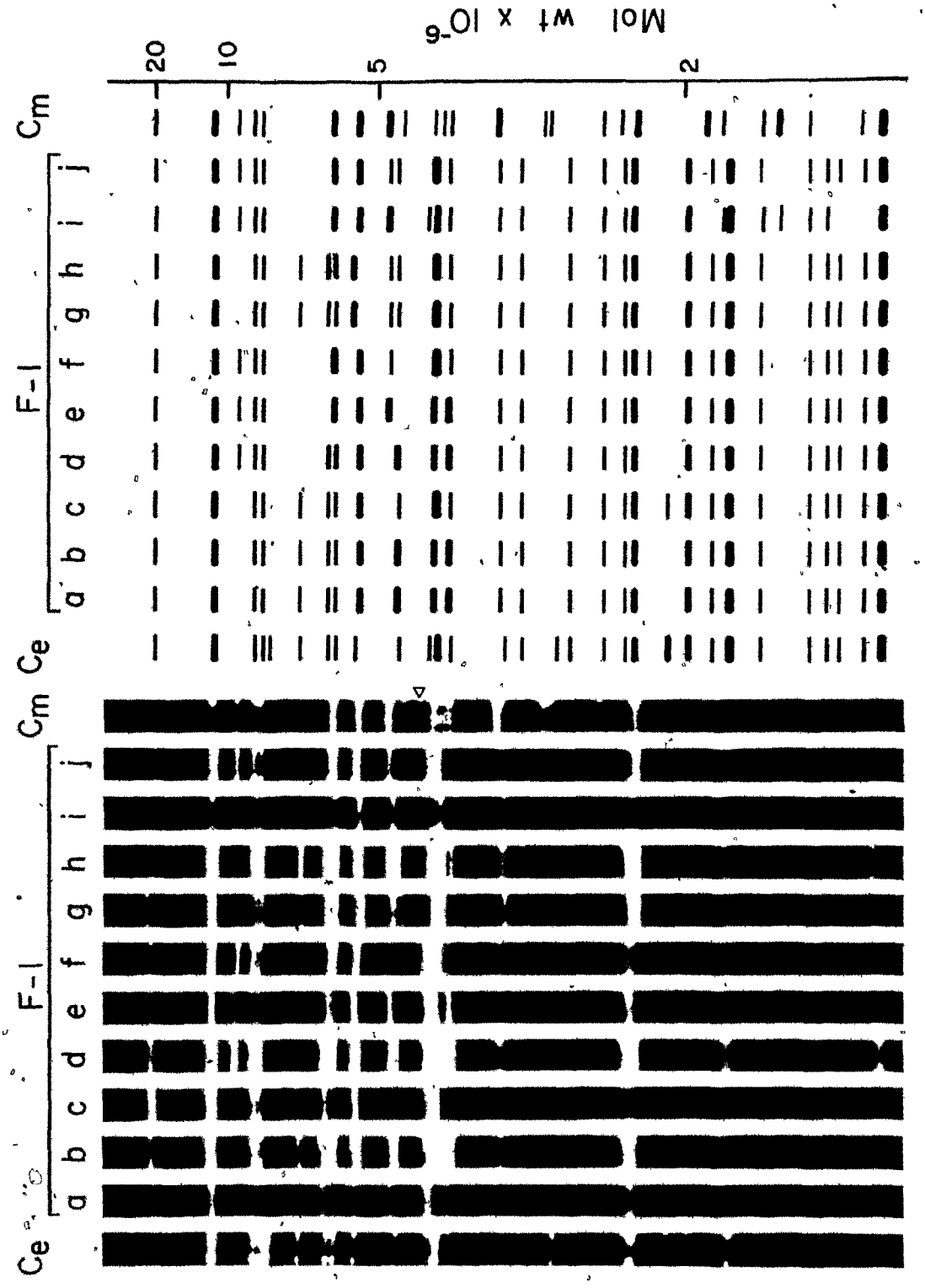

Mol wt x 10^{-6}
20
10
5
2
Cm
F-1
a b c d e f g h i j
Ce
Cm
F-1
a b c d e f g h i j
Ce

FIGURE 17. Electrophoretic patterns of *Bst*EII digests of cpDNA from subclones of ten F_1 hybrid meiotic products (F-1-a through -j) and from the *C. eugametos* (C_e) and *C. moewusii* (C_m) parental strains. F-1 (-a through -f) were recovered from the cross *C. eugametos* (*sr*-2 *mt*+) X *C. moewusii* (*pab*-1 *mt*-) while F-1 (-g through -j) were recovered from the cross *C. eugametos* (*sr*-2 *mt*+) X *C. moewusii* (*er-nM*1 *mt*-). The *Bst*EII cpDNA restriction pattern of *C. moewusii* (*pab*-1 *mt*-) was indistinguishable from that of *C. moewusii* (*er-nM*1 *mt*-). All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. Increasing line-thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA.

