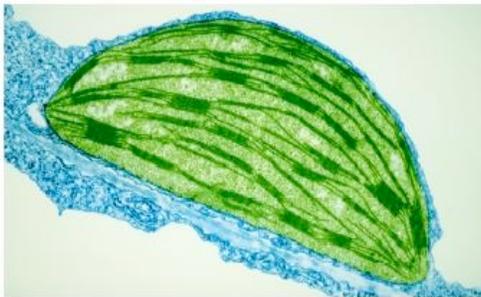
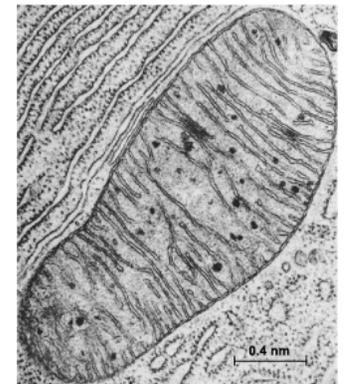


## Section 17

# Organelle Genetics



## Genetics of Mitochondria and Chloroplasts



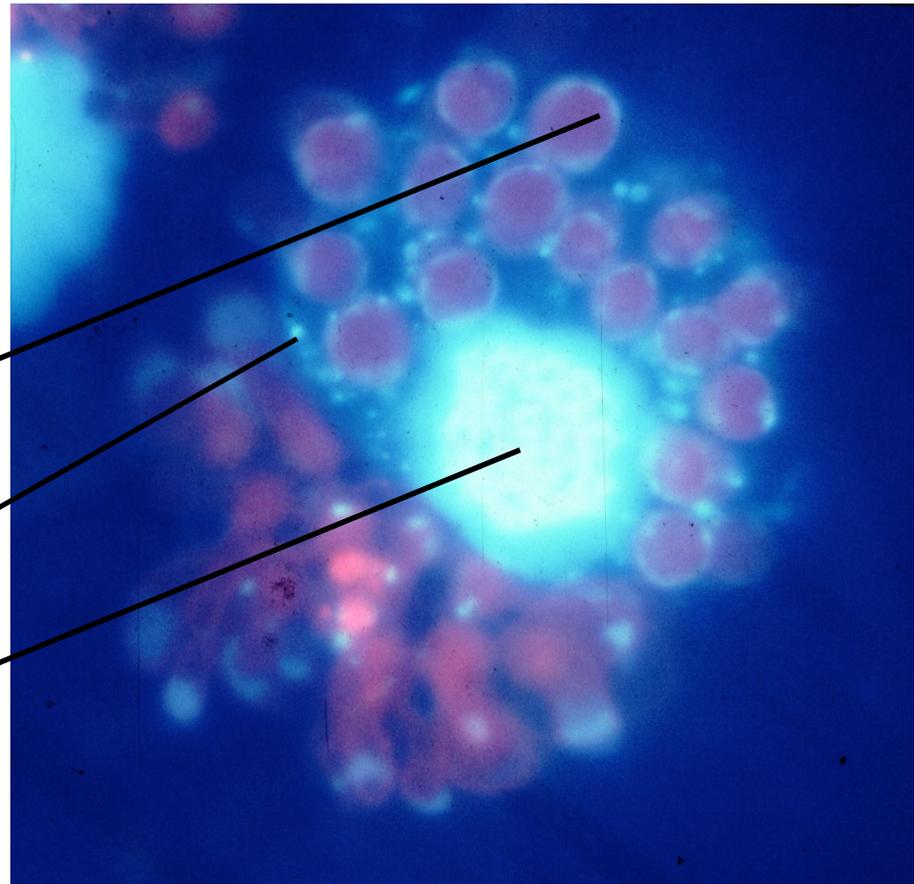


Flourescence micrograph of alga *Olisthodiscus*. Chlorophyll autofluoresces red. DNA stained with DAPI fluoresces white.

