

Gene Ecology Guide to: Measuring Horizontal Gene Transfer

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This paper summarizes a critical analysis of the effectiveness of attempts to measure horizontal gene transfer (HGT) from genetically modified/engineered plants (GMO/GEOs or LMOs) to soil microorganisms, and extrapolations of those studies to conclusions about the risks of HGT from GMOs in general. The focus of the paper is detection of HGT from GMOs, because national and international laws require that such risks be assessed.

Monitoring HGT from GMOs is sometimes required by law or contractual obligation. In New Zealand, for example, monitoring for HGT has been imposed on approved GMO developments to provide an additional biosafety margin. Is the effectiveness of monitoring such that it would prevent or heal a harm that might come from HGT at frequencies known to occur in nature?

Our results suggest that it is not. First, HGT happens in nature at frequencies high enough to create threats to human health and the environment. Second, claims that HGT can confidently be ignored in an assessment of the risk involved in release of transgenic plants are based on improper experiments.

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Introduction

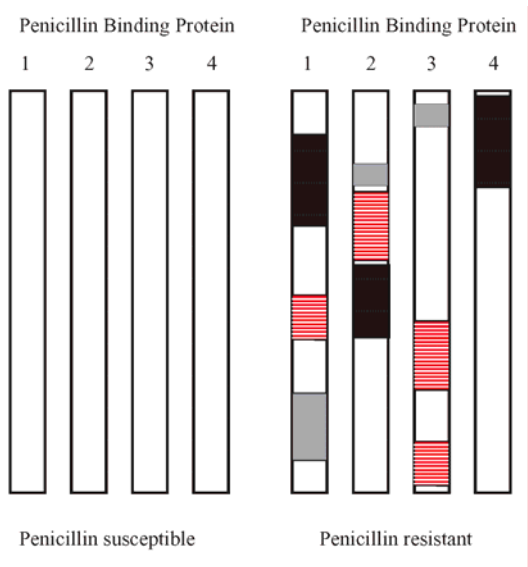
How unfit for task are current assessments and ongoing monitoring experiments? Available techniques for monitoring HGT in the environment are too crude to detect it before it can create relatively large numbers of recombinant organisms. Only a dramatic increase in investment in developing new approaches to monitoring HGT and understanding how it works will permit regulatory authorities and scientists to make sound assessments.

The best available measurements of frequencies of gene transfer have been made using bacteria that have acquired resistance to antibiotics by HGT. Resistance to antibiotics among disease-causing bacteria has evolved only since the adoption of antibiotics to medicine about 50 years ago. The evolution of antibiotic resistance in bacteria provides insight both into how, and how fast, genes evolve by HGT.

The evolution of penicillin-binding protein genes in one species of bacteria, *Streptococcus pneumoniae*, is a particularly informative example of genetic change by HGT. This single example is powerful enough to draw critical conclusions about the level of certainty regarding the risks of HGT from GM plants.

The HGT Uncertainty Principle

HGT from plants to other organisms is known to occur because, for example, viruses infect many different species of plants and even cross between animals and plants. The elusiveness of HGT “in the wild” is reminiscent of the electron whose position and velocity cannot be simultaneously known. Something similar to Heisenberg’s Uncertainty Principle may therefore be needed to understand the limitations of contemporary techniques used to monitor both the pace and location of HGT under natural conditions.



S. pneumoniae did not evolve resistance by mutation. It did so by replacing segments of its PBP genes with corresponding segments of genes from other species (Figure 1).

Until all four genes changed in specific ways, the bacterium would still be susceptible to penicillin. Acquiring all the changes at once is highly unlikely, because each transfer event, when re-created in laboratory studies, occurred at a frequency of one in a million. The combinatorial probability of the minimum four recombination events would be a staggeringly low 1 in 10^{24} .

Figure 1. Open rectangles represent the genes for four proteins that contribute to *S. pneumoniae*’s susceptibility to penicillin. Those genes can be changed following exposure to the DNA from other species of bacteria that have one or more PBP proteins that do not bind penicillin. In the figure, up to three different species (black, grey and striped fills) can contribute to each protein. The striking lesson is that this recombination is between species that are, on average, as different as some plants and animals and involve segments of genes rather than whole genes.

What *S. pneumoniae* has taught us about HGT

Penicillin should be the perfect antibiotic for treating infections caused by *S. pneumoniae* and would still be effective today if HGT were truly rare.

Penicillin kills *S. pneumoniae* by inactivating four proteins, aptly named PBPs (for penicillin binding proteins). The probability that *S. pneumoniae* would evolve resistance to penicillin by random mutation is very low, because approximately five mutations are necessary for resistance.

