Sampling the mobile gene pool: innovation via horizontal gene transfer in bacteria

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organisms to acquire novelties. As with mutations, transferred genes can be neutral or deleterious, and many of the agents that facilitate HGT are entities evolving in their own right. Their interactions with the donors and recipients of HGT can set the scene for evolutionary conflicts.

HGT can occur across vast phylogenetic distances, and these events may have important ecological consequences. For example, *Hypothenemus hampei*, a species of beetle, appears to have acquired a gene for mannanase degradation from *Bacillus*, enabling it to become an economically-important pest of coffee plantations [8]. In other cases, HGT appears more opportunistic, and the adaptive consequences are less clear. For example, some strains of the human pathogen *Neisseria gonorrhoeae* have acquired a 685 bp region of the Long Interspersed Nuclear Element (LINE1), a fragment of a retroelement gene found in the human genome that has no clear function in the recipient bacterium [9]. Inter-kingdom gene transfer can occur surprisingly often given the right ecological conditions, as is shown by the independent acquisition of the bacterial gene *acdS* by 15 different lineages of fungi and other eukaryotes [10]. The amount of DNA transferred can be considerable — in one case, almost an entire bacterial genome was found to have transferred into the nuclear genome of a *Drosophila* [11]. Events like these demonstrate that species boundaries can be more permeable than is often assumed, and that genetic information can in principle move between even highly divergent lineages. It has even been proposed that no insurmountable barrier to HGT exists [12]. HGT into metazoan genomes is striking, the more so because it has disrupted long-held assumptions about the nature of inheritance and evolution in complex organisms. However, while metazoan HGT clearly occurs, the complexity of eukaryotic genomes, and the ease by which samples can become contaminated with bacterial DNA creating false positives, have thrown some of the more extreme claims into doubt [13]. The consensus therefore is that successful HGT into metazoan taxa is relatively rare, albeit with potentially huge impact for phenotype and fitness where it does occur [14].

By contrast, in the microbial world, HGT is a fact of life. For bacteria in particular, HGT is a major mode of adaptation, making a significant contribution to genome evolution and structure [1,15,16]. Bacterial HGT has a central role in adaptation to environmental challenges, like colonisation of new environments, exploitation of novel carbon sources, and resistance to toxins [17]. The increasing evidence placing humans in the midst of an essentially microbial world [18-20], where bacteria have fundamental roles in biogeochemical cycles, health and disease, and food security, make it all the more important

to understand their evolution and ecology. Furthermore, bacteria are increasingly used as model systems for understanding general evolutionary processes [21]. In this review we will focus on HGT between bacteria, although many of the themes we discuss are likely to apply when considering the horizontal transfer of traits more generally.

Horizontal gene transfer is central to bacterial evolution

Bacteria have several features that may make them especially well-suited for HGT-mediated evolution. All cells are generally reproductively proficient, i.e. germline, meaning that mutations and acquired genes can be easily passed down to subsequent generations. Unlike eukaryotes, bacteria lack membrane-bound nuclei, meaning that their genomes are more accessible to incoming DNA. This can enhance the acquisition and integration of new genes [12]. Bacteria can evolve rapidly, thanks to their potential for huge population sizes and short generation times, which means that infrequent gene transfer events are more likely to occur and selectively advantageous events less likely to be lost due to drift. Bacteria have a truly cosmopolitan distribution, inhabiting and adapting to a vast range of environments and performing reactions the benefits of which may be limited spatially, such as degradation of exotic carbon sources. Migration is thought to occur readily [22,23], and consequently myriad bacterial species can coexist in a community [24]. This represents diverse genetic material that can potentially transfer between, and be of value to, its members.

Comparative analyses have revealed the pervasiveness of HGT in bacterial evolution and genome dynamics. Even closely-related bacterial strains can vary greatly in genome content [25], and increased sequencing shows that only a minority of genes carried by a species might be shared across all members [25,26]. This set of genes represents a ‘core genome’, and can be contrasted with the ‘variable’ or ‘accessory’ genome which represents genes present only in a subset of strains [27]. The total of all the unique genes in a species, termed the ‘pan genome’, generally increases in size with each new strain sequenced (though this can vary between species [28]), a pattern which emerges because different lineages within a species acquire and lose variable genes from other species in their local communities. The size of the pan genome, and its distribution amongst strains, hints therefore at a large ‘pool’ or ‘library’ of genetic material that is available for acquisition — a genetic resource on which evolving bacteria can draw for adaptation. This library is apparently well-used. Comparative studies show that in several bacterial lineages, genes are acquired and lost at rates comparable to or even greater than nucleotide substitutions [29,30].

