In summary, Strauss and colleagues state that there is a low risk from the consumption of transgenic plants "where no novel biochemical or enzymatic functions are imparted." The question is, of course, how can one know if a novel and potentially harmful molecule has been created unless the testing has been done? How can one predict the risk in the absence of an assay? Because of the high mutagenicity of the transformation procedures used in GE, the assumptions made by Strauss and colleagues and by the US Food and Drug Administration[33] about the precision and specificity of plant genetic engineering are incorrect. Nonetheless, it appears that the position of Strauss and colleagues and the agbiotech industry, as well as the current US regulatory framework for the labeling and safety testing of transgenic food crops, is to maintain the status quo and hope for the best.

The problem is that there are no mandatory safety testing requirements for unintended effects[1] and that it may take many years before any symptoms of a disease arising from a transgenic product to appear. In the absence of strong epidemiology or clinical trials, any health problem associated with an illness caused by a transgenic food is going to be very difficult, if not impossible, to detect unless it is a disease that is unique or normally very rare. Therefore, although GE may enhance world health and food crop production, its full potential may remain unfulfilled unless rigorous prerelease safety testing can provide some assurance to consumers that the products of this new technology are safe to eat.

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**Strauss and colleagues respond:** Wilson *et al.* claim on the one hand that their report "did not specifically 'argue for rejection if even a single base pair is changed,'" while recommending that "transgenic lines containing genomic alterations at the site of transgene insertion be rejected." In addition, in their original report, they further state that they "recommend that both the transgene insertion event (including all transferred DNA and a large stretch of flanking DNA) and the original target site be sequenced and compared as the only known way to definitively determine whether gene sequences have been disrupted." In the context of their discussion, even a single base pair change is clearly considered to be a "genomic alteration," so we believe that we have accurately represented the implications and rationale of their position.

Regarding the possibility that some genomic changes occur due to transformation, we never denied that this occurs, and in fact cited their study as a source for our statement that "unknown mutations and chromosomal translocations can occur during the transformation and regeneration process." Where we differ with Wilson *et al.* is in their opinion that such mutations will "lead sooner or later to harmful consequences." There is no documentation of such harmful consequences in their report for products that have undergone phenotypic screening for commercial release.

A central point of our Perspective was that a very large number of genomic and gene differences already exist within crop cultivars, and even among individual plants within a cultivar, without producing any harmful consequences (for another striking example, see ref. 1). Thus, the assumption of the inevitability of harmful consequences from genomic differences associated with gene transfer ignores the ubiquity of extensive genome sequence variation within existing food crops.

Although Wilson *et al.* agree with us that "analysis of the phenotype is the one true measure of safety," they nonetheless state that phenotypic analysis is of "unproven effectiveness" and suggest that genomic sequence data would be more reliable or effective. Both of these arguments are flawed. First, phenotypic analysis has been extremely effective in the development of many thousands of commercial cultivars in a wide range of crops for several generations. Second, how Wilson *et al.* propose to distinguish the toxicologically silent genomic differences that are abundant in crop plants from ones that might actually have phenotypic consequences is addressed neither in their original report nor in their comment.

In his letter, Schubert raises several issues, many of which have been addressed extensively in published literature. For completeness, we address these issues here in summary fashion:

***Alleged lack of precision in genetic engineering (GE).*** The lack of precision due to random gene insertion and genomic alteration is often raised as a criticism of GE. However, conventional breeding is based on essentially random induction or assembly of mutations, followed by selection among a multitude of unpredictable and often imprecise natural recombinations between

genomes. The expression profile of genes is often changed in ways that are not well understood, and with multiple phenotypic consequences (that is, pleiotropy), by inbreeding and wide crosses, as further discussed below. This lack of 'precision' has not prevented plant breeding from developing improved crops, as the focus has been primarily on the resulting phenotypes, not on their genomic basis. Similarly, ancillary genomic changes accompanying GE may occur, but are irrelevant so long as the expected and desired phenotype is produced without unacceptable side effects.

***Basic research versus cultivar development.*** Schubert cites extensive "unintended effects," but many of these result from failing to distinguish between the use of transgenes in basic research and the development of improved cultivars using GE. Unexpected changes in phenotypes, usually due to overexpression or knockouts, are a routine part of basic research using GE. However, these events are not subjected to the phenotypic, biochemical and often molecular selection demanded in breeding of competitive crop varieties. Breeders, whether working with conventional methods or transgenes, conduct years of intensive laboratory, greenhouse and field screens so phenotypically abnormal, unstable or undesirable genotypes or events are discarded.

