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THE INHERITANCE OF GENES IN MITOCHONDRIA AND CHLOROPLASTS: Laws, Mechanisms, and Models

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■ **Abstract** The inheritance of mitochondrial and chloroplast genes differs from that of nuclear genes in showing vegetative segregation, uniparental inheritance, intracellular selection, and reduced recombination. Vegetative segregation and some cases of uniparental inheritance are due to stochastic replication and partitioning of organelle genomes. The rate and pattern of vegetative segregation depend partly on the numbers of genomes and of organelles per cell, but more importantly on the extent to which genomes are shared between organelles, their distribution in the cell, the variance in number of replications per molecule, and the variance in numerical and genotypic partitioning of organelles and genomes. Most of these parameters are unknown for most organisms, but a simple binomial probability model using the effective number of genomes is a useful substitute. Studies using new cytological, molecular, and genetic methods are shedding some light on the processes involved in segregation, and also on the mechanisms of intracellular selection and uniparental inheritance in mammals. But significant issues remain unresolved, notably about the extent of paternal transmission and mitochondrial fusion in mammals.

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INTRODUCTION

The literature on the inheritance of genes in mitochondria and chloroplasts (hereafter, organelle genes) has changed, grown, and advanced tremendously since I first reviewed it for the *Annual Review of Genetics* (13). That review focused on the new discoveries made with *Saccharomyces cerevisiae* (hereafter, yeast or budding yeast) and on the models developed to account for these data. Some of these models have long since been discarded and others have been modified in light of new information from yeast and an increasing number of other organisms. Fortunately, this paper can build on more recent reviews that, together, cover various aspects of the subject in greater depth than is possible here (15–18, 32). The focus is on the search for molecular and cellular mechanisms responsible for the patterns of organelle gene inheritance. The aim is to explain the unique features of organelle inheritance, just as the chromosome theory of heredity and its extensions explain the most basic features of the inheritance of nuclear genes.

Some of the most exciting advances since the previous reviews have been made in understanding the molecular and cellular mechanisms of organelle division and distribution between daughter cells (partitioning) in yeast, animals, and plants; genetic studies of segregation and within-generation selection of mitochondrial genes in mammals and *Drosophila*; and the controversial subject of mitochondrial bottlenecks in mammals. Other exciting discoveries dealt with the mechanisms of uniparental inheritance in *Chlamydomonas* and mammals, and a controversy over whether there is a low level of biparental inheritance and recombination in humans. The growing excitement about mitochondrial genetics in humans and mammals has been driven in large part by their application to human diseases caused by mitochondrial mutations, and by the widespread use of mitochondrial genes to study the population genetics and evolution of humans and other animals. These subjects have also been reviewed in the past three years, but mainly as separate subjects and not in the context of organelle heredity in general.

The review begins with a brief reminder of the basic rules of inheritance of nuclear genes (Mendelian genetics) and the cellular mechanisms behind them. These are contrasted with the non-Mendelian rules of inheritance of organelle genes. This is followed by discussions of the molecular and cellular mechanisms of non-Mendelian inheritance: vegetative segregation, uniparental inheritance, and limited recombination. Besides satisfying our curiosity about the mechanisms of heredity, an understanding of these phenomena is essential for plant and animal breeding, including genetic engineering of organelle genomes; for diagnosing inherited mitochondrial diseases and counseling patients; and to enable the use of organelle genomes to study population genetics and evolution, including human evolution.

MENDELIAN VERSUS NON-MENDELIAN GENETICS

Mendel's Five Laws and the Mechanisms of Mendelian Inheritance

Mendel's model of heredity is still the accepted description of most of the important features of heredity for nuclear genes. As biological models go, Mendel's has great generality, although we now know of many exceptions (e.g., linkage). Textbooks commonly speak of Mendel's first and second laws, but the model that he used to explain his data actually had five components all of which are explicit or implicit in his paper (82). In 1909, Erwin Baur (5) showed that chloroplast genes in *Pelargonium* (geraniums) violate four of Mendel's five laws:

1. During asexual reproduction, alleles of nuclear genes do not segregate: Heterozygous cells produce heterozygous daughters. We now know that this is because nuclear genomes are stringent genomes (16) in which (*a*) all chromosomes are replicated once and only once in interphase; and (*b*) mitosis ensures that both daughter cells get one copy of each chromosome. In contrast, alleles of organelle genes in heteroplasmic cells segregate during mitotic as well as meiotic divisions to produce homoplasmic cells. This vegetative segregation occurs because organelles are relaxed genomes (15, 16) in which some copies of the organelle genome can replicate more often than others by chance or in response to selective pressures or intrinsic advantages in replication, and alleles can segregate by chance during cytokinesis.
2. Alleles of a nuclear gene always segregate during meiosis, with half of the gametes receiving one allele and half the other ("Mendel's first law"). Alleles of organelle genes may or may not segregate during meiosis; the mechanisms are the same as for vegetative segregation.
3. Inheritance of nuclear genes is biparental. Organelle genes, in contrast, are often inherited from only one parent (uniparental inheritance).
4. Alleles of different nuclear genes segregate independently ("Mendel's second law"), as a result of the independent segregation of chromosomes at meiosis and of recombination between genes on the same chromosome. In

contrast, organelle genes are nearly always on a single chromosome and recombination is often severely limited by uniparental inheritance or failure of organelles to fuse and exchange genomes.

5. Fertilization is random with respect to the genotype of the gametes. This is the only part of Mendel's model that applies to organelle as well as nuclear genes.

VEGETATIVE SEGREGATION

Vegetative segregation is the most general characteristic of the inheritance of organelle genes, occurring in both mitochondria and chloroplasts in all individuals or clones of all eukaryotes. I emphasize four systems that have been the focus of much of the research: chloroplast genes in plants and the alga *Chlamydomonas*, and mitochondrial genes in yeast and mammals. However, the conclusions about mechanisms of organelle heredity apply to many, and probably all, eukaryotes.

Plant Chloroplasts: Vegetative Segregation Due to Stochastic Partitioning of Organelles

AN ASIDE ON TERMINOLOGY Organelle genomes are physically divided up between daughter cells at every cell division, but alleles do not necessarily segregate at every division. To avoid confusion, I use partitioning for the physical separation of genomes or alleles, and reserve segregation for those cases in which different alleles end up in different cells.

STOCHASTIC PARTITIONING OF ORGANELLES The first, and simplest, formal model of organelle inheritance was applied to *Epilobium* and other plants with two or more chloroplasts per cell (62). This model assumes that the plastid is the unit of mutation and inheritance (Figure 1). (We speak of plastids because that term includes the proplastids in the embryo plants as well as fully differentiated chloroplasts.) Each plastid is assumed to divide once per cell cycle. At cytokinesis, the two daughter cells are assumed to receive equal numbers of chloroplasts but a strictly random sample of the chloroplast genotypes. This physical model of partitioning corresponds to the mathematical model of sampling without replacement and the hypergeometric probability distribution (62, 95). Although it was developed for plant plastids, the physical model and the hypergeometric distribution have been applied to mitochondria, to mitochondrial and chloroplast genomes, and to other organisms. It has been known for three decades that no part of this simple physical model is strictly correct (for reviews see 11, 13, 15, 20). However, it is a reasonable approximation for many plants, correctly predicting that vegetative segregation will be complete within about one plant generation, given the number of proplastids seen in plant embryo cells (62). Although plastids do fuse (59), this is so rare in plants that it can be ignored. Of course, the plastid cannot always be

the unit of inheritance because each plastid has tens to hundreds of genomes, and a new mutation only affects one. However, a genome with a new mutation increases in frequency in one or more plastids until they become homoplasmic for the mutant allele, after which the plastid is the unit of inheritance. We also know that daughter cells do not always receive equal numbers of organelles, and consequently, some plastids may have to divide more often than others to restore equality, but partitioning is numerically equal in most cases (14). It is to be expected that mechanisms have evolved to ensure that a cell has a better-than-random chance of receiving half of the parent cell's organelles (or organelle genomes), because this reduces the chances of its receiving no organelles.

