Horizontal Gene Flow in Microbial Communities

The dynamic microbial gene pool compensates for microbial species clonality, presenting us with both threats and promises

Tamar Barkay and Barth F. Smets

Genetic exchanges among prokaryotes, formerly considered only a marginal phenomenon, increasingly are being viewed as profoundly affecting evolution. Indeed, some researchers argue for utterly revamping our concept of microbial speciation and phylogeny by replacing the traditional "tree" with a newer "net" to account for these horizontal transfers of genes (see p. 401). This conceptual ferment is occurring while molecular biologists reveal how horizontal gene transfers occur even as microbes protect the integrity of their genomes. Other studies reveal the number and diversity and abundance of genetic elements that mediate horizontal gene transfers (HGTs) or facilitate genome rearrangements, deletions, and insertions.

Taken together, this information suggests that microbial communities collectively possess a dynamic gene pool, where novel genetic combinations act as a driving force in genomic innovation, compensating individual microbial species for their inability to reproduce sexually. These microbial genomic dynamics can present both environmental threats and promise to humans.

One major threat, for example, comes from the spread of antibiotic resistance and virulence genes among pathogenic microbes. Another less-documented issue involves transgenic plants and animals, whose uses are being restricted because of concerns that genes may be transferred to untargeted organisms where they might cause harm. A possible benefit from HGT comes from its potential to enhance the functional diversity of microbial communities and to improve their performance in changing or extreme environments. Such changes might be exploited, for example, as part of efforts to manage environmental pollution and might be achieved by spreading genes into resident microbes to confer specific biochemical activities.

Discrete Steps Needed for Stability of Gene Transfer

Stably incorporating horizontally transferred genes into a recipient genome involves five distinct steps (Fig. 1). First, a particular segment of DNA or RNA is prepared for transfer from the donor strain through one of several processes, including excision and circularization of conjugative transposons, initiation of conjugal plasmid transfer by synthesis of a mating pair-formation protein complex, or packaging of

- Microbial communities possess a dynamic gene pool, and novel genetic combinations act as a driving force to shape genomes.
- The newly realized extent and importance of horizontal gene transfers will need to be integrated with 20th-century explanations of Darwinian evolution.
- Horizontal gene transfers can affect human and environmental health and productivity— for example, by enabling pathogens to develop resistance to antibiotic treatments.
- Little is known about accessory and ORFan genes, even though they likely are involved when microbial communities adapt to changing environments.

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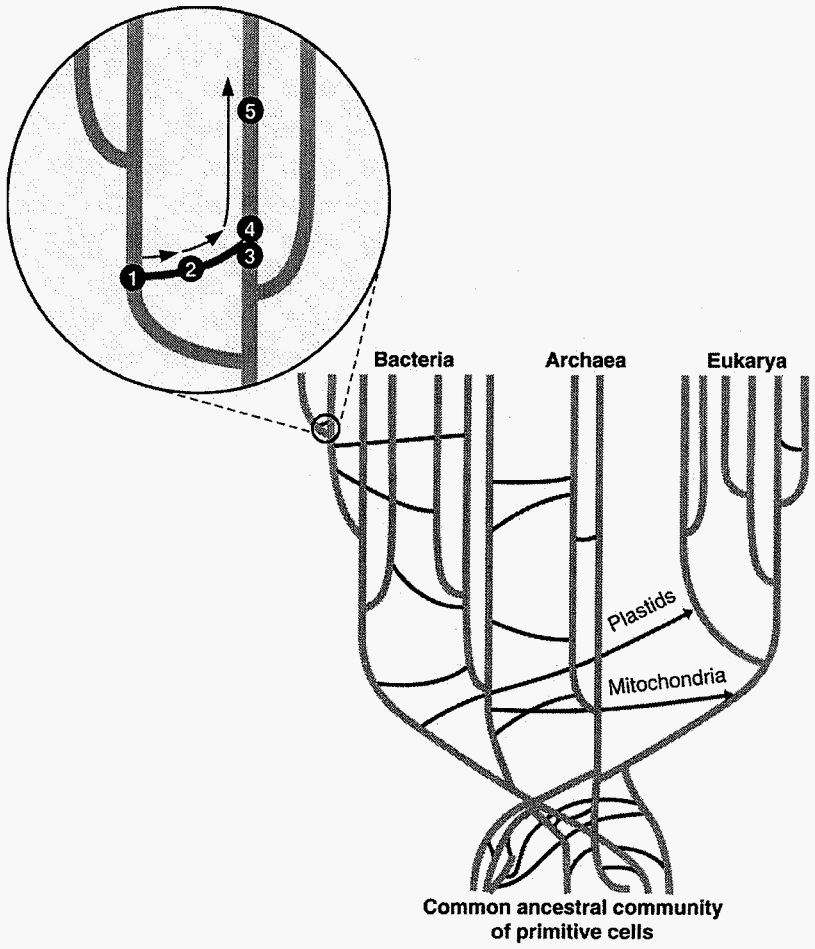
nucleic acids into phage virions. Next, the segment is transferred either by conjugation, which requires contact between the donor and recipient cells, or by transformation and transduction without direct contact.

