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Organellar genes
why do they end up in the nucleus?

Many mitochondrial and plastid proteins are derived from their bacterial endosymbiotic ancestors, but their genes now reside on nuclear chromosomes instead of remaining within the organelle. To become an active nuclear gene and return to the organelle as a functional protein, an organellar gene must first be assimilated into the nuclear genome. The gene must then be transcribed and acquire a transit sequence for targeting the protein back to the organelle. On reaching the organelle, the protein must be properly folded and modified, and in many cases assembled in an orderly manner into a larger protein complex. Finally, the nuclear copy must be properly regulated to achieve a fitness level comparable with the organellar gene. Given the complexity in establishing a nuclear copy, why do organellar genes end up in the nucleus? Recent data suggest that these genes are worse off than their nuclear and free-living counterparts because of a reduction in the efficiency of natural selection, but do these population–genetic processes drive the movement of genes to the nucleus? We are now at a stage where we can begin to discriminate between competing hypotheses using a combination of experimental, natural population, bioinformatic and theoretical approaches.

Endosymbiosis, the persistence of one organism within another, can lead to the melding of two organisms into one. At least two common eukaryotic organelles, the mitochondrion and the plastid, are derived from bacteria whose fate became linked with unicellular eukaryotes some two billion years ago. One of the most obvious features of organelle evolution has been the reduction in genome size that occurred following endosymbiosis. Some organellar genes became expendable in the internal environment of the host, and nuclear genes have replaced the

function of some organellar genes, but much of the reduction occurred through the transfer of organellar genes to the nucleus[1,2].

Many genes might have been transferred to the nucleus early on, but we now realize that there are different rates of gene transfer and loss in eukaryotic lineages[1,3]. The most extreme cases lie within the cradle of eukaryotic diversity, the protists. The mitochondrial genome of the retortomonad protist, *Reclinamonas americanus*, codes for 97 genes[4] and the plastid genome of the red alga,

Jeffrey L. Blanchard
jlb@ncgr.org

Michael Lynch*
mlynch@
oregon.uoregon.edu
....
National Center for Genome Resources, 2935 Rodeo Park Drive East, Santa Fe, NM 87505, USA.
*Biology Department, University of Oregon, Eugene, OR 97403, USA.

Calculating mutation rates is a difficult task and usually involves some assumptions. For instance, nucleotide substitutions in a coding region that do not change the amino acid are often referred to as having no effect on organismal fitness (neutral). We know that this is not exactly true, but to a first approximation it does provide a reasonable measure. We also know that mutations do not occur randomly in the genome. CG dinucleotides are an example of a mutational hotspot in the nuclear genome and can decrease in frequency following the insertion of an organellar DNA fragment into the nucleus[52].

Mutation rate
The number of mutations occurring in germ-line cells per nucleotide site, per gene or per genome per unit of time or cell division.

Mutational effect
The effect of a mutation on organismal fitness; often referred to as the selection coefficient.

Substitution rate
The rate at which a mutation is fixed in a population. If the mutational effect is zero (neutral) then the substitution rate is equal to the mutation rate.

Width of the selective sieve
The substitution rate per gene divided by the mutation rate per gene.

DNA insertion rate
The number of mutations involving the insertion of organelle DNA into the nucleus (or vice versa) per genome per unit of time. This rate is analogous to the mutation rate.

DNA integration rate
The rate at which a DNA insertion is fixed in a population. This rate is analogous to the substitution rate.

Porphyra purpurea, encodes 253 genes[5]. At the other extreme, the apicomplexans, a group of parasitic protists (which include the malarial parasites, coccidia, piroplasms and Toxoplasma), have a mitochondrial genome of only 6 kb with just five genes and a plastid genome of 35 kb with only 30 protein-coding genes[6].

