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Early Vegetative Segregation of Mitochondrial Genes in *Saccharomyces cerevisiae*

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The vegetative segregation of seven mitochondrial gene loci was studied in yeast. At various times after mating antibiotic resistant and sensitive strains, samples of the diploid progeny were examined to determine the segregation rates of the alleles at each locus in three- and four-factor crosses. The rate of segregation was approximately the same for the *cap1*, *ery1*, *oli1*, *oli2*, and *par1* loci, which are scattered over about two-thirds of the mitochondrial DNA molecule. Differences in segregation rates were found but showed no consistent relationship to the map positions of the loci. This is in contrast to the segregation of chloroplast genes in *Chlamydomonas*, where loci segregate at rates proportional to their distance from an "attachment point" which appears to govern the partitioning of chloroplast DNA molecules between daughter chloroplasts when the chloroplast divides. Our data are compatible with a model in which the mitochondrial DNA molecules in a cell occur in a small number of groups corresponding to individual nucleoids or mitochondria. Most or all of the molecules in a group carry the same allele at any given locus. These genetically homogeneous groups of molecules may thus be the units of segregation, and may be partitioned randomly between mother cell and bud at each division.

The vegetative segregation of alleles is an important phenomenon of cytoplasmic inheritance, which clearly differentiates the behavior of genes in mitochondria and chloroplasts from that of eukaryotic nuclear genes. Consider for example a cross between two yeast strains, one sensitive to chloramphenicol and erythromycin (mitochondrial genotype C^SE^S) and the other resistant to both antibiotics (C^RE^R). The zygotes contain a mixture of mitochondrial DNA (mtDNA) molecules of genotypes C^SE^S and C^RE^R and are said to be heteroplasmic at the C and E loci. They are also heterozygous for any nuclear markers involved in the cross. Recombination produces mtDNA molecules with additional genotypes (C^SE^R and C^RE^S). The zygotes reproduce vegetatively by budding; since all divisions are mitotic, the progeny are all diploid and heterozygous for the nuclear markers. But at each division some progeny are produced which are homoplasmic for one mitochondrial allele or genotype. After a number of generations, virtually all of the diploid progeny of the heteroplasmic zygotes have become homoplasmic for one or another of the mitochondrial genotypes (here, C^SE^S, C^RE^R, C^SE^R, or C^RE^S) and also for one allele or the other at each locus. This segregation of alleles during vegetative (mitotic) reproduction is an almost universal characteristic of genes in mitochondria and chloroplasts (Birky, 1978; Gillham, 1978).

The mechanism of vegetative segregation of organelle gene alleles is not fully understood, but is widely believed to involve an element of randomness in the physical partitioning or segregation of the organelles themselves or of the organelle DNA molecules during cell division. In plants, the random segregation of green and mutant white chloroplasts has been postulated to account for the production of variegated progeny following a cross, and for the pro-

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duction of sectors of pure white mutant tissue following mutation in a single chloroplast (Kirk and Tilney-Bassett, 1978). Random assortment of plasmid DNA molecules has been postulated to explain cases of plasmid incompatibility, the production of progeny bacteria which have lost one or the other of a pair of genetically similar plasmids present in the parent cells (Novick and Hoppensteadt, 1978; Ishii *et al.*, 1978).

Random models of segregation have been proposed for yeast mitochondrial genes. Dujon *et al.* (1974) assumed that the mtDNA molecule itself is the randomly segregating unit. In other words, the bud receives a sample of mtDNA molecules from the mother; the probability that a particular molecule in that sample will be of a particular genotype is determined solely by the frequency of that genotype in the mother. To account for the rapid rates of segregation observed, Dujon *et al.* postulated that only two or three mtDNA molecules enter the bud, out of the 100 or more molecules in the mother cell. However, the pedigree data of Birky *et al.* (1978b) and the biochemical evidence of Sena *et al.* (1976) suggest that the bud must receive a large number of mtDNA molecules (see also Callen, 1974; Birky, 1975; Strausberg and Perlman, 1978). Birky (1975; see also Dujon and Slonimski, 1976; Birky *et al.*, 1978b) suggested that the large number of mtDNA molecules in a yeast cell might be organized into a small number of genetically homogeneous groups, possibly corresponding to the nucleoids observed by Williamson *et al.* (1978) or to the mitochondria themselves. These groups of molecules might then segregate randomly. The partial pedigree data of Birky *et al.* (1978b) would fit such a model if the number of groups in the mother cell were quite small, usually less than a dozen, with only one or two entering the bud.

In Birky's model it is a *group* of molecules which is the randomly segregating unit. Individual molecules and gene loci are segregating *non*randomly: like molecules tend to segregate together. They tend to be found in the same group (nucleoid or mitochondrion), because they originated from the same parent cell when the zygote was formed. In other words, molecules segregate nonrandomly because their spatial distribution within the zygote is nonrandom. During the first division or two of the zygote, the groups of molecules themselves are probably not randomly distributed, as shown by cytochemical staining experiments and by differences in the genotypes of first buds which come from one parental end or the other of the zygote (Callen, 1974; Strausberg, 1976; Strausberg and Perlman, 1978; Waxman and Birky, unpublished data). However, nonrandom spatial distribution affects all loci on a molecule equally, and the position of a locus on the mtDNA molecule should have no effect on the segregation rate of alleles at that locus.