During the third step, genetic material enters the recipient cell, where cell exclusion may abort the transfer. Otherwise, during the fourth step, the incoming gene is integrated into the recipient genome by legitimate or site-specific recombination or by plasmid circularization and complementary-strand synthesis. Barriers to transfer during this step come from restriction modification systems, failure to integrate and replicate within the new host genome, and incompatibility with resident plasmids. In the final step, transferred genes are replicated as part of the recipient genome and transmitted to daughter cells in stable fashion over successive generations.

Researchers from different disciplines tend to focus on specific stages within this five-step sequence. Thus, evolutionary biologists who examine microbial genomes for evidence of past transfers tend to look at HGTs from the perspective of step five. Molecular biologists are more likely to examine the details of the transfer events, while microbial ecologists look more broadly when they describe the magnitude and diversity of the mobile gene pool, sometimes called the mobilome.

We believe that these perspectives need to be integrated if we are to develop an understanding of how HGT affects microbial speciation, diversity, and competence across the multitudes of ecological niches found on Earth. Indeed, embracing HGT may force us to revisit and revamp 20th-century efforts to explain Darwinian evolution in the context of Mendelian genetics, particularly its central tenet regarding the vertical inheritance of genes.

FIGURE 1



Horizontal gene transfer and its impact on the evolution of life is represented by a web connecting bifurcating branches that complicate, yet do not erase, the tree of life. The inset illustrates the continuum of events that lead to the stable inheritance of a transferred gene in a new host.

What Genomes Say about HGT and Its Impact on Microbial Communities

Comparing microbial genomes for evidence of HGT is a retrospective approach, detecting such events based on marks they made on DNA sequences and on gene distributions, deletions, and insertions. Also, the presence of conflicting phylogenies between specific genes and a consensus phylogeny representing the majority of the genes in the genomes, when supported by robust statistical analyses, suggests inheritance of the former by HGT. The presence of atypical genes—those deviating in their codon usage patterns or G+C content—may indicate transfers so recent that the protein synthesis machinery of the new host has not yet affected these biases.

While such studies are consistent with rampant HGT, their implications for microbial spe-

For Barkay, Science Combines Dreams, Craftsmanship, and Love of Nature

Asked about what she finds appealing about science, Tamar Barkay mentions its "element of dream," her term for the creative process of developing and testing ideas to reveal the unrecognized or unknown. "Science is full of discoveries, driven by such 'dreams,' and I believe that, without the dream part, we would not know as much as we do," she says. "The important point is to keep on dreaming, even if 9 times out of 10 you either cannot experimentally test your idea—or you find out it is wrong. As long as we can master the means to examine our ideas experimentally, the only limit to where science can take us is our imagination."