To get a sense of how low this number is, consider that a single bacterium would have to be sampling genes from many bacteria at a rate of one million times a second for 20 billion years (the estimated age of the universe)! In fact, these events occurred in under 50 years. So how could such massive frequencies of gene transfer have been overlooked?

Most likely, the assembly of the PBP genes in penicillin resistant *S. pneumoniae* did not occur all at once from one lucky scoop out of the gene pool, but did so incrementally (Figure 2). Each single nucleotide that changed in each of the most important PBP genes had some probability of making a recipient of that DNA marginally more resistant to penicillin, slightly increasing its

representation in the population in environments where penicillin was present and lethal to fully susceptible bacteria, but at concentrations that were not lethal to the recombinant bacteria. Such intermediate strains would continue to increase in number and go through further rounds of exposure to HGT until fully resistant strains began to emerge.

Contrast this scenario with how monitoring for gene transfer is done around GM crops. All published experiments so far have required large pieces of DNA to be taken up

which genes to focus on (as happened with penicillin resistant *S. pneumoniae*). Detection occurs well after recombinant populations reach sizes that could result in ecological harm.

The lessons for monitoring GMOs that we can take from understanding penicillin resistance are:

- most “gene” transfers are actually much smaller than a gene, but nevertheless can, at some frequency, significantly change the biochemical properties of the genes into which they transfer;

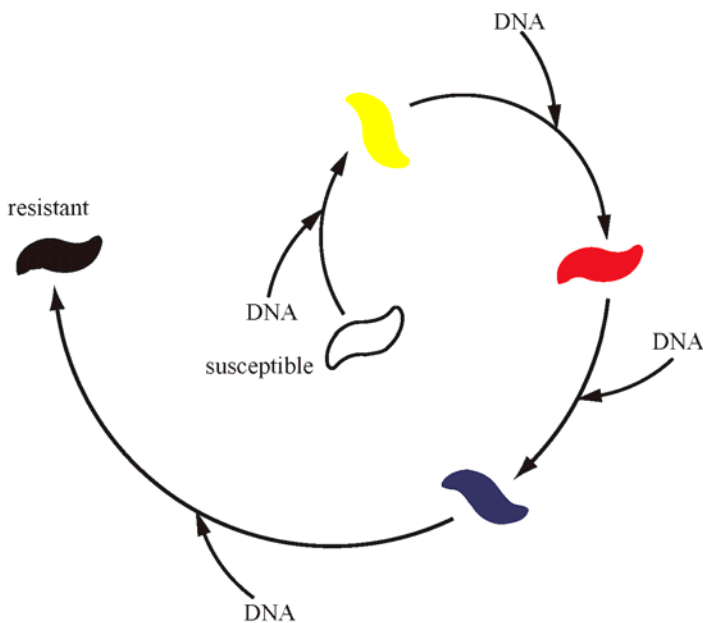


Figure 2. PBP evolution cycle is illustrated from fully penicillin susceptible bacterium (open shape) stepwise (increasingly dark shapes) through encounters with DNA from other species and waves of selection with increasing concentrations of penicillin.

all at once by a relatively small number of bacteria in a matter of hours to days or months. The limit of these experiments is a detection of between 1 event in one million to an estimated 1 event in 10^{17} . What limits the experiments is that only between 0.1 and 10% of all bacteria can be cultured, so most bacteria that would receive DNA around crops will not show themselves to investigators. In addition, techniques for detecting changes in the DNA of genomes cannot see small changes, of the type that built up the PBP genes of *S. pneumoniae*. Recombinants can only be detected after they become very common or we know

- the frequency of HGT is very high but our ability to detect it, especially when most events do not immediately change the characteristics of the recipient organism, makes it appear to be infrequent.

How big would field trials have to be?

To base claims that HGT can be dismissed as a risk factor on sound science, they would have to have come from extremely large field trials testing for HGT. In fact, we can calculate just how large they would

have had to be. From this, we can evaluate the reliability of such claims.

The risk assessment community considers an HGT frequency of 1 in 10^{17} to be the absolute highest frequency possible in soil. With GM crops now planted on nearly 70 million hectares of land worldwide, as many as a trillion recombinant bacteria would be created by DNA from GMO plants. To find one of these recombinants would require sequencing the genomes of 2 billion bacteria per gram of soil, in 3 billion metric tonnes of soil.

11.4 million hectares of land is planted in one type of recombinant corn, Bt11. This GM crop was recently re-evaluated by the US EPA and its approval was renewed in the absence of evidence of gene transfer from Bt11 to soil microbes. However, to properly test if any transfers had occurred would require the EPA to have examined all the genomes in approximately 500 million metric tonnes of soil.