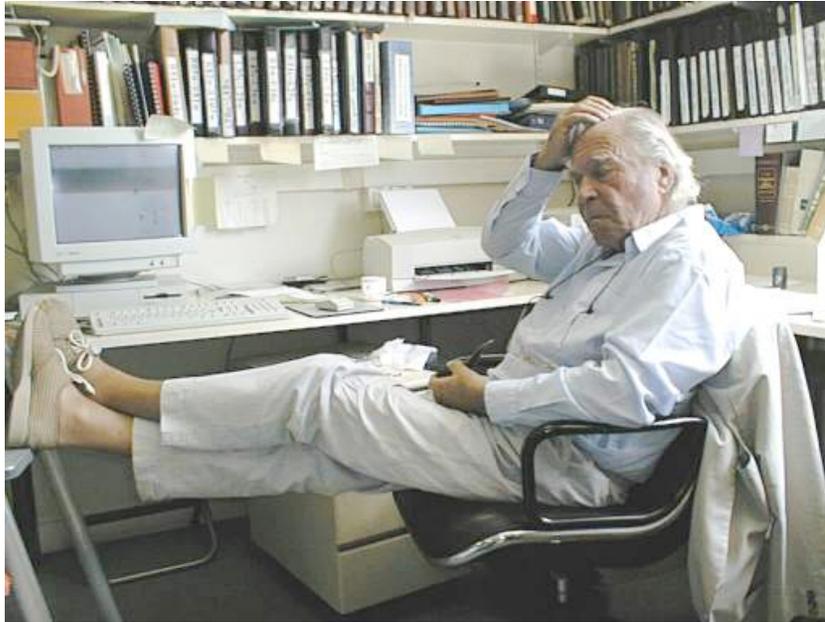
- Chloroplasts and cpDNA

- Mitochondria and mtDNA

- Nucleus and nuDNA



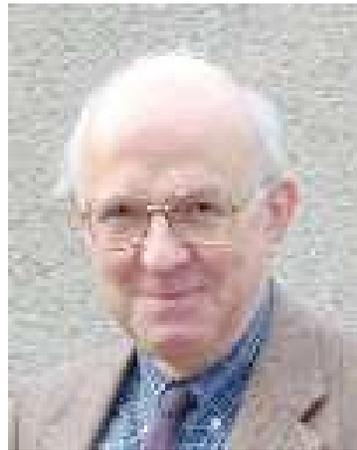
**Yeast mitochondria**



**Piotr Slonimski**



**Tony Linnane**



**David Wilkie**

***Chlamydomonas* chloroplast**



**Ruth Sager**



**Nick Gillham**

Originated as intracellular symbionts:

Eukaryotic cell --phagocytosis of alpha-proteobacterium--> cell + symbiont --  
genes lost or transferred to nucleus--> cell + mitochondria

Cell + mitochondrion --phagocytosis of cyanobacterium→ cell + symbiont--genes  
lost or transferred to nucleus→ cell + chloroplast

In subsequent evolution, mito and cp retained some traits of their symbiotic  
ancestors:

- self-replication
- some genes
- protein-synthesizing machinery.

BUT organelles never evolved

- mechanism to ensure that every copy of the genome replicated once per cell  
cycle
- mitotic apparatus to partition copies

They never evolved the machinery necessary for Mendelian inheritance.

- **Mitochondria and chloroplasts are self-replicating organelles.**

They are produced only by growth and division of pre-existing mitochondria or chloroplasts. They cannot be formed *de novo* or from other organelles or pre-existing membranes. They grow by the insertion of molecules in to their membranes.



(a) Mitochondria and chloroplasts contain DNA genomes with a small number of functional genes.

mitochondrial DNA = mtDNA

chloroplast DNA = cpDNA

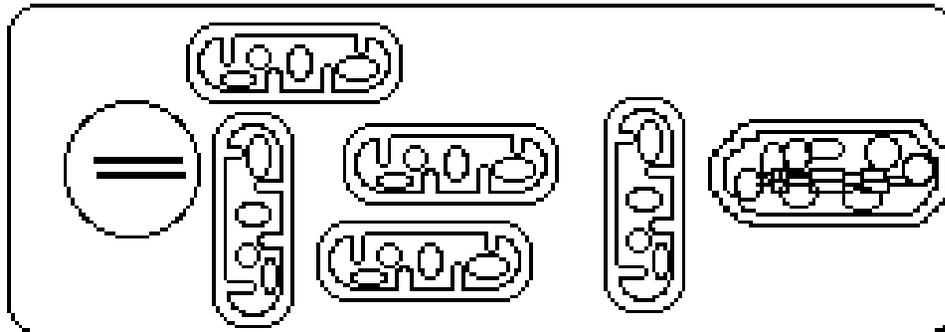
- Very few genes remain from the symbiont ancestors.

Compare and contrast to nuclear genes (orders of magnitude):

	<u>number of genes</u>	<u>C value in kbp</u>
eukaryote nuclear genome	$10^4 - 10^5$	$10^4 - 10^8$
euk. mitochondria & chloroplasts	$40 - 10^2$	$1 - 10^2$
prokaryote cell genome	$500 - 10^4$	$500 - 10^4$

Number of genes ranges from 37 genes in human mito to  $\approx 130$  genes in plant mitochondria.

