

**The Risks and Consequences of
Gene Transfer from
Genetically-Manipulated micro-
organisms in the Environment**

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The Risks and Consequences of Gene Transfer from Genetically-Manipulated micro-organisms in the Environment

A Review by

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Introduction

Our aim in this review is to provide a short and concise summary of the issues involved in the transfer of genetic material from genetically modified micro-organisms (GMMs) in the environment. After consultation with the DETR this report is aimed at the level of the informed biologist; i.e. this is neither a document designed for the lay reader, nor for a specialist microbial ecologist or geneticist. As the authors represent the disciplines of microbiology and population ecology, we have made a special effort to interpret the issues in the light of current population theory.

We begin with a brief summary of the means by which genetic material can be exchanged amongst bacteria. In the second section we review the importance of gene transfer in natural populations and then in the third section address issues concerned with the movement of genes inserted in genetically modified micro-organisms (GMMs). The final section discusses the consequences of the spread of novel genes in bacterial populations.

Natural Mechanisms of DNA Transfer in Bacteria

Most species of eukaryotes, "higher organisms", engage in sexual reproduction and consequently genes move freely within the interbreeding gene pool. Bacteria do not have sex in the eukaryote manner but nevertheless there are a number of mechanisms that allow gene exchange between different individuals, their significance varies considerably among different bacterial groups. Importantly, these mechanisms may also facilitate transfer of DNA between species and higher taxonomic groups. Any movement of modified DNA from GMMs in the environment would almost certainly occur via one of these methods, and hence because of their importance to all questions of bacterial bio-safety we begin by a brief description of the main modes of natural gene transfer.

Transformation

Bacteria frequently take up free DNA from their immediate surroundings, and this is probably a common form of gene transfer in the natural environment. The process by which the genetic material of a bacterium is changed by DNA uptake is called *transformation* and for this to occur the bacterium must be in a *competent* state. DNA uptake is an active rather than a passive process, and the molecular mechanisms involved vary amongst bacterial taxa but have been characterised in a number of

cases. In general, DNA is absorbed onto the cell surface and then transferred to the interior via a protein channel spanning the cell envelope. There are a limited number of binding sites on the surface of the bacteria (c.50 in *Bacillus subtilis*) and the degree to which they recognise specific types of DNA varies markedly between species. Some species (e.g. *B. subtilis*) appear to take up any DNA while others (e.g. *Neisseria*, *Haemophilus*) appear only to take up conspecific DNA. A third class appear to be intermediate between these extremes of specialisation. Lorenz and Wackernagel (1994) provides a thorough review of natural genetic transformation in the environment. A number of bacterial genera have been shown to be “naturally competent” under appropriate environmental conditions. Bacteria that fall in this class include species of *Bacillus*, *Acinetobacter*, *Pseudomonas*, *Micrococcus*, *Flavobacterium* and *Alcaligenes spp.* (Timms-Wilson *et al.*, 1999, 2000). Such active release of DNA may accelerate the rate of adaptation of those organisms that occur in a competent state in the vicinity of the releasing organisms. In a detailed study of *B. subtilis*, Dubnau (1991a, 1991b) found that competence depended on the stage in the replication cycle, on the nutritional composition of the immediate environment and also varied amongst bacterial clones. Competence for DNA uptake was greatest when cells were exposed to intermediate levels of nutrient availability. In nutritionally limited environments (i.e. soil) cells can be at low metabolic states, or form resting stages, which effectively prevents their involvement in the active process of transformation.

Most DNA taken up by bacteria is destined to be degraded by the cellular machinery. Nearly all bacteria possess enzymes that cut DNA at specified sequences i.e. restriction nucleases which evolved as defensive mechanisms against incoming viral and other foreign DNAs. Host DNA is protected either through methylation of nucleotides in the DNA or through the absence of the restriction enzyme target sequences. Thus the most likely DNA to be successfully transformed and integrate into the recipient bacteria’s genome will come from closely related bacteria.

Interestingly, some bacteria actively release large amounts of DNA into the environment, presumably this has been positively selected as a means of horizontal gene transfer during evolution. Where the release DNA survives and once transformed avoids degradation in the competent cell, its chances of successfully

recombining with the host chromosome or plasmid are directly dependent on its sequence homology. The more similar the sequence the greater the chance of successful incorporation. Without incorporation in the recipient genome the DNA will be lost; unless it can self-replicate, as is the case with plasmids (extra-chromosomal genetic elements) and bacteriophage (bacterial viruses).

DNA taken up from the environment may provide *de novo* genetic material of potential adaptive significance. Experiments have shown that the uptake of DNA can provide a novel phenotype, influence the regulation of gene expression and increase the level of antigenic variation. More generally, conspecific DNA taken up from the environment provides a source of nutrients which may contribute directly towards DNA repair or provide a source of nucleotides for new DNA synthesis, or of carbon, nitrogen and phosphorous for general metabolism.

Conjugation

Conjugation is the process by which genes spread from one bacterium to another through the agency of a self-replicating mobile genetic element (Frost 1992). The major part of the bacterial genome consists of a single or small number of essential DNA molecules, bacterial chromosomes. In addition to the chromosome, many, perhaps most, bacteria support other circular or linear DNA molecules called *plasmids*. Plasmids may impart a selective advantage to the host bacteria, i.e. the ability to grow on a novel substrate or detoxify an antibiotic. In some cases plasmids can be large, representing up to 10% of the total genome, and contain a substantial number of (novel) genes. All plasmids carry the genes required for their own replication, although they may be dependent on a number of host specific factors to be maintained with a cell. In this respect certain plasmids exhibit a very specific host range. Other plasmids, termed broad host range plasmids, are able to replicate in a diversity of bacteria. Furthermore, many plasmids carry genes that mediate their transfer from one cell to another through the process of conjugation. Although the molecular details of conjugation are complex and reasonably well characterised, they are beyond the scope of this review. Briefly two bacterial cells have to come into direct physical contact, and following surface interaction they form a *conjugative pilus* which mediates the active transfer of a copy of the plasmid from the donor to the recipient cell. Successful transfer requires that the recipient is permissive of plasmid

replication and that both donor and recipient cells be metabolically active as DNA replication is a part of conjugation.

Not all plasmids contain the genes that code for the conjugation machinery (transfer proficient and non-proficient plasmids are referred to as tra^+ and tra^- respectively). However, some plasmids can be mobilised (mob^+), that is transferred between bacteria, as long as a tra^+ plasmid is simultaneously present in the same cell to complement this activity by providing the missing genetic functions. Once a link is made between the recipient and donor (which carries the tra^+ plasmid), it is also possible for plasmids in the recipient to move into the donor, a process called plasmid retrotransfer (Sia *et al.*, 1996).

