Think again

# Horizontal gene transfer in eukaryotes: The weak-link model

*Jinling Huang*[1)2)]

**The significance of horizontal gene transfer (HGT) in eukaryotic evolution remains controversial. Although many eukaryotic genes are of bacterial origin, they are often interpreted as being derived from mitochondria or plastids. Because of their fixed gene pool and gene loss, however, mitochondria and plastids alone cannot adequately explain the presence of all, or even the majority, of bacterial genes in eukaryotes. Available data indicate that no insurmountable barrier to HGT exists, even in complex multicellular eukaryotes. In addition, the discovery of both recent and ancient HGT events in all major eukaryotic groups suggests that HGT has been a regular occurrence throughout the history of eukaryotic evolution. A model of HGT is proposed that suggests both unicellular and early developmental stages as likely entry points for foreign genes into multicellular eukaryotes.**

**Keywords:**
endosymbiosis; eukaryotic evolution; gene acquisition; genome evolution; organellar gene transfer

## Introduction

About a decade ago, Doolittle et al. raised a question about the number of bacterial genes in protists, speculating that many bacterial genes should have accumulated in genomes of protists through feeding activities [1, 2]. Back then, horizontal gene transfer (HGT) had been documented widely as a mechanism to gain foreign genetic materials in prokaryotes, but remained largely an exotic concept in eukaryotes, with little substantial evidence. It is now clear that HGT has occurred in all major eukaryotic lineages. Horizontally acquired genes are not only frequent in unicellular eukaryotes [3–5], but also found in various multicellular eukaryotes, including cnidarians [6, 7], mites [8], insects [9–12], nematodes [13–15], fish [16], and land plants [17–22]. Although reports of HGT in eukaryotes are still frequently met with skepticism, evidence for HGT throughout eukaryotic evolution is abundant and increasing.

In this paper, I discuss issues related to HGT in eukaryotes. Because most foreign genes reported in eukaryotes thus far are from bacteria, I will focus on bacterial genes. I argue that many bacterial genes in eukaryotes cannot be explained simply as gene transfers from mitochondria or plastids; rather, HGT in eukaryotes should be widespread and expected. Further, I propose a mechanism for integration of foreign DNA into eukaryotic genomes during unicellular or early developmental stages, when their nuclear DNA is relatively exposed to potential sources of HGT.

## No barrier to HGT in eukaryotes is insurmountable

To understand the scale of HGT in eukaryotes, it is important to consider the presumed barriers to gene acquisition. Historically, protists were thought to be more likely to acquire genes than multicellular eukaryotes [1, 3]. Many protists are intimately associated with bacteria or microbial eukaryotes, which serve as food sources, pathogens, or symbionts. Certain species (e.g. *Acanthamoeba* sp.) can harbor a wide range of bacterial endosymbionts; in effect, they are training camps for bacterial adaptation to the intracellular environments of eukaryotic hosts [23, 24]. Although mitochondria and plastids – derived from α-proteobacterial and cyanobacterial endosymbionts, respectively – often receive the most

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[1)] Department of Biology, East Carolina University, Greenville, NC, USA
[2)] Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China

**Corresponding author:**
Jinling Huang
E-mail: huangj@ecu.edu

**Abbreviation:**
**HGT,** horizontal gene transfer; **OGT,** organellar gene transfer.

prominent recognition, their bacterial ancestors likely represented only a portion of microbial diversity present within their ancient host cells. Temporal, transient, or obligate endosymbionts occur in many organisms [25], and differences between obligate endosymbionts and organelles can be marginal. For example, there has been heated debate over whether *Prochlorococcus*-related cyanobacterial endosymbionts (chromatophores) in *Paulinella* should be considered organelles [26–28]. Although not to the same extent as in mitochondria and plastids, chromatophore genomes are highly reduced [29], with some essential genes transferred to the nucleus [30]. Translocation machinery also has evolved to reimport the protein products of transferred genes back into chromatophores [31, 32].

