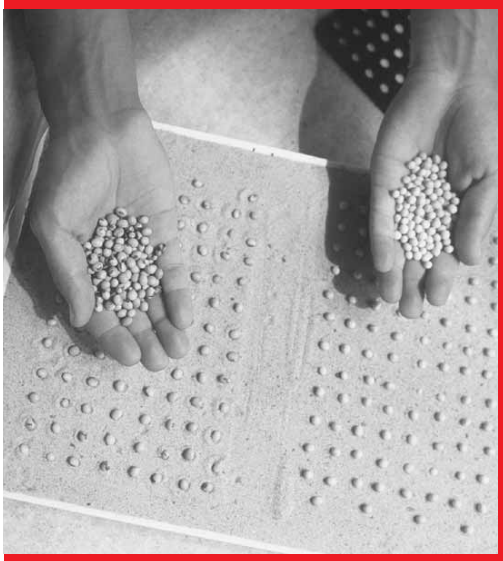

Risk Underestimated

Interviews with nine scientists on the subject of genetically modified plants

Genetics

GREENPEACE

Published on the web by Greenpeace e.V., Große Elbstraße 39, 22767 Hamburg, Germany. Tel. ++49 (0)40 30618 100. Editor responsible Christoph Then. Berlin political unit, Marienstraße 19-20, 10117 Berlin. E-mail mail@greenpeace.de. Internet at www.greenpeace.de. Authors Florianne Koechlin, Blauen-Institut, Basel; Katja Moch, Öko-Institut, Freiburg; Christoph Then. Interviews by Florianne Koechlin. Scientific editing by Sue Mayer and Janet Cotter. Texts edited by Anja Oeck and Markus Nitsch. Translation by John Saul. Cover design by Ulrike Hemme and Nicole Krohn. Photos by G Gilabert (2), A Kirchhof, all © Greenpeace. Berlin political unit, Marienstraße 19-20, 10117 Berlin. E-mail mail@greenpeace.de. Internet at www.greenpeace.de.

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Foreword

These accounts attempt to give greater clarity to the genetic manipulation of plants in the light of current knowledge of the complexity of gene regulation and the uncertainties arising from this.

Many new aspects have emerged in this area of genetics in the last few years, but their overall effects have not yet been presented in overview.

Against this background, Greenpeace has collaborated with the Institute for Applied Ecology in Freiburg (Germany) and Blaueninstitut in Basel (Switzerland) in a project in which various leading scientists have been interviewed about their current findings and insights. The interviews, conducted by Florianne Koechlin, bring a new, unique collection of material making it possible not only for experts, but also for a broader public, to understand why the technology of genetic manipulation on plants has so many uncertainties and risks attached to it.

In their overviews, Katja Moch, Florianne Koechlin and Christoph Then, endeavour to summarise the state of knowledge today and give the reasons why new insights mean evaluation of the risks involved must be revised.

To enliven the debate, this collection of texts contain diverse, controversial opinions as well as those that see no special risk in genetically modified organisms. (Of course, none of the interviews with the scientists should be interpreted as giving the position of Greenpeace, the Institute of Applied Ecology or of the Blaueninstitute).

The project also includes an extensive compilation of literature by Katja Moch from the Öko-Institut (Das überholte Paradigma der Gentechnik[1], Institute for Applied Ecology, Freiburg, 2004) and a congress, at which some of the scientists interviewed here will present their findings and insights (Epigenetics, Transgenic Plants and Risk Assessment, Frankfurt, 2005).

It still has to be said that as a result, 30 years after the famous Asilomar conference at which scientists warned for the first time about the risks of technologically recombining genetic material, and over 20 years after plants were genetically modified for the first time, that, to use the well-known dictum, "they (still) know not what they do".

The aim of the project is to intensify discussion on the risks of genetically manipulated seed, prompt new debates, and contribute to the idea that plants can be produced and cultivated in new, non-GM, ways which will lead to real sustainable innovations.

