Hijackers, hitchhikers, or co-drivers?
The mysteries of microbial mobilizable genetic elements

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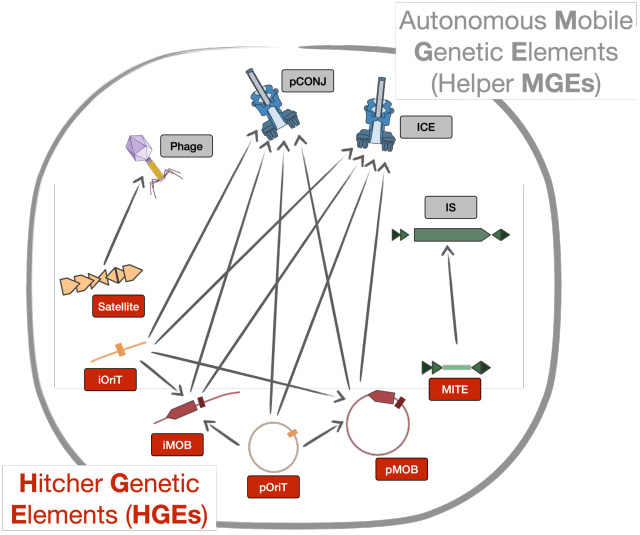
Abstract

Mobile genetic elements shape microbial gene repertoires and population dynamics, but their mechanisms of horizontal transmission are often unknown. Recent results reveal that many, possibly most, bacterial mobile genetic elements require helper elements to transfer between (or within) genomes. We refer to these non-autonomous, albeit mobile, elements as Hitcher Genetic Elements (hitchers or HGEs). They could constitute a large fraction of pathogenicity and resistance genomic islands, whose mechanisms of transfer have remained enigmatic for decades. Together with their helper elements and their bacterial hosts, hitchers are in tripartite networks of interactions that evolve within a parasitism-mutualism continuum, with advantages and costs to each party. The emerging view of microbial genomes as networks of interacting mobile genetic elements brings to the fore many mysteries. Which elements are being moved, by whom, and how? How often are hitchers costly hyper-parasites or instead beneficial mutualists to their helpers and to the bacterial hosts? What is the evolutionary origin of hitchers? Are there key advantages associated with hitchers' lifestyle that justify their unexpected abundance across genomes? Or is their frequency largely the result of selfish spread across communities? Understanding the principles, origin, mechanisms, and impact of Hitcher Genetic Elements will lead to key insights in bacterial ecology and evolution.

Graphical abstract:



Introduction

The past few decades have transformed our understanding of microbial evolution. It is now clear that there are vast intra-species variations in the gene repertoires of microbial populations and a strong suspicion that such differences explain local adaptation. This diversity leads to large species pangenomes, often much larger than the average individual genome, and to closely related strains differing markedly in gene number and type (1,2). These variations are caused by processes of gene gain *via* horizontal gene transfer driven by **mobile genetic elements** (**MGEs**, see Glossary) (3). The latter encode core functions required for their horizontal and/or vertical mobility, as well as accessory functions that favor the MGE by increasing its host's growth or survival. The transfer and stabilization of MGEs often incurs in a fitness cost to the bacterium: **bacteriophages (phages)** kill the host, **transposable elements** may disrupt host genes, and **conjugation** impacts the growth dynamics of bacteria (4–6). These deleterious effects to the host may or may not be compensated by MGE-encoded accessory traits such as antibiotic resistance, immune defense, or virulence. Hence, the interests of MGEs and their hosts are sometimes aligned and other times misaligned, resulting in a shifting balance in the parasitism-mutualism continuum (7,8). There is a rich literature on these wavering interactions between single MGEs and their hosts. Yet, recent results strongly suggest that such interactions can only be fully understood on a broader context because MGEs are rarely alone within cells.

Some MGEs – bacteriophages, **conjugative elements** – transfer autonomously between cells. Other MGEs, that we will refer to as Hitcher Genetic Elements (**hitchers** or **HGEs**), cannot transfer autonomously and must use functions of autonomous **helper** elements to transfer. Importantly, the term "helper" does not necessarily imply altruism. It merely describes an MGE that is involved in the mobility of a hitcher. As we will describe below, some hitchers require other hitchers for their mobilization (beyond also strictly requiring a helper). For simplicity, we will name both types of hitchers in the same way, since both types of elements depend on a helper, which we define as an element that can transfer autonomously. Recent works have shown that hitchers are very abundant and may be key to understand the mobility of many bacterial genes (9,10). The view of the interactions between bacterial genomes and MGEs is thus evolving. If traditionally one would think of pairwise interactions between a bacterium and its MGE, the focus is now shifting towards MGEs forming, together with their hosts, a network of complex functional interactions, ranging from antagonism to mutualism. This brings to the fore major unsolved mysteries. First, it highlights the need to unravel the mechanisms of interaction shaping the mobilization of MGEs and, ultimately, understand who moves whom and by which mechanisms. Second, while it is common to regard HGEs as hyper-parasites, it is unclear what are their actual costs, and whether these could be offset by the advantages they provide to both helpers and the bacterial host. Third, there is little data on the evolution of the mechanisms of co-mobilization leading to the emergence of HGEs. Finally, a better understanding of their emergence, interactions, costs, and benefits could explain why they evolved to be mobilizable and not autonomously mobile. Here, we put forward these mysteries and contextualize them with the goal of sketching the way ahead.

Who are the known hitchers, and who's helping them move?

Phages are viruses that infect bacteria and can drive DNA exchanges between cells (11). Phages encode genes to replicate their DNA, to produce, assemble and package the viral particle containing the DNA, and to lyse the bacterial cell to release the newly formed viral particles in the environment, from where they can infect new bacterial hosts. Virulent phages nearly immediately replicate in the host cell (lytic cycle) resulting in cell death, whilst temperate phages can either follow a lytic cycle or a lysogenic cycle. In **lysogeny**, the phage DNA either integrates into the bacterial chromosome (as a **prophage**) or remains episomal (as a **phage-plasmid**) being vertically transmitted until it re-enters the lytic cycle and

eventually kills the host cell. Sometimes bacterial DNA is mistaken as phage DNA and is packaged in the viral particles, being then possibly transferred to other bacterial cells (**transduction**). **Phage satellites** are a diverse group of HGEs that package their genome, instead of the one of the helper phage, in the viral particles encoded by the latter (**Figure 1B**). The first described phage satellite, P4, was the only one known for decades, and is now the reference of a characteristic family of satellites known as P4-like (12). In the last few years, other families of phage satellites were uncovered, such as the phage inducible chromosomal islands (PICI) (13,14), capsid-forming PICI (cfPICI) (15), PICI-like elements (PLE) (16) and phage inducible chromosomal minimalist islands (PICMI) (17). Additional families of phage satellites in marine (VEIMEs (18) and Tycheposons (19)) or soil bacteria (20) have been recently proposed. Some elements are satellites of ssDNA phages, e.g. the plasmid pDolos (21). Phage satellites depend on helper phages to produce all or parts of the viral particle or even to replicate the satellites' DNA. They can hijack viral particles by manipulating the capsid size (e.g., (22)) or by redirecting packaging towards their own DNA (e.g., (23)). Some satellites, like the *Staphylococcus aureus* satellite SaPI3, are not induced by the helper and requires another satellite for that (24). This suggests that satellites are involved in a complex hierarchy of functional dependencies within the bacterial cell (**Figure 1B**).