Relative to protists, HGT is often assumed to be rare in complex multicellular eukaryotes because the physical isolation of germ cells from somatic cells may prevent foreign genes from being transmitted to offspring [33, 34]. Despite this assumption, recent HGT events have been frequently reported in both animals and plants [8, 11, 19–21, 35, 36]. Some of these horizontally acquired genes in animals and plants are derived from symbionts, either inside or outside germ cells [7, 10, 36], whereas others are from free-living organisms [16]. The magnitude of HGT from obligate bacterial endosymbionts to their insect hosts can be staggering [10]. In some other cases, genes even were acquired from other sources to help maintain obligate endosymbionts [37]. HGT appears to be particularly frequent in plant mitochondria [38–40], which often harbor mitochondrial genes from distantly related plants. It has been speculated that the acquisition of foreign genes in plant mitochondria was mediated through parasitism, transfer agents (e.g. insects, pathogens, or viruses), illegitimate pollination or other mechanisms [38, 41]. In any case, these foreign genes must ultimately pass through germ cells to be transmitted to the mitochondria of offspring. These data suggest that, although isolated germ cells may indeed be barriers to HGT in animals and plants, they are not insurmountable.

## Bacterial genes in eukaryotes: How many are of organellar origin?

Given that barriers to HGT clearly are not insurmountable, we can examine available data to contemplate the number of HGT-derived genes in eukaryotes. It is well known that eukaryotic genomes contain many bacterial genes [42]. Because of the α-proteobacterial and cyanobacterial origins of mitochondria and plastids, respectively, these genes are often presumed to predominantly be mitochondrial or plastid derivations [43–45]. Indeed, a supertree analysis of 185 genomes from all three domains of life revealed that the strongest phylogenetic signals among eukaryotic genes come from cyanobacteria, α-proteobacteria, and archaebacteria. Presumably these are attributable to plastids, mitochondria and an archaeon that was likely involved in the origin of eukaryotic cells [46]. However, strong signals also exist for different proteobacterial groups (16.7%) as well as for various other bacteria (13.8%), raising the question: how many of these remaining bacterial genes are indeed of mitochondrial or plastid origin?

It can be argued that all these bacterial genes are possibly derived from mitochondria or plastids. Prokaryotic genomes are fluid and shaped by constant gene acquisition and gene loss [47, 48]. Over time, such fluidity could erase the α-proteobacterial or cyanobacterial signal of an organellar gene [45, 49, 50]. This scenario is not unlikely, particularly if the ancient progenitor of mitochondria or plastids was phylogenetically basal to extant α-proteobacteria or cyanobacteria; a single homologous replacement in either the endosymbiotic ancestor or its sister taxon could completely obliterate the true phylogenetic identity of an organelle-derived gene. Fluid prokaryotic genomes, however, appear not to be a significant issue for identifying plastid-derived genes. The majority of functional genes in plastid genomes are either closely related or highly similar to cyanobacterial sequences [51], despite evidence for HGT to the cyanobacterial progenitor of plastids [52]. Similarly, cyanobacterial signal remains the strongest for all bacterial genes in eukaryotes [46], even though some plastid-derived genes might have evolved rapidly as a result of functional decoupling from plastids [53]. Therefore, it is doubtful that the fluidity of prokaryotic genomes could account for all or the majority of genes of other bacterial origins.

The argument that all or most bacterial genes in eukaryotes are of organellar origin is inherently linked to the dynamic process of DNA transfer from organelles to the nucleus [43, 54, 55]. During the evolution of mitochondria and plastids, many organellar genes were either gradually lost or transferred to the nucleus. I here call the latter process organellar gene transfer (OGT) to distinguish it from endosymbiotic gene transfer (EGT), which also frequently involves other endosymbionts. Indeed, OGT has led to the incorporation of numerous genes of organellar origin into the nuclear genome. However, it is rarely mentioned that the transfer of functional genes from mitochondria or plastids to the nucleus is constrained by the gene pool of these organelles. Genes acquired from mitochondria and plastids in any eukaryotic genome must be fewer in number than the original gene contents of the α-proteobacterial and cyanobacterial progenitors of two organelles. Although ongoing DNA transfer from organelles to the nucleus has been demonstrated experimentally [56, 57], functional genes are rarely involved. Conversely, frequent gene loss events during organellar evolution, occurring independently or as a result of OGT, led to increasingly reduced transferrable gene pool in organellar genomes [54, 58]. Major reductions of genomes are common features of many endosymbionts, bacterial, or eukaryotic [58, 59]. Although the scale of gene loss versus transfer to the nucleus is not entirely clear, the loss of organellar genes can be significant in some organisms [60, 61]. For instance, the tremendous loss of organellar genes is particularly evident in apicomplexans, both the plastid and mitochondrial genomes of which are highly reduced or completely lost [62, 63]; their nuclear genomes, with genes presumably derived from five genomes (including plastid, mitochondrial, and nuclear genomes of an algal endosymbiont), sometimes contain less than 4,000 genes [62, 64, 65].