Katja Moch, Öko-Institut, Freiburg
Florianne Koechlin, Blauen-Institut, Basel
Christoph Then, Greenpeace Germany

[1] The outdated paradigm of genetic engineering

I. The Outdated Paradigm of Genetic Engineering

Katja Moch
Öko-Institut - Institute for Applied Ecology, Freiburg (Germany)

After the structure of DNA was discovered in the 1950s, the paradigm of a linear "one gene, one protein" sequence, where the flow of information is directed only from gene to protein usually triggering an effect that could be defined, became established in molecular biology. However, in the last few years understanding of how the genome is structured and regulated has altered fundamentally. Decoding of the genome of diverse organisms has shown a high similarity at the DNA level. In addition, generally genomes have been found to contain fewer genes than the large number of proteins might suggest. This means that regulation of the genome is far more complex than the "one gene, one effect" paradigm assumes and a lot of scientists have recently declared this reductionist, deterministic paradigm to be at an end. A new defining paradigm has yet to be found.

Can Epigenetics produce the New Paradigm?

Epigenesis is described as a self-organising regulatory network in which the selection, switching on or off, and extent to which genes are activated, is co-determined by interlinkage with signals from the environment. Cellular processes which lead to a specific phenotype being developed, and determine an organism's ecological properties, are then set in motion. (See here interview with Strohman and Buiatti.)

Geneticists define epigenetics more narrowly so as to enable the molecular biological processes to be described more precisely. According to Russo et al. (1996), epigenetics is the study of effects which can be passed on mitotically and/or meiotically and cannot be traced back to a change in DNA. Meins & Binns (1979) define epigenetic changes as stable, potentially reversible changes in the expression of a gene. But these definitions leave little room for gene regulation's complex interactions and, most notably, interactions between the genome and the outside world. They also fail to include interactions in the secondary metabolism, a complex subject which the interview with Richard Firn is devoted to.

Transgenic Plants – What is examined?

The paradigm that a gene codes for only one protein and causes only one effect still continues to form the basis of all genetic engineering work in the laboratory and field. While a series of side effects inherent in the methods used regularly occurs when plant genomes are genetically modified, these unintended changes are not adequately depicted in the relevant literature. The questions posed in published studies are usually framed very narrowly, excluding any analyses of unintentional changes. Changes are mentioned but not analysed further. Plants with undesirable effects or poor performance are furthermore often rejected in the laboratory (see the interview with Seralini).

The causes or effects of certain unintended changes in genetically modified plants have thus generally been inadequately studied. Genetically modified herbicide-resistant soybeans are a good example of puzzling findings of unintended effects. In 2001, a Belgian group found out that foreign DNA was integrated in the soybean genome and several rearrangements in the sequence occurred at a flank region, with the plant DNA having probably been rearranged at the point of integration. An additional, abbreviated version of the herbicide-resistance gene, with 254 base pairs, was also found. The adjacent DNA segment of 534 base pairs in length and without sequence homologies to soybeans or other plant DNA was also discovered which Windels et al (2001) suspect is part of the vector or other foreign DNA. Recent studies have found that the superfluous DNA is partly transcribed. Different RNA variations are produced as a result of post-transcriptional modifications which could code for previously unknown fusion proteins from the protein responsible for herbicide resistance (Rang et al. 2005).

In other studies, soybean plants resistant to Roundup have shown themselves more heat-sensitive and generally more sensitive to stress than conventional varieties (Gertz et al. 1999; Vencill 1999). The Roundup Ready soybeans were also smaller and had a lower chlorophyll content and fresh weight. The plants were moreover more branched. At normal temperatures, Roundup Ready-resistant soybean plants have a 13 per cent higher lignin content than conventional soybeans (Gertz et al. 1999), which the authors believed was due to disruption by the Roundup resistance gene of the shikimic acid pathway that results in the synthesis of lignin.

Hormone management of phytoestrogenes has also been found to have changed , with the content of various plant hormones falling by as much as 14 per cent in Roundup Ready soybeans (Lappe et al. 1999). Studies by Manuela Malatesta (Malatesta et al. 2002) also point to altered composition. As related in her interview, Malatesta fed mice with transgenic Roundup Ready soybeans and compared them to mice fed normal feed. This illustrates the complexity of plant substances and throws a light on the difficulty of testing the transgenic plants' safety for food.

Although each of the changes described here does not, when considered in isolation, necessarily present a risk, when taken in sum they demand extensive investigations to be made. The range of unintended effects seen with transgenic soybeans should trigger its re-evaluation together with the other transgenic plants authorised in the EU.

