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Reducing the genome size of organelles favours gene transfer to the nucleus

Marc-André Selosse, Béatrice Albert and Bernard Godelle

Endosymbiotic organelles exhibit strong genetic erosion during their evolution as a result of the loss of unnecessary genes and of gene transfer to the nucleus. The reasons for this erosion are much debated. Unidirectionality of DNA exchange between cell compartments could favour biased gene transfer, but selection might also act to favour nuclear localization of genes, for example, because organelles accumulate more mutations than do nuclei. Selection for rapid replication might be a general cause of organelle genome reduction. This selection also accounts for the compactness of organelle genomes.

Mitochondria and plastids are remnants of free-living organisms, engulfed in the eukaryotic cell[1], whose genome has been drastically eroded. The various plastid lineages have lost >95% of the gene content present in free-living cyanobacteria[2] (Table 1). The genome is even smaller in animal mitochondria (about 50 kb, 100-fold less than in free-living bacteria), whereas it is entirely lost in hydrogenosomes, recently been demonstrated to be the modified mitochondria of anaerobic eukaryotes[3].

The genes missing from endosymbiotic organelles have been either lost or transferred. Some genes were lost because they became dispensable (such as those required for cell-wall building or motility), and others were lost because nuclear genes took over their function (gene 'transfer'), as described for some plastid ribosomal proteins[4] and for mitochondrial aminoacyl-tRNA synthetase[5]. This transfer corresponds to a series of rare events (Box 1) that replace an organelle gene with a copy of itself, situated in the nucleus of the host and targeting its product to the organelle with a TRANSIT PEPTIDE (see Glossary; Fig. 1). The resulting nucleo-cytoplasmic redundancy is followed by the loss of the organelle gene. Many other organelle genes have been transferred to the nucleus, where they still code for the organelle protein[1] (Fig. 1b) – up to 5000 genes might have been relocated during plastid evolution[2]. An example of a gene transferred from plastids to the nucleus in plants is *rbcS*, the gene encoding the small subunit of the ribulose 1-5-bisphosphate carboxylase oxidase (RuBisCO).

Researchers have examined why organelle genes should stay in organelles[6,7], but have paid less attention to why the organelle genomes should be eroded[2,8,9]. Here, we examine the various hypotheses

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Table 1. The prokaryotic-derived plastidial genomes in various plastid lineages show genetic reduction in comparison with free-living cyanobacteria

Species	Systematic position	Genome size (kb)	Total gene number[a]
Free-living cyanobacteria			
Synechocystis PCC6803	Unicellular	3573	3168
Anabaena PCC7120	Filamentous	6400	?
Primary endosymbiosis			
Cyanophora paradoxa	Glaucocystophyta	136	170
Porphyra purpurea	Rhodophyta	191	220
Mesostigma viride	Prasinophyceae[b]	119	135
Chlorella vulgaris	Chlorophyta[b]	151	111
Nephroselmis olivacea	Chlorophyta[b]	201	127
Pedinomonas minor[c]	Chlorophyta[b]	98	104
Marchantia polymorpha	Hepatopsida[b]	121	119
Pinus thunbergii	Coniferopsida[b]	120	108
Nicotiana tabacum	Magnoliopsida[b]	156	113
Epifagus virginiana	Magnoliopsida[b,d]	70	42
Oryza sativa	Liliopsida[b]	135	110
Zea mays	Liliopsida[b]	140	132
Secondary endosymbiosis			
Cryptomonas Φ	Cryptophyta	112	~140
Guillardia theta	Cryptophyta	122	180
Odontella sinensis	Heterokonta	120	156
Euglena gracilis	Euglenophyceae	143	88
Plasmodium falciparum	Alveolata[d]	30	48

[a]Gene number includes tRNA genes and putative ORFs only when they are conserved in at least two sequenced plastids.

[b]Viridaeplantae.

[c]M. Turmel *et al.*, unpublished data.