Conjugative elements transfer copies of their genomes into neighboring recipient cells using a conjugation system that is costly but does not usually entail donor cell death (25,26). **Conjugative plasmids (pCONJ)** are extrachromosomal fragments of DNA that replicate independently of the bacterial chromosome. They encode a relaxase that initiates the transfer of plasmid DNA at their origin of transfer (*oriT*), and a mating pair formation system that connects the donor and recipient cells and serves as channel for the plasmid transfer. **Integrative Conjugative Elements (ICEs)** are also transferred by conjugation, but they encode an integration and excision module to integrate into the bacterial chromosome, thereby replicating with it. Some MGEs do not encode a functional conjugative apparatus and thus require those encoded by autonomous conjugative elements to transfer horizontally. These conjugative HGEs may be plasmids that encode a relaxase and an *oriT* (e.g. **pMOB**), plasmids that carry only the *oriT* (e.g. **pOriT**), or **Integrative Mobilizable Elements (IMEs)** carrying an *oriT* with a relaxase (iMOB) or without it (iOriT). Since the conjugation machinery of ICEs and plasmids is homologous, they can potentially use each other's relaxases or assembled pilus (27). Therefore, pMOBs, pOriTs and IMEs can be potentially mobilized by both types of conjugative elements (28–30) (**Figure 1A and C**). Conjugative HGEs may use proteins from multiple elements, some of which may also be hitchers themselves, thereby establishing an hierarchy of interactions within the cell that is necessary for their transfer (31). Such plasmids are common: a third of those having only an *oriT* transfer between cells using a relaxase from one plasmid and a conjugative system from another (9).

The focus of this text is on co-mobilization in horizontal gene transfer. Yet, while most known mobilizable elements are horizontally transferred between cells, relations of functional dependency are also observed for MGEs involved in intra-genomic mobility. The best described case concerns the association between transposable elements and miniature inverted repeat transposable elements (**MITES**), which are encoded by more than 50% of bacteria (32). These short elements (ca. 300nt) lack protein coding genes and are transposed by transposases encoded in **Insertion Sequences** or other transposable elements (33). They can thus be considered HGEs of transposable elements (**Figure 1E**). **Integrons** are elements encoding a specific integrase that mediates recombination between *attC* sites flanking gene cassettes (34). This mechanism results in the integration of novel cassettes and shuffling of old ones. While not strictly speaking an MGE, integrons can exchange cassettes with other integrons and thus participate in intra-genomic genetic mobility. Cassette arrays lacking an integrase are called **CALIN** (35), can be mobilized in trans by complete integrons, and may thus also be thought of as HGEs of integrons (**Figure 1D**). Of note, the interactions between agents of intra-genomic mobility are important for the MGEs involved directly on inter-genomic mobility (horizontal transfer). This is especially true for conjugative HGEs and their helpers, which contain numerous transposable

elements, MITEs, integrons and CALINs (36,37). These elements are key for the transfer of other genes between plasmids and/or integrative MGEs, e.g. for exchanges of antibiotic resistance genes between conjugative and mobilizable plasmids (38). While they do not allow horizontal transfer, they lead to novel assemblies of potentially adaptative functions in the helpers and hitchers transferring between bacteria.

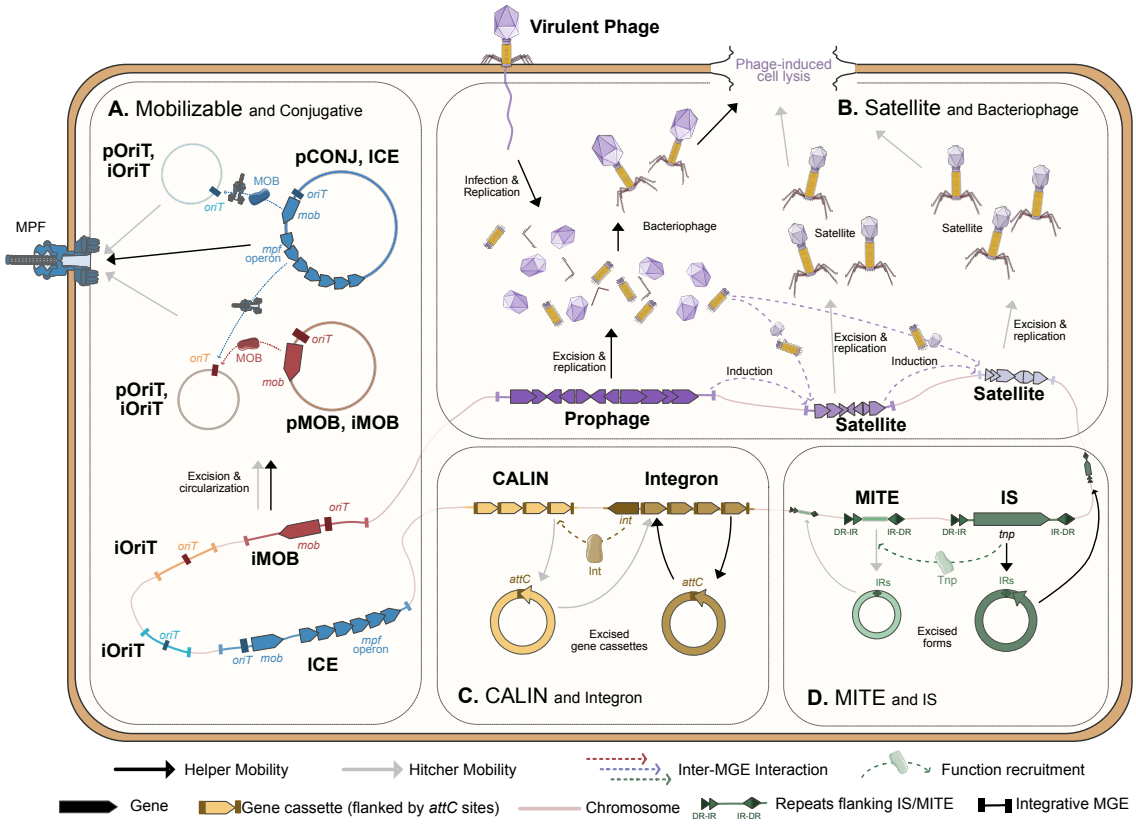


Figure 1. Diversity of hitchers and helper elements, and their interactions within the cell. The black continuous arrows indicate the mobility of the helper MGE, whereas the grey continuous arrows represent the hitcher mobility. Colored dashed arrows indicate interactions between different types of MGE. **A.** The helper conjugative plasmid (pCONJ) assembles the mating pair formation (MPF) system for its own mobility. pMOB plasmids encode for their own relaxase (MOB) and *oriT*, sufficient to be mobilized by the MPF encoded by a pCONJ. pOriTs carry their own *oriT* but require a MOB encoded by a pCONJ (up) or a pMOB (bottom). **B.** Bacteriophages produce all the elements required for the assembly of viral capsids, the genome packaging, and the cell lysis. Phage induction may induce satellites, which use resources provided by the phage (e.g. orange satellite). Likewise, the induction of some satellites may trigger the induction of additional satellites and their mobility (e.g. purple satellite). Some satellites shrink the capsids of phages (PLE, P4), whereas others produce their own smaller capsids (cfPICI), which results in phage particles carrying only the satellite DNA in small capsids. **C.** ICEs assemble the MPF system for their own transfer. Some IMEs, iMOB, encode their own relaxase and *oriT* and can therefore use the ICE's MPF. iOriTs only carry their own *oriT*, and need the MOB encoded by an ICE (bottom) or by an iMOB (up), to be mobilized by the MPF. Before conjugation, ICEs and IMEs need to be excised and circularized. To avoid confusion, this step has been removed from the figure. **D.** Integrons use their own integrase (Int) to shuffle their gene cassettes array. CALINs are cassette arrays that lack an integrase and depend on an integron's integrase to be mobilized. **E.** Insertion Sequences (IS) encode a transposase (Tnp) which mediates their intragenomic mobility. Here, it is exemplified only one of many different mechanisms of transposition. MITEs are hitchers that utilize the transposase of their helpers to be mobilized within the genome. DR: Direct Repat. IR: Inverted Repeat.

