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MiniReview

The ecology of transfer of mobile genetic elements

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Abstract

The ecological aspects of the transfer and spread of mobile genetic elements (MGE) are reviewed in the context of the emerging evidence for the dominant role that horizontal gene transfer (HGT) has played in the evolutionary shaping of bacterial communities. Novel tools are described that allow a refined analysis of HGT in natural settings. The occurrence of HGT processes in soil and water, as affected by environmental factors, is then discussed. Examples are provided that illustrate how MGE can influence the behavior of microorganisms in their natural habitats. The occurrence of microorganisms as groups of cells in structured communities, such as those found in biofilms, is used as a framework in order to review the data and pose further questions on the evolutionary role and significance of contemporary gene transfer processes in nature. Selection by the environment is likely to be the dominant force in shaping the genetic make-up of bacterial communities. In fact, selective force can act as an apparent accelerator of gene transfer processes, mainly as a result of the enhancement of survival and persistence of favorably selected products of gene transfer processes (genes, metabolic pathways, microbial cells and communities). However, the current understanding of the triggering and impact of HGT in nature remains limited by our lack of understanding of the very nature and variety of the selective forces that act on microorganisms in situ. Hence, the relevant questions with respect to these triggers acting in natural habitats need to be answered using advanced approaches for studying HGT processes in nature, such as those discussed in this review.

Keywords: Gene transfer; Mobile genetic element; Ecology

1. Introduction

Horizontal gene transfer (HGT) between bacteria can be defined as the non-parent-to-offspring exchange of genetic material between donor and recipient cells. During the 1930s and 1950s the three essential mechanisms for gene transfer, transformation, transduction and conjugation, were discovered. The involvement of mobile genetic elements (MGE) such as bacteriophages, plasmids, and transposons in these processes has since gained wide acceptance [1]. It is currently apparent that these MGEs provide key vehicles for gene transfer between bacteria [2] and contribute directly to their evolution and, potentially, to bacterial speciation [3–7]. MGEs have also been linked, in recent times, to the spread of adaptive and symbiotic traits involved in host survival [8,9]. Thus, a significant amount of information on the microbiological and molecular mechanisms of gene transfer has been assembled. However, although data on gene transfer processes in natural habitats have accumulated, the direct ecological and evolutionary impact of these processes has often remained elusive [10].

The first exploratory studies on HGT between bacteria in natural or semi-natural habitats were performed in the 1970s (e.g. [11,12]). Since that time, there has been a progressive increase in the number of studies addressing HGT in the environment, using microcosm-based and field studies [10]. Until relatively recently, the majority of such studies have focussed on determining how key environmental factors such as temperature, moisture, nutrient availability, and the presence of grazing, competing or antagonistic organisms, affect the rate at which gene transfer processes take place [10,13]. The basic conjecture here was that these ecologically highly relevant factors could act as determinants of natural gene transfer rates, and thus, if under-

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stood, could help in predicting the frequencies with which HGT occurs [13]. A main impetus to the field was also given by societal questions about the impact of genetically modified organisms in nature as well as on the spread of antibiotic resistance genes in natural habitats. However, the intricacies of HGT processes in natural settings, including the in situ phenomena of signalling, recognition, cell-to-cell and cell-to-MGE contact, the molecular triggers in these processes and their dependence on the environment, and the way in which these processes impact the microbial communities in their adaptation to natural habitats, has received much less attention.

The development, over the last decade, of a large range of advanced molecular techniques applicable to microbes in natural settings [14] has enabled researchers to interrogate environmental gene transfer processes at much more refined levels of resolution than ever before. For instance, the information contained in complete sequences of plasmids [15] or bacterial genomes (e.g. [16]), when employed in DNA microarrays, allows the study of the expression of specific plasmid and/or host genes in relation to HGT in the natural habitat. In addition, the resolving power offered by reporter gene technology, in particular on the basis of the green fluorescent protein (GFP), has allowed detailed analyses of the patterns of gene transfer in microcolonies or biofilms to be made [17]. The impetus of these developments in fostering our understanding of environmental HGT and its impact on natural microbial communities is only beginning to emerge.

This review examines the current understanding of HGT processes in natural settings. We briefly review the impact of novel methodological developments and the effects of key environmental factors on HGT in soil, the phytosphere and aquatic environments. We then focus on the role of MGE, the importance of structured microbial consortia and of selective forces, and how these affect lateral gene transfer processes and adaptive responses of bacterial communities.

2. Tools

Table 1 shows an overview of approaches to studies on environmental gene transfer, including traditional as well as more advanced strategies. Traditional studies on gene transfer in the environment have mainly relied on cultivation-based techniques, by which donor, recipient and transconjugant, transductant or transformant colonies have been detected following their dislodgement from the environmental setting (reviewed in [13]). Key to the success of these methods has been the ability to select for donor, recipient and transconjugant cells using a combination of appropriate markers, including antibiotic resistances [13,14]. In addition, researchers investigating HGT in natural habitats have complemented these approaches with a range of molecular methods such as PCR typing, DNA:DNA hybridization, and sequencing, and applied them directly to habitat-derived DNA or RNA [14,18,19] and to bacterial isolates. Recent developments in bioinformatics have led to the analysis of the sequences of MGE and bacterial hosts [15,16]. A major objective has been the identification of specific sequence signatures and functions relevant to HGT and the persistence of MGE as they respond to environmental conditions or triggers. Novel methods, such as the reverse transcription-PCR analysis of environmental mRNA followed by hybridization, or DNA-based hybridization using microarrays, should ultimately facilitate the study of the expression of specific plasmid, phage, transposon or host genes in relation to HGT in the natural environment. Thus, the distribution and activity of genes and transcripts can be determined to identify processes associated with the interaction between MGE, bacteria and the environment. However, for the study of environmental samples, these methods are by definition system-disruptive, that is, they all rely on the prior extraction of material (DNA, RNA or bacterial cells) from the environment.