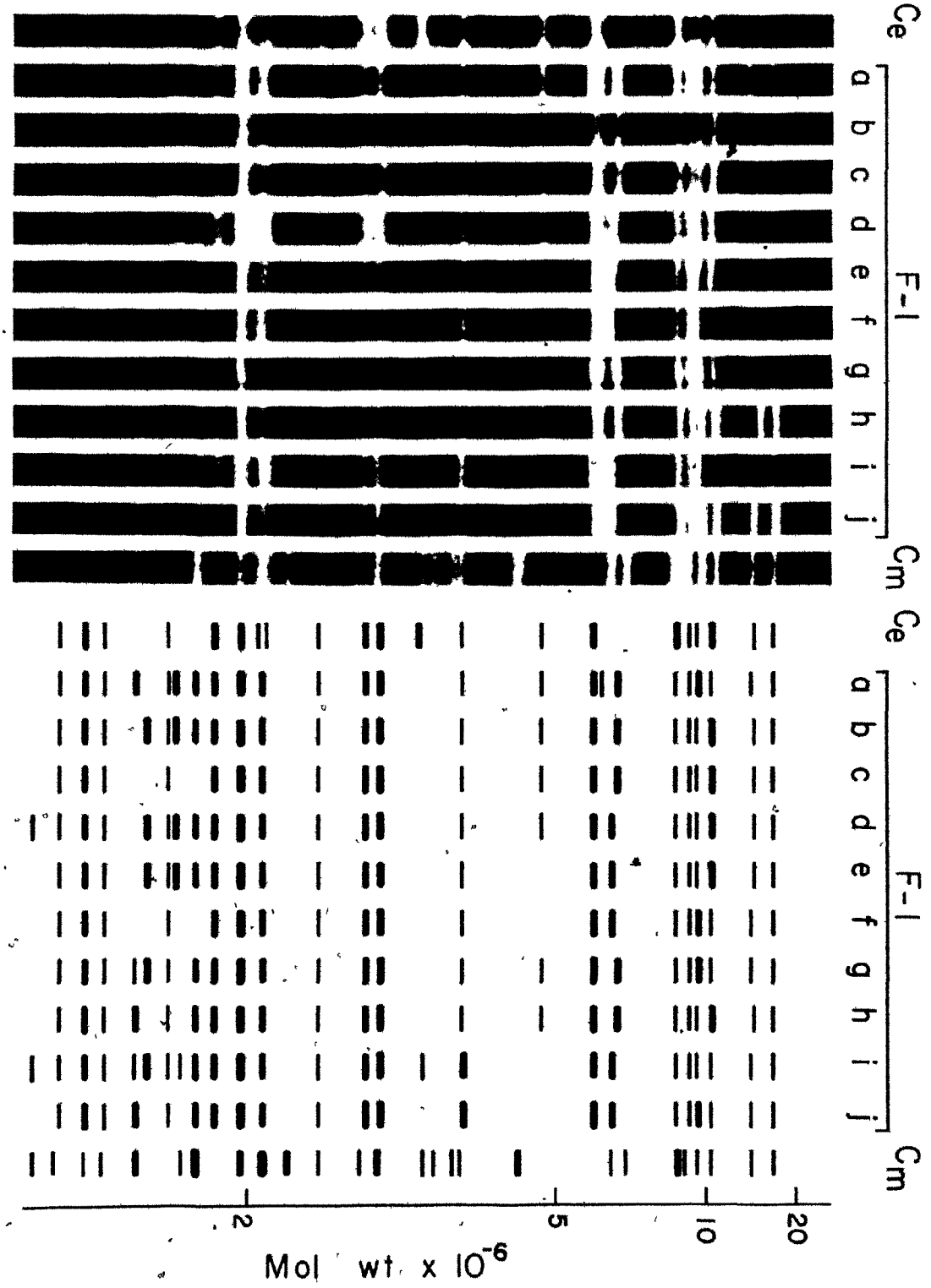

Ce
a b c d e f g h i j
F-1
Cm
Ce
a b c d e f g h i j
F-1
Cm
2
5
10
20
Mol wt x 10^{-6}

FIGURE 18. Electrophoretic patterns of *Sma*I digests of cpDNA from subclones of five F_1 hybrid meiotic products (F-1-a, F-1-c, F-1-d, F-1-e, F-1-f) and from the *C. eugametos* (C_e) and *C. moewusii* (C_m) parental strains. The F_1 products were recovered from the cross *C. eugametos* (*sr*-2 *mt*+) X *C. moewusii* (*pab*-1 *mt*-). All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. The 3.9 megadalton DNA band visible in the *C. moewusii* digestion pattern (see arrow) is not represented in the schematic diagrams because this DNA is not considered to be a digestion product of cpDNA (see Chapter I). Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA.

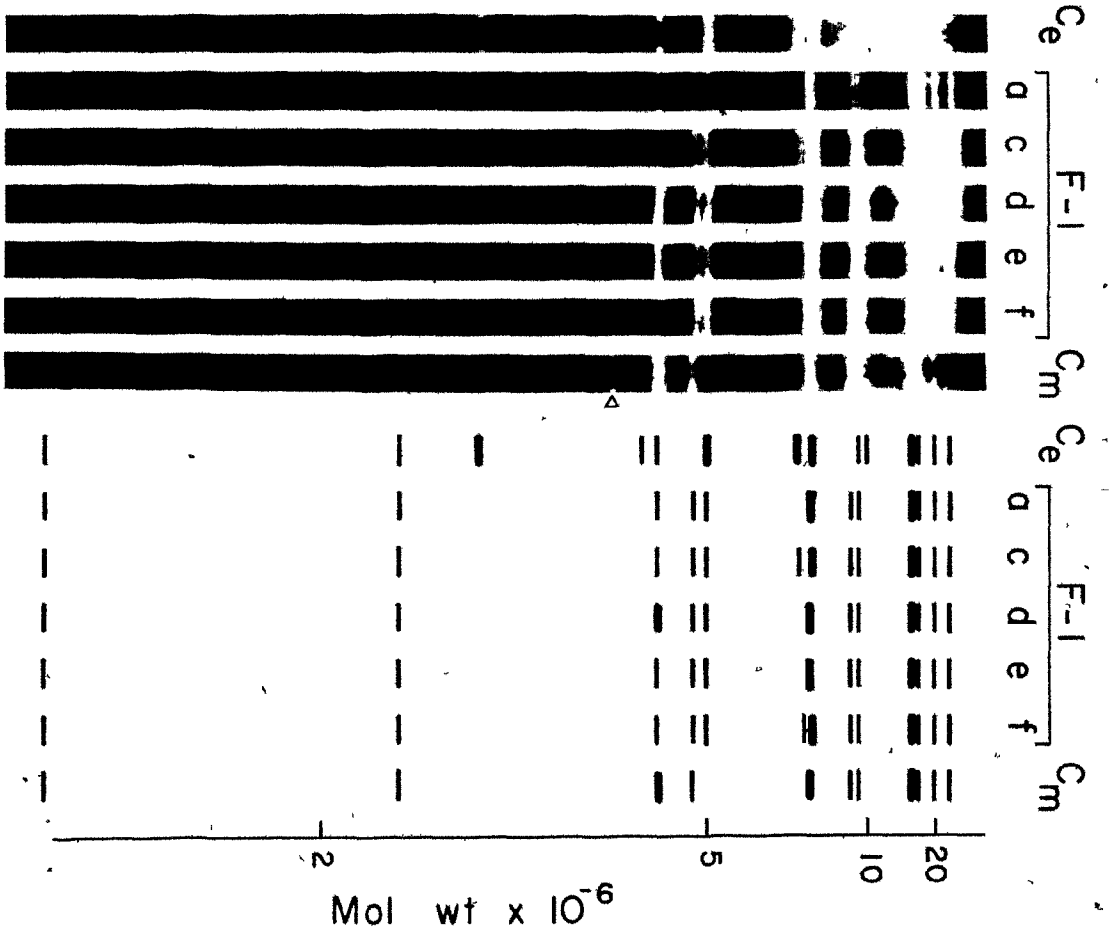

Ce
a
c
d
e
f
F-I
Cm
Ce
a
c
d
e
f
F-I
Cm
2
5
10
20
Mol wt x 10-6

FIGURE 19. Electrophoretic patterns of *Ava*I digests of cpDNA from subclones of seven B_1 hybrid meiotic products (B-1-a through -g) and from the F_1 hybrid (F-1-a) and *C. moewusii* (C_m) parental strains. B-1 (-a through -c) were recovered from the cross F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*mt*-) while B-1 (-d through -g) were recovered from the cross F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*er*-*nM*1 *mt*-). The *Ava*I cpDNA restriction pattern of *C. moewusii* (*mt*-) was indistinguishable from that of *C. moewusii* (*er*-*nM*1 *mt*-). All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. The 3.9 megadalton DNA band visible in the *C. moewusii* digestion pattern (see arrow) is not represented in the schematic diagrams because this DNA is not considered to be a digestion product of cpDNA (see Chapter I). Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA.