The ways HGEs attain helper-mediated genetic mobility differ widely. Some hitchers encode sophisticated mechanisms to actively subvert their helpers (viral particles or conjugative apparatus).

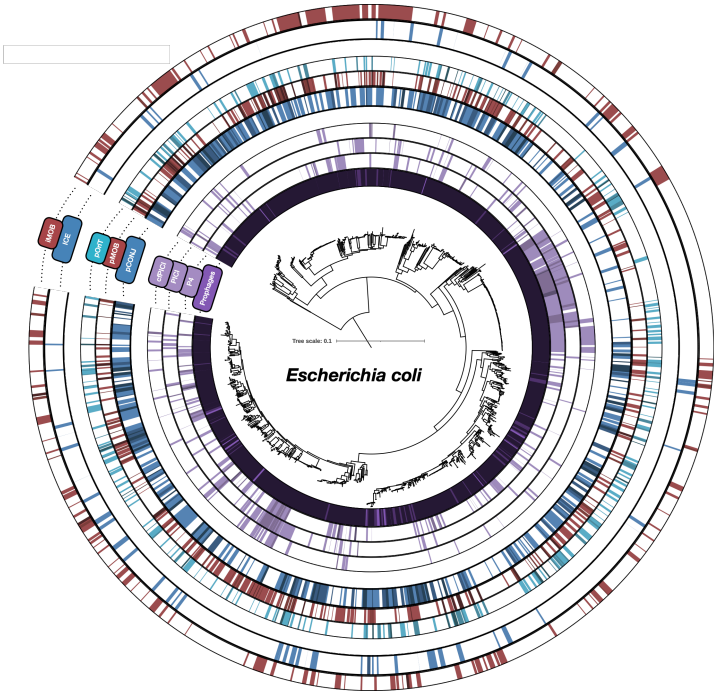
This was more frequently identified in phage satellites, many of which encode genes dedicated to physically redirect the packaging of viral particles towards the satellite genome (23), to reshape or exclude the viral capsid to fit the satellite DNA (22), and/or exclude the packaging of helper phage genomes in viral particles (39). HGEs mobilized by conjugation often encode their own relaxases and coupling proteins to facilitate the interaction with the type IV secretion system of the conjugative element (40,41), but without excluding its previous or subsequent use by the helper. An example of a more complex mechanism of subversion involving conjugation is given by IME SGI1 which encodes a gene (*traG*$_S$) that reshapes the pilus of its helper conjugative plasmid to enhance its transfer at the expense of the latter (42). These are sophisticated molecular mechanisms of subversion that require proteins encoded by the hitcher. Other hitchers tend to lack such genes and may be more passive, in that they depend only on the presence of DNA motifs in their genome that are recognized by the helpers' transfer machinery. For instance, the mobilization of PICMI satellites seem to depend only on the presence of a packaging DNA motif to be packaged in viral particles (17), and many plasmids only encode an origin of transfer to be mobilized by conjugative plasmids (9). This is also the case of MITES, which lack protein coding genes and whose mobilization relies solely in the opportunistic use of transposases from helper transposable elements. As we will discuss later, these distinctions might reflect the evolutionary paths that led to each hitcher and may contribute to their impact in the fitness of their helpers.

Who else might be a hitcher?

Prokaryotic genomes have plenty of MGEs. More than half encode prophages, transposable elements, MITES, and nearly as many have plasmids. Phages and conjugative elements have been widely studied, but might be a relatively small fraction of the MGEs present in genomes. The search for genes coding the key mechanisms of plasmid mobility revealed that only 25% are autonomously conjugative (43,44) and 7% are phages (45). Hence, more than two thirds of the plasmids are either mobilizable or non-transmissible. Recent studies in the model species *E. coli* and *S. aureus* strongly suggest that most of these plasmids are HGEs mobilized by autonomous conjugative elements (9,46), and many could be passively mobilized by phage transduction (47), especially those at high copy number (48). The analysis of MGEs integrating bacterial genomes is more difficult because delimiting poorly known ones is challenging. Yet, bacterial chromosomes have a small number of regions ("hotspots") where most genetic exchanges with other bacteria take place. One of the reasons of existence of these hotspots is that they are flanked by sequences targeted by the site-specific recombinases of MGEs (49). Hence, hotspots are expected to have many MGEs, and some are indeed well-known to be targeted by phages or ICE (50). Intriguingly, many of these hotspots have one or several MGEs broadly called **Genomic Islands (GIs)** lacking identifiable phage or conjugative elements (49). These MGEs have been identified for decades and intrigued researchers because they lacked recognizable mechanisms of transfer.

Many of these GIs are now being revealed as phage satellites or IMEs. Until recently only a handful of satellites were known. In just one year, two new families were discovered and characterized in detail (15,17), three additional novel types of satellites were uncovered in Cyanobacteria and Actinobacteria (18–20), a phage-plasmid-satellite was described (21) and several thousands of novel satellites of these families were identified in genomes (10). Likewise, novel IMEs are regularly uncovered across various Phyla, even if they have diverse names such as Genomic Islands (e.g. SGI1 mobilized by IncA/C plasmids) (51), transposons (e.g. Tn*4451* mobilized by the plasmid RP4) (52), or Nonreplicating Bacteroides Units (e.g. NBU1 mobilized by the plasmid R751 or the ICE Tcr-ERL) (53). Indeed, despite largely unknown, ICEs are more numerous than conjugative plasmids among sequenced genomes (54), so one can expect that a lot of IME diversity remains to be explored. These hitchers may account for a large fraction of the genomic islands whose mechanisms of mobility have remained unknown for decades (55–57).

Knowledge about HGEs remains concentrated in a limited number of elements from a few bacterial species, notably in *E. coli*. Most genomes of this species harbor at least one prophage (and up to 16) and 43% have at least one satellite. Around 52% of the genomes carry at least one fully autonomous conjugative element (and up to 5) and 58% carry at least one conjugative hitcher (**Figure 2**). Hitchers can be numerous in individual bacteria, with some genomes carrying up to 6 phage satellites and others carrying 10 elements mobilizable by conjugation. Other genomes have multiple mobilizable elements *of each type*, e.g., a single genome (*E. coli* O157:H7 strain USDA5905) has 3 phage satellites, 5 mobilizable plasmids and 1 iMOB. Despite the abundance of hitchers in certain bacteria, the frequency of HGEs may vary widely between closely related genomes. This might result from the frequent gain and loss of these elements, resulting in a scattered distribution across the phylogenetic tree of the species.



