From Trading Genes to Crafting New Tricks: How Horizontal Gene Transfer Potentiates the Emergence of Novel Functions

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Abstract

Horizontal gene transfer (HGT), the set of processes by which genetic information is transferred between individuals, has shaped life's evolution. It is particularly frequent in microbial organisms, where it has driven numerous remarkable adaptations to extreme conditions, anti-microbial agents, or biotic interactions. Its role in spreading novel functions is now documented by countless examples of dramatic phenotypic changes. It is however less clear how frequently HGT favours functional innovations, i.e. the emergence of novel biochemical, physiological or ecological functions. Here, I will argue that very high rates of gene acquisition by HGT favour functional innovation by providing a constant flow of genes encoding a large diversity of processes. Part of this genetic information is under little, if any, purifying selection, paving the way for evolutionary tinkering that may ultimately result in their co-option for novel functions. Such processes have been described for the evolution of regulatory networks, metabolic pathways, antibiotic resistance, and co-option of complex machineries. Mobile genetic elements provide a lot of the material for functional co-options and then spread them across microbial communities. They have the key role of preventing that functional innovations remain private to the lineages where they emerge. Consequently, when microbial communities encounter new challenges or opportunities, they function as a collective of agents that innovate to address them.

Keywords: Microbiology; horizontal gene transfer; mobile genetic elements; molecular evolution

Introduction

The mechanisms by which DNA can be transferred between genomes of different individuals, resulting in Horizontal gene transfer (HGT), have been recognized for almost a century. The discoveries of these processes were foundational moments for modern molecular biology. Natural transformation, a process whereupon Prokaryotes uptake naked environmental DNA [1], provided the first clear proofs that DNA is the genetic material [2,3]. This is one of the rare processes of genetic exchanges that is not driven by mobile genetic elements (MGEs). Transformation has a high impact in gene flow that leads to allelic recombination within species [4], but seems less important than other mechanisms for the spread of novel genes between species (HGT) [5]. Bacteriophage(phage) kill producing cells to transfer their DNA, or the bacterial DNA by transduction [6]. The discovery of phage-driven DNA transfer provided the definitive proof that DNA carries the cell's genetic material [7]. The discovery of conjugation, a mechanism by which MGEs produce a mating pair formation system to transfer their own DNA between cells [8], spurred the understanding of the mechanism of homologous recombination [9]. The study of these processes has also profoundly shaped our understanding of evolutionary mechanisms. HGT allow genetic exchanges independently of sexual reproduction and can take place between lineages that diverged billions of years ago [10]. This allows the spread of functional innovation across the tree of life. Research on the evolution of phage–bacterial interactions confirmed that adaptation proceeds through random mutations that are subsequently filtered by natural selection [11]. Work on HGT also drove biotechnological innovations in genetic engineering, including the use of plasmids or phages and the discovery of restriction enzymes and CRISPR [12–15]. Finally, soon after the introduction of the first antibiotics, it was discovered that HGT can have a key impact on public health by driving variations in virulence and antibiotic resistance within microbial populations [16,17].

General acceptance that HGT drives many of the rapid changes of phenotypes in prokaryotes took much longer. In the 1990s it was proposed, to some uproar, that more than 10% of *Escherichia coli* K12 was the result of recent HGT [18]. When multiple genomes of the same bacterial species became available, these earlier analyses were found to be correct, even under-estimates, of the real amount of HGT [19]. It is now known that the diversity of gene repertoires in many bacterial species, their pangenome, exceeds the average genome size by more than an order of magnitude [20]; and that it is largely the result of HGT [21].

Prokaryotes can engage in intra-species allelic exchanges by gene conversion [22]. These are different from exchanges occurring in sexual reproduction, because they are small and unidirectional: a variant locus from one genome becomes also the variant locus of another. While the literature often separates HGT from recombination, the distinction between the two processes is not always straightforward [5]. Homologous recombination events at core genes may result in allelic conversion of these genes and HGT of the genes between them (for reviews on these mechanisms see [23–25]). Here, I shall use the term HGT to denote the acquisition of novel genetic information but other processes concomitant with HGT, like allelic exchanges by recombination, can occur.

Most past studies focused on how HGT enables instantaneous phenotypic shifts by transferring unmodified functions between individuals [26]. This requires the expression on a novel microbial host of a pre-existing function whose type of molecular activity is assumed to remain constant. There are many examples of such plug-and-play functions in the evolution of virulence [16], adaptation to extreme environments[27], mutualistic interactions [28], antiviral defenses [29], cellular protection [30], or antibiotic resistance [31]. Mobile genetic elements (MGEs), such as phages or conjugative elements that are abundant in microbial genomes [32,33], depend on such plug-and-play functions to spread across microbial populations [25]. Here, I will focus on another aspect of HGT that is less detailed in the literature (but see [34–36]): by incorporating a novel genetic background, the recently acquired genetic systems may foster the emergence of novel functions.

