References and Notes

1. Thomas A. Mutch, a professor of geological sciences at Brown University and an associate administrator for space sciences at the National Atmospheric and Space Administration, was killed in a mountain climbing accident in the Himalayas in October 1980. Professor Mutch was the leader of the lander imaging team during the primary and extended missions. Contact was lost with the station after uplinking a command sequence. Repeated attempts to uplink new commands did not establish contact.
2. A sol is one martian day with a duration of 24.66 hours. When capitalized, Sol refers to a lander event time: the sol of landing is Sol 0; Sol 2238 is 2238 sols after landing. A martian year is nearly twice as long as a terrestrial year because the revolution period of Mars is 686.980 days, compared to 365.256 days for Earth.
3. Previous analyses of Viking Lander imaging data were made by E. A. Guinness, C. E. Leff, and R. E. Arvidson [*J. Geophys. Res.* **87**, 10051 (1982)]; K. L. Jones *et al.* [*Science* **204**, 700 (1979)]; H. J. Moore, R. E. Hutton, R. F. Scott, C. R. Spitzer, and R. W. Shorthill [*J. Geophys. Res.* **81**, 4497 (1977)]; H. J. Moore *et al.* [*ibid.* **84**, 8365 (1979)]; J. B. Pollack *et al.* (*ibid.*, p. 2929); and C. Sagan, D. Pieri, P. Fox, R. E. Arvidson, and E. A. Guinness [*ibid.* **82**, 4430 (1977)].
4. The Viking Lander meteorology experiment was described by S. L. Hess, R. M. Henry, C. B. Leovy, J. Ryan, and J. Tillman [*J. Geophys. Res.* **82**, 4559 (1977)].
5. The lander camera system was described by W. R. Patterson, F. O. Huck, S. D. Wall, and M. R. Wolf (*ibid.*, p. 4391). There are two identical cameras on each lander, spaced 0.8 m apart and standing 1.3 m above the surface. The cameras work by acquiring one vertical scan line with a nodding mirror. The light is focused onto a photodiode array, and the image is constructed by rotating the assembly about a vertical axis between each scan line. Six diodes were used for multispectral imaging in the 0.5- to 1.0-μm region, with an angular resolving power of 0.12°, while another suite of diodes was used for broadband (peak in red) imaging at 0.04° angular resolving power. Another diode was used for broadband, low-resolution (0.12°) imaging. In addition, one diode was used to obtain atmospheric optical depth by imaging of the sun. The sensor output voltages were coded to a range of 64 brightness units and the data were either telemetered directly back to Earth or stored for later relays.
6. Images acquired in support of sampler activities were described by S. Liebes, Jr., and A. A. Schwartz (*ibid.*, p. 4421).
7. J. A. Ryan, R. D. Sharman, R. D. Lucich, *Geophys. Res. Lett.* **8**, 899 (1981); P. B. James and N. Evans, *ibid.*, p. 903.
8. To detect changes in brightness and contrast during the observations the radiometric responses of the lander cameras must not drift or the drift must be checked. Drift in camera preamplifier voltages for the blue, green, red, and three infrared diodes was monitored on a regular basis for the first 921 sols on Mars, until the station was commanded to an automatic state. On command, the camera lens was covered by a black flag and the diode responses were measured. Next, each diode response to a lamp was measured. Except for the infrared channels, the change in preamplifier voltage, corrected for diode temperature, was negligible for this period. Extrapolation to the end of the mission suggests that the drift would be less than one brightness unit.
9. The brightness in shadowed areas varied between the frames by only 1 to 2 units, while the cameras recorded a range of 64 units for each frame.
10. C. Leovy, *J. Atmos. Sci.* **38**, 39 (1981); J. A. Ryan and R. D. Sharman, *J. Geophys. Res.* **86**, 3247 (1981).
11. Soil in this case refers to fine-grained debris, with no connotations as to organic content. Particle sizes for the soils exposed at the station are thought to be in the clay to silt size range. Clods composed of these fine-grained soils up to centimeters in width can be seen in the images, and their cohesion varies. Thus, a spectrum of particle sizes exists. See H. J. Moore *et al.* [*J. Geophys. Res.* **84**, 8365 (1979); *ibid.* **87**, 1043 (1982)] for a summary of soil properties.
12. C. Leovy, personal communication.
13. The friction velocity is the square root of the ratio of wind shear stress to atmospheric density. The wind velocity at some height above the surface is related to the friction velocity and the distribution of surface roughness elements. Theoretical and experimental work [R. Greeley, R. Leach, B. White, J. Iversen, J. Pollack, *Geophys. Res. Lett.* **7**, 121 (1980); J. Iversen, R. Greeley, J. Pollack, *J. Atmos. Sci.* **33**, 2425 (1976)] was used to compute the threshold friction velocity quoted in text for atmospheric conditions in the late winter.
14. J. L. Sutton, C. B. Leovy, and J. Tillman [*J. Atmos. Sci.* **35**, 2346 (1978)] suggested that surface roughness values from 0.1 to 1.0 cm might be appropriate. These estimates and the Von Karman relation for a fully turbulent boundary layer indicate that winds with velocities of 25 to 30 m/sec are needed to erode loose soil. An equally plausible interpretation is that the 4- to 5-mm particles were moved directly by strong winds between Sols 1720 and 1757. In this case, threshold friction velocities of 3 to 5 m/sec and wind velocities of 40 to 90 m/sec would be needed.
15. J. B. Pollack, R. H. Aberle, R. Greeley, J. Iversen, *Icarus* **29**, 395 (1976).
16. B. White, *J. Geophys. Res.* **84**, 4643 (1979).
17. O. B. Toon, J. B. Pollack, W. Ward, J. A. Burns, K. Bilski, *Icarus* **44**, 552 (1980).

18. We are indebted to the small but highly motivated crew at Jet Propulsion Laboratory that operated the Mutch Memorial Station and provided data. We thank G. N. Gianopulos, A. Britting, Jr., J. P. Brinkle, Esche, D. Pieri, and K. L. Jones. Thanks are also extended to S. LaVoie, whose perseverance in the face of limited funds provided us with data. Support was provided by the Planetary Geology and the Mars Data Analysis Programs, NASA Headquarters. Finally, deep thanks are extended to J. Boyce and H. Brinton for continued interest in the findings of the Mutch Memorial Station.

Relaxed Cellular Controls and Organelle Heredity

C. William Birky, Jr.

DNA molecules carrying small but vitally important sets of genes have been demonstrated in the chloroplasts of plants and algae, and in the mitochondria of protists, fungi, plants, and animals (*1–3*). The inheritance of these mitochondrial and chloroplast genes has been studied in all of the eukaryotic kingdoms, so that it is now possible to identify the truly general features of organelle gene transmission, segregation, and recombination (*4*). Of special interest and importance are phenomena that are characteristic of mitochondrial and chloroplast genes and distinguish their behavior from that of genes in the nucleus. They are often transmitted from only one parent, and alleles segregate during mitotic cell divisions. Much effort has been devoted to analyzing the cellular and molecular mechanisms which underlie and explain these phenomena.