By contrast, another suite of techniques has been developed that allow direct in situ observations to be made on HGT and selection in a non-disruptive manner. These approaches have been made possible by exploiting the potential of fluorescent markers, such as the *gfp* gene, for studying the self-transfer or mobilization of plasmids [17,20,21]. The studies by Molin et al. [20–23] have been pivotal in this respect. In particular, the in situ monitoring of plasmid transfer and microbial community physiology

Table 1
Conceptual approaches for studying environmental gene transfers

Type/level[a]	Method[a]	System	Examples [references]
I – Direct, disruptive	Extractive, cultivation-based coupled to molecular analysis	Microcosms	Plasmid transfer in soil and rhizosphere [11,29,30,33–36,44,47] Transformation in soil and plant [42,43]
II – Direct, disruptive	Extractive, cultivation-based coupled to molecular analysis	Field: (soil)	Plasmid transfer in the phytosphere [10,25,32,46]
III – Retrospective	Plasmid isolation, PCR, sequence analysis	Field: soil, aquatic, clinical	Detection of (sequences of) genetic elements providing evidence of gene transfer (potential) [3,5,9,18,19,39]
IV – Direct, non-disruptive	Donor-repressed *gfp*	Biofilm, marine	Detection of plasmid transfer in biofilms [17,20,22]

[a]Approaches are divided in direct experimental and retrospective (indirect) approaches. The direct approaches can be based on a system-disruptive (extractive) or a non-disruptive (*gfp* based) methodology.

in structured microbial communities (through the combined use of fluorescent reporter systems and confocal laser scanning microscopy) has provided a greater understanding of these complex processes [17,22]. Using molecular tools, such as the monitoring of the expression of unstable fluorescent reporter proteins introduced into chromosomes or on MGE, in conjunction with assembled biofilm communities, plasmid transfer and cellular activity could be directly recorded [23]. These in situ observations of gene transfer processes underpin the advances in our knowledge base beyond earlier efforts that were largely system-disruptive [10]. For example, the use of a donor strain containing a plasmid carrying a *gfp* gene that is repressed for expression in the donor genotype provides the means to screen for transfer of the plasmid to non-culturable bacteria where the *gfp* is expressed. To date, however, no direct evidence has been produced to support this assumption (Hermansson, pers. comm.).

Recently, the adaptation of in vivo expression technology (IVET), developed for plant-associated bacteria [24], to studies on plasmid gene expression in soil and the phytosphere was described ([7]; Fig. 1). This approach is an extension of a promoter trapping system in which recombinants are screened for the complementation of an essential growth factor (EGF). Chromosomal deletions of a gene involved in the biosynthesis of an EGF, i.e. diamino pimelic acid or pantothenate, were obtained. Thus, organisms with this deletion would only grow when the missing substrate is added or when the intact gene is introduced into the cell. Vectors (denoted pIVET), were constructed that carry a promoterless EGF biosynthetic gene. In this example, libraries of an environmental plasmid, pQBR103 [7], were constructed by insertion of small fragments of pQBR103 upstream of the promoterless EGF gene, and host bacteria, e.g. *Pseudomonas fluorescens* SBW25ΔESG (pQBR103), were transformed. A pIVET vector-library insertion duplicates the promoter and does not interrupt the function of the gene in the plasmid. Environmentally induced promoters are selected for, as the in vivo-expressed EGF is required for growth. A second promoterless marker, *lac*Z, was also included as an in-frame fusion downstream of the promoterless EGF on pIVET. It is possible to differentiate between promoters active only at the plant surface or in soils and those that are active both on plants and in vitro using isolation agar containing X-gal and the EGF. Plasmids such as pQBR103 appear to carry a number of genes that are uniquely expressed in the phytosphere at different periods of colonization during plant growth. These genes are distinct from those similarly expressed by the host bacteria [9,24] and share little or no homology with the sequences available in data bases. These genetic data support previous data that plasmids can provide periodic fitness advantages to their hosts [25]. The challenge is to identify the phenotypes of these plasmid-associated, ecologically significant genes and the contribution they make to host ecology and evolution.