Once a gene has been lost from the organellar genome, it is likely gone forever unless a homolog is acquired again from another source, a very rare phenomenon. Over time, the gene pool available for OGT becomes increasingly smaller. In this sense, the process of OGT represents a closed system; it will eventually approach a dead end when the transferable gene pool is depleted. While this does not diminish the significance of OGT in eukaryotic genome evolution, it also suggests that mitochondria and plastids should not be granted unbound power to explain all bacterial genes in eukaryotes.

Compared to OGT, HGT represents an open system, one theoretically allowing genes to be acquired from virtually unlimited sources. Given the ultimate constraint on OGT, many genes of other bacterial origins may at least be equally explained by HGT. Importantly, the large numbers of proteobacterial and cyanobacterial genes in eukaryotes are also consistent with the observation that proteobacteria and cyanobacteria are the most common endosymbionts in many eukaryotic groups (e.g. amoebae [23, 66], fungi, [67], plants [68], insects, nematodes, and other animals [69, 70]). In particular, *Wolbachia* and *Rickettsia* are not only common endosymbionts, but also potentially closely related to the bacterial ancestor of mitochondria [43, 71]. In many respects, they are similar to mitochondria in having reduced genomes resulting from both gene loss and transfer to the nucleus [72, 73]. In other cases, they might have been secondarily lost, leaving only their genes in the host nucleus [74]. If similar proteobacterial endosymbionts existed during early eukaryotic evolution, it would be nearly impossible to distinguish their phylogenetic signal from that of mitochondria.

## HGT occurs continually in major eukaryotic lineages

There are many straightforward cases of HGT in eukaryotes that involve recently acquired genes [10, 18, 36, 75, 76]. Because phylogenetic signal from their donors remains clear, these recently acquired genes can be readily identified. There are also cases of convergent gene acquisitions or recurrent transfers of the same genes. For instance, acquisition of genes encoding enzymes for plant cell wall degradation occurred independently in multiple major lineages [15, 77, 78]. Recurrent HGT events involving bacteria as ultimate donors have been observed in plants, choanoflagellates, amoebae, and others [17, 78, 79]. Not only can HGT lead to acquisition of individual genes, but also entire metabolic pathways [11, 14, 76, 80, 81]. The proficiency of some eukaryotes in acquiring foreign genes is further evidenced by their stunning ability to recycle plastids [82, 83]. In some cases, protein products of horizontally acquired genes function in permanently established plastids [84, 85]; in others, transient plastids were stolen from algal prey and genes were acquired for plastid maintenance [86]. These observations showcase HGT as a dynamic process in eukaryotes, and one that is usually under-appreciated.

The dynamic nature of HGT is also reflected in its continual occurrence over time. Although complex multicellular eukaryotes appear to have fewer recently acquired genes than protists, this does not diminish the possibility that they acquired many genes more anciently. Unicellularity is the most common form of eukaryotic life, and it is known that unicellular eukaryotes are prone to HGT [3, 4]. In fact, acquired genes can be found in numerous unicellular eukaryotes including many obligate intracellular parasites [76, 87–89], which often have streamlined genomes and retain fewer foreign genes. The fact that all multicellular eukaryotes descend from unicellular ancestors points to potentially more frequent ancient HGT [20, 90]. Indeed, foreign genes were introduced regularly at major historical stages during the evolution of primary photosynthetic eukaryotes [17, 20, 91–96]. In some unicellular eukaryotes such as rumen ciliates and red alga *Galdieria sulphuraria*, acquired genes account for 4–5% of the total genome [77, 96]; similarly, HGT has contributed to over 3% of the nematode genome [97] and at least 8–9% of the gene content in bdelloid rotifers [98]. These numbers may still be underestimations because of the loss of phylogenetic signal in many anciently acquired genes.