At this juncture, it is important to briefly clarify two concepts that could merit an entire article. What is a function? I will consider that a function is a cellular activity, structure, or product that has a positive contribution to fitness to the genome encoding it when averaged across generations [37]. When genes or other genetic elements are conserved across very long periods of time, relative to the rates of mutation and deletion, they are most likely performing a function. It is also not simple to define what differentiates a function from another, even if this notion is at the core of this article. In some cases, the difference is intuitive: enzymes with different activities have different functions. Yet, as we shall see, enzymes often have secondary or promiscuous activities rendering the distinction less clear. Here, I will consider that a function changes when its contribution to fitness differs in a qualitative manner. For example, an enzyme's function changes when there are changes in its set of relevant biochemical activities (among those contributing positively to fitness). This leads us directly to Gould's taxonomy of fitness, where exaptations are characters previously shaped by natural selection for a particular function, that are coopted for another [38]. Processes of exaptation are poorly understood, but are thought to involve what François Jacob called evolution by tinkering with existing genetic elements [39]. When genes are transferred between cells they usually already have a function. Cooption and tinkering are key to subsequent functional innovation.

Change in genome repertoires by HGT

Most gene families are either very frequent or extremely rare in a typical species pangenome [40,41]. Only a few gene families are found at intermediate frequencies (but see [42]). The gene families present in almost all genomes of the species, their core genome, encode the most fundamental systems of the cell. While they may be strongly affected by allelic recombination [43], they tend to be transferred vertically at the macro-evolutionary scale [44]. Other gene families are present in very few genomes; they include accessory functions, most MGEs, and numerous genes of unknown function [45]. These genes present in very few genomes of a species, but with homologs across other species, were for the most part acquired by HGT [46,47]. The origins of genes lacking homologs in other species and encoded outside MGEs are more difficult to uncover. They may have been acquired from unsampled genomes or by *de novo* gene

emergence in the species. The latter is thought to be rare in Prokaryotes given the integrated effects of a bias towards gene deletion [48,49] and the presence of small intergenic regions that are under purifying selection [50]. The U-shaped spectrum of gene frequencies within species seems almost universal among prokaryotes, while varying extensively in degree, i.e. in the ratio of the frequencies of core and rare genes [51,52]. Some species are more enriched in core gene families, whereas others contain a preponderance of rare ones [53]; the relative proportion of the two reflects numerous aspects of microbial ecology and evolution. As a result, the pangenome of a species can be orders of magnitude larger than the average genome of its individuals, contributing to explain phenotypic variability in microbial populations [54].

While the presence of gene families at low frequency in a species is a strong indication that the family was acquired by HGT, the variation in the number of genes within a given gene family can occur by either HGT or intra-chromosomal duplication. The latter process is dominant in many Eukaryotes, but not in Prokaryotes. The frequency, dynamics and short-term consequences of HGT and duplication are remarkably different (Figure 1). While HGT can lead to very sharp phenotypic shifts, *e.g.* immediate high level of antibiotic resistance, duplication processes are thought to lead to smoother fitness changes[55]. This is because genes duplicated since the last common ancestor of a species tend to be very similar which results in similar genetic networks and similar proteins that have the same physical interactions in the cell [56]. Duplication processes are therefore expected to initially generate genetic redundancy [57]. In contrast, homologs originating from different species by HGT are more likely to generate less redundant regulatory and physical interactions networks because they are very different from the native ones. Gene expression also differs markedly between duplicated or horizontally transferred homologous genes. Duplications are more frequent among highly expressed genes [56]; confirming that high gene dosage may drive initial stages of natural selection for keeping gene duplicates [58]. In contrast, genes acquired from distantly related species tend to be weakly expressed [59,60], presumably because their regulatory sequences are poorly recognized by the novel host machineries. Furthermore, horizontally transferred genes [61], and especially those in MGEs [61,62], tend to be A+T rich which makes them targets for gene expression silencing by host nucleoid-associated proteins [63]. Overall, gene duplication-mutation processes or HGT can bring into cells novel genes, but their immediate consequences for the cell biology are very different.

Most recently acquired genes are rapidly lost. This is true for duplicated genes and for genes acquired by HGT [56]. This can be easily explained by mutation and selection processes. First, tandem gene duplicates tend to be genetically unstable because of intrachromosomal recombination [64,65], while MGEs are also rapidly lost by multiple mechanisms [65,66]. Second, duplicated genes tend to be lost without fitness impact because they are often redundant. Genes acquired by HGT are often poorly or not expressed at all. Third, genes acquired by HGT can be very costly due to the expression of non-adaptive functions, cytotoxic effects, and the cost of the MGEs carrying them [67,68]. In the end, existing data suggests that gene family amplifications caused by HGT are less likely to get lost than those arising from intra-chromosomal duplications [56,69]. Hence, HGT tends to produce more lasting gene family amplifications.