There is still no general agreement about these mechanisms or about their evolutionary significance. Several different hypotheses have been proposed, none of which is sufficiently general to encompass the phenomena seen in different organisms. However, recent genetic studies have suggested an underlying theme that relates the inheritance of organelle genes to a lack of stringent cellular control over the behavior of organelles and organelle DNA.

C. William Birky, Jr., is a professor in the Department of Genetics and the Program in Molecular, Cellular, and Developmental Biology at Ohio State University, Columbus 43210.

Phenomena to Be Explained

The most important and singular features of organelle heredity are uniparental inheritance and vegetative segregation (*4*). These phenomena are illustrated by crosses of green and mutant white chloroplasts in the geranium (*3*) (Fig. 1A). When a plant with green plastids in its germ-line cells is crossed to a plant with mutant white germ-line plastids, three kinds of zygotes are produced, in varying proportions: (i) uniparental (maternal) zygotes, which develop into plants with only green plastids; (ii) uniparental (paternal) zygotes, which give rise to plants with only white plastids (these plants die as seedlings); and (iii) biparental zygotes, which produce variegated plants having sectors of green and white cells. The first two classes show uniparental inheritance in that they develop into plants that contain chloroplast genes from only one parent.

The young plants arising from biparental zygotes have mixed cells that contain both green and white plastids; but each cell in the mature variegated plant is homoplasmic (homozygous for cytoplasmic genes), containing only green or only white plastids. These cells are the result of vegetative segregation—that is, the segregation of wild-type and mutant al-

leles of chloroplast genes into different cells during vegetative (mitotic) cell division. The relative sizes of the green and white sectors (equal to the frequencies of wild-type and mutant plastids) vary greatly among the progeny of a single mating.

A more extreme form of uniparental inheritance of chloroplast genes is seen in about two-thirds of all plant genera, which produce only maternal zygotes (classical maternal inheritance) (*3*). Mitochondrial DNA (mtDNA) also shows strictly maternal inheritance in animals (Fig. 1B), in at least some plants, and in some fungi [references in *1–5, 6*)]. In these organisms heteroplasmic cells usually occur only as the result of a mutation affecting one of the many copies of an organelle gene in a cell. The mutant and wild-type alleles rapidly segregate during subsequent vegetative cell divisions.

Uniparental inheritance also occurs in organisms with undifferentiated gametes (isogametes). Mitochondrial gene inheritance is being intensively studied in baker's yeast, *Saccharomyces cerevisiae* (*2, 4, 5, 7*). Figure 1C illustrates a cross involving the mitochondrial alleles E^R (erythromycin resistance) and E^S (erythromycin sensitivity). Haploid parent cells of mating types *a* and α (determined by a pair of nuclear gene alleles) and homoplasmic for the E^S and E^R alleles, respectively, fuse in pairs to form diploid zygotes. These zygotes are heteroplasmic E^R/E^S and heterozygous a/α. Each zygote reproduces by budding and mitosis to form a colony or zygote clone consisting of diploid cells. After about 20 cell generations, each of these cells is still heterozygous for nuclear genes (a/α) but is now homoplasmic E^R or E^S as a consequence of vegetative segregation of the mitochondrial alleles. Some zygote clones contain only E^S cells and are thus uniparental E^S, while others are uniparental for the E^R allele. The biparental zygote clones, like the variegated geraniums, show varying frequencies of mutant E^R and wild-type E^S mitochondrial genes. The fission yeast (*Schizosaccharomyces pombe*) behaves similarly, except that vegetative segregation is slower (*8, 9*).

The unicellular alga *Chlamydomonas reinhardtii* has played a major role in studies of chloroplast gene inheritance. Although this species is isogamous, most zygotes transmit chloroplast genes from only one parent (mating type mt^+) to their progeny (*1–4*). This effect of the nuclear mating-type genes on organelle gene inheritance can be overcome in several ways, in which case the pattern of chloroplast gene inheritance is very similar to that seen for mitochondrial genes (*10*).

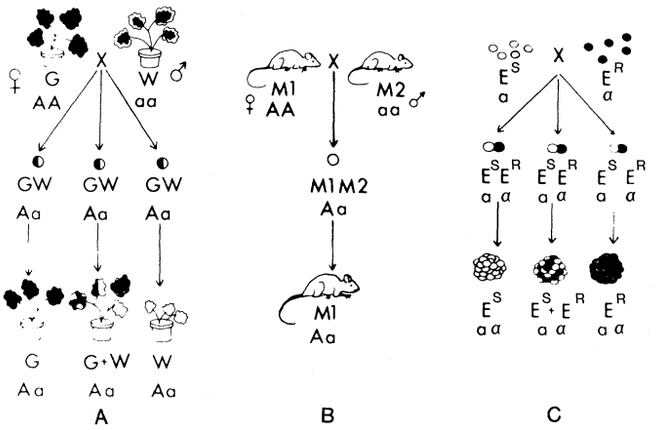


Fig. 1. Inheritance of organelle and nuclear genes contrasted. (A) Cross between geraniums (*Pelargonium*) with wild-type green (G) and mutant white (W) chloroplasts; the male parent is a chimera with W chloroplasts in the germ line. *A* and *a* are nuclear genes. (B) Cross between two mammals with mtDNA's of different restriction patterns M1 and M2. *A* and *a* are nuclear genes. (C) Cross between yeast cells with erythromycin-sensitive (E^S) and -resistant (E^R) mtDNA, and nuclear gene mating-type alleles *a* and α. In each cross the first line shows the parents, the second line shows the zygotes, and the third line shows the progeny (zygote clones consisting of diploid cells when yeast is used).

In isogamous microorganisms, and in plants like the geranium, it is probable (in some cases certain) that every zygote initially contains organelle genes from both parents. But the alleles contributed by one parent or the other literally disappear from the uniparental zygotes. Genes from both parents are retained in biparental zygotes, but to varying degrees, and are quickly sorted out into separate daughter cells. Nuclear genes behave very differently, as is well known. Biparental inheritance is the general rule, although there are exceptions such as genes on sex chromosomes and those exhibiting meiotic drive. Alleles of nuclear genes segregate during meiosis but only rarely during mitotic cell divisions. In a cross between homozygous mutant and wild-type animals or plants, every cell in every individual offspring is heterozygous. These are the differences that require explanation.

Summary. Genes in mitochondria and chloroplasts behave quite differently from genes in the nucleus: they are often inherited from only one parent, and they segregate during mitotic cell divisions. Cells contain many copies of each mitochondrial or chloroplast gene, and the replication, recombination, and partitioning of these genes at cell division are much less stringently controlled than is the case for the one or two copies of each nuclear gene. Relaxed control results in random changes in gene frequencies inside single cells or lineages. This may have been the primitive mechanism behind the uniparental inheritance as well as the vegetative segregation of cytoplasmic genes and is still an important factor in many organisms.

Traditional Explanations

Vegetative segregation is usually attributed to random partitioning of mitochondria, chloroplasts, or their DNA molecules at cell division (*1–4, 11, 12*). For example, when a plant cell containing both green and white plastids divides, a daughter cell may by chance receive only green plastids, or only white (Fig. 2). The resulting homoplasmic cells produce only homoplasmic progeny; heteroplasmic cells may produce homoplasmic progeny again in the next generation, so that eventually nearly all cells are homoplasmic. Stochastic models of this kind, involving random sorting out of organelles or organelle DNA molecules, are appealing because there is no known cellular mechanism to prevent random partitioning. Moreover, they have been successful in explaining many of the data from plants (*11*) and, with modifications, from yeast and *Chlamydomonas* (*12*).