While plasmids normally exist independently of the chromosome, if there is homology between parts of the chromosomal and plasmid DNA, then recombination can occur leading to plasmid integration within the chromosome. Though integrated, the genes coding for conjugation proteins on the plasmid can still be expressed and the plasmid with the attached chromosome may transfer from one cell to another. In practice, because the size of the chromosomal DNA, only a portion of the genetic material transfers. The adoption of this basic activity provided the tools on which classical bacterial genetics was founded in the era preceding the development of molecular biology. Under certain conditions of very intimate contact, non-conjugative plasmids and other mobile genetic elements may be transferred between cells in a manner similar to transformation, perhaps after the donor cell has died and released its DNA.

Plasmids very rarely carry genes that are absolutely essential for the bacteria, but very frequently carry genes that improve the fitness of the host under certain circumstances (Coplin 1989). As has been known for nearly forty years, many cases of antibiotic resistance involve plasmid-borne genes, as do many other cases of adaptations for resistance against toxins, against other stresses, and that allow the utilisation of unusual metabolic substrates. We return to the question of plasmid fitness and its relationship with its host below, when we discuss the spread of introduced genes.

Another class of mobile genetic elements have been found to be widely distributed, *transposons*. Transposons are mobile genetic elements that are present in both chromosomes and plasmids. Typical transposons carry genes and aligned DNA sequences that allow their excision and transfer to other sites in the genome. Transposition plays an important role in the transfer of DNA within and between chromosomes and mobile genetic elements such as plasmids. A subclass of these elements, *conjugative transposons*, has been identified which also carry the genes for the conjugation machinery and can thus move from one bacteria to another. Conjugative transposons have been infrequently described and are limited to a small group of bacteria.

Transduction

Bacterial viruses (*bacteriophages*) can have very important consequences for bacterial population dynamics. A bacteriophage in its extracellular phase consists of a DNA molecule surrounded by a protein sheath (the viral *capsid*). On contact with a susceptible bacterium, the protein binds to recognition sites and the DNA is transferred into the interior of the cell. After this adsorption and infection phase, one of two things can occur. The virus, can enter a *lytic cycle* in which it replicates rapidly, synthesises new coat proteins, and eventually ruptures and kills the host bacterium, releasing the next generation of bacteriophages into the environment. Alternatively, the virus, can persist in the cell, *lysogeny*, often integrating into the chromosome. In this form, the virus causes relatively little harm to the host, and could conceivably increase host fitness if it carried a beneficial gene. The virus may remain in this “dormant” form through many host generations until a lytic cycle is induced and more phage are introduced into the environment.

Individual phages are specific to certain taxa or even strains of bacteria. Specificity is determined both by the phages' ability to adsorb to the bacterium prior to infection, and by its ability to escape the host's defensive system such as the DNA restriction endonuclease and modification enzymes discussed above.

Bacteriophages can effect the transfer of bacterial DNA through processes termed *generalised* or *specialised transduction* that differ fundamentally at the molecular level (Chiura, 1997; McKane & Milkman, 1995; Ripp *et al.*, 1994; Wild *et*

al., 1996). In the former, essentially random segments of host DNA become encapsulated (i.e. incorporated in the viral capsid) either with (coencapsidation) or without viral DNA. Both chromosomal and plasmid DNA may be encapsulated. A phage carrying non-viral DNA is able to attach to a new host, and release the packaged DNA which may then replicate or be integrated into the bacterium's genome. Exactly how much DNA can be transferred by generalised transduction depends on the geometry and size of the viral capsid, i.e. packaging constraint.

Specialised transduction occurs during the lytic cycles of phages that have integrated themselves within the chromosomal genome. At the initiation of the lytic cycle, the prophage is excised from the bacterial chromosome, a process that may accidentally result in some host DNA being taken up with the viral DNA (as a single DNA segment). Integrated host DNA may lie adjacent to the phage in the chromosome or be acquired by the phage via transposition. The host DNA can be packaged within the viral capsid and transferred to a new host.

The potential importance of transduction in bacterial population genetics has been studied in continuous cultures of *Pseudomonas aeruginosa* (Replicon *et al.* 1995). A gene that is able to spread through transduction can be maintained in a population from which it would otherwise have been lost. Ripp *et al.* (1994) have also shown how transduction can play an important role in spreading genes through a freshwater bacterial community. In this study, transduction frequencies were markedly enhanced by the addition of particulate matter, probably because this facilitated the concentration of bacteria and phage leading to increased rates of infection. Transduction has also been observed in phylloplane pseudomonad communities (Kidambi *et al.* 1994) and rhizosphere communities (Ashleford *et al.*, 2000). These studies demonstrate how the two-dimensional (and high nutrient) nature of the habitat facilitates the study of the importance of the phage-host interaction in the population dynamics of natural bacterial communities.

The Importance of Gene Transfer in Natural Environments

There are at least three ways to ascertain the importance of gene transfer in natural environments: (i) direct experimental study in the field or in semi-natural

conditions; (ii) determination of the genetic linkage structure of the population; and (iii) comparative genomic investigation. We review each of these in turn

Experimental investigation

There is ample evidence that transformation is a common occurrence in natural environments; see for example the major review by Lorenz & Wakernagel (1994). Estimations of transformation frequencies in natural conditions overlap with those obtained in microcosm studies, both of which tend to be much lower than those obtainable under optimised conditions in the laboratory. In general, transformation frequencies in natural and semi-natural conditions are difficult to predict and depend on numerous environmental parameters such as temperature, pH, nutrient levels and fluxes, as well as the composition of the local microbial flora. One generalisation that can be made is that rates of transformation are greater in aquatic environments than in the soil and other terrestrial microhabitats. This has been attributed to the higher diffusional movement and reduced absorption to solid particles of cells and DNA in aqueous suspensions (Fry and Day, 1992)

Microcosm and field studies have shown that plasmids can be transferred between different strains and species within the same genus (e.g. *Enterobacter*, *Escherichia*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, & *Bacillus*) as well as between genera (e.g. between *Escherichia* and *Pseudomonas*, *Alcaligenes*, *Rhizobium* or *Erwinia*; and between *Xanthomonas* and *Erwinia* or *Pseudomonas*) (van Elsas *et al.*, 1990; van Elsas *et al.*, 1989; Top *et al.*, 1990; Richaume *et al.*, 1992; Glew *et al.*, 1993; Lilley *et al.*, 1994 and Björklöf *et al.*, 1995). As with transformation, a wide variety of abiotic factors such as temperature and pH have been shown to influence plasmid transfer, as have more biological factors such as bacterial population densities, and the availability of surfaces upon which bacterial densities can be concentrated.