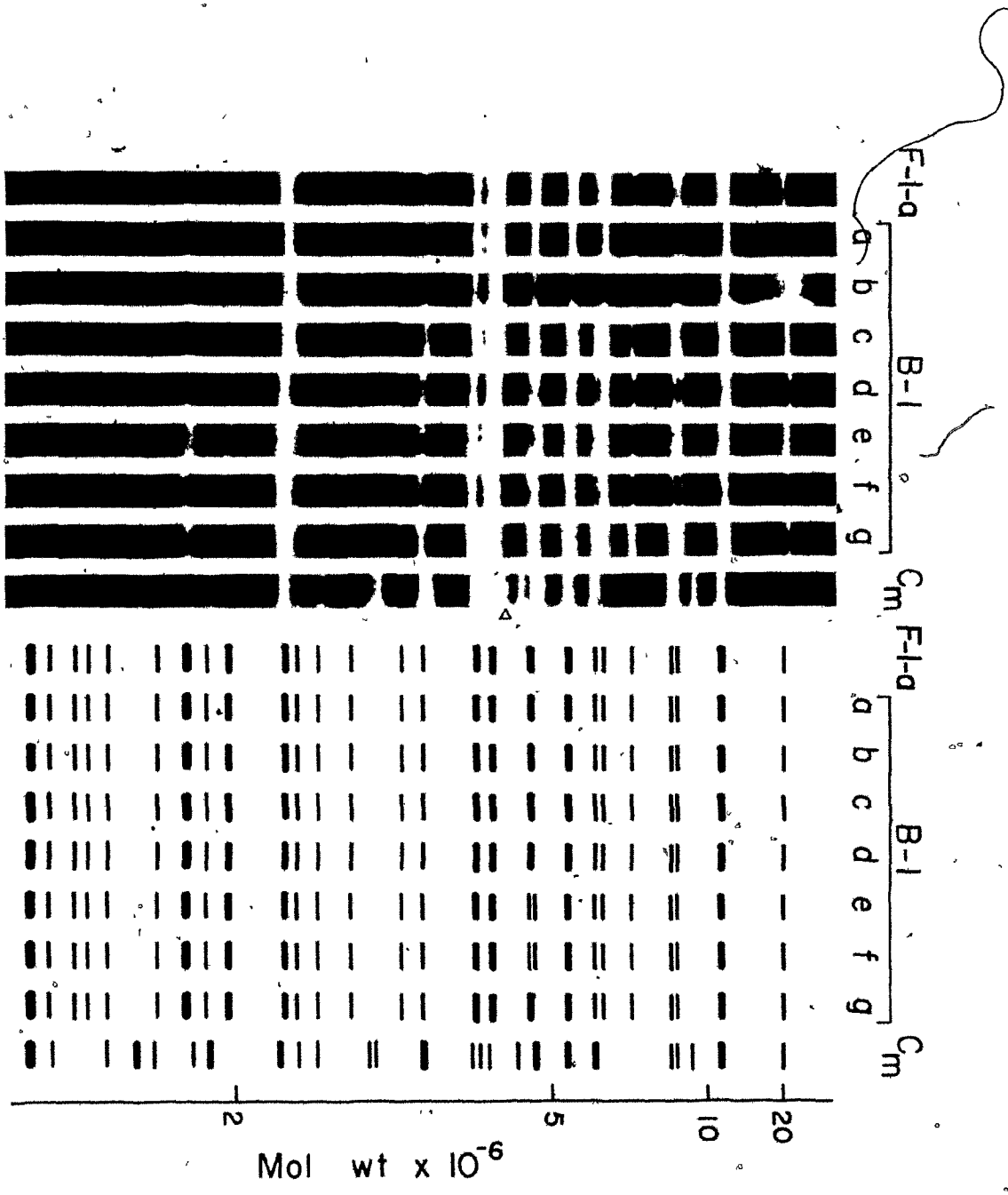

F-1-a
a
b
c
d
e
f
g
B-1
Cm
F-1-a
a
b
c
d
e
f
g
B-1
Cm
2
5
10
20
Mol wt x 10^{-6}

FIGURE 20. Electrophoretic patterns of *Bst*EII digests of cpDNA from subclones of seven B_1 hybrid meiotic products (B-1-a through -g) and from the F_1 hybrid (F-1-a) and *C. moewusii* (C_m) parental strains. B-1 (-a through -c) were recovered from the cross F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*mt*-) while B-1 (-d through -g) were recovered from the cross F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*er-nM*1 *mt*-). The *Bst*EII cpDNA restriction pattern of *C. moewusii* (*mt*-) was indistinguishable from that of *C. moewusii* (*er-nM*1 *mt*-). All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA.