There are four popular hypotheses to explain the migration of genes to the nucleus. The first is 'insertion happens', but insertions occur more often in the nuclear genome than in organellar genomes (Box 1)[7]. The second is that once integration of an organellar gene into the nucleus happens, the gene will disappear from the organellar genome because a leaner organellar genome will have an advantage in intraorganellar competitions[8]. The third postulates that the frequent organellar bottlenecks that occur during germ-line divisions and/or a haploid asexual lifestyle lead to the fixation of deleterious mutations in organellar genomes[9,10]. The fourth suggests that deleterious mutations are not the determinant, but that beneficial mutations can be fixed more rapidly in the recombination-proficient nuclear genome. These hypotheses are not mutually exclusive, and we have reached a point where we can begin to evaluate their relative roles in the transfer of organellar genes to the nucleus.

Steps in time – building a functional organellar gene in the nucleus

The process of gene transfer begins with the movement of copies of organellar DNA to the nucleus (Fig. 1). DNA can apparently move from the mitochondria into the nucleus at the rate of 5×10^{-6} events per cell per generation in yeast[11]. However, most of the mitochondrial DNA remained extrachromosomal and less than 1% of this DNA inserted into the yeast nuclear genome[11]. Because many of the insertions would disrupt nuclear genes (73% of the genome is protein coding in yeast), the integration rate is likely to be much less than the insertion rate. Surveys of yeast nuclear genomic sequence found few copies of mitochondrial DNA, none comprising a complete mitochondrial gene[12,13].

As in yeast, most organellar-derived sections found in nucleus of plants and animals are fragments of genes often less then 150 bp (Refs 12, 14). However, multigene blocks of plastid and/or mitochondrial genomes exist in plant and animal nuclear genomes, and might provide fodder for the transfer process[15,16]. In felines, a 7.9 kb mitochondrial segment is found in a tandem array of 38–76 repeats[17], and the sequencing of *Arabidopsis* chromosome II turned up a 270 kb segment comprising 75% of the large *Arabidopsis* mitochondrial genome[18]. Multigene chunks of mitochondrial DNA have also been uncovered by the Human Genome Sequencing Project (Ref. 12 and J.L. Blanchard, unpublished). However, because of the extensive editing of plant chloroplast and mitochondrial sequences, and because of changes in the animal mitochondrial genetic code it is not clear whether these large blocks will lead to new genes in the nucleus. Recent gene transfers in plants appear to have been through an RNA intermediate[19–22]. Thus, an important parameter for determining the transfer rate is not simply the quantity of organellar-derived DNA in the nucleus, but the potential of the segment to give rise to a functional gene.

We also need to consider that some groups, like vertebrates and plants, have a much larger proportion of noncoding DNA, and that these groups appear to have more organellar-derived DNA in their nuclear genomes than compact genomes like those of *Saccharomyces cerevisiae* and *Caenorhabditis elegans*. It is not clear if this is a result of an increase in the likelihood that the insertion would disrupt a functional nuclear gene in compact genomes or is a result of an increased rate of deletion of noncoding DNA as is apparent in *Drosophila*[23]. Other impediments of gene transfer and a discussion of 'why organelles have retained genomes' have been reviewed recently[24].

Cases studies of natural and experimental gene transfer

Once the organellar gene is assimilated into the nuclear genome, there are still a number of steps to becoming an active nuclear gene and returning to the organelle as a functional protein. The gene must be expressed and its protein product must acquire a transit peptide to allow access to the organelle. As the copy number of organellar genomes and organelles can be quite high in some tissues, simply providing enough of the protein is likely to be a chore for the nucleus. Finally, the problem comes of coordinating the expression to different light levels or energy needs. The complexity of this process will be dependent on the individual gene. Although it seems an insurmountable challenge to move an organellar gene to the nucleus, the