Uniparental inheritance, in contrast, is most often explained by deterministic mechanisms of two kinds.

1) Some cases of purely maternal inheritance may be due to failure of the parental gametes to transmit organelle genomes to the zygote [reviewed in (*4, 5, 13*)]. I have called this mechanism monogametic organelle transmission (*5*). In many animals where the sperm do contribute mitochondrial genes to the zygote, they are so few in relation to those in the egg that they would be undetectable [references in (*5*)], and this may also

be the case for many plants and some fungi such as *Neurospora*.

2) The organelles or organelle DNA from one parent may be destroyed, as appears to be the case for chloroplasts or chloroplast DNA in some algae and plants (*1, 13–15*) and for sperm mitochondria of some animals (*4, 5*). It is also conceivable that the organelle DNA from one parent might be phenotypically inactivated, or might fail to be replicated and hence be diluted to the point where it cannot be detected, but there is no direct evidence for this. In this article, I follow Sager and Kitchin (*16*) and refer to any mechanism which consistently eliminates genes of one parent from the zygote as selective silencing, but without necessarily endorsing their proposed molecular mechanism for all cases.

These hypotheses were designed for cases of strictly maternal inheritance; they do not explain the production of two different classes of uniparental progeny, pure for alleles from either parent, as described above for some plants, yeast, and some *Chlamydomonas* crosses. These cases clearly require a different approach. Realizing this, Tilney-Basset (*17*) and Gillham *et al.* (*18*) suggested that uniparental inheritance in geraniums and *Chlamydomonas* is due to competition of parental organelles or their DNA for replication in the zygote. Their hypotheses were forerunners of the more general stochastic hypothesis described below.

The Cell Viewed as a Population of Organelle Genes

Eukaryotic nuclei are usually haploid or diploid, containing only one or two copies of each gene. In contrast, the cytoplasm of each cell contains on the order of 10^2 to 10^4 molecules of mtDNA or chloroplast DNA (cpDNA), and each molecule carries a complete genome (*2, 4, 19*). Each organelle gene is thus present in many copies per cell. This requires that we think about organelle genes in a completely different way: a cell contains a population of organelle genomes and genes, to which the concepts of population genetic theory can be applied (*4*). Especially important is the concept of allele frequency. Consider a yeast cell having, for example, 100 molecules of mtDNA, and suppose that 43 of these carry a mutant allele E^R of a gene conferring resistance to erythromycin, while the remaining 57 carry the wild-type E^S allele. The cell is heteroplasmic, but this does not suffice to describe it. We must also state that the frequency of the E^R allele in the cell is 0.43 or 43 percent; the E^S allele frequency is 0.57 or 57 percent. The concept may also be extended to allele frequencies in a clone or other collection of cells. This is easiest to do when vegetative segregation is completed and all the cells are homoplasmic, some E^R and some E^S. The intracellular E^R frequency in these two types of cells is 1 (100 percent) and 0 (0 percent), respectively. If a clone consists of 1000 cells, of which 391 are resistant and 609 are sensitive to erythromycin, the frequency of the E^R allele in the clone is approximately 0.391 or 39.1 percent, if it is assumed that resistant and sensitive cells have, on the average, the same numbers of mtDNA molecules.

Allele frequencies can be used to describe uniparental inheritance and vegetative segregation, and thus provide a picture of what happens to organelle genes in a cross. Yeast is a simple example which does not involve monogametic transmission or selective silencing. Let us suppose that two haploid strains are mated; one is E^R and has an average of about 55 mtDNA molecules per cell, while the other is E^S and has an average of about 45 mtDNA molecules per cell. Pairwise fusion of these cells produces zygotes in which the average starting or input frequencey of the E^R allele is $r = 0.55$ and the E^S frequence is $1 - r = 0.44$. There is some variation around this mean value for different zygotes, since there is some variation in the mtDNA content of the parent cells. This variation is difficult to measure directly. However, we would expect a priori that the values of r would show an approximately Gaussian frequency distribution as in Fig. 3A, and there is indirect evidence for this (*5*).

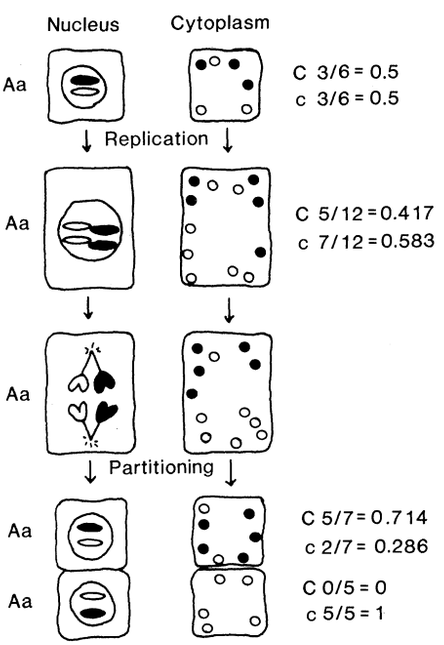


Fig. 2. Replication and partitioning of nuclear chromosomes and alleles (*A* and *a*) and of organelles or organelle DNA molecules and alleles (*C* and *c*) during mitosis. Different alleles are represented by solid and open symbols. Numbers on the right represent intracellular frequencies of the organelle genes. In interphase each nuclear chromosome and gene is replicated exactly once, while organelles or organelle DNA molecules and genes are selected more or less at random for replication resulting in intracellular drift of gene frequencies. At cell division the nuclear chromosomes are partitioned regularly so that each daughter cell is heterozygous; organelles or organelle DNA molecules are partitioned randomly, resulting in homozygosity (in only one daughter in this case).

After about 20 cell divisions, each zygote has produced a zygote "clone" of homoplasmic progeny. In the absence of selection we would expect the mean frequency of the E^R allele in these clones to be 0.55 as it was in the zygotes, and there is in fact good evidence that the output frequency of an allele in a large collection of zygote clones is greater, the greater the input allele frequency in the zygotes (*4, 7*). But a plot of the frequency distribution of r among individual clones no longer shows a tight Gaussian distribution (Fig. 3, B to D). In some crosses the distribution is very broad but retains a distinct peak at the mean value (Fig. 3B). This cross produced very few uniparental zygotes (that is, zygotes that form clones with allele frequencies of 0 or 1). In other crosses (Fig. 3C) the distribution of allele frequencies shows much greater variance and there are substantial numbers of uniparental E^R or E^S zygote clones in which one or the other allele, E^S or E^R, has disappeared. Moreover, at least some of the biparental zygotes have also undergone shifts in allele frequencies, some increasing r and some decreasing r to varying extents. When the input allele frequencies are biased, that is, very different from 0.5, the output frequency distribution is skewed and many zygotes are uniparental for the majority allele (Fig. 3D). Similar frequency distributions are seen for mitochondrial genes in the fission yeast *Schizosaccharomyces pombe* (*8, 20*).