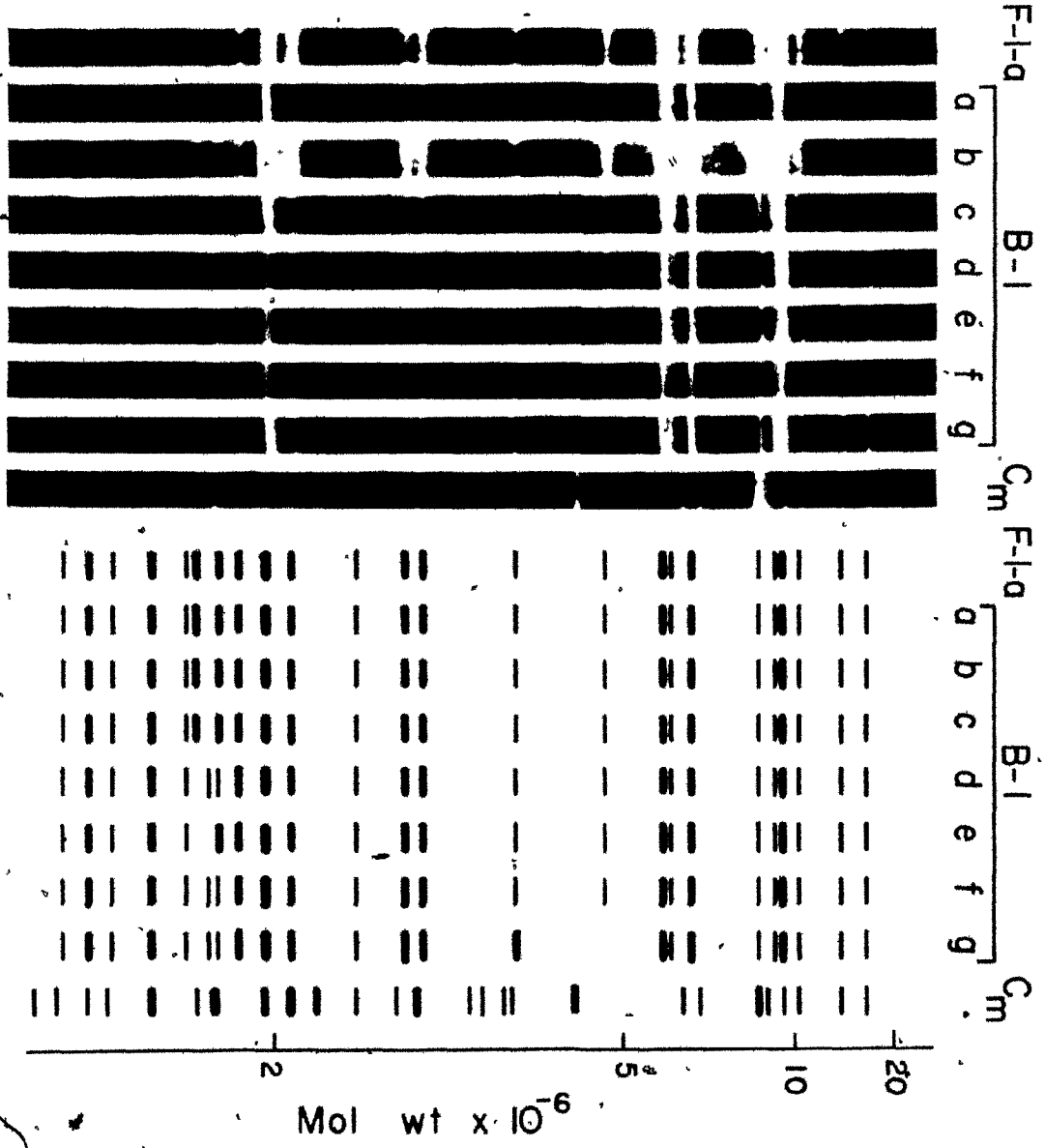

F-I-a
a
b
c
d
e
f
g
B-I
Cm
F-I-a
a
b
c
d
e
f
g
B-I
Cm
2
5
10
20
Mol wt x 10⁻⁶

FIGURE 21. Electrophoretic patterns of *Ava*I digests of cpDNA from four homoplasmic mitotic segregants derived from an F_1 biparental hybrid meiotic product (F-1-k). This hybrid product was recovered from the cross *C. eugametos* (*sr-2 mt+*) X *C. moewusii* (*er-nM1 mt-*). The non-Mendelian phenotypes of the segregants analysed were parental (S, E) or non-parental (+, ES). The electrophoretic patterns of *Ava*I digests of cpDNA from the *C. eugametos* [C_e(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. The 3.9 megadalton DNA band visible in the *C. moewusii* digestion pattern (see arrow) and in some hybrid patterns is not represented in the schematic diagrams because this DNA is not considered to be a digestion product of cpDNA (see Chapter I). Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA. Abbreviations: S = resistant to streptomycin and sensitive to erythromycin; E = resistant to erythromycin and sensitive to streptomcyin; + = sensitive to streptomycin and to erythromycin; ES = resistant to streptomycin, to erythromycin and to a mixture of erythromycin and streptomycin.