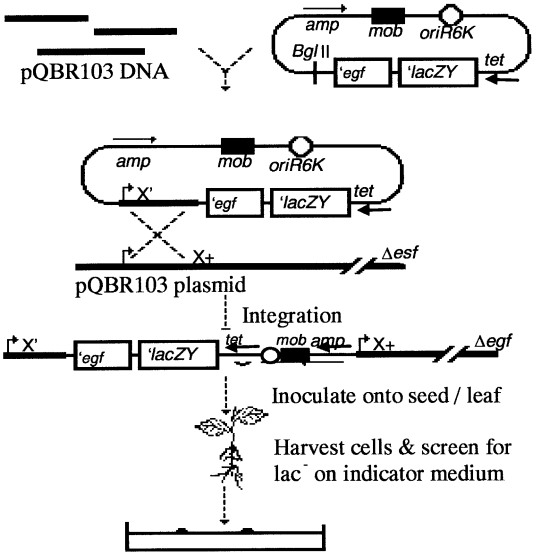

Fig. 1. A random plasmid or genome fragmented library of ca. 1–1.5 kb generated by partial digestion (*Sau*3A) or by sonication of genomic DNA is inserted into the cloning site upstream of the promotorless *egf* gene (figure adapted from [7]). Following library construction in pIVET-EGF, the recipient bacteria are transformed. In the example discussed here, the library has been constructed from fragmented DNA of the purified mega-plasmid pQBR103 (330 kbp, tra$^+$ [25]) and transferred to the host bacterium *P. fluorescens* SBW25-Δ*egf* carrying pQBR103. Recombinants are selected on plates containing tetracycline (vector) and mercuric chloride (pQBR103 phenotype). In vitro expression is determined by the presence of blue colonies on media containing X-gal. The pool of pIVET-library fusions were introduced directly onto seeds, leaves or roots of sugar beet. Growth (and colonization) can only occur where the EGF is genetically complemented as a result of a plant-induced (or plant-environment-induced) pQBR103-plasmid promoter activating transcription of the promoterless *egf* gene. In order to select those promoters induced specifically during colonization of the plant (and not in vitro), bacteria are recovered from the phytosphere and plated on minimal X-gal indicator medium supplemented with the EGF and tetracycline (this enables SBW25Δ*egf*(pQBR103::pIVET-EGF). Blue colonies, which display a lacZ$^+$ phenotype, are ignored (because the promoters remained active in vitro), but those which are lacZ$^-$ (white) are likely to contain fusions to plasmid genes and their promoters activated on plants. These are then isolated, sequenced and characterized.

3. Ecological factors affecting HGT processes in natural environments

The majority of natural environments – soils and aquatic systems – are often very restricted by the abundance of resources for microbial growth, which can severely limit population densities and activity. This, in turn, restricts those microbial processes that are dependent on density and activity, such as all HGT mechanisms [10,13]. However, a number of particular sites in these natural habitats, mostly related to soil or plant surfaces or surfaces in aquatic environments, have been shown to provide conditions for bacterial colonization, mixing and

activity, resulting in the occurrence of locally enhanced densities of active cells. These sites are often conducive to HGT processes [10], and have been denominated 'hot' spots for bacterial gene transfer activity. The conditions in these hot spots that together determine the HGT-conduciveness can be dissected into single factors, divided into abiotic (e.g. temperature, pH, moisture content, micro- and macronutrient availability, presence of surfaces, O_2) and biotic factors (grazing, predatory, antagonistic, competing or syntrophic organisms and plants). As natural environments are heterogeneous and dynamic, conditions vary in time and space. The conditions that reign locally at each point in time control HGT processes.

The key abiotic and biotic factors that affect the extent of HGT in hot spots in natural settings have been reviewed [10]. It is difficult to predict the extent to which these factors affect natural conjugation, transduction and transformation, as they have differential effects under different other conditions. A range of hot spots for HGT processes in soil and aquatic environments [26] is presented in Table 2, and discussed in detail below.

3.1. Factors affecting HGT in soil and phytosphere

The presence of large surfaces composed of mineral and organic phases in soil plays a key role in determining the physiological status of soil-dwelling bacterial cells. Soil is heterogeneous with regard to the distribution of gaseous, liquid or solid compounds [27]. Clay-organic matter complexes are important sites for soil microorganisms, due to their negatively charged surfaces and enhanced nutrient availability [27]. Availability of water in soil is a second important factor driving microbial activity. In bulk soil, bacterial cells occur mainly adsorbed to surfaces, which often results in microcolonies that are refractory to movement or to contact with cells at different locations. Hence, most bacterial cells in soil can interact only with partners in their immediate vicinity. Conditions that reign locally, i.e. at the level of the site where bacterial cells are localized, will affect the cells and their involvement in HGT. In spite of the grossly nutrient-poor status of soil [10,13], nutrients can become concentrated in hot spots (Table 2), primarily plant phytospheres, decaying organic material of animal or plant origin, and guts of soil animals like earthworms [28] and *Collembola* [29]. The rhizosphere of many plants represents a region in soil with a (transient) high availability of organic carbon and potentially also N, P and S. Moreover, water flow in soil induced by plant roots may enhance bacterial movement. Both mechanisms promote cellular activities and cell-to-cell contacts. Moreover, above-ground plant parts (the phyllosphere) can also provide nutrient-rich surfaces, resulting in similar hot spots [30]. Further, as mentioned in the foregoing, the guts of a range of soil animals represent another class of hot spots, as the mixing of cells and MGE is enhanced, cells are activated and cell-to-cell contacts are stimulated [28,29]. Finally, the importance for HGT processes of easily available substrate in soil, such as those provided by manure, has been indicated by Götz et al. [31].

Thus, soil, on the one hand, poses physical barriers to cell-to-cell contacts and nutritional limitations, whereas, on the other hand, nutrient upshifts and alleviation of translocation or contact barriers may be found in soil hot spots. HGT rates in soil are certainly affected by the combination of these phenomena.