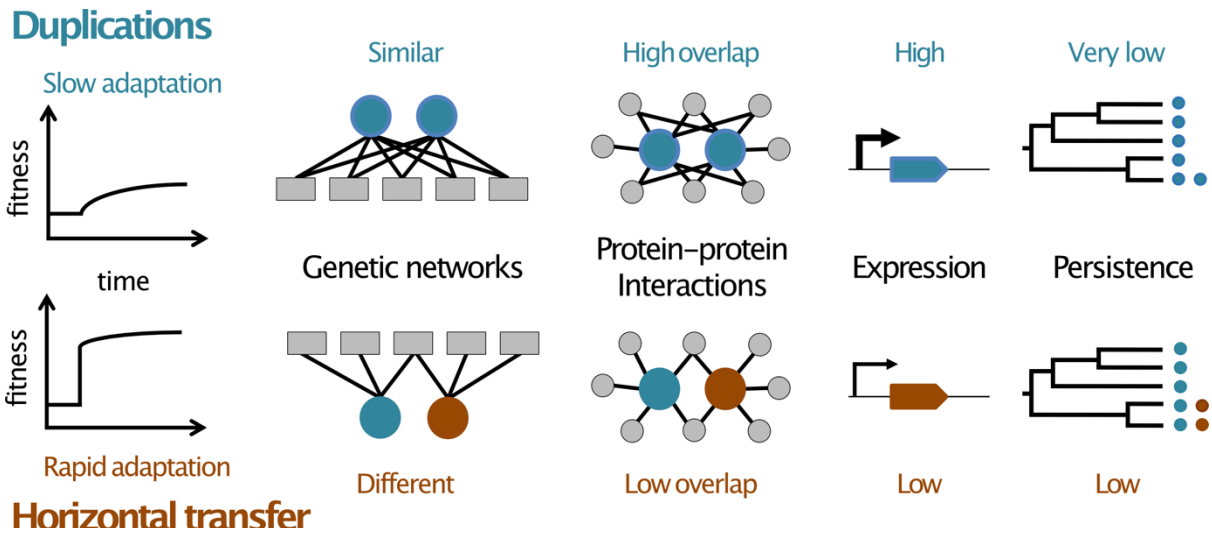

Figure 1. Gene duplications and HGT have different evolutionary dynamics, because whereas the former tends to produce initially highly redundant genetic, physical and biochemical networks, the latter often bring novel genes into genomes. Levels of expression and persistence may vary between the genes resulting from the two processes.

Horizontal gene transfer spurs evolvability

Many proteins are multi-functional or have one major function while "moonlighting" other accessory functions, which are also under natural selection. Proteins may also lack in specificity, i.e. they generate promiscuous activities that are not selected for. This promiscuous activity is most often seen as a nuisance, resulting is less efficient biological systems. Yet, it may favour the emergence of novel functions because it implies that different, potentially adaptive, activities are accessible only a few mutations away. This facilitates the shift between functions without passing through intermediate non-functional variants that could be deleterious or lost by genetic drift [70]. Along this process the protein may acquire novel specific traits. Moonlighting or promiscuous proteins are very abundant in bacteria [71] and also occur in temperate phages [72]. For example, a third of *E. coli* enzymes account for two thirds of the known metabolic reactions because they catalyze multiple reactions [73]. A screen of 104 single-gene knockout *E. coli* strains revealed that 20% of these auxotrophs were rescued by the overexpression of at least one other gene [74]. The multiplicity of activities of genetic systems means that gene functions may change without requiring unlikely mutational pathways.

The replacement of an existing gene by a homolog acquired by HGT seems rare [69] (but see [75]), suggesting that in most cases either both persist or the novel gene is lost. But genetic redundancy, while it lasts, provides opportunities to evolve novel functions, because the genetic material is less constrained by natural selection[76] and evolution of one copy does not affect the original function that can be provided by the other copy [77]. Genetic material acquired by HGT can thus be further modified by processes of duplication, deletion, and mutation, like it has been described for duplicate genes [78,79]. As reverse engineers know well, complex machineries have multiple functions performed by multiple modules. The individualization of a functional module may

ultimately result in sub-functionalization because the machinery produces a different function in its new context. The sub-functionalization of genes might be particularly frequent in multi-copy multi-functional genes [80], since the presence of multiple copies facilitates their divergent specialization (Figure 2). This process may only require loss of genetic material, although, as discussed below, most often this also involves mutations and even accretion of other cellular components. HGT may also facilitate neo-functionalization, i.e. the emergence of a novel function in an existing gene. Such processes are hazardous, since there is a high likelihood that the gene is lost before a novel function emerges. Yet, as mentioned above, a novel function may be just a few mutations away in a gene with promiscuous functions. The integration of both processes by transient duplication-divergence of genes acquired by HGT may rapidly generate genes with distinct activities [81].