- Most proteins, and all lipids, etc. in organelles are synthesized in the cytoplasm and imported.
- Genes required for aerobic respiration/electron transport (mitochondria) and photosynthesis (chloroplasts).
- Organelle genomes almost always have all their genes on a single circular DNA molecule.
- Each cell contains many organelle DNA molecules, order of  $10^2 - 10^4$  (more in big eggs), hence many copies of each gene.
- These are packaged in  $1 - 10^3$  organelles (more in big eggs).



e.g. alga *Chlamydomonas*, haploid: ca. 100 cpDNA molecules in one chloroplast

e.g. yeast: ca. 50-100 mtDNA molecules in 1 to 50 mitochondria (depending on genotype and physiological state of cells; mito fuse and divide)

e.g. mammalian cells in culture: ca.  $10^3$  mtDNA molecules in several hundred mitochondria

1909 Erwin Baur and Carl Correns found first cases of non-Mendelian heredity, in plants. But only Baur interpreted them correctly.

*Pelargonium* (geranium) Studied inheritance of wild type green and mutant white (no chlorophyll, no photosynthesis) variegated leaves:



Violated two of Mendel's laws:

- Some plants inherited genes from only one parent, usually female, sometimes male.
- Alleles segregated during vegetative (asexual) growth.

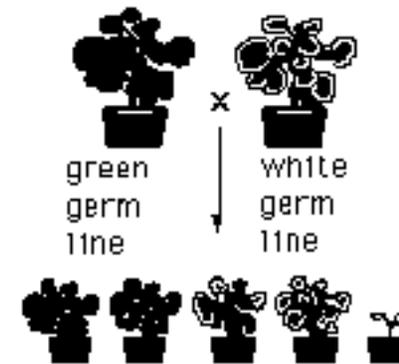
## Laws of Organelle Genetics

- Vegetative segregation: alleles of organelle genes segregate during mitotic as well as meiotic divisions.
- Uniparental inheritance: organelle genes are often transmitted from only one parent.