This does not appear to be the case for the genes in the single chloroplast of Chlamydomonas reinhardtii. Sager (1972; Sager and Ramanis, 1976a,b) studied the frequency of "Type III" segregation events, those in which a heteroplasmic cell divided to produce two homoplasmic daughter cells. She found that the frequency of these events is different for different gene loci on the chloroplast genetic map. The farther a locus is from a fixed point on the map, called the "attachment point" or *ap*,[3] the more often do alleles at that locus show Type III segregation. To explain this result, Sager postulated that the segregation of chloroplast DNA (cpDNA) molecules is governed by the *ap*, which functions like the centromere (kinetochore) on a nuclear chromosome at mitosis. At each cell division, the *ap*'s of sister cpDNA molecules (derived by replication from one molecule) segregate into different chloroplasts and hence into different daughter cells. In this model, a heteroplasmic cell will produce two homoplasmic daughter cells only if there is a crossover between the *ap* and the locus which is segre-

[3] Abbreviations used: *ap*, attachment point; MMD, minimal medium with glucose; RG, complete medium with glycerol; HETS, heteroplasmic diploids.

gating, just as alleles on nuclear chromosomes segregate during mitosis only if there is a crossover between the locus of the alleles and the centromere. The farther a gene locus is from the *ap*, the more often there will be a crossover between them and the greater will be the rate of segregation of alleles at that locus.

Sager's model requires that there be only two cpDNA molecules per cell, which replicate to produce four prior to crossing over and cell division. However, there is substantial evidence that *Chlamydomonas* contains many cpDNA molecules in its chloroplast (Gillham, 1978), organized into a small number of nucleoids (Birky and Lorenz, unpublished data). To accommodate these observations as well as the genetic data of Sager and her colleagues, VanWinkle-Swift (1980) proposed a model which retains the concept of attachment points but also assumes that the nucleoids of a zygote are nonrandomly segregated at division due to nonrandom spatial distribution within the chloroplast.

Differences in the rates of segregation of different yeast mitochondrial gene loci have also been reported (Callen, 1974, 1975; Uchida and Suda, 1976) and led Callen to propose an attachment point model for yeast, but these differences have not been correlated with map position. At least three loci must be followed in a cross in order to establish such a correlation. In this paper we report on three- and four-factor crosses in which the segregation of alleles at each locus was followed from approximately the time of the first zygotic division until segregation is complete. We find no evidence to suggest that there is an attachment point that plays an important role in the segregation of mtDNA molecules in yeast. We also show data which describe the process of segregation in terms of changes in the distribution of allele frequencies among cells.

MATERIALS AND METHODS

The stocks, their sources, and their genotypes are listed in Table 1. The abbreviated symbols C^R/C^S, E^R/E^S, O^R/O^S, and P^R/P^S are used for pairs of mitochondrial alleles determining resistance/sensitivity to chloramphenicol, erythromycin, oligomycin, and paromomycin, respectively. Figure 1 shows the map positions of the loci. All crosses were homopolar ($\omega^+ \times \omega^+$). Media are described by Birky *et al.* (1978a); the incubation temperature was 30°C.

TABLE 1

STRAINS USED IN SEGREGATION EXPERIMENTS

Strain	Nuclear genotype	Mitochondrial genotype[a]	Source
A11-1	*α leu*	$\omega^+C^SE^SO^SP^S$	Callen
A11-5	*a trp*	$\omega^+C^SE^SO^SP^S$	Callen
A15-295A	*α arg met*	$\omega^+C^RE^RO^R_{II}P^R$	Treat
A21-112	*α iso val*	$\omega^+C^RE^RO^R_IP^R$	Treat
A21-36	*a ade*	$\omega^+C^RE^RO^R_IP^R$	Treat
2-36	*α trp*	$\omega^+C^RE^RO^SP^R$	Kleese
D22A15	*a ade*	$\omega^+C^SE^SO^R_{II}P^S$	Griffiths
D22A19	*a ade*	$\omega^+C^SE^SO^R_{III}P^S$	Griffiths
D22A21	*a ade*	$\omega^+C^SE^SO^R_IP^S$	Griffiths
LM870	*a his*	$\omega^+C^SE^SO^R_VP^S$	Slonimski
41	*a leu*	$\omega^+C^SE^SO^SP^S$	Forster
4120E	*a leu*	$\omega^+C^SE^RO^SP^S$	Forster
51	*α his trp met*	$\omega^+C^SE^SO^SP^S$	Forster
LT70	*α lys*	$\omega^+C^RE^SO^R_IP^R$	Treat
3-2/1	*α his*	$\omega^+C^RE^RO^R_IP^S$	Perlman
4810	*a lys*	$\omega^+C^SE^SO^SP^R$	Kleese

[a] The abbreviated symbols represent the following loci: C^R, *cap^{r}1*; E^R, *ery^{r}1*; O^R_I, *oli^{r}1*; O^R_{II}, *oli^{r}2*; O^R_{III}, *oli^{r}3*; O^R_{IV}, *oli^{r}4*; and P^R, *par^{r}1*.

To measure the rate of vegetative segregation we do crosses to produce heteroplasmic zygotes (e.g., C^R/C^S) and monitor the appearance of homoplasmic cells (C^R or C^S) among their progeny after varying numbers of generations, until segregation is complete (virtually all of the diploid progeny are homoplasmic for one allele or the other at each locus studied). Cells are classified as homoplasmic or heteroplasmic by plating them on nonselective medium where they produce a clone in which segregation is completed if the parent cell was heteroplasmic. Homoplasmic-sensitive (e.g., C^S) cells are easiest to identify; they produce a clone containing