[d]Achlorophyllous, parasitic species.

that have been proposed to explain genome erosion. Some explanations focus on the transfer probability from one compartment to another, arguing that it could be easier for a gene to move from an organelle to the nucleus than vice versa. Others refer to the properties of organelles (such as the production of mutagenic compounds and the lack of sexuality) that selectively favour a nuclear location for their genes. However, not all data can be explained by these arguments alone. We provide an alternative explanation that, independently of the mechanisms of GENE TRANSFER, selection for small cytoplasmic genomes is likely to promote genetic loss. This accounts satisfactorily for facts that cannot be explained by the preceding hypotheses, mainly the low content of noncoding DNA in organelles. Finally, we outline some considerations of relevance to selective pressures explaining the physical and genetic size of organelle genomes.

The unidirectional transfer hypothesis

The unidirectional transfer hypothesis suggests that genes can move only from organelles to the nucleus, because one or more of the steps described in Box 1 are more likely to occur in one direction (towards the nucleus) than in the other. For example, Step 1 (sequence transfer to the nucleus) could be unidirectional: organelles would be unable to import nucleic sequences, perhaps because transfer is more frequent from a multicopy genome to a single copy genome than the reverse[10]. A slightly different argument is that damage or lysis of the genomic compartment would be required for the release of DNA during the transfer[11,12]. Several observations support this hypothesis. Long organelle sequences can be transferred to the nucleus (up to 250 kb in *Arabidopsis*[13]) and, in yeast, escape of DNA from the mitochondria to the nucleus depends on vacuolar lysis of the mitochondria[14]. In any case, the existence of multiple organelles favours transfer to the nucleus without damage to the cell, whereas the uniqueness of the nucleus would preclude the reverse transfer.

However, in COENOCYTIC eukaryotic lineages (some fungi, brown and green algae) which harbour several nuclei within a single cytoplasm, lysis of one nucleus does not preclude survival. Conversely, some cell lineages, such as Cryptophyta[15] or Apicomplexa[16], harbour a single plastid and are not exempt from organelle genome erosion. In addition, in *Arabidopsis thaliana*, some transfer occurs via RNA intermediates, as revealed by the similarity between the translocated copy of the gene and the edited sequence in the organelle[17]. Translocation of RNA does not require organelle genome damage and can be achieved without organelle lysis, as exemplified by tRNA imported from the cytosol into some mitochondria[18].

The continuous membranes surrounding the organelles might also impede DNA transfer to the organelle[11], whereas the nuclear pores allow a topological continuity between the cytosol and the nucleoplasm. However, organelles can receive exogenous nucleic sequences, that is RNA (Ref. 18), that can be reverse transcribed[19], or even DNA (Ref. 20), as exemplified by BIOLISTIC METHODS used for organelle transformation. The integration of exogenous sequences is well documented by the presence of plastid and nuclear sequences in plant mitochondria[19]. Thus, Step 1 itself is not necessarily unidirectional, even if transfers from both sides are not equally probable.

A second group of hypotheses states that endosymbionts are not able to perform Step 2 (transcriptional activation; Box 1) on transferred sequences possibly because of the prokaryotic characteristics of their genome. However, genetic innovations are allowed in those genomes, leading to new open reading frames (ORFs) and new functions. For example, in plant mitochondria, various original ORFs are associated with cytoplasmic male sterility[21] and the activation of plastid-derived tRNA has been reported[22]. Step 2 cannot strictly explain the unidirectionality and a functional gene of nuclear origin has recently been found in cnidarian mitochondria[23].

Moreover, explanations focusing on the prokaryotic nature of the endosymbiont are inadequate, because GENETIC REDUCTION also occurs when the endosymbiont is a former eukaryote (the so-called 'secondary ENDOSYMBIOSIS'). In some algal lineages, plastids are surrounded by four membranes, indicating that they originated from eukaryotes, such as red or green algae, engulfed by another eukaryotic

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Box 1. Gene transfer to the nucleus: a scenario in three steps.

Many genes from organelles have been transferred to the nuclear genome during eukaryotic evolution. This phenomenon is still taking place; for example, mitochondrial genes are undergoing transfer to the nucleus in extant plant lineages[a,b]. Analysis of the models of gene transfer suggests a transfer in three steps (Fig. I: reviewed in Ref. c).