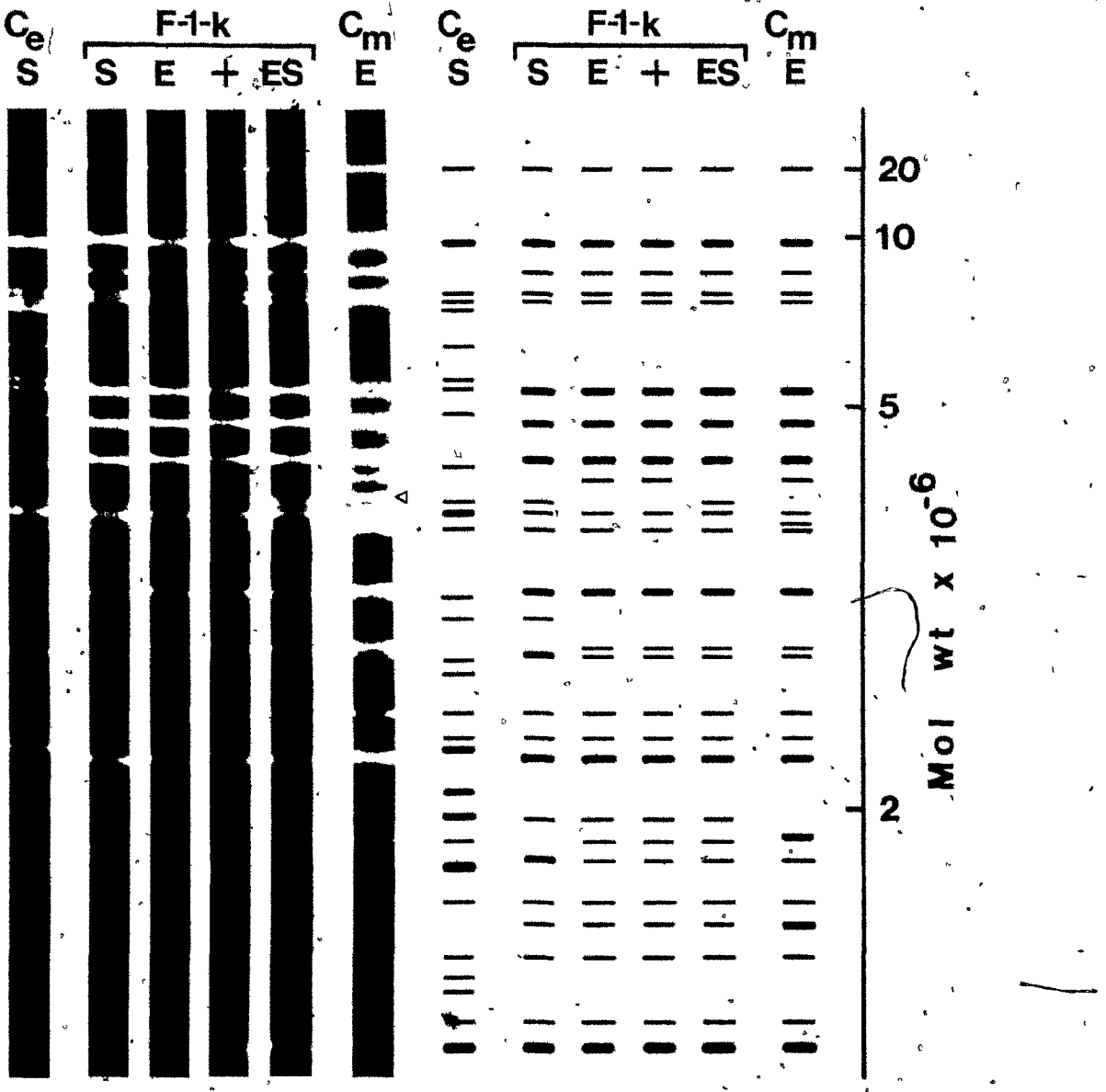

Ce
S
F-1-k
S E + ES
Cm
E
Ce
S
F-1-k
S E + ES
Cm
E
20
10
5
2
Mol wt x 10-6

FIGURE 22. Electrophoretic patterns of *Bst*EII digests of cpDNA from four homoplasmic mitotic segregants derived from an F_1 biparental hybrid meiotic product (F-1-k). This hybrid product was recovered from the cross *C. eugametos* (*sr*-2 *mt*+) X *C. moewusii* (*er-rM*1 *mt*-). The non-Mendelian phenotypes of the segregants analysed were parental (S, E) or non-parental (+, ES). The electrophoretic patterns of *Bst*EII digests of cpDNA from the *C. eugametos* [C_e(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA. Abbreviations: S = resistant to streptomycin and sensitive to erythromycin; E = resistant to erythromycin and sensitive to streptomycin; + = sensitive to streptomycin and to erythromycin; ES = resistant to streptomycin, to erythromycin and to a mixture of erythromycin and streptomycin.

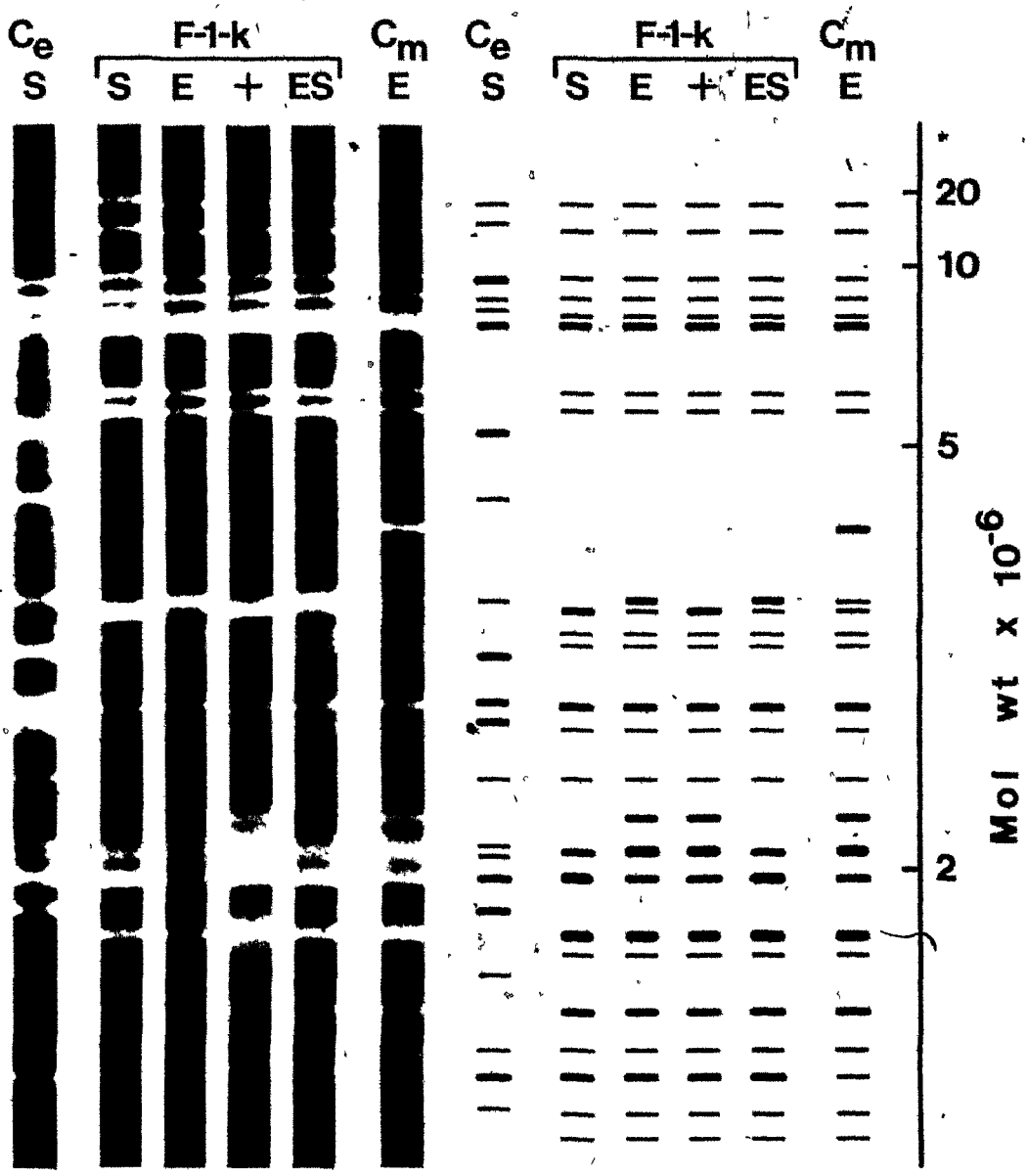

Ce
S
F-1-k
S
E
+
ES
Cm
E
Ce
S
F-1-k
S
E
+
ES
Cm
E
20
10
5
2
Mol wt x 10-6

FIGURE 23. Electrophoretic patterns of *Ava*I digests of cpDNA from homoplasmic mitotic segregants derived from two B_1 biparental hybrid meiotic products (B-1-i and B-1-j). These hybrid products were recovered from the cross F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*cr-nM*1 *mt*-). The non-Mendelian phenotypes of the segregants analysed were parental (S, E) or non-parental (+, ES). The electrophoretic patterns of *Ava*I digests of cpDNA from the F_1 hybrid [F-1-a(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. The 3.9 megadalton DNA band visible in the *C. moewusii* digestion pattern (see arrow) and in some hybrid patterns is not represented in the schematic diagrams because this DNA is not considered to be a digestion product of cpDNA (see Chapter I). Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA. Abbreviations: S = resistant to streptomycin and sensitive to erythromycin; E = resistant to erythromycin and sensitive to streptomycin; + = sensitive to streptomycin and to erythromycin; ES = resistant to streptomycin, to erythromycin and to a mixture of erythromycin and streptomycin.