The example of transgenic soybeans also shows that unexpected effects have yet to be satisfactorily defined/classified. Classification has been mainly of effects at genetic level and pleiotropic or unexpected effects. In relation to epigenetic effects in transgenic plants, studies have mainly been on gene silencing, i.e. the silencing of foreign genes introduced. The reason is simple – gene silencing is the biggest obstacle to stable transgene expression and would impede possible commercial introduction of genetically modified plants. Interestingly, however, silencing foreign genes makes it possible to see how plants react to environmental influences. Particular crop conditions, light, and high temperatures cause greater silencing of foreign genes in genetically modified plants.

Effects at Genetic Level

When genes are modified, effects at the genetic level are caused by the method itself. It is basically not possible to control where transgenes integrate in the plant genome. The function and regulation of a gene depends, amongst other things, on its position in the genome. Integration of the genetic structure into the plant genome – called insertion – is undertaken with Agrobacterium methods or by particle bombardment by illegitimate recombination and subsequent repairing mechanisms for broken double strands in the DNA. Changes in the sequence and deletions often occur at the point of insertion. Insertion can produce "filler" DNA. This filler DNA may come from distant parts of the plant genome but can also stem from the plasmid backbone. The origin of filler DNA remains partly unexplained.

Insertions of fragments of the genetic construct, vigorous rearrangements, inverse (repeating) sequences, deletions and, together, the formation of "complex transgenic loci", regularly occur when genetic changes are made. T-DNA "backbone" sequences, i.e. the backbone of the plasmid, which is outside the T-DNA border and ought not to be integrated, also manages to enter the genome when both the particle bombardment and Agrobacterium methods are used. Unfortunately, studies on the resultant effects seldom go beyond genetic changes. Effects, such as those on the formation of secondary substances, for example (see the interview with Firn), or on fitness, are not routinely investigated.

Pleiotropic Effects

"Pleiotropic effects" is a wholesale term meaning that the unexpected development of several characteristics arising from one gene. The term is thus used to describe the most diverse processes in transgenic plants. The European Food Safety Authority prefers to use the term "unexpected effects“ – meaning potential differences in the kind and amount of plant substances that arise following genetic modification. The genome changes described in genetically modified soybeans are, for example, usually summarised as pleiotropic effects. The option of describing pleiotropic effects as epigenetic, and then explaining causes in the genome regulation, has not yet been considered.

Outlook

On the whole, research into epigenetic changes and possible risks has been rare. There is an urgent need for research on epigenetics and the effects of genetic change on the complex and dynamic organisation of the genome, and on possible risks that may arise. In general, the range of the limitations and uncertainties in methods using genetic engineering ought to be fully elucidated. Not only genetic changes can cause changes in morphology and plant physiology. Epigenetic changes, alternative splicing, the influence of the secondary metabolism at the gene expression level, or unexpected functions of proteins, can also have effects on a plant's behaviour. The possible changes in the composition of plant substances, or possible formation of new or altered plant substances must be fully explained. The effects of fluctuating environmental influences on transgenic plants should be studied more extensively. How different transgenic plants can react, and what ecological effects this can have, should be at the forefront of research. Above all, however, plant genetics needs a new paradigm, a systems paradigm, which takes into consideration not only genes but also the cells, the environment, conditions for development and the dynamic network of genome regulation. A systems paradigm will, therefore, recognize the plastic genome of plants.

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II. Nine Interviews on Epigenetics and Transgenic Plants

Florianne Koechlin
Blauen-Institut, Münchenstein (Switzerland)

"A gene can have a lot of different functions; the number of its functions has no upper limit. A gene can also acquire new functions. This applies to all genes, those of humans, flies or plants. When I learn Chinese, genes which play a role in my language centre will have new functions. If I then ask what functions these genes have, I have to ask if this is before or after I learnt Chinese. My learning Chinese will certainly have bestowed particular genes in my language centre with new functions." (Martin Heisenberg, Germany.)

Genes are ambivalent and dynamic, and epigenetic regulation mechanisms are more complex than had been assumed for a long time. Nine experts were interviewed on the subject. The issue and problem areas that are presented here do not constitute a uniform or complete picture, but rather a kaleidoscope of different aspects relevant to the risks involved with GM organisms.

All those interviewed agreed that the new findings and insights in epigenetics and related areas meant there was a need for research. The majority rejected the idea of commercial releases of GMOs being allowed at this time.

