Scrambling and Gambling with the Genome July 2005
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"With genetic engineering, transferring genes from one species' DNA to another is just like taking a page out of one book and putting it between the pages of another book." This popular analogy is used often by advocates of genetically modified (GM) food. The words on the page are made up of the four letters, or molecules, of the genetic code, which line up in "base pairs" along DNA. The inserted page represents a gene, whose code produces one or more proteins. The book is made up of chapters, which represent chromosomes—large sections of DNA.

The analogy makes the process of genetic engineering appear to be as simple and precise as inserting a new page. A groundbreaking report, however, shreds the book analogy. *Genome Scrambling – Myth or Reality?*, written by three scientists at the UK-based Econexus, reveals that the process of genetic engineering results in widespread mutations—within the inserted gene, near its insertion, and in hundreds or thousands of locations throughout the genome—and that these are overlooked by many scientists and regulators. [1]

The report is an extensive review of research that overturns the central arguments by biotech advocates—that the technology is precise, predictable, and safe, and that current studies are adequate. On the contrary, it demonstrates that GM crops represent a significant gamble to public health and the environment (see www.econexus.info).

Gene Insertion Methods Create Mutations, Fragments, and Multiple Copies There are two popular methods for creating GM crops; both create mutations. The first method uses *Agrobacterium*—bacteria that contain circular pieces of DNA called plasmids. One section of this plasmid is designed to create tumors. Under normal conditions, *Agrobacterium* infects a plant by inserting that tumor-creating portion into the plant's DNA. Genetic engineers, however, replace the tumor-creating section of the plasmid with one or more genes. They then use the altered *Agrobacterium* to infect a plant's DNA with those foreign genes.

The second method of gene insertion uses a gene gun. Scientists coat thousands of particles of tungsten or gold with gene sequences and then shoot these into thousands of plant cells. Years ago, the sequences that were shot into cells usually included both the genes that were intended for transfer (gene cassette) as well as extraneous DNA from the plasmid used in the creation and propagation of the cassettes in bacteria. This is true for most GM foods currently on the market. These days, many scientists take the added step of eliminating the extraneous, mostly bacterial DNA and coat the particles just with the cassette.

With both methods of gene insertion, scientists speculate that the process triggers a wound response in the plant cell, which helps its DNA integrate the foreign gene. With the gene gun technique, only a few cells out of thousands incorporate the foreign gene.

According to the book analogy, a single, intact, foreign page (gene) is inserted. That's the intention. In reality, most transformed DNA end up with multiple copies of the foreign gene, incomplete genes and/or gene fragments. Sections of the inserted genes are commonly changed, rearranged, or deleted during the insertion process. In addition, extraneous pieces of plasmid DNA sometimes end up interspersed within and around the inserted gene or scattered throughout the genome.

Mutations Near the Site of Insertion In addition to the changes made in the material that is inserted, the sections of the plant's DNA near the insertion site are almost always messed up in some way. This effect, called insertional mutagenesis or insertion mutation, has been known for years, but it wasn't until 2003 that a large-scale systematic analysis was conducted. Researchers looked at insertions into 112 *Arabidopsis thaliana* plants—a species used often in

plant research. [2] Although the study may not accurately reflect what happens in edible crop plants, it is the only large study at this point.

Plants were selected that had single copies of the foreign gene, which were inserted using *Agrobacterium*. Of the 112 plants, 80 (71%) had small mutations near the insertion site. These included deletions of 1-100 base pairs and/or insertions of 1-100 extraneous base pairs. The inserted sequences came from the foreign gene, extraneous parts of the plasmid, or other parts of the plant's DNA.

The remaining 32 plants (29%) had large scale insertions, rearrangements, duplications and/or deletions. In two plants, parts of whole chromosomes had broken off and translocated into another section of the DNA.

Another study using the same plant species also found that a section of DNA at least 40,000 base pairs long had translocated from one chromosome to another. In fact, that long section had duplicated itself, since it was also found intact in its original position. [3] A third study identified a deletion of 75,800 base pairs, which probably contained 13 genes. [4]

The above studies used the *Agrobacterium* insertion method. There have been astoundingly few studies analyzing insertion mutations resulting from the gene gun method, but the research that has been conducted consistently demonstrate large scale disruptions of the DNA. According to the Econexus report, "The vast majority of the insertion events created via particle bombardment [gene gun] are extremely complex, with multiple copies of transgenic DNA inserted at a single insertion-site."[1] They contain large amounts of extraneous DNA, including multiple fragments of the foreign gene and/or small or large fragments of plant DNA interspersed with the inserted genes. In one study, scientists found 155 separate breaks indicating recombinations of the inserted genetic material. [5] According to the Econexus report, in the rare cases where only a single copy of the foreign gene is inserted, they "turn out to contain fragments of superfluous DNA and/or they appear to be associated with large deletions and/or rearrangements of the target plant DNA." [1]