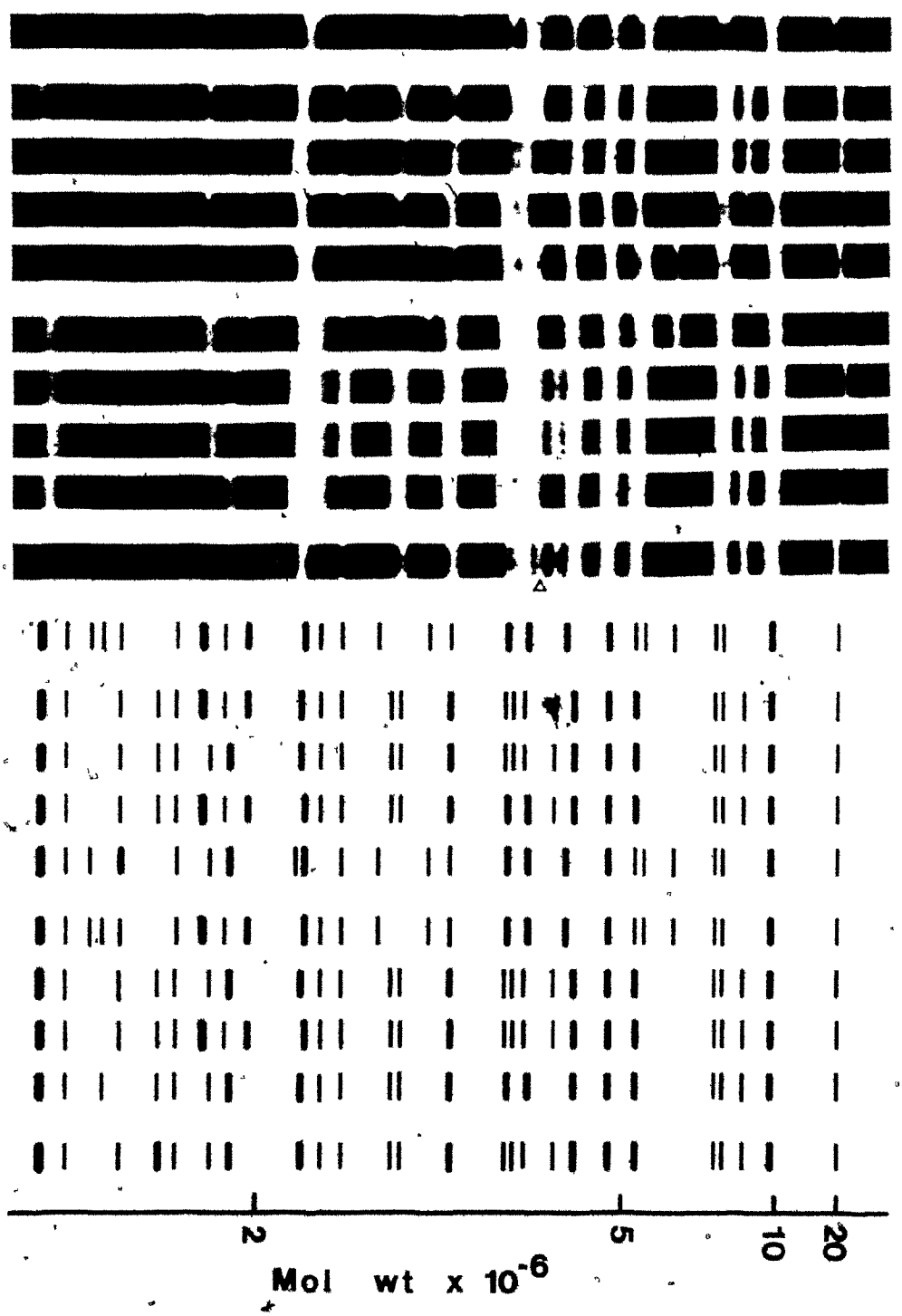

F-1-a
S
B-1-i
S
E
+
ES
B-1-j
S
E
+
ES
Cm
E
F-1-a
S
B-1-i
S
E
+
ES
B-1-j
S
E
+
ES
Cm
E
2
5
10
20
Mol wt x 10-6

FIGURE 24. Electrophoretic patterns of *Ava*I digests of cpDNA from homoplasmic mitotic segregants derived from two B_1 biparental hybrid meiotic products (B-1-k and B-1-l). These hybrid products were recovered from the cross F-1-a (*sr*-2 *pab*-1 *mt*+) X *C. moewusii* (*cr*-*nM*1 *mt*-). The non-Mendelian phenotypes of the segregants analysed were parental (S, E) or non-parental (+, ES). The electrophoretic patterns of *Ava*I digests of cpDNA from the F_1 hybrid [F-1-a(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. The 3.9 megadalton DNA band visible in the *C. moewusii* digestion pattern (see arrow) and in some hybrid patterns is not represented in the schematic diagrams because this DNA is not considered to be a digestion product of cpDNA (see Chapter I). Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA. Abbreviations: S = resistant to streptomycin and sensitive to erythromycin; E = resistant to erythromycin and sensitive to streptomycin; + = sensitive to streptomycin and to erythromycin; ES = resistant to streptomycin, to erythromycin and to a mixture of erythromycin and streptomycin.