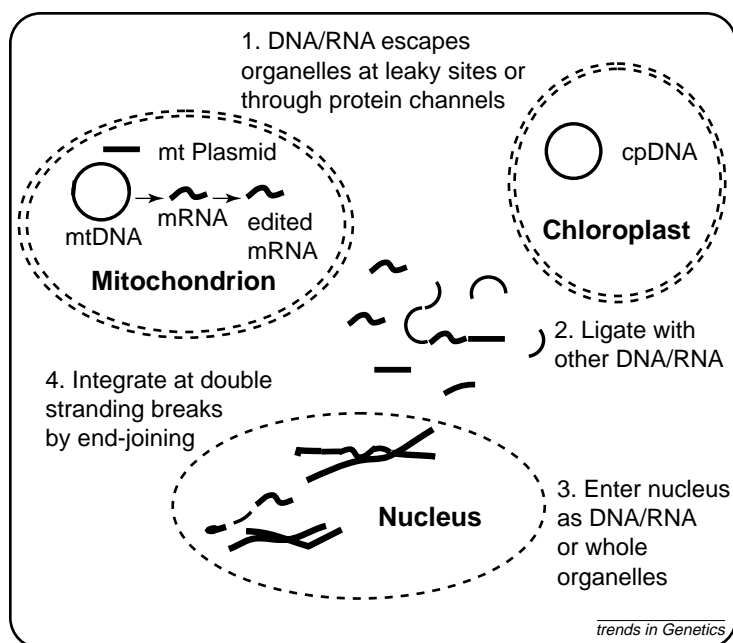challenge has been met both naturally and through human intervention.

Following the origin of land plants, at least eleven mitochondrial genes (*coxII, coxIII, rps2, rps7, rps10, rps11, rps12, rps14, rps19, rpl6, sdhC*) and one plastid gene (*rpl22*) have made a successful trek to the nucleus[1,16,25]. Kadowaki and colleagues have recently developed two remarkable gene transfer stories. In both cases the mitochondrial gene acquired the N-terminal region of a pre-existing mitochondrial targeted protein, but the pathways were different. A plant mitochondrial *rps11* became functional in the plant nucleus after integration through a recombination–duplication event, positioning the N-terminal region of a cytochrome oxidase subunit in front of the *rps11* protein-coding region[26]. A second copy of the *rps11* gene is also found in the nucleus but with the N-terminal region of a mitochondrial ATPase subunit. The mitochondrial copy of *rps11* is no longer functional (it contains an internal stop codon), but it is transcribed and edited, indicating that gene expression is halted at the translational stage.

The mitochondrial *rps14* gene integrated into an intron in the nuclear gene encoding succinate dehydrogenase and now both proteins are produced in the nucleus through alternative splicing in rice[27]. A similar gene organization and alternative splicing pattern was discovered independently in maize[28]. In both of these cases the plant organellar gene has become an active nuclear copy by acquiring the N-terminal region of a pre-existing organelle targeted gene, thus providing an instant solution to expression, targeting and regulatory problems.

Palmer *et al.* have recently developed a framework for studying the gene transfer process in action[29]. In legumes, the integration and activation of the mitochondrial *coxII* gene appears to be closely linked, as in the two previous examples. Intense sampling of 392 legume genera revealed that in some legume lineages the mitochondrial copy has been lost or become nonfunctional, other lineages retained the mitochondrial copy but the nuclear copy has become nonfunctional, and in some lineages both copies are expressed at varying ratios. Further investigation is under way to test whether there is tissue-specific expression in the plants that express both copies. Differential expression of nuclear and mitochondrial copies of the *atp9* gene has been observed in *Neurospora* (Ref. 30). The possibility also exists of regulation at the translational level (both the nuclear and organellar transcripts might exist but only one copy makes a functional protein) or at the protein level (targeting, modification, proper assembly into the cytochrome oxidase complex).

The above natural transfer pathways are similar to the approaches taken by scientists to experimentally manipulate organellar genes into the nucleus in fungal and plant systems. In fungi, the mitochondrial genetic code is different from the nuclear code and the mitochondrial genes need to be modified substantially to create an active nuclear gene. In spite of these difficulties, a nuclear transgene was constructed with roughly 70% of the wild-type activity[31]. In plants, the process is somewhat simpler. For the tobacco *rbcL* gene, the plastid gene was inserted into an expression cassette containing the upstream targeting sequence and regulatory regions of the nuclear *rbcS* gene[32]. The *rbcL* nuclear transgene complements the plastid null mutant, but has only 3–10% of the wild-type levels of RUBISCO activity. The *rbcL* gene is highly