3.1.1. HGT in soil – evidence for the involvement of hot spots

Numerous investigations have shown that the rhizosphere is indeed a major hot spot for bacterial activity and increased gene transfer when compared to bulk soil [10]. This may be due to a number of factors, for example the enhanced nutrient input and water fluxes in the rhizosphere may stimulate bacterial metabolic activities and the elicitors of conjugative plasmid transfers between rhizosphere inhabitants, such as pseudomonads [32–35]. In addition, the phyllosphere of plants has been shown to be equally conducive to conjugative plasmid transfer [30]. Recent studies have further indicated that the horizontal gene pool in the phytosphere is highly mobile and directly linked to fitness of the host [7,25]. Using a *gfp*-marked plasmid to follow plasmid transfer in situ in membrane

Table 2
Hot spots in environmental settings conducive to gene transfers[a]

Habitat	Hot spot	Mechanism	Key examples in:
Soil	rhizosphere and plant tissue	conjugation	[10,25,32–35]
		transformation	[13,42,43]
	phyllosphere	conjugation	[10,30,35,43]
	manured soil	conjugation	[31]
	guts of soil animals	conjugation	[28,29,44]
Aquatic	epilithon	conjugation	[10,45,48]
	sewage/sludge	conjugation	[10,48]
	sediment	conjugation	[52]
		transformation	[60]

[a]These hot spots are sites of enhanced gene transfer activity, which is often based on an enhancement of cell densities, of cell-to-cell contacts, of cellular movement or activity. They have been extensively discussed in [26].

biofilms, peak transfer rates were recorded, at 1:1000 to 1:1500 cells per hour, for the environmental plasmid pQBR11 (Lilley and Bailey, unpublished). Secondary transfers, from transconjugant cells to other cells, played a minor role in plasmid establishment. Key factors for plasmid establishment were found to be the sizes and activities of donor populations and the effect that plasmid carriage had on host fitness. When the actual host range achieved by plasmid transfer was assessed in colonized plants 74 days after sowing seeds inoculated with donors, a variety of indigenous *Pseudomonas* spp. were shown to have acquired the introduced plasmid [36]. In related field work, the natural associations formed between plasmid and host types were evaluated from different crops, grassland pasture and herbaceous plants. Spatial and temporal variation in genotype for the isolated plasmids was observed. Representatives of single plasmid types were found on a wide range of plants in a background of diverse pseudomonad hosts. The plasmid types exhibited stronger niche and temporal distribution patterns than their patterns of association with hosts. A temporal component was found in the transfer activity and frequency of occurrence for each of the plasmids [37]. This seasonal or plant-development-based transfer activity and selection for the plasmids may be better understood when the traits conferred to their hosts have been identified.

The phytosphere is also a habitat in which HGT by transduction can be stimulated. Stephens et al. [38] first showed that naturally occurring phage was responsible for the decline of introduced pseudomonads in the sugar beet rhizosphere, indicating an enhanced productive infection rate of the strain studied. Mendum et al. [39] recently focussed on the potential for transduction between rhizobia in rhizosphere and soil. Two *Rhizobium leguminosarum* biovar *viciae* bacteriophages, under which transducing phage RL1RES, were isolated. Lysogeny was indicated to provide a common effective phage survival strategy, but phage RL1RES possibly also persisted as a phage particle in soil. Results also indicated that where phage and susceptible bacteria coincide, especially in regions of enhanced growth such as the rhizosphere, infection could occur, making transduction possible. Phages were further suggested to provide a reservoir of bacterial genes in conditions where the host might not survive [39]. On the other hand, Burroughs et al. addressed the dynamics of phage–host interactions in soil using *Streptomyces lividans*, *Streptomyces coelicolor* and actinophage ΦC31 [40]. Strikingly, under the growth conditions of the host in soil, ΦC31 showed a burst size (average number of phage released per bacterial cell) of up to 300 as opposed to 130 found in vitro. Moreover, only recently-germinated spores were susceptible to phage infection in soil. These factors have a profound influence on the frequency of gene transfer and lysogeny, as they indicate that predictions made on the basis of in vitro experiments may actually underestimate HGT frequencies in nature. In a related study, phages obtained from phytosphere bacteria were able to infect different closely related bacteria as they colonized the plant surface. The apparent overlap observed in phage susceptibility was interpreted as a demonstration of an extended gene pool [41]. Interestingly, not only did individual bacterial types have a specific range of phages that infected them, but also did their susceptibility vary temporally over the growing season. These apparent successions in abundance of host bacteria and infecting phage genotypes were recorded for both enterobacteria and pseudomonads. The interaction between bacteria and their phages was the most relevant factor driving bacterial persistence, succession and population density [41].

Transformation can also be enhanced by a range of different compounds exuded by plant roots into soil, as was recently shown by Nielsen and van Elsas [42]. Specific organic acids and amino acids had a significant stimulatory effect on the transformation of *Acinetobacter* sp. BD413. Other plant parts also seem conducive to transfer, as was recently indicated by Kay et al. [43]. The experiments showed that strain BD413, when co-infecting tobacco with the plant pathogen *Ralstonia solanacearum*, was able to capture plant-derived DNA [43]. The presence of homology between the captured DNA and the recipient genome was an absolute prerequisite for this transfer.

The role of soil animals, including *Collembola* and earthworms, as providers of hot spots, has been indicated in the above [28,29]. Specifically, work by Thim et al. [28], following pioneering work of Daane et al. [44], confirmed this hot spot for HGT in soil, by providing evidence that earthworms, in particular *Lumbricus rubellus*, can facilitate the spread of plasmids from *Escherichia coli* to soil bacteria. Marker-tagged plasmids with different transfer properties, i.e. narrow and broad host range replication, conjugative, mobilizable, and non-mobilizable, were used. In microcosm studies with soil at 12°C, transconjugants were only detected in the casts of *L. rubellus*, indicating that gut passage was required for plasmid transfer. Plasmid RP4 marked with a luciferase (*luc*) gene was transferred to indigenous bacteria at higher frequencies than those detected in filter matings. Transconjugants were identified as the following gamma-*Proteobacteria*: *Pseudomonas putida*, *Serratia proteomaculans*, *Achromobacter* sp. and *Stenotrophomonas maltophilia*.