	Total (all genomes)	Average (per genome)	Maximum (per genome)	% encoding genomes
Prophages	9512	6	16	99.9%
P4	663	0.42	3	30.9%
PICI	408	0.26	6	21.7%
cfPICI	177	0.11	2	10.6%
pCONJ	1268	0.8	5	49.8%
pMOB	853	0.53	6	34.7%
pOriT	565	0.36	6	25.7%
ICE	118	0.07	2	7.4%
iMOB	398	0.25	2	23.8%

Figure 2: Distribution of MGEs (helpers and hitchers) in *E. coli* genomes. Phylogenetic tree built from the core genes of 1,585 *E. coli* complete genomes retrieved from RefSeq (May 2021) is annotated for the presence of prophages (detected with VirSorter2) (58), phage satellites P4, PICI and cfPICI (detected with SatelliteFinder) (10), conjugative plasmids, ICEs and conjugative-based HGEs (identified with CONJScan v.2) (59). In each circle, darker shades correspond to higher numbers of the respective elements in the corresponding genomes.

If *E. coli* has so many HGEs, and some may even remain to be identified, it is likely that other species contain at least as many. But how can we find them? Novel elements resembling known HGEs can often be identified by sequence similarity, as above. For instance, PICIs were detected in 35% of Staphylococcus genomes (10), and 34% of *S. aureus* genomes carry known pOriTs (9). The true difficulty lies in identifying elements that differ from the known HGEs. One approach is to study the mechanisms of mobilization of helpers and find other MGEs by homology. The presence of some genes or DNA motifs might be sufficient to identify potential mechanisms of mobilization, e.g. the presence of genes encoding relaxases and *oriTs* suggest that the hitcher is mobilized by conjugation and the presence of genes encoding terminases or *cos*/*pac* sites suggest the element is mobilized by phages. Yet, some conjugative elements and phages are poorly known, some HGEs lack protein coding genes homologous to the helper, and DNA motifs such as *oriTs* or *cos/pac* are often unknown or hard to identify (60). It is also likely that the mobilization of completely novel HGEs differs from the known mechanisms. In such cases, the identification of novel hitchers will require the integration of experimental and computational approaches to analyze mobilized DNA in bulk, e.g., by identifying satellites packaged in viral particles through sequencing of purified phage lysates (18).

Who moves whom?

Now that thousands of HGEs are being unraveled, one must identify their helpers to infer the networks of interactions between mobile genetic elements within cells. The co-occurrence between helpers and HGE could, in principle, provide clues to their transfer compatibility, i.e. in this context, meaning the ability of a given helper to mobilize a given hitcher. Yet, as shown above (**Figure 2**), genomes may have multiple hitchers and helpers, and there is no guarantee that they match together. For instance, phages HK106 and HK446 were found to mobilize the satellite cfPICI EcClEDL933, but neither is native to the *E. coli* strain where the satellite was discovered. Strikingly, this strain encodes 17 prophages, none of which mobilized the satellite at detectable frequencies (15). Inversely, some genomes have hitchers without having any possible helper. In the *E. coli* analysis described above, 24% of the genomes have hitchers for conjugative elements but not the latter. Hence, co-occurrence has not yet been shown to allow systematically matching hitchers with helpers. In some cases, the elements are expected to co-occur rarely if ever. This is the case of hitchers whose helpers are virulent phages, which will not be stable in a cell. It is also the case of hitchers that block their helpers since in this case only one of the elements will transfer.

Another approach to find pairs of compatible helpers and hitchers is to search for homologous DNA motifs that are present in the genomes of both elements, such as *oriTs* for conjugation or *cos/pac* sites for packaging in viral particles. In these cases, the similarity of DNA motifs between hitchers and helpers is essential for the former to be mobilized by the latter. Hence, pairs of elements with identical sequences should make a pair of compatible hitchers/helpers. Unfortunately, these DNA sequences are often small, degenerate, and most are yet unknown.

Finally, co-integration of helpers and hitchers suggest the two elements are compatible. Some conjugative hitchers can transfer between cells by conduction, i.e., by co-integrating the genome of the helper in the donor cell and excising once they are in the recipient cell. In this case, the hitcher does not need to encode any function related to conjugation, not even an origin of transfer, since it is now part of the conjugative helper and transfers with it, in a way resembling ISs that translocate into a conjugative plasmid. Examples of this mechanism include the co-integration of pSC101 and the conjugative plasmid R1-19, or of pML21 and R64-11 (61). Of note, in these cases conduction is an active mechanism, i.e. the hitcher encodes genes that seem to have evolved to integrate and then excise the other element. In many cases, it may be difficult to distinguish this mechanism from casual co-integration between MGEs, e.g. driven by transposable elements. In theory, phage satellites could also integrate conjugative elements and transfer by conduction, but satellites known so far rarely integrate conjugative plasmids (10). Conduction is expected to be rare or inexistent between phages and satellites, because their co-integration creates a larger genome that can only be packaged in the original phage particle if the satellite is very small. A different process is sometimes observed in phages and satellites (and even in conjugative elements): some HGEs integrate their helpers after transfer. For example, some satellites were observed to integrate within prophages (62), and some IMEs within ICEs (30). These processes are different from conduction because the co-integrate is split before transfer and the two elements are transferred independently. Nevertheless, they can also provide information on the compatibility of helpers and hitchers, since available data suggests that co-integration allows the HGE to take control of the transmission of their helper (30). Hence, co-integration of hitcher and helper provides strong evidence that the two are a compatible pair, i.e. the former moves the latter.

What are the host-ranges and helper-ranges of hitchers?

There are two types of host ranges for MGEs: the set of bacterial hosts they can infect and the set of bacterial hosts from where they can further transfer. The first is often broader than the latter because MGEs may be able to transfer their DNA to a host, but the DNA cannot further transfer from the novel host. For example, some conjugative elements can transfer into eukaryotic cells, i.e. they can produce a MPF in a bacterial cell allowing their transfer into an eukaryotic cell. But once in the latter, their MPF cannot be formed because the cell structure is too different, resulting in an element that cannot further transfer (63). Both ranges are important for the horizontal transfer of genetic information. The range of bacteria that can be infected by helper MGEs is extremely diverse because of the variations in the molecular mechanisms of transfer, integration, and replication of MGEs and because of the differences in bacterial physiology and immune defenses. Phages interact specifically with cell receptors and tend to have relatively narrow host ranges, often limited to a clade within a species or genus (64). In contrast, the molecular interactions of conjugative elements with the recipient cell tend to be either less specific or more tolerant to the absence of specific receptors (65), and this results in broader host ranges (64). What about HGEs? The study of these elements requires a further extension of the traditional concepts of host range because the hitcher depends on the bacterial host and on the helper. A simple prediction is that these additional constraints would further narrow the host range of HGEs. The available data suggests a more complex and intriguing picture.