Today Barkay's scientific reveries take shape in the Department of Biochemistry and Microbiology in Rutgers University's Cook College, where her research focuses on interactions of microbes with mercury and on horizontal gene transfers among microbes and their role in natural microbial communities. Her work often deals with microbial transformations that affect mercury.

Barkay was born on a kibbutz in the northern part of Israel, where her parents still live. At age 14, she began working with dairy cattle, an experience that, when she completed her military service several years later, prompted her to enter agricultural school. "All kids on the kibbutz at that time were to spend a couple of hours a day helping out on the farm, or in providing services to the community," she explains. "I simply chose the cows instead of working in the kitchen, or other such duties."

During her sophomore year in college, she became "totally enchanted" with biochemistry and genetics, particularly when the two were merged to describe gene expression and protein synthesis. "I was struck by the beauty of it all, by the logic and efficiency of how such complex processes work," she says. "I could see how the two fields complement and feed each other—remember that this was in the '70s, when molecular biology was in its infancy."

When an opening became available in the department of plant pathology, she joined as an undergraduate assistant. "From the beginning I liked the combination of intellectual activity with craftsmanship, and with the love of nature which, to me, are at the core of what I love about my profession," she says.

Barkay is one of three daughters of a historian—her father—and a teacher—her mother, who, since retiring, has owned a small bookbinding shop. There are no other research scientists in the family; one sister develops computerized education systems, and the other is a psychotherapist.

Barkay says that her attraction to science came relatively late. "I wish I could say that I knew that I wanted to be scientist since kindergarten, but really this was not the case with me," she says. "In fact, up until I finished high school, I was totaly focused on the social sciences, and I still remember how much I dreaded lab classes, during which I would wander around the lab being totally bored."

Nevertheless, she became interested in the academic life dating from a 9th-grade class visit to Hebrew University in Jerusalem. "When the tour guide asked who among us kids was planning to go to university, I was the only one who raised her hand," she recalls. "One has to realize that, at that time, you were not expected to become a scholar if you were born on a kibbutz and, indeed, only a few among the 40 students in my high school class ever pursued higher education."

She received her B.Sc. degree from the school of agronomy at the Hebrew University in Rehovot in 1974, followed by her M.Sc. in environmental health from the Hebrew University in Jerusalem two years later. She left for the United States soon after, and has been here ever since.

"When I was about to finish my master's degree, I wrote to about 12 people asking if I could work with them for a limited period of time," she says. "Rita Colwell, one of only two who responded, wrote: 'I have money for you for six months, if you want to come, come.' Those initial six months stretched—first to five years, during which I finished my Ph.D. at the University of Maryland—and now to almost 30 years."

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ciation are hotly contested. Not all genes are treated equally by HGT. For annotated open reading frames (ORFs), there seems to be a relationship between predicted phenotypes and degree of inheritance by HGT. Accessory genes, those often found in the mobilome, are trans-

ferred often. These genes are subject to strong selective pressure, undergo frequent transfers, and often do not produce phylogenies that can be related to those of their host genomes.

Among genes that are less frequently transferred than those in the mobilome are those encoding major metabolic functions, such as the synthesis of amino and fatty acids. These "operational" genes, in turn, have more frequently evolved by HGT than have "informational" genes, those encoding DNA replication, transcription, and translation. This distinction between HGT of operational and informational genes was developed by Jim Lake and his collaborators at the University of California, Los Angeles, who analyzed 312 groups of orthologous genes in four bacterial and two archaeal genomes. Informational functions are mediated by complex structures, such as ribosomes, requiring interactions among large numbers of proteins, while operational functions involve proteins that partake in fewer interactions. Hence, the impact of HGT on the evolution of a particular gene is related to the complexity of the interactions of its product.

Gene transfers are more likely when they occur between closely related organisms with similarities in genome structure and the mechanisms that control recombination, replication, and gene expression, according to Jeffrey Lawrence and his collaborators at the University of Pittsburgh in Pittsburgh, Pa. However, this barrier may be weakened in mutator strains having a defective DNA mismatch repair, according to Jeffrey Townsend, now at the University of Connecticut in Storrs.