Step 1

The genomic sequence is copied and integrated in the nucleus as a pseudogene. Such integrations are frequent in the nuclear junk DNA (Refs d,e).

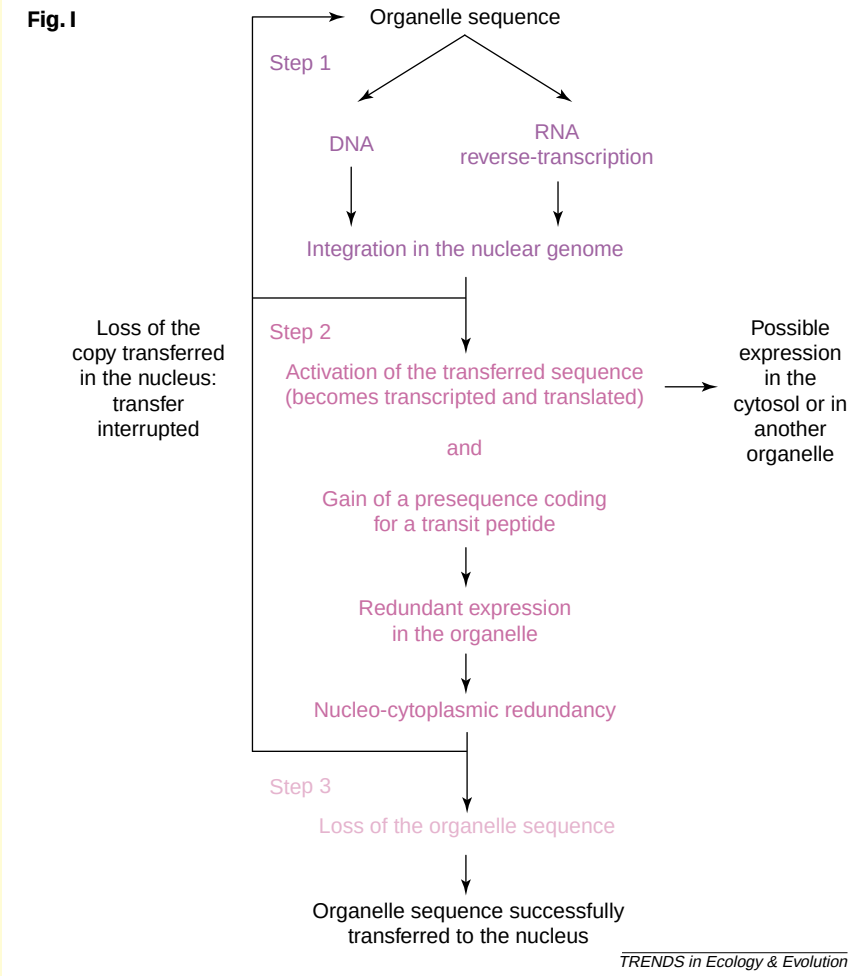
Step 2

While organelle gene continues to encode the relevant protein, the transferred sequence acquires various features that transform it into an active nuclear gene, for example, it adapts to the nuclear gene code, gains a promoter and a pre-sequence coding for a transit peptide. This transit peptide, which allows the targeting of the protein product to the organelle, is often added by EXON SHUFFLING[f]. This leads to genetic redundancy, with the two gene copies both allowing the presence of the protein in the organelle.

Step 3

Until this point, the nuclear copy of the gene can be lost without damage. However, if the organelle gene copy is lost after redundancy, the transfer process is completed and the nuclear copy can no longer be lost without damage.

Fig. I



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cell. The nuclear genomes of those eukaryotic endosymbionts also undergo genetic reduction, leading to a degenerated nucleus (the 'NUCLEOMORPH'), in Cryptophyta[24,25] and Chlorarachniophyta[15], or to a complete nuclear disappearance, such as in photosynthetic Euglenes and Heterokonta (diatoms and brown algae)[26]. In those lineages, the eukaryote–eukaryote confrontation also implies a gene transfer from the nucleus of the endosymbiont to the nucleus of the host[10,25,27]. All organelles undergo a genetic reduction, independently of their phylogenetic origin.