Frequency distributions of chloroplast alleles in the geranium and *Chlamydomonas* show more uniparental zygotes (Fig. 4, A to C). In the case of *Chlamydomonas* the excess of zygotes uniparental for alleles from the mt^+ is probably due to degradation of cpDNA molecules from the mt^- parent in the zygote (selective silencing) (*1, 15*), but this does not account for paternal zygotes or the variance of allele frequencies among the biparental zygotes (*21*). In some zygote

clones, the frequency of the chloroplast allele from the mt^+ parent has actually decreased. This becomes apparent only when one looks at the distribution of allele frequencies among all the zygote clones (*21*), rather than just classifying zygotes as uniparental or biparental (*1*). The bidirectional changes in allele frequencies can be seen clearly if the overriding unidirectional effect of cpDNA degradation is compensated by inactivating cpDNA from the mt^+ parent with ultraviolet irradiation as in Fig. 4B. Alternatively, it is possible to select and study the rare vegetative zygotes, in which the mating-type effect is much less pronounced (*22*) (Fig. 4C), or protoplasts of the same mating type can be fused so that there is no mating-type effect (*10*).

Frequency distributions can also be used to study vegetative segregation, as illustrated in Fig. 5 for a yeast cross. In this case it is allele frequencies in individual diploid cells, not zygote clones, that are being estimated. Successive distributions are from samples of cells taken at successive times after a mating. The distributions show the gradual disappearance of heteroplasmic cells from the population, as they divide and produce homoplasmic progeny.

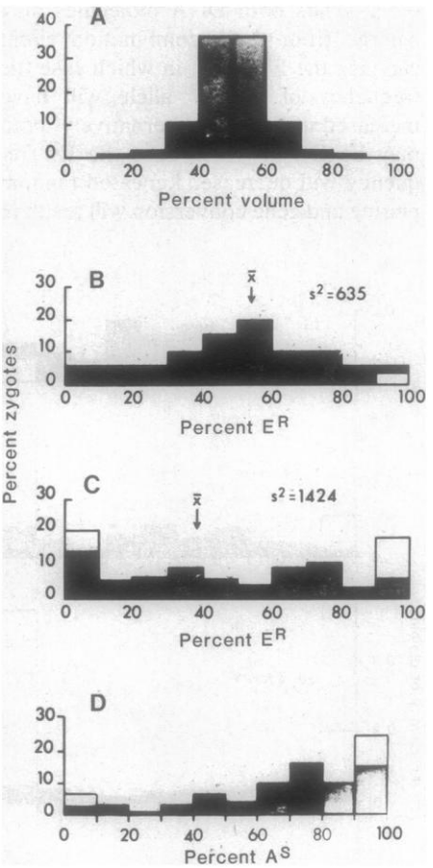
Control of DNA Replication, Recombination, and Partitioning

The behavior of chromosomes, DNA molecules, and genes in the nucleus is stringently controlled. In contrast, control over these events is relaxed for genomes in mitochondria and chloroplasts (Fig. 2).

Information about the partitioning of cytoplasmic elements at cell division is scarce (*23*). Although mitochondria may be closely associated with the mitotic apparatus and cytoplasmic organelles may be associated with elements of the cytoskeleton, there is no visible mechanism to control partitioning. In the case of mitochondria in certain scorpion spermatocytes and chloroplasts in the alga *Olisthodiscus* (*23*), it has been shown that each of the two daughter cells usually receives half of the organelles in the mother cell, but not always. Partitioning is stochastic, that is, unpredictable, and one daughter may receive more organelles than the other by chance.

It is generally accepted that partitioning is also genetically stochastic. In other words, cells do not distinguish organelles or organelle DNA molecules carrying different alleles may be segregated by chance into different daughter cells. This hypothesis accounts for vegetative segregation of chloroplast genes in plants (*3*, *11*) and is at least partly responsible for vegetative segregation of mitochondrial alleles in yeast (*4*, *5*, *12*). Sager (*1*) has postulated that cpDNA molecules in *Chlamydomonas* are partitioned regularly, like nuclear chromosomes at mitosis. Her model is applicable to cells having a single mitochondrion or chloroplast, but it has not been universally accepted for *Chlamydomonas* (*4*, *12*) and has been ruled out for mitochondrial genes in yeast (*24*).

In cultured mouse cells, mtDNA molecules are selected at random for replication, one or a few at a time, until the total number has been doubled (*25*). Some molecules may be replicated more than once, and others not at all, in a cell cycle. Density shift experiments suggest a more uniform replication of mtDNA molecules in yeast (*26*), but more sensi-



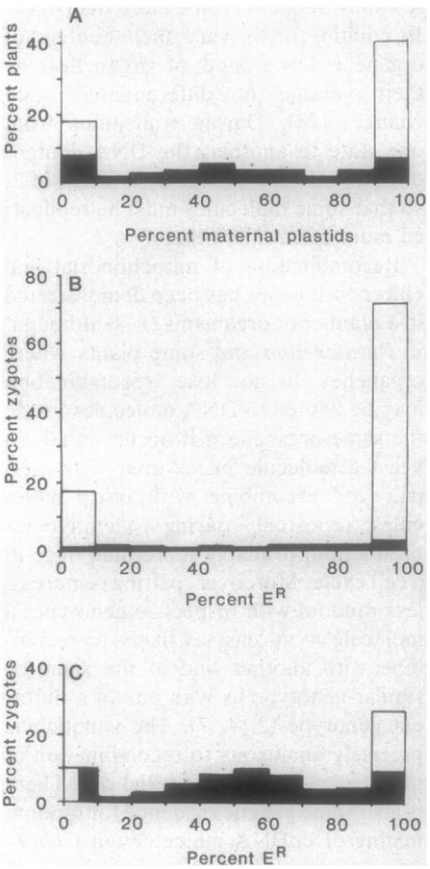


Fig. 3 (left). Distributions of mitochondrial gene frequencies among zygotes or zygote clones in *Saccharomyces cerevisiae*. (A) Cross of erythromycin-resistant and -sensitive strains [$E^R \times E^S$; experiment DW8 in (*8*); reprinted by permission of Cold Spring Harbor Laboratory]. The input frequencies of the E^R allele were estimated by volume measurements of 53 newly formed zygotes as described (*5*). (B) Same cross as in (A); output frequencies of the E^R allele in 50 zygote clones. After about 20 cell divisions of the zygotes, when nearly all of the diploid progeny cells are homoplasmic as a result of vegetative segregation, each zygote clone was subcloned, and the frequency of E^R and E^S cells in each clone was determined by replica plating. This gives an estimate of the allele frequency in each zygote clone as a whole. Open bars represent uniparental zygote clones, with 0 or 100 percent E^R alleles. The mean ($\bar{x}$) and variance (s^2) of the allele frequencies are shown. (C) Output distributions for 100 zygote clones from the same cross as in (A) and (B), but performed with a different mating procedure that gives more uniparental zygotes and higher variance of allele frequencies. (D) Output distribution for the cross ρ5 $C^SE^SO^S \times$ ID4/1 $C^RE^RO^R$, a three-factor cross involving mitochondrial genes for sensitivity and resistance to chloramphenicol, erythromycin, and oligomycin. A^S represents the mean of the frequency distributions for the C^S, E^S, and O^S alleles. Fig. 4 (right). Distributions of chloroplast gene frequencies in plant and algal crosses. (A) *Pelargonium*, cross between wild-type (green) female and mutant (white) male. For each progeny seedling the percentage of tissue with maternal (green) chloroplasts was estimated. Open bars represent uniparental plants with all green or all white plastids [data from (*45*), last cross in table 3]. (B) *Chlamydomonas*, cross mt^+ $E^R \times mt^-$ E^S. Frequencies of the maternal E^R chloroplast allele in individual zygospore clones were determined by subcloning the homoplasmic haploid cells and replica plating [data from (*21*), figure 3]. Open bars represent uniparental zygote clones with allele frequencies of 0 or 100 percent. (C) *Chlamydomonas*, cross mt^+ $E^R \times mt^-$ E^S. Frequencies of the maternal E^R allele were determined in individual clones from vegetative zygotes by subcloning the homoplasmic diploid cells and replica plating (*46*).