A number of workers have explored plasmid dynamics using the transfer proficient plasmid RP4 and its close relatives which can replicate in a variety of phylogenetically distinct taxa and hence have a broad host range. Many studies have generally failed to detect plasmid transfer between bacteria except in sterile soil or when nutrients were added. Nutrient addition increases the number of metabolically

active donors and recipients, while soil sterilisation both releases nutrients and reduces competition from other bacteria (Klingmüller *et al.*, 1990; 1993; Rafii *et al.*, 1988; Rafii & Crawford, 1989, Wellington *et al.*, 1990; Smit *et al.*, 1991).

These results suggest that the frequency of plasmid transfer will be low in the bulk of the soil. However, the rhizosphere, the root and its immediate surroundings, may act as a hot spot of plasmid transfer (Lilley *et al.*, 1994). The exact mechanisms through which roots influence plasmid transfer frequencies are not fully resolved, but almost certainly include the provision of nutrients from root exudates, and elevation in bacterial densities both through the direct results of nutrients on population growth, and the indirect results of chemotactic migration to roots and bacterial concentration on the root surface.

The majority of the studies reviewed above have involved relatively atypical bacterial strains, plasmids or biological conditions. Sundin *et al.* (1989), however, using both an indigenous pseudomonad and a naturally isolated conjugative plasmid detected transfer on bean plants, but at a rate 0.01% of that obtainable on optimised bacteriological media. Our own field experiments (Bailey *et al.*, 1997, 2000) have made use of the marked strain, *Pseudomonas fluorescens* SBW25EeZY6KX, and have been designed to mimic natural field conditions as closely as possible. This strain was originally isolated from a sugar beet crop at an Oxfordshire field site and has been marked with both selective and colorimetric traits (*lacZY*, *aph*, *xyIE*) by the vectorless insertion of gene cassettes at two chromosomal loci (Bailey *et al.*, 1995). Permission to release the strain was granted for two years of field studies. These established that the strain is an effective through-the-season coloniser of sugar beet roots and leaves when released as a seed dressing (Thompson *et al.*, 1995).

An interesting bio-safety question is the extent to which an engineered, but plasmid-free, bacterium may acquire plasmids from indigenous bacteria that may then act as vectors for the introduced gene. Lilley & Bailey (1997a) explored this question by screening SBW25EeZY6KX that had been introduced into field crops for mercury resistance. A variety of plasmids in natural pseudomonad populations confer mercury resistance (though why is uncertain as mercury is not found in these field sites) and hence this screen identifies some but not all transconjugant events. Transconjugants

were isolated from both root and leaf samples, and plasmid transfer was a comparatively frequent event. Molecular characterisation of the acquired plasmids showed that they were genetically diverse and represented most of the mercury-resistant plasmid groups previously identified in natural bacterial populations in the field site. Interestingly, most plasmid-transfer occurred in a relatively restricted temporal window, perhaps related to a pulse of bacterial growth associated with a specific plant developmental stage. The relative frequency of plasmid-bearing bacteria changes over the field season, and this suggests that the plasmid may increase host fitness at certain times, again probably related to some substrate release by the plant at a particular growth stage.

The studies highlighted above confirm the need to understand the complexity presented when considering the population dynamics and population genetics of assemblages of bacterial strains and their plasmids under natural conditions. In the light of observations of the movement of mobile genetic elements, the apparent ease with which engineered bacteria can obtain these elements from the surrounding indigenous community, that plasmids carry adaptive traits that may enhance the fitness of the host and as mobile elements are the vectors by which an introduced genes could, under exceptional circumstances, be transferred, it is essential that we develop our understanding of the genetic structure of bacterial populations. Only by this understanding will it be feasible to predict not only the likelihood of transfer, but also the frequency with which it may occur. Without this basic understanding it is not possible to even consider the consequence of such a transfer event, particularly if the likelihood is rare and ecologically insignificant.

Linkage structure

To understand population structure, it is important to know the frequencies with which genes recombine (move) in nature and the factors affecting this. Population genetics has developed measures of population structure which allow genetic recombination (movement) to be measured and different species to be compared. Consider two extremes of bacterial population structure. In the first there is no genetic exchange between individuals and the population is made up of a set of independent clones. Thus if we sequenced two adjacent genes in one bacterial strain and called the genes *A* and *B*, we may then sequence the equivalent genes of another

bacterium and find different versions (alleles) of these genes which we will call *a* and *b*. A lack of genetic exchange means we would never expect to see individuals with *Ab* or *aB* combinations. Now consider a bacterial population where genetic exchange is more common. Recombination (gene flow) between *AB* and *ab* individuals would now give rise to the *Ab* and *aB* genotypes. If recombination was very common then the frequency of the four different genotypes (*AB*, *Ab*, *aB* and *ab*) would be determined purely by the frequencies of the different alleles (*A*, *a*, *B* and *b*). The frequency then of any bacterial genotype, say *Ab*, would be a product of the frequencies of the two alleles, i.e. the frequency of *Ab* bacteria = the frequency of *A* alleles in the population x the frequency of *b* alleles in the population. The last state is termed linkage equilibrium and deviations from it, linkage disequilibrium, are a measure of the clonality of the population. This issue is significant for GMMs because we would expect lower frequencies of fixation of horizontally spread genes in a clonal or near-clonal bacterial population (though of course introduced GMMs could still increase in frequency by out-competing other bacteria).

Determining whether genetic exchange has occurred amongst bacteria can be done by direct comparison of gene sequences (see next section) or by statistical estimation of linkage disequilibrium. For the latter, a phylogenetic tree is constructed from sequence data and the number of homoplasies (sites whose identity must be due to gene exchange or convergence) compared with that expected under a null model with no recombination. There are many technical intricacies in this method (codon-bias, the possibility of hypervariable sites etc.) and computer simulations suggest that only fairly broad estimates of linkage disequilibrium are possible.

These techniques have to date been generally applied to pathogenic bacteria. Some species have been shown to be effectively clonal (at most very rare recombination), for example *Haemophilus influenzae* and *Borellia*; while others seem to be at effective linkage equilibrium, for example *Neisseria meningitidis*, *N. gonorrhoea* and the soil bacterium *Bacillus subtilis*. The population structure of *Escherichia coli* and *Salmonella* spp. is intermediate.