One might think that if a hitcher is mobilized by a single helper, then they should have the same bacterial host range. This seems to be the case of the satellite PLE that is known to exploit only the virulent phage ICP1 (16) whose only known host is *Vibrio cholerae* (66). As a result, this hitcher is only found within the triplet PLE-ICP1-*Vibrio cholerae*. Yet, hitchers and helpers may have different host ranges if their ability to function in the new host differs (e.g., if only one has a functioning replication initiator) or if the novel host defense systems target one element and not the other (e.g. small MGEs escape restriction more easily (67)). Furthermore, some hitchers can be mobilized by a wider diversity of helpers. For example, P4 can be mobilized by at least five different P2-like phages (68). The range of helpers used by elements mobilizable by conjugation can be even broader. Relaxases of mobilizable elements (e.g. MOB_{Q1} or MOB_{P5}) interact with multiple diverse conjugative systems (69). Moreover, some mobilizable plasmids carry multiple *oriTs* (e.g. pEC156) (70), or encode coupling proteins (e.g. CloDF13) (40) that expand the range of helpers that can mobilize them. The ability of a HGE to be mobilized by different helpers may dramatically increase their bacterial host range both in terms of infection (as they can hijack helpers that infect different hosts) and their ability to transfer from the novel recipient cell (since the likelihood of coinciding there with a helper for subsequent transfer is higher). Indeed, many of the plasmids with the broadest host range are mobilizable, like RSF1010/R1162 (71), pLS1 (72) or pBI143 (73). For instance, RSF1010 can be mobilized by the plasmid R388 in *Escherichia* (69), by pAtC58 in *Agrobacterium* (74), and by RP4 in the very distantly related *Mycobacterium* and *Streptomyces* (75). Likewise, nearly identical P4-like satellites can be found in *E. coli* and *Klebsiella* spp. (12), corresponding to phylogenetic distances rarely crossed by phages. In theory, although this remains to be demonstrated, hitchers using different populations of helpers in distinct clades may have a key role in transferring genes across distant bacteria which their helpers, individually, cannot reach. Hence, HGEs can access broad host ranges if they have a large panel of helpers.

Importantly, the helper range of some HGEs might be dynamic, since only a few mutations are required, either in the hitcher or the helper, to create a functional association. For instance, in *S. aureus*, a single amino-acid substitution in the relaxase of an IME allows it to recognize divergent *oriTs* (76). Likewise, a common point mutation in the relaxase accessory protein SmpO allows the helper conjugative plasmid pWBG749 to mobilize a broader range of mobilizable plasmids (76). Hence, not only some HGEs can be mobilized by a large range of helpers, but some might be just a few point mutations away from accessing a different group of helpers, and thus potentially a new set of bacterial hosts.

The costs and benefits of hitchers: Hijackers, hitchhikers or co-drivers?

It is usually assumed that hitcher mobilization decreases the fitness of their helpers, as the hitchers compete for a common pool of resources and hijack components of the latter (e.g., conjugative pili or viral particles). This is indeed the case for some HGEs. The pMOB CloDF13 reduces the rate of transfer of its helper by competing for the conjugation machinery (77), the IME SG1 diminishes the conjugation of its helper plasmid pVCR94 (42), and PLEs abolish the helper phage reproduction (16). Yet, other HGEs have little or no effect on the transfer of the helper. For instance, the satellites cfPICI EcClEDL933 (15) and $PICMI_{115}$ (17) had no significant effect on the production of the viral particles carrying the DNA of their helpers. Similarly, integrative elements mobilized by ICEs related to ICE*St3* (78), as well as plasmids mobilized by the conjugative plasmid pWBG749 (79), have a negligible impact in the efficiency of transfer of their helpers. Nevertheless, the cost of hitcher mobilization to its helpers probably depends on the hitcher and on the helpers. This was observed, for instance, with some PICI where the cost of the satellite, in terms of the reduction of the number of phage containing particles, differs between helpers (39). While these results were obtained in laboratory conditions that may not represent accurately the costs of hitchers on their helpers in natural conditions, other observations further suggest that hitchers are often not costly to helpers. For instance, although some conjugative plasmids encode CRISPR-Cas systems to target other plasmids, the targets are typically other conjugative plasmids and not their hitchers, suggesting the latter are not the major targets of competition within the cell for the helpers.

If hitchers have little or no impact on the helper's mobility, are there hitchers that *increase* the mobilization of helpers? CTX is a filamentous phage encoding the toxin CTX that makes *V. cholerae* a deadly pathogen. RS1 is its satellite and counteracts the phage repressor promoting the expression of itself and of its helper phage, resulting in increased expression of the cholera toxin genes and increased virulence of *V. cholerae* (80). This could be a case of cooperation, where a satellite increases its helper transferability to improve the chances of both co-transferring into novel recipient bacteria. Indeed, the regulatory networks responsible for repressing and inducing MGEs are sometimes shared by hitchers and helpers, even those with different evolutionary histories (e.g. the SgaD/C and AcaC/D protein-homologs in SGI1 IME and pVCR94X pCONJ respectively (81), or the protein E in P4 satellites and P2 phages (82)). But the actual benefits for the helper of increased helper mobility promoted by their hitchers remains to be shown, since the effects of an untimely mobilization might be counterproductive for the helpers. Cases where parasites increase the growth of hosts while decreasing their fitness have been described in other contexts, e.g., some virulent phages of Cyanobacteria encode photosynthesis genes that increase their hosts' growth rate during a lytic infection but still kill their hosts at the end (83). Nevertheless, if there are co-induction mechanisms between helpers and hitchers and the two elements respond to distinct environmental queues for induction, this might increase each other's transferability, and hence survivability. A key question for future research is thus if genetic interactions affecting the induction of the helper are beneficial to the latter.

In the tripartite relation between hitchers, helpers and the host, the costs of mobility for the latter can be high. Regardless of their interactions with HGEs, the horizontal transfer of helpers is almost always costly to the donor bacterium: novel phage particles usually require cell lysis for dispersion and conjugation affects growth rates in both donor and recipient bacteria (6,84). But **does HGEs mobilization carry additional costs for the bacterial host?** Interestingly, it often might not. Satellites themselves have no mechanisms to lyse the cell. They require phages to transfer, and the mobilization of the latter kills the cell whether satellites are present or not. Although this is costly for the cell, the presence of the satellite might even impart a benefit for the bacterial population, since if the satellite

diminishes the helper reproduction it may benefit the survival of related bacteria. Of course, mechanisms such as the one described above where satellites increase the induction of the helper may effectively lead to an increased cost to the bacterial host for encoding hitchers. Conjugative HGEs may also incur in little or no additional cost to the bacterial host because the conjugative machinery is expressed and assembled by the helper whether the hitcher is present or absent. Even if DNA transfer requires energy (85), the small size of mobilizable elements (see below) allows this cost to be kept low. Hence, given that hitchers only transfer when the helpers are present and induce transfer, their costs of transfer might often be negligible to the host already carrying a helper MGE.

Could hitchers be hyper-mutualists?

Parasitism is defined as an antagonistic symbiotic relationship in which one partner is harmed, while the other benefits (7). In contrast, mutualism is a symbiotic relationship in which both partners benefit, or are perceived to benefit (7). These definitions can be applied to interactions between MGEs and the host. Because transfer and carriage of MGEs have been usually assumed to be costly, they are often considered *genetic parasites* of bacteria (86). Likewise, since the mobilization of HGEs may be costly to other MGEs (helpers) which may be costly to the bacterial host, they could be considered as *genetic hyper-parasites* (i.e., parasites of the parasites) (87,88). But is this really the case? Can hitchers be neutral, or even beneficial to their helpers and their hosts? To understand this, we must look at the costs and benefits of HGEs in two ways: in terms of their mobilization (discussed above), and of their stability.

The fate of autonomous MGEs hinges on a trade-off between their rate of horizontal transmission (usually costly to the host) and the frequency at which they are vertically inherited. The latter may be increased if the MGE carries adaptive traits (89,90). If HGEs have a reduced horizontal transmission, which remains to be shown, then they might require improved chances of vertical transmission to persist in microbial populations. Hence, hitcher-associated fitness costs or gains, for both the bacterial host and the helper, can be key for hitchers' success. The maintenance of hitchers can be costly to the bacterial host even in the absence of transfer, especially in the case of multicopy plasmids (91,92) or when they encode highly expressed proteins that are not adaptive to the host or helper. Interestingly, the co-occurrence in the cell of both hitchers and helpers may lower the cost of the former: co-residence of small plasmids with large plasmids can in some cases cost the host less than the sum of their individual costs (94), favoring their co-existence within the cells (9,94). The mechanisms involved in these epistatic interactions remain poorly understood, but these observations further suggest that hitchers may have little impact on hosts when the helpers are also present.