Duplication-divergence processes may result in periods of within-cell polymorphism. MGEs favour these processes because they are major actors of gene amplification in genomes. In Prokaryotes, this is especially the case for plasmids and transposable elements (reviewed in [82,83]). The genes of high copy number plasmids are in high dosage in the cell and therefore are more likely to have at least one copy mutated. When novel variants are adaptive and dominant, this accelerates the rate of their fixation [84,85]. For example, high-level enzymatic resistance to ceftazidime obtained by mutation-selection processes was observed when the corresponding gene was on a high copy number plasmid, but not when it was located on the chromosome [86]. In contrast, recessive adaptive mutations may take longer to be fixed, or even be lost, when occurring in high copy number MGEs [87]. Such processes also facilitate protein sub-functionalization. For example, evolution of the enzyme *bla*TEM-1 towards ceftazidime resistance occurs predominantly through the acquisition of a single mutation that reduces its activity against ampicillin [88]. This effectively limits multi-drug resistance. However, when the two alleles are encoded in different plasmids, they may coexist within the cell for hundreds of generations, as long as there is selection for both functions, relieving the constraints of the trade-off in the evolution of antibiotic resistance [89].

High evolvability, including high recombination or mutation rates, may be favoured when individuals are maladapted [90], resulting in a higher likelihood of functional innovation when it is under high demand. Genes acquired by HGT may actively increase genome evolvability by increasing recombination or mutation rates that potentially affect all genes in the genome. Transposable elements are very often transferred horizontally within other MGEs [91]. They have the ability to change genome repertoires by inactivating or duplicating genes [92]. They also spur structural changes in genomes [93], including gene transfer between different types of MGEs or between these elements and the rest of the bacterial genome [94–97]. Conjugation induces the SOS response in *E. coli* and *Vibrio cholerae* [98], which increases mutation rates [99]. Phages and conjugative elements encode specific recombinases that mediate homologous recombination between more divergent sequences than the typical cellular mechanisms [100,101]. This increases the chances and the genetic diversity of recombination events. HGT can also result in the acquisition of alternative DNA repair mechanisms that affect the evolvability of the genome. Some MGEs encode non-homologous end joining systems [102], which by ligating non-homologous sequences can produce novel chimerical genes. Finally,

some MGEs encode error-prone polymerases that increase mutation rates upon their establishment in the recipient cell[103]. For example, a *umuCD* cassette in a plasmid, encoding an error-prone DNA polymerase, was found to increase mutagenesis, and accelerate the evolution of free-living soil bacteria into rhizobial symbionts [104]. Such cassettes are present in many plasmids encoding symbiotic functions, suggesting their co-transfer is under selection and facilitates the accommodation of complex functions in a novel genetic background. If so, beyond providing cellular genomes with complex functions, MGEs may also bring genes that accelerate the accommodation of such functions to the new genetic background.

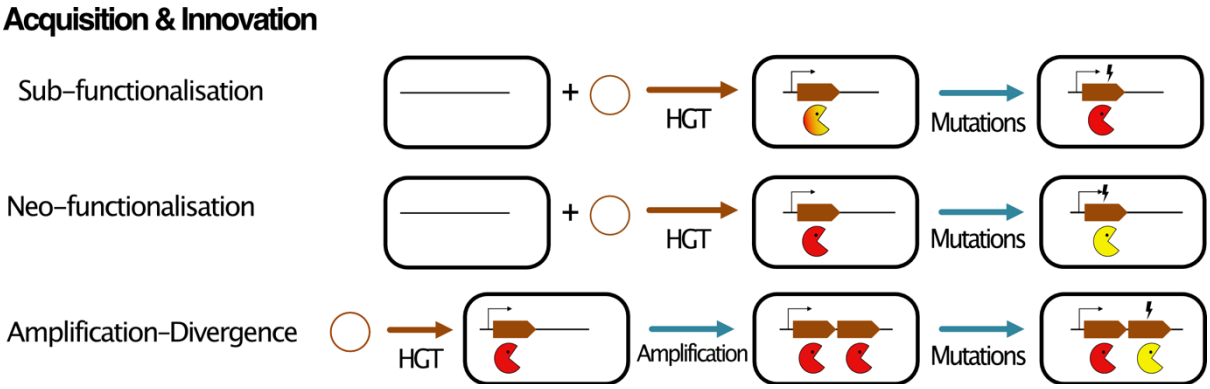

Figure 2. The interplay between HGT and duplication/mutation process facilitates functional innovation. The acquisition of a gene encoding a multi-functional protein when one of the functions is already present in the genome (or vice-versa) potentiates sub-functionalization. Genes arriving in a novel genetic background may acquire novel functions by processes of mutation and recombination (neo-functionalization). Gene amplification of horizontally acquired genes expands mutational targets that may facilitate subsequent selection of novel functions (eventually leading to the loss of genes encoding previous functions if no longer under selection).