One study on gene gun insertion revealed that DNA of an oat plant contained the full sequence of the foreign gene plasmid, a small stretch in which oat DNA was mixed up with foreign plasmid DNA, a partial copy of the plasmid, and another section with oat and plasmid sequences scrambled together. [6] Analysis also indicated that the plant's DNA on either side of the insertion contained rearrangements or deletions. There were also two other insertions elsewhere in the DNA. One included a rearranged section of the plasmid (296 base pairs), scrambled plant DNA on either side, and the deletion of 845 base pairs. The study employed DNA sequence analysis, the most thorough method for evaluating insertion mutations. In practice, it is rarely used. Instead, genetic engineers traditionally rely on the less precise Southern blot test, which picks up only major changes in DNA sequence. When this test had been applied to the oat DNA above, it indicated the presence of only a single intact inserted gene. It failed to identify the other two insertions and all of the mutations and fragments. This means that on the whole, biologists who create GM plants have no idea of the extent to which their creations may produce unintended side effects do to scrambled DNA.

Location, Location, Location Neither gene insertion method can "aim" the foreign gene into a particular location in the DNA. Furthermore, scientists rarely conduct experiments to find out where exactly the inserted genes end up. But in the real estate of the DNA, location is vital. The functioning of the foreign gene can change dramatically, depending on where in the genome it is located. The side-effects of gene insertion can be significantly influenced by location as well.

Even though only an estimated 1-10% of plant DNA constitutes the genes, Agrobacterium insertions end up inside functioning gene sequences between 35%-58% of the time. (The percentage for gene guns is unknown.) Genes are also inserted in other areas that influence gene expression. In either case, insertions can significantly disrupt the normal functioning of the

plant's genes.

(One reason why insertions end up inside genes so often is that in order for the foreign genes to function, they need to be located within the regions of the host DNA that are "active," that allow gene expression. To figure out which inserted genes end up in these portions of the DNA, scientists typically add an antibiotic resistant marker (ARM) gene to the genetic cassette. After insertion, they apply antibiotics to all the cells, killing those that don't have a functioning ARM gene in their DNA. Since the active region of the DNA is also where the plant's functioning genes are located, those that survive this selection process are more likely to have foreign genes lodged inside the host genes.)

Mutations Throughout the DNA Once genes are inserted into a plant cell's DNA, scientists typically grow the cell into a fully functioning plant using a method called tissue culture. Unfortunately, this artificial method of plant propagation results in widespread mutations throughout the genome. In fact, *tissue culture is sometimes used specifically to create mutations* in plant DNA. These mutations can influence the crops' height, resistance to disease, oil content, number of seeds, and many other traits. [7],[8]

Genetically modified cells that undergo tissue culture can have even more mutations throughout the genome than cultured non-GM cells. It is unclear why gene insertion has this effect, but scientists speculate that it may, in part, come from unsuccessful insertions or insertions of small fragments.

Taken together, the process of gene insertion combined with tissue culture typically results in hundreds or thousands of mutations, including small deletions, substitutions, or insertions in the genetic code. The changes are vast. Two studies suggested that 2-4% of the genome of a GM plant was different than non-GM controls.[9],[10] Furthermore, estimates are based on detection methods that miss many mutations such as short deletions and insertions and most base pair substitutions. Thus, the actual degree of gene disruption is probably greater.

These genome-wide mutations are found in every GM plant analyzed. Astoundingly, these types of mutations are not evaluated in commercially released GM food crops.

If the original GM plant is crossed (mated) with other lines over and over, many of these small, genome-wide mutations will get corrected. It is unknown, however, how many mutations still persist in food crops. Furthermore, the propagation of certain species, such as the GM potato that was on the market years ago, probably did not undergo any outcrossing, and it is likely to contain all of the mutations created during insertion and tissue culture.

Mutations Can Have Serious Consequences Mutations and extraneous insertions carry risk. They can permanently turn genes on or off, alter their function, and/or change the structure or function of the protein that they create. A single mutation can influence many genes simultaneously. Thus, the insertion process might cause the over production of toxins, allergens, carcinogens, or anti-nutrients, reduce the nutritional quality of the crop, or change the way that the plant interacts with its environment. Because of our limited understanding of the DNA, even if we knew which parts of it were disrupted, we wouldn't necessarily know the consequences.

In addition, the insertion of bacterial plasmid DNA into plant DNA creates another serious risk. Similarities in the genetic sequence between the plasmid and the DNA of bacteria found in the gut of humans or animals or in the soil might significantly increase the likelihood of horizontal gene transfer. This means that genes from the plant may transfer into the DNA of the soil or

gut bacteria. The only human feeding study on genetic engineering confirmed that the genes inserted into GM soybeans *do* transfer into the bacteria inside human intestines.

Advocates of biotechnology often defend the safety of their products by claming that modern methods of plant breeding *other* than genetic engineering are used on a wide scale, have a history of safe use and create comparable mutations. The Econexus report reveals that everything about this argument is pure speculation and is not supported by scientific literature. There is *no* evidence that these modern methods are used widely, are consistently safe, or create mutations of the same kind or frequency as genetic engineering.