tive tests will be required to prove that each molecule replicates exactly once. In several cases there is evidence for rapid turnover of mtDNA (*27*). It is likely that degradation is random, so that some molecules will by chance have more surviving replicas than others. Moreover, if partitioning of organelle DNA or organelles at cell division is not equal, it must be compensated for by unequal replication (*23*). Cells or cell lineages with too many organelles or genomes must replicate only a sample of them; cells with too few must replicate some more than once. In addition, cells vary their content of organelle DNA (and of organelles) as their volume or differentiated state changes (*28*). During transitions from one state to another, the DNA content does not always change by a factor of 2, so that some molecules must be replicated more often than others.

Recombination of mitochondrial and chloroplast genes has been demonstrated in a number of organisms (*1–4*) although, in *Paramecium* and some plants where organelles do not fuse, recombination may be limited to DNA molecules inside the same organelle if it occurs at all. In yeast a molecule may "mate," that is, pair and recombine with other molecules, repeatedly during vegetative reproduction, probably more than once in a cell cycle. Moreover, pairing is more or less random with respect to genotype; a molecule is at least as likely to recombine with another one of the same or similar genotype as with one of a different genotype (*2, 4, 7*). The situation is precisely analogous to recombination of phage in an infected bacterial cell. There is also some genetic evidence for random mating of cpDNA molecules in *Chlamydomonas* zygotes (*29*).

Consequences of Relaxed Control

Randomness in the replication, recombination, and partitioning of organelle DNA molecules has a far-reaching effect on genotypes and phenotypes: it causes random changes in allele frequencies within cells (Fig. 2), cell lineages, and clones or whole organisms. In a heteroplasmic cell with alleles E^R and E^S where, by chance, E^R molecules are selected for replication more often than E^S, the frequency of the E^R allele would increase in that cell. Conversely, chance selection of more E^S molecules for replication in another cell would decrease the E^R frequency in that cell. Random selection of molecules for degradation, as during turnover, will do the same. The result will be that in any one cell the frequency of an organelle gene allele will undergo repeated unpredictable (stochastic) changes.

Repeated random pairing of DNA molecules for recombination will also result in random walks of gene frequencies (*30*), because recombination will involve gene conversion as well as classical crossing over. When two molecules pair in the vicinity of a gene for which they carry different alleles, such as E^R and E^S, one allele may be converted to the other. Thus both DNA molecules may emerge from the recombination event carrying the E^R allele, in which case the frequency of the E^R allele will have increased in the cell. Alternatively, both may be E^S, in which case the E^R frequency will decrease. Repeated random pairing and gene conversion will result in repeated changes of allele frequencies, increasing or decreasing unpredictably in different cells.

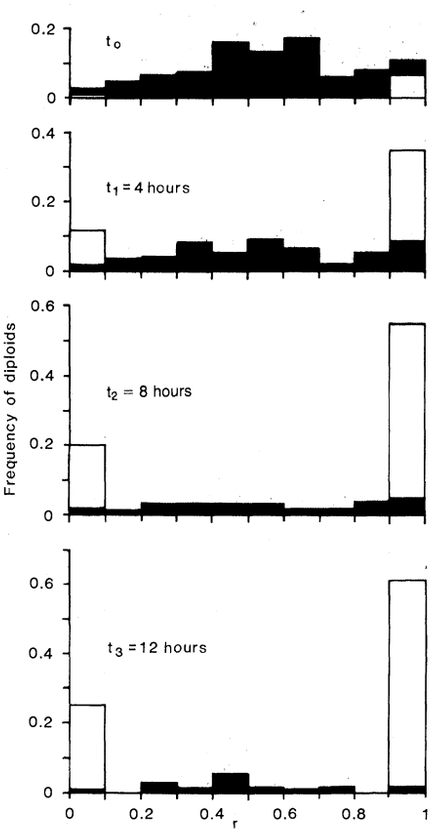


Fig. 5. Vegetative segregation of mitochondrial genes in *Saccharomyces cerevisiae*. After a mating A21-112 $C^RE^RO^R \times$ A11-5 $C^SE^SO^S$, samples of 50 diploid cells were plated at various times. The cells plated at t_0 were undivided zygotes; at t_4, t_8, and t_{12}, they were diploid progeny after about two, five, and eight generations of vegetative (mitotic) reproduction. The abscissa is the mean frequency of the C^S, E^S, and O^S alleles in the diploids, determined by the frequency of homoplasmic sensitivity progeny which they produce after subcloning. Open bars are homoplasmic diploids (gene frequency = 0 or 1). [Data from (*47*), table 34, with permission]

Random replication, degradation, and recombination all result in random walks of mitochondrial or chloroplast allele frequencies inside interphase cells, analogous to random drift in Mendelian population genetics. Mathematical analysis and computer simulations (*30, 31*) show that the rate of random drift depends on the number of molecules in the cell, and in general is quite low for cells with 100 or more copies of organelle DNA. However, more rapid drift would result from the combined effects of these processes. Also, it is possible that groups of genetically identical molecules are selected for replication or degradation, which would lower the effective population size. For example, a polymerase molecule may replicate a DNA molecule and then preferentially replicate the daughter DNA molecules because both the polymerase and the products of its action tend to remain in the same organelle, as suggested by studies on mtDNA in *Xenopus* oocytes (*32*). In any event, stochastic gene conversion and replication will increase the variance in allele frequencies among zygotes. If continued long enough, it will make zygotes (or zygote clones) uniparental by fixing one allele and eliminating the other; this can explain the frequency distributions described above.

Intracellular random drift will be especially efficient at eliminating one allele if that allele is present in low frequencies. This will be the case in fertilized eggs of animals where the egg contains 10^6 to 10^8 mtDNA molecules while the sperm contributes about 100 molecules (*33*). Random drift will rapidly eliminate the parental mitochondrial alleles in most of the eggs, leading to maternal inheritance. In fertilized rat eggs the initial frequency of parental mtDNA molecules is about 1/3000; a restriction fragment analysis capable of detecting the parental molecules in this frequency showed none in the mature offspring (*34*). Their absence could be due to selective silencing, but it could also be due to random drift, which would eliminate the paternal allele in all but about 1 out of 3000 progeny. Also a new mutant allele arising in a cell will initially be present at very low frequency; if the cell contains 10^3 molecules, the mutant allele frequency will be $1/10^3$. It will be lost, very quickly, with a probability of 999/1000 and will be fixed (become homoplasmic), with a probability of 1/1000.