Tracing this dynamic process back to the inception of eukaryotic evolution leads to the following conjecture. If the ancestral eukaryote was indeed chimeric and derived from a symbiosis of bacteria and archaea as often suggested [99, 100], then we should expect that the earliest eukaryotes were similar to extant prokaryotes in proficiency of DNA uptake. Thus, they probably acquired many genes from external sources. Similarly, if the host cell or the ancestral eukaryote was able to engulf the proteobacterial progenitor of mitochondria, it might engulf other bacteria or bacterial DNA as well. Assuming the nucleus or other unique eukaryotic features did not appear in a sudden event, specific barriers to HGT now present in eukaryotic cells would have evolved gradually. Therefore, HGT during early eukaryotic evolution might occur as frequently as in modern bacteria and archaea, allowing foreign genes to trickle into early eukaryotes continually. Even with a low fixation rate, many foreign genes could have accumulated in extant eukaryotes over a long evolutionary time period [1, 2]. This process theoretically is open-ended and could have introduced miscellaneous genes independently in different lineages through time. Conceivably, genes acquired later from other sources might have replaced mitochondria- or plastids-derived homologs in the nucleus, explaining in part the observation that many proteins of non-organellar origin function in organelles in various lineages [84, 85, 94].

The assumption that organelles are the sole or primary source of bacterial genes not only contradicts many apparent cases of HGT in eukaryotes [3–5, 35], but also provides little explanation for eukaryotic adaptation to diverse habitats. Adaptation to shifting environments is often accompanied by acquisition of new genes and loss of others [47, 101]. Bacteria and archaea are able to adapt to their environments by sampling from a large global gene pool and maintaining fluid genomes [47, 48]. If novel genetic information cannot be achieved, the adaptability of any eukaryote with a limited gene pool will be hampered. Particularly for early eukaryotes, the task for surviving in various niches and further diversifying into major lineages was probably

daunting. Given the fact that point mutation, recombination, gene duplication, and genome rearrangement only operate on pre-existing genes, it would have been disadvantageous for early eukaryotes to completely abandon their ability to acquire ready-to-use genes from other sources.

## The weak-link model explains frequent HGT in eukaryotes

One of the most popular models for HGT in eukaryotes is the gene ratchet mechanism proposed by Doolittle [1, 2]. Under this model, bacteria phagocytized by protists as food sources are lysed within host cells, allowing their DNA to be incorporated into host genomes. Though elegant, this model does not explain the widespread existence of foreign genes in eukaryotes that do not engage in phagocytosis. Given the occurrence of HGT in eukaryotes with miscellaneous lifestyles [3–5, 35], I here offer a different perspective on HGT mechanisms in eukaryotes.

For any foreign gene to be acquired and stably inherited by a recipient organism, it must (i) enter recipient cells, (ii) be integrated into the recipient genome, and (iii) be transmitted to offspring. Foreign genes could enter cells of the recipient organism at any weakly protected stage of the lifecycle in natural environments. This process could be facilitated if the recipient and donor organisms maintain an intimate physical association through symbiosis, parasitism, infection, or other known forms of contact [18, 35]. For unicellular eukaryotes, the HGT process could be similar to the gene ratchet mechanism proposed by Doolittle [1], but does not specifically require feeding activities for foreign genes to enter recipient cells. Once inside the cell, integration of foreign genes into recipient genomes does not appear to be particularly difficult, given the still frequent movement of organellar DNA fragments into the nucleus in different organisms [56, 57]. Even if the initial attempt to integrate into the nuclear genome fails, as long as foreign genes can enter recipient cells without significant difficulties, successive attempts may eventually lead to successful integration of foreign genes into the recipient genome [1]. Subsequently, transmission of integrated foreign genes to offspring can be accomplished simply through mitosis in these unicellular organisms (Fig. 1A).