Machines for spreading genes

In principle, HGT requires two physical processes. First, genetic information must cross biological membranes into the recipient species. Second, the genes must be linked to a functioning origin of replication in a germline cell to ensure subsequent vertical transmission in the recipient. There are several well-defined mechanisms that facilitate gene transfer between bacteria by enhancing one or both of these processes (figure 1), with novel mechanisms still being characterised and, in all likelihood, more yet to be discovered.

Traditionally there are three canonical mechanisms of DNA transfer in bacteria: conjugation, transduction, and transformation. Conjugation occurs when a donor bacterium expresses a multi-component macromolecular membrane-traversing structure — a 'conjugative pilus' — which provides a physical link for DNA to move between donor to recipient [31,32]. Transferred DNA contains an 'origin of transfer' (oriT), a sequence of bases which is recognized by the conjugative machinery [33]. Transduction occurs when bacteriophage from a donor bacterium package non-phage DNA in viral particles which then infect other bacteria [34,35]. Both conjugation and transduction provide protection for DNA from environmental damage after it leaves the donor cell. Natural transformation is the process by which bacteria take up DNA from their environment. This occurs by the retraction of cell surface fibres (pili) which pull double-stranded DNA close to the cell membrane, allowing uptake via a conserved membrane pore. The DNA substrates for transformation may be actively secreted into the environment by donors [36], or released by dying bacteria (e.g. following lysis by phages, [37]). It has been recorded that bacteria can take up genes released by neighbours they themselves have killed [38]. More recent research has identified other mechanisms by which DNA can transfer between hosts. Gene transfer agents (GTAs) are DNA-containing particles that resemble phages, but are incapable of carrying the genes for particle production [39]. Genes can also be transferred between bacteria that form intercellular connections via nanotubes [40] or membrane fusion [41]. Some bacteria release DNA-containing membrane-bound vesicles that can carry genetic information to new hosts [42]. *Mycobacteria* undergo an unusual form of conjugation that appears to be regulated in part by the recipient, doesn't require oriT, and results in transconjugants whose genomes are a patchwork of its parents [43,44].

Once DNA has entered a recipient, it must replicate to avoid loss by segregation during cell division. Incoming DNA can carry its own origin of replication thus replicating separately from the chromosome of its new host. Such is the case for plasmids: pieces of DNA, usually

circular, which remain physically distinct to the chromosome [45]. Alternatively, the incoming DNA must recombine with a resident element to gain access to an origin of replication, either on the chromosome or an extrachromosomal replicon like a plasmid. This can happen via general mechanisms of recombination, but is enhanced by an assortment of enzymes, which catalyse the integration, excision and recombination of DNA [46].

Although the machineries discussed in this section enable the horizontal transfer of genetic innovations, it should be noted they have not necessarily evolved 'for' that purpose. For example, transformation tends to cause the replacement of longer stretches of DNA with shorter ones, so is more prone to reducing, rather than expanding, genome content [47]. Various restrictions on assimilating DNA from unrelated strains suggests that transformation may tend towards being a more conservative than innovative process [48,49]. But perhaps more importantly, many of these mechanisms are not in fact under the control of the bacteria but are instead controlled by semi-autonomous segments of DNA which, far from being functional tools for bacterial gene exchange, have their own self-interest at heart.

