

Monitoring horizontal gene transfer

To the editor:

Two articles by Heinemann and Traavik (*Nat. Biotechnol.* 22, 1105–1109, 2004) and Nielsen and Townsend (*Nat. Biotechnol.* 22, 1110–1114, 2004) use different arguments to reach a similar conclusion, namely that present methods of monitoring the potential transfer of antibiotic genes from transgenic plants to the bacterial population lack the necessary sensitivity by many orders of magnitude. They argue that horizontal transfer may arise at frequencies vastly lower than are presently measurable and that unintended effects may follow depending on the possible selective advantage of the resulting antibiotic-resistant bacterium. Although it is not known, because it is impossible to know, the level at which transgene uptake ceases to be of ecological importance, I feel it necessary to mention some additional points.

First, Heinemann and Traavik put a highly personal interpretation on the results of *Acinetobacter* ADP1 model system for transfer of transgenic neomycin resistance (*nptII*) gene from plants to bacteria. They conclude that “horizontal gene transfer does occur, *even if it is influenced by the method used to observe it*” (my italics). In reality, these experiments demonstrate that horizontal gene transfer does not occur, within the limits of detection of the experiment, unless the experimenter has already artificially manipulated the bacterial strain to contain sequences homologous to the incoming transgenic DNA. This manipulated strain is simply an elegant positive-control showing that the bacterial cells are indeed competent and that the donor DNA is functional. Without it, the negative result for the wild-type bacterium would be meaningless. Such bacteria are highly unlikely to occur in nature unless the bacterium already contains the *nptII* gene (in which case the problem does not arise). In addition, it should be noted that these experiments are designed to maximize transgene detection because, in addition to homologous sequences, they use purified DNA and a highly-transformable strain of *Acinetobacter*. Transformation would not succeed, even in the presence of DNA homology, with more common bacteria, such as *Escherichia coli* or *Rhizobium leguminosarum* or *Pseudomonas putida*.

Second, both articles compare the large-scale cultivation of transgenic plants,

carrying antibiotic resistance genes, to the wide-scale use of antibiotics that ended the pre-antibiotic era and resulted in the spread of antibiotic-resistance genes. This comparison is unrealistic, because, in the pre-antibiotic era, antibiotic-resistance genes existed at a low frequency, if at all. In contrast, in the present world, antibiotic-resistance genes are ubiquitous and are carried by highly perfected, wide host range, horizontal transfer machines (plasmids, transposons and integrating conjugative elements) that enable efficient horizontal transfer between species¹. It is in this world that the transgenes escaping from genetically engineered plants must compete. Thus, the important question is whether, to use their example, the *nptII* gene, if transferred from transgenic plants to bacteria at the extremely low frequency that the authors envisage, will have a selective advantage, relative to its ubiquitous wild relatives. In other words: will anything happen that is not already happening?

Third, the authors of both articles advocate developing new detection methodologies to monitor events that may occur at a trillionth of the present detection capability. With present methodology this is impossible and in any case it is illogical because detection is not prevention and, as the authors say, the time scale may be considerable. In contrast, the question of antibiotic resistance in transgenic plants has been examined by a variety of prestigious study groups at the World Health Organization (Geneva)/Food and Agriculture Organization (Rome), the European Commission (Brussels), the

Jack A. Heinemann and Terje Traavik reply:

Our article points out that the relevance of transgene transfer to biosafety has been dismissed, *as if its irrelevance were experimental fact*. Davison misses the point by confusing the example of antibiotic-resistance gene transfer from transgenic plants to bacteria with our illustration of resistance evolution by gene transfer. We showed that based on experimental observations of resistance evolution, sound biosafety experiments have been impossible. Either the biosafety claims are experimentally verified or they are based on intuition and educated guesses. The standards of the science behind claims of safety

International Council for Science (Paris), the UK Royal Society (London), the Belgian Biosafety Council (Brussels), the National Academy of Sciences (Washington, DC, USA) and the Nuffield Council of Bioethics (London) (URLs available on request), and all propose that future transgenic plants be constructed without antibiotic-resistance markers. Modern methods exist for the construction of transgenic plants using nonantibiotic markers, or no markers at all, whereas site-specific excision methods allow the removal of superfluous DNA, including antibiotic-resistance genes. In the absence of antibiotic-resistance genes in transgenic plants, the problem of their transfer to bacteria ceases to exist.

Finally, we come to the most difficult question. The authors, together with various environmentalist groups, fixate their attention upon transgenes in general and on antibiotic-resistance genes in particular. However, DNA of all kinds (viral, microbial, plant, animal and human) is common and rather stable in the environment. If it is believed that antibiotic-resistance genes can be transferred from transgenic plants to microbes, then it is only logical to also believe that any gene or DNA fragment can be incorporated. Thus, the problem they outline, if real, may be unrelated to transgenic plants.

1. Davison, J. *Plasmid* 42, 73–91 (1999).

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should not be adjusted in the name of transgenic organisms simply because the claim is too difficult to verify.

Antibiotic-resistance evolution arose because of undetectable frequencies of horizontal gene transfer (HGT) that grew into discernable threats. This exemplifies the general uncertainty over how the many different transgenes, including those for antibiotic resistance, will affect the characteristics of organisms that subsequently receive them. Resistance genes and antibiotics were not rare before human use of antibiotics¹, as Davison says, but they were effectively non-existent among the organisms that cause disease in people. A change in human prac-