Lake and his group used parsimonious tree reconstructions for 20,000 genes drawn from the genomes of four bacteria and four archaea to show that organisms that share ecological niches are more likely to exchange genes than those that do not. The discovery of archeal genes in the genome of the thermophilic bacterium *Thermotoga maritima*, and conversely, the extensive presence of bacterial genes in the genome of the mesophilic methanogen *Methanosarcina mazei*, support the importance of a shared ecological niche as a boundary to HGT.

Other evidence for the extensive role of HGT in shaping microbial communities comes from environmental metagenome sequencing projects. For example, Forest Rohwer and colleagues from California State University in San Diego find many mobile elements of archaeal and bacterial genes in environmental viral genomes (step 2 in Fig. 1). The involvement of phage-mediated HGT and recombination is also suggested by phage signatures and frequent recombination sites in the metagenome of an acidophilic community currently assembled by Jillian Banfield of the University of California, Berkeley. Likewise, the genomes of the Sargasso Sea community include several large plasmids, according to Craig Venter of the J. Craig Venter Institute in Rockville, Md., and his collaborators.

Glossary

Accessory genes: Genes that specify functions that are required under unique conditions rather than for the general metabolism of the organism. Often such genes encode for resistance to antimicrobial agents, utilization of exotic growth substrates, or pathogenic traits.

Operational genes: Genes encoding central metabolic functions such as the synthesis of macromolecules, cell wall components, secondary metabolites, and energy production.

Informational genes: Genes specifying information storage and processing, such as DNA replication, transcription, and translation.

ORFan gene: An open reading frame whose putative product has no homolog in databases and whose function is unknown.

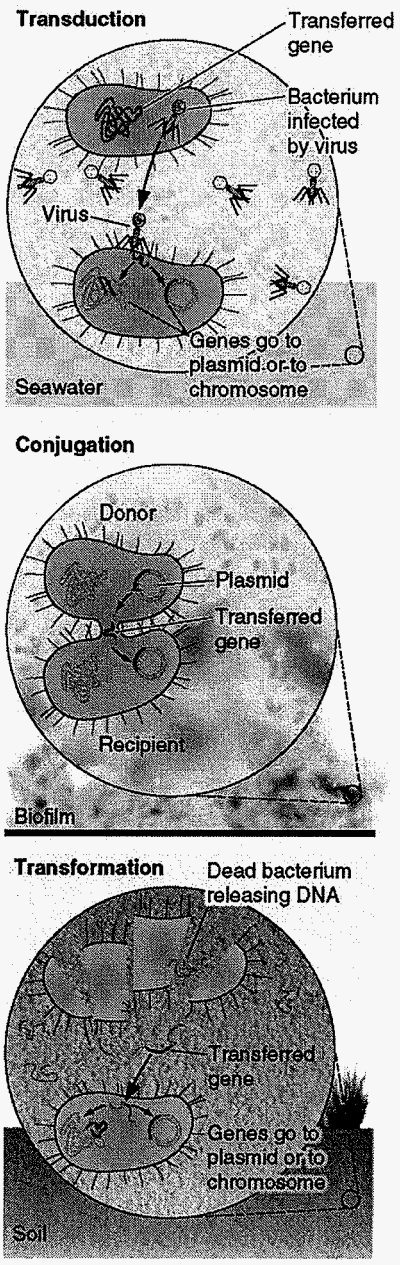
Transferant: An organism whose genome has been modified by the acquisition of foreign DNA or RNA regardless of the mechanism by which this gene transfer occurred. It is inclusive of several terms, including transconjugant, transformant, and transductant.

Mobilome: That part of the genome that is intrinsically mobile (e.g., plasmids), can easily be made mobile (e.g., integrated conjugative elements, genomic islands), or is the result of identifiable horizontal transfer event (e.g., genomic island).

How Are Genes Transferred within Communities?