Mobile genetic elements: perpetrators of HGT

From the perspective of a gene, HGT represents another opportunity for reproduction, and thus is subject to natural selection. The microbial world is teeming with mobile genetic elements (MGEs), genetic entities that are adapted to transferring between strands of DNA and between different bacterial hosts [50]. The bestiary of MGEs is rich, and the elements involved are dynamic, modular, and nested. For example, transposable elements (TEs), DNA sequences which carry genes enabling them to hop between DNA strands, can be found on larger elements such as plasmids which carry an origin of replication [51]. Plasmids may carry their own set of genes for conjugative transfer (i.e. conjugative plasmids), or, if they have a compatible oriT, may utilise the conjugative machinery encoded by a different replicon (i.e. are mobilizable) [33,52]. Plasmid gene content is dynamic, and plasmids that acquire new genes from their hosts, perhaps through the activity of TEs, carry these genes onwards when they conjugate [53,54]. Integrative and conjugative elements (ICEs) resemble plasmids in many ways, except they carry enzymes that catalyse insertion into the host chromosome and thus do not need to carry their own origin of replication [55]. Bacteriophages can be either purely virulent, killing their hosts quickly in order to reproduce, or 'temperate' phages, that, similarly to ICEs, can insert their genomes into the bacterial chromosome. Both types of phages can mediate HGT. For temperate phages there is an opportunity for bacterial genes or transposons to become integrated into the phage genome

and thus be co-inserted into the bacterial genome when they integrate, called lysogenic conversion [56]. However for all phages, bacterial DNA or other MGEs can be packaged in phage particles and become transferred by transduction [57]. Indeed, some integrative elements specialise in repurposing phage capsids for their own transfer [58]. Collaborations and conflicts between MGEs can therefore enhance their ability to spread within and between hosts.

MGEs can carry genes other than those necessary for transfer and replication. These genes — or where they can be functionally grouped, 'modules' [59] — may help the selfish vertical transmission of the element. For example, partitioning systems segregate plasmids between daughter cells, reducing the frequency of plasmid-free offspring, and toxin-antitoxin systems impose a large (usually lethal) cost on daughter cells that have lost the MGE, favouring MGE carriers by removing such competitors from the population [60]. The evolutionary benefits of such modules are easy to appreciate. Many MGEs also carry 'accessory genes', which do not play a direct role in their vertical or horizontal transmission. Instead, accessory genes may have effects at higher organisational levels, boosting the success of the element indirectly. For example, the spread of antimicrobial resistance (AMR) genes is facilitated by the carriage of these genes on elements such as transposons, ICEs and plasmids [61]. Acquiring these accessory genes can allow a bacterial host to flourish in otherwise deadly environments, with concomitant positive effects on the elements that it carries. The benefits of accessory gene carriage demonstrated by the success of integrons, elements first identified on MGEs that appear adapted for the acquisition, assembly, and expression of accessory genes [62,63]. Yet, the function of many accessory genes is not known [64], and the selective factors that favour their mobility and co-occurrence are similarly unclear. Nevertheless, MGEs likely constitute the means by which most genes travel through bacterial communities, and are therefore potent agents of HGT [65,66].

Thus, there exist many potential routes by which genes can transfer between bacteria, and although rates for processes have been measured experimentally (e.g. [67-69]), understanding the relative importance of these mechanisms *in situ* is complicated by the fact that efficacies are likely to vary between species and environments [45,70]. This can stem from physical limitations, for example, mechanical agitation can inhibit conjugative transfer [71] and different environments are more or less harsh to extracellular DNA, affecting opportunities for transformation [72]. It is also likely to be driven by the ecology of both bacteria and MGEs, for example lysogenic conversion is more common among bacteria with fast growth

rates [73], which likely reflects the conditions which favour the life history strategy of temperate phages. Genome analyses suggest that plasmids are better-connected 'hubs' in networks of gene exchange than phages, for example, [66], but it can be difficult to identify how genes have moved by analysing their sequences [74]. A clear priority for future research is to quantify the relative importance of HGT mechanisms and how such rates vary with taxonomy and ecology.