Intracellular random drift of allele fre-

quencies may also play an important role in vegetative segregation. In yeast, segregation may be studied by isolating buds from a dividing zygote and allowing them to produce colonies. Some of these produce colonies that are uniformly of one parental genotype, suggesting that the buds received mitochondria of only that genotype at cell division. However, fluorescent staining suggests that these buds always receive mtDNA molecules of both parental types, although one may be in the minority (*5*). If so, these buds are like uniparental zygotes, starting with two mitochondrial alleles but losing one (usually the minority one) by intracellular drift. Thus vegetative segregation in yeast, and presumably in other organisms as well, is due to a combination of random partitioning of organelle genomes at cell division and random replication, degradation, or gene conversion taking place between divisions.

Analogous phenomena are seen in bacterial plasmids. Some plasmids have been shown to replicate randomly, and some may also be partitioned randomly at cell division. The net effect of random replication and partitioning is plasmid incompatibility, the inability of a bacterial cell line to retain two similar (but not identical) plasmids in every cell. Incompatible plasmids are analogous to mitochondrial or chloroplast genomes carrying different alleles, and this kind of incompatibility is analogous to vegetative segregation (*35*).

Experimental Tests of Drift

As a zygote divides, random drift of the mean allele frequency in the clone as a whole slows and eventually stops. This is partly because random partitioning of organelle genomes produces homoplasmic cells in which the allele frequency is fixed at 1 or 0. Also, allele frequencies in the daughter cells drift independently, often changing in different directions and tending to cancel out. This leads to a strong prediction: if uniparental zygotes and the high variance of allele frequencies among zygote clones are due to intracellular random drift, then delaying division of the zygotes should allow more drift and increase both the frequency of uniparental zygotes and the variance among zygote clones. This prediction has been verified for mitochondrial genes in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (*8*), and for chloroplast genes in *Chlamydomonas reinhardtii* (*22, 36*). An example is shown in Fig. 6. Similar experiments have demonstrated that random drift plays a role in eliminating, or more rarely fixing, new mitochondrial mutations in yeast cells (*37*). An analysis of recombination frequencies in delayed division experiments suggests that random gene conversion does not play a major role in producing uniparental zygotes in yeast and focuses our attention on stochastic replication and degradation (*5, 8*). However, conversion may play a significant role in *Chlamydomonas* zygotes (*36*).

Why Nuclear and Organelle Genes Are Inherited Differently

It is both convenient and instructive to incorporate the phenomena of uniparental inheritance and vegetative segregation into a single, more general rule: heteroplasmic cells are rare; when they are formed, by whatever process, they or their progeny usually become homoplasmic within a single sexual generation. Like many other generalizations in biology, this one must be stated rather loosely (we cannot make it quantitative because rates of vegetative segregation and proportions of uniparental zygotes vary greatly) and there are exceptions (*38*). Nevertheless, it is true for a wide variety of organisms and it describes precisely those phenomena which serve to identify cases of organelle inheritance and distinguish them from Mendelian genetics. It also describes the fixation of a new organelle mutation in cells, the phenomena of invasiveness or suppressiveness which thus far seem to be limited to yeast and some other fungi (*2*), and the recombinational polarity of mitochondrial genes in yeast (*2, 4, 7*). This general rule operates effectively at the population level: recent studies of mtDNA in animal populations show that individuals are rarely detectably heteroplasmic even when the population is genetically polymorphic for restriction sites on mtDNA (*39*). In contrast, heterozygosity for nuclear genes is common in natural populations.

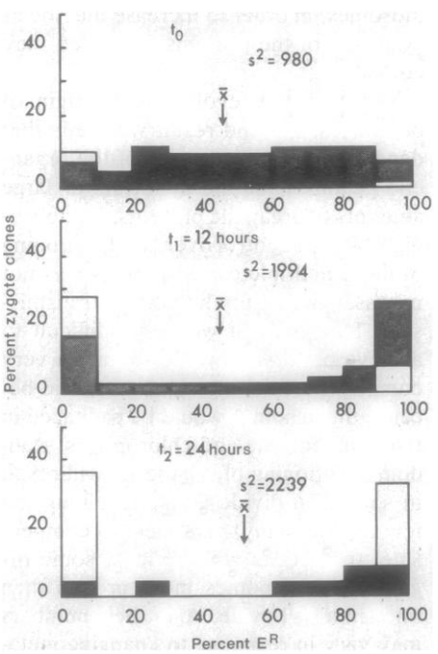


Fig. 6. Effects of delaying cell division on uniparental inheritance and variance of gene frequencies among zygote clones of *Saccharomyces cerevisiae*. The cross was α *cdc24* E^R × *a cdc24* E^S, where *cdc24* is a nuclear temperature-sensitive mutation that blocks cell division at 36°C. Zygotes were held at 36°C for 0, 12, or 24 hours, then moved to 23°C to allow division to begin. Gene frequencies in zygote clones were determined as described for Fig. 3B; symbols as in Fig. 3. [Courtesy of Cold Spring Harbor Laboratory]

Heterozygosity for organelle genes is not a stable condition, because random drift leads to fixation of one allele or one genotype in each cell, and often in an entire cell lineage or organism, within a single sexual generation. This can happen because organelles are intracellular population systems, and the events of organelle genetics are not only stochastic but they can be repeated many times within a single sexual generation. Many rounds of organelle DNA recombination can occur in one cell generation; in yeast and possibly other organisms there is time for many sequential replications of organelle DNA molecules in one cell generation or in one dormant zygote; and finally, organelle genomes can segregate at each of the many vegetative cell divisions that occur between sexual reproduction events, as well as during meiotic cell divisions.

In contrast, nuclear genotypes do not show random drift and thus do retain heterozygosity because there are no repeated stochastic events within a single sexual generation (Fig. 2). Chromosomes are replicated repeatedly, once in every cell generation, but every chromosome is replicated precisely once each time. Recombination generally occurs only once, at meiosis, and is nonrandom in the sense that it involves pairs of chromosomes from different parents. Random partitioning leading to segregation of alleles occurs only once, at meiosis. In very general terms, organelle and nuclear genomes differ in the degree of control exerted over their replication, recombination, and segregation; more is left to chance for mitochondria and chloroplasts.

Sex and Organelles

From the viewpoint of genetics, sexual reproduction consists of combining genes from two different individuals in a single cell, followed by recombination of alleles from the two parents to produce new genotypes. Genes in eukaryotic nuclei are most often sexual. Both parents usually contribute one complete set of nuclear genes to the zygote. Moreover, the stringent control over chromosome replication and partitioning ensure that diploid cells will retain alleles from both parents until recombination occurs at the next meiotic division. Genes in mitochondria and chloroplasts, in contrast, are asexual in organisms with monogametic transmission because only one parent contributes genes to the zygote. In species where zygotes do receive alleles from both parents, random drift (and sometimes, selective silencing) quickly makes cells homoplasmic and thus greatly reduces the opportunity for recombination.