Novel functions derived from transferred genes

Past studies on functional innovation by HGT have focused on a narrow set of functions. This is a consequence of the interests of the scientific community, but also of the difficulty of the task of identifying functional changes, especially when most transferred genes have unknown or poorly known functions. It may also be difficult to identify the role of HGT when the novel function is encoded in DNA that bears little resemblance with the original one. It has been shown that point mutations, translocations, and indels can lead to the emergence of novel regulatory regions from existing or random sequences [105,106]. They can also result in small genes with the ability to control microbial functions, *e.g.* resulting in anti-MGE activity [107]. The genomes of Prokaryotes are very dense in coding sequences and even intergenic sequences are often under strong selective constraints. HGT provides a constant inflow of genetic sequences devoid of selective constraints that may favour innovation.

While most past studies have focused on the horizontal transfer of genes, or more complex loci, regulatory regions can be transferred independently of the genes they regulate and thereby rewire genetic networks in the recipient genome [108]. Hence, **regulatory innovation** can arise by HGT. Gene expression data suggests that at least 16%

of the expression divergence between *Escherichia coli* strains can be explained by the horizontal transfer of regulatory regions [108]. Transposable elements acquired by HGT have been involved in cases of regulatory rewiring of chromosomal genes [109]. For example, ISaba is a transposable element with a promoter directed towards the outside of the element, providing *Acinetobacter baumannii* with resistance the antibiotic ceftazidime by overexpressing a neighbouring cephalosporinase [110]. In *Salmonella enterica*, the chromosomal excision of the ROD21 pathogenicity island, acquired by HGT, is associated with the systemic phase of infection, because it affects the expression of key virulence factors in other genomic islands (also acquired by HGT) [111]. The frequency of chromosomal excision of the genomic island varies across organs of the human body, thereby shaping the virulence of the pathogen. Many other gene expression switches associated with the acquisition of MGEs have been described. They most often work by inactivation of a gene or a regulatory sequence upon chromosomal integration of the MGE and its subsequent re-establishment by MGE excision [112–114]. The emergence of novel regulatory functions by activity expressed *in trans* have also been described. For example, a conjugative plasmid of *Rhodococcus equi* encodes regulators that, upon transfer into the cell, reshape the regulatory network of the bacterial host changing the expression of 18% of the chromosomal genes. This has a positive impact on the physiological adaptation of the pathogen to macrophages [115]. In some cases, the MGE was shown to take advantage of its ability to manipulate host gene expression. For example, an integrative conjugative element was shown to change the regulatory network of the *Pseudomonas syringae* chromosome to maximise its growth [116]. In summary, the acquisition of novel genetic information provides ample opportunities to change the shape and function of genetic networks.

Novel **metabolic functions** can arise by transfer, integration and recombination with extant pathways. Non-ribosomal peptides have very diverse roles in bacterial physiology and ecology [117]. HGT followed by recombination in specific domains of the genes encoding their synthesis pathways results in structural changes generating novel non-ribosomal peptides [118]. More broadly, it has been proposed that as evolutionary processes led to the progressive accumulation of novel biochemical activities, extant metabolic pathways were built by patchwork [119], *i.e.* successive rounds of accretion of enzymes, where HGT can play an important role. The evolutionary assembly of the chlorobenzene degradation pathway of *Pseudomonas* sp. strain P51 provides an interesting example since the first enzymes of the pathway are encoded on a transposon located on a plasmid and are homologs of enzymes involved in toluene and benzene degradation [120]. Many examples of metabolic novelty favoured by HGT are currently emerging due to the selective pressures imposed by pesticides and other pollutants in natural environments [121,122]. Carbaryl is an insecticide widely used in agriculture and a *Pseudomonas* strain evolved the ability to metabolize it by combining three sets of enzymes encoded in three mobile genetic elements [123]. Pathways created by HGT are sometimes lacking in efficiency because they suffer from poor substrate specificity [124]. This may stem from the fact that recently co-opted enzymes often retain vestigial activities from their ancestral biochemistry [120]. In the long term, such activities can be lost, especially when the function is already provided by other proteins in the genome or when it is not selected for [125]. Hence, HGT re-assorts enzyme combinations into novel

metabolic pathways which have a collective different function and this evolutionary process may result in different individual chemical activities [126].

Functional change may come with trade-offs. Enzymes degrading antibiotics were recently acquired by many nosocomial pathogens where they sometimes evolve further. For example, the Tet(X) protein provides tetracycline resistance and is frequently transferred within MGEs. It was shown to rapidly evolve in the lab to provide resistance to Tigecycline [127] and in nature to provide broad resistance to all tetracyclines in clinical use [128]. The pathways of evolution of these enzymes reveal that significant changes of function are often only a few mutations away. Yet, pathways of functional innovation can also be narrow. For example, the increase by five orders of magnitude resistance to cefotaxime, a third-generation cephalosporin β-lactam, involves five mutations in a TEM β-lactamase, but the trajectories resulting in viable intermediate proteins are restricted by strong epistatic interactions[129]. Numerous other cases of evolution of antibiotic genes have been found to result in extended spectrum of activity [130,131], but they may involve trade-offs with the original function, which becomes less efficient [88]. Hence, genes acquired by HGT may be close, from the mutational point of view, of acquiring novel functions for antibiotic resistance, but that may incur costs to the original function.