ORGANELLES CAN BE DIVIDED INTO APPROXIMATELY EQUAL PARTS Plastids must divide into two equal parts if they are to be considered units of inheritance that are identical in all respects except genotype; recent studies are beginning to show how this happens. In many organisms, division of mitochondria or chloroplasts is preceded by the appearance of a filamentous plastid division ring, or mitochondrial division ring, around the division furrow at the middle of the organelle (48). Recent studies detected two different molecular systems for the division of organelles in different organisms. One is FtsZ, which is used to divide bacterial and archaeal cells (73) as well as mitochondria and chloroplasts in plants and at least some eukaryotic protists (7, 45, 57; reviewed in 6, 70). In bacteria and archaea, the FtsZ protein polymerizes as a ring of filaments similar to tubulin around cells at their midpoint and constricts during cell division. In plants and algae, there are probably two FtsZ genes, one each for the division rings on the inner and outer plastid membranes (70, 71).

In bacteria, several proteins are necessary for positioning the ring made by FtsZ in the middle of the cell (reviewed in 25). Homologues of two of these are encoded by plastid genes in the green alga *Chlorella* (94). A nuclear gene encodes one in *Arabidopsis*, where it is required for correct positioning of the division ring at the midpoint of the plastid (25).

ANOTHER ASIDE ON TERMINOLOGY: DETERMINISTIC, STOCHASTIC, AND RANDOM MODELS The study of organelle genetics has been plagued by confusion about the roles of chance and determinism in genetics. Scientists tend to favor deterministic models (hypotheses, laws; e.g., first and fourth Mendelian laws) because they make unambiguous predictions: A specific event is invariably succeeded by a specific outcome. But much of the world is unpredictable and must be described by stochastic models, which give only the probability of a specific outcome. Such models are also commonly called random, but that term is also used to describe a specific kind of stochastic process in which all outcomes have the same probability. I use strictly random to describe this specific class of models. The third and fifth Mendelian laws are strictly random. We now know that the segregation of different genes is not always strictly random because of linkage, but it is still stochastic.

Chlamydomonas Chloroplasts: Stochastic Replication and Partitioning of Genomes

VEGETATIVE SEGREGATION IS RAPID IN *CHLAMYDOMONAS* CHLOROPLASTS *Chlamydomonas reinhardtii* has been used extensively for chloroplast genetics since the pioneering studies of Sager (74, 75) and Gillham (31, 33). In contrast to the plants discussed above, *Chlamydomonas* cells have one chloroplast, which divides into two equal parts just before the cell divides; consequently, vegetative segregation cannot be explained by the partitioning of chloroplasts. Most data come from crosses of antibiotic-resistant by sensitive clones. Vegetative segregation can be studied in vegetative zygotes, which divide by mitosis instead of meiosis, or in the meiotic and early mitotic divisions of the small percentage of zygospores that show biparental inheritance. In either case, segregation is complete within a few cell generations. This is much too fast to be accounted for by random partitioning of the approximately 50–100 genomes.

GENOME PARTITIONING IS PROBABLY STOCHASTIC BUT NOT STRICTLY RANDOM One possible explanation for rapid segregation is that when the two gamete chloroplasts fuse in the zygote, the plastid genomes from the parents tend to remain in different parts of the chloroplast and consequently tend to segregate together rather than strictly randomly (92). The chloroplast genomes are grouped in about 5–15 nucleoids, and it is possible that the 10 or more genomes in each nucleoid tend to be replication products of one genome. In other words, genome partitioning is stochastic but not strictly random; like molecules tend to segregate together because they are joined in nucleoids and/or the nucleoids from the gametes are not completely mixed in the zygote.

GENOME REPLICATION IS STOCHASTIC Different *Chlamydomonas* zygotes from the same mating give rise to clones with very different frequencies of alleles from the two parents. Some zygote clones are uniparental, with organelle genomes from only one parent or the other. Frequency distributions of gene frequencies in a large number of zygote clones bear a striking resemblance to the gene frequency distributions of Mendelian populations undergoing random genetic drift (21). When the mitotic division of vegetative zygotes (93), or the meiotic divisions of zygospores (76), was delayed for a time by starvation, the variance in gene frequencies increased and more uniparental zygote clones were produced. These data suggested that plastid genomes continue to replicate during starvation and that replication is stochastic, with some genomes replicating more often than others by chance. The result is that gene frequencies within cells undergo stochastic changes, which I called intracellular random drift by analogy to random drift of nuclear gene frequencies in populations of organisms (13). Stochastic replication by itself will not completely eliminate an allele from a cell or clone, but may reduce it to a frequency too low to detect. Alternatively, there may be some degradation of organelle DNA molecules, which will then be replaced by additional replications

of other molecules (turnover). Note that the stochastic replication of genomes, and the stochastic partitioning of genomes into daughter organelles when an organelle divides, can also explain how a mutant genome becomes homoplasmic in plant plastids. Figure 2 illustrates vegetative segregation due to a combination of stochastic replication and partitioning of organelle genomes.

Yeast Mitochondria

Much has been learned about organelle heredity from the study of another model genetic system, mitochondrial genes in budding or baker's yeast (*Saccharomyces cerevisiae*). The best markers are mutant genes conferring antibiotic resistance; respiration-deficient mutants (*petites*) are also used but their inheritance is strongly affected by selection. When heteroplasmic zygotes are produced by mating yeast strains that differ in one or more mitochondrial alleles, the majority of diploid progeny are homoplasmic after no more than 20 cell generations. Strictly random partitioning could only explain this rate of segregation if there were no more than 2 to 5 segregating units (19). This is much smaller than the number of mtDNA molecules in diploid cells [approximately 100] and slightly smaller than the number of nucleoids. Mitochondria from the two parents cannot be the segregating units because they fuse in the zygote. Consequently, vegetative segregation in yeast must be explained by some combination of the same factors that were invoked above for chloroplast genes in *Chlamydomonas*: (*a*) partitioning of genes that is stochastic but not strictly random, with similar molecules tending to remain together; (*b*) stochastic replication; or (*c*) turnover. There is experimental evidence only for the first two processes, but it is likely that all three are involved.