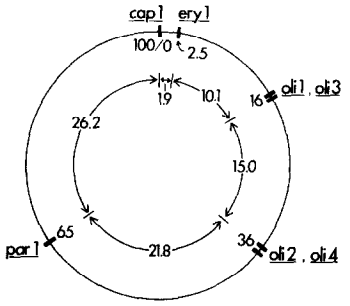

FIG. 1. Map of the mitochondrial gene loci whose segregation was studied. The outer circle represents the mtDNA molecule of *S. cerevisiae* divided into 100 units. The entire molecule contains about 75 kilobase pairs; the inner circle shows the distances between adjacent markers in kbp. The positions of the *cap1*, *oli1*, *oli2*, and *par1* loci are indicated as determined by coretention of markers in *petite* deletion mutants (Schweyen *et al.*, 1978); the positions agree well with those determined by physical mapping with overlapping deletions and restriction analysis. The position of the *ery1* locus, mapped by these physical methods, is from Jacq *et al.* (1977), based on a *cap1-ery1* distance midway between their lower and upper estimates of 1.3 and 2.5 kbp for ω^+ strains. The *cap1* and *ery1* loci are in the gene coding for the 21 S rRNA; *par1* is in or near the gene for 15 S rRNA; *oli1* is in the gene for subunit 9 of the oligomycin-sensitive ATPase; the very closely linked *oli3* is either in the gene for subunit 9 or that for subunit 6; *oli2* (and the very closely linked *oli4*) are in the gene for another subunit of the same complex.

only sensitive cells and that clone will not grow when replica-plated onto medium containing the antibiotic (chloramphenicol in the example). Homoplasmic resistant (C^R) cells produce pure resistant clones which will grow when replica-plated onto the antibiotic. Heteroplasmic (C^R/C^S) cells produce clones containing a mixture of homoplasmic-resistant (C^R) and -sensitive (C^S) cells, and such clones will also show growth on antibiotic replica plates. These last two classes of cells can only be distinguished by replica-plating subclones.

Log phase haploid strains in liquid RD (complete medium with glucose) were mated for 2 h in a shaker at a concentration of approximately 10^7 cells/ml. At the end of the 2 h, aliquots were taken from the suspension and diluted to a concentration of approximately 10^3 cells/ml in liquid MMD (minimal medium with glucose). The dilution stops the initiation of new mating. The haploid parents in each cross carried complementing auxotrophic mutations (e.g., *ade* and *trp*); only the zygotes and their diploid progeny are prototrophic (e.g., *ade trp*$^+$/*ade*$^+$ *trp*) and can grow in minimal medium. Samples designated t_0 were taken immediately from the MMD flasks and plated on MMD plates. At various times after mating (4, 8, 12, 22, and in some crosses 26 or 28 h) additional samples were taken from the MMD flasks and plated on MMD. After several days of growth, colonies were picked and patched on fresh MMD plates. These master plates were incubated overnight and then replica-plated to RG (complete medium with glycerol) and RG plus appropriate antibiotics. After 2 days' growth the percentage colonies containing only sensitive cells was scored for each locus in the cross. This method, called *clonal analysis*, gives the percentage homoplasmic-sensitive cells.

An unbiased cross is one in which the *a* and α parent cells contribute approximately equal numbers of mtDNA molecules to the zygotes, and in consequence the final frequencies of alleles from the two parents are approximately equal. For such crosses it can be assumed that equal frequencies of homoplasmic-resistant and homoplasmic-sensitive cells are present at each time; then the total percentage homoplasmic cells at any given time is twice the percentage homoplasmic-sensitive cells estimated by the above method. Then the percentage heteroplasmic diploids (percentage HETS) = 100% − 2 (percentage homoplasmic-sensitive diploids). In a biased cross, where alleles from one parent have a frequency greater than 50%, those majority alleles will initially segregate more rapidly than the minority alleles from the other parent and at any given time the homoplasmic-resistant cells may be more or less frequent than the sensitive. Consequently the percentage HETS cannot be calculated from the percentage homoplasmic-sensitive cells.

A method (called *subclone analysis*) which avoids this problem was used for three crosses (A11-5 × A15-295A, A11-5 × A21-112, and A11-1 × A21-36). Samples of 50 or 100 clones were selected at random from MMD plates from each time t_0–t_{12}. These clones were individually suspended in liquid, streaked on MMD plates to subclone them, and replica-plated after 2 days' growth. In this way homoplasmic-resistant and heteroplasmic cells as well as homoplasmic-sensitive cells were identified and the exact percentage HETS determined.

The output frequencies of the various alleles in each cross were determined by standard random diploid analysis, as follows. The t_0 plates contained several hundred colonies, most of which were derived from single unbudded zygotes. All of these zygote clones were washed off a plate, suspended in distilled water, diluted, and about 100 cells plated on each of several MMD plates. Most of these cells were homoplasmic, since segregation was essentially complete by the time the zygote clones were harvested, and each produced a colony of resistant or sensitive cells after incubation. The percentage resistant and sensitive clones, determined by replica-plating these master plates, thus gives the frequency of the resistant and sensitive alleles in the cross.

RESULTS

Control Experiments

In these experiments it was important for mating to be confined to a short interval (2 h) so that cell division and segregation should be approximately synchronous. This was insured by diluting the mating mixture to 10^3 cells/ml immediatey after the 2 h mating period. To see if this dilution was sufficient to prevent further mating, immediately after the parent strains were mixed a sample was taken and diluted to 10^3 cells/ml in MMD. Samples were plated from this control and from experimental flasks at the same time. No diploid progeny grew on the control plates in any of the experiments, showing that this dilution was sufficient to stop mating.

A control experiment was done to see if one allele of a gene might have a selective advantage over the other during the period of growth in liquid. Homoplasmic diploids from the crosses LT70 × 4120E and A11-5 × A21-112 were combined in a 1:1 ratio and inoculated in MMD flasks at a concentration of approximately 10^3 cells/ml. For example, combinations from the LT70 × 4120E cross were $C^SE^S + C^RE^R$ and $C^RE^S + C^SE^R$. Samples were taken at 0, 4, 8, and 12 h from the flasks and plated. These master plates were incubated and then replica-plated to classify the clones by genotype. The output genotype frequencies in this reconstruction experiment were not significantly different from the inputs (50% of each genotype). These data indicate that there was no significant selective advantage of any allele at the *cap1*, *ery1*, or *oli1* loci, or of any combination of alleles.

The parent strains in each cross were tested just before mating to determine the frequency of spontaneous *petite* mutants and prototrophic revertants; if these were high enough to affect the results, the mating was discarded.