Genetics and Epigenetics

Marcello Buiatti (Florence University) has described how a gene can perform many different functions. “The human genome has approximately 30,000 so called “coding genes” and these can code for more than 500,000 different proteins. Therefore, gene “ambiguity” – more proteins coded by a single gene – is very high. Ambiguity, leading to plasticity of responses is made possible by the presence of many sophisticated mechanisms fixed throughout evolution – that’s part of epigenetics. … Additional ambiguity sources at the cell and organism level are somatic mutation, methylation, amplification processes and RNA interference all occurring in specific regions of the genome and affecting gene regulation. Some of these processes may be transmitted to cell progenies. When they occur in germinal cell lines, they can be transferred to subsequent generations.”

Richard Strohman (emeritus professor at Berkeley University, USA) has explained the links between genes, epigenetics and cells saying: "DNA has been called the Book of Life by Human Genome Project scientists, but many other biologists consider DNA to be simply a random collection of words from which a meaningful story of life may be assembled. In order to assemble that meaningful story, a living cell uses a second informational system. It is “dynamic” because it regulates changes in products over time, and it is “epigenetic” because it is above genetics in level of organization. And some of these changed products feed back to DNA to regulate gene expression. The key concept here is that these dynamic-epigenetic networks have a life of their own — they follow network rules not specified by DNA. And we do not fully understand these rules.”

In his interview, Strohman notes that epigenetic systems form a bridge between the genome and the phenotype. They "sense" changes in the organism's environment and bring this information into the system. The information is then translated into signals which end up in the genome. “It’s a search for some kind of a genomic change in patterns of expression which might give the organism a way out of the environmental problem or a way to react more efficiently to the environment so that their survival is enhanced.”

Strohman further emphasises that epigenetics and genetics can be complementary. Genetics alone explains little, but when supplemented by these dynamic epigenetic systems, a meaningful picture forms. “For the first time in some years we are able to march forward with a complementarity between these dynamic epigenetic systems, these informational systems on the one hand and the genome on the other.”

Epigenetics plays an even greater role with plants than with animals, Buiatti adds. Plants, like animals, have evolved a number of mechanisms with which they can display an enormousamount of variability, both genetic and epigenetic. Parts of their DNA are hyper-variable – they mutate often. And in contrast to animals, plants can change their number of chromosomes. They can also cross with some other plant species and produce fertile hybrids. Epigenetic variation is also greater with plants than in animals. ” First of all, the plant cells belonging to one individual do not necessarily have, as is usually the case in animals, the same genotype – the same number and kind of genes. On the contrary, there are tissues within the same plant which have different chromosome numbers, bear different mutant genes, and quite often have different copies of the same genes.” “ This means plants, at any time in their life, and under various environmental conditions, have been able to select the variations advantageous to them from a much bigger pool than animals. Additional instabilities arise in producing transgenic plants.

Transgenic Plants

Cesare Gessler (ETH Zürich / Swiss Federal Institute of Technology, Zurich) notes, "Genetic engineering has not been fully developed. The products of genetic engineering today are still at the level of dinosaur technology. We use genes which are foreign to a species, not knowing where they are inserted or what else will change in the whole chain from gene to protein. We don't know which regulatory relationships we're intervening in. And another point: today we know a lot of genes which for the moment have no function. We know, for example, whole clusters of resistance genes. For the time being they have no function; they have been shut down. But when a pathogen alters its identifying proteins, it can happen that the plant, thanks to a resistance gene which until then has not been functional, nonetheless recognises the pathogen. This gene then has a function even if it hadn't been active for a long time.“

Gessler argues for the great knowledge of genetics to be used for marker assisted breeding, i.e. using genetic markers in conventional breeding. Breeding could then be swifter and more precise. He uses molecular assisted breeding in breeding apples. However, he is open minded about transferring a species' own genes in the future if the techniques of genetic engineering are perfected.

Many of those interviewed have the same or similar objections to the production of genetically modified plants today. The fact that few studies on instabilities and epigenetic changes in transgenic plants have been published does not surprise Gilles-Eric Seralini (Caen University). "When you study the role and regulation of genes," he says, "you undertake a lot of trials and you only select the GMOs that do not have these kind of problems. That means that 98% of all the GMOs that you produce do not function just because the gene might be methylated or it might be present but is not used normally by the organism which received it. All the organisms that are modified but do not express the gene are put in the garbage. So you find very few studies on these issues. There are some papers about insertional mutagenesis or transgenicsis in plants showing that artificial gene constructs may be more unstable than others. This has led to the discovery of antisense RNA or RNAi. So I think that we should bear in mind that the study of the composition and the analysis and the substantial equivalence is far below the level of sufficiency to be able to predict any toxicity or any unintended effect of a plant.“