MITOCHONDRIAL FUSION AND FISSION A yeast cell may contain a single large mitochondrial network, or a network plus a few small separate mitochondria, or many small discrete mitochondria, depending on its physiological state. Yeast mitochondrial genomes undergo multiple pairings with recombination in zygotes, showing that genomes from the two parents can interact extensively. Considerable progress has been made in understanding mitochondrial fusion and fission in yeast. Fission is accomplished by the dynamin system in yeast and animals (reviewed in 23). The dynamin Dnmp1p localizes to mitochondria at division sites and tips and is required for normal mitochondrial morphology. Mitochondrial fusion requires the *fzo1* (*fuzzy onion*) gene, a homologue of the *fuzzy onion* gene that is required for mitochondrial fusion in *Drosophila*. In yeast, normal mitochondrial morphology requires a balance between the activities of Dnm1p and Fzo1 (78).

BUD POSITION EFFECTS: NONRANDOM PARTITIONING Early models of mitochondrial gene inheritance in yeast assumed that fusion was so frequent that a cell is effectively a single population of freely interacting genomes. That this could not be strictly true was demonstrated by pedigree studies of zygotes (19, 22, 83), which showed that (*a*) when the first bud comes from one end of the zygote, the majority

of its mitochondrial genes come from the parent which formed that end of the zygote; and (*b*) buds that arise from the neck of the zygote receive markers from both parents, as well as a higher frequency of recombinant genotypes. This indicates that the mixing of mitochondrial genomes from the two parents is incomplete when the first bud is formed; later buds usually include markers from both parents, indicating more complete mixing. This interpretation was verified by showing that labeled mtDNA from one parent failed to enter the opposite side of the zygote until some time after the first bud was formed, although it did enter first center buds (68). The mitochondrial membranes from the two parents fused quickly, so delayed mixing of mtDNA was not due to delayed mitochondrial fusion; evidently, the movement of mtDNA across the zygote involves a different mechanism from the movement of mitochondria. Mitochondrial proteins also move more quickly through the mitochondrial network than does mtDNA (3, 68, 69).

MITOCHONDRIAL MOVEMENT FROM MOTHER TO BUD Because *Saccharomyces* cells bud rather than undergoing binary fission, a mechanism is required to move mitochondria and their genes from the mother into the growing bud. The experimental studies of this process have been reviewed (23). Mitochondria are actively transported from the mother cell into the bud, where they are immobilized at the tip of the bud until cytokinesis is complete. Mitochondria probably move along actin filaments by a motor that depends on actin polymerization (80), and movement also requires intermediate filaments encoded by the MDM gene (58). It is not surprising that a mechanism evolved which ensures that buds receive at least some mitochondria, which are required for survival, and mitochondrial genomes, which are required for respiratory competence.

STOCHASTIC REPLICATION As was the case for *Chlamydomonas* chloroplast genes, yeast cells can become homoplasmic for mitochondrial genes without dividing, owing to random genetic drift of gene frequencies within the cell (reviewed in 16). This was demonstrated using delayed division experiments with both budding and fission yeast (91), analogous to those in *Chlamydomonas*. Birky and colleagues (20) reported that many first central buds are uniparental, producing clones with mitochondrial genes from only one parent; however, when wild-type cells were mated with ρ^o mutants that have mitochondria but no mtDNA, all first central buds receive mtDNA. They suggested that all first central buds probably receive mtDNA from both parents but that stochastic replication (possibly combined with turnover) eliminates genes from one parent or the other. Stochastic replication is almost certainly a major contributor to the production of homoplasmic cells during asexual reproduction in yeast, i.e., to vegetative segregation.

NUCLEOID STRUCTURE AFFECTS MITOCHONDRIAL GENE INHERITANCE It was suggested that the segregating units in yeast mitochondria might be nucleoids (19), and recent studies suggest that nucleoid structure does affect the inheritance of mitochondrial genes. The mtDNA molecules in a nucleoid appear to be held

together by Holliday structures (46, 51, 53, 97), perhaps because mtDNA replication is initiated by recombination (8, 56, 77) as it is in T-even phage (66). Mutations that affect the resolution of the Holliday structures also modify the inheritance of neutral ρ^- genomes in $\rho^- \times \rho^+$ crosses (54, 98).

Mammalian Mitochondria

Vegetative segregation is difficult to study in humans and other mammals with uniparental inheritance, because the only sources of heteroplasmic cells are new mutations. Early studies of mitochondrial genetics in mammals bypassed uniparental inheritance by fusing cultured animal cells or enucleated cytoplasts of different mitochondrial genotypes and following the proportions of the two genotypes over time. The interpretation of these studies is complicated by the use of human-rodent and other interspecific hybrids that may have been affected by incompatibility of nuclear and mitochondrial genes, or by the use of antibiotic resistance mutants that were subject to selection. The discovery of mitochondrial mutations segregating in a herd of dairy cattle showed that a mitochondrial mutation can be fixed in a few generations (1, 47). More recently, vegetative segregation has been studied in heteroplasmic mice created by cytoplast fusion. The offspring of such mice can have dramatically different levels of heteroplasmy; this is not due to selection because the mean allele frequency among the progeny is the same as that in the mother (24). Single-cell PCR has been used to demonstrate that the increase in the variance of allele frequencies takes place in maturing oocytes and in dividing germline cells (41). Colonic crypts were used for another elegant demonstration of drift in heteroplasmic mice (42). Each crypt is derived from a single founder cell that produces stem cells, which in turn continually divide to replace crypt cells. The proportion of donor mitochondrial genomes was determined in individual crypts from heteroplasmic embryos aged 4 and 15 months. The mean gene frequency was about 4% at both times, indicating the absence of selection, but the variance of donor mtDNA proportion among crypts increased greatly. The frequency distributions of genotype frequencies at the two times strongly resemble frequency distributions of allele frequencies in populations of organisms undergoing random genetic drift.

There is no way of telling to what extent vegetative segregation in animals is due to stochastic partitioning as opposed to stochastic replication. There is some interesting evidence that the choice of mtDNA molecules for replication is not strictly random but is biased in favor of molecules near the nucleus (26, 61, 63).

The studies on cattle and on mouse models show that vegetative segregation requires one or a few organismal generations to complete. If there are about 20 cell generations per organismal generation, segregation in animals is substantially slower than in yeast, *Chlamydomonas*, and plants. Paradoxically, a clone of animal cells carrying wild-type and respiration-deficient genomes produced almost no homoplasmic cells (39, 50). Unrealistic models were proposed to explain this as a case of no, or very slow, vegetative segregation. However, the apparently stable

heteroplasmy may actually represent an equilibrium between intracellular selection tending to increase the frequency of respiration-deficient genomes (see Paradoxical Intracellular Selection . . . below) and intercellular selection against cells with very high frequencies of respiration-deficient genomes.

Do Mammalian Mitochondria Fuse?