Thus, the mechanism of gene transfer is not fully unidirectional *per se*. The previously listed factors can imply that gene transfer to the organelle will be rare, compared with nuclear transfer. It has been reported that the insertion rate of organelle DNA in the nucleus of yeast is as high as the mutation rate ($>2.10^{-5}$ per generation[11]). However, the rate of transfer to the organelle remains largely unknown and the low value suggested ($<10^{-10}$ per yeast generation[11]) might also reflect a counterselection of insertions in organelle genomes. In plant mitochondria, where insertions of foreign DNA

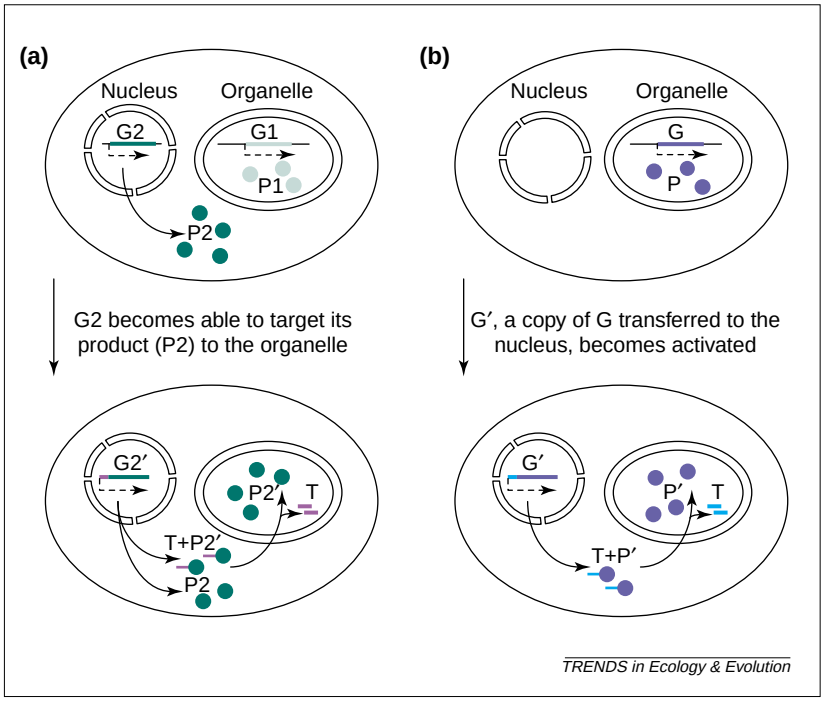

Fig. 1. Gene substitution and gene transfer are ways in which the genome size of organelles can be reduced. (a) Gene substitution can occur when a nuclear gene (G2) encodes a protein (P2) whose function is analogous to that of an organelle protein (P1, encoded by G1). Duplication or alternative splicing could enable G2 to encode a modified form of P2 (P2′) targeted to the organelle by a transit peptide [T, encoded by a presequence (purple) in G2′]. (b) Gene transfer occurs when an organelle gene (G), which encodes a protein (P), is transferred to and activated in the nucleus (see Box 1). The transferred copy (G′) can produce a protein, P', with a transit peptide (T) targeting it to the organelle. In both (a) and (b), the organelle gene (G1 or G, respectively) is functionally replaced by a nuclear gene and can, therefore, be lost.

represent 6.2% of the genome[19], the transfer rate to the organelle is not limiting.

Free radical and Muller's ratchet hypotheses

Once the genetic redundancy following Step 2 is established, any copy can be lost (Box 1). Repeated sequence transfers, followed by random loss at Step 3, could lead to gene transfer (the 'gene transfer ratchet')[12]. However, the expression of the new nuclear copy is likely to be less accurately regulated and less adapted to (eco)physiological needs than that of the original organelle copy[9]: why then does it survive? A role for selection at this step can be addressed by evaluating the outcome of coexistence between a fully functional nuclear copy and an organelle copy. Any selective pressure favouring organelle copy loss could also explain the functional substitution of organelle genes by redundant nuclear genes (Fig. 1a), a phenomenon that is not predicted by statements on the mechanism of gene transfer. Several hypotheses argue that higher mutation rates and/or fixation in organelles favour transfer – a nuclear location would reduce the genetic load on cytoplasmic genes. Modelling experiments suggest that higher mutation rates in organelles can amplify the biased transfer rates, and promote gene transfer to the nucleus[8].