Early Segregation Rates by Subclone Analysis

Table 2 summarizes the results from the three crosses in which subclone analysis was used to determine the exact frequencies of heteroplasmic and homoplasmic diploids, 0, 4, 8, and 12 h after mating. In each cross the frequency of heteroplasmic cells decreased and the frequency of homoplasmic cells increased with time at each locus. Segregation was not complete at t_{12} (about eight generations), since there were still 2–30% HETS remaining. There appear to be variations in the segregation rates for the various loci, but the differences show no consistent relationship to map order and are probably due in large part to the small numbers of diploids that can be examined

TABLE 2

RESULTS FROM DETERMINATION OF EXACT PERCENTAGE HETS FOR $C^SE^SO^SP^S \times C^RE^RO^RP^R$ CROSSES BY SUBCLONE ANALYSIS

Cross $C^SE^SO^SP^S \times C^RE^RO^RP^R$	Time	Percentage HETS for C	E	O	P	N
A11-5 × A21-112	$_0$	91.9	92.9	89.9	88.9	99
(*oli1*)	t_4	59.0	61.0	52.0	53.0	100
	t_8	33.0	39.0	21.0	28.0	100
	t_{12}	11.0	14.0	7.0	13.0	100
A11-5 × A15-295A	t_0	60.0	84.0	78.0		50
(*oli2*)	t_4	58.0	74.0	58.0		50
	t_8	18.0	38.0	6.0		50
	t_{12}	22.0	30.0	4.0		50
A11-1 × A21-36	t_0	80.0	80.0	74.0	70.0	50
(*oli1*)	t_4	36.0	54.0	40.0	48.0	50
	t_8	18.0	30.0	24.0	16.0	50
	t_{12}	8.0	16.0	6.0	2.0	50

by subclone analysis. The viable cells plated at t_0 were almost entirely unbudded zygotes, including those that were classified as homoplasmic. Since each zygote begins with mitochondrial alleles from both parents and must be heteroplasmic at all loci, one might expect all the t_0 cells to be scored as HETS for all markers. In fact, about 10–20% of the zygotes produced clones in which one allele or the other, at the locus being scored, has disappeared. These are uniparental zygotes (Birky *et al.*, 1978a), in which vegetative segregation cannot be studied.

One can determine the frequency of any allele, e.g., C^S, among the progeny of any heteroplasmic cell identified by subclone analysis. This in turn is an estimate of the frequency of that same allele in the population of mtDNA molecules in the heteroplasmic cell itself. Figure 2 presents a representative sample of these data in the form of frequency distributions (complete data and figures for all loci and crosses are in Treat, 1978). The height of each bar gives the percentage cells which have allele frequencies within a 10% interval; solid bars represent allele frequencies of 0% (homoplasmic C^R) or 100% (homoplasmic C^S). At t_0 the solid bar represents uniparental zygotes which transmitted only the C^S allele to their progeny. The distributions show a gradual shift of cells from heteroplasmic to homoplasmic. There are more homoplasmic C^S than homoplasmic C^R cells at each time because the cross is biased, with an output of the C^S allele greater than 50%. The distribution of allele frequencies among the heteroplasmic cells in these experiments was nearly uniform from time t_4 or t_8 on; i.e., all allele frequencies are about equally common among the HETS.

Segregation Rates by Clonal Analysis

The results of clonal analysis of three- and four-factor crosses are given in Table 3. The first six crosses in the table are essentially unbiased, with mean allele frequencies in the random diploids ranging from 47 to 57% (not far from 50%). The last five crosses are highly biased, with the frequencies of the minority alleles ranging from 13 to 35%. Figures 3 and 4 show representative results from unbiased and biased crosses, respectively (figures for all crosses are in Treat, 1978). When biased outputs were obtained only the rates of segregation of homoplasmic-sensitive alleles from the *same* parent

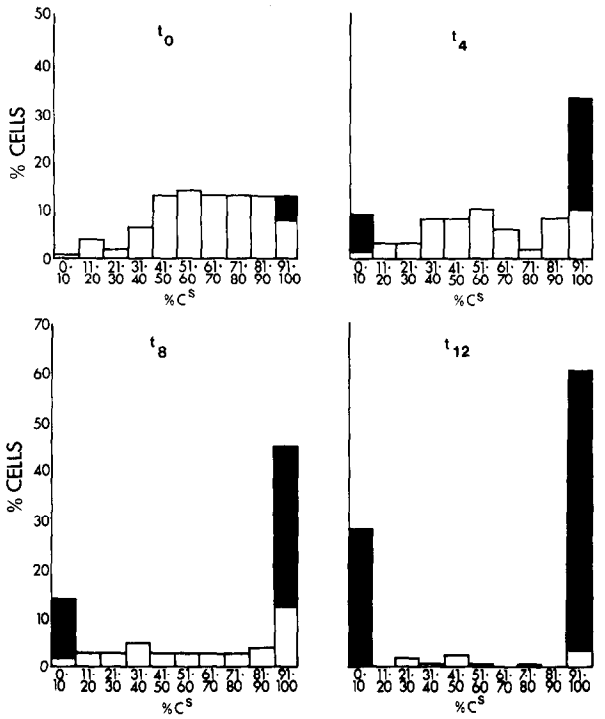

FIG. 2. Subclone analysis of 100 cells selected at random for times 0, 4, 8, and 12 h to determine proportion of HETS for the cross A11-5 × A21-112. C^R/C^S HETS given. Height of shaded bars gives percentage cells which are 0% C^S or 100% C^S.

could be compared. In an unbiased cross the rates of formation of homoplasmic-sensitive cells could be compared for all loci, irrespective of which parent contributed the sensitive allele.