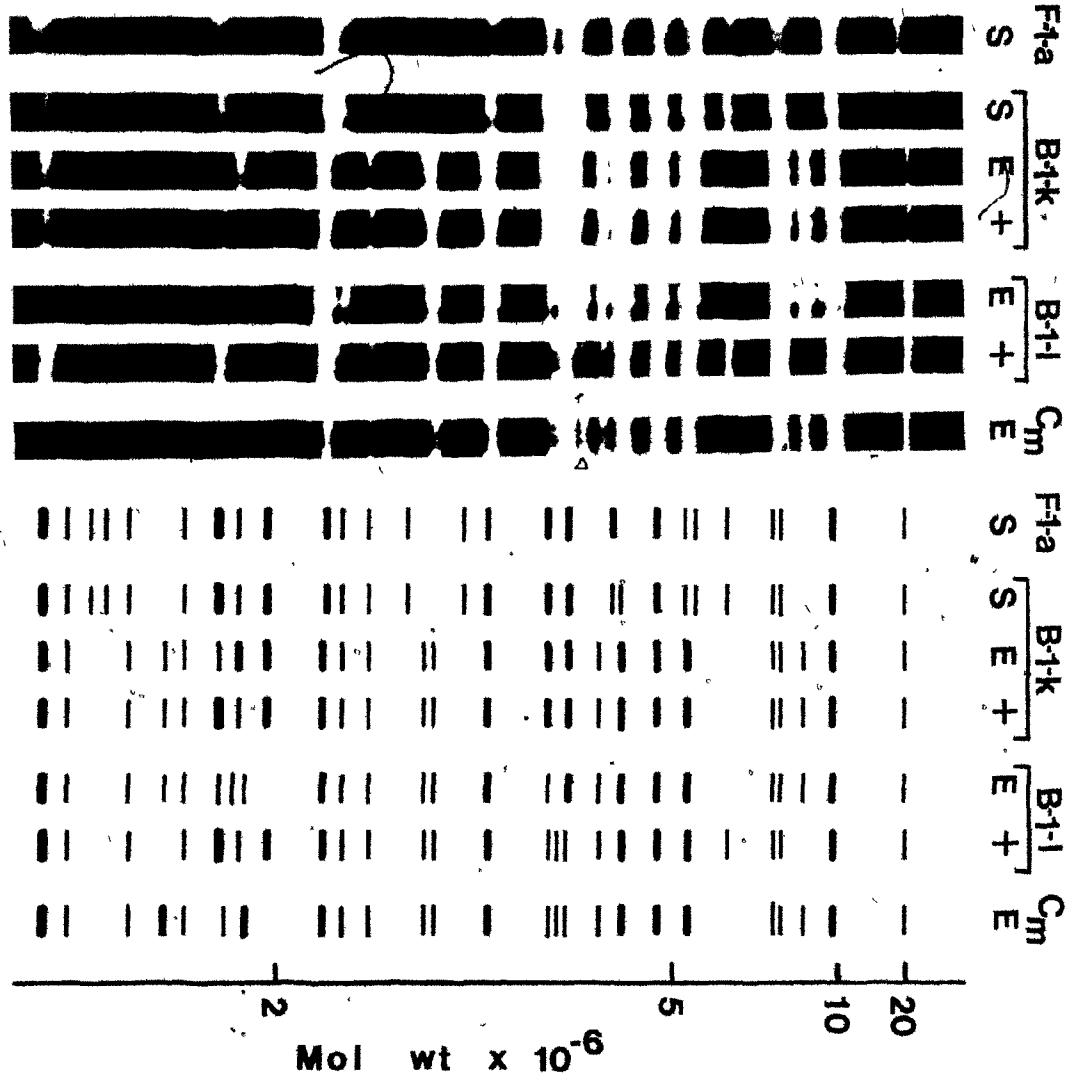

F-1-a
S
B-1-k
S
E
+
B-1-l
E
+
Cm
E
F-1-a
S
B-1-k
S
E
+
B-1-l
E
+
Cm
E
2
5
10
20
Mol wt x 10-6

FIGURE 25. Electrophoretic patterns of *Bst*EII digests of cpDNA from homoplasmic mitotic segregants derived from two B_1 biparental hybrid meiotic products (B-1-i and B-1-j). These hybrid products were recovered from the cross F-1-a (*sr-2 pab-1 mt+*) x *C. moewusii* (*er-nM1 mt-*). The non-Mendelian phenotypes of the segregants analysed were parental (S, E) or non-parental (+, ES). The electrophoretic patterns of *Bst*EII digests of cpDNA from the F_1 hybrid [F-1-a(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA. Abbreviations:
S = resistant to streptomycin and sensitive to erythromycin;
E = resistant to erythromycin and sensitive to streptomycin;
+ = sensitive to streptomycin and to erythromycin;
ES = resistant to streptomycin, to erythromycin and to a mixture of erythromycin and streptomycin.