One approach for measuring HGT within microbial communities is retrospective and does not directly address the dynamics of the HGT

FIGURE 2



The three major mechanisms of HGT as they occur among microbes in the environment. Environments where each process has been documented and considered to affect microbial community fitness are depicted.

process. A second approach detects ongoing HGT in intact or manipulated microbial communities, including conjugal plasmid transfer from donor bacteria to recipients in microcosms (Fig. 2). For instance, *Acinetobacter* spp. serve as model recipient organisms for both intra- and interspecies gene transfers by transformation involving biofilms and simulated soil communities. Other studies suggest that competency—the phase during which cells can take up exogenous DNA—is much more common than was once surmised, highlighting transformation as a mechanism of HGT in the environment. Meanwhile, transduction is another process leading to genetic innovation in aquatic and terrestrial environments where viral abundance exceeds that of the prokaryotic biomass by 10 to 1.

Other efforts focus on compiling inventories of the environmental mobilome. For example, John Fry from Cardiff University "captures" conjugal and mobilized plasmids from microbes in undisturbed communities by "seeding" their environment with marked recipient strains. Others use this approach to collect novel plasmids from various environments. Similar tools are used to capture transposons and integrons. The emerging picture shows an immense, possibly ecosystem-specific, diversity. For example, Patricia Sobecky and collaborators at the Georgia Institute of Technology in Atlanta describe novel origins of replication in plasmids from marine bacteria. Likewise, Hatch Stokes and his collaborators from Macquarie University in Sydney, Australia, studying integrons in soil DNA extracts, suggest that these elements are key players in the evolution of microbial communities. How these elements may modulate microbial evolution is exemplified by the Tn*4371* element in *Ralstonia* sp. A5, which was recently reclassified as a genomic island by Adriane Toussaint and coworkers at the Université Libre de Bruxelles in Belgium. This 55-kb element carries genes for biphenyl and chlorobiphenyl degradation (*bph* operon) and flanking genes encoding conjugal transfer functions, such as those specifying mating pair formation and plasmid replication. The Tn*4371* flanking motifs also are found in the chromosomes of several proteobacteria, suggesting a wide distribution of such genomic elements. Genomic islands themselves can retain different degrees of horizontal mobility, and their patchwork composition suggests an assembly process driven by HGT.

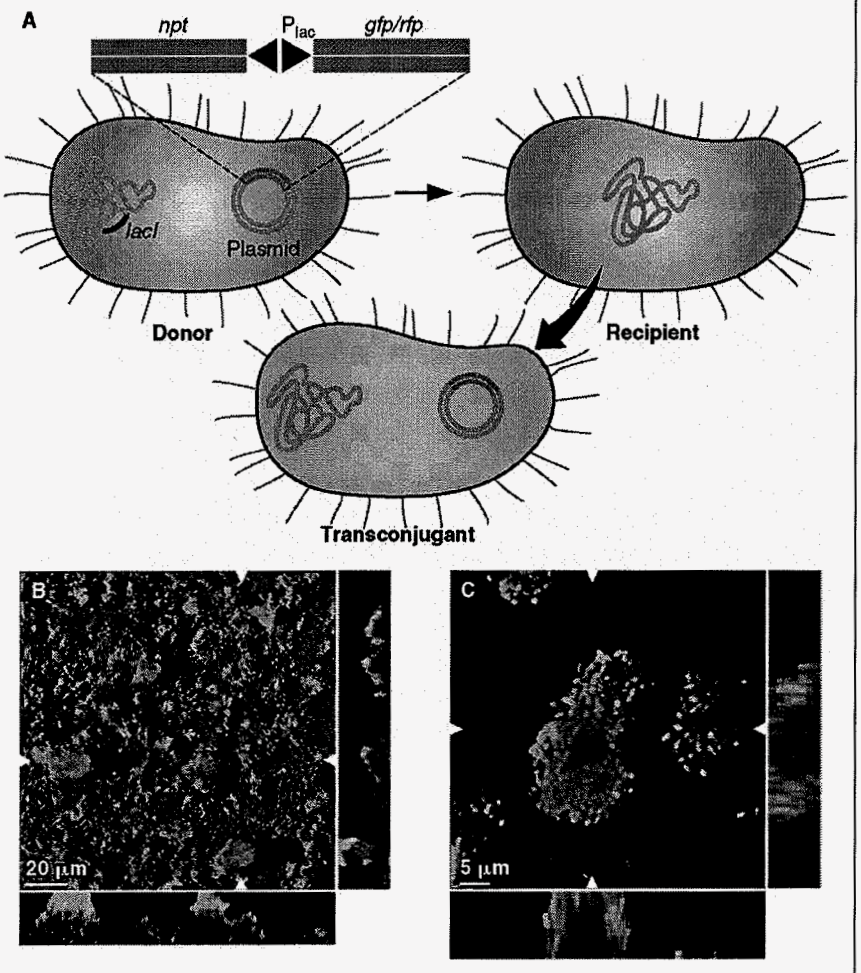
Approaches and Best Niches for Estimating HGT