FIGURE 1. The cellular melting pot

1. DNA/RNA escapes organelles at leaky sites or through protein channels

mt Plasmid
mtDNA → mRNA → edited mRNA
Mitochondrion

cpDNA
Chloroplast

2. Ligate with other DNA/RNA

4. Integrate at double stranding breaks by end-joining

Nucleus

3. Enter nucleus as DNA/RNA or whole organelles

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Through comparative and experimental approaches a picture of intracellular DNA transfer has gradually emerged. Yeast mutants with increased rates of mitochondrial DNA escape appear to result from leaky mitochondrial membranes[11], but nucleic acids can also be shuttled out of mitochondria through membrane transporters[56]. Either RNA or DNA can serve as transfer intermediates as evidenced by the transfer of reverse transcribed edited mRNA transcripts and nontranscribed DNA (Refs 12, 14, 16, 22, 57). Experimental studies also suggest that mitochondrial DNA can find its way to the nucleus independent of an RNA intermediate[58]. In spite of extensive editing of plant plastid transcripts, no edited plastid sequences have been found in the nucleus yet. Whole mitochondrial genomes have been observed in the nucleus[59], but the presence of co-ligated plastid, mitochondrial and mitochondrial plasmid sequences in nuclear genomes[12,14] is evidence that intracellular pools of organellar RNA and/or DNA can accumulate. These organellar DNA fragments integrate into the nuclear genome by nonhomologous recombination at double-stranded breaks in the chromosome[12,13].

expressed in the wild-type plant under photosynthetic conditions, and as plants contain multiple copies of the nuclear *rbcS* gene (whose product interacts with a one to one stoichiometry with the RBCL protein) a single copy might not be sufficient to restore wild-type activity.

Transfer of genetic information into organelles

Although the transfer of genes is highly polar, organellar genomes are not refractory to exogenous DNA. Plant mitochondrial genomes have long been known to harbor partial and complete copies of plastid and nuclear genes and the recent sequencing of the *Arabidopsis* mitochondrial genome has revealed a wonderful mixture of exogenous sequences[25,33]. In the genome can be found 16 sections of plastid DNA, fragments of nuclear genes, retrotransposons and sequences similar to plant-pathogen RNA viruses: 49% of the genome is still unaccounted for. The integration of plastid DNA has resulted in six functional tRNAs. Thus, the *Arabidopsis* mitochondrial tRNA repertoire now includes 12 'native' tRNAs, six plastid derived tRNAs (now mitochondrial encoded), and probably another 10 or so that are imported from the nucleus (and derived from eukaryotic type nuclear tRNAs)[25]. There are also well-documented insertions of introns into plastid and mitochondrial genomes (for a recent example see Ref. 34).

FIGURE 2. A hypothetical model for how both copies become essential
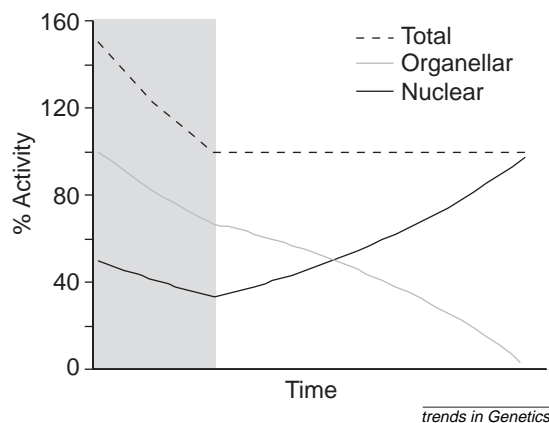
This graph starts with organelle gene activity at 100% and with duplicated nuclear copy activity at 50%. Initially, there is an excess of enzyme activity (protein quantity). As soon as the organelle gene's activity dips below 100%, both copies are essential. Deleterious mutations will continue to occur through genetic drift until the total efficiency of two genes is reduced to the level of the initial singe copy (end of gray area). If the organelle gene continues to fall in fitness because of Muller's ratchet (or a decreased efficiency in selection), beneficial mutations could accumulate in the nuclear copy until the activity of the nuclear copy reaches 100%. At this point the organelle gene can be lost without consequence to the cell or host.