Comparative genomics

Over the last five years the sequence of almost 50 entire bacterial genomes has been published that complement and underpin hypotheses relating to the role of the horizontal gene pool in the ecology and evolution of bacterial populations (Lawrence & Ochman 1998; Thomas, 2000; Ochman *et al.*, 2000; Levin and Bergstrom, 2000). Furthermore these data have allowed a systematic investigation of the importance of horizontal gene transfer in mediating the diversification and evolution of prokaryotes. For example, *Pseudomonas* sp. strain P51, isolated from soil heavily contaminated with the man-made pollutant chlorobenzene, is able to degrade this toxin, a property regarded as an evolutionary novelty (Werlen *et al.* 1996). The genes for the enzymes are organised in a cluster (an operon) located on a transposon integrated within a plasmid. Analysis of the operon structure revealed a high degree of homology with the plasmid-borne genes that allow *Pseudomonas putida* F1 to detoxify benzene and toluene, and more distantly with other operons involved in biphenyl, naphthalene and benzoate degradation. The most parsimonious explanation for this data is that the genes for carbon ring-compound degradation has been horizontally transferred between host bacteria, and then selected to detoxify particular substances.

A second example involves soil bacteria with the ability to detoxify the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) (James & Williams 1988). This trait has been isolated in numerous soil bacteria and has a complex and variable genetic basis, the genes responsible being normally, though not always, found on plasmids. Different genes in the degradative pathway sequenced from the plasmids and chromosomes of phylogenetically distinct bacteria show significant levels of homology, indicating both horizontal transfer by plasmids and movement of genetic material from the plasmid to the chromosome. A particularly clear example is provided by the plasmids pKA2 and pKA4 that were isolated from *Alcaligenes paradoxus* and *Pseudomonas pickettii* strains from contaminated soils. The plasmids, which carry an important gene in the 2,4-D degradative pathway, are highly similar and could only have appeared in different genera of bacteria by horizontal transfer.

Without doubt some of the best evidence for horizontal gene spread comes from antibiotic resistance in pathogenic bacteria. For example, the same or closely related genes for antibiotic resistance are known from many bacteria. Sequencing

studies have shown these genes to be carried by a variety of different plasmids and be present in phylogenetically distinct bacteria. In some groups such as *Neisseria*, antibiotic genes are carried on the chromosome, and comparisons of gene sequences from different members of the genus show that the gene is often a mosaic with different parts derived from different species. For a useful summary of the important issues in the spread and persistence of antibiotic resistance see the 1997 Ciba Foundation Symposia (Vol 207)

There are now complete data for almost 50 whole-genome sequences of bacteria, and the numbers will undoubtedly grow in the near future. The availability of this type of information allows a more systematic survey of genetic recombination. To determine if a gene is derived from a horizontal transfer event, it has to be distinguishable from "indigenous" genes. One way this can be done is through base composition ratios. Directional biases in mutation rates from G/C to A/T lead to base composition ratios that to at least some extent are characteristic of different bacterial species. A gene that has been acquired through horizontal transfer may thus have a different base composition, though over time this will change as mutation and selection adjusts the base ratio to that characteristic of the recipient (a process termed amelioration).

Using these methods, Lawrence & Ockman (1998) estimated that 755 of the 4288 (17.6%) of the genes (open reading frames) of *E. coli* had untypical base composition and hence had been acquired from other genetically distant taxa (note, this is an underestimate as genes acquired from bacteria with a similar base composition are invisible to this technique). They estimated that some 755 genes were acquired in at least 234 individual events in the course of the evolution of *E. coli*. By comparing amelioration rates at synonymous and non-synonymous codon sites it is possible to estimate the average age of an acquired gene which is 6.7 million years, and this in turn can be used to calculate the rate at which genetic material is acquired via horizontal transfer which is 64 kilobases per million years (kb/Myr). However, much of the most recently acquired DNA are insertion sequence elements, prophage remnants etc., which are unlikely to last long in the genome. Excluding very recently acquired DNA, the average age of the horizontally transferred genes is 14.4 Myr and the accumulation rate of "useful" and functional whole genes estimated at 16kb/Myr, where one gene approximates to 1kb. Comparisons of genome size within

E. coli strains and with near relatives such as *Salmonella* indicates that they diverged 100Myr ago. Therefore it is reasonable to assume that despite the (slow) accumulation of novel genetic material into the chromosome, the genome is not increasing in size. This implies that genes are being lost at the same rate at which they are being gained. At least on these relatively long evolutionary time scales. A dynamic model applies for bacterial genome development. Comparison of *E. coli* and *Salmonella* also reveals that the phenotypic differences between the two bacteria are not due to changes in gene function brought about by a combination of mutation, duplication and reorganisation followed by selection- the traditional model. Rather variation (phenotypic plasticity) results almost exclusively through the horizontal acquisition of new genes (or the loss of ancestral DNA). Similar conclusions have been reached from the study of more limited sequence data from other bacteria (for example the study of "pathogenicity islands" in disease-causing species). These insights underline the importance of horizontal gene transfer to adaptation. But that rates appear low, and dependant of fitness advantage and selection, either for the individual or by group selection.

It must be emphasised that not all classes of genes are subject to horizontal transfer. Operational genes that perform "housekeeping tasks" appear to move comparatively frequently between bacteria, informational genes such as those involved in transcription, translation and related processes are more conserved. By the analysis method used to provide the above data conserved "essential" genes have become genetically fixed, and demonstrate limited variation, thus transfer and recombination rates cannot be determined. A good example of this is the sequence of 16S ribosomal RNA genes, these are universal and the subtleties in variation have been exploited to develop phylogenies which to some extent infer evolutionary relationships between taxa. A possible explanation for the "conservation of essential genes", is that a single housekeeping gene or operon can perform a useful function in a recipient cell whilst an informational gene acts only as one cog in a very complicated machine and is unlikely to confer a selective benefit if transferred alone (the complexity hypothesis, Jain *et al.* 1999).

Will Introduced Genes Spread?

A critical issue in assessing whether an introduced gene will spread is how the gene is inserted into the bacterium. In order of decreasing likelihood of spread, we can rank genes inserted on (i) transfer proficient plasmids, (ii) non-conjugative (tra^-) plasmids, or introduced into the in the bacterial chromosome via (iii) a competent transposon, (iv) a disarmed transposon and (v) through site-directed homologous recombination. The choice of insertion strategy will depend on a balance of the costs and risks of the different strategies. Regulatory agencies would rightly insist that GMMs designed for planned release into the environment employ stable constructs to minimise the likelihood of transfer. Where a gene is inserted into the bacterial chromosome spread is likely through transformation or mobilised by a conjugative plasmid with chromosomal mobilising ability or by transducing phage (Bailey *et al.*, 1995).

In the following sections we consider the fate of extracellular DNA in the environment and the probability of spread of introduced genes by transformation in the field (in the example given the transfer of genes from genetically modified plants to bacteria naturally competent for DNA uptake). We will then present data derived from other experiments investigating gene exchange via plasmids and phage, and consider the relevance of procedures that have engineered “suicide” systems to limit the dispersal of both the genes and the initial inocula. Finally, issues concerning the spread of transgenic organisms are discussed in relation to analysis using quantitative population genetics and population dynamics frameworks.