One elegant hypothesis suggests that, in plastids and mitochondria, redox reactions produce oxygen free radicals that can mutate DNA (Ref. 28). Nucleus-transferred genes would, therefore, undergo decreased mutation. However, this hypothesis fails to explain the gene loss previously mentioned for the nuclei of secondary endosymbionts situated out of the plastidial stroma, which produce radicals, and in plastids of nonphotosynthetic organisms[16,29], which are devoid of such radicals.

It has been proposed that the effects of MULLER'S RATCHET could favour loss of the functional organelle copy[2,30]. Once fixed in the nucleus, a gene escapes from the ratchet – any lineage with a nuclear gene would be fitter than lineages with an organelle gene. However, the importance of Muller's ratchet in endosymbionts is far from clear[31] and organelles that undergo recombination after biparental inheritance are not truly asexual. Studies of mtDNA polymorphism in natural populations have revealed the existence of recombination in fungi[32] and, unexpectedly, in human and chimpanzee[33,34], where a decline of linkage disequilibrium as a function of the distance between sites was demonstrated. This can be best explained by the occurrence of recombination (perhaps because of leakage of paternal mtDNA during PLASMOGAMY). In addition, endosymbiotic genomes are often polyploid within an organelle[12] (a feature also reported in strictly endosymbiotic bacteria[35]) and can undergo intermolecular recombination, thus behaving as sexual populations. Alternatively, efficient repair or alternative strategies (e.g. no mutation repair, but purifying elimination of severely mutated organelles[36]) could counteract the deleterious effects of mutation.

The final outcome might be a nucleotide substitution rate lower in organelles than in nuclei, as demonstrated in several mitochondria[23] and plastids[37]. In such lineages, where the nuclear copy should mutate (and therefore silence) more frequently when redundancy occurs, gene transfer cannot be explained by Muller's ratchet or free radical hypotheses alone. It can be stated that gene loss occurred before the evolution of a low mutation rate: but gene transfer was probably continuous during eukaryotic evolution[38] and is still an ongoing process in some eukaryotes[39].

Selection for small genome size as an alternative explanation

Loss of organelle genes could be favoured if, in intracellular life forms, there is selection for smaller organelle genomes. The size of the eukaryotic host genome is less constrained[40], as shown by the presence of noncoding sequences. No selective pressure specifically favours nuclear loss and asymmetric gene loss could be selected after genetic redundancy has been achieved by gene transfer (Fig. 1b) or GENE SUBSTITUTION (Fig. 1a). There is a long-recognized trend towards genome size reduction in organelles[8,41] and recent sequencing data have highlighted the genetic reduction operating in various obligate intracellular bacteria[34,42], including *Mycoplasma*, *Rickettsia*, *Buchnera* and various bacterial endosymbionts of animals. It has been suggested that competition exists among cytoplasmic genomes (Ref. 43) and that this entails selection for smaller genomes that, we suggest, generally contributes to the elimination of redundant cytoplasmic genes, by selecting for deletions in organelles.

Box 2. Small is successful

Deleted organelle genomes have an advantage in numerous heteroplasmic situations where competition with nondeleted genomes occurs, at least when this deletion is not deleterious. This phenomenon is often observed *in vitro*.

The so-called 'rho-' yeasts, which carry a deleted mitochondrial genome[a], can survive in nonrespiratory conditions. Some of them exhibit a 'hypersuppressiveness' phenotype: in crosses with strains having wild-type mtDNA, deleted mtDNA is inherited in 90–100% of the progeny[a], although both mitochondrial types are transmitted to the zygote. This is interpreted as a replicative advantage, because hypersuppressive rho- retain at least one replication origin of the mtDNA. Although the remnant DNA fragment is often tandemly repeated, the rho-genome has a higher density of replication origin[a].