In the unbiased cross 4810 × 3-2/1 the three sensitive alleles C^S, E^S, and O^S originated from 4810 while the P^S allele came from 3-2/1. The rate of appearance of homoplasmic-sensitive progeny from all four loci was essentially the same; that is, there was no difference in segregation rates among the *cap1*, *ery1*, *oli1*, and *par1* loci. Note that these loci are distributed throughout the mitochondrial map (Fig. 1).

The unbiased cross A11-5 × A21-112 also involved the *cap1*, *ery1*, *oli1*, and *par1* loci with the sensitive alleles originating from A11-5. The cross showed similar frequencies of cells homoplasmic for C^S, E^S, O^S, and P^S at each time point (Fig. 3), indicating that the segregation rates of the four loci are similar. These four loci did not show identical percentages of homoplasmic cells at every time point, but there was no relationship to map position that was consistent at every time point. This confirms the results obtained in the repetition of this cross which was analyzed by subclone analysis (Table 2). Cross A11-1 × A21-36 involved the same four loci, but the sensitive alleles were contributed by the α parent rather than the a parent. Nevertheless, the segregation rates were similar for all loci in these two crosses. The cross A11-5 × A15-295A also involved the *cap1*, *ery1*, and *par1* loci, this time with the *oli2* locus. In this cross the frequency of homoplasmic P^S cells was significantly lower at each time point, but the remaining three loci showed no significant differences in segregation rates. Comparison of the segregation rate of the *oli2* locus in this cross with that of the *oli1* locus in the preceding two crosses cannot be made because of the

TABLE 3

EARLY SEGREGATION DATA OF VARIOUS HOMOPOLAR CROSSES, FROM CLONAL ANALYSES

Cross	Percentage homoplasmic for:	Time					Random diploids
		t_0	t_4	t_8	t_{12}	t_{22}	
4810 × 3-2/1	C^S	8.3	19.2	42.7	41.2	44.7	47.2
$C^SE^SO^SP^R$ ×	E^S	7.5	11.7	37.1	32.8	38.0	46.4
$C^RE^RO^RP^S$	O^S (*oli1*)	6.1	12.5	29.7	32.9	34.7	48.4
	P^S	12.8	17.1	31.1	37.5	31.0	53.2
N		360	385	569	400	300	250
A11-5 ×	C^S	10.3	34.8	52.2	60.3	61.8	61.7
A21-112	E^S	6.4	26.3	43.8	52.0	58.7	56.2
$C^SE^SO^SP^S$ ×	O^S (*oli1*)	6.7	32.2	48.9	54.4	57.3	55.6
$C^RE^RO^RP^R$	P^S	9.7	29.2	41.5	50.2	51.3	55.8
N		1469	1617	1750	1818	2400	2000
A11-1 ×	C^S	13.8	42.1	63.3	67.3	63.1	55.3
A21-36	E^S	7.9	31.4	52.2	58.5	60.2	50.4
$C^SE^SO^SP^S$ ×	O^S (*oli1*)	7.9	34.4	56.0	62.5	56.2	47.0
$C^RE^RO^RP^R$	P^S	9.5	16.0	41.2	50.7	47.0	50.0
N		391	337	1135	1340	1829	2490
A11-5 ×	C^S	8.7	16.7	37.6	36.2	44.0	42.1
A15-295A	E^S	5.0	7.9	20.6	25.7	37.8	41.4
$C^SE^SO^SP^S$ ×	O^S (*oli2*)	7.7	17.1	39.8	36.6	46.0	37.6
$C^RE^RO^RP^R$	P^S	23.2	28.7	33.9	28.1	32.9	66.3
N		323	216	505	751	1455	1000
2-36 ×	C^S	14.0	28.1	43.2	49.9	56.9	57.1
D22A21	E^S	7.9	17.0	34.7	43.0	52.0	55.4
$C^RE^RO^SP^R$ ×	O^S (*oli1*)	4.5	21.9	35.3	40.5	34.5	45.6
$C^SE^SO^RP^S$	P^S	10.6	19.5	32.2	33.9	41.3	41.2
N		265	406	1000	1000	1000	1400
2-36 ×	C^S	13.3	34.1	46.3	60.7	57.8	51.4
D22A19	E^S	5.4	18.7	29.4	34.3	53.0	48.2
$C^RE^RO^SP^R$ ×	O^S (*oli3*)	5.0	21.4	36.6	38.2	36.6	47.2
$C^SE^SO^RP^S$	P^S	6.9	21.6	27.3	33.7	44.0	40.6
N		686	921	1000	1000	1000	1400
2-36 ×	C^S	31.1	42.7	54.3	60.6	68.8	70.6
D22A15	E^S	18.3	32.7	46.8	54.4	67.1	71.1
$C^RE^RO^SP^R$ ×	O^S (*oli2*)	2.5	12.1	29.6	30.8	32.2	25.7
$C^SE^SO^RP^S$	P^S	23.7	31.5	51.2	57.1	68.0	65.6
N		367	330	818	1180	1600	2118
2-36 ×	C^S	7.5	19.9	36.2	43.4	39.7	28.6
LM870	E^S	3.8	15.1	28.7	33.6	40.8	36.8
$C^RE^RO^SP^R$ ×	O^S (*oli4*)	26.0	35.9	49.4	50.4	41.1	38.0
$C^SE^SO^RP^S$	P^S	3.4	11.8	15.5	32.5	38.2	36.8

TABLE 3—*Continued*

Cross	Percentage homoplasmic for:	Time					Random diploids
		t_0	t_4	t_8	t_{12}	t_{22}	
N		213	332	1037	1600	1600	1000
41 × LT70	C^S	2.0	7.3	14.4	16.1	18.0	18.6
$C^SO^SP^S$ ×	O^S (*oli1*)	0.2	2.9	6.2	8.2	6.7	9.3
$C^RO^RP^R$	P^S	1.1	4.0	7.8	9.2	7.6	14.2
N		1078	1200	1200	1200	1000	1698
4120E ×	C^S	3.8	7.0	11.2	15.6	20.0	14.0
LT70	E^S	24.3	42.6	62.2	69.1	74.6	84.4
$C^SE^RO^SP^S$ ×	O^S (*oli1*)	1.4	2.9	4.8	9.5	12.6	10.8
$C^RE^SO^RP^R$	P^S	1.6	3.5	5.5	11.8	11.6	12.6
N		1117	1189	1200	1200	1000	1674
4120E × 51	E^S	29.7	43.6	61.4	70.4	75.7	82.4
E^R × E^S, *N*		721	1000	1000	1000	1000	1230

Note. Every experiment was repeated at least twice, with two replicates in each experiment.

marked differences in transmission frequencies of the sensitive alleles.