Costs, benefits, and conflicts in HGT

Horizontally-transferred genes have the potential to provide their recipients with striking benefits. But HGT is not a benign process, and gene exchange can impose significant fitness costs on both donor and recipient. Indeed, the distribution of fitness effects of HGT has been proposed to be more dispersed than that of nucleotide mutations [29], with potential costs, as well as potential benefits, likely to be more extreme. Costs emerge in the short term from a variety of mechanisms [75]. Incoming genes represent additional DNA that draws on cellular resources for replication, transcription, and translation. The sequence composition of acquired DNA may be poorly optimised for the host's expression machinery, resulting in stalled ribosomes, misfolded proteins, and triggering of stress responses, while expressed genes may interfere with cellular homeostasis by disrupting metabolic or signalling processes. Acquired TEs can proliferate in the chromosome, damaging the genes into which they insert and causing gene loss through recombination. Donors are also affected. The production of conjugative machinery is metabolically expensive, and exposes its bearers to 'male-specific' bacteriophage that recognize and use the conjugative pilus as a receptor [76]. The release of capsids to secrete genetic material, through generalized transduction or GTAs, can require lysis [39]. Unlike the metaphors that refer to transferred genes as swappable 'smartphone apps' [77], HGT may in reality be a more traumatic experience.

Several mechanisms have evolved that can inhibit HGT, allowing cells to escape this disruption [45]. For example, DNA restriction-modification systems and CRISPR-Cas loci represent 'immunity' systems that recognize and selectively degrade foreign DNA. Though they likely evolved as a means of resisting highly antagonistic agents, such as bacteriophage, these systems may also impact potentially beneficial MGEs [78,79]. They therefore have the potential to cut lineages off from the flow of adaptive innovations. Experimental studies exploring this tension show that where pressure to acquire plasmid-borne genes is strong enough, bacteria tend to jettison their CRISPR-Cas immunity loci completely [80], enabling gene acquisition. However, immunity loci are also horizontally transferred, so it is possible to

re-acquire them. Indeed, comparative studies show no correlation between the degree of CRISPR-Cas immunity and recent HGT acquisitions [81], suggesting that immunity to HGT is likely to be dynamic, with transient periods of susceptibility and resistance. Besides physically degrading foreign DNA, bacteria can also exert some control over the expression of recent acquisitions. Newly-arrived DNA tends to be relatively AT-rich in comparison with the resident chromosome, possibly reflective of an itinerant lifestyle [82]. The histone-like nucleoid structuring protein (H-NS), a regulator encoded by bacteria, binds to and silences AT-rich DNA, preventing costly and maladapted expression of foreign genes [83,84], and representing a form of 'censorship' by the established genome.

An important aspect of the MGEs that facilitate gene exchange is that they themselves reproduce and mutate, and are subject to natural selection. They therefore have their own fitness 'interests', which may not necessarily be aligned with those of their hosts. For example, plasmids are under selection to increase copy number within a cell, but high copy number imposes a high cost on that cell [85]. This can generate significant evolutionary conflict between hosts and MGEs. Plasmid carriage, for example, can exert a considerable toll on host fitness, and selection might favour hosts which have managed to shed their plasmid burden [86]. Meanwhile, to prevent their loss, MGEs acquire modules to ensure their maintenance, such as those involved in plasmid partitioning, or genes which disable the host's CRISPR-Cas immunity loci [87]. Vertical and horizontal modes of MGE transmission are likely to trade off against one another: adaptations that improve the ability of an MGE to move across lineages are likely to make that MGE costlier to the host it is in, while decreases in cost are likely to come from repressing horizontal transfer [88]. Hosts are under pressure to 'domesticate' or shed fractious MGEs, while MGEs are under pressure to maintain autonomy. In this context, it is interesting that of the three canonical mechanisms of DNA transfer, only one (natural transformation) is under the direct control of bacteria, the others are encoded by the semi-autonomous MGEs that inhabit them. The mobile gene pool may be akin to a library, but the books are alive.

Considering the potential for conflict between MGEs and their hosts, carriage by MGEs of potentially useful accessory genes, such as those involved in antimicrobial resistance or virulence, is difficult to explain. The benefits of accessory genes are likely to be highly context-dependent, varying with chemical, physical, and social environment [89-91]. Plasmids, for example, carry genes for resistance to environmental pollutants even in pristine habitats [92]. Under such conditions, where accessory genes are not beneficial, plasmids

persist as parasitic entities, and would be expected to become more efficient parasites, streamlining their genomes through accessory gene excision and increased transfer rate. Positive selection for accessory genes could offset the costs of plasmid carriage, but under such conditions selection would favour integration of the beneficial traits into the host chromosome and loss of the plasmid backbone. Regardless of selective conditions, accessory gene carriage by MGEs, though widespread, appears problematic. This puzzle has been termed the 'plasmid paradox' [93], but it can be generalised to include other MGEs such as transposons and integrative elements which maintain accessory gene mobility.