Although plant plastids are not dispensable, deletions that retain the replication origin can accumulate under *in vitro* conditions or at some developmental stages (e.g. pollen formation in plants with maternal plastid inheritance[b]). In *Euglena*, a unicellular alga that is facultatively phototrophic, accumulation of deleted plastids is easily observed on carbon substrates[c].

Progressive accumulation of organelle deletions is common in tissues of multicellular organisms. It has been claimed that deleted mtDNAs accumulate in ageing animal tissues[d]; for example, deleted mtDNA level increases 10 000-fold in muscles over a human lifespan[e]. Above a certain threshold, the deleted mtDNA can behave as selfish elements by increasing their number at the expense of the cell or the organism. In mitochondrial diseases resulting from mtDNA deletions, the delayed onset and progressive course of the disease in heteroplasmic patients[f] is often linked to the increasing accumulation of deleted mtDNA (Ref. d). Small DNA molecules derived from the mitochondrial chromosome also accumulate during ageing in several fungi[g].

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Table 2. The size of nucleomorphs from secondary endosymbionts

Species	Size	Chromosome number	Gene density	Intron features	Refs
Cryptophyta[a]					
Guillardia theta	555 kb	3	1 per 0.8 kb	Nearly none	25
Pyrenomonas salina	660 kb	3	Unknown	Unknown	24
Chlorarachniophyta[b]					
Chlorarachnion reptans	380 kb	3	1 per 1.2 kb	Size <20 bp	15

[a]Plastid derived from a red alga.

[b]Plastid derived from a green alga.

Although, to our knowledge, direct experimental data are lacking, the size of the genomic molecule is obviously correlated with the time and/or energy necessary to complete its duplication[8]. This replicative advantage of small organelle genomes can be selected at various levels, because cytoplasmic genes are involved in a multilevel selection process[44]. Owing to organelle polyploidy (up to 100 copies per organelle[12]), competition among genomic molecules can occur; the shortest molecule can invade the organelle as a result of its faster replication. In HETEROPLASMIC situations, such as following drift in HETEROGENOMIC organelles or biparental inheritance, interorganelle competition could also favour the fastest replicating organelle – as long as they are not deleterious, smaller genomes can accelerate organelle duplication. The duration of organelle division is also likely to affect the mitotic proliferation and thus contributes to intercellular and interorganism competition. In situations implying a single organelle with a single genome per cell (e.g. the nucleomorph of Cryptophyta), neither intergenomic nor interorganelle competition can take place, however, replicative advantage can act through the interorganism selection level.

It has also been claimed that organelles were selected for economy and efficiency[40,45]: reducing the gene content also reduces the space occupied by the DNA and ribosomes involved in its expression. This increases the enzyme concentration in the organelle matrix and, therefore, improves metabolic efficiency. In addition, at the cellular level, a single gene copy in the nucleus rather than hundreds of copies in organelles, is more economic and reduces the need for nutrients. Various selective pressures can thus favour smaller organelle genomes, explaining why they are the most successful in many cases (see Box 2).

Organelle genome compactness

Selection for small genomes also accounts for organelle genome compactness. Pseudogenes are uncommon in organelle genomes and intergenic spacers are rather short. Unnecessary genes often undergo deletions; for example, the fast elimination of photosynthetic genes from nonphotosynthetic plastids of *Epiphagus* (a parasitic angiosperm of recent origin[29]) and *Plasmodium* (the causal agent for malaria, which possesses a highly derived plastid[16]) (Table 1). Note that the loss of pseudogenes in organelles after gene transfer or gene substitution strongly argues against any neutralistic process (mutation or transfer bias) as the sole explanation for these phenomena.