Another group of experiments involved crossing the oligomycin-sensitive strain 2-36 to each of four strains carrying four different oligomycin resistance mutations, *oli1*, *oli2*, *oli3*, and *oli4*. Each of these loci segregated in its cross at about the same rate as the *cap1*, *ery1*, and *par1* loci which were followed in the same crosses. The crosses of 2-36 by D22A21 and D22A19, carrying mutations at *oli1* and *oli3*, respectively, are unbiased crosses and show comparable segregation rates for all loci. Unfortunately the segregation rates for *oli1* and *oli3* seen in these crosses cannot be compared with the rates for the distant markers *oli2* and *oli4* which are segregating in the crosses of 2-36

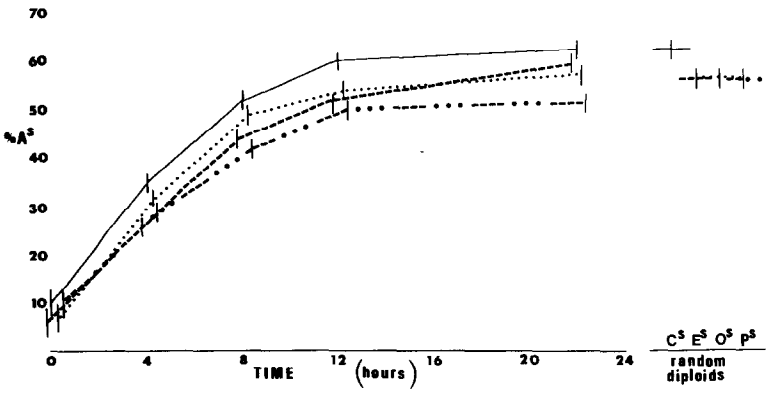

FIG. 3. Early segregation rates for the cross A11-5 × A21-112. The percentage cells homoplasmic for the C^S (——), E^S (– – –), O^S (· · · · ·) and P^S (– – · ·) alleles was determined at 0, 4, 8, 12, and 22, h by clonal analysis. Vertical lines indicate the 95% confidence intervals. Random diploid results are also shown.

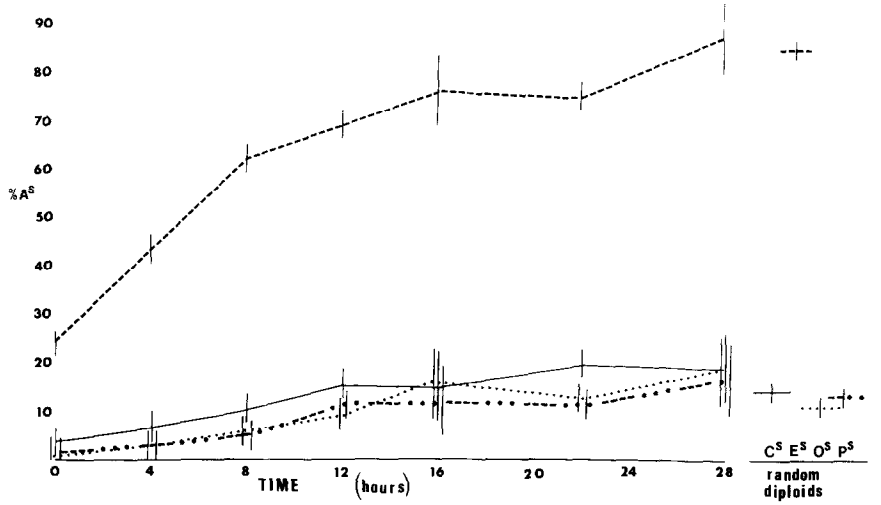

FIG. 4. Early segregation rates for the cross LT70 × 4120E. The percentage cells homoplasmic for the C^S (——), E^S (– – –), O^S (· · · · ·) and P^S (– – · ·) alleles was determined at 0, 4, 8, 12, and 22 h by clonal analysis. Vertical lines indicate the 95% confidence intervals. Random diploid results are also shown.

by D22A15 and LM870 because the latter crosses are highly biased. However, the segregation rate of *oli1* in the cross 2-36 × D22A21 can be compared with the segregation rate of *oli2* in the cross A11-5 × A15-295A which is also unbiased; these two loci show similar segregation rates even though they are quite far apart on the mtDNA molecule.

The last three crosses in Table 3 involve strains for which vegetative segregation was studied by Forster and Kleese (1975) using pedigree analyses. They reported that in most crosses more than 90% of all zygote clones contained only homoplasmic cells after 16 generations. However, only about 70% of the progeny from cross 4120E × 51 were homoplasmic by 16 generations. The 4120E strain is a spontaneous E^R mutant derivative of strain 41, which also showed somewhat slower than normal vegetative segregation when crossed to a mutant derivative of strain 51. We repeated the cross 4120E × 51, and also crossed both 4120E and its parent strain 41 to LT70. As Forster and Kleese (1975) found, all three crosses are highly biased, showing less than 20% transmission of markers from 41 and 4120E. Samples were taken from these crosses at 28 h as well as at earlier times (data for 4120E × 51 in Fig. 4). Whereas in most crosses, segregation was essentially complete by about 12 h, in the cross 4120E × 51 it was not complete until 22 or 26 h. This cross does seem to produce diploids which segregate more slowly than others, although we did not find the high frequency of heteroplasmic cells remaining after 16 generations (about 24 h) which was seen by Forster and Kleese (1975). The progeny of the cross 4120E × LT70 also seemed to segregate somewhat more slowly than usual, as seen in Fig. 4. In this cross and in the cross 41 × LT70, the C^S, O^S, and P^S alleles appear to segregate at about the same rate, although it is difficult to judge because of the low transmission of these alleles.