The presumably low cost of many HGEs, because they tend to be small (see below) and induce low transfer costs, opens the opportunity of establishing mutualistic interactions with the host. This may explain why some hitchers carry functional genes that are not directly implicated in the core functions of the MGE (mobility, replication and/or integration) and may improve the host fitness (95). For instance, mobilizable plasmids are usual carriers of bacteriocins (96,97) and have the highest densities of antimicrobial resistance genes in *E. coli* and *S. aureus* (9). Under bacteriocin or antibiotic pressure, cells carrying these HGEs will be strongly selected, and so will the bacterial host. Phage satellites rarely carry antibiotic resistance genes, but some *S. aureus* PICIs encode virulence factors (98) and many satellites encode anti-phage defense systems (17,99,100), allowing the bacterial population to better withstand phage predation.

Co-residence of hitchers and helpers in a host provides opportunities for tripartite mutualisms on the basis that the growth and survival of the host benefits all of them. For example, many of the defense systems encoded by phage satellites target multiple phages but not their helper (99), thus increasing

the survival of both the host and the helper. Such cooperative strategies are consistent with longstanding co-evolution between hitchers, their helpers and their common host, because they all share, to a certain extent, interest in the survival of the latter. The above-mentioned example of mobilizable plasmids providing antibiotic resistance to the bacterial host may also favor helpers indirectly when favoring their common host. Hence, there is extensive potential for shifts towards mutualism in hitcher-helper-host interactions, especially when helpers are not very virulent to the host and hitchers are not very costly to the helpers.

Yet, these mutualistic interactions may be ephemeral. The contribution to host fitness of HGE-encoded accessory traits (e.g. antimicrobial resistance or anti-phage defense), may be positive under certain circumstances (e.g. presence of antimicrobials or phages), and negative under different conditions (101,102). The mutualistic interactions between hitchers, helpers and the host thus depend on specific physiological and environmental conditions that may change rapidly. When alliances break the consequences can be brutal, e.g., phages and their satellites will be transferred between cells at the cost of killing the host. We favor the view that such tripartite interactions evolve in a shifting equilibrium between mutualism and antagonism. Understanding the relative frequencies of each type of interaction and the determinants of the shifts will be instrumental to understand the dynamics of these intracellular ecological interactions.

When and how did hitchers emerge?

Dating the origin of HGEs is difficult because it is a process that accumulates the problems of dating the bacterial hosts (deep phylogenies, lack of fossil record) with the specific properties of the evolution of MGEs: rampant recombination, rapid turnover of gene repertoires, and pervasive horizontal gene transfer between bacterial cells. Available data suggests that some HGEs are very ancient. For example, P4-like satellites are distributed across the order Enterobacterales (12) with some evidence of isolation between the most distant bacterial clades, which might set their origin to hundreds of millions of years ago. Likewise, some relaxases specific of mobilizable plasmids ($MOB_{P5/HEN}$) show distinctive conserved motifs compared to the relaxases of conjugative elements, suggesting they emerged a long time ago (103).

Despite the difficulties in dating their origins, it is clear that hitchers arose many times independently in natural history. This is obviously the case for elements with different, unrelated types of helpers (phages vs conjugative). It is also the case within each type of HGE and even within families. Conjugative hitchers have paraphyletic or even non-homologous relaxases, the key proteins involved in mobilization by conjugation. Among the nine relaxase families, five harbor a canonical HxH motif (MOB_F, MOB_Q, MOB_P, MOB_V, MOB_B) (103,104). The others are unrelated and likely arose from different enzyme classes: the MOB_H family is related to HD-hydrolases (105), the MOB_C family is related to restriction endonucleases (106), the MOB_T family related to *Rep_trans* rolling-circle replication proteins (107) and the MOB_M family is related to tyrosine recombinase (108). Crucially, the mobilization of hitchers by conjugation through these evolutionary unrelated MOB families suggests they have emerged several times independently (103,104). Likewise, phylogenetic trees of the capsid genes of cfPICI revealed three distinct clades, suggesting these elements emerged from phages three times independently (15). Similar processes were suggested for HGEs implicated in intra-genomic mobility: available evidence suggests that MITES and CALIN emerge regularly in bacterial genomes (35,109–111). Hence, some types of hitchers emerged a long time ago and did so multiple times, which raises the question of the underlying evolutionary process. We propose two alternative non-exclusive models: the reductive evolution model and the *de novo* evolution model (**Figure 3**).

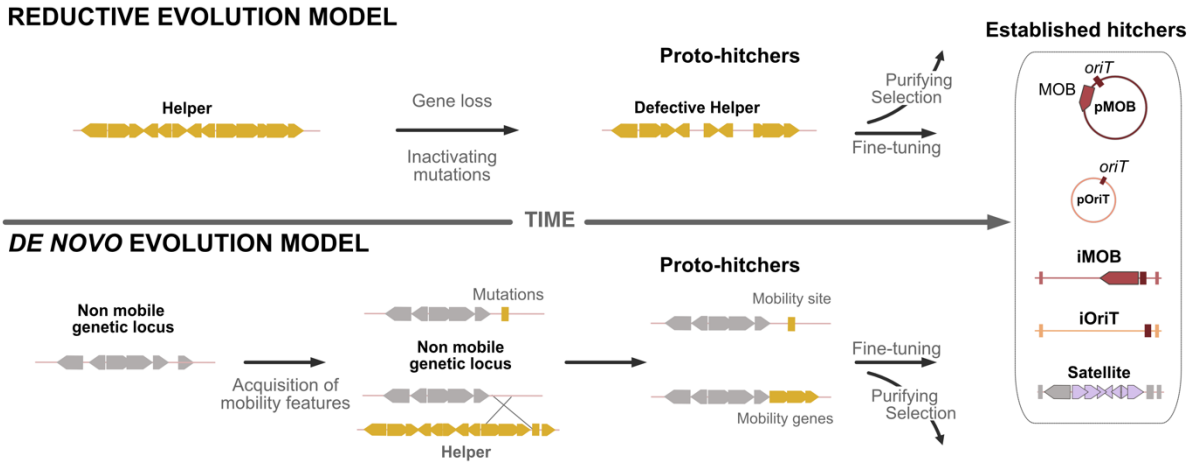


Figure 3: Evolutionary models leading to the emergence of HGEs. Reductive evolution model: Autonomous elements evolve towards proto-hitchers by gene losses and/or inactivating mutations. After fine-tuning proto-hitchers eventually become established HGEs. *De novo* evolution model: Non-mobile Elements and/or genomics islands evolve towards proto-hitchers by acquisition of mobility features. In both scenarios, after fine-tuning proto-hitchers eventually become established hitchers. pOriT: plasmid carrying *oriT*, *oriT*: origin of transfer, MOB: relaxase.