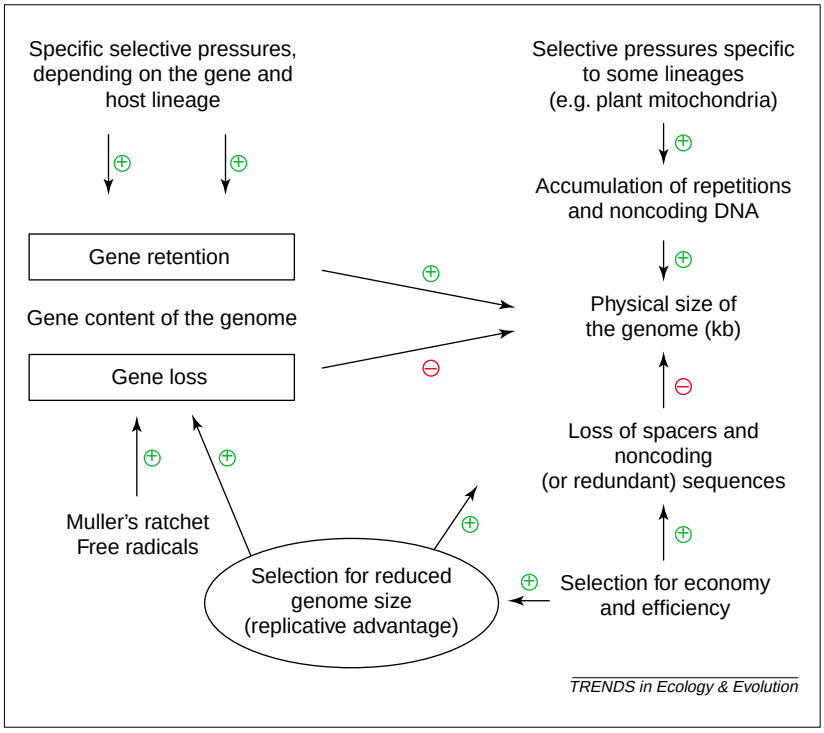

Fig. 2. The effect of selective forces on genome size and gene content. Some reduce the physical size of the genome (replicative advantage and selection for economy[45]), whereas others reduce the gene content by favouring gene transfer and gene substitution. The Muller's ratchet[2,30] or the mutagenicity of free radicals might contribute to gene content reduction in some organelles but, generally, the replicative advantage tends to reduce the gene content to the benefit of the nucleus. Those pressures are counteracted by others that depend more on the lineage considered, favouring the retention of some genes[7] or even an increase of genome size (e.g. in plant mitochondria[19]). + indicates a positive (increasing) effect and – a negative (decreasing) effect.

As a result, many organelles have a compact genome. In the mitochondria of animals, fungi and protists, few or no noncoding sequences persist[41,46] and chloroplastic DNA has a low noncoding content[47,48]. Eukaryotic genomes in plastids acquired by secondary endosymbiosis, independently of their phylogenetic origin, also undergo genetic reduction[15,24,25] (Table 2) and exhibit various features of compactness; for example, unusually short spacers (average <65 bp in *Chlorarachnion*[15]), shared promoting sequences (leading to polycistronic RNA in *Chlorarachnion*) and overlapping genes.

The disappearance of noncoding sequences is accounted for by selection for small genomes, but not by Muller's ratchet or the free radical hypotheses, which only predict minimization of the loci number and do not impede accumulation of intergenic spacers or pseudogenes. In addition, selection for small genomes predicts gene transfer in organelles where biparental inheritance occurs, followed by recombination, that is, organelles that are not subject to Muller's ratchet. This also explains why, although DNA transfer to the organelle is possible, gene fixation and activation in organelles remains a rare event.

Selection pressures acting on organelle genome size

Selective pressure for small size is probably not continuous, but it might act at some stages of the life cycle, mainly those requiring fast division. Some limited stages, such as the zygotic bottleneck[36] or gametogenesis[49], can be of major importance for cytoplasmic organelle populations. Similarly, accumulation of deleted mitochondria during ageing varies among human tissues[50], indicating different selection pressures.