For complex multicellular eukaryotes such as plants and animals, foreign genes must be passed through specialized reproductive cells (germ lines) to be transmitted to offspring. Therefore, the isolation of germ cells from somatic cells is often considered to be the major barrier to HGT in animals and, to a lesser extent, higher plants [33, 34]. Overcoming this barrier is certainly not easy, but possible. For example, the close association of *Wolbachia* with germ cells of arthropods or acquisition of bacterial endosymbionts during embryonic development could promote stable HGT from these endosymbionts to their hosts [9, 10]. Similarly, if a plant or an animal is exposed to and readily incorporates foreign DNA during its very early developmental stages, subsequent cell proliferation and differentiation will spread these foreign genes to other tissues, including germ cells (Fig. 1B). For instance, in nonvascular and seedless vascular plants, female gametes are weakly protected in archegonia and exposed to external environments during fertilization, and male gametes generally are exposed completely prior to reaching an oocyte. External fertilization occurs in animals inhabiting aquatic environments, meaning gametes and zygotes are, likewise, freely exposed to foreign sources of DNA. Structurally internalized gametes in seed plants and animals in terrestrial environments may be protected from mechanical damages, but not necessarily foreign DNA from symbiotic bacteria, pathogens, or other microbes [8, 10, 19, 38]. Foreign genes introduced during zygotic or embryonic development will be propagated through mitosis into germ cells and, therefore, next generation. Propagation of foreign genes also is possible through gene transfer among neighboring cells, as demonstrated in natural plant grafts [102, 103]. In these respects, the entry points in early developmental stages represent the weak link in recipient organisms for initiating foreign gene transfer; as such, they ultimately control the transmission of foreign genes to offspring. Once foreign genes are passed onto offspring, they can be fixed in the population through drift or positive selection on newly acquired functions.

This model of gene acquisition critically depends on the presence of weak or unprotected points for foreign genes to enter recipient cells. Other than early developmental stages (e.g. zygotes, embryos, or spores) of multicellular eukaryotes, weak-link entry points for foreign genes include all lifecycle stages in unicellular eukaryotes. In multicellular eukaryotes with complex sexual reproduction, the propagation of foreign genes through cell proliferation and differentiation does not require direct contact between the donor and differentiated reproductive tissues of the recipient (Fig. 1B). In multicellular eukaryotes with asexual reproduction, this process allows foreign genes to be transmitted directly to offspring by mitotic propagation of cells carrying these genes (Fig. 1C). Because foreign genes are expected to decay into pseudogenes if not selectively advantageous to the recipient organism, the actual number of acquired genes should vary among organisms of different lifestyles. However, given the potential role of HGT in allowing organisms to explore new resources and niches [47, 48, 101, 104], foreign genes with novel functions could be fixed more frequently in recipients under resource limitation or in shifting environments.

This model also makes the following specific predictions regarding the occurrence or overall frequency of HGT in eukaryotes of different lifestyles:

(i) Frequent HGT in unicellular eukaryotes. Since all developmental stages of unicellular eukaryotes represent weak-link entry points, there are ample opportunities for foreign genes to be integrated and, therefore, transmitted to offspring.
(ii) Occurrence of foreign genes in multicellular eukaryotes with fully exposed unicellular or early developmental stages (e.g. spores, zygotes, or embryos) in their lifecycles (see above).
(iii) Frequent HGT in asexual multicellular eukaryotes. The absence of specific germ cells means that any cell carrying foreign genes may