Transformation: fate of extracellular DNA in the environment

The rate at which transformation occurs will be strongly influenced by the half life of naked DNA in the environment, and this in turn will be strongly influenced by the biotic and abiotic conditions of particular microhabitats. Estimated DNA half lives from different natural environments are shown in Table 1.

Table 1. Estimated DNA half life in various environments.

Location	Half-life (h)	References
<i>Aquatic environment</i>		
<i>Wastewater</i>	0.017-0.17 ^a	Phillips <i>et al.</i> 1989
	0.23 ^b	Fibi <i>et al.</i> 1991
<i>Freshwater</i>		
Oligotrophic	4.2 ^c	Paul <i>et al.</i> 1989
Eutrophic	5.5 ^b	Paul <i>et al.</i> 1989
<i>Marine water</i>		
Estuarine	3.4-5.2 ^c	Paul <i>et al.</i> 1987, 1989
	5.5 ^b	DeFlaun & Paul 1989
<i>Ocean surface</i>		
Oligotrophic	12.8 ^c	Paul <i>et al.</i> 1989
P Limited	4.5 ^c	Turk <i>et al.</i> 1992
Not P limited	45.0-83.0 ^c	Turk <i>et al.</i> 1992
<i>Marine sediment</i>		
	235 ^d	Novitsky 1986
	140 ^c	Maeda & Targa 1974
<i>Terrestrial environment</i>		
<i>Soil</i>		
Loamy sand soil	9.1 ^e	Romanowski <i>et al.</i> (1992)
Silty clay soil	15.1 ^e	Romanowski <i>et al.</i> (1992)
Clay soil	28.2 ^e	Romanowski <i>et al.</i> (1992)

^a Conversion of supercoiled into relaxed-circular or linear plasmid DNA.

^b Loss of hybridization signals of plasmid DNA in Southern transfers or dot blots.

^c Loss of acid-precipitated material (colourimetric DNA determination or ³²P-labelled plasmid DNA).

^d In dead cells degradation measured as in footnote c.

^e Loss of transformation activity of plasmid DNA.

Many bacteria produce substances that degrade foreign DNA. Over 90% of bacterial isolates from soil and aquatic environments possess DNA-degrading activities, and are likely to cause the destruction of a large fraction of extracellular DNA. However, despite all the factors that can cause DNA degradation in the environment, large quantities of high molecular weight DNA can be extracted from soil and aquatic habitats indicating that a proportion of extracellular DNA either resists or is protected from degradation (Romanowski *et al.* 1992, 1993). In soils, some protection of DNA may occur by absorption to particulate matter. DNA complexes with several soil minerals such as clay, quartz and feldspar, as well as with humic acid. DNA complexed in this manner is likely to be protected from degradation, though it may also be unavailable for bacterial uptake (Aardema *et al.*, 1983; Blum *et al.*, 1997). Soil is an extremely complex microbial habitat compared with the fluid and surface habitats normally studied by microbial ecologists, and our understanding of DNA fluxes and the factors affecting bacterial DNA uptake is still in its infancy.

Transformation in the field

Nielsen *et al.* (1997a) have performed a series of experiments to study the transformation of *Acinetobacter calcoaceticus* (strain BD413) in the soil. Homologous DNA containing an antibiotic resistance marker was used as the potential transforming agent and hence these experiments are useful in assessing the degree to which naked DNA derived from an introduced GMM might transform related bacteria in the soil. The availability of introduced DNA for transformation declined quite quickly in the soil as the DNA was degraded, though the speed with which this happened was influenced by soil type (silt versus loam), moisture content, and ionic composition. *A. calcoaceticus* cells introduced into the soil tended to be metabolically inactive and hence transformation incompetent. Adding nutrients strongly increased the likelihood of transformation as this resulted in more cells becoming metabolically active and transformation competent (Nielsen *et al.*, 1997a, 1997b).

The source of DNA for transformation need not only be other bacteria but can potentially be any organism. An issue that has been raised in general debates about the safety of GMOs is the possibility that genetic material from genetically-

engineered plants might be taken up by bacteria. Sometimes antibiotic-resistance markers are introduced into plants, and these could conceivably be of a selective advantage to a micro-organism. Nielsen *et al.* (1997b, 1997c, 2000) and Gebhard and Smalla (1998) designed a sensitive assay to examine this issue. First, they modified sugar beet by introducing an antibiotic (kanamycin) gene. They then engineered a bacterium so that it too had the same marker, except that the bacterial gene had a 317 base pair deletion so that the enzyme responsible for antibiotic resistance could not be expressed. This design specifically optimised the detection of transformation events if they were to occur as, in theory, plant DNA containing the marker taken up by bacteria would have a high probability of recombining with homologous DNA around the deletion site in the bacterial chromosome and restore the antibiotic resistance phenotype. The bacteria used was *Acinetobacter calcoaceticus* which is normally associated with plant roots. Even under optimised conditions in the laboratory with extracted plant DNA transformation occurred at frequencies of less than 10^{-13} . No transfer of DNA in the field or directly from plant material was observed (Nielsen *et al.*, 2000).

Experimental studies of the transfer of chromosomal genes in GMMs

Several experimental systems have now been created to study the spread of genes that have been artificially inserted into bacterial chromosomes. Troxler *et al.* (1997) examined the extent to which a chromosomal gene might be spread by a conjugative plasmid. They worked with a strain of *Pseudomonas fluorescens* (CHA0) which carried a plasmid (IncP) which itself bore a transposon (Tn5). This raised the possibility that the transposon would mediate the transfer of chromosomal genetic information to the plasmid (transposon-facilitated recombination) and thence via conjugation to other bacteria. Gene transfer via this route was demonstrated *in vitro* and in sterile soil but not under more realistic conditions in the soil. This group also studied a strain of *Pseudomonas aeruginosa* (PAO) containing a plasmid (IncP R68.45) that has a chromosomal insertion site and hence as described above is capable of mobilising part of the bacterial chromosome. Transfer of chromosomal markers was detected both under laboratory conditions but significantly also in "natural" microcosm soils where the constructs were designed so that the recombinants had a trophic selective advantage. This will have elevated gene transfer

frequencies, but the experiments clearly show the possibility of gene flow through this route.