To get a sense of the scale of the experiment, consider that 500 million tonnes is 6,000 times the weight of the largest pyramid of Giza, and 4 million times the weight of the Eiffel tower. Researchers would have required a train of 500 million boxcars to transport the soil to the laboratory. Such a train would be long enough to wrap itself around the equator 192 times.

Normally, safety engineers would develop tests which incorporated additional safety margins. 1 in 10^{17} is a frequency 10 million times higher than was needed to create penicillin resistant *S. pneumoniae*, as measured by tests comparable to those used to measure HGT in soils around GMOs. So a safety engineer would base claims of safety on experiments with detection limits 10-100 times below 1 in 10^{24} , approximately a billion times more sensitive than can be extrapolated from a field trial

that examined all the DNA in 500 million tonnes of soil.

Clearly, an experiment of this magnitude has not been done. So confidence is low in claims that HGT has been studied and found to not be a significant risk. In this case, the absence of evidence of risk could easily be explained by extrapolations from the results of experiments that were not suited for making a proper assessment of risk.

What could be done?

One alternative to extrapolating risk from existing knowledge is to generate better knowledge. Changes in the science of monitoring would only marginally increase sensitivity, if at all. For example, applying the technique of PCR to examining the DNA from soil microorganisms would at best increase detection by 10- to 100-fold, far short of the more than trillion-fold necessary.

The most obvious alternative, then, is not a technique but a decision to consider the scientific uncertainty surrounding bio-applications that introduce HGT risks and adjust the pace of releasing these products to match developments in our ability to monitor at relevant sensitivities.

This choice is not an improvement in science but a change in policy, recognizing that the technology of safety monitoring lags behind the technology of genetic engineering. Meanwhile, governments must re-direct funding priorities to research that is relevant to understanding HGT rather than *ad hoc* measures to perform experiments on released GMOs.

Concluding remarks

What has been learned in the field of medical microbiology over the past several decades should inform those who assess risk about how to design experiments and why current approaches have yielded uniformly uninformative (negative) results. Those

lessons are not effectively incorporated into the attempts to detect gene transfer in the soils surrounding GM plants at present. Furthermore, more investment in these flawed approaches will not increase confidence in risk assessments. On the contrary, scientists who persist with such approaches risk building a possibly false confidence in the claims that HGT creates few risks.

Whereas gene transfer is a natural phenomenon, as it was with the creation of antibiotic resistant bacteria, human activities influence it, creating outcomes that may not

best serve our interests. GM crops currently in production carry antibiotic resistance genes, but even those concerns may be eclipsed by modifications under development. We cannot assume that current GMOs have created additional evidence for the safety of crops engineered to produce pharmaceutical grade compounds, or for animals that might produce vaccines. With regard to GMO bio-applications, we have the luxury of time to conduct verified and proper assessments before release, if we should choose to take it.

Glossary

antibiotics and antibiotic resistance

Antibiotics are natural compounds that have been isolated from bacteria, fungi and other sources. They may be further modified and concentrated for use in medicine and as dietary supplements. Resistance refers to a decrease in susceptibility to antibiotics among the disease causing bacteria.

DNA, nucleotides

A DNA molecule is composed of two polymers, or 'strands', consisting of a linear sequence of nucleotides called A, T, G and C (abbreviations of their chemical names). DNA is normally double-stranded, with the strands bound together by bonds between complementary nucleotides. The weak bonds between nucleotides on different strands are formed optimally when A pairs with T and G pairs with C.

Heisenberg Uncertainty Principle

In quantum mechanics theory, it is impossible to know an object's position and its velocity (actually, momentum) simultaneously because any attempt to hold one variable, either position or velocity, constant makes the error in the measurement of the other variable infinite. Thus, in the Principle we can talk only in probabilities.

horizontal gene transfer

Gene transfer from parent to offspring (reproduction) is technically referred to as vertical gene transfer. Any occurrence of genes transferring independently of, or asynchronously with, the reproduction of the organism is horizontal gene transfer. It is most commonly recognized as infectious transfer, such as the reproduction of a virus between people.

polymerase chain reaction, PCR

These principles are the basis of the polymerase chain reaction. In the PCR, discrete and short sequences of DNA from an organism are selectively amplified by a runaway, or chain, reaction concentrated on those particular sequences. The reaction is both initiated and bounded by even shorter sequences of DNA (usually <20 nucleotides) called primers. Primers are synthesized to be complementary to each end of a sequence of DNA to be amplified. The match between primer and 'template' (organism DNA) is a critical determinant of the efficiency of the PCR

reaction. PCR efficiency is the minimum concentration of a target template in a mixed sample that can be reliably amplified.

Based on research by:

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