*Prevalence of mutagenized cultivars.* Schubert states "mutagenesis was used in the United States during the middle part of the past century, but food crops made by this technique now constitute less than a few percent of US production, with sunflowers being the major representative," citing ref. 2. This is a rather disingenuous summary of the cited paper, which documents the extensive use and enormous economic impact of the more than 2,275 varieties of 175 species that have been derived either as direct mutants or from their progenies. Many currently popular varieties of numerous crops contain mutagenized progenitors in their pedigrees. The widespread production and consumption of mutation-derived varieties without ill effect over the past 50 years is evidence that these do not need to be regulated differently from varieties developed via other methods.

*Wide crosses and ploidy manipulation.* Schubert goes on in his letter to argue that conventional breeding is inherently safer than GE, stating that "in wide crosses and other forms of ploidy manipulation, there are clearly changes in gene dosage, and proteins unique to only one parent can be produced in the hybrid, but there is no a priori reason to assume that mutations are going to occur simply because there is a change in chromosome or gene number." Rather than relying on a priori assumptions, a large body of evidence indicates that complex and as yet poorly understood genetic changes often accompany wide crosses and ploidy manipulation, including gain and loss of DNA, gene silencing, translocations, epigenetic modifications and mobilization of transposable elements (e.g., refs. 3–6). Schubert's statement that "only GE and mutagenesis introduce large numbers of mutations" is grossly incorrect. In addition, introgression of genes via wide crosses most often occurs via recombination and substitution of chromosomal segments, not via increases in ploidy, as Schubert claims.

*Dangerous nature of genetic changes?* Schubert writes that "Strauss and colleagues correctly state that plants normally contain the same *Agrobacterium tumefaciens* and viral DNA sequences that are used to create GE transfection constructs, but fail to point out that with GE these pieces of DNA are part of a cassette of genes for drug resistance along with strong constitutive viral promoters… which are used to express foreign proteins at high levels in all parts of the plant—hardly a natural event." This argument has several problems. First, strong promoters are not restricted to viral DNA; plants also naturally contain many strong, near-constitutive promoters (e.g., ref. 7), and some of these are now used to aid plant transformation (e.g., refs 8,9). Second, the viral promoters/enhancers Schubert is concerned about act over very limited distances on a genomic scale, and thus have very limited potential to cause random increases of gene expression. The fourfold repeated cauliflower mosaic virus enhancer element (the source of its constitutive promoter activity) influences gene expression predominantly over 5 kb[10], or about the size of a single genomic locus in plants. Third, the use of tissue-specific, plant-derived promoters, rather than constitutive promoters, is becoming increasingly common in GE programs (e.g., refs 11,12). Fourth, those transgenic crops that express antibiotic resistance genes (not all transgenic crops do) express only those genes whose expression is already widespread in bacteria found in the human gut (e.g., refs 13–15). Finally, with respect to drug resistance marker genes generally, an in-depth review recently concluded " that there are no objective scientific grounds to believe that bacterial AR [antibiotic resistance] genes will migrate from GM plants to bacteria to create new clinical problems[16]."

*Retrotransposons.* Schubert claims that our statement that "retrotransposons continuously insert themselves between genes" is incorrect because these high copy number elements are transpositionally inactive in normal modern food plants. The latter statement is not supported by experimental results. Expressed sequence tag databases reveal that retrotransposon RNA is present in plants[17–19], from which it can only be inferred that their expression continues. The rate of transposition is likely to be highly variable depending on species, developmental stage and inducers, such as environmental and genomic stress. Common non-GE procedures such as tissue culture, which is used routinely for dihaploid production and propagation, are known to substantially increase the rate of transposition (e.g., ref. 20), and many tissue culture–derived, non-GE varieties have been in the food supply for some time.

*Screening for unexpected molecules.* The high diversity of "nonessential small molecules that provide adaptive benefits under conditions of environmental or predator-based stress" that Schubert refers to are also produced in complex and unpredictable ways during normal crop management, shipping, storage, processing and food preparation. Cheeses, plant-derived beverages and many other processed foods are known to contain vast numbers of biochemicals of diverse types (e.g., refs. 21), the great majority of which have never been tested for safety. Should all the molecules produced by each new type of cheese be subject to detailed toxicological assessments? This also underlines the general, rather than specific, basis of human adaptation to diverse plant chemistries. Human digestive systems routinely deal with vast numbers of natural chemicals present at low concentrations in food, many of which can be shown to be mutagenic at high concentrations[22].