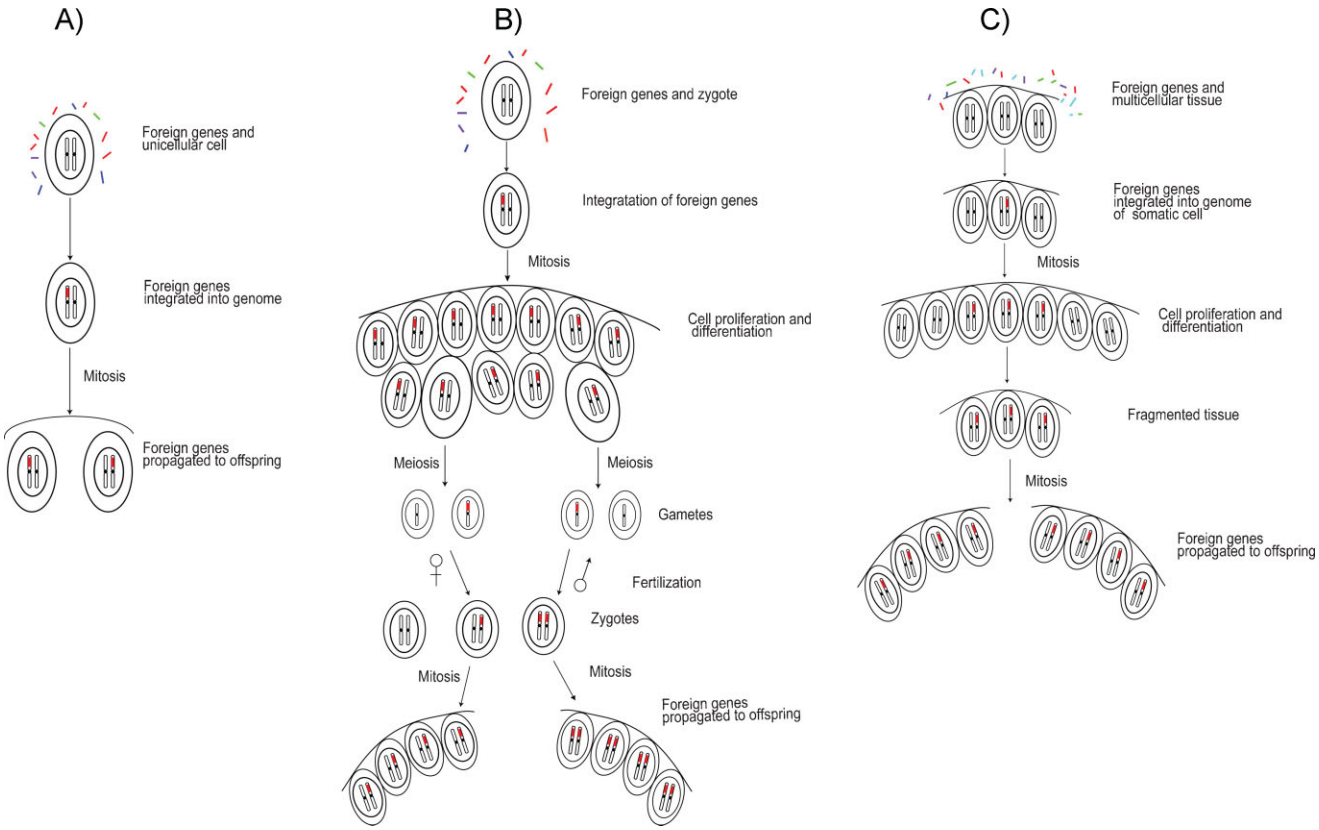


**Figure 1.** Illustration of the weak-link model of HGT. The unicellular or early developmental stages (spore, zygotes, embryos, etc.) are exposed to foreign genes. These weakly protected stages allow the entry and integration of foreign genes. **A:** In unicellular eukaryotes, foreign genes may be directly transmitted to offspring through mitosis. **B:** In multicellular eukaryotes with sexual reproduction, cell proliferation and differentiation spread the foreign genes to all cells including germ cells, which then give rise to male and female gametes. Subsequent fertilization allows foreign genes to be transmitted to offspring. **C:** In asexual multicellular eukaryotes, propagation of cells carrying foreign genes allows the foreign genes to be transmitted to offspring.

propagate them into offspring. The frequency of HGT should be even higher if bacterial endosymbionts exist in asexual structures, such as spores and hyphae in fungi [67].