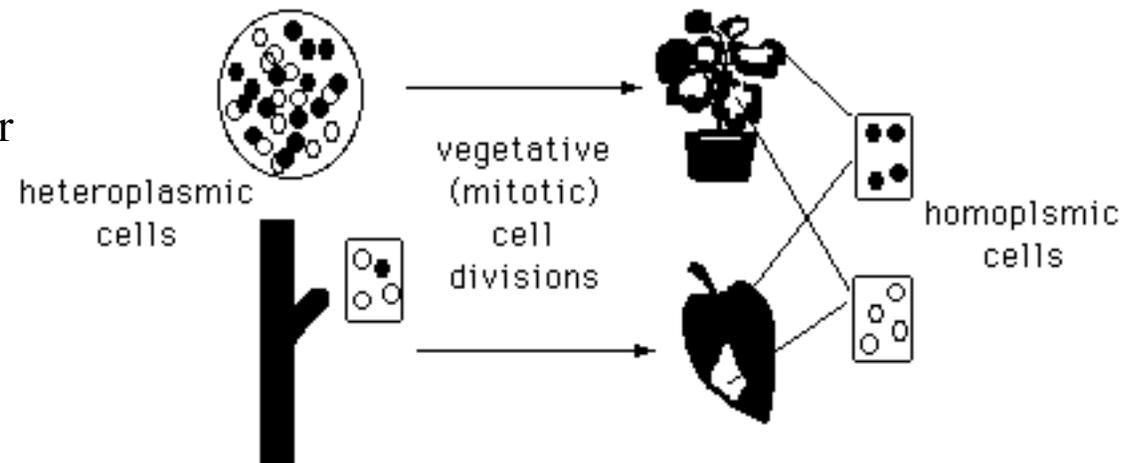
## Mechanisms of Vegetative Segregation

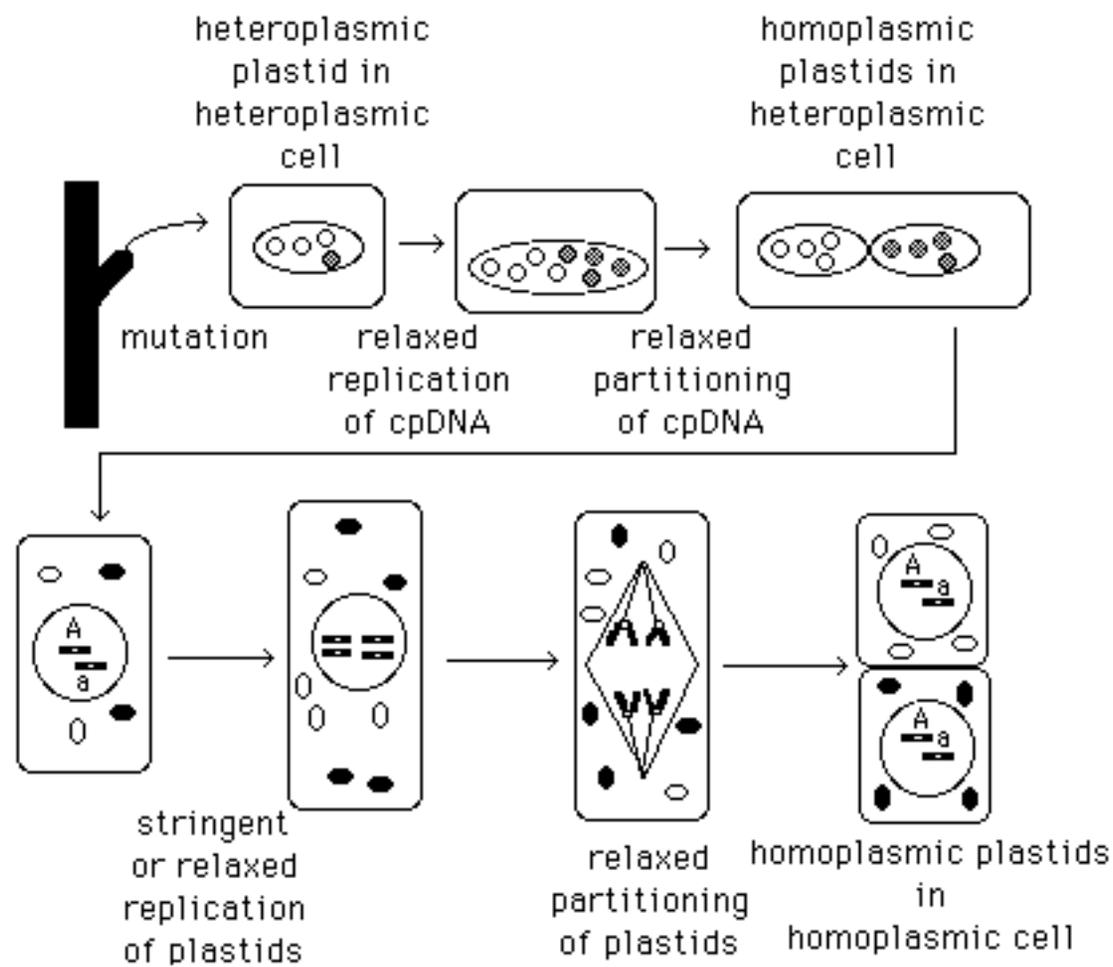
- Many copies of genome per cell and per organelle.
- Genomes selected ca. randomly for replication, so some may replicate more than others.
- Genomes partitioned ca. randomly when organelle divides.
- Organelles partitioned ca. randomly when cell divides.