Transferants, organisms whose genomes are altered by gene transfers regardless of mechanism, traditionally were detected by selection against both donor and recipient strains. However, this approach fails when the microbial community at large is the "recipient." Recent approaches that rely on the conditional expression of phenotypes prove robust when used with proteins such as green fluorescent protein (GFP, Fig. 3). For instance, when *gfp* is repressed by a strong repressor that is present in the donor chromosome but not among potential recipients, transferants can be readily detected by fluorescence, according to Sørin Molin and collaborators at the Technical University of Denmark in Lyngby.

When Søren Sørensen and his coworkers at the University of Copenhagen combined this GFP approach with flow cytometry to search recipient communities for rare fluorescent transconjugants, they found 20- to 100-fold higher conjugal transfer rates of an Inc P1 plasmid compared to when they estimated rates by selective plating. Furthermore, when transconjugants were identified following fluorescence-activated cell sorting, the Inc P1 plasmid that was thought to be limited to gram-negative bacteria was found also in gram-positive actinobacteria.

Examining cells from natural communities that are subject to HGT by confocal scanning laser microscopy helps to show how biofilm architecture and other cellular activities affect transconjugant populations (Fig. 3). However, although extremely rare transfer events in complex bacterial communities might well be the norm in natural environments, those events can be detected only when transferant populations become dominant.