The nucleic acid or proteomic tests of large numbers of gene expression products that were proposed by Schubert are extremely sensitive and extremely expensive. They may detect hundreds or even thousands of changes in a novel variety, whether conventionally bred or produced using GE, if compared with their progenitors under a full range of growth environments, stresses and developmental stages. How would such data be interpreted with respect to risk? Simply obtaining more data via mandated mass spectrometry, microarray evaluations or the like, without a means to

evaluate them with respect to benefit/risk of whole foods, does not add to knowledge and safety but to chaos and controversy.

Schubert backs up his argument by noting that Kuiper *et al.*[23] called for metabolic profiling of each transgenic event. However, coauthors of that paper now agree[24] that "further research is required to validate profiling methodologies...The safety assessment of [genetically modified] GM crops should focus primarily on the intended novel traits (target gene(s) and product(s)). Unintended effects occur in both GM and non-GM crops; however, GM crops are better characterised. It may be suggested that the two should be treated the same in safety assessments, bearing in mind that safety assessments are not required for non-GM crops. Profiling techniques should not at present be an official requirement[24]."

Finally, because random mutations and alterations in gene expression occur widely in all plants during breeding, if perturbations of biosynthetic pathways could readily give rise to important toxins from commonly grown crops their effects should already be widely observed. Experience indicates, however, that phenotypes and metabolic pathways tend to be highly buffered from the effects of mutations. This is likely to be the reason that most loss-of-function mutations show only minor, if any, phenotypic changes. For example, in a screen for insertional inactivation in *Arabidopsis thaliana*, only 3% of the T-DNA insertions among a population of 55,000 events showed a visible phenotype[25]. This buffering appears to be due to the immense number of interactions and feedback mechanisms in higher organisms[26], which can occur at the levels of gene expression, enzymatic pathways, cellular processing and multicellular development.

*Unintended changes in plant composition.* To support his contention that unintended consequences can arise from GE, Schubert cites one study that found higher lignin levels in transgenic *Bt* maize. However, those results were not reproduced in a more extensive study[27]. Numerous studies document the equivalent performance of animals fed silage from *Bt* and non-*Bt* corn[28–30] (reviewed in ref. 31), which would not be expected were their lignin compositions substantially altered.

Likewise, Schubert cites the claim that isoflavone levels are altered in transgenic soybeans. This claim has been roundly criticized because it did not compare soybeans of the same genetic background or grown in the same environment, two factors that are known to have a large effect on isoflavone content (see http://www.soybean.com/gmsoyst1.htm). The example of isoflavone variability in soybean also illustrates the fallacy behind testing for metabolites; merely finding a difference in the amounts of metabolites is biologically irrelevant without additional information on the beneficial versus deleterious effects of specific metabolites in whole plants and on the range of metabolite levels that can occur within different genotypes grown under a wide range of environmental conditions[24].

*Value of mutagenicity tests.* Schubert suggests use of the Ames test, apparently to examine whether "unexpected changes in small-molecule metabolism" are of mutagenic significance. However, it is widely known that this high-dose test gives a greatly inflated rate of false discovery of nontoxic minor compounds in food (e.g., approximately half of the compounds in coffee do not pass this test[22]). The results of these tests are also known to be very poor predictors of the potential for mammalian carcinogenicity[32]. Compounds that are harmful at the high concentrations used in such tests may even be beneficial to health at low concentrations. Given the hundreds of metabolites that may be altered via conventional or GM breeding (not to mention by environmental conditions, or the presence of pathogens or insects), it is exceedingly unlikely that screening them via the Ames test would contribute to the goal of producing more healthful foods.

Our article attempted both to put recombinant DNA modification in a genomic context with respect to traditional breeding methods and the diversity of wild progenitors and to propose a regulatory framework where the benefits from use of gene transfer approaches are not lost amidst excessive attention to collateral genomic changes. Unintended genomic changes can be significant for all forms of breeding, including gene transfer. Yet the preponderance of scientific research, and experience from plant breeding and applied biotechnology, suggests that the effects of these genomic changes on food safety are modest and manageable by paying attention to plant phenotypes. The technical and ethical challenge is to distinguish important risks from trivial ones so the many tangible benefits that can be provided by GE are not stifled by burdensome regulatory requirements that do not enhance safety of the food supply.

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