Keeping genes moving — resolving the plasmid paradox

Experimental evolution studies are providing some answers to this problem, at least for plasmids. Co-evolution between plasmid and host can rapidly ameliorate the major costs of plasmid carriage, reducing the effects of purifying selection and maintaining gene mobility. Compensatory evolution can occur on the chromosome [94-96] or to the plasmid [97,98], and may be specific to that host-plasmid pairing or represent a more general adaptation. Interestingly, in some cases plasmid cost emerges from conflicts with other horizontally-transferred elements. In *Pseudomonas aeruginosa* PAO1, cytotoxic gene expression from the small plasmid pNUK73 is induced by two recently acquired chromosomal genes. Disruption of one or both of these genes alleviates plasmid cost, resulting in maintenance of the plasmid and the antibiotic resistance gene it carries [99]. Some plasmids reduce their burden by deploying their own H-NS-like genes, which reduce burden by repressing plasmid gene expression [100]. Evolution of gene regulators may prove to be a general theme in the accommodation of acquired genes, as comparative analyses show that gene regulatory regions tend to correlate with the accessory compartment rather than the core genome [26].

Alternatively, where rates of conjugation outweigh the costs of carriage and imperfect transmission to daughter cells, plasmids can be maintained in a population through infectious transfer [101]. Though there has been considerable debate over whether they are achieved in nature, high infection rates have been shown to sustain carriage in several laboratory experiments, at least over short periods [102-104]. Persistence through infection leads to a more antagonistic relationship between plasmids and their hosts: hosts are predicted to develop adaptations for actively resisting (re)-infection, whereas plasmids are likely to lose accessory genes to become better parasites.

Whether and how conflict with MGEs is resolved varies between hosts, as differing gene content between strains offers different opportunities and constraints to conflict resolution. In some species, plasmids are highly unstable due to poor vertical transmission, in others they are unstable due to a high cost [86], with differing evolutionary outcomes. For example, the IncP-1 plasmid derivative pMS0506 evolved increased stability in *Shewanella odeidensis* through mutations in *trfA*, which reduced plasmid cost [105], but in *Pseudomonas moraviensis* stability was increased by the acquisition, from another plasmid, of a transposon carrying a toxin-antitoxin system, which effectively reduced plasmid loss [106] (an example of mobile elements interacting to enhance plasmid maintenance). Moreover, although beneficial accessory genes can become 'captured' by the chromosome under positive selection, with consequent loss of the plasmid, this phenomenon varies between species [107].

MGEs are not just the traits they carry, and the relationship between these elements and their hosts may be multifaceted and more subtle than the effects of their accessory genes. Temperate phages, for instance, can be effective 'weapons' in bacterial warfare [108] while plasmid encoded conjugative pili may can help bacterial hosts to form biofilms [109]. Besides these ecological effects, MGEs can alter bacterial evolution and gene regulation in ways beyond gene acquisition. Integrative elements like temperate phages, transposons and other IS elements can jump into genes and regulatory regions. This sledge-hammer approach to gene disruption can lead to rapid adaptation of the host to new environments [110,111]. Recent advances may also suggest that integrative elements may actively integrate into and excise out of bacterial genes to act as functional 'switches' in turn disrupting and restoring gene function [112]. Furthermore, the multicopy nature of many of these elements both increase the opportunity for mutations in the genes they carry [113], and, in the case of plasmids, constitutes a responsive platform for altering gene dosage by varying copy number. During infection, *Yersinia* requires an increased gene dose of its type 3 secretion system (T3SS) for efficient colonisation. This is achieved by a transient increase in copy number of the virulence plasmid pIBX, which carries the T3SS, from 1 to 3 per cell [114]. *Aureimonas* species carry plasmid-borne ribosomal RNA (rRNA) genes, potentially enabling rapid change in copy number which might provide selective benefit under changing environmental conditions [115].