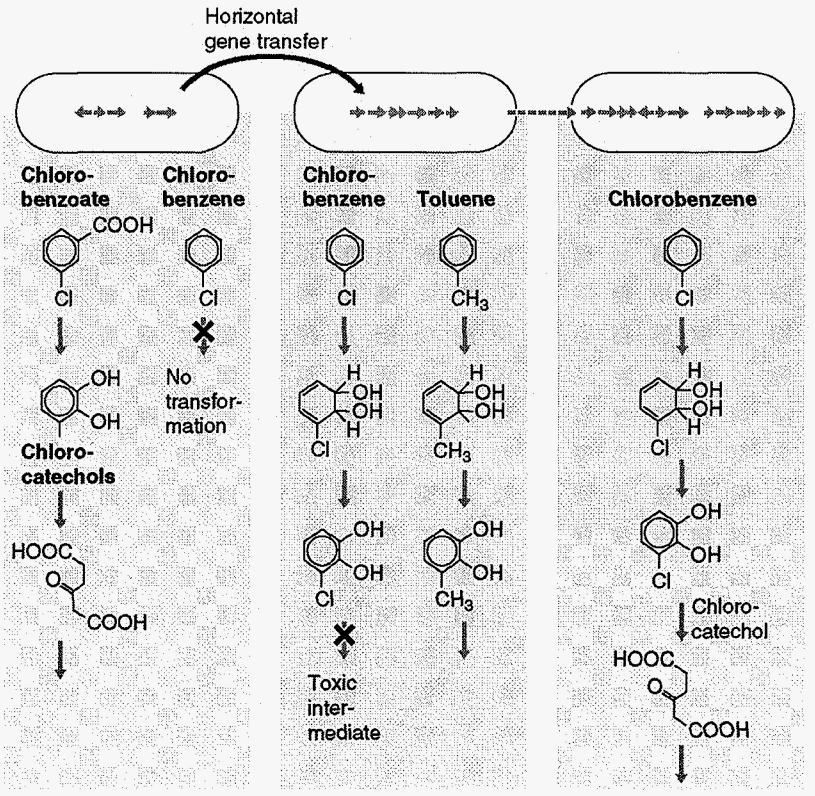
FIGURE 3



Use of conditional GFP expression to facilitate on-line and single-cell resolved detection of HGT. A schematic of the approach is shown in panel A, where expression of the plasmid-encoded *gfp/rfp* is repressed in the donor cell. Upon transfer of the plasmid to a recipient that does not carry *lacI* in its chromosome, *gfp/rfp* is expressed and the transconjugant strain is distinguished by its fluorescence. The right panel shows the application to study conjugal plasmid transfer in biofilms. Panels B and C show composite x-y projections at different resolutions of x-y image stacks acquired using confocal scanning laser microscopy. Shown to the right and below are vertical sections through the biofilm collected at position indicated by the white arrows. Spatial distribution of green-fluorescent transconjugants (green or yellow) is revealed relative to plasmidless *P. putida* RI and *Acinetobacter* sp. strain C6 recipient cells in a model biofilm. The organisms *P. putida* (red) and *Acinetobacter* sp. strain C6 (purple) were identified by 16S rRNA targeted in situ hybridization. After hybridization, green-fluorescent transconjugants appear as either yellow or green, depending on the ratio between the green Gfp signal and the red hybridization signal. The micrographs suggest that transconjugants are predominantly found at the periphery of biofilm structures, where contact with donors may be highest and growth conditions most favorable (from Bjarke B. Christensen et al. Appl. Environ. Microbiol. **64**:2247–2255, 1998).

Some microbial ecologists are examining how nutrient availability, temperature, salinity, soil composition, and other factors influence rates of conjugation, transduction, and transformation. Their results suggest that environments characterized by high bacterial density, energy, and diversity support HGT. Such

FIGURE 4



Vertical expansion of a catabolic pathway through horizontal gene transfer. Left panel: Chlorobenzoates and chlorocatechols can be efficiently metabolized by microorganisms (e.g., via *clc* operons) that, however, cannot initiate transformation of chlorobenzenes. Middle panel: Chlorobenzenes can be attacked but not efficiently degraded by microorganism that carry the typical aromatic ring dioxygenase-meta cleavage pathway (e.g., via *mcb, tod, xyl* operons) because chlorocatechol dioxygenation would lead to toxic intermediates. Right panel: By transferring of the chlorocatechol gene cluster, a new productive pathway is created that permits growth on chlorobenzenes.

environments include interfaces between the solid, liquid, or gaseous phases, as well as the external and internal surfaces of plants and animals.

Why and Where Does HGT Matter?

HGT in microbial communities can affect human and environmental health and productivity. In terms of public health, HGT shapes the evolution of pathogens that resist the immune system and antibiotic treatments. For example, Anne Summers and colleagues at the University of Georgia found that class 1 antibiotic resistance integrons, previously thought limited to the *Enterobacteriaceae*, are widely distributed among gram-positive bacteria in poultry litter. This finding raises the possibility that drug resistance and pathogenic traits persist and may be distributed among indigenous microbes, even though pathogens may not survive.

Rampant transfers of antibiotic resistance traits among members of microbial communities led some researchers to suggest that HGT is an adaptive response induced by exposure to antibiotics. At least in one case, tetracycline can stimulate the transfer of conjugative and mobilized transposons that specify tetracycline resistance in *Bacteroides* spp., according to Abigail Salyers at the University of Illinois and her collaborators. The generality of this mechanism is not known.