Complex machineries acquired by HGT can be repurposed to perform novel functions. Many cases have been described of co-option of MGE machineries, possibly because they are numerous, complex, and susceptible to domestication when integrated in the genome (Figure 3). For example, let's examine a tailed bacteriophage. It is a complex machinery encoding multiple functional modules that can be co-opted by bacteria. Phages punch holes in membranes using their tails. Accordingly, numerous tail-derived loci have been described in bacteria that are not part of phages anymore and allow bacteria to kill other bacteria [132]. Phages package their DNA in a capsid. This function may be co-opted to spread bacterial DNA to other cells by transduction, as shown for gene transfer agents [133]. Phages inactivate other phages using anti-phage systems, which are useful to protect the cell [29]. Temperate phages can regulate gene expression by integrating and excising chromosomes (see above), and this active lysogeny has been shown to regulate virulence and other processes [134]. Individual phage enzymes may also be co-opted for novel functions. For example, a phage peptidoglycan hydrolase gene was co-opted by *Caulobacterales* for a completely different function [135]. It started as a phage toxin and ended up as a protein shaping cell morphology and development. Hence, a decaying phage can be the origin of multiple sub-machineries that get re-purposed to become relevant to the cell [136]. One of the most impressive machineries exapted from phages is the type 6 secretion system (T6SS), a tool allowing direct secretion from the bacterial cytoplasm to the intracellular space of other bacteria or eukaryotes [137]. Co-option of this system involved the loss of many genes, the acquisition of an ATPase, of a complete membrane complex, and a myriad of protein effectors that are secreted by the system. While phage components are still clearly identifiable in the T6SS, the overall architecture of the machinery and its function are now very different.

Other MGEs also contain complex machineries that can be extensively repurposed. Plasmid replication machineries were co-opted to secondary chromosomes in *Vibrio* spp

[138]. The type 4 secretion system (T4SS) at the core of the mating pair formation system in conjugative elements transfers their DNA to the other cell [8]. This has been co-opted repeatedly for the secretion of protein effectors to eukaryotic and prokaryotic cells [139]. *Helicobacter pylori* is a naturally competent species, like many other bacteria, but uses a unique system to capture DNA. A T4SS, typical of conjugative systems, evolved in the species not to transport DNA to another cell but to import DNA from the environment [140]. In conjugation, the DNA interacts with a relaxase in the cytoplasm of the donor to be exported by the T4SS into the recipient's cell [8]. Here, the DNA is captured in the extracellular space, presumably by a pilus or pseudo-pilus of the T4SS, and brought into the periplasm by the T4SS.

Fascinating examples of functional innovation can be found in **exchanges between the different domains of life**. For example, two genes with the key function of producing acetyl-CoA in methanogenesis from acetate in *Methanosarcina* (Archaea) acquired this function after being transferred from *Clostridia* (Bacteria) where the homologs are involved in the production of acetate in fermentation [141]. The latter pathway is performed by another enzyme in the Archaea. RomA is an effector of *Legionella pneumophila* secreted into human cells, where it localizes to the cell nucleus and produces a novel histone modification. Phylogenetic analyses suggests it was acquired from the protozoan hosts of *Legionella*, and then changed to produce the novel methylation modification allowing the bacteria to control the host gene expression[142]. In the same bacterial species, the LpSpl protein prevents the increase in sphingosine levels in infected host macrophage cells that could induce autophagy. This protein secreted by *L. pneumophila* was acquired from Eukaryotes and while it maintained its original biochemical activity, it is now used by bacteria for a very different function: subvert host cell's responses [143]. HGTs have been more frequently found in the opposite direction, from prokaryotes to eukaryotes[144], where they resulted in such diverse novel functions as protection from Bacteria[145], chlorophyll degradation[146], novel metabolic activities [147], reversion to free-living lifestyle[148], and feeling gravity's pull [149]. It is likely that most transfers between domains of life are unproductive. But when they work, the radically different genetic, physiological and ecological contexts may be particularly prone to favour the emergence of novel functions.