### UNIPARENTAL INHERITANCE



### VEGETATIVE SEGREGATION



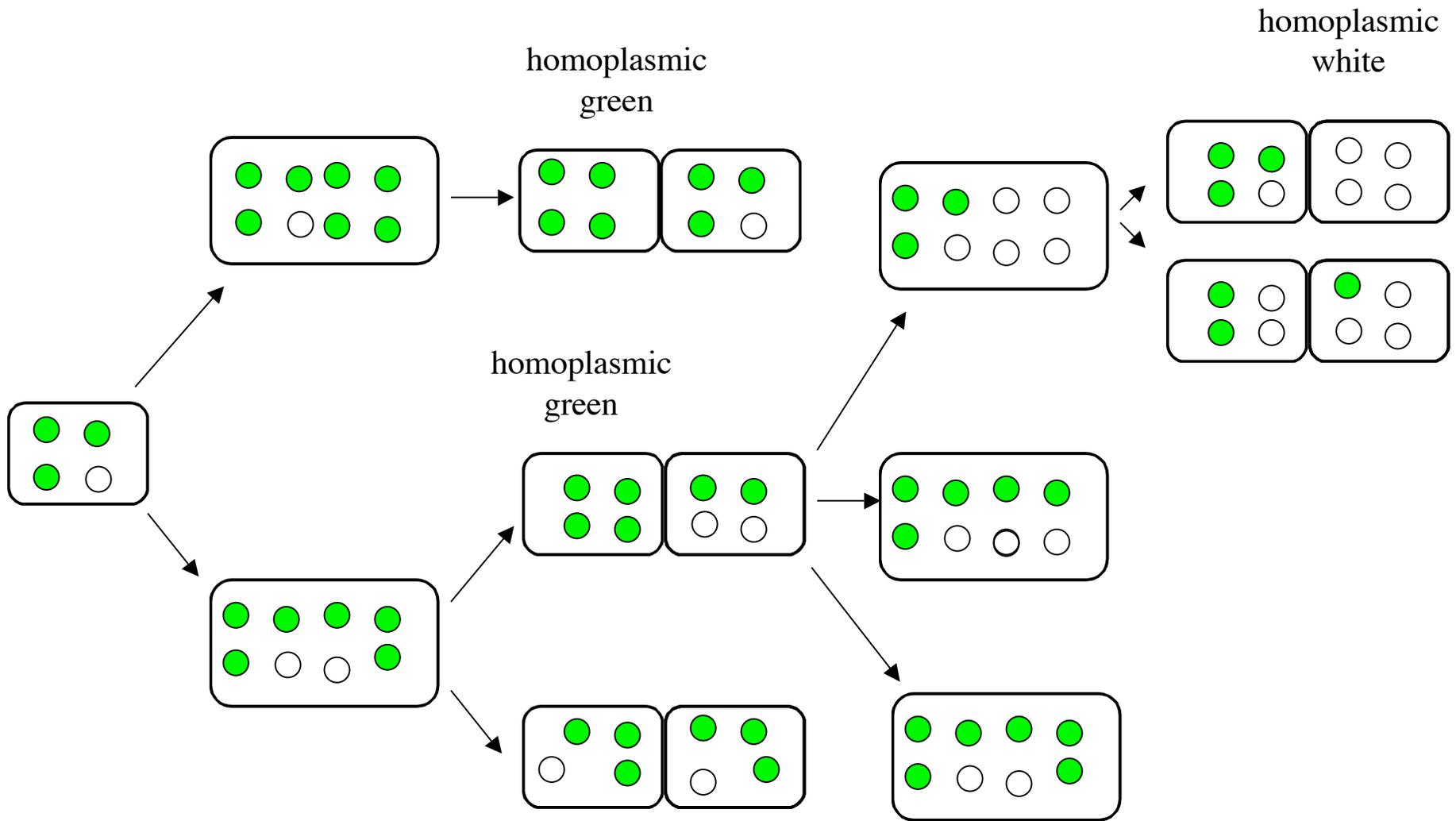


# MECHANISMS OF VEGETATIVE SEGREGATION

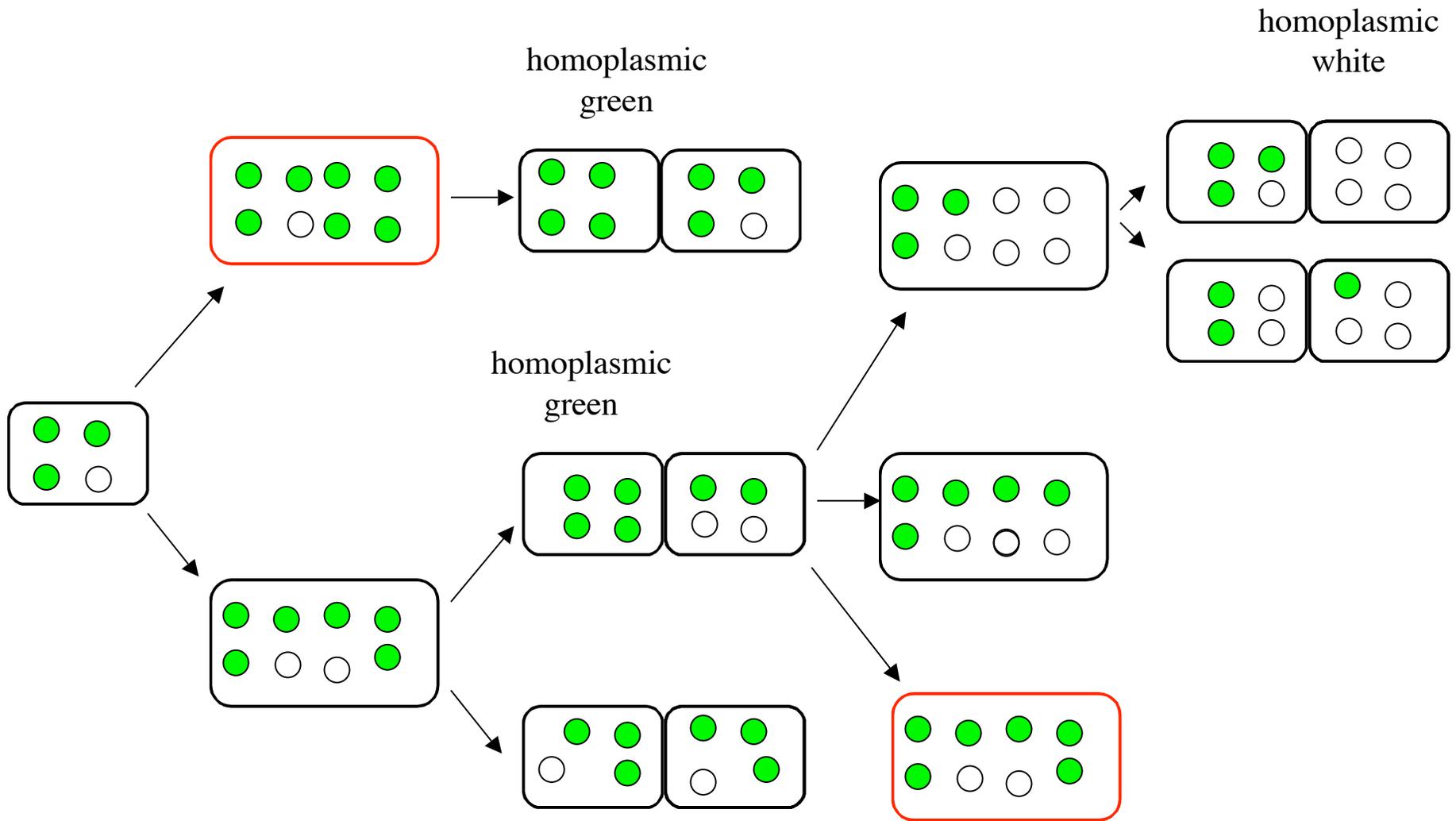
- Homoplasmic: cell, organelle, or organism has only one allele of an organelle gene (cf. homozygous)
- Heteroplasmic: cell, organelle, or organism has  $\geq 2$  alleles of an organelle gene (cf. heterozygous)
- Alleles in heteroplasmic cell can be in different proportions (frequencies), e.g. 1/100, 23/100, etc.
- Random replication: Organelle DNA molecules are selected randomly (stochastically) for replication, so one allele often replicates more than the other, just by chance.
- Random partitioning of genomes: When organelle divides, genomes partitioned randomly with respect to genotype, so one or both organelles are often homoplasmic.
- Random partitioning of organelles: When cell divides, organelles are partitioned randomly between daughter cells, so one or both daughters are often homoplasmic.