Seralini also explains that the foreign genes in almost all genetically modified plants are in a different form than described in the patent or marketing applications of the companies involved. This might suggest instability in the transgenes. He points to a further problem: almost a hundred per cent of all GMOs tolerate or produce pesticides and 75–80 per cent of all GMOs are made resistant to only one herbicide – Monsanto's Roundup. However, Seralini says that this pesticide, is not harmless. "We showed in our lab that human placental cells are very sensitive to Roundup, to concentrations lower than the agricultural use. This could explain miscarriages and premature births in North America in farmers. We noticed some other effects too.“

Manuela Maltesta (University of Urbino, Italy) investigated mice which had been fed with genetically modified Roundup Ready soybean and compared them with those fed normal feed. She conducted ultrastructural morphometric and immunocytochemical studies. “We found significant modifications of some nuclear features in GM-fed mice. In particular, GM-fed mice

showed irregularly shaped nuclei – the nuclei looked like they were corrugated – whereas the nuclei of the control animals had a smooth and roundish shape. An irregular shape generally represents an index of high metabolic rate, and a higher number of nuclear poressuggests an intense molecular trafficking. So our conclusion is that the GM-diet can influence nuclear features of liver cells in young and adult mice. The liver of a mouse fed on genetically modified soybean seems to have cells working harder than a liver of a mouse fed on natural soybean."

Secondary Metabolism

Richard Firn (University of York, UK) describes how secondary metabolites in plants present an additional source of uncertainty that was ignored until recently. The theory that enzymes are substrate-specific, and only catalyse a specific reaction, is only correct for enzymes involved in primary metabolism. In contrast, enzymes of secondary metabolism can be multifunctional, much like genes. "In the Grand Fir (*Abies grandis*) two enzymes can make multiple products from a single substrate. One enzyme can produce 52 and the other 34 different products."

Plants produce roughly half a million different molecules. Firn explains that this enormous chemical diversity is an important strategy for plants, enabling them to react quickly to new threats. Only seldom does a molecule have just one biological activity. Like our immune system, a plant's biochemistry has great redundancy"We have to understand that the rules for primary and secondary metabolism will be very different. The enzymes of the primary metabolism must be substrate specific; there is little space for diversity. But the enzymes of the secondary metabolism are not substrate specific because it is the lack of substrate specificity that gives the opportunity for generating and retaining chemical diversity. Primary metabolism has evolved to be fairly predictable while secondary metabolism has evolved to be unpredictable."

If a gene which affects secondary metabolism is inserted, this can have highly unpredictable consequences. Firn demonstrates this with three examples:
"First, the introduction of an enzyme expected to produce a single new chemical could also produce other new compounds owing to the substrate tolerance of existing enzymes. Second, the introduction into a new organism of a gene encoding an enzyme involved in secondary metabolism could produce more than one product owing to the substrate tolerance of the introduced enzyme. Third, the introduction of a gene into an organisms could disturb secondary metabolite production simply as a consequence of the random gene insertion, with unplanned and unexpected increases in the content of some compounds, owing to changes in the metabolic flux through matrix pathways."
And he adds, "Of major concern is the fact that the secondary metabolite profiles of individuals in a population can vary considerably and can vary depending on the prevailing conditions. So the effect of the introduction of a gene into a plant might be predictable only under defined conditions that may not be achievable in the field. The secondary metabolite profile is complex, and extremely small amounts of highly potent compounds could have profound biological consequences."

Almost all transgenic plants at present cultivated throughout the world are either herbicide-tolerant or insect resistant, Bt plants. The industry would certainly welcome other additional properties, not least because of growing public opposition in Europe and elsewhere. It is mooted by Firn that the reason for the success of these two properties is that their metabolic pathways are relatively isolated and do not interact with other ones. This is not the case with most other traits – hence the many failures. And what does not tend to function in the lab, often goes wrong in the field.

Firn points to the blind spots in science. For example, he is astonished that biochemists have never taken an interest in the evolution of biochemistry. Only individual enzymes are examined – and that the major role of chemical diversity in the evolution of plants is ignored. This is also of relevance in discussing risk assessment of GMOs.