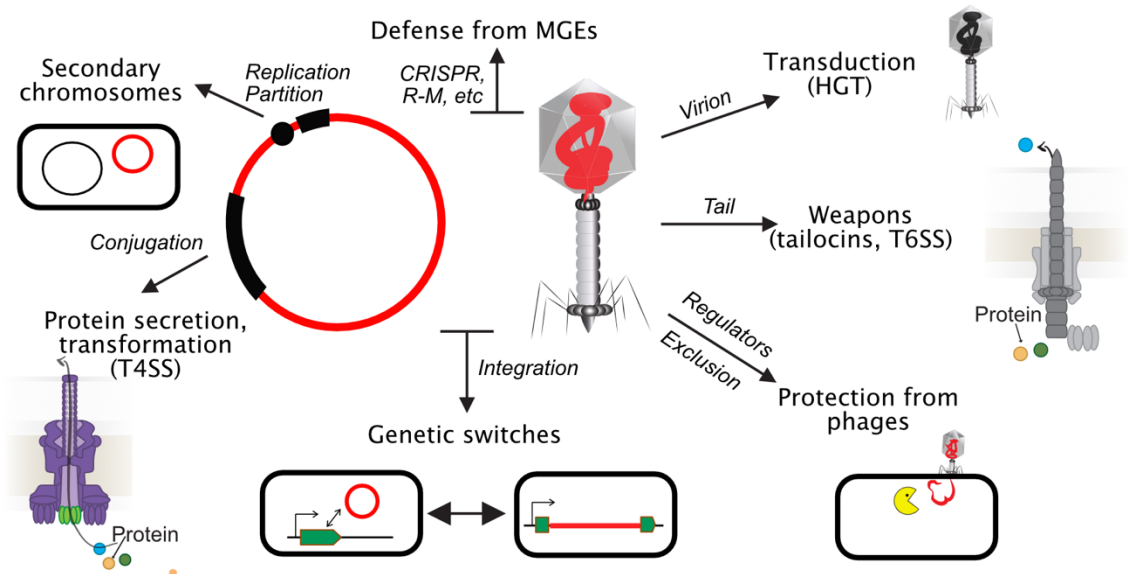

Figure 3. Paths of domestication of functions of MGEs.

The lifecycle of functional innovation by HGT

The interplay between endogenous mechanisms of genetic change and the action of MGEs creates a positive feedback loop that drives functional innovation (Figure 4). MGEs are constantly infecting Prokaryotes and integrating their genomes despite the presence of numerous anti-MGE defence systems. If a MGE carries an adaptive function, its expression produces an immediate phenotypic shift. The initial genetic linkage between this focal function and the MGE can be broken by genetic deletions or by chromosomal translocations. The function encoded in the newly stabilized genetic locus will progressively integrate the cell machinery and its genetic regulatory network. If its adaptive, it may not endure any further significant change; it just adds to the individual's functional toolbox.

The presence of certain selective pressures may result in processes of functional innovation in the acquired genetic information. While each individual process of innovation may be unlikely, genomes are constantly flooded with MGEs, which encode sophisticated biochemical activities that can be tinkered with. Occasionally, this results in functional innovation, which may be facilitated by hyper-mutagenesis or hyper-recombination encoded by the MGEs themselves.

Once a novel function has emerged, it would often be on the individual's best interest to keep it private. In practice, microbial systems appear indifferent to the concept of intellectual property. MGEs, especially plasmids, frequently acquire genetic material from chromosomes by the action of transposable elements. When the locus encoding the novel function is captured by an MGE, the latter is endowed with the capacity of increasing the fitness of recipient cells by infecting them. If the novel function increases the recipient's fitness, this results in more efficient propagation of the MGE, which thus gets a direct benefit from carrying it. The cycle of functional innovation closes by the transfer of the novel function to other genomes.

This schema raises several questions.

Why can't individuals resist transfer of their novel functions to other genomes? Prokaryotes encode a vast arsenal of anti-MGE systems [150]. But MGEs also encode a vast arsenal of anti-anti-MGE systems [151]. This co-evolutionary process has lasted for billions of years and there is probably no magic bullet able to stop all MGEs infecting a cell. It is also possible that, should such a mechanism exist, it would not be selected for. If a genome could become immune to all MGEs, it would also be incapable of acquiring novel functions by HGT, which could be deleterious in the long-term. Accordingly, sexually isolated microorganisms tend to have small genomes and depend on other organisms to grow.

Why aren't innovations homogeneously spread within communities, i.e. why aren't all functions everywhere? While functional adaptation in one genome may often translate in benefits in a novel genetic background [152], many functions are selected only under very specific ecological circumstances. For example, antibiotic resistance was quite rare in human pathogens before the use of antibiotics in the clinic. The advantage of specific cellular functions may vary with genetic backgrounds. Functions often have epistatic interactions with others, e.g. a metabolic pathway is linked to others, a membrane protein may not work in another species with different envelope structure, some metabolites are toxic in specific contexts. This restricts the relevance of novel functions to the set of compatible genomes. Finally, transfer rates vary widely across species because of envelope composition[153], defence systems[154], ecological constraints [155,156], and the mechanisms of mobility of MGEs [23]. This may slow down significantly the spread of novel functions.