One surprising example of gene transfer was encountered during the sequencing of an octocoral mitochondrial genome. This animal mitochondrial genome contains a putative DNA repair protein (MutS-like)[35] that is not found in any other mitochondrial genomes outside the octocorals, even in the closely related scleractinian corals[41]. If the scenario presented by Culligan et al.[36] is correct, then the octocoral gene might originally have been mitochondrial, then moved to the nucleus billions of years ago, where it diversified into a family of six msh genes, and finally a copy of msh3 integrated into the mitochondrial genome in an ancestor of the octocorals.

How does the organelle gene become superfluous?

Does a successful replacement event depend on the fitness of the nuclear copy starting near the fitness of the organellar gene? It is likely that, as in the experimental systems, the nuclear copy would initially be less fit than an established organellar gene, and only through a number of mutations or recombination events can the nuclear gene rise up and replace the organellar gene. One solution requires that both the organellar and nuclear copy become essential until the nuclear copy becomes as fit as the organellar gene. This could happen through one of three different routes. (1) The establishment of the nuclear copy could be a beneficial event, making up for a suboptimal organellar gene. This would increase the integration rate as the gene would be fixed by positive selection instead of through drift. (2) Both genes might become essential, owing to the accumulation of deleterious mutations in one or both copies to the extent that the loss of one copy is not tolerated (Fig. 2). A similar model of deleterious mutation accumulation has been proposed to explain the persistence of genes following genome duplications[38]. (3) A dosage–compensation mechanism triggered by the import of the nuclear protein could lead to the downregulation of the organellar gene and/or protein. Once both copies become essential (at least temporarily), the population–genetic processes, such as the efficiency of selection on beneficial or deleterious mutations, will determine the winner.

Drift, bottlenecks and the ratchet

The image of asexual organellar genomes rising up to many hundreds of copies per cell then crashing down to a few copies during germ-line divisions invokes analogies with Muller's ratchet: the progressive loss of fitness in finite populations because of genetic drift[39]. If organellar genomes are effectively haploid and inherited uniparentally, then they will be more subject to random genetic drift than nuclear genes, because of a fourfold decrease (twofold because it is haploid and twofold because of uniparental inheritance) in their effective population size. Furthermore, although there is recent evidence for recombination between mitochondrial halpotypes in natural populations[40], organelles still appear to be more vulnerable to genetic drift through linkage disequilibrium effects. Because organelles are a linked set of genes, fixation of a beneficial mutation might result in a deleterious mutation hitchhiking along at another site, or the rate of fixation of a beneficial mutation might be slowed by deleterious mutations at other sites. The important consequence of these population–genetic processes is that a mutation will be less visible to natural selection (more subject to genetic drift) in an organellar gene than in an identical nuclear gene.

One 'click' of Muller's ratchet corresponds to the loss of the original genotype and the fixation of a less fit genotype. The click rate of the ratchet depends on the effective population size, mutation rate, recombination rate and the distribution of mutational effects. One way in which this can be visualized experimentally is by picking a bacterial colony at random and streaking it onto a new plate. With each transfer, the population goes through a bottleneck of one and the result over time is a steady accumulation of deleterious mutations[41,42].

Muller's ratchet has been cited as playing a role in the transfer of organellar genes to the nucleus[9,10], reduction in organelle and bacterial genome sizes[43], changes in animal mitochondrial genetic code[10], occurrence of mRNA editing[44] and a role for organelle genomes in extinction processes[45]. An important assumption in invoking a ratchet-like process is that the population cannot regain its original fitness because compensatory mutations are rare and have small positive mutational effects.