In several of our crosses there were more zygotes uniparental for the C^S allele than for sensitive alleles at other loci. This is seen as a higher frequency of t_0 cells, i.e., zygotes, which were scored as homoplasmic sensitive in crosses A11-5 × A21-112, A11-1 × A21-36, 2-36 × D22A21, 2-36 × D22A19, and 2-36 × D22A15. Thus, at any given time point prior to the completion of segregation, more cells were homoplasmic for C^S for the other alleles, but the *rate* of segrega-

tion was approximately the same for all alleles. This phenomenon was also seen by Forster and Kleese (1975) in some crosses, but not in other studies (e.g., Birky *et al.*, 1978a,b). The most extreme cases of early C^S segregation reported by Forster and Kleese (1975) are due to polarity in $\omega^+ \times \omega^-$ crosses (Dujon *et al.*, 1974).

Another anomaly in our data is seen in crosses 2-36 × D22A21 and A11-5 × A15-295A, where the P^S allele at the *par1* locus did not show coordinate transmission with alleles linked to it. This might be due to nuclear modifiers of mitochondrial resistance or sensitivity to paromomycin which sometimes make it difficult to score unambiguously the growth of colonies on replia plates. Waxman *et al.* (1979) reported nuclear suppressors of *par^{r}1*, the resistant allele at this locus.

Linear Regression Analysis of Segregation Rates

Sager (1972; Singer *et al.*, 1976) used linear regression to map chloroplast genes in *Chlamydomonas* relative to *ap* which is postulated to govern segregation of cpDNA molecules. After plotting the log of the ratio of the percentage HETS at time t to the percentage HETS at time 0, they drew linear regression lines. The line for each chloroplast gene locus had a unique slope, i.e., a unique segregation rate which is greater the farther the locus is from *ap*. Although we did not find differences in segregation rates by plotting percentage homoplasmic cells vs time, it seemed worthwhile to use linear regression analysis as an additional test and to make the analysis more nearly parallel in the two systems.

The data from the crosses analyzed by subclone analysis (Table 2) were plotted and linear regression analysis was done. The slopes are given in Table 4. For Sager's data a log transformation gave straight lines with origins at log 2, but a log transformation was not sufficient to give straight lines with origins at log 2 for the *cap1*, *ery1*, and *par1* loci in our crosses. This may indicate a lack of constant variances of values at each time. For example, the variance of values at t_4 is different from the variance of values at t_8.

It is instructive to try to use these slopes for mapping, as did Sager (1972; Singer *et al.*, 1976). The order of loci from the slopes in Table 4 is *ery1* − *cap1* − *par1* − *oli1* − *oli2* indicating that *ery1* would be close to a hypothetical *ap* while *oli2* is farthest away. This order would be roughly compatible with the known mitochondrial map if *par1* and *oli1* were approximately equally distant from *ery1* but on opposite sides of the circular map. But in fact *oli1* is closer than *par1* to *ery1* as determined by molecular mapping techniques (Linnane and Nagley, 1978; Schweyen *et al.*, 1978; Faye *et al.*, 1979). A test of homogeneity of regression coefficients (Li, 1964) was done on the data; the F value is 1.40. This indicates that there are no statistically significant differences in the slopes of the five lines: the lines must be considered parallel.

Table 4 also gives the slopes for the same crosses obtained by clonal analysis (data from Table 3), using a mean point for each cross at each time point. The slopes indicate the following order: *oli2* − *par1* − (*cap* ≃ *oli1*) − *ery1*. But if all points from these crosses are used in the linear regression analysis without averaging, the resulting order is *par1* − *ery1* −(*cap1* ≃ *oli2*) − *oli1*. These results from the same set of crosses do not indicate a consistent order of segregation rates and are not compatible with the known map.

The pooled data from all crosses in this study obtained by clonal analysis were also used for regression analysis (Table 4). According to these slopes, the order is *par1* − (*ery1* ≃ *oli1* + 3) − (*cap1* ≃ *oli2* + 4). This order is entirely different from any of the others presented above and is incompatible with the known mitochondrial map. Regardless of the method used for analyzing the data or obtaining percentage HETS, the map orders were not consistent—various

TABLE 4

RESULTS OF LINEAR REGRESSION ANALYSIS OF PERCENTAGE HETS VS TIME

Source		Locus				
		C	E	O_I	O_{II}	P
$C^SE^SO^SP^S \times C^RE^RO^RP^R$ crosses (Table 2, % HETS by subclone analysis)	Slope	−0.07	−0.06	−0.09	−0.11	−0.08
	Intercept	2.02	2.03	2.08	2.42	2.05
$C^SE^SO^SP^S \times C^RE^RO^RP^R$ crosses (Table 3, % HETS by clonal analysis)	Slope	−0.10	−0.14	−0.10	−0.05	−0.08
	Intercept	2.00	2.17	2.00	1.99	2.05
All crosses (Table 3, % HETS by clonal analysis)	Slope	−0.06	−0.04	−0.04[a]	−0.06[b]	−0.03
	Intercept	1.99	1.98	1.92	1.96	1.98

[a] Pooled data for closely linked loci O_I and O_{III}.
[b] Pooled data for closely linked loci O_{II} and O_{IV}.

methods gave different orders, none compatible with the known mitochondrial map.