A continuing controversy about mammalian mitochondrial genetics is the question of the extent to which mitochondria fuse and share genomes and other components. Fusion is difficult to prove using the static pictures from electron microscopy, and light microscopy cannot distinguish between permanent fusion and transient contacts of these tiny organelles. A number of authors have created cells heteroplasmic for two different mitochondrial genotypes and looked for complementation, which would indicate sharing of genes or gene products. Complementation was found in some but not all cases. I pointed out that in the experiments where no complementation was observed, there was no independent evidence that the two mutants could complement each other (18). This potential problem was highlighted by a recent paper (27). Human cells with two different mutations, one in tRNA(Lys) and one in ND4, were mixed and treated to promote fusion. Fused cells were selected using nuclear drug-resistant mutant genes in glucose medium, which does not select for respiratory competence. Cells with complementation of the mitochondrial mutations were selected in galactose-containing medium in which respiratory competence is required for growth. The frequency of cell fusion was much greater than the frequency of complementation, leading the authors to conclude that no more than 1.5% of the fusion products showed complementation. But the cells showing complementation grew slowly, suggesting that complementation might not be complete enough to be detected in many of the cells in which it occurred. This potential problem was avoided in another experiment by creating cybrids that were heteroplasmic for a genome with a deletion of several tRNAs and a genome with a point mutation in another tRNA (87). The cybrids did not have sufficient respiratory competence to grow in selective medium, but complementation was demonstrated in medium that did not select for respiratory competence, by finding fusion peptides that could only be transcribed from the deletion genome and translated with the help of normal tRNAs from both genomes. It has been suggested that when complementation was found, it might be due to transient fusion of mitochondria induced by the PEG used to fuse the cells (27). But this does not explain why complementation was found only in medium that did not select for respiratory competence (87). I conclude that the evidence for complementation continues to be more convincing than the evidence against it. Unfortunately, these studies do not indicate the frequency of mitochondrial fusion. Mitochondrial DNA from wild-type cytoplasts spread throughout mitochondria from ρ^o cells within 6 h after fusing the cells (35). However, we do not know if such rapid fusion and sharing of genomes would be seen between ρ^+ mitochondria.

The Simplest Model of Vegetative Segregation

It is apparent from the discussion of vegetative segregation that stochastic replication and partitioning of genomes during the division of organelles and cells, and stochastic partitioning of organelles at cell division work together to produce homoplasmic daughter cells from heteroplasmic mothers (Figure 3). Stochastic replication of organelles may also play a role but this has not been clearly demonstrated. Two other stochastic processes, gene conversion and turnover, can assist in making homoplasmic cells heteroplasmic even in the absence of cell division. Evidently, the rate of vegetative segregation is determined by many factors: the number of organelles and the extent to which they share organelles; the number of genomes per organelle and per cell; the variance in number of times a genome replicates; the variance in numbers of genomes that are partitioned into daughter organelles and daughter cells; and the degree of mixing of organelles of different genotypes in organelles and in cells. It is extremely unlikely that we will ever know all of these parameters exactly for any organism. This is especially true for animals and plants, where the parameters probably vary among different cell types and at different stages of development. Even if we did know all of the relevant parameters, an exact mathematical model would be impossible to solve and computer simulations would be tedious at best. Fortunately, we can borrow a simple mathematical model that requires only two measurable parameters from Mendelian population genetics. In this model, the cell has an effective number n_e of organelle genomes. Stochastic replication and partitioning are modeled by giving each daughter cell a strictly random sample with replacement from the mother cell. The allele frequencies in the daughter cells follow the binomial distribution. Starting with a cell in which the allele frequency is p_0, binomial sampling is continued for a number c of cell generations, at which time the variance in the frequency of the allele among the cells is given by

$$V_c = p_0(1 - p_0)[1 - (1 - 1/n_e)^c] \qquad 1.$$

The literature reflects a great deal of misunderstanding about the parameter n_e. This is an effective number, which is an unspecified function of the real number of genomes in a cell. For example, if the increase in variance were due entirely to random partitioning, n_e could be replaced by the hypergeometric distribution and the real number n of genomes per cell, as in the simplified plant model described earlier. The important point is that n_e is not the number of genomes or of any other biological entity in any cell, and in fact it is unlikely ever to correspond closely to the number of anything. Its utility lies in the fact that it can be estimated from V_c, p_0, and c using Equation 1, after which it can be used to predict the rate of vegetative segregation or to compare the rate of segregation in different systems. It should be measured using neutral alleles so that it reflects drift alone, not intracellular or intercellular selection. After that it can be used as a null model; if other alleles segregate more rapidly, one can suspect that the alleles are subject to selection or have some effect on other factors that

can affect segregation such as the number of genomes or their distribution in the cell.

Bottlenecks: Are they Real, and Do We Need Them?

The literature on mammalian mitochondrial genetics is full of references to bottlenecks, most of which reflect a misunderstanding of the effective number of genomes in Equation 1. Many authors have used this equation or something analogous to estimate n_e from the variance in gene frequencies in a clone of cells or the offspring of a single female. Then they compare this to the real number of genomes, nucleoids, or organelles per cell to see if any real number matches n_e; this is then taken to be the effective segregating unit. But n_e does not represent any real physical entity. For example, if n_e is smaller than the real number of genomes per cell (which it always is), one possibility is that genome replication is strictly random but partitioning is not because genomes are not well mixed in the cell. We do not need bottlenecks to explain why n_e is smaller than the number of genomes. But this does not mean that bottlenecks are not real. A review of published electron micrographs led to the conclusion that there are fewer than 10 mitochondria per primordial germ cell (40); these eventually give rise to primary oocytes with many hundreds of mitochondria.

INTRACELLULAR SELECTION

Intracellular Selection Based on Phenotype

Birky (4, 10) showed that mitochondrial genomes carrying antibiotic-resistance point mutations replicate and replace wild-type genomes when cells are exposed to antibiotic. In the absence of the antibiotic, wild-type genomes out-replicate those with resistance markers. Selection within an organism has also been demonstrated in mice that are heteroplasmic for mitochondrial genomes from different strains (e.g., 42, 88). Unfortunately, studies on whole animals cannot distinguish between intracellular selection and intercellular selection, i.e., selective growth of cells that acquired more copies of the favored genotype. Clear evidence of intercellular selection has been seen in plants that are heteroplasmic for green and white plastids.

Intracellular Selection Based on Genome Structure

A superficially similar phenomenon was seen in the earliest experiments on yeast mitochondrial genetics, in which wild-type genomes are out-replicated by highly suppressive *petite* genomes. A highly suppressive *petite* genome consists of a small segment of the wild-type genome, repeated to produce a molecule of approximately normal size. It is now almost certain that these genomes out-replicate wild-type genomes because they have more copies of a replication origin (52). Molecules

with more replication origins may also have an advantage in other systems such as cultured human cells (89). To distinguish cases such as this from intracellular selection based on the phenotypes of the molecules, I suggest that the two phenomena be called phenotypic intracellular selection and structural intracellular selection.

DO SHORT GENOMES HAVE A REPLICATIVE ADVANTAGE? It is often assumed that shorter genomes will generally have a replicative advantage over longer ones in the same cell because they can complete replication and re-initiate more quickly. The experimental evidence is mixed: in heteroplasmic *Drosophila*, selection can favor either shorter (72) or longer genomes (44, 81). However, it is not clear whether selection was intracellular or intercellular in these cases, or whether it is based on some phenotypic effect of the deletion. Even without experimental evidence, it is not necessarily expected that smaller molecules can complete replication faster that larger ones. In human cells from a KSS patient, heteroplasmic for wild-type mtDNA and genomes with a deletion, the same number of wild-type genomes and deletion genomes were synthesized during a 5-h period (65). This suggests that the original amplification of the deletion mutant genome in the patient (or the patient's mother) was not due to the more rapid completion of replication of the smaller genome. The authors suggested that the overall rate of replication might be limited by the rate of initiation rather than the rate of completion of mtDNA synthesis. Perhaps the structure of the replicating genome is modified so as to prevent additional initiations until the first replication is completed.