Direct comparison of nuclear, organellar and bacterial tRNA structures suggests that organellar tRNAs are less thermodynamically stable than their nuclear counterparts and eubacterial ancestors, and a greater variance in the organellar tRNA loop and stem regions indicates that more sites are subject to drift[46,47]. Thus, the relatively greater width of the selective sieve in organelles (Fig. 3) is not simply a balance of deleterious and compensatory mutations that results in no net change in fitness or a faster recycling of the same sites. The reduction in fitness from Muller's ratchet is a very long-term process and is not likely to endanger species on timescales of less than a million years[45].

Other organelles, including bacteriocytes (non-cell autonomous organelles found in many insects), show signs of decreased rRNA stability and high rates of substitution relative to their free-living and nuclear counterparts[48]. Selection might be relaxed against some organellar genes, as in nonphotosynthetic plastids, but some simple calculations suggest that the reduced fitness is primarily a result of the smaller effective organellar population sizes[45]. However, theoretical models suggest that the germ-line bottlenecks, instead of facilitating Muller's ratchet, can act to slow any ratchet-like process by increasing genetic variability in the population thereby making selection more effective[49].

Some organellar genes do not show signs of the accumulation of deleterious mutations, but are subject to greater genetic drift than their nuclear counterparts. Plant chloroplast tRNAs are indistinguishable in their stem stability from their nuclear counterparts, but have wider sieve widths[47] (Fig. 3). Therefore, in plant chloroplast tRNAs, deleterious mutations might be fixed by drift followed by compensatory mutations. The result is that relatively more organellar sites are subject to drift, but with no net change in fitness. Thus, although genetic drift is a component of Mullers' ratchet, increased genetic drift does not implicate a role for Muller's ratchet.

How quickly is the race run?

Several groups have compared mutation and substitution rates of organellar genes with either functional[50] or nonfunctional copies in the nucleus[51,52]. In each study, the organelle sequences in the nucleus now conform to the nuclear mutation rate and pattern. The rate at which genes become silenced is dependent on the mutation rate. If all else is equal, the copy in the genome with the lower mutation rate will persist longer. In plants the nuclear mutation rate is orders of magnitude higher than the mitochondrial mutation rate, thus the nuclear copy should be inactivated much more frequently than the mitochondrial copy if the process is driven solely by the mutation rate. Therefore, we would expect to see few, if any, plant mitochondrial gene transfer events, which is in contradiction to the many events previously referenced. Furthermore, Adams et al.[29] have reported a similar number of separate mitochondrial and nuclear coxII gene inactivations within legumes. Thus, the mitochondrial copy seems to be losing more often than the mutation rates would dictate. Further investigation of the legume coxII system should provide valuable information on rates of synonymous versus non-synonymous substitutions, and should provide a test of whether the mitochondrial and/or nuclear copy is subjected to elevated drift.

Selection for smaller organellar genomes either at the organismal or intraorganellar level could tilt the scales towards the nuclear copy by speeding up the rate at which an organellar deletion is fixed. Large mitochondrial deletions can confer a replicative advantage to the smaller mitochondrial genome, in spite of the negative consequences for the host. Case studies of gene transfer in plants suggest that a mitochondrial gene can become nonfunctional due to −1 frameshift mutations and/or the formation of stop codons. Some of these nonfunctional organellar genes are still expressed and edited, and some have no obvious defect in the protein-coding sequence[16,26]. Thus, the first step in nonfunctionalization of an organellar gene often does not involve a large deletion. However,