Why are such innovations perceived as rare? We don't really know their frequency, since there is no systematic study on the frequency with which horizontally transferred genetic material changes of function. The likelihood of each individual innovation event may be low, because novel functions may require unlikely evolutionary paths[129], and prokaryotes endure deletion biases that diminish the interval of time allowed for non-adaptive DNA to acquire a novel function [48]. While any single functional breakthrough may hinge on a rare sequence of events, the planet-wide census of over 10^{30} prokaryotic cells [157] provides an enormous arena for parallel evolution that can be recombined by the action of HGT, making the emergence of novel functions nearly inevitable.

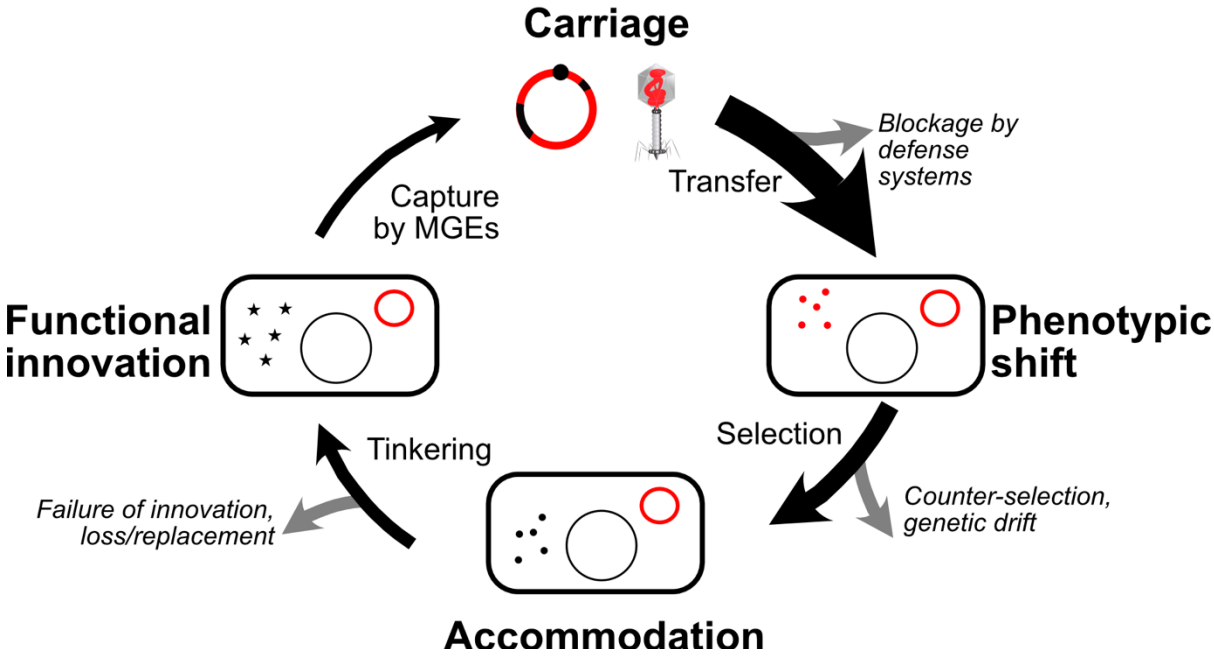

Figure 4. The lifecycle of functional innovation. MGEs carry novel functions to prokaryotic genomes, which may either lose them, fix them, or change them. When these processes result in functional innovation this may impact fitness in a significant way. This recipient has now the property of a novel function. Yet, MGEs will sooner or later capture such genes and spread them across microbial populations because by doing so they increase their own reproduction rate, closing the cycle.

Conclusion

The interplay of HGT and MGEs on functional innovation carries several key implications. Functional evolution can't usually remain private. This means that new functions will almost necessarily spread across the microbial world. In this sense, functional innovation in microbes can be viewed as a massive, parallel-distributed process that constantly generates novel traits to meet challenges and exploit emerging opportunities. The rates of generation and spread of innovations depend on the networks of gene transfer across individual genomes. Microbes engaging in high rates of HGT take risks, because infection by MGEs can be deadly, but they are also more likely to rip rewards by acquiring novel functions faster. It remains unclear which conditions favour individuals that are highly receptive to acquiring new genes and which favour their counterparts. It also remains unclear how much choice microbes really have, considering that MGEs may spread selfishly across populations, despite the best efforts of cellular organisms to control them. Because of HGT, microbes are less dependent on endogenous processes of functional innovation by duplication and change than humans. This may explain why despite high frequency of genetic exchanges their genomes have extremely high coding densities and tightly packed and organised genomes[158]. When odd, cumbersome, multicellular organisms separated their germinal from soma lines they must have paid a cost in terms of limited functional innovation by HGT. At this stage, endogenous processes of functional innovation became the key substance of their evolutionary

genetics. Maybe this is part of the raison-d'être of all the complex, junkyard-like genetic loci that make our own genomes.

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