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 species[1]. 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 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).

John Davison

Laboratory of Cellular Biology, Institut National de la Recherche Agronomique, Route de St Cyr, Versailles 78026, France.
e-mail: davison@versailles.inra.fr

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 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 antibiotics[1], as Davison says, but they were effectively non-existent among the organisms that cause disease in people. A change in human prac-

© 2004 Nature Publishing Group http://www.nature.com/naturebiotechnology

tices influenced the transition of these genes into "horizontal transfer machines," leading ultimately to their introgression into new species. The lesson here is that different applications of genetic engineering may create new opportunities in evolution, some of which are worth anticipating before we invite them.

For example, the US National Research Council (Washington, DC, USA) recently considered 'suicide genes' for containing recombinant microorganisms[2]. We predict that such genes easily become horizontal transfer machines[3,4], making their use as a containment tool a potential mistake.

Unless we have a basis for predicting when genes make the transition to horizontal reproduction[5], we cannot know *a priori* that particular transgenes will be uncompetitive, contrary to what Davison suggests. We similarly cannot assume that transgenes are equivalent in their abilities to make this transition, particularly as they are neither the same DNA sequence nor in the same context as their natural counterparts. They are also not released into the same world that their natural counterparts evolved in, because they are maintained through human-assisted breeding programs and very often reach novel concentrations in the environment through the use of co-technologies (e.g., herbicides) that may have unanticipated effects on the evolution of these genes by HGT.

Contrary both to Davison and to Nielsen and Townsend, we do not assume that the only biosafety-relevant outcome of HGT is introgression. We also question the latters' assertion that an "overwhelming majority of HGT events in nature are *known* to be deleterious to the bacterial transformant" (our emphasis) on the basis of only two laboratory studies using *Escherichia coli*. First, there is no evidence that the majority of HGTs result in genomic insertion. Second, not all biosafety risks are from the growth and spread of organismal recombinants and transgene introgression into organismal lineages. Some horizontal transfer machines pose relevant risk without having introgressed into a genome, as viruses and plasmids and some transposable elements demonstrate[6].

1. Baquero, F. & Blazquez, J. *Trends Ecol. Evol.* **12**, 482–487 (1997).
2. Committee on the Biological Confinement of Genetically Engineered Organisms, NRC. *Biological Confinement of Genetically Engineered Organisms.* (National Academies Press, Washington, DC, 2004).
3. Cooper, T.F. & Heinemann, J.A. *Proc. Natl. Acad. Sci. USA* **97**, 12543–12648 (2000).
4. Cooper, T.F. & Heinemann, J.A. Selection for plasmid post-segregational killing depends on multiple infection: evidence for the selection of more virulent parasites through parasite-level competition. *Proc. R. Soc. Lond. B Biol. Sci.*, in press.
5. Heinemann, J.A. & Silby, M.W. in *Multiple Drug Resistant Bacteria.* (ed. Amábile-Cuevas, C.F.) 161–178 (Horizon Scientific Press, Wymondham, 2003).
6. Syvanen, M. & Kado, C.I. (eds.) *Horizontal Gene Transfer*, edn. 2 (Academic Press, San Diego, 2002).

Kaare M. Nielsen and Jeffrey Townsend reply:

Davison's considerations may be relevant to citizens and policymakers as they decide upon policy toward the usage of plant marker genes; however, it is up to the scientific community to provide unbiased and accurate methods for assessing risk, which is what our article advocates. Our article did not discuss the specific effects of plant marker gene flow, but the general failure of current studies to detect HGT if it occurred, and the absence of population genetic considerations in these studies. Thus, none of the comments made by Davison is relevant to the scientific issues we point out. We wish to make our own clarifications of Davison's assertions.

First, he argues that HGT studies conducted with bacteria with inserted sequence-similarity to plant transgenes are not relevant to understanding HGT processes in natural environments, presumably because naturally occurring bacteria lack sequence-similarity to transgenes. In fact, a recent study shows that many commonly occurring bacteria have high sequence similarity with plant marker genes, suggesting homology-based recombination can occur[1]. Moreover, the plant marker gene can provide the anchor sequence necessary to initiate transfer of adjacent transgenes into bacteria. For instance, another recent study demonstrates that short stretches of DNA sequence similarity facilitate the incorporation of larger (>1 kb) heterologous DNA fragments, including entire plant genes, into naturally transformable bacteria[2]. These observations suggest that additive integration of transgenes can take place in bacteria after homology-initiated recombination; and additive integration is known to occur at high frequencies in bacteria[3]. More than ten peer reviewed studies now demonstrate that bacteria, including *Acinetobacter baylyi* and *Pseudomonas stutzeri*, take up either purified plant DNA or DNA naturally present in colonized whole plants when sequence similarity is present[4]. Natural transformation has also been demonstrated in *Escherichia coli*[5].

Second, Davison suggests that *nptII* genes and other plant marker gene homologs are widespread in nature and that rare HGT from transgenic plants would add insignificantly to the dissemination of these. However, no reference to peer reviewed studies is given providing evidence for the uniform and geographically widespread occurrence of *nptII* genes in soils exposed to this transgene.

Third, he only considers the risk of HGT with regard to plant marker genes that represent only a subset of the transgenes in use. However, a main argument in our article is that novel transgenes that do not have natural counterparts are those that require particular attention in a HGT context. These are transgenes, including novel combinations of regulatory elements and toxin protein domains (e.g., for vaccines or biopharmaceuticals), that may differ substantially from those arising by natural evolutionary processes[6].

We hope these considerations, along with our perspective and the methods suggested therein, focus interested parties on the issues important to resolving the long-term effect of transgenes exposed to complex microbial communities.

1. Bensasson, D. *et al. Heredity* **92**, 483–489 (2004).
2. De Vries, J. *et al. Mol. Microbiol.* **53**, 323–334 (2004).
3. Nielsen, K.M. *et al. Appl. Environ. Microbiol.* **66**, 206–212. (2000).
4. Kay E. *et al. Appl. Environ. Microbiol.* **68**, 3345–3351 (2002).
5. Woegerbauer, M. *et al. Appl. Environ. Microbiol.* **68**, 440–443 (2002).
6. Nielsen, K.M. *Nat. Biotechnol.* **21**, 227–228 (2003).

© 2004 Nature Publishing Group http://www.nature.com/naturebiotechnology