Meanwhile, other microbial ecologists are seeking to manage contaminated environments by transferring catabolic genes among resident microbial communities. For example, Eva Top and her collaborators at Ghent University, now at the University of Idaho, seeded soils with *Pseudomonas putida* carrying the 2,4-D degradation plasmid pJP4 and showed extensive transfer of the plasmid and a complete degradation of added 2,4-D in 19 days, whereas in unseeded soils the herbicide was stable for as long as 89 days. This approach is inspired by the occurrence of modular catabolic operons in nature.

Jan Roelof van der Meer, now at the Université de Lausanne in Switzerland, and collaborators describe the "natural history" of such recent HGT events in bacterial isolates from a contaminated environment. A bacterial strain with a *clc* genomic island encoding enzymes that degrade chlorocatechols and chlorobenzoate had acquired a gene cluster encoding an aromatic ring dioxygenase/dehydrogenase, which was found in other bacterial strains from the same environment. The resulting strain contains a functional chlorobenzene catabolic pathway (Fig. 4). The *clc* island, furthermore, readily disseminates via HGT from *Pseudomonas* sp. B13.

Open Questions To Be Addressed

The microbial gene pool is dynamic, and HGT is an important force in microbial evolution.

However, little is known about how selectively advantageous is gene swapping itself and how HGT affects accessory genes, even though they seem likely to be involved when microbial communities are adapting to changing environments. Moreover, less is known about the role of "ORFan genes," which often are linked to these accessory genes.

Insights into these questions will be forthcoming as we better characterize the mobilome and learn more about how environmental conditions affect its composition and dynamics. Microbial communities appear to be awash with mobile elements, and many are likely to be found in still-uncharacterized genomes. Although innovative approaches are enabling researchers to detect HGT in undisrupted microbial communities, they need to move towards predicting HGT. Doing so will require them to develop a strong quantitative framework and models for calculating HGT rates when interrogating microbial communities. They also need to determine the role of selection and fitness cost in maintaining the mobilome.

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SUGGESTED READING

Breitbart, M., P. Salamon, B. Andresen, J. M. Mahaffy, A. M. Segall, D. Mead, F. Azam, and F. Rohwer. 2002. Genomic analysis of uncultured marine viral communities. Proc. Natl. Acad. Sci. USA 99:14250–14255.

Christensen, B. B., C. Sternberg, J. B. Andersen, L. Eberl, S. Møller, M. Givskov, and S. Molin. 1998. Establishment of new genetic traits in a microbial biofilm community. Appl. Environ. Microbiol. 64:2247–2255.

Dejonghe, W., J. Goris, S. El Fantroussi, M. Höfte, P. De Vos, W. Verstraete, and E. M. Top. 2000. Effect of dissemination of 2,4-dichlorophenoxyacetic acid (2,4-D) degradation plasmids on 2,4-D degradation and on bacterial community structure in two different soil horizons. Appl. Environ. Microbiol. 66:3297–3304.

Eisen, J. A. 2000. Horizontal gene transfer among microbial genomes: new insights from complete genome analysis. Curr. Opin. Genet. Dev. 10:606–611.

Gogarten, P. J., and J. P. Townsend. Horizontal gene transfer, evolution, and genome innovation. Nature Rev. Microbiol., in press.

Jain, R., M. C. Rivera, and J. A. Lake. 1999. Horizontal gene transfer among genomes: the complexity hypothesis. Proc. Natl. Acad. Sci. USA 96:3801–3806.

Lawrence, J. G., and H. Hendrickson. 2003. Lateral gene transfer: when will adolescence end? Mol. Microbiol. 50:739–749.

Nandi, S., J. J. Maurer, C. Hofacre, and A. O. Summers. 2004. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. Proc. Natl. Acad. Sci. USA 101:7118–7122.

Springael, D., and E. M. Top. 2004. Horizontal gene transfer and microbial adaptation to xenobiotics: new types of mobile genetic elements and lessons from ecological studies. Trends Microbiol. 12:53–58.

van der Meer, J. R., and V. Sentchilo. 2003. Genomic islands and the evolution of catabolic pathways in bacteria. Curr. Opin. Biotechnol. 14:248–54.