**Intracellular random genetic drift** (Thraillkill, Birky, Lückermann, and Wolf 1980 mitochondria; Birky et al. 1981 chloroplasts): **random changes in allele frequencies in cell.**

**Intracellular selection** (Birky 1973): Some molecules can replicate more often than others, not only by chance but also because they are smaller and can replicate faster, or because they confer increased fitness on their mitochondria.



When all cells are homoplasmic, expected ratio homoplasmic green:homoplasmic white = 3:1  
 Frequency green genomes =  $f(G) = 0.75$        $f(W) = 0.25$

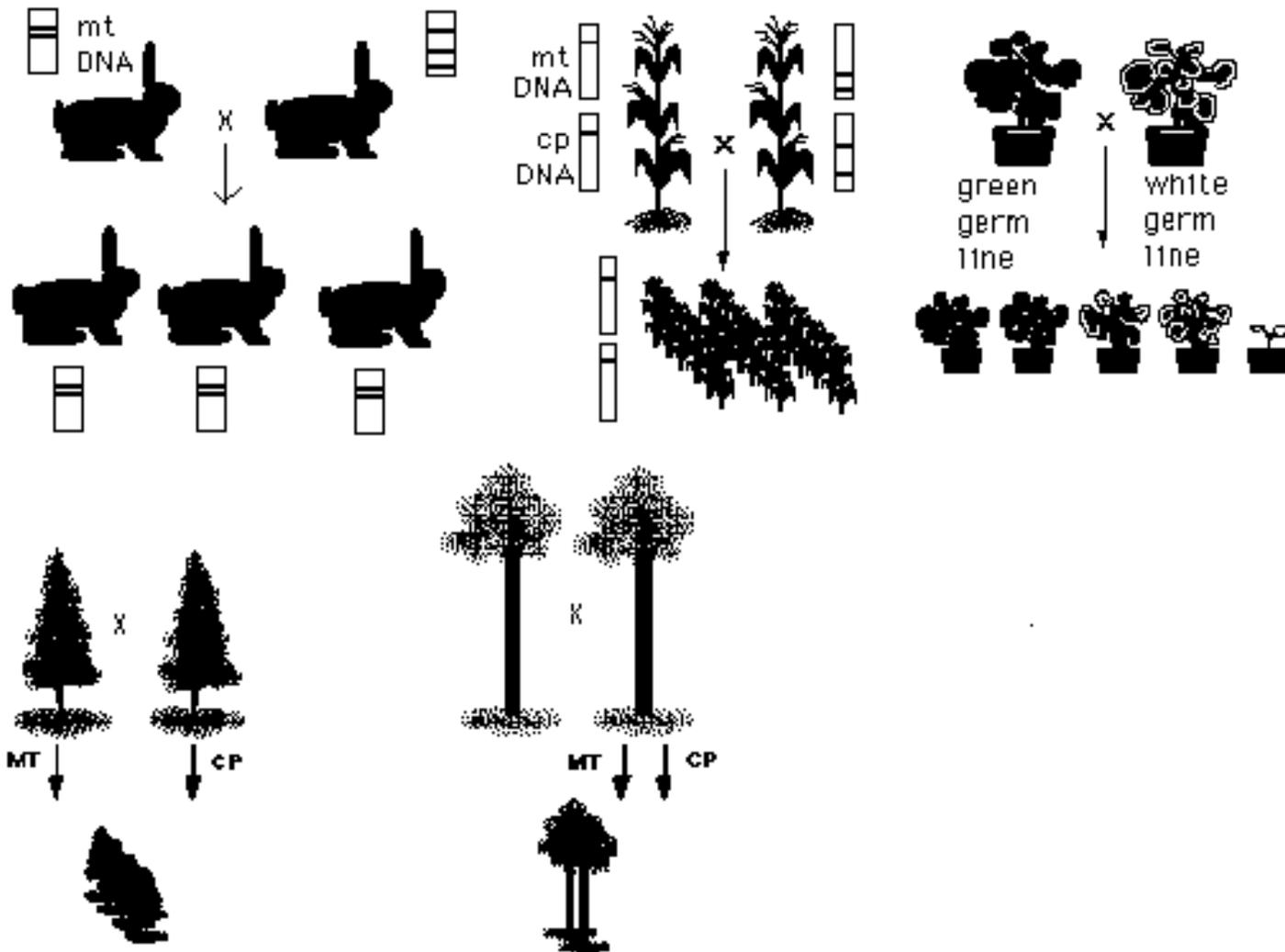


Intracellular selection for green plastids: replication stochastic, not strictly random. Red outcomes favored.  $f(G)O > 0.75$

# Uniparental Inheritance

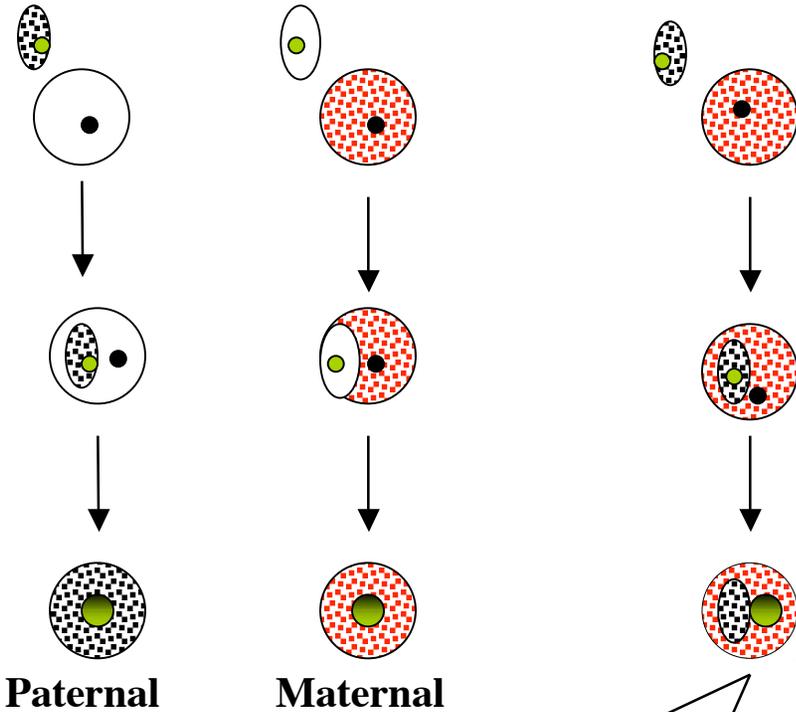
Inheritance in animals and many plants is exclusively from female parent, therefore often called maternal inheritance. But this isn't the way to state a general rule of organelle inheritance; uniparental inheritance is better.