Finally, the addition of organic matter to soil, in the form of manure, was clearly indicated as a stimulator of the persistence of *P. putida* hosts and their mobilization of MGE [31]. This finding identified recent manuring of soil as another 'temporal' hot spot conducive to HGT. When considered together, the above data indicate that HGT, mediated by conjugation, transduction and transformation can be promoted in soil under conditions where host bacterial activity is stimulated. These data also underline the caution needed in the extrapolation of laboratory studies to predict events that may occur to different extents in agricultural or natural environments.

3.1.2. Plasmids conferring gene mobilizing capacity to soil systems

As indicated above, plasmids are key mediators of gene transfer in specific environments in soil [10]. However, there still is a paucity of information on the diversity of these MGE in soil and phytosphere. Depending on the plasmid isolation procedure, different plasmids, with diverse characteristics with respect to Inc group, host range, avidity to transfer and the type of accessory genes present, can be obtained. One of the most effective methods of obtaining plasmids with transfer proficiency is (bi- or triparental) exogenous isolation. These methods capture transfer-proficient plasmids directly from environmental samples into recipient strains that can be grown in the laboratory [45], and have been successfully applied to soil and phytosphere habitats [32,46]. One plasmid, denoted pIPO2, was shown to self-transfer and mobilize IncQ plasmids to a range of diverse Gram-negative bacteria in the wheat rhizosphere in the field [46]. Moreover, plasmids carrying mercury resistance determinants that were able to mobilize IncQ plasmids like RSF1010 were also found [10]; the prevalence of these plasmids was suggested to be enhanced under conditions of mercury stress. Recently, other plasmids obtained by exogenous isolation from soil were tagged with *gfp* and their transfer into soil bacteria observed in vitro [47]. Three plasmids showed high transfer frequencies and very broad host ranges, whereas five others transferred at low rate. Moreover, analysis of the full sequence of plasmid pIPO2 allowed the conclusion that a type IV secretion system probably represents the mechanism behind its conjugational ability [15]. On the basis of specific primers and probes applied to environmental DNA, it was suggested that this plasmid occurs primarily in the rhizospheres of a variety of plants such as wheat, grass, potato and tomato [15]. Although pIPO2 clearly can be a mediator of in situ transfer processes in rhizospheres [46], its role and significance to the host are still unclear. Together, these results point to the natural occurrence of a broad diversity of plasmids that can serve as mediators of HGT in soils and related environments. However, we are far from understanding the full diversity of plasmids in these habitats and the extent of their roles as transfer mediators.

3.2. Factors affecting HGT in aquatic environments

The extent of HGT between bacteria in aquatic systems is similarly related to the presence of hot spots [10]. In spite of the fact that aquatic systems appear to be relatively homogeneous, they do contain specific structured (micro)habitats. Conceptually, aquatic systems can be divided in (1) the free (bulk) water phase, (2) the colonizable suspended matter, (3) sediment or sewage, (4) stones and other surfaces that carry biofilms (called epilithon [48]), and (5) aquatic animals. These habitats offer very different conditions to their bacterial inhabitants. The presence of nutrients as well as colonizable surfaces is particularly important, as such sites are known to support large densities of metabolically active microorganisms [48]. In contrast, bulk water can be a nutrient-poor environment which may induce a (starvation) stress response in bacterial cells. Particular environments such as sewers, with high inputs of organic matter, form an obvious exception to this generalization. On the other hand, suspended particles of varying sizes, as well as sediment and stone surfaces, represent nutrient-rich, more hospitable habitats that support microbial communities. Further, sediments, which are often rich in organic material, typically support bacterial populations exceeding those found in bulk water by several orders of magnitude [10]. Biologically diverse and metabolically active communities can also be found in the epilithon of stones in rivers or lakes [10,48] and within other biofilms that form at solid/water interfaces. Microorganisms within the epilithon are components of the extensive polysaccharide matrix which protects cells and adsorbs dissolved and particulate organic matter from the overlying water. Similar biofilms can be found in the percolating filterbeds used in sewage treatment processes [49]. Finally, a range of aquatic animals provide internal and external surfaces that are colonized by varied microorganisms.

As bacterial hosts are likely to accumulate at surfaces where nutrients are concentrated, their distribution in aquatic systems is generally not even. In particular the bulk water phase may contain relatively few bacterial cells that are able to participate in HGT processes. On the other hand, aquatic systems tend to provide excellent possibilities for mixing of bacterial cells and MGE, and thus, for cell-to-cell and cell-to-MGE contacts. These contacts occur mainly in biofilms at surfaces rather than in the bulk water. The tendency of bacterial cells to stick to suspended particles, to sediment or to stones (epilithon) in aquatic systems may even lead to the development of separate communities, ultimately resulting in the formation of separate species (speciation). Nevertheless, given the capability of many bacteria to occur in either sessile forms in microcolonies or biofilms, or in motile forms, and thus to potentially connect spatially separated biofilms, aquatic habitats provide important sites for cell-to-cell contacts resulting in HGT between bacteria [10,48] (see Table 2).