The pressure might not even be constant at the phylogenetic level and seems to be relaxed, at least partly, for plant mitochondria, whose genomic size reaches 200–2500 kb (Refs 19,47). In plant mitochondria, accumulation of noncoding DNA has been proposed to be a by-product of a recombination strategy aimed at lowering the mutation rate[48]. Proliferation of repeated sequences appears to be favoured to enhance recombination and leads to a eukaryotic-like genome structure: the size is less constrained and allows the accumulation of exogenous, nuclear or plastidial sequences[19]. However, gene transfers to the nucleus also occur[17,51], showing that, in this particular organelle-nucleus relationship, gene exchange is reciprocal. In plant mitochondria, the organelle copy is often inactivated by point mutations rather than by deletions[39], suggesting that, when nucleo-cytoplasmic redundancy occurs, random mutations drive the process. This might be because of the suppression of selection for small size – plant mitochondria, useful models for studying the gene transfer mechanism, might not reflect the general role of selection in this process.

Conclusions

Selection for small genomes, through replicative advantage and/or increased metabolic efficiency, contributes to the genetic reduction of endosymbionts, independently of their eukaryotic or

Glossary

Biolistic methods: experimental genetic transformation using tungsten bullets and a gun to enhance DNA penetration in the cells.
Coenocytic: cell with numerous nuclei within a single cytosolic space.
Endosymbiosis: mutually beneficial association of two organisms, with one living in the cells of the other. [Primary endosymbiosis: symbiotic engulfment of a bacterium by a eukaryotic cell. It can lead to organelles surrounded by two membranes (the outer one deriving from the phagocytotic membrane). Examples include plastids of Green Algae and mitochondria. Secondary endosymbiosis: symbiotic engulfment of an alga by a eukaryotic cell. It can lead to organelles surrounded by more than two membranes (the outer one putatively deriving from the phagocytotic membrane)]: plastids with four membranes (e.g. Chlorarachniophyta, Cryptophyta and Brown Algae) or three membranes (with possible secondary loss of one membrane, e.g. Euglenes, some Alveolata).]
Exon shuffling: gene creation by assembly of separate exons from pre-existing genes.
Genetic reduction (or genetic erosion): regressive evolution of a genome that reduces its size and/or coding content.
Gene substitution: evolutionary process by which a nuclear gene substitutes for an organelle gene (Fig. 1a).
Gene transfer: evolutionary process by which an organelle gene is replaced by a nuclear copy of it (Fig. 1b).
Heterogenomic: organelle containing at least two different sub-populations of genomic DNA.
Heteroplasmic: cell containing at least two different sub-populations of a given organelle (mitochondrium or plastid).
Muller's ratchet: irreversible accumulation of deleterious mutations likely to occur in small, clonal populations. It could act on endosymbiotic genomes if recombination (either within or between organelles) never occurs.
Nucleomorph: in some secondary endosymbiotic plastids, the degenerated nucleus of the primary symbiotic host. It persists in Chlorarachniophyta and Cryptophyta.
Plasmogamy: first step of the mating process, namely the fusion of gametic membranes.
Transit peptide: N-terminal extension of a protein that targets it to an organelle.

prokaryotic origin, either through gene transfer or through substitution of nuclear genes (Fig. 1). Combined, it leads to the increasing control of organelles by the nucleus. Acquisition of new genes by organelles, although genetically and cytologically possible, is greatly hampered by this selection in most lineages. The extant coding content of organelle genomes (Fig. 2) is the outcome of several selective forces acting in the same direction or antagonistically.

A main problem is to assess the quantitative significance of selective forces in gene transfer and the possible contribution of other factors, such as the probability of transfer from one compartment to another. Several model organisms that offer opportunities to study the mechanism of transfer, such as yeasts[11,14] or plants[39], could allow microevolutionary experiments. In yeast especially, it would be interesting to assess the respective effects of transfer rate and selection in the rare acquisition of exogenous DNA by mitochondria. Phylogenetic studies and organelle genome comparisons could also allow us to further investigate the history of gene transfers in various groups[38], for example by comparing the transfer rates in lineages of recombinant organelles with those of truly asexual organelles. Theoretical analyses and modelling experiments[8,44] will probably be of help to assess the respective weights of those various selective forces.

Acknowledgements
We wish to thank A. Atlan, G. Dujardin, A. Rötig, P. Saumitou-Laprade and B. Sears for helpful discussions, and S. Loiseaux-De Goër for help in preparing Table 1. We also acknowledge two anonymous referees for their comments on this Opinion.

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