Plasmid maintenance in a species, through amelioration or infectious transfer, may resonate through a community. In species-rich microbial communities, a subset of members able to

maintain plasmids may act as a ‘source’ species for otherwise unfavourable hosts [107], a pattern that is reflected in the fact that the ability of a plasmid to invade a microbial community is correlated with existing plasmid maintenance [116]. Plasmids can still conjugate from hosts that have completely ameliorated their cost [96], and the ability of a plasmid to invade a diverse fraction of a community [117] means that a few source species could maintain community-wide gene mobility.

The impacts of HGT on genome evolution

Genes can be rapidly lost as well as gained, and gene loss frequently acts to pare down bacterial genomes [118]. This balance between acquisition and loss gives rise to the patterns of HGT that become apparent in large-scale genome analyses. These infer HGT by identifying genes shared between lineages and thus detect both recent gene transfer events and those which occurred longer ago [30]. Shared genes represent not only successful transfer, but also maintenance in the donor and recipient lineages [119]. There is a bias in the types of gene detected by these studies, leading to theories about why certain genes are overrepresented amongst shared genes (i.e. are more ‘transferable’ than others). ‘The Complexity Hypothesis’ suggests that a gene’s associated biological process is the main determinant of its transferability, with ‘informational’ genes (involved in transcription, translation and replication) less likely to be successfully transferred than ‘operational’ genes (involved in functions such as metabolism and regulation) [15]. This hypothesis has since been refined to show that the primary factor impacting transferability is the number of protein-protein interactions the gene product is involved in [120]: genes highly integrated with many partners in one cell will be unlikely to provide benefit in a different cellular environment. Innovations that perform distinct, specific tasks are thus more likely to be maintained in a new lineage.

The apparently high rates of gene gain and loss detected when comparing recently diverged lineages relative to more ancient branches [29], suggests that transferred genes ‘live fast, die young’, undergoing constant turnover [30]. Those which are beneficial are retained by selection, whilst others, excised by the pervasive razor of gene deletion, are lost [118]. A study of HGT amongst human-associated microbes showed that although genes with plasmid-, phage- or transposon-related functions were identified, they comprised only a small fraction of the transfers detected [121]. This suggests that while MGEs can enhance HGT, they aren’t required for long-term maintenance of transferred genes in the recipient. Longer timescales are likely to see retention of beneficial genes and loss of their means of entry

[122]. Indeed, the genetic context of transferred genes varies considerably between individuals and between populations [74] due to recombination breaking linkage. This is consistent with a model of HGT whereby horizontal gene spread is lubricated by the activity of MGEs, but over longer periods the signatures of these elements is gradually erased as the functional genes become integrated into the physiology of their new hosts.

Where there is sufficient HGT, selection appears to act on genes, and sweeps can carry a particular allele to fixation in a population without purging other loci of their diversity, as was observed in a marine *Vibrio* population [123]. However, if advantageous alleles arise where there is a relatively low rate of HGT, genetic diversity is lost in a 'genome-wide' selective sweep, resulting in a much more clonal population. A nine-year study of 30 bacterial populations in a freshwater lake found gradual purging of genome-wide diversity in one species of green sulphur bacteria due to a selective sweep [124], though these dynamics were not shared by other species at the same site. Interestingly, propensity to undergo gene-specific or genome-wide selective sweeps may be a stable trait, with low-diversity populations with evidence of prior genome-wide sweeps more likely to undergo future diversity-purging sweeps [125]. This suggests that the flow of genes varies within and between species, and is structured by consistent barriers. These barriers are likely determined in large part by the peculiarities of the elements involved, for example carriage and compatibility of MGEs, or presence of cognate restriction-modification systems [45,126].

HGT shapes bacterial populations

The opportunity for gene transfer can have significant effects on the genetic structure of bacterial populations. Related bacteria end up with fewer genes in common, as lineages acquire different sets of genes from their local neighbours [127]. Meanwhile, horizontally-transferred genes are associated more with ecological conditions or geographical locations rather than the phylogenetic lineages in which they are found [25,74,121]. In some cases these genes have a clear relationship to the local environment, encoding, for example, degradation of locally-occurring carbohydrate sources [74,128], or biofilm formation and host colonisation [123]. Where bacteria can migrate, MGEs may be under selection to maintain mobility of these 'niche-specific gene pools' [129], perhaps by transfer to and assembly on a readily-exchangeable plasmid or ICE, since transfer of these locally-beneficial traits to potentially competitive newcomers benefits the success of the MGE [101,130].