Using both microcosm and in situ experiments, HGT between different bacterial hosts has been shown to occur in drinking water, river water and epilithon [46,48], lake water, seawater, marine sediment and waste water [10]. HGT appears to be a common process in aquatic environments, particularly in specific niches where nutrients are more abundant.

The ecological role of plasmids in marine bacterial communities and the factors that may contribute to the selection of bacteria that maintain them was recently studied [21,50]. Several transfer-proficient plasmids, exogenously isolated into *P. putida*, were also able to mobilize RSF1010. Detailed studies allowed the detection of in

situ plasmid transfer, at the single-cell level, as the plasmid had been modified to carry a *gfp* gene that was repressed for expression in the donor strain. Transconjugants, resulting from the transfer of a derivative of a natural isolate, pBF1::*gfp*, from *P. putida* to indigenous seawater bacteria, were detected using epifluorescence or confocal laser scanning microscopy [50]. A variety of indigenous bacteria received the plasmid. Gene transfer frequencies were relatively high, at 2.3×10^{-6} to 2.2×10^{-4}. Other plasmids with broad host ranges, which were able to transfer between representatives of the alfa and gamma proteobacteria and the *Planctomycetes*, were also isolated from marine systems; these possibly highlight the prevalence and importance of HGT in this aquatic environment. In a related investigation, the transfer of the broad host range plasmid RP1 was recorded between *Vibrio* cells that had been starved of nutrients by incubation for 100 days in filtered seawater; transfer was not recorded between *E. coli* cells that had been similarly starved [51]. This study indicated that *Vibrio* cells have an energy maintenance mechanism that allows successful transfers to occur long after blocking of the energy resources, whereas *E. coli* does not possess such a system. Thus, the host/plasmid combination is a crucial determinant of transfer rate and success.

4. Ecological consequences of HGT

It is a long-standing dogma that selective pressure is a key factor that can exacerbate the success and impact of gene transfer processes. Such effects of selective pressure are most easily seen in cases in which the MGE transferred confer some type of selective (growth) advantage to their hosts. Top et al. [52] recently reviewed the issue of selection acting on gene transfer in soils. Briefly, several, diverse, lines of evidence have invariably indicated strong effects of selection [52]. Thus, a catabolic plasmid encoding a 2,4-dichloropropionate (DCPA)-degradative gene was shown to be transferred from an *Alcaligenes xylosoxidans* donor to members of the indigenous community in soil, but this was only seen in soil treated with DCPA. In addition, *Enterobacter agglomerans* carrying the self-transmissible biphenyl-degradative plasmid RP4::Tn4371 was introduced as a (non-expressing) donor into soil with or without added biphenyl. The introduced donor strain declined to extinction very quickly. In contrast, indigenous transconjugants belonging to the genera *Pseudomonas* and *Comamonas* able to degrade biphenyl and those carrying RP4::Tn4371 were shown to thrive, but, again, only in the soil that had received biphenyl [52]. Furthermore, the transfer of the 2,4-D-degradative plasmid pJP4 from *Ralstonia eutropha* JMP134 to *Variovorax paradoxus* was only detectable in the presence of high levels of 2,4-dichlorophenoxyacetic acid (2,4-D) in soil [52], and the transfer frequency of pJP4 to indigenous *Pseudomonas* and *Burkholderia* spp. increased as 2,4-D concentrations increased [52]. Interestingly, the donor strain, JMP134, died out rapidly under these conditions. Finally, Top and co-workers attempted to transfer the 2,4-D-degradative plasmids pJP4 and pEMT1 to indigenous bacteria of two different soil layers, defined as horizons A and B [52]. No 2,4-D degradation was observed in uninoculated control soils, whereas inoculation with JMP134, and the subsequent transfer of pJP4, resulted in complete degradation of 2,4-D within 19 days. Proliferation of the new transconjugants formed in soil was noted, which included representatives of *Burkholderia graminis*, *Burkholderia caribensis* and *R. eutropha*. Overall, this work very clearly demonstrated that the products of gene transfer in soil can be stimulated by the presence of driving forces such as a utilizable carbon source, resulting in bioaugmentation [52]. Also, as a part of this study, it was indicated that a naphthalene-catabolic plasmid had transferred in a coal tar-contaminated field site. The acquisition of novel pathways by HGT resulted in the adaptation of the indigenous bacterial communities to utilize the xenobiotic compounds as sources of nutrients [52]. In related studies, evidence has also been provided of a direct role for genetic recombination in the adaptation of bacterial aquifer communities to chlorobenzenes [53]. These and related studies [54] demonstrate the central role that HGT plays in the adaptation of bacterial communities to changing resources and environmental pressures, such as novel substrate utilization, antibiotic resistance and toxin production.

Studies on plasmid ecology performed by Wellington and co-workers have focussed on the impact of selection on plasmid transfer in streptomycete hosts in soil habitats. The spread of selectable traits, in particular drug resistance, was studied. Transfer was detected in soil, and frequencies were affected by the presence of selection for plasmids that coded for antibiotic resistances [54]. These investigations clearly confirmed the key role of the overriding forces exerted by selection for either a utilizable carbon source or a resistance to antimicrobial compounds, in shaping bacterial populations in nature as a result of HGT.