Sexual reproduction has potential disadvantages as well as advantages for individuals and populations (*40*). In particular, recombination may break up adaptive combinations of alleles. Also biparental inheritance facilitates the spread of detrimental mutant genes or genomes that replicate more rapidly than the wild type. Several authors have focused on these disadvantages and suggested that monogametic transmission and selective silencing may have been selected to reduce organelle gene recombination (*1, 41*). These suggestions do not consider the possible role of random drift in the evolutionary history of organelle genes.

Separate Evolutionary Pathways for Organelle and Nuclear Genomes

I suggest that nuclear and organelle genomes have followed divergent evolutionary pathways, respectively, as sexual systems under stringent control and asexual systems under relaxed control, from the time they were established as autonomous genomes in primitive unicellular organisms. The principal genome of most cells, including prokaryotes, is present in low copy number (1 to 2 per cell) and is under stringent control; this is likely to have been a very primitive condition. It is widely believed that mitochondria and chloroplasts arose from intracellular symbionts (*42*). Initially, the replication of the symbiont DNA and of the symbionts themselves would be under their own control, not that of the host. When the host cell divided, the distribution of symbionts to daughter cells would be random, and symbionts present in more copies per cell would be more likely to be represented in both daughters.

Natural selection acting on the symbionts would thus tend to increase the number of symbionts per cell (up to the point where the host was damaged). The number of symbiont genomes per cell would remain large even if the number of symbionts was subsequently reduced by their fusion. As the host came to rely on the symbionts for photosynthesis or respiration, the host (nuclear) genome would exert some control over the replication and partitioning of the symbionts and their genomes. However, the high copy number would ensure the effective hereditary transmission of symbiont genomes even if nuclear control was not stringent.

Alternatively, the autogenous theories of organelle evolution hold that they arose by separate packaging of nuclear and organelle genomes native to the same cell (*42*). It may be that the organelle genes were released from stringent control when they were separated from the nuclear genomes. Their copy number may have been high, like amplified ribosomal RNA genes released from chromosomes, in order to increase the rate of synthesis of the proteins for which they coded.

Whatever the evolutionary origin of organelles, the end result was cells that depend on multiple copies of the organelle genomes for the synthesis of large amounts of organelle proteins. Establishing stringent control over the partitioning of these multiple-copy genomes was not necessary to ensure hereditary transmission. Moreover, it would be difficult to achieve because it would require a very complex partitioning apparatus, and because the genomes would be packaged in the mitochondria and chloroplasts. Random partitioning of organelles will result in some just-divided cells receiving too few (or too many) genomes; to compensate, these cells will replicate some organelles or genomes more or less than once in a cell cycle. Also their numbers may vary in response to changing metabolic needs of the cell. Stringent replication control in which each molecule replicates exactly once in every cell cycle would not be possible. The end result is random drift of gene frequencies.

Because random drift limited recombination frequencies even in these primitive cells, organelles may never have experienced the full advantages or disadvantages of sex. This permitted selection for oogamy (*43*), in which large female gametes contribute more organelle genes than small male gametes and the opportunity for recombination was further reduced. At the extreme, this led to monogametic transmission. Selection for selective silencing would also be permitted, as for instance if zygotes formed under starvation conditions needed to scavenge raw materials by degrading some organelle DNA, as postulated (*22*). Whatley (*14*) has noted that these and other mechanisms that determine uniparental inheritance of chloroplasts are all used by one or another species of algae and plants, without any apparent evolutionary trends; she also suggests that these mechanisms may be accidental results of other evolutionary events, rather than being selected for causing uniparental inheritance. Organelle fusion, which is required for recombination, could also be lost if recombination was not effective; this may have occurred in some plants (*13, 14, 38*) and in *Paramecium* (*44*). In this view, monogametic transmission and selective silencing are evolutionary consequences of random drift, in the sense that they are permitted to evolve in genetic systems that are already effectively asexual.

Conclusion

Organelle and nuclear genomes have probably followed separate evolutionary paths from the time of origin of eukaryotic cells. The former began, and remained, as multicopy systems under relaxed control; the latter have been one- and two-copy systems under stringent control. It is these fundamental differences at the molecular level which result, directly or indirectly, in their different modes of inheritance. The most general and important consequences are that nuclear genomes can show high levels of heterozygosity and enjoy the advantages of sexuality to the fullest, while organelle genes cannot. In spite of these differences, the two systems perform their respective tasks well and in harmony. How they achieve this harmony is a major question for the future.

References and Notes

1. R. Sager, *Cytoplasmic Genes and Organelles* (Academic Press, New York, 1972).
2. N. W. Gillham, *Organelle Heredity* (Raven, New York, 1978).
3. J. T. O. Kirk and R. A. E. Tilney-Bassett, *The Plastids* (Elsevier/North-Holland, Amsterdam, ed. 2, 1978).
4. C. W. Birky, Jr., *Annu. Rev. Genet.* **12**, 471 (1978).
5. _____, A. R. Acton, R. Dietrich, in *Mitochondrial Genes*, G. Attardi, P. Borst, P. P. Slonimski, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), p. 333.
6. R. A. Lansman, J. C. Avise, M. B. Huettel,