Bailey *et al.* (1995) worked with a different strain of *Pseudomonas fluorescens* (SBW25) associated with the phylloplane. Two sets of marker genes were placed in the bacterial chromosome, one at an insertion site that was highly specific to this strain of pseudomonad, the other at a site that is found commonly in field isolates of fluorescent pseudomonads. The GMM colonised beet plants in the field and was not excluded by the indigenous phylloplane flora. No transfer of the marker genes was found from the GMM to native bacteria. However, evidence for DNA movement in the opposite direction was found: the introduced bacterium acquired a number of large conjugative plasmids similar to those that had previously been isolated from natural phylloplane fluorescent pseudomonads.

Suicide systems

In proposing the use of suicide systems, two considerations should be kept in mind. First, any suicide system is unlikely to be 100% effective and hence it can contribute to but not constitute the whole of a containment strategy. Second, it is conceivable that part or all of the suicide system could move into indigenous bacteria and result in their impairment or suicide under certain environmental stimuli.

Several proposals have been made to engineer safety features into GMMs, in particular molecular mechanisms that result in cell auto-destruction under certain stimuli. For example, *hok* genes cause a lethal collapse of the trans-membrane potential in *Escherichia coli*. Molin *et al.* (1987) put a *hok* gene under the control of the *fimA* promoter which is switched on during pilus formation. As a result, the bacterium kills itself when it tries to form a conjugative pilus prior to plasmid transfer. Molina *et al.* (1988) describe a system in which a recombinant microbe engineered to make use of a novel substrate such as a toxin subject to bioremediation survives provided the substrate is present but commits suicide once the toxin is exhausted.

Quantitative analysis

Many of the issues associated with the regulation of GMMs concern whether populations of introduced bacteria will establish and spread in the environment (often but not always a good thing) and whether the genes introduced by genetic

manipulation will spread to other bacteria of differing degrees of relatedness. Essentially, both questions concern the probability of invasion, a subject that has been intensively investigated by population biologists, population geneticists and by epidemiologists. The methodological tools developed in these other subjects are now being used to explore issues concerning GMMs.

Perhaps the most fundamental concept in the quantitative analysis of population and genetic invasions is the invasion criterion which has many alternative names in different fields, for example R_0 in epidemiology. The invasion criterion in its simplest form is the number of copies left by an individual/gene when rare: the population of a bacterium will increase if every individual leaves more than one offspring; a disease will spread if each original case of infection leaves more than one daughter case; and a gene will spread if a rare allele contributes more, in terms of fitness, than simply replicating itself in the next generation. While the definition of the invasion criterion itself is a truism, analysing how the different elements of an individual's biology or a gene's phenotype combine to determine the invasion criterion can be extraordinarily useful in understanding whether spread will occur. Moreover, simple calculations of the invasion criterion in homogeneous, time-invariant environments, can be extended to include both temporal and spatial heterogeneities, though at the cost of greater mathematical complexity and difficulty in parameterisation.

Population dynamics. An initial consideration might be whether an introduced bacterium will increase in density following its open release to the environment. In different circumstances such spread may be advantageous or disadvantageous, though perhaps most frequently this question is context-dependent (for example a bacterium engineered for pollutant bioremediation should increase in the presence of the toxicant and decrease in other circumstances). Determining whether populations will increase when rare is a classical problem in population ecology and is solved by writing down an equation for population change and determining the parameter combinations that lead to positive growth rates. The success of this exercise is determined by how well one understands the population dynamics of the organisms under study. For bacteria, a relatively large number of studies have successfully modelled the growth of pure cultures in liquid media (e.g. chemostats), or of pure cultures on simple solid media

such as the surface of agar. A smaller number of studies have considered populations of more than one species of bacterium, and of interactions between bacteria and phage. In these cases, the invasion criterion can often be determined with substantial precision. Studies of bacterial population dynamics in more complex environments are much fewer and normally involve pathogenic species. For example, Levin & Bull (1996) have modelled the population dynamics of bacteria in mammalian infections and how they might be controlled by phage therapy. A substantial body of work concerns epidemiological models of bacterial diseases instead of the bacterial densities themselves, the numbers of infected individuals are modelled (the microparasite model of classical epidemiology) (e.g. Anderson & May 1992). The disease spreads if the number of secondary infections that result from an initial infection in a susceptible host population is greater than one ($R_0 > 1$).

These studies of the population dynamics of bacteria in simple laboratory systems or of pathogenic species are valuable in suggesting what might happen in more complex environments such as the phytosphere or the soil. However, the systematic study of population dynamics in these situations is still in its infancy. For a more detailed consideration of the topic the reader is recommended Baumberg *et al.*, (1995). The major problems include the following: (i) Coping with the heterogeneity of the natural environment. Population dynamic models of bacteria in simple environments work so well because the systems are mixed and homogenised. We do not yet know enough about the structure of bacterial populations in more realistic environments to model them successfully. In developing this theory, progress is likely to be most swift in studying communities in the phytosphere, and slowest in the very complex environment of the soil. (ii) The diversity of species in natural systems. It is only in recent years that the full diversity of bacteria in natural systems has been fully appreciated, as well as the importance of bacteriophage-bacteria interactions in the ocean and elsewhere. We still have only a rudimentary understanding of how bacteria partition resources in natural communities, and of the fine structure of microbial food webs. (iii) The technical and logistic difficulties of studying bacteria in complex environments are great, though rapid progress is being made in developing new means of study microbial communities *in situ*. In particular, "gene-chip" and related technologies offer the real prospect of the quantitative sampling of large assemblages of different genotypes in the near future.

Population genetics. There are two questions here: will a gene spread to other members of its own "species", and will it spread to and through the population of other "species". The two questions are not as clear cut as they would be in higher organisms as bacterial species are often poorly defined. Consider first a purely clonal species (see above under linkage structure); in the absence of any genetic exchange questions of population dynamics and population genetics are identical though with the potential complexity that an introduced modified clone may be competing with bacteria that have effectively identical ecological niches. Spread of the modified clone requires the invasion criterion to be greater than one, which in the competitive situation means that the genetic modification must impart a fitness advantage to the bacterium.

Now consider populations that depart from pure clonality, and suppose that gene transfer can occur through transformation. Whether a gene spreads is now determined by two factors: the fitness consequences to the bacteria of carrying the gene, and the frequency of transformation. The mathematics of this case is well-understood as part of classical bacterial population genetics (Baumberg *et al.* 1995). As long as the initial inoculum is large enough that the stochastic loss of the gene can be ignored, the modified gene will spread if it imparts a fitness advantage, and the speed of the spread will be determined by the magnitude of the advantage and the frequency of transformation. A gene that reduces a bacterium's fitness may still spread if it has a high frequency of transformation (a selfish gene). In practice, a major regulatory concern is how the gene is engineered into the bacterial chromosome and will this lead to elevated rates of transformation. A further concern is the difficulty of predicting the fitness of an engineered gene in a novel genetic background after a transformation event to a more distantly related bacteria (and possibly after selection has acted on the gene).