5. HGT as a community phenomenon – advantages to bacterial populations or to MGE?

In spite of the considerable body of knowledge relating to the ecology and genetics of some MGE, we still know relatively little of the processes involved in the role and response of HGT in natural habitats. In particular, the relation of HGT to key natural processes such as biofilm formation, cell-to-cell signalling, habitat sensing and the orchestrated regulation of genes involved in mating pair formation or gene exchange in nature, are enigmatic. In addition, how individuals respond to selective pressures within highly structured microbial communities, such as biofilms, has only recently been investigated in any detail

[55]. These studies provide a practical system to the collective response and behavior of natural bacterial communities in relation to MGE. Current evolutionary concepts dictate that every function in (population) biology has a cost in addition to a potential benefit, and that biological systems evolve towards minimalization of costs and maximalization of (fitness) benefits. Several outcomes are possible, i.e. (i) the host gains a net selective disadvantage from the interaction with the MGE, (ii) the host–MGE interaction remains neutral, i.e. there is no obvious advantage or disadvantage due to carriage of the MGE, or (iii) the host gains a clear net selective advantage from carriage of the MGE. For instance, carriage enables it to actively utilize a specific carbon source or to withstand specific antimicrobial compounds such as antibiotics, and this outweighs the extra energetic costs of MGE maintenance. However, the net outcome of host–MGE interactions may not be predictable as local biotic and abiotic factors, host physiology and the genetic interaction between MGE and host background have a net effect. The natural fluctuations within any ecosystem will impose diverging selective pressures that act on host/MGE combinations and drive them to proliferate or form new associations. As hosts and MGE have probably co-evolved, they may share or complement strategies for interaction and mutual survival. In addition, it is probable that no two host cells are physiologically identical, as populations, even of the same bacterial type, exist as either collections of interacting and structured communities or as dispersed single cells. Such variance in the life cycle of bacterial cells probably results in the largely divergent outcomes observed when cells and MGE interact. These range from the very rapid (infectious) spread of plasmids like RP4 through a bacterial community, which can eventually occupy up to 100% of potential recipient cells in just a few hours under the appropriate conditions, to the apparent reluctance of structured microbial communities to accept exogenous DNA or active MGE. Different populations can respond in varying ways to different plasmids and the outcome is therefore difficult to predict. Hence, the interactions that bacteria exhibit with, and in response to, their environment and upon contact with potential donors of MGE are both intimate and complex.

5.1. HGT from the perspective of an MGE

Whereas the fitness gain from HGT for populations of bacterial cells may often be apparent, there is less clarity about the strategies that have evolved in MGE to assure their self-perpetuation. MGE (plasmids, bacteriophages) will be confronted with the varying strategies, including concerted differentiation events, developed by their potential hosts to colonize and explore the environmental niches they occupy. It seems obvious that MGE, in order to persist within microbial consortia, have developed strategies that enable them to either be replicated and maintained within growing cells in an ecologically relevant way (vertical transmission), or to be transferred at ecologically optimal rates (horizontal transmission), or both [1]. Vertical transmission may be dependent on plasmid replication by plasmid-encoded replicative systems, or on integration into the host chromosome. Horizontal transmission is equally dependent on plasmid-encoded functions, and sometimes requires functions from other sources like the host or other MGE. Individual plasmids have adopted different strategies that facilitate their self-perpetuation, and apply them in accordance with the eco-physiological status of host cells [1,2]. Hence, it is plausible that, at the level of the structure of the host cell community, different types of interactions, including vertical transmissions, horizontal transfers and even integration or excision events, can take place simultaneously.

However, a mechanistic description of the factors that influence the behavior and function of plasmids and their response to the strategies adopted by their host remains unexplored until more sensitive methods are available that allow the study of gene expression and the phenotype of single cells. Hence, novel research is needed to gain an understanding of the genetic solutions developed by plasmids to combat or complement their hosts' response to ensure survival and self-perpetuation. Such advances will certainly require novel and more sensitive tools that will be derived from two emerging fields, (1) the explosive development of bacterial genomics, in which whole genomes, including plasmids, can be sequenced and compared, and (2) the potential offered by the use of promoter trapping and related approaches to evaluate gene expression and determine phenotype.

5.2. HGT in biofilms

There is emerging evidence that microbial life in natural settings mainly takes place in microbial consortia in the form of microcolonies or biofilms rather than as isolated cells [55]. Biofilms or even microcolonies are highly structured communities of cells that co-exist as consortia [55]. In these structured communities, cell-to-cell signalling (quorum sensing), cellular differentiation, perception of the local environment via two-component regulatory systems, and responses to environmental stresses play key roles in determining the behavior of individuals and the community as-a-whole. Thus, interdependency of the role and activity of free cells, cells in biofilms or those in microcolonies produces populations that are morphologically, physiologically and even genetically very different [56]. For instance, a biofilm consisting of *P. putida* cells, once established on a glass slide, differentiated into several sections, each one of which could support distinct morphotypes (Haagensen, unpublished). Subsequent challenge of the biofilm with sublethal concentrations of the antibiotics kanamycin and tobramycin, followed by vital staining, revealed that the biofilm consisted of a heterogeneous

collection of quiescent to metabolically active cells that defined both the age and the structure of the developing biofilm.