Proc. Natl. Acad. Sci. U.S.A. **80**, 1969 (1983).
7. B. Dujon, in *Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1981), p. 505.
8. K. M. Thrailkill, C. W. Birky, Jr., G. Lückemann, K. Wolf, *Genetics* **96**, 237 (1980).
9. R. Egel, J. Kohli, P. Thuriaux, K. Wolf, *Annu. Rev. Genet.* **14**, 77 (1980); L. Del Guidice, K. Wolf, G. Seitz, G. Burger, B. Lang, F. Kaudewitz, *Mol. Gen. Genet.* **152**, 319 (1977).
10. R. F. Matagne and M.-P. Hermesse, *Curr. Genet.* **1**, 127 (1980); *ibid.* **3**, 31 (1981). The *mat-1* mutation also uncouples the mating-type locus from chloroplast gene inheritance; but in the absence of detailed studies of gene frequency distributions among the biparental zygotes, it is not clear how closely the result resembles mitochondrial gene inheritance in yeast [see R. Sager, C. Grabowy, H. Sano, *Cell* **24**, 41 (1981)]. Uniparental inheritance is also seen in other *Chlamydomonas* species, including the homothallic *C. monoica*. [K. P. VanWinkle-Swift and B. Aubert, *Nature (London)* **303**, 167 (1983)].
11. P. Michaelis, *Nucleus (Paris)* **10**, 1 (1955).
12. M. F. Waxman and C. W. Birky, Jr., *Curr. Genet.* **5**, 171 (1982); K. P. VanWinkle-Swift, *ibid.* **1**, 113 (1980). These models are only partly random, with a tendency for like DNA molecules to stay together when cells divide.
13. B. B. Sears, *Plasmid* **4**, 233 (1980).
14. J. M. Whatley, *Biol. Rev. City Coll. N.Y.* **57**, 527 (1982).
15. W. Burton, C. T. Grabowy, R. Sager, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1390 (1979); T. Kuroiwa, S. Kawano, S. Nishibayashi, C. Saito, *Nature (London)* **288**, 481 (1982); A. W. Coleman and M. J. Maguire, *Curr. Genet.* **7**, 211 (1983).
16. R. Sager and R. Kitchin, *Science* **189**, 426 (1975).
17. R. A. E. Tilney-Bassett, *Genet. Res.* **16**, 49 (1970).
18. N. W. Gillham, J. E. Boynton, R. W. Lee, *Genetics* **78**, 439 (1974). These models have been reevaluated on the basis of further evidence (*21, 45*).
19. In some plants the mitochondrial genome may be distributed over several different sizes of mtDNA molecules, but each molecule and hence each gene is still present in many copies per cell [W. M. Spruill, Jr., C. S. Levings III, R. R. Sederoff, *Dev. Genet.* **1**, 363 (1980); B. L. Wood, R. S. Anderson, A. J. Bendich, *Cell* **25**, 793 (1981)].
20. K. Wolf and F. Kaudewitz, in *Mitochondria 1977*, W. Bandlow *et al.*, Eds. (de Gruyter, Berlin, 1977), p. 65.
21. C. W. Birky, Jr., K. P. VanWinkle-Swift, B. B. Sears, J. E. Boynton, N. W. Gillham, *Plasmid* **6**, 173 (1981).
22. K. P. VanWinkle-Swift, *Nature (London)* **275**, 749 (1978).
23. C. W. Birky, Jr., *Int. Rev. Cytol.* **15** (Suppl.), 49 (1983); T. Butterfass, *Planta* **84**, 230 (1969).
24. L. G. Treat and C. W. Birky, Jr., *Plasmid* **4**, 261 (1980).
25. D. Bogenhagen and D. A. Clayton, *Cell* **11**, 719 (1977).
26. D. H. Williamson and D. J. Fennell, *Mol. Gen. Genet.* **131**, 193 (1974); E. Sena, J. Welch, S. Fogel, *Science* **194**, 433 (1976); J. Leff and T. R. Eccleshall, *J. Bacteriol.* **135**, 436 (1978). For *Chlamydomonas* cpDNA, see M. Turmel, C. Lemieux, and R. W. Lee [*Curr. Genet.* **4**, 91 (1981)].
27. O. C. Richards and R. S. Ryan, *J. Mol. Biol.* **82**, 57 (1974) (this paper on *Euglena* mtDNA includes a review of rat mtDNA).
28. B. J. Stevens, *Biol. Cell.* **28**, 37 (1977); T. Butterfass, *Patterns of Chloroplast Reproduction* (Springer-Verlag, New York, 1979); B. B. Sears, J. E. Boynton, N. W. Gillham, *Genetics* **96**, 95 (1980); M. N. Conrad and C. S. Newlon, *Curr. Genet.* **6**, 147 (1982); G. S. Michaels, W. W. Hauswirth, P. J. Laipis, *Dev. Biol.* **94**, 246 (1982); see also (*33*).
29. J. E. Boynton, N. W. Gillham, E. H. Harris, C. L. Tingle, K. VanWinkel-Swift, G. M. W. Adams, in *Genetics and Biogenesis of Chloroplasts and Mitochondria*, Th. Bucher, W. Neupert, W. Sebald, S. Werner, Eds. (North-Holland, Amsterdam, 1976), p. 313; C. W. Birky, Jr., M. Lorenz, A. S. Albel-Hennis, E. A. Clines, unpublished data.
30. C. W. Birky, Jr., and R. V. Skavaril, *Genet. Res.* **27**, 249 (1976); T. Ohta, *ibid.* **30**, 89 (1977).
31. C. W. Birky, Jr., and R. V. Skavaril, *Genetics* **88**, s11 (Abstr.) (1978).
32. M. Tourte, F. Mignotte, J.-C. Mounolou, *Dev. Growth Differ.* **23**, 9 (1981).
33. I. B. Dawid and A. W. Blackler, *Dev. Biol.* **29**, 152 (1972); L. Piko and L. Matsumoto, *ibid.* **49**, 1 (1976).
34. M. Simpson, personal communication.
35. R. P. Novick and F. C. Hoppensteadt, *Plasmid* **1**, 421 (1978); K. Ishii, T. Hashimoto-Gotoh, K. Matsubara, *ibid.*, p. 435.
36. B. B. Sears, *Curr. Genet.* **2**, 1 (1980); C. W. Birky, Jr., M. Lorenz, A. S. Albel-Hennis, E. A. Clines (unpublished data). Recombination frequencies increased along with uniparental zygote frequency in the former experiment, suggesting a role for gene conversion; but there was no increase in recombination frequency in the latter experiment, which dealt with vegetative zygotes rather than zygospores.
37. J. S. Backer, thesis, Ohio State University (1980).
38. K. C. Vaughn, *Curr. Genet.* **3**, 243 (1981).
39. J. C. Avise, R. A. Lansman, R. O. Shade, *Genetics* **92**, 279 (1978); J. C. Avise, C. Giblin-Davidson, J. Laerm, J. C. Patton, R. A. Lansman, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6694 (1970); S. S. Potter, J. E. Newbold, C. A. Hutchison III, M. H. Edgell, *ibid.* **72**, 4496 (1975); W. B. Upholt and I. B. Dawid, *Cell* **11**, 571 (1977); K. Buzzo, D. L. Fouts, D. R. Wolstenholme, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 909 (1978). An exception is discussed by W. W. Hauswirth and P. J. Laipis [in *Mitochondrial Genes*, G. Attardi, P. Borst, P. P. Slonimski, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), p. 333].
40. J. Maynard Smith, *The Evolution of Sex* (Cambridge Univ. Press, Cambridge, 1978); G. Bell, *The Masterpiece of Nature* (Croom Helm, London, 1982).
41. P. Grun, *Cytoplasmic Genes and Evolution* (Columbia Univ. Press, New York, 1976).
42. M. W. Gray and W. F. Doolittle [*Microbiol. Rev.* **46**, 1 (1982)] review this controversial subject.
43. G. A. Parker, R. R. Baker, V. G. F. Smith, *J. Theor. Biol.* **36**, 529 (1972).
44. R. A. Maki and D. J. Cummings, *Plasmid* **1**, 106 (1977); A. Adoutte, thesis, University Sud-Paris, Centre-d'orsay (1977).
45. R. A. E. Tilney-Bassett and C. W. Birky, Jr., *Theor. Appl. Genet.* **60**, 43 (1981).
46. Unpublished data from C. W. Birky, Jr., M. Lorenz, A. S. Albel-Hennis and E. A. Clines.
47. L. G. Treat, thesis, Ohio State University, Columbus (1978).
48. I am grateful for helpful discussion, commentary on versions of this paper, and the sharing of unpublished data, ideas, and manuscripts to numerous colleagues, especially Michael Adams, John Boynton, James Crow, Nicholas Gillham, Philip Perlman, John Preer, Michael Savageau, Barbara Sears, Tracy Sonneborn, Kathryn Thrailkill, Karen VanWinkle-Swift, and Klaus Wolf. I also thank the anonymous reviewers for valuable suggestions. Supported by PHS grants GM19607 and GM21896.