Consequences for Risk Assessment

Seralini, Malatesta and Tappeser give requirements that they believe should be included in the authorisation procedures for GMOs .
Seralini proposes that GMOs should, like pesticides, be tested for toxicity using long-term feeding trials. "The pesticide directive, CEE/91/414, requires a much more thorough assessment. When

you assess a new pesticide, you have to give the new pesticide to three species – generally rats, mice and dogs – for three months. It further prescribes that the new pesticide is given in food for one year to one species – generally dog – and for two years to another one – generally rat. There is absolutely no scientific reason to avoid these kind of experiments for actual GMOs.“ Today obligatory toxicity tests are not prescribed for GMOs. “I think it is stupid to give GMOs to people for an entire life time," he says, "when at the same time there is no requirement to undertake toxicity tests even for three months. So we should force industry to publish their results and we should enforce such long term tests.“

Manuela Malatesta (University of Urbino, Italy) also calls for long-term trials with animals fed GMOs. She believes multidisciplinary studies, an integration of biochemistry, microbiology and electron microscope histology, are needed in order to investigate the safety of transgenic plants in food. “It needs long-term trials with GM-fed animals. The ones required now are too short-term. Then it needs multidisciplinary studies, combining biochemistry, electron microscopic histology and also microbiology. In fact, we should also think about the intestinal bacteria. They could change due to diet changes, and we do not know very much about them. And only after these studies could we accept transgenic food in our markets. Now, I’m not sure.”

However, although these scientists felt long-term animal studies were needed, public discussion will be necessary to to find out if the potential benefits of GM crops are considered to be great enough to justify animal experiments.

Seralini also considered that the transgene should be re-sequenced after insertion into the genome which would give pointers to possible genomic instability arising from from the genetic modification.

Beatrix Tappeser (Federal Nature Protection Agency, Germany) lists the criteria used to evaluate a GMO release in her department. This includes three levels: molecular characterization, characterization of the plant as a whole and the system level.

What is important in each case is that there are data drawn from several years and under differentclimatic conditions. But the documents received rarely contain adequate data in the areas being examinedso there is a deficit.

All the scientists and researchers interviewed are convinced that the new insights in epigenetics and metabolism underline the need more research and adaptations of safety regulations.

Matthias Fladung (Institut für Forstgenetics and Forstbaumzucht institute for forestry research and tree breeding, Grosshansdorf, Germany), a proponent of transgenic plants, is of the view, however, that Bt maize is the best-investigated maize variety ever produced. "A relatively great deal is known about it. Were an epigenetic change indeed to have occurred induced by genetic transfer, if, for example epigenetics played a role in some regulating mechanism, this would have been discovered." Nevertheless he regards it as premature for transgenic trees to be released at present.

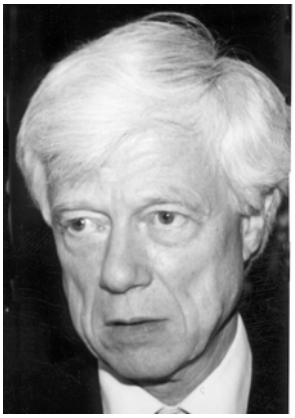
Gessler – as most of the others interviewed – thinks it would be wrong to release today's transgenic plants. He does not believe this is necessary, because the priority should be for more research in greenhouses. "Not until everything functions and we have really tested it," he says, "can we take something out onto the field. We aren't nearly that far along. As long as we still need this 35S promoter, for example, I just don't see why we should go from there onto the field.“ He is thus in favour of a moratorium for the commercial release of GMOs.

"I'm certain of the fact that risk assessments are insufficient today," notes Seralini, "not only because of epigenetics, but also because a lot of the GM plants are supposed to make pesticides that are not tested on health. And then the transgenics are all artificial gene-constructs. Often it is said that they are natural ones, but in fact some of them are very different, for instance in the case of Bt maize 176.”

"Risk assessments," notes Firn, "are all about using knowledge and understanding to predict outcomes. The growing understanding of the mechanisms underlying epigenetic effects reveals a complexity that must inevitably mean that risk assessments being made of GM plants will carry

greater uncertainty than one might have liked.” With regard to releases, there is not just one answer except that the simple views of the last two decades have, to some extent, to be be revised. "It would be nice," he says, "if there was a greater humility and more experts would admit the limits of their knowledge.”

1. Interview with Professor Martin Heisenberg



Institute of Neurobiology and Genetics
Würzburg University (Germany)

FK: Mr Heisenberg, what does your research focus on?