The loss of one or more mobilization-associated genetic components in a helper element may result in them becoming a HGE, i.e. to the emergence of a partially defective MGE that is functionally dependent on another element. The latter becomes its helper by providing the missing functions. This is the basis of the ***reductive evolution model*** for the emergence of HGEs. In this model, the proto-hitcher initially resembles its helper and will progressively diverge from it. Since processes of gene loss occur at high frequency, and can be accompanied by genetic exchanges, they have the potential to create very intermingled evolutionary histories between hitcher and their helpers. This has been most extensively described in mobilizable plasmids which can emerge by the loss of conjugation-related genes. The phylogeny of the most frequent relaxases shows that conjugative elements are ancestral, but very often, by gene loss, give rise to plasmids with defective conjugation systems that can only be mobilized *in trans* (44,112). As for MITES and CALIN, the current paradigm is that they emerge by gene deletion: loss of the transposase for the former (113) and IS-mediated translocation of the integrase for the latter (35). Many bacterial genomes encode defective prophages that could be proto-hitchers (114). For example, DLP12-like and Rac-like cryptic prophages are found in 74% of *E. coli* strains (115). Detailed analyses of an *E. coli* O157:H7 revealed 18 prophages, most of which are inducible and capable of packaging DNA but only 2 are fully functional (116). Some of these elements have defective structural genes, suggesting these functions might be provided by other prophages. It remains to be understood how frequently the defective elements can be mobilized by helpers and whether they survive in the long term to become efficient HGEs.

There is reason to believe that many defective elements that work as proto-hitchers are lost in the process. Phylogenies of relaxases reveal many more transitions of conjugative to mobilizable plasmids than the inverse (44), suggesting source-sink evolutionary dynamics where most transitions are quickly lost, i.e. most novel mobilizable plasmids are poorly adapted to their novel role and disappear. These novel elements lack many of the characteristics that would favor their efficient mobilization by a helper. Notably, their non-functional mobility systems may be costly for the cell (due to, e.g., production costs, toxicity of aggregation of non-assembled protein components) and may interfere with the one of the helpers (since they are homologous). Their cost to the cell, when it exists, and less efficient transfer may result in their frequent extinction. Some proto-hitchers might also be *too* related to the helper for their stable maintenance in the population. For example, a defective conjugative plasmid is initially incompatible with its closely related helper due to their similar replication and partition systems, meaning that they cannot stably co-reside in the same cell (117), which would favor

extinction of the hitcher. Accordingly, genome analyses suggest that plasmids successfully transitioning from conjugative to mobilizable rapidly acquire novel replication initiators (44). Similar problems may arise when phage satellites emerge from defective prophages and the original element has superinfection exclusion systems (118). In such cases, the newly formed hitchers might rarely co-reside with the suitable helper, increasing their chances of extinction. A key bottleneck of the reductive evolution pathway may thus be the transition from being a defective helper (proto-hitcher) to become an efficient hitcher.

Alternatively, existing autonomous MGEs may have acquired the ability of being mobilized by other unrelated MGEs. This ***de novo* evolution model** predicts that hitchers may be very distinct from the helpers in the first place. This fits well with some types of satellites, like the P4-like satellites and PLE, which have very few homologs to their helper phages (12,16), and none concerning the proteins responsible for subverting the helpers. Likewise, the relaxases of many mobilizable plasmids are rarely found among conjugative plasmids (103,104). How could these functions emerge to generate a hitcher? One possibility is that potential proto-hitchers acquired them through genetic exchanges. This is the case of the pOriTs pCERC7 and pBuzz, which have acquired the *oriT* region of the conjugative plasmids R64 and p838B-R, respectively (119,120). Likewise, PICI satellites have phage-like DNA packaging systems (14), and the capsids of cfPICI satellites form multiple distinct clades suggesting they were independently coopted a long time ago from phages (15). Such genetic exchanges may be followed by mutations or genetic reassortments resulting in fine-tuning the functions to their new role in the novel hitcher.

Another possibility is that such functions evolve *de novo* in mobile genetic elements that were initially non-transmissible between cells. For example, short DNA motifs such as *oriTs* for conjugation or *cos/pac* sites for transduction might emerge by random mutations or recombination events. This process might not be too unlikely given the small size and the low specificity of some of these DNA motifs (76,121). Recently, it was shown that phages and satellites may transduce plasmids in *S. aureus* (47), an indication that packaging signals with sufficient efficiency may arise easily in MGEs. Since MGEs evolve fast and mobility can be under strong selection, relatively inefficient DNA motifs may quickly evolve and improve their ability to mediate the novel hitcher mobility. A mix of mutation and recombination events can also generate novel hitcher-specific genes. For instance, MOB_T relaxases, encoded by many elements mobilized by conjugation in Firmicutes, are a combination of two domains present in other proteins, one related to RCR initiator proteins of the Rep_trans family and another to helix-turn-helix proteins binding DNA (107). It is also possible, albeit less likely, that new protein coding genes are fully created *de novo* as recently shown for other functions (122).

Regardless of the evolutionary pathway leading to the emergence of HGEs, their subsequent success requires further evolution. Newly formed hitchers might need to improve their efficiency of mobilization and acquire the ability to sense and manipulate helpers. This occurs in a context where helpers may evolve to avoid the interference of hitchers (if the latter are costly to the former). The ability to be in a *transferable state* during the self-mobilization of its helper might be one of the most important features for a successful hitcher, especially for those that must excise from the chromosome before transfer. Proto-hitchers may initially be both “mute and blind” regarding when (or if) transfer might occur. How hitchers subsequently acquire – and fine tune – these traits is a promising path of research to understand the emergence and evolution of these elements.

Less is more?

One intriguing property common to all types of HGEs is that they tend to be much smaller than those of their helpers. For example, conjugative elements have a median size more than five times larger

than the mobilizable elements (43,88), even if a few exceptions have been described (123). The genomes of phage satellites are also typically much smaller (6-18Kbs) (10,17) than those of their helpers (dsDNA, usually >> 25kb). One may assume that hitchers have smaller genomes than their helpers simply because they do not need to encode mobility functions. Conjugation systems require at least a dozen genes and often many more, whereas genes encoding viral particles are a substantial fraction of the gene repertoires of temperate phages. Yet, these reasons do not seem enough to explain the hitchers' small sizes. There is extensive evidence that conjugation can transfer long replicons, e.g. the historical HFr strains can conjugate the entire *E. coli* chromosome. Furthermore, the size difference between mobilizable and conjugative plasmids (>100 kb) (43) is much larger than the average size of loci encoding the conjugative system (124). Although some of the mechanisms used by satellites to hijack viral particles do constrain the size of their genomes (e.g. P4 and PLE shrink the capsids of their helpers so that they can only package themselves (22,125)), other satellites do not re-size the capsid of their helpers and even package multiple copies of their DNA within the viral particle (17). The cfPICI satellites even produce their own capsids (15), which could provide them with the possibility of having larger genomes than their helpers (which is not the case (10)). Hence, their mobility mechanism does not always require a smaller size. Recent data shows that when one compares old lineages of mobilizable plasmids with recent ones, the former are smaller, suggesting that natural selection favors the streamlining of HGEs (44). All these facts suggest a pervasive trend for hitchers to be smaller than their helpers even when there are no obvious mechanistic reasons for that.