UNIPARENTAL INHERITANCE IN ANIMALS AND PLANTS

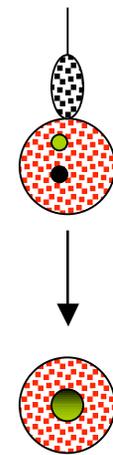


# UPI HAS MANY MECHANISMS

No organelles in gamete

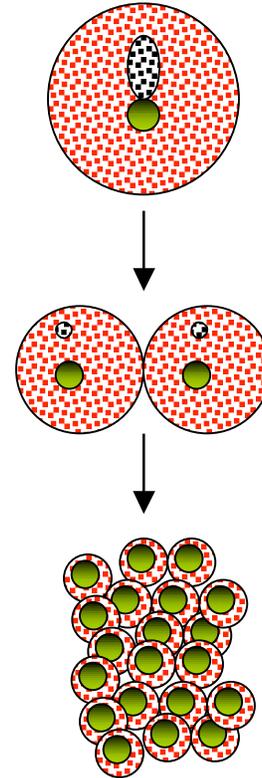


Organelles excluded from zygote

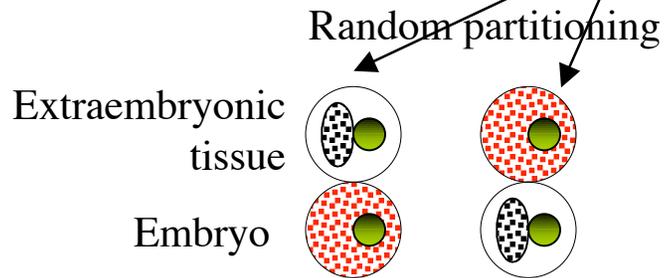
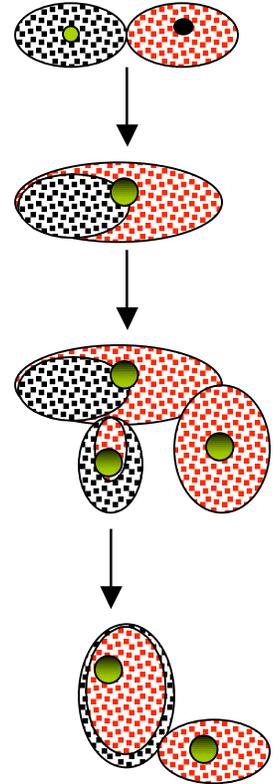


Maternal

Input bias + random replication

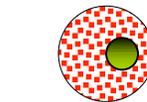


Mixture



Maternal Paternal

Selective silencing (degradation)



Maternal

## Selective silencing in *Chlamydomonas reinhardtii*

Sager: reciprocal crosses using cpDNA gene determining sensitivity vs. resistance to streptomycin:

$mt^+ str-r \times mt^- str-s$



all tetrads  $2 mt^+ : 2 mt^-$

most tetrads  $4 str-r : 0 str-s$

$mt^+ str-s \times mt^- str-r$



all tetrads  $2 mt^+ : 2 mt^-$

most tetrads  $4 str-s : 0 str-r$

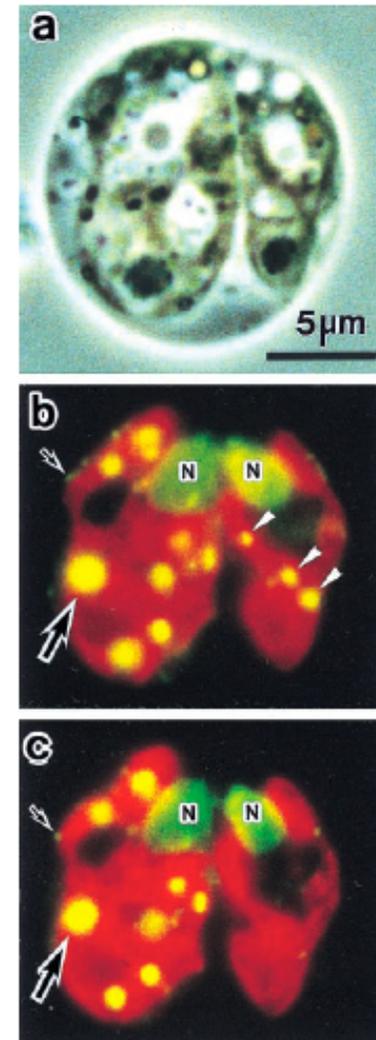


Fig. 1. Preferential disappearance of  $mt^-$  cp nucleoids visualized in a living zygote of *C. reinhardtii*. The living zygote was stained with SYBR Green I, and the preferential disappearance was observed under B irradiation. Phase-contrast (a) and fluorescent images of identical zygotes before (b) and after (c) the preferential disappearance are shown. To distinguish the  $mt^+$  and  $mt^-$  chloroplasts, small  $mt^-$  gametes were used. (b and c) The  $mt^+$  (Left) and  $mt^-$  (Right) chloroplasts are emitting red autofluorescence. Cell nucleus (N), cp nucleoids (big fluorescent spots; large arrow), and mitochondrial nucleoids (small fluorescent spots; small arrow) all are visualized with SYBR Green I staining. The cp nucleoids in the  $mt^-$  chloroplasts (b, white arrowheads) disappeared completely within 10 min (b and c).



**Doug Wallace**

## **MITOCHONDRIAL DISEASES**

- **A number of inherited diseases in humans are due to mitochondrial mutations.**
- **Homoplasmic mutant cells (with only mutant mito genomes) are unable to do aerobic respiration, so they die.**
- **Heteroplasmic cells survive, but have reduced respiration -> defects in tissues requiring hi level of respiration, e.g. muscle, eye.**
- **Mitochondrial mutations may be involved in aging.**

# MITOCHONDRIAL GENES USED TO TRACK HUMAN GENEALOGIES

Described in text; will discuss after Spring Break.



## SUMMARY

Organelle genes differ from nuclear genes:

- Many copies per organelle and per cell: homoplasmic or heteroplasmic, allele frequencies
- Replication random with respect to genotype (but final number counted); *unless* have intracellular selection.
- Partitioning of genomes and organelles random (stochastic) with respect to genotype.

Organelle genes don't obey Mendel's laws:

- Vegetative segregation
- Uniparental inheritance (maternal in humans and most other animals)