A gene that has been engineered into a plasmid, or has moved there naturally, is more likely to spread horizontally through conjugation. Prompted by questions of plasmid-borne antibiotic resistance, the mathematics of this situation has been studied quite intensively. The basic invasion criterion were derived by Stewart & Levin (1977; see also Levin & Stewart 1977, 1980; Macken *et al.* 1994). Consider a

population of bacteria containing a mixture of bacteria with and without plasmids (pla^+ and pla^-) and denote their densities as P and B respectively. Let their rates of increases be I_P and I_B which implies that the plasmid increases the fitness of the bacteria if $I_P > I_B$. Similarly, denote the mortality rates of the two strains as m_P and m_B ; the probability that the plasmid is lost at cell division (segregation loss) as t ; and the conjugation rate as g . With these assumptions the condition for spread is:

$$\frac{I_P(1-t) + gB}{m_P} > 1.$$

This is easy to interpret in words. For the plasmid and the gene it carries to spread, the expected number of daughter copies produced during a pla^+ bacterium's lifetime must be greater than 1. To estimate this we multiply the life expectancy of a pla^+ bacterium ($1/m_P$) by its instantaneous rate of reproduction. The latter has two components: the rate at which pla^+ bacteria multiply (devalued by segregation loss: $I_P(1-t)$) and the rate at which conjugational transfer occurs (gB). An important point is that the last term depends on the frequency of pla^- bacteria (B) in the environment.

We have discussed this example in a little detail, not because it will apply to the spread of all plasmid-borne genes, but because it is illustrative of how the invasion criterion can be used to assess the relative importance of different aspects of the bacterium's ecology and genetics in promoting the spread of a gene. A variety of extensions can be made to the arguments presented above. For example, if the pla^+ bacterium is spreading through a stable population of pla^- bacteria and we assume that mortality rates are the same in the two strains then we can substitute $m_P = m_B$ and find that in the absence of conjugation spread is purely determined by whether the pla^+ bacteria has a fitness advantage, but when conjugation occurs a deleterious plasmid is able to spread, especially when the density of pla^- bacteria is high (perhaps through local concentration in the phytosphere) (Levin & Stewart 1977). However, Simonsen (1991) has argued from what is known about the values of the relevant parameters that the maintenance of "selfish" plasmids is most likely not to occur in the field. Essentially, the product gB in the invasion criterion described above would be too small to offset the known fitness disadvantages of carrying plasmids that confer no benefits. Simonsen (1990) has also pointed out that the basic equations underlying these calculations of the invasion criterion all assume mass action, in effect that the

bacteria are suspended in liquid. Her experiments on plasmid transfer amongst bacteria growing on a two-dimensional surface indicate that the mass action assumption may apply, at least approximately, in these situations, provided bacterial densities are not too low.

A more complex extension is to examine the question of whether a non-conjugative plasmid can spread through the agency of a conjugative plasmid. Levin & Stewart (1980), who examined this question showed that this was possible when the non-conjugative plasmid increased the bacterium's fitness, and that even when this was not true spread was possible, though the conditions were stringent and probably unlikely to occur in the field. Using a related approach, van der Hoeven (1984, 1986) has explored the coexistence of multiple bacterial plasmid types, and argued that no more than two may exist. One caveat about the use of the invasion criterion should be made. There are circumstances, explored by Macken *et al.* (1994), when the invasion criterion predicts no spread but where a plasmid introduced in a sufficiently large inoculum can establish itself and spread. Their paper, which deals with the long-term dynamics of $plac^+$ and $plac^-$ bacteria interactions, explores a model that demonstrates "a dizzying array" of mathematical behaviours, though we as yet know too little about plasmid/bacteria biology to assess whether this dynamic behaviour will occur in the field. Finally, once a plasmid has established itself in a population, both theoretical arguments (Levin & Lenski (1983) and some ingenious experiments with long term cultures (Bouma & Lenski 1988, Modi & Adams 1991) suggest there may be evolution or coevolution leading to a more mutually beneficial relationship. Whether such evolutionary change might involve an engineered gene has yet to be explored.

The spread of genes from GMMs should also be considered in terms of vertical proliferation of carrier populations which may colonise new habitats as well as horizontal gene transfer. One area where the invasion criterion has been applied to both horizontal and vertical spread is the spread of antibiotic resistance in clinical environments (Lipsitch *et al.*, 2000; Bonhoeffer *et al.*, 1997; Austin *et al.*, 1997, 1999a, 1999b). These assessments, and others, have identified and evaluated targets for strategies against the spread of antibiotic resistance genes. These have included treating with single antibiotics, sequential use of different antibiotics, giving parts of a population different antibiotics and treating patients with combinations of antibiotics.

The disproportionate importance of general dispersal of bacteria within a hospital to the spread and persistence of antibiotic resistance has also been highlighted. The local isolation of GM or other bacterial populations can be an important limitation to the spread of genes. Conversely the availability of dispersal mechanisms may considerably increase the opportunity for gene transfer to new populations and the colonisation of new habitats.

Many of the modelling approaches applied to plasmids can also be used to investigate transduction, phage-mediated gene transfer. The added complexity here is that phages may kill bacteria and be a major influence in determining bacterial densities (Levin *et al.* 1977). The most important issues in determining spread are (i) the frequency with which host DNA is incorporated in the viral capsule; (ii) the dynamics of phage infection; and (iii) the factors determining whether a phage enters a lytic cycle or remains dormant in the cell or integrated within the genome.

What are the Consequences of Introducing New Genes into the Environment?

There are two broad classes of problems potentially associated with the introduction of GMMs into the environment. The first concerns the actual gene introduced into the GMM. Could it, either in the original bacteria or after recombination in a different micro-organism cause harm to man, man's crops, livestock or pets, or to wild plants and animals. The answer to this question depends critically on what the introduced gene is, and the likelihood that it will spread. The second type of problem concerns the community ecology of micro-organisms in the environment. Might the introduction of a novel bacteria with a potentially different natural history influence the structure of the natural microflora with detrimental effects to biodiversity and ecosystem functioning. We discuss each in turn.