Within communities HGT promotes diversity, rescuing unrelated species from purifying selection by the spread of ecologically relevant traits [101]. At the same time, HGT increases relatedness at specific loci, creating conditions conducive to the evolution and success of 'cooperative' traits (which may likewise be niche-specific). Cooperative traits, in this context, are costly actions that provide a benefit not only to the individual performing them, but also to their neighbours. Where neighbours do not reciprocate, co-operative traits are difficult to explain, because co-operators, burdened by the cost of their actions and sharing the benefits, are out-competed. Cooperative traits are thus expected to succeed where the recipients of the co-operative action are likely to be co-operators too. By spreading the genes involved in cooperation between otherwise unrelated individuals, plasmids and other MGEs can favour cooperation. In other words: from a plasmid's perspective, inducing cooperative behaviour in their hosts is beneficial, because it enhances the success of neighbours that are likely to become plasmid hosts too, through HGT [131]. Consistent with this, plasmids and other MGEs are overrepresented in accessory genes that encode traits regarded as cooperative: secreted functions the benefits of which are shared as 'public goods' amongst neighbours [132]. Experimental and modelling studies on a synthetic plasmid system also suggest that HGT can favour cooperative traits [133].

The acquisition of new traits by HGT can have decisive effects on the evolutionary trajectory of the recipient lineage with consequences that extend into human society. Pathogenic lineages often owe their devastating behaviour to genes harboured on MGEs. The *ymt* gene, which provided the agent of plague, *Yersinia pestis*, with an arthropod vector by allowing it to colonize the guts of fleas, was acquired when a TE transposed into a plasmid sometime in the late Bronze age [134]. The lysogenic phage CTX-phi converts *Vibrio cholerae* hosts from non-pathogenic to pathogenic by the expression of cholera toxin, which was acquired horizontally by CTX-phi, exemplifying the nested levels of gene mobility in microbes [135]. Meanwhile the efficiency and flexibility of HGT, amplified by this nested structure, can be observed in the spread of AMR genes in hospital-acquired infections, where resistance genes carried on transposons are able to hop between different resident plasmids each able to infect multiple host lineages [54]. On a larger scale, HGT between more divergent participants lies at the root of key evolutionary transitions — although a matter of current debate in the literature, it has been suggested that major phylogenetic transitions in Archaea are associated with HGT from Eubacteria [136,137]. New acquisitions causing dramatic shifts in phenotype space offer opportunities to take up a very different lifestyle.

Concluding remarks

HGT amongst microbes may prove to be a good model for understanding the spread of innovations at other scales. Certainly, microbes have various idiosyncrasies that make them exceptionally susceptible to HGT as an engine of evolutionary change, and harbour well-described elements that are adapted for the job. But though the mechanisms facilitating it remain unclear (though some candidates have been described, [4,138]), the occurrence of HGT within and between multicellular eukaryotes is becoming increasingly apparent [14]. It will be interesting to see how far the ecological drivers of HGT in microbes similarly promote gene exchange amongst other lifeforms. The interaction between selection and drift [127] and the ability for HGT to facilitate rapid adaptation of migrants [130] may have particular relevance.

Generalising further, bacterial HGT demonstrates that, for the spread of innovations, 'the medium is the message' [139] — at least in the short term. High impact innovations are usually carried into cells by selfish MGEs, and though their signal may eventually be masked by gene loss, recombination, and domestication, the peculiarities of these strange semi-autonomous biological entities affect both the ways that genes flow through communities, and the consequences of this flux. Understanding the negotiations between MGEs and their hosts may therefore be as important as understanding the ecological or clinical significance of the genes that they transfer.

Figure 1. The many routes for horizontal gene transfer. DNA can be transferred between individuals by multiple mechanisms falling broadly into three categories. In transduction (blue text) DNA is transferred either on as part of the phage genome itself or as additional DNA packaged into phage particles or gene transfer agents. In conjugation (yellow text) donor cells form conjugative pili, typically encoded on plasmids or integrative conjugative elements, through which DNA is transferred. Finally transformation (red text) is the process by which DNA in the environment is actively taken up by the donor cell. In addition, recent studies have shown that bacteria can also transfer DNA fragments in membrane bound vesicles and via nanotubes.