In reality, many biotech scientists are unaware of the massive quantity of mutations that are generated by the GM transformation process (gene insertion and tissue culture). In fact, the regulatory agencies that approve GM foods operate as if the insertion process has no impact on safety. [11],[12] They do not require extensive evaluation of the mutations and therefore the extent of these in approved GM food crops has not been identified. The few studies that have been conducted revealed many significant problems. GM varieties contain truncated or multiple fragments of the inserted gene and extraneous or scrambled DNA. One GM corn variety contained a fragment from a gene that was supposed to be inserted into a different GM variety. The protein produced by the foreign genes can also be truncated, altered, or fragmented. And many significant differences between GM and non-GM crops have been observed, which may result from the insertion process. An approved GM squash, for example, contains 68 times less beta-carotene and four times more sodium than non-GM squash. GM soybeans have much higher levels of a potential allergen and anti-nutrient. But GM crops are tested for only a handful of nutrients or known toxins, and therefore the true impact of gene mutations is not known. Furthermore, GM plants are grown in vast amounts. Undetected alterations may result in harm to the environment or human health on an unprecedented scale. With so little known about the impact of gene insertion, and with so much at risk, applying genetic engineering to food and crops is a huge gamble.

Revised Book Analogy With genome scrambling in mind, let's revise the book analogy as follows:

The DNA is like a large book with the letters consisting of the four molecules that make up the genetic code. Located throughout the book are special one- to two-page passages, called genes, which describe characters called proteins (including enzymes). The book is divided into chapters called chromosomes.

When a single foreign page (gene) is inserted through the process called genetic engineering, the book goes through a profound transformation. There are typos throughout, in hundreds or thousands of places. Letters are switched here and there; words and sentences are scrambled, deleted, repeated or reversed. Long and short passages from one part of the book may be relocated or repeated elsewhere, and bits of text from entirely different books show up from time to time. As you get close to the inserted page, things get really strange. The story becomes indecipherable. The text includes random letters and sections of inserted foreign text, and several pages are missing. The inserted page may actually be multiple identical pages, partial pages, or small bits of text, sections that are misspelled, deleted, inverted, and scrambled. As a result of changes in the story line throughout the book, several characters (proteins) act differently, sometimes switching roles from heroes to villains, or vice versa. It all makes you wonder about the comment made by the biotech advocate as he handed you the volume, "It's just the same old book, only with a single page added."

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[1] Allison Wilson, PhD, Jonathan Latham, PhD, and Ricarda Steinbrecher, PhD "Genome Scrambling -Myth or Reality? Transformation-Induced Mutations in Transgenic Crop Plants Technical Report - October 2004, www.econexus.info. The references below were cited in this report.[2] Forsbach A, Shubert D, Lechtenberg B, Gils M, Schmidt R (2003) A comprehensive characterisation of single-copy T-DNA insertions in the *Arabidopsis thaliana* genome. Plant Mol Biol 52: 161-176.[3] Tax FE, Vernon DM (2001) T-DNA-associated duplication/translocations in *Arabidopsis*. Implications for mutant analysis and functional genomics. Plant Physiol 126: 1527-1538.[4] Kaya H, Sato S, Tabata S, Kobayashi Y, Iwabuchi M, Araki T (2000) *hosoba toge toge*, a syndrome caused by a large chromosomal deletion associated with a T-DNA insertion in *Arabidopsis*. Plant Cell Physiol 41(9): 1055-1066.[5] Svitashev SK, Pawlowski WP, Makarevitch I, Plank DW, Somers DA (2002) Complex transgene locus structures implicate multiple mechanisms for plant transgene rearrangement. Plant J 32: 433-445.[6] Makarevitch I, Svitashev SK, Somers DA (2003) Complete sequence analysis of transgene loci from plants transformed via microprojectile bombardment. Plant Mol Biol 52: 421-432.[7] Dennis ES, Brettell RIS, Peacock WJ (1987) A tissue culture induced *Adh*2 null mutant of maize results from a single base change. Mol Gen Genet 210: 181-183.[8] Brettell RIS, Dennis ES, Scowcroft WR, Peacock WJ (1986) Molecular analysis of a somaclonal mutant of maize alcohol dehydrogenase. Mol Gen Genet 202:235-239.[9] Bao PH, Granata S, Castiglione S, Wang G, Giordani C,Cuzzoni E, Damiani G, Bandi C, Datta SK, Datta K, Potrykus I, Callegarin A, Sala F (1996) Evidence for genomic changes in transgenic rice (*Oryza sativa* L.) recovered from protoplasts. Transgen Res 5: 97-103.[10] Labra M, Savini C, Bracale M, Pelucchi N, Colombo L, Bardini M, Sala F (2001) Genomic changes in transgenic rice (Oryza sativa L.) plants produced by infecting calli with *Agrobacterium tumefaciens*. Plant Cell Rep 20: 325-330.[11] NRC/IOM: Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health (2004) *Safety of Genetically Engineered Foods: Approaches to assessing unintended health effects*. The National Academies Press, Washington, DC.[12] Kessler DA, Taylor MR, Maryanski JH, Flamm EL, Kahl LS (1992) The safety of foods developed by biotechnology. Science 256: 1747-1832.