HOW IS MTDNA REPLICATION CONTROLLED? The outcome of intracellular selection in heteroplasmic cells could be affected not only by the rate of replication initiation or the time to completion, but also by the mechanism used by cells to stop replication when the appropriate number of genomes has been reached. Recall that genomes are selected stochastically for replication until the number of genomes is doubled (or reaches some other predetermined value). It is likely that either the number of genomes or the mass of organelle DNA is counted, directly or indirectly. In yeast, studies on *petite* genomes suggest that mass, or the total number of base pairs, is titrated (30, 37, 67). Human cell lines containing wild-type mtDNA or mtDNA mutants with complete or no impairment of respiration, very different sizes, and different numbers of replication origins all had the same total mass of mtDNA per cell (90). The authors suggested that mtDNA may be replicated until tightly regulated dNTP pools are depleted.

If cells limit mtDNA replication by titrating total DNA mass, then there is an alternative explanation for the replicative advantage of smaller genomes. Consider a simple model in which mtDNA molecules are selected randomly in a series of rounds of replication until the total mass of genomes is increased to a certain value. If long genomes are chosen for replication more often than short ones, by chance, the final mass will be reached in a smaller number of replication events.

If short genomes are chosen more often, they will have to undergo more rounds of replication to reach the same mass. After two or more doublings, the result will be an increase in the proportion of short genomes. The behavior of this model needs to be confirmed mathematically or by simulations, but it is easily shown to be correct for a simple case of cells that are heteroplasmic for two genomes, one half as long as the other (C.W. Birky, Jr., unpublished).

Paradoxical Intracellular Selection Based on Respiration

Many patients with respiration-deficient mitochondria acquired the mutant genomes as a new mutation early in embryogenesis or in their maternal germline (e.g., 49). This mutation must have been amplified in the cells to the point where it causes clinical symptoms. Many of these are deletions, and one could suppose that this gives them an advantage, as discussed above. However, respiration-deficient point mutations can also have a selective advantage (96). In a review of data from a large number of human pedigrees in which one or another of six common pathogenic point mutations were segregating, at least three point mutations showed significant selection in favor of the mutant allele (24). Mutant and wild-type genomes have the same mass in this case, but the replicative advantage of this mutant can be explained if it is respiration that is titrated. The model for this case is formally the same as the one for mass differences: It takes more mutant molecules than wild type for a cell to achieve any specific level of respiration, so mutant molecules replicate more often. This explanation may also apply to respiration-deficient mutations such as *kalilo* in *Neurospora* (9), which increase in frequency as a mycelium ages, until they kill it.

UNIPARENTAL INHERITANCE

Patterns of Uniparental Inheritance

MATERNAL INHERITANCE IS NOT A GENERAL FEATURE OR LAW OF ORGANELLE HEREDITY Baur's work on plants showed that maternal inheritance was not a general law of organelle heredity, since some plants produce a mixture of maternal, paternal, and biparental progeny. More recently, uniparental inheritance has been seen in organisms that have no differentiation of maternal or paternal sexes. Moreover, in plants and algae, mitochondrial and chloroplast genes may be inherited preferentially from different sexes or mating types. The most general statement we can make about uniparental inheritance is that in most organisms, some or all progeny inherit organelle genes from only one parent.

UNIPARENTAL INHERITANCE IS OFTEN A QUANTITATIVE PHENOMENON When there is some degree of biparental inheritance, one can estimate the frequency of one allele in a large number of offspring from a single mating or from a group of matings of the same genotypes. A frequency distribution of the numbers of progeny with

different allele frequencies usually shows a continuous distribution, which may be unimodal, bimodal, or occasionally trimodal but is without sharp discontinuities (Figure 4).

LARGE SAMPLE SIZES ARE NEEDED TO DEMONSTRATE STRICT UNIPARENTAL INHERITANCE This is a consequence of the variation among the progeny of a mating in the degree of biparental transmission. For example, assume that an animal transmits paternal mitochondrial genes such that 1% of all of the mitochondrial genes in the progeny of a cross come from the father. If there are few or no biparental zygotes, only about 1% of the progeny will have the paternal genotype and a sample of about 300 progeny with no paternal genotypes would be required to demonstrate that there was less than 1% paternal inheritance (64). A more likely scenario would be that all of the paternal genes were in biparental progeny, but in that case it is possible that there are only a few such individuals, or that most of the biparental progeny have very low frequencies of paternal genes. Furthermore, most of the minority markers may be localized in one or a few tissues. The best approach is to use sensitive molecular methods to detect the marker in the pooled tissue of many progeny; if paternal transmission is found, one should then screen individual progeny to determine how the paternal markers are distributed. Selection for streptomycin-resistance genes was used to detect paternal transmission in 1/1500 progeny of a cross in *Nicotiana* (60), but this method is rarely practical.

Mechanisms of Uniparental Inheritance

THERE ARE MANY DIFFERENT MECHANISMS OF UNIPARENTAL INHERITANCE The striking variation among different organisms in the extent and pattern of uniparental inheritance is mirrored in a remarkable diversity of mechanisms. The transmission of organelle genes from one parent to the offspring can be blocked at any stage of sexual reproduction [see (12, 17) for evidence and examples]:

1. Gametogenesis: organelles may be segregated from the gamete during premeiotic or meiotic divisions; organelles or organelle DNA may be degraded in the gamete.
2. Fertilization: organelle DNA is shed from gametes before fertilization, or does not enter the egg.
3. Postfertilization: selective silencing (degradation) of organelles or organelle DNA in the zygote; stochastic or directed segregation of organelles into extraembryonic tissues during early cleavages; or loss of alleles from one parent due to stochastic replication and/or turnover of organelle genomes.

SELECTIVE SILENCING OF PATERNAL MITOCHONDRIAL GENES IN MAMMALS In mussels, mtDNA occurs in two separate lineages that are inherited differently; this is not reviewed here because the mechanism remains a mystery. Apart from this

exception, mtDNA is always inherited maternally in crosses in all the animals that have been studied to date. However, in most cases too few offspring have been examined to detect low levels of paternal inheritance. This is especially true in studies of human pedigrees in which fewer than 2500 offspring have been examined in all of the available pedigree data (28). This seems like a lot, but because the sperm contains about 1/1000 times as many mtDNA molecules as the oocyte, one expects to find fewer than 2.5 uniparental paternal individuals in this sample, or somewhat more biparental individuals. When different species of mice were crossed and the hybrids were repeatedly backcrossed to the male parent to amplify small paternal contributions, paternal mtDNA was detectable by PCR (34). Another group used PCR to detect small paternal contributions in both inter- and intraspecific crosses (43). In intraspecific matings, paternal mtDNA was detected in the majority of embryos at the early pronucleus stage in intraspecific crosses but disappeared in all of 48 embryos by late pronucleus through blastocyst stages. But in interspecific crosses, paternal mtDNA was detected in some embryos at every stage, including 24 of 45 neonates. Subsequent studies of an interspecific mouse cross by this group (79) used a PCR method sufficiently sensitive to detect a few molecules of paternal mtDNA in a background of 10^8 molecules of maternal mtDNA. They again detected paternal mtDNA in embryos but when the hybrid mice were reared to maturity, paternal mtDNA could be found in only one or a few tissues in each individual; this is expected if the paternal and maternal genomes segregated during development. Few hybrid animals had paternal mtDNA in the ovary and none had it in their unfertilized eggs, nor was any detected in backcross progeny. The investigators conclude that some mechanism recognizes and specifically destroys paternal mtDNA in eggs of intraspecific crosses, but partially fails in interspecific hybrid eggs so that a small number of paternal genomes escape degradation.