The community lifestyle of most microorganisms in biofilms should be taken into account when generalizing the role and interaction of host and MGE, particularly those linked with HGT processes. In fact, the presence of plasmids in bacterial cells may even stimulate cellular aggregation, leading to biofilm formation [57]. An as yet conceptual model dictates that in young biofilms, HGT mediated by conjugation is related to the growth of donor and recipient colonies [58]. Predictably, nutrient concentration was a key determinant of HGT rates. However, more complex models are needed to describe activity and HGT in respect of the physiological and spatial heterogeneity typical of more mature biofilms. Christensen et al. [17,20], studying the spread of a gfp-marked TOL plasmid in a mature biofilm, noted that transfer of the plasmid was confined to the outer (upper) regions of the biofilm which presumably coincided with the location of cells with the greater metabolic activity. Cells deeper in the biofilm did not act as plasmid recipients. In such mature biofilms, HGT may, thus, depend on the physiological state of donor and recipient cells, on the ability of cells and plasmids to move within the biofilm or on the ability of bacteria to perceive local conditions to either attract or repel transfer.

We know little of the factors that promote or repress HGT in natural settings, or whether such functions are co-ordinated between the complex of recipient, donor and plasmid. If they are co-ordinated, do control mechanisms act on the population or only on the individual cells that represent the mating aggregates? Are there basic principles in respect of the potential recipient that hold for transduction and transformation as well as conjugation? For example, HGT between streptococci is dependent on local signalling and perception of the cell's environment mediated by a small peptide analogous to a pheromone in function [59]. Plasmid transfer is thus a highly orchestrated event, involving multiple partner cells at least in the signalling phase. To further illustrate this point, in the stimulation of Ti plasmid transfer between *Agrobacterium* cells, apparently the *traR* product, which is regulated by opines, plays a central role (Farrand, pers. comm.). An autoinducer co-acts with the *traR* product in the induction of transfer, and thus, cell density is crucial for transfer. This complexity of the regulatory circuits of Ti plasmid transfer gene expression demonstrated that further understanding of gene transfer requires the study of the signals that regulate the expression of conjugative systems.

It is apparent that cellular perception and response in/to the environment dictate HGT as a consequence of the physiological state of donor and recipient cells. To be able to predict how and whether such events occur and how frequently they take place, it is essential that studies be undertaken in situ. Bioinformatics can provide insight in respect of the conservation of sequence motifs or related functions. For example, it is tempting to speculate that the *Agrobacterium* transfer system is widespread and has developed primarily for transfer of mobile genetic material, but how certain can we be of this? The transfer apparatus encoded by the Ti plasmid involves a type IV secretion system, and similar systems have been found on other environmentally isolated conjugative plasmids like pIPO2 [15]. However, type IV systems are also common in bacterial pathogens, and may reflect a common process for cell-to-cell location and the export of large biomolecules. Is the involvement of such systems in gene transfer processes a colateral or an 'intended' (selected) effect of evolution?

6. Concluding remarks

To elucidate and predict the outcome of gene transfer processes in nature, it is required that consideration of the ecological principles that affect transfer, such as temperature, host nutrient status, cell-to-cell contact rates and selective pressure be supplemented by other measures at a more refined level. These include assessments of how bacterial host cells sense the environment and respond accordingly, how they interact with other similar or dissimilar cells (by mechanisms such as signalling, competition, antagonism, predation or parasitism), and how these community-level processes affect HGT. In addition, and perhaps most importantly, there is a need to identify and define the fitness-affecting traits carried by the horizontal gene pool. This potential is largely still cryptic and may pertain to functions that are difficult or impossible to mimic in the laboratory, yet are of extreme importance for the survival of the host and MGE alike. On the other hand, the combination of molecular biology, genomics and ecology is bound to reveal many of the best kept plasmid secrets. For example, novel gene clusters containing open reading frames, which probably encode small proteins of unknown function, have been described on many plasmids and chromosomes (e.g. [15]). It may well be that these proteins play a vital role in the cell-to-cell contact mechanism leading to gene (plasmid) transfer in the natural habitat. Alternatively, they may function in the interaction between the plasmid host, with the rhizosphere as its preferred niche, and the plant, and plasmid carriage may thus be advantageous to the host dwelling in this natural setting. This is supported by the observations that the carriage or transfer of large plasmids by *Pseudomonas* spp. appeared to be advantageous only at a very particular period during growth of the host plant [25]. This may well suggest that carriage of these plasmids and expression of plasmid functions promote fitness of the host under these conditions. Thirdly, plasmid pSym carriage was shown to represent a disadvantage in rhizobia surviving in bulk soil in the field, yet to enhance fitness in the rhizosphere [9]. This pointed to a function other

than just nodulating capacity as the mechanism behind the fitness-enhancing trait. These three observations open up very interesting leads for future fundamental research on the ecology of plasmid transfer and maintenance. In particular, there seems to be a much more intimate relationship, in ecological terms, between plasmid transfer and host fitness than previously thought. In other words, the mechanism and triggering of plasmid transfer may have very intricate links to host fitness and/or sensing of conditions in the natural habitat. Finally, it is axiomatic that the accessory traits carried by MGE, that (positively) affect host fitness, are of ecological and evolutionary importance. However, we need to better understand the ecological aspects of plasmid transfer, particularly in respect to the local conditions, compounds or signals that stimulate plasmid transfers and how MGE and hosts interact in response to these stimuli. Resolving those environmental conditions that affect cellular metabolism and cell-to-cell contact remains the key focal point for many researchers. Therefore, the application of new molecular tools, such as reporter genes, DNA arrays and bioinformatics, will allow the study of in situ expression of transfer-related genes, thus shedding more light on the factors in natural habitats that trigger the events leading to cell-to-cell contact (mating pair formation) and plasmid (gene) transfer.

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