One possible explanation is that larger genomes might express a wider diversity of functions, which renders them more likely to conflict with their hosts. Hence, the streamlining of hitchers' genomes could decrease carriage costs and favor vertical propagation within bacterial lineages, which would eventually increase the chances that the element co-occurs with a helper. This would be especially important if hitchers are less mobile than their helpers implicating that their fitness impact on the bacterial host is more important for their own fitness. Streamlined hitchers may also have fewer conflicts with other MGEs. In HGEs mobilized by conjugation, the size of the element may affect the cost of its transfer to the helper (if there is one). A smaller conserved gene repertoire might also provide fewer targets for anti-MGE defenses. For example, small plasmids can more easily escape restriction-modification systems without encoding anti-restriction (67). Available data also suggests that helpers are more specifically targeted by defense systems, hinting that they, and their mobilization machinery specifically, might be more costly to the cell and other MGEs. For example, host-encoded defense systems usually target phage-capsid genes (126), and plasmid-encoded CRISPR-Cas systems target conjugative systems of other conjugative plasmids (127). Hitchers, because of their small size, might be more likely to evade defense mechanisms of both hosts and other MGEs. The hitchers' simplicity might thus be the result of a trade-off between autonomy in genetic mobility and size, where the smaller gene repertoire of HGEs increases their chances of persisting by transfer (lower cost to helper), by vertical inheritance (lower cost to bacterial host), or by evasion of bacterial immune defenses.

Conclusion: A broader view of Hitcher Genetic Elements across the tree of life

The recent observation that many MGEs (hitchers) are mobilized by other MGEs (helpers), challenges our understanding of the processes underlying horizontal gene transfer. Hitchers carry traits that influence the complex network of interactions between cells within populations (e.g. bacteriocins, antibiotic resistance). They are also key components of the networks of interactions between MGEs within cells: they modulate the stability, mobilization, and transferability of other MGEs. As such, they have very diverse impacts on the fitness of the bacterial host and of the helper. Whether they are hyper-parasites or hyper-mutualists may depend on the specific triplet host-helper-hitcher, on the accessory traits they carry, and on the circumstances. The last point is essential since it implies these

assemblages host-helper-hitcher can make temporary and niche-dependent alliances that may latter fall apart. These alliances are quite striking regarding defense systems. It was previously pointed out that many, possibly most, "bacterial" defense systems are encoded in MGEs which means that defense systems are best understood in the context of interactions between MGEs within cells (128). The recent results showing how satellites protect cells from phages, but not from their helpers whose infection is important for the spread of satellites (99,100), are a clear indication of the limits of alliances between hitchers and hosts. These ecological networks of interactions have evolutionary consequences. As much of bacterial evolution depends on the genetic exchanges promoted by MGEs, HGEs have an important role on the gene flow within and between bacterial populations.

Hitchers have been known for decades but only recently their relevance has started to become fully appreciated. This is because it has become clear they are not just rare defective MGEs on their way to extinction. Instead, hitchers are a category of mobile elements that is distinctive, ancient, and diverse. Our recent ability to identify them has shown that they are very numerous, often outnumbering their helpers. It is not yet clear if this abundance is the result of their selfish spread across communities, of their ability to provide adaptive functions to helpers and hosts, or a mixture of the two. Still, the remarkable functional, structural, and evolutionary parallelisms between very different hitchers, coupled with their abundance across some bacterial clades, suggest that becoming mobilizable by other MGEs is a successful evolutionary strategy. The lack of hitchers in many clades suggests that we have just started to uncover them.

Hitchers can also be found in the genomes of Eukaryotes and Archaea. MITES are frequent in plants, where they play a key role in promoting genomic plasticity (129,130), and in other eukaryotic organisms (131). In Archaea, ca. 20% of the genomes contains MITEs (32). Many eukaryotic viruses are satellites of autonomous viruses. One well-described example is the Hepatitis Delta Virus (HDV), a small, “defective” RNA virus. HDV is the smallest known virus that infects humans, and causes the most severe form of viral hepatitis. HDV does not encode the surface antigens that allow it to infect human cells, but the Hepatitis B Virus (HBV) does. Hence, HDV requires HBV to move between cells (132). Since the generation of defective interfering particles is common during viral infection (133,134), many eukaryotic virus-like hitcher emerge, at least temporarily, through reductive evolution (**Figure 3**). Another type of HGE in Eukaryotes are virophages, elements that encode structural genes but require the viral particle factory of giant viruses for their own replication (135). Archaeal viruses also have their satellites (136). While conjugation is unknown in Eukaryotes, it is present in Archaea and hitcher mobilizable by conjugation might exist, although their identification is difficult because we ignore many of the proteins with a relaxase function (137). It is thus likely that many of the challenges and outlooks that we describe here apply to HGEs in other domains of life, many of which may remain undiscovered. This suggests that the strategy of being mobilizable, instead of autonomously mobile, might have been a key, perhaps inevitable, part of the evolution of life.

Glossary

- Mobile genetic element (MGE). Genetic elements that encode enzymes and other proteins mediating the mobility of DNA within genomes or between bacterial cells.
- Helper MGE. MGE that can mobilize another MGE.
- Transduction. Process whereby a viral particle transfers into another bacterium DNA that does not encode the phage particle (bacterial, satellite).
- Bacteriophages (phages). Bacterial viruses.
- Lysogeny. Process whereby a temperate phage integrates the host genome (integrating the chromosome or remaining as a plasmid) and replicates with it. During lysogeny only a few of the phage genes are expressed.
- Prophage. Integrated phage during lysogeny.
- Phage satellites. MGEs that are not phages but encode molecular mechanisms or DNA signals favoring their packaging in viral particles totally or partially produced by phages.
- Phage-plasmid. A phage that stays in cells as a plasmid during lysogeny.
- Conjugation. A molecular process allowing the transfer of (usually single stranded) DNA between cells using a mating pair formation system (such as a conjugative pilus), a relaxase and an origin of transfer.
- Conjugative elements. MGEs capable of autonomous conjugation (conjugative plasmids or integrative conjugative elements). Conjugative plasmids (pCONJ). Plasmids that encode all the major components for conjugation.
- Mobilizable plasmids. Plasmids that are not conjugative but can be mobilized by conjugation when the mechanism is encoded in part or completely in another MGE.
- Origin of transfer (*oriT*). Origin of transfer by conjugation is a small DNA motif that is recognized by the relaxase in the beginning of the process of conjugation.
- pMOB. Mobilizable plasmids encoding a relaxase (and presumably an OriT, which may be unknown).
- pOriT. Plasmids encoding an origin of transfer for conjugation, but no relaxase or mating pair formation system.
- Integrative conjugative elements (ICEs). MGEs that integrate the chromosome and encode all major components for conjugation.
- Integrative mobilizable elements (IMEs). MGEs that integrate the chromosome and encode a relaxase and an origin of transfer by conjugation, but not the mating pair formation system.
- iMOB. IMEs encoding a relaxase (and presumably an OriT, which may be unknown).
- iOriT. IMEs encoding an origin of transfer for conjugation, but no relaxase or mating pair formation system.
- Insertion Sequences (IS). The smallest autonomous transposable elements, including a gene for a transposase and flanked by repeats for its mobility.
- Miniature Inverted Transposable Elements (MITES). Same as Insertion Sequences, but lacking the transposase.
- Integrons. Genetic elements containing a site-specific recombination system (integron-integrase IntI) that integrates or shuffles gene cassettes delimited by recombination (*attC*) sites.
- Clusters of *attC* sites lacking integron-integrases (CALIN). Same as integrons, but lacking the site-specific recombination system.
- Genomic Islands (GIs). There is an ambiguity in the literature concerning these islands. For some, they are regions of chromosomes highly variable in gene content. For others they are genetic regions in tight linkage in highly variable chromosome regions that seem to be MGEs, in the sense that they co-transfer between bacteria (often using unknown mechanisms of mobility). We here use the latter definition .

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