In fact, electron microscopy indicates that the entire sperm midpiece degenerates in the mammalian egg (e.g., 36, 84, 86). Recent studies have provided strong evidence that sperm are marked for degradation by ubiquitination. Ubiquitin is a protein that binds to other proteins and marks them for degradation by the 26S proteasome. It also marks for engulfment and lysis by lysosomes or vacuoles. Sutovsky and collaborators (85) demonstrated that ubiquitin, detected by fluorescence-labeled anti-ubiquitin antibodies, is bound to sperm mitochondria during spermatogenesis and in the oocyte of cows and rhesus monkeys. Ubiquitin was not detected in sperm on the surface of the egg or soon after entering the egg; the authors proposed that ubiquitin is masked in these sperm, but they could not rule out the possibility that the ubiquitin on the sperm is lost somewhere between spermatogenesis and fertilization, then is re-established in the egg. The ubiquitinated sperm subsequently disappear, typically between the third and fourth cell division. The authors proposed that ubiquitin marks the mitochondria for subsequent degradation by proteasomes and lysosomes. Ubiquitination is evidently required for this degradation, because degradation is prevented by injecting anti-ubiquitin antibodies into the fertilized egg, or by treating the egg with ammonium chloride,

which is "lysosomotropic" (84). Ubiquitination of sperm was not observed when cow eggs were fertilized with sperm of the wild gaur, and the ubiquitin-labeled sperm could be detected in eight-cell embryos (85). The results of this interspecific cross parallel the transmission of paternal sperm in crosses between *Mus musculus* and *M. spretus* (43).

RECOMBINATION

Between-Lineage Recombination of Organelle Genes is Limited

The majority of animals and plants, and many or most fungi and eukaryotic protists, reproduce sexually at least occasionally. During sexual reproduction, nuclear genes are inherited biparentally and genes from different parents recombine due to crossing-over, gene conversion, and independent segregation of chromosomes. But organelle genes from different lineages rarely or never recombine in most of these same organisms. This is because organelle genomes are usually inherited uniparentally; and if they are inherited biparentally, the organelles from the two parents fail to fuse and share genomes. In many angiosperms, for example, mitochondrial and chloroplast genes from different individuals do not recombine in crosses where they are inherited uniparentally. If cells from two parents are fused, recombinant genotypes are readily detected for mitochondrial genes, but recombinant chloroplast genomes are rare and can only be detected after stringent selection. When recombinants for two different antibiotic resistance genes are selected, other markers on the selected genomes show extensive recombination (59). This shows that plant chloroplasts do have the enzymatic machinery required for recombination but the chloroplasts rarely fuse.

Although plant organelle genomes from different lineages rarely have an opportunity to recombine, intramolecular and intermolecular recombination within a lineage can still occur. These forms of recombination can be very important in rearranging genomes. Intramolecular and intermolecular recombination of cpDNA maintains the sequence identity of the inverted repeats, inverts the order of genes in the single-copy regions, and produces dimeric genomes; while inter- and intramolecular recombination in mtDNA produces subgenomic circles (reviewed in 32). Repeated rounds of random pairing and gene conversion could cause intracellular random drift (12), but so far it has not been possible to determine how important this is, relative to stochastic replication. One might imagine that when organelle genes are inherited biparentally, they would still show less recombination on average than nuclear genes because they are all on one chromosome. But this is not necessarily so: In *Saccharomyces cerevisiae*, the genomes in a zygote and its early buds undergo repeated rounds of pairing and recombination, resulting in recombination frequencies of about 1% per 100 bp (97).

Do Hominid Mitochondrial Genes Have a Low Level of Biparental Inheritance and Recombination?

A potentially more powerful approach to detect paternal inheritance is to analyze population and evolutionary genetic data for evidence of recombination between different mtDNA lineages. In principle, this approach could detect paternal inheritance and recombination because it analyzes the pooled results of very large numbers of matings taking place over long time periods. But the results have been controversial. Eyre-Walker et al. (29) argued that the substantial homoplasy seen in mtDNA trees was more likely due to recombination between different lineages than to multiple mutations at a site, as was previously assumed. However, their data sets contained significant errors, and when these were corrected the argument that homoplasy is due to recombination was weakened (55). Awadalla et al. (2) then provided more compelling evidence for recombination, showing that the amount of linkage disequilibrium between pairs of mitochondrial sites decreased as the distance between the sites increased. Although the logic of this test is correct, the results were challenged on methodological grounds, and some additional data sets were found not to show a significant negative correlation between distance and disequilibrium (see discussion at http://www.sciencemag.org/cgi/content/full/288/5473/1931a). The subject was nicely reviewed by Eyre-Walker (28), who noted that only 9 of 14 human data sets show the negative correlation and none was significant. On the other hand, a chimpanzee data set did show a significant negative correlation, and the majority of the data seem to point in that direction. If this is confirmed by future studies, the assumption that mtDNA can be used as a clonal maternal lineage in studies of human evolution will have to be reconsidered. Another important consequence of paternal leakage would be that mitochondria would not be strictly asexual and would be less susceptible to the accumulation of detrimental mutations (Muller's ratchet) than has been assumed.

Do human mitochondria contain the enzymes necessary for recombination? Tang et al. (89) found that a human cell line homoplasmic for dimeric mtDNA molecules gave rise to monomeric wild-type and deletion genomes, as expected if there were intramolecular recombination of the dimers. They review biochemical evidence that mammalian mitochondria contain at least some of the enzymes required for intramolecular recombination. However, when they cultured cells that were heteroplasmic for the wild-type and deletion genomes, they found none of the dimers that would result from intermolecular recombination. They suggest that this may be because the two genomes were initially in separate cells that were fused to make heteroplasmic cybrids; consequently, they may have remained physically isolated from each other, in different organelles or different regions of an organelle. On the other hand, they argue that the dimeric mutants were almost certainly originally formed by a combination of intramolecular and intermolecular recombination between two molecules in the same cell. The data of Tang et al. (89) suggest that the conclusion that mammalian mitochondria do not recombine

(18) is incorrect, at least for genomes in the same organelle. These authors (89) also point out that triplicated mitochondrial genomes found by Holt et al. (38) in cell cultures initially homoplasmic for duplicated genomes probably arose by intermolecular recombination.