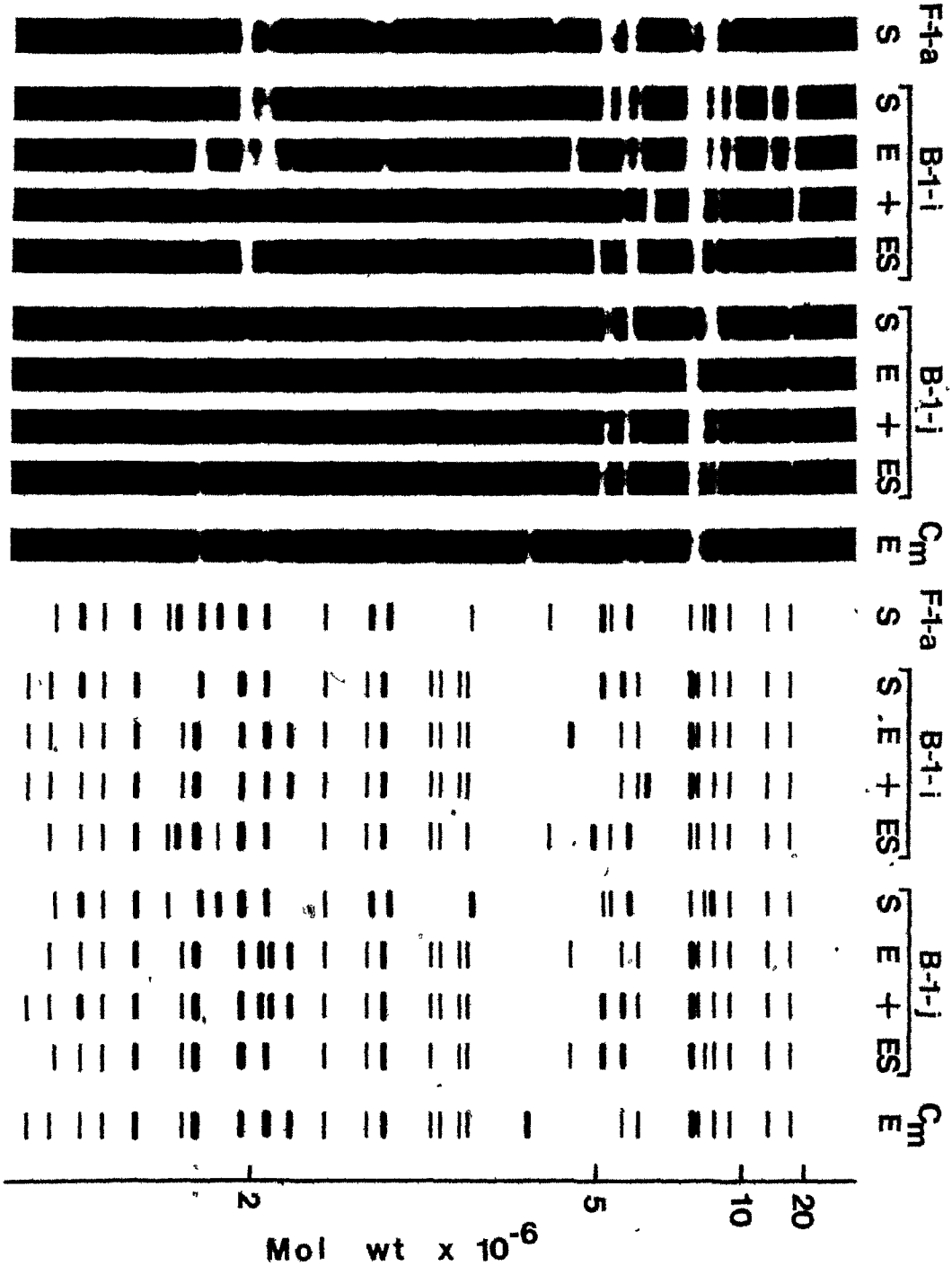
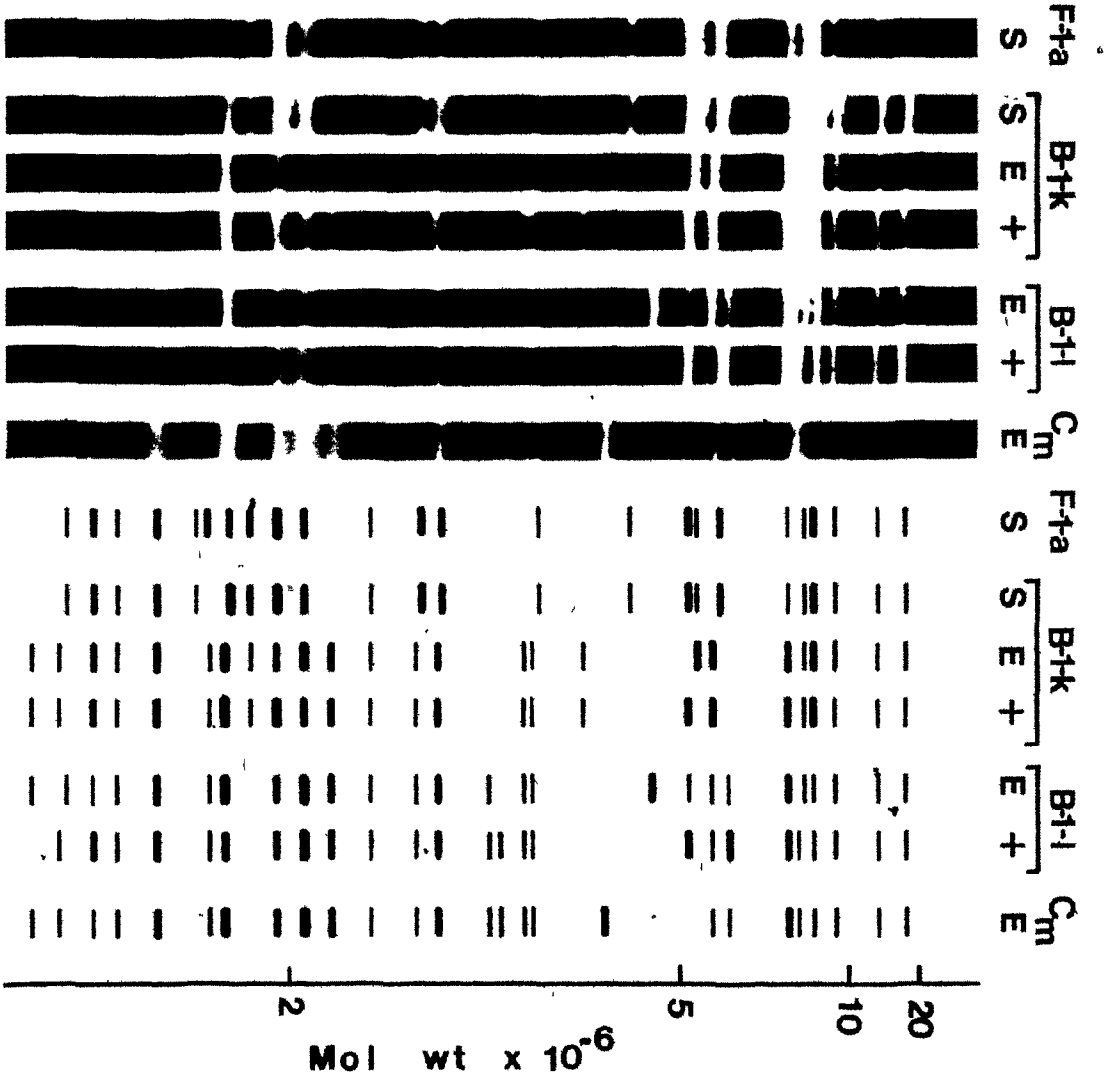

F-1-a
S
B-1-i
S
E
+
ES
B-1-j
S
E
+
ES
Cm
E
F-1-a
S
B-1-i
S
E
+
ES
B-1-j
S
E
+
ES
Cm
E
2
5
10
20
Mol wt x 10-6

FIGURE 26. Electrophoretic patterns of *Bst*EII digests of cpDNA from homoplasmic mitotic segregants derived from two B_1 biparental hybrid meiotic products (B-1-k and B-1-l). These hybrid products were recovered from the cross F-1-a (*sr-2 pab-1 mt+*) X *C. moewusii* (*er-nM1 mt-*). The non-Mendelian phenotypes of the segregants analysed were parental (S, E) or non-parental (+, ES). The electrophoretic patterns of *Bst*EII digests of cpDNA from the F_1 hybrid [F-1-a(S)] and *C. moewusii* [C_m(E)] parental strains are also shown for comparison with the progeny. All cpDNA digests were not run on the same gel; minor differences in mobility of the patterns are corrected in the schematic diagrams. Increasing line thicknesses represent bands containing one, two or three fragments. The molecular weight scale is based on the electrophoretic mobility of reference fragments from the *Bam*HI and the *Eco*RI digests of *C. reinhardtii* cpDNA. Abbreviations: S = resistant to streptomycin and sensitive to erythromycin; E = resistant to erythromycin and sensitive to streptomycin; + = sensitive to streptomycin and to erythromycin; ES = resistant to streptomycin, to erythromycin and to a mixture of erythromycin and streptomycin.


F+a
S
B-1k
S
E
+
B-1-l
E
+
Cm
E
F+a
S
B-1k
S
E
+
B-1-l
E
+
Cm
E
2
5
10
20
Mol wt x 10^-6

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LITERATURE CITED

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ADDENDUM

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Since the completion of this thesis research the preliminary results of Mets (1977, 1979) have been published (Mets 1980) as have some of the results reported in this dissertation (Lemieux et al. 1980, 1981).

Mets, L.J. (1980) Uniparental inheritance of chloroplast DNA sequences in interspecific hybrids of *Chlamydomonas*. Curr. Genet. *2*: 131-138.

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