Functions in Genetics

MH: We are researching the brain of the Drosophila fly. There have been major breakthroughs in the last five to ten years so we are beginning to understand how genes are switched on or off in the right cells at the right time. So we are at the centre of epigenetics. We can now manipulate these processes in such a way that they switch certain groups of cells in the brain on or off as directed. Then we are trying to connect these changes in the brain to changes in behaviour.
In the last few years we have come to see where in the fly's brain particular acts of memory are stored. Flies can remember things and their memory must be filed somewhere. So we are studying the epigenetic regulation of genes in the brain and connecting the functions of the genes to the functioning of the brain.

FK: How can the functions of genes be defined?
MH: That is without doubt one of the most open questions there is. A gene can have a lot of different functions – the number of its functions has no upper limit. A gene can also acquire new functions. This applies to all genes, those of humans, flies or plants.
When I learn Chinese, genes which play a role in my language centre will have new functions. If I then ask what functions these genes have, I have to ask if this is before or after I learnt Chinese. My learning Chinese will have bestowed particular genes in my language centre with new functions.
The Chinese function of a gene can be recognised by the fact that it makes a difference to my Chinese whether or not this gene is there. Imagine, for example, that I can speak Chinese, and then for some reason a particular gene in all my language centre cells which is important in the ability to speak Chinese stops working – say the ability to distinguish between particular sounds which is important in Chinese. When this gene stops working I can't distinguish between these sounds. So the gene has a function in my ability to understand Chinese and so to speak it.

FK: So the function of a gene goes far beyond coding particular proteins. Genes influence various regulating mechanisms and communicate with one another.
MH: It goes a lot further. Take this language gene for Chinese. The gene was already there naturally, before human languages were developed, but it is used in a quite specific new way in Chinese language. But this was, of course, only a hypothetical example.

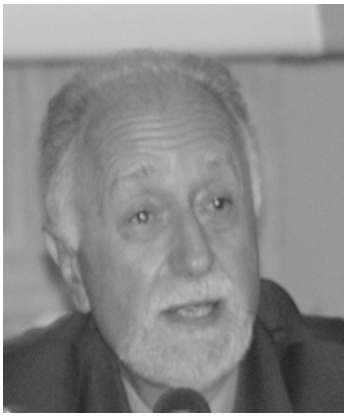
On the Question of Epigenetics and "Not knowing"

FK: A lot of epigenetic mechanisms regulating the expression of genes are known today. The question then is: ‘what regulates and controls epigenetics?‘. And what controls this? The molecular biologist Richard Strohman says this is a kind of infinite regress, and the answer left to us today is ‘the cells‘. But this is rather vague. Another problem, says Strohman, is that while we have discovered a great deal of complexity, this is not the same as understanding it.
MH: I would like to modify this. If I put myself in the place of a contemporary of Gregor Mendel interested in biology, I wouldn't have had the slightest idea about all these processes involved in the regulation of genes and genetic products. I wouldn't have known anything at all of what I didn't know.

I remember well the time when, in developmental biology, it wasn't possible to imagine how a highly differentiated organism with extremities and a front, back, top and bottom came into existence from an amorphous egg. It had to work somehow, it wasn't voodoo – but it was so unimaginable that it couldn't even be properly formulated how it might proceed. Now we have a basic idea and a feeling that this arsenal of ideas can take us quite a long way. But all these black holes in our knowledge are not perceptible at all, like the blind spot in our eye is not perceptible, except when we do certain exercises. We don't see what we don't know; we don't know all that we don't know. So we simply can't say there must be still be a lot to know. Of course this is not true on an abstract level but this does not help us much. We have discovered a lot of new mechanisms in the last few years. And we are all agreed that the regulatory mechanisms known so far are not nearly sufficient to describe how the whole might really function. An enormous amount will be learned at every level. Even then, though, we will not come to an end; you can never come to an end in biology.

2. Interview with Marcello Buiatti

Professor of Genetics
University of Florence (Italy)



FK: Marcello Buiatti, you are a molecular geneticist at the University of Florence. What is your research area?
MB: a) Modeling evolutionary dynamic processes and analysing deviations from randomness in DNA sequences through the evolution of the genomes.
b) Studying the effects in plants, animals and humans of mutations in hypervariable sequences in key genes affecting development and/or relevant illnesses in humans.
c) Studying the effect of the insertion of animal genes related to development plants.
d) Developing GMO detection methods and analysing molecular instability both in experimental plants and in commercially approved GMOs.
e) Working with a network of philosophers and biologists on the epistemology of contemporary biology.