WHAT NEEDS TO BE DONE

Most of the genetic phenomena unique to organelles have been known for over a decade, and the possible cellular and molecular mechanisms have been identified. Finally, the time is ripe to apply a combination of genetic, molecular, and cytological methods to determining the relative importance of these mechanisms in specific cases. New genetic methods such as directed mutagenesis and transfection and new selection methods now enable us to obtain mutants that are defective in a variety of processes affecting the transmission of organelles and organelle DNA. New molecular and cytological tools such as green fluorescence protein, fluorescence in situ hybridization, and confocal microscopy enable us to measure parameters such as organelle and genome number and visualize the effects of mutants. Now we need to apply these methods. For example:

1. It is not yet clear to what extent vegetative segregation in plant plastids is due to strictly random partitioning of plastids. For this, the new molecular and cytological techniques must be used to determine the numbers of organelles in eggs, embryos, and meristem cells where most segregation occurs. Plastids need to be counted in pairs of daughter cells to determine how often partitioning really is numerically equal.
2. *Saccharomyces cerevisiae* is the only organism in which extensive data on the inheritance of mitochondrial genes can be matched with detailed cytological pictures of the movement of mitochondrial membranes and mtDNA and molecular genetic analysis of the role of specific proteins. The combined use of mutants and molecular genetic methods for which yeast is famous, coupled with new high-resolution cytology, could lead to a detailed picture of the mechanisms underlying vegetative segregation and uniparental inheritance in yeast. Surprisingly, this has not been done. There have been almost no studies of the effect of mutants defective in fusion or fission, or mitochondrial and mtDNA movement in zygotes, on the inheritance of mitochondrial genes. Much of what has been done used *petite* mutants whose inheritance is too strongly affected by intracellular and intercellular selection to give a clear picture of mtDNA inheritance.
3. Before we can understand intracellular selection, which is so important in human mitochondrial gene diseases, we must understand how cells control organelle DNA replication. We cannot expect a single model to suffice. The available data suggest that replication control mechanisms differ not only between organisms, but also between cell types in the same organism. It also

appears that different kinds of mutants may have a replicative advantage for different reasons; perhaps one cell type can measure organelle genome replication in more than one way.

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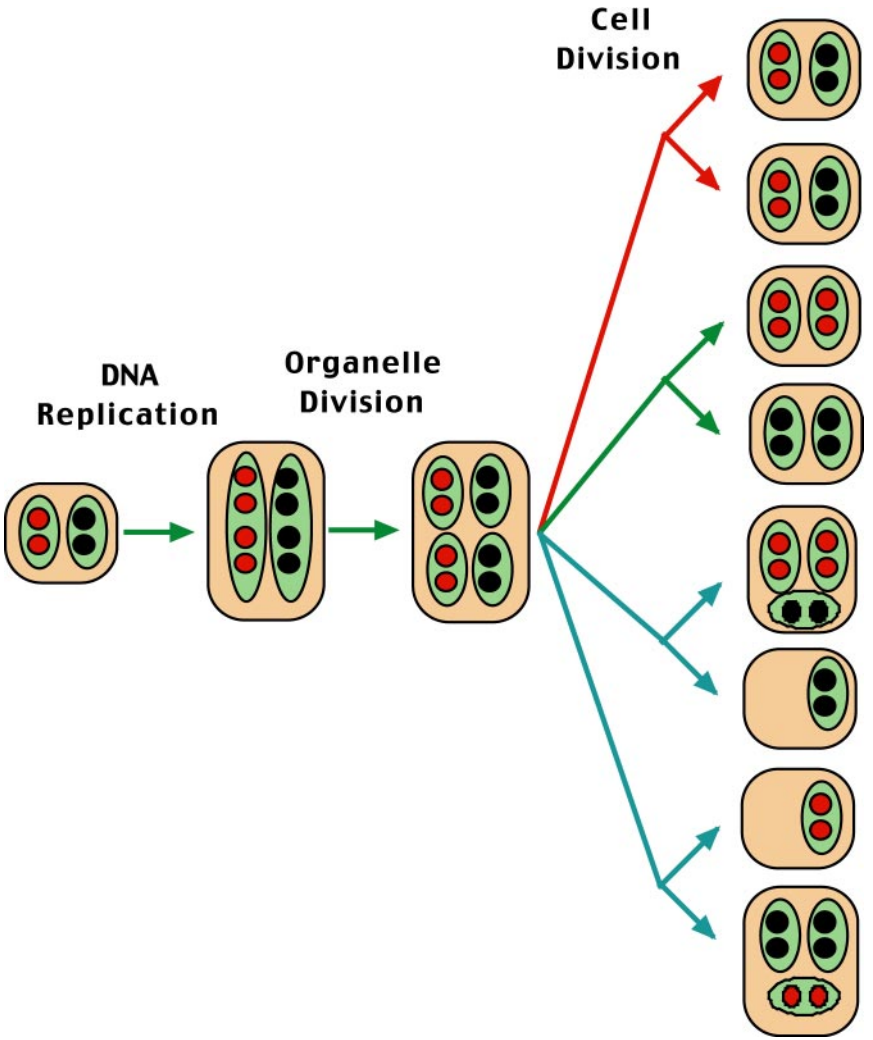

Figure 1 Vegetative segregation: simple plant model. A heteroplasmic cell has two organelles, one with two wild-type genomes (*black circles*) and one with two mutant genomes (*red circles*). DNA replication is stringent (each genome replicates once). When the organelles divide, the genomes are partitioned equally. When the cell divides the organelles are partitioned numerically equally (*red and green arrows*) or unequally (*blue arrows*). Organelle partitioning is genetically stochastic (relaxed) and can produce cells that are homoplasmic. If partitioning were deterministic with sister organelles always going to different cells (stringent partitioning; *red arrows*), there would be no vegetative segregation.