DISCUSSION

Non-random spatial distributions of mtDNA molecules are known to influence the segregation of yeast mitochondrial genes. Strausberg (1976; Strausberg and Perlman, 1974, 1978), Callen (1974), and Aufderheide and Johnson (1976) have shown that mitochondria from the two parents are not completely mixed in the zygote until after the first one or two buds are formed. Consequently early buds coming from one end of the zygote have a higher probability of being homoplasmic for mitochondrial alleles contributed by the parent which formed that end of the zygote than early buds from the center where mixing is more rapid. Nonrandom factors such as these bud position effects favor the separation of molecules from different parents during the first two divisions of the zygote, but they are not expected to cause distinct loci to segregate at different rates.

Map-related differences in segregation rates among loci would result from nonrandom segregation mechanisms such as proposed by Sager (1972; Sager and Ramanis, 1976a) and Callen (1974). In these models the segregation of each organelle DNA molecule is governed by a specific site (*ap*) on the molecule, just as centromeres on nuclear chromosomes determine the meiotic or mitotic segregation of the chromosomes. Sager's model does not take into account the presence of many cpDNA molecules in each cell, or the packaging of those molecules into separate nucleoids within the single chloroplast of *Chlamydomonas*. VanWinkle-Swift (1980) has described a model which includes these features together with attachment points. A similar model could be designed for yeast, although at present we have no hard information concerning the number of mitochondria or nucleoids in yeast zygotes and their buds. However, there seems to be no necessity for such a model. The observations of Callen (1974), Forster and Kleese (1975), and Uchida and Suda (1976) showed that mitochondrial loci in yeast differ in segregation rates, but did not show that the rates are correlated with map position; hence, the variation in rates could be accounted for by other factors, such as differences in the rates of gene conversion among loci. In this study we were unable to find significant rate differences among loci, or to correlate the variations which were found with the known map positions of the mitochondrial loci.

It should be noted that locus-specific

variations in segregation rates might not be detected if the reciprocal recombination events which generate Type III segregation in Sager's model were much less frequent in yeast than in *Chlamydomonas*. This appears to be the case. Waxman and Birky (unpublished data) found that less than 1% of all mitochondrial segregation events were of Type III in the first three divisions of *Saccharomyces* zygotes, compared to about 6–12% for chloroplast genes in the first two mitotic divisions of the *Chlamydomonas* zygospores (Sager and Ramanis, 1976b). Thus, if there is an *ap*, it cannot play an *important* role in determining overall segregation rates. The data presented here are particularly strong in that they involve loci whose map positions are accurately known from molecular mapping procedures, and which mark major sectors of the mtDNA molecule (Linnane and Nagley, 1978; Schweyen *et al.*, 1978; Faye *et al.*, 1979). In contrast, the chloroplast genetic map has not been verified by molecular methods; and in fact, the cosegregation map of Sager and Ramanis (1976b) does not agree with the conventional recombination map of Harris *et al.* (1977).

Our data also do not support models in which the mtDNA molecules in a cell segregate randomly with respect to genotype at each cell division. If a bud received a large random sample of mtDNA molecules from the heteroplasmic mother, e.g., 50 molecules, the probability that all of those molecules would be of the same genotype would be vanishingly small and the bud would rarely be homoplasmic. To account for the high frequency of homoplasmic buds actually observed, one must postulate that a bud receives only two or three mtDNA molecules. But in that case a bud could have only a limited number of allele frequencies in the population of mtDNA molecules it contains, and when its progeny became completely homoplasmic due to further segregation, they could only show a small number of phenotype frequencies. For instance, if a bud received two molecules, the possible allele frequencies would be 0, 50, and 100%; three molecules would give 0, 33.3, 66.7, or 100%. We found that heteroplasmic diploid cells produced a wide variety of allele frequencies. The distribution of frequencies among heteroplasmic cells (Fig. 2) is continuous and essentially uniform after t_0, rather than having one or two discrete modes at 50% or at 33.3 and 66.7%. This verifies the previous observations of Birky *et al.* (1978b) from partial pedigree data. Physical evidence that the first buds of zygotes receive a large number of mtDNA molecules was presented by Sena *et al.* (1976).

Our data are compatible with a random segregation model in which the units of segregation are *groups* of mtDNA molecules, possibly corresponding to individual mitochondria or nucleoids (Birky, 1975; Dujon and Slonimski, 1976; Birky *et al.*, 1978b). All of the molecules in one group would usually, but not always, carry the same allele at any given locus. Segregation is rapid because the number of these units in a cell is small. Although these units must exchange mtDNA molecules to permit recombination, their genetic homogeneity could be maintained by random drift of gene frequencies due to gene conversion or other mechanisms (Birky and Skavaril, 1976; Birky, 1978). The units are not completely homogeneous, as indicated by the continuous distribution of gene frequencies as discussed above. The exact shape of the distribution would depend upon a number of factors, including the size and number of the units, the rate of drift within each unit, and the rate at which molecules are exchanged between units.

The situation is further complicated by the occurrence of random drift of allele frequencies within an entire cell (Thrailkill *et al.*, 1980), which could convert a heteroplasmic cell to the homoplasmic state by fixing one allele or the other at the locus being studied without the intervention of cell division. It is in fact possible that in some cases, when a heteroplasmic cell produces a homoplasmic daughter, that daughter was

initially heteroplasmic but became homoplasmic due to drift occurring during interphase. The delayed-division experiments of Thrailkill *et al.* (1980), which provide the best evidence for random drift, also show that vegetative segregation cannot be entirely due to drift, but also requires cell division.

This model says, in effect, that the segregation of mtDNA molecules at cell division is nonrandom, since genetically similar molecules tend to segregate together. But this happens not because of centromere-like attachment points but because genetically similar molecules tend to be in the same nucleoid or mitochondrion.

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