(iv) Existence of many anciently acquired genes in multicellular eukaryotes. Because multicellular eukaryotes are ultimately derived from unicellular ancestors, it is expected that many foreign genes acquired by their unicellular ancestors remain in the genomes of their multicellular descendants.

## HGT may still be underestimated in eukaryotes

Despite potentially frequent HGT in many eukaryotes, identification of acquired genes often is complicated. Although foreign genes may gradually accumulate in recipient genomes, their phylogenetic signal tying them to specific source taxa may be muted or completely erased by substitutions over time. Additionally, HGT from uncultivated or extinct bacterial lineages may not be properly identified [105]. Even if phylogenetic signal is retained, recovery of accurate phylogenies can be complicated. In particular, many gene families are patchily distributed in prokaryotes and eukaryotes, and explanations of such patchiness can be controversial [106–108].

Interpretations of patchy distributions hinge on underlying assumptions [2]. For researchers who view vertical inheritance as the sole or dominant genetic paradigm, HGT rarely offers a satisfying explanation. In such cases, a patchy distribution is best explained by differential gene losses, misidentification of genes, or simply phylogenetic artifacts. Although these factors can create patchy distributions, indiscriminately resorting to them as the chief explanation not only discounts the obvious existence of HGT in many eukaryotes, but also ignores the gene pool constraints from the common ancestor of eukaryotes and progenitors of organelles. Clearly, some reported cases of HGT turn out to be artifacts [4, 35], but the existence of some established artifacts does not discount the likelihood of HGT in many other cases.

On the other hand, patchy distributions are easily explained based on current knowledge of HGT. For examples, HGT from prokaryotes, sometimes involving the same genes independently and recurrently [78, 109, 110], can spread prokaryotic genes among unrelated eukaryotes. Further, the bacterial

ancestry of mitochondria and plastids, the widespread distribution of secondary, tertiary, or transient plastids, and the presence of bacterial endosymbionts (e.g. *Wolbachia* and *Rickettsia* in animals) in many eukaryotes, are all known to lead to gene transfer and, therefore, bacterial genes in eukaryotic genomes. In such cases, patchy distributions not only are expected, but also clearly reflect the very nature of HGT in eukaryotes [111].

Given the difficulties and complications discussed above, it is important that putative cases of HGT in eukaryotes be investigated carefully. To do so, independent lines of evidence and alternative scenarios should be considered. Many cases of patchy distribution probably reflect combined effects of duplication, gene loss, HGT and other processes [80, 112, 113]. Nevertheless, as long as vertical inheritance remains the null hypothesis, HGT in eukaryotes will likely be underestimated. Therefore, it is useful to bear in mind that HGT, although difficult to "prove" in every individual case, offers a valid explanation for many of the atypical gene distributions in eukaryotes.

## Conclusions and outlook

A large percentage of eukaryotic genes are unquestionably of bacterial origin. Because mitochondria and plastids represent fixed gene pools, from which many genes have been lost completely during their evolution, OGT alone cannot adequately explain the large number of bacterial genes in eukaryotic genomes. The occurrence of recent HGT events in all major eukaryotic groups indicates that there are no insurmountable barriers to HGT, even in complex multicellular forms. Additionally, the finding of many anciently acquired genes in eukaryotes suggests that HGT is a dynamic process that has operated continually throughout the history of eukaryotic evolution. The weak-link model of HGT hypothesizes that unicellular and early developmental stages are the most likely entry points for foreign genes into recipient cells. Given the universal existence of these weak-link entry points, HGT is expected to occur frequently, on an evolutionary time scale, in all groups of eukaryotes. The weak-link hypothesis makes several explicit predictions that can be tested either by genome analyses or by experiments under controlled conditions. Future work is critically needed to understand the overall scale of HGT, but also the contribution of HGT, compared to other genetic mechanisms such as de novo gene generation and duplication, to the expansion of gene pool in different eukaryotic lineages throughout evolutionary time. Such work can be accomplished through careful evolutionary genomic analyses and will benefit our understanding of the role of HGT in the innovation and evolution of eukaryotes.

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