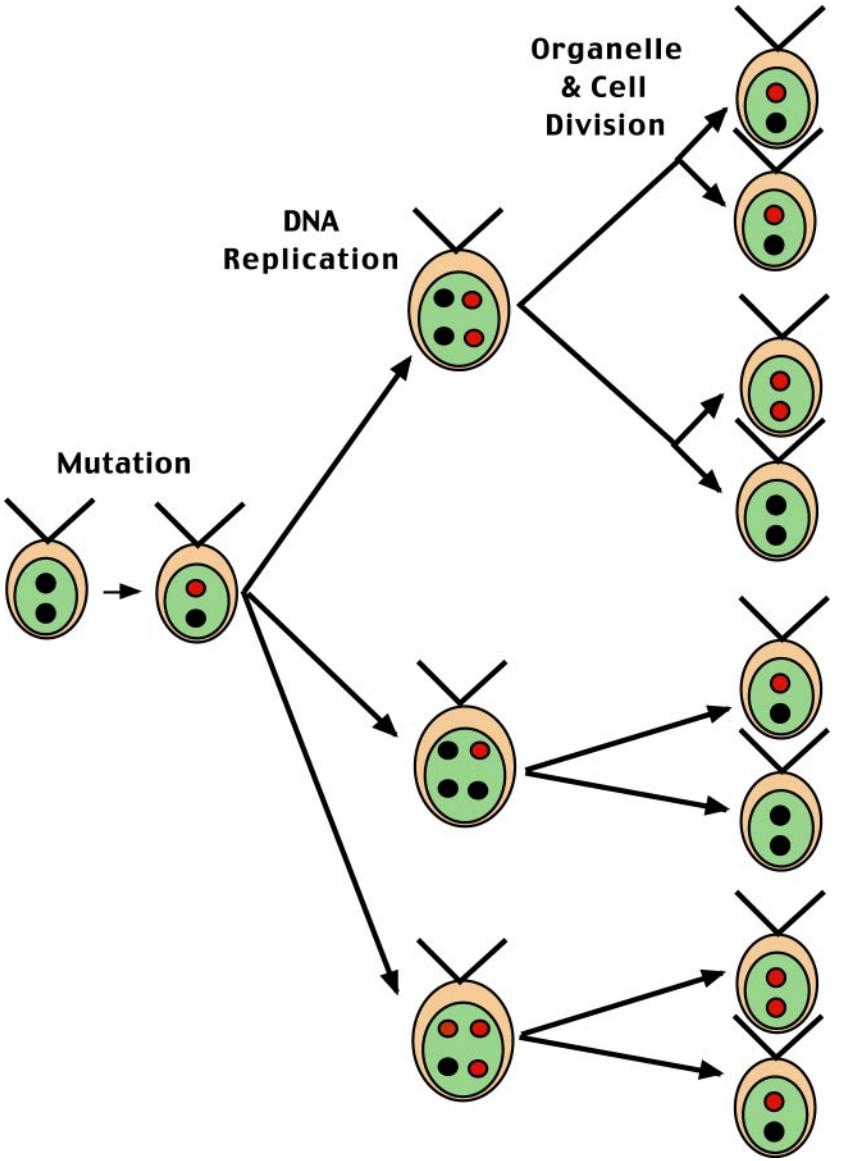

Figure 2 Vegetative segregation in Chlamydomonas. A cell has one chloroplast with two wild-type genomes; mutation produces a heteroplasmic cell. DNA replication is stochastic (relaxed). Genome partitioning is numerically equal but genetically stochastic. Relaxed replication increases the probability that a daughter cell will be homoplasmic because it produces cells with 3:1 or 1:3 ratios of wild-type:mutant and these always produce a homoplasmic daughter.

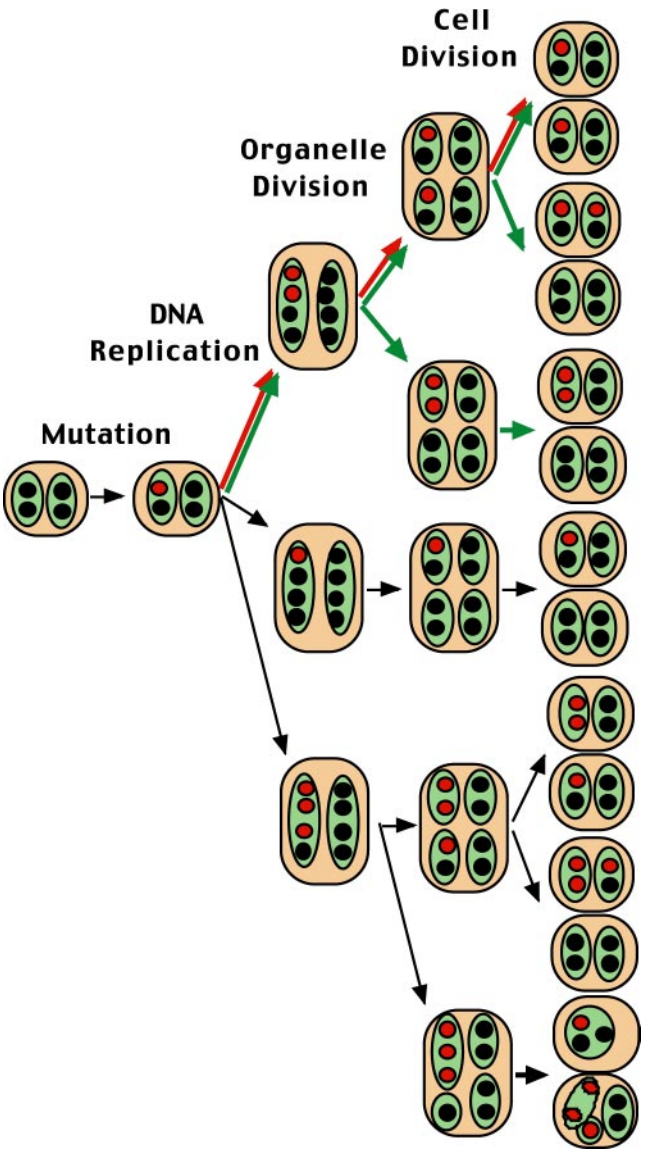

Figure 3 Vegetative segregation with discrete organelles: a more sophisticated model. After a mutation produces a heteroplasmic organelle, relaxed replication and partitioning of genomes produces homoplasmic organelles; thereafter the organelle is the unit of segregation. Red and green double arrows show the results of stringent replication and partitioning; green arrows collectively show the simple plant model; the black arrows add the additional cases that are possible if genome replication is relaxed and if organelle partitioning is relaxed with respect to number.

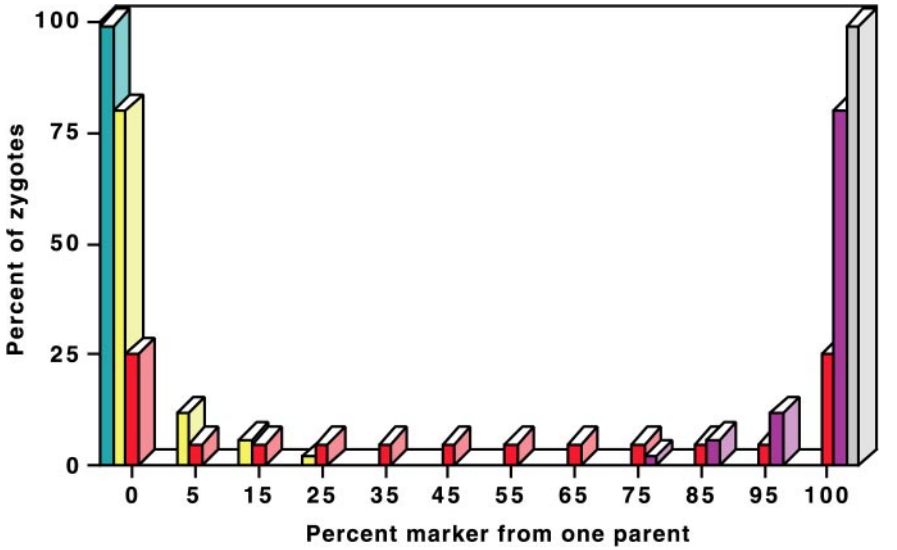

Figure 4 Uniparental inheritance is a quantitative phenomenon. Frequency distributions of the frequency of alleles from one parent (e.g., the paternal parent) in the zygotes produced by matings of different organisms. Blue graph shows strict uniparental maternal inheritance; yellow graph shows mixture of maternal and biparental zygotes; red graph shows maternal, biparental, and paternal zygotes; purple graph is paternal plus biparental zygotes; and grey graph illustrates strictly paternal inheritance.