FIGURE 3. Accelerated substitution rates in organellar genomes
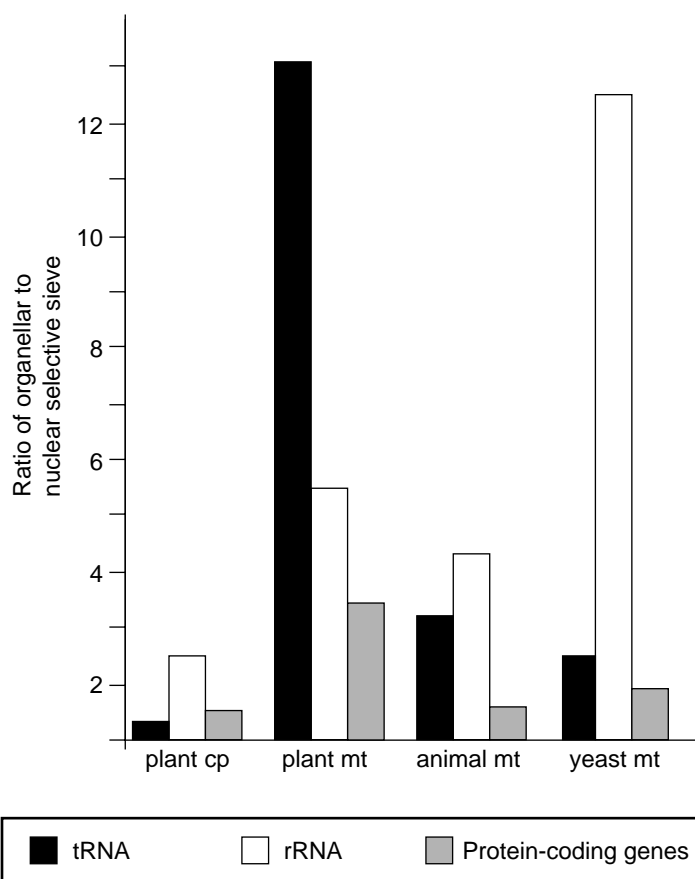
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The relative width of the selective sieve is the ratio of organellar or nuclear substitutions standardized by their respective mutation rates. The ratio of the organelle to nuclear selective sieve widths is shown. In all categories, the ratio is greater than one, indicating the mutations in organellar genes are relatively less visible to selection. This is in spite of the tremendous variation in substitution rates that run from an order of magnitude higher in vertebrate mitochondria to an order of magnitude lower in plant mitochondria relative to their respective nuclear genes. Thus, mutation rates are not the determinant of whether a gene is relatively more prone to genetic drift. Data are taken from Refs 45–47. The black, white and gray bars are tRNA, rRNA and protein-coding genes, respectively.

point mutations, like a single substitution in a tRNA gene, can put the mutant mitochondrial genome at a replicative advantage[53]. Recent computer simulation studies have suggested parameters important in the deletion of unnecessary mitochondrial genes[54].

In plants, because of the high nuclear mutation rate, a frameshift mutation or other types of gene-inactivating mutations would probably occur within a few million years. Thus, both the 'integration happens' and the small-genome-size models predict that the race would be run in or under a few million years. However, the process of gene transfer for coxII and the plastid rpl22 gene has been ongoing for at least 60 million years[21,55] (but these estimates might need downwards revision; see Ref. 29). Why is the process taking so long? It is likely that, as in the experimental systems, the nuclear copy would initially be less active than an established organellar gene and the loss of the organellar copy would be selected against. Thus, the models mentioned above that require the retention of both copies would need to be invoked.

During the timecourse when both copies are present, an organellar gene might lose fitness because of drift, setting the stage for compensatory mutations to come in through the nuclear gene as in Fig. 2. Then beneficial mutations could accrue in the nuclear copy to a level where the organellar gene can be lost with no fitness consequence to the host. Thus, a high mutation rate in the nuclear copy (as in plants) might no longer be a handicap, but might hasten the generation of beneficial mutations.

Time to test hypotheses

Currently, we cannot provide a satisfactory answer to why the nucleus is the preferred location for organellar genes or why there are different rates of gene transfer in different taxon groups. In the next few years, we can expect progress in defining and estimating the parameters needed to explain why organellar genes end up in the nucleus. Genome projects should allow us to estimate the rate and size of organelle DNA insertions in nuclear genomes, allow the detection of other functional nuclear copies of organellar genes and allow us to determine whether flanking regions of DNA insertions are derived primarily from pre-existing genes. As there are many recent examples of gene transfer in plants, surveys of plant species should reveal the time periods over which the organellar gene and the nuclear copy are expressed. Experimental approaches can be used to test directly for dosage compensation. There is also a need to develop theoretical models, and to test the role of deleterious and beneficial mutations in organelle evolution. Together these efforts will help provide answers to a question that is often subject to speculation.

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