The introduced gene

One commonly voiced concern is that any GMM released might acquire genes, or mutate to become a plant or animal pathogen. This is highly unlikely, in fact probably impossible. Many pathogenic bacteria are known, and have been the subject of intensive molecular investigations, in fact the majority of bacteria for which complete genome sequence are available fall into this class. Furthermore pathogenic

groups also contain strains of bacteria, such as *E. coli*, that are mutualistic commensals which appear not to cause harm, and as normal gut flora may even be essential to the host. Therefore a greater definition than species assignment is required to define a pathogen. Molecular analysis of pathogenic bacterial strains show that pathogenesis requires a suite of genes, often arranged together on plasmids and chromosomes in ‘pathogenicity islands’. Pathogenicity is a complex trait, and not the result of simple mutation, or the acquisition of a single gene. Studies on *Salmonella typhimurium* show that at least 10-20 genes are obligately required for the colonisation and infection of mice, but that over 100 genes are expressed during infection that are not expressed *in vitro*. This suggests that the type of genes, the candidate bacterial recipients of those genes and the legislation in place currently being considered for GMM applications would not result in animal or plant disease.

Of all categories of genes that have been used in GMM technology, the greatest concern attaches to antibiotic resistance. Historically antibiotic resistance genes have been used in the genetic modification procedures to facilitate the isolation of the recombinant constructs from the background unmodified cells. They also have the advantage of providing a ready phenotype, and genetic marker, with which to identify GMMs. The reason for the concern has been with the appearance of antibiotic resistance in clinical isolates. Natural resistances have been acquired as a result of plasmid transfer from indigenous populations under the selection since the application of antibiotics as therapeutics. Variants of most if not all clinical pathogens carry antibiotic resistant genes, and certain strains such as MR-*Staphylococcus aureus* are resistant to virtually all commonly available antibiotics.

The potential problems of antibiotic resistance gene spread from GMOs have been considered by the regulatory agencies. The British Advisory Committee on Novel Food Processes (ACNFP) refused permission for transgenic corn containing an ampicillin resistance gene because of the potential risk that would result if the gene transferred to human gut bacteria. The precautionary line adopted by ACNFP for GMOs is in contrasted with the European Scientific Committee for Animal Nutrition’s approval of the use of the antibiotic avoparcin as a prophylactic or growth supplement in animal feeds. Avoparcin is an analogue of vancomycin, the antibiotic of last resort against multiple drug resistance *S. aureus*.

Ecological perturbation

Much of the current disquiet about GMO's, especially in the public domain but to a lesser extent also within the scientific community, is that their irreversible introduction into the natural environment will cause a perturbation to the ecological community that will propagate and possibly grow in magnitude as it ripples through the ecosystem, as one species or strain interacts with another. This perturbation might be caused by the introduction of a more vigorous strain of bacteria, or may be caused by an introduced gene changing the phenotype of endemic bacteria that acquire the gene. These questions are extraordinarily difficult to answer because by their very nature they involve the non-linear behaviour of complex systems. There are perhaps three different approaches to answering this question: study of the deliberate or accidental release of non-manipulated organisms, experimental approaches, and theoretical modelling.

It is clear that the release of pathogenic micro-organisms can have major effects not only on their host, but indirectly on ecosystem structure. Two good examples, albeit not involving bacteria, are the myxoma virus of rabbits and the fungal pathogen of Dutch elm disease. Not only did these pathogens kill hosts, but they led to major changes in community structure consequent on the destruction of a major herbivore or an important component of European deciduous woodland. Rabbits and elm are species which have a major structuring role in natural communities, in ecological jargon keystone species. Clearly any GMM introduction that affected such a species might lead to a major ecological perturbation. In contrast to this, numerous pathogens of specific insect herbivores have been used in pest management with no major detectable ecological perturbation.

Were a pathogenic GMM to be considered for licence, it would clearly be important to consider the role of the host within the ecosystem. This may be less of an issue, for example, if the GMM was a avirulent vaccine derivative or a modified strain used as a vector in gene therapy. In these instances a degree of containment of the recipient patient may be possible. For open environmental release however the most likely candidate GMMs will be natural, non-pathogenic bacteria that will enhance bioremediation, or colonisation the phytosphere for plant growth promotion. At issue have been concerns that the introduction of a non-pathogenic bacteria may have major knock-on effects within the microbial or wider community. Despite many

small scale releases world-wide no adverse effects have been recorded. Investigations were typically directed at resolving whether any effect might be mediated by the GMM out-competing endemic micro-organisms, or through their acting as a reservoir for shared bacteriophages (apparent competition). Most biologists would argue that this is unlikely, because it is difficult to envision a microbial equivalent of a keystone species (though root-nodulating *Rhizobium* might conceivably fill this role). However, it is reasonable that advances be made that provide a better understanding of the dynamics of microbial communities in natural habitats, which involving both experiments and theory. For example, a question that is currently being investigated with higher organisms is the extent to which ecosystem functions are degraded as community biodiversity is reduced: does the loss of a small proportion of the community destroy its functionality (the rivet hypothesis) or is the loss of certain species compensated for by others (the buffering hypothesis); alternatively do ecosystem functions change linearly with biodiversity, or is the relationship completely idiosyncratic (Lawton 1994, Naeem 1998). Experimental investigation of these questions with micro-organisms would immeasurably help us assess the risks of propagating ecological disturbances.

Conclusions

An understanding of the consequences of introducing new genes into bacteria requires consideration of bacterial population genetics and population ecology, as well as understanding the regulation and expression of the gene and the activity and functional persistence of protein coded for. Clearly, a crucial factor is the nature and stability of the construct, many aspects of which can be assessed in the laboratory using molecular and cellular techniques. The more difficult questions concern the fate of the gene and the GMM in the environment. Important issues relate to the probability that the gene may spread through the environment through the processes of transformation, transduction and conjugation. We need to know how much can be learnt about the risk of gene transfer through study of the linkage structure of natural bacterial populations, comparative genomics, and controlled experiments with model GMMs. Harder still, we need to develop and build a predictive science of microbial community ecology.

It is almost a cliché that to call for more research. Nevertheless, there is a need for a greater understanding of the processes involved. The good news is that exactly the same technologies that have allowed the genetic manipulation of micro-organisms to take place also provided the tools to enable the microbial ecology of bacteria to be studied at the same level of resolution as higher organisms. Moreover, the field will be further transformed in the next decade as gene chip and related technologies become available and are applied to environmental questions. These technical advances, coupled with the great intellectual challenges in this area, should attract some of the best scientists into this field, and its present skills base means the United Kingdom is in an excellent position to be at the forefront of this research.

It is not possible to make blanket statements about the safety or otherwise of GMMs; any opinion must refer to a particular construct in a specific situation. The precautionary approach adopted that deals with each release on a case by case basis. Knowing the type of GMMs that are currently under consideration for development and ultimate release into the environment, we are guardedly optimistic that no major environmental or health risks are involved. But as recent events have proved, such technology is treated by the general public and media with enormous suspicion, overcoming this fear, and reaping the undoubted benefits that the new technologies offer, requires a much greater effort to understand the fate and effect of GMMs in natural environments.

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