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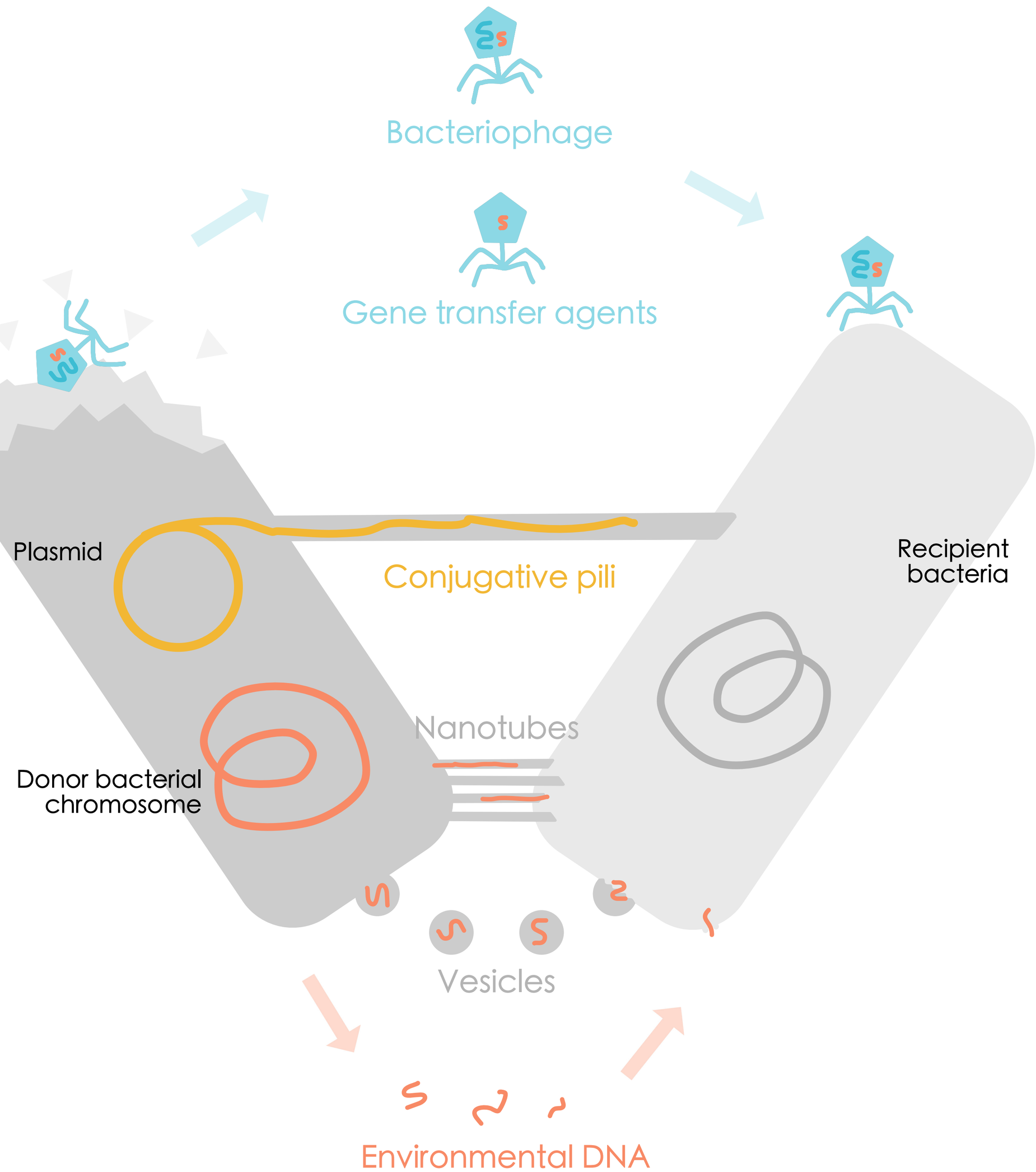
Bacteriophage
Gene transfer agents
Plasmid
Conjugative pili
Recipient bacteria
Nanotubes
Donor bacterial chromosome
Vesicles
Environmental DNA