Epigenetics

FK: Could you, for a start, give us a broad definition of epigenetics?
MB: I would define epigenetics in operational terms: all that happens to the phenotype which is not directly defined and determined by genes.

FK: Then it also includes changes on the genetic level which can be inherited through generations but which are basically reversible?
MB: This is part of what I mean. When talking about epigenetics, I often go back to Conrad Hal Waddington back in the 1960s. He was a well known embryologist who postulated that the genotype paradigm was not enough to understand how the development from a zygote to organism could function. He proposed a new phenotype paradigm based on his own evidence – he introduced epigenetics.
He didn’t know about the regulation of gene expression, like methylation – it was the early 1960s. For him, epigenetics was all the regulation which is not written in the genome. The zygote, at the very beginning of life, has many possibilities: within a certain range all paths are possible. This was what he called the epigenetic landscape. The zygote will eventually choose one path, choose several paths and move from one path to another. But it will become more restricted. And, at the end of its life, it won’t be able anymore to change the paths so easily.

FK: Like a skier on the top of a mountain: on top she or he has plenty of different slopes to choose from. The farther down, the less possibilities there are; there are hills and obstacles between the different slopes; choices are being narrowed down.
MB: Exactly.

FK: Could you briefly outline some epigenetic mechanisms we know so far?
MB: The human genome has approximately 30,000 so called “coding genes” and these can code for more than 500,000 different proteins. Therefore, gene “ambiguity” – more proteins coded by a single gene – is very high, and is present at the level of transcription, between transcription and translation, and also after translation due to post-translational modifications of proteins .
Ambiguity, leading to plasticity of responses is made possible by the presence of many sophisticated mechanisms fixed throughout evolution – that’s part of epigenetics.
Firstly, the same gene can be “read” starting from different transcription starting points. Transcribed RNAs are spliced into fragments which may be assembled in different orders to give different “mature” RNA sequences containing the information for different proteins coded by different combinations of the fragments. Variation in proteins coded by the same gene may be extremely high like that found in neurexins, a class of proteins involved in neural connections. In that case, more than 2,000 proteins can be produced on the basis of the information contained in only three genes. Additional ambiguity sources at the cell and organism level are somatic mutation, methylation, amplification processes and RNA interference – all occurring in specific regions of the genome and affecting gene regulation. Moreover, single proteins can have different shapes leading to different functions and be complexed with different substances, again changing activity. Some of these processes, and particularly methylation and amplification, may be transmitted to cell progenies. When they occur in germinal cell lines, they can be transferred to subsequent generations.

Exploratory Strategies and Benevolent Disorder

FK: An expression you always use in your talks and articles is “exploratory strategies”. What do you mean by that?
MB: All living beings, all cells, and also molecules like DNA or proteins, have a tremendous amount of potential variability or plasticity. This allows them to choose the part of variability to be used following signals at any moment. Exploratory strategies means a strategy based on exploration. When Columbus arrived in America, he went around to explore the different areas. He had the possibility of going to many different places; he settled for some and left others out. And this is similar to what we are doing in our lives. We explore, and this capacity allows us to choose different strategies in different situations.

FK: Another favourite expression of yours is “benevolent disorder”.
MB: You can also say plasticity. It gives you the possibility of not having only one program, but a very wide range of programs within the limits of the tools you have to make the programs. Of course, I will never become an elephant, but I will become a different person at every second, which makes me very happy.
Now the fascinating thing is that we find “exploratory strategies” and “benevolent disorder” at every level of life: organisms, organs, cells and also molecules like DNA or proteins. That’s the essence of life.

FK: And applied to plants and genomics?
MB: First of all, plants are living beings and therefore they follow the general rules of all living beings. So they also utilise "explorative strategies" to survive . This means, very simply, that during evolution they have developed a number of mechanisms by which they can display a tremendous amount of variability both genetic and epigenetic. The first is due to the fact that, just as in animals, part of their DNA is hypervariable – it mutates very frequently. Unlike animals, plants can also change the number of their chromosomes and are interfertile with different species. So while animal species cannot cross with each other to give fertile hybrids, plants have been using interspecific crosses spontaneously for millions of years as a tool for the creation of new species.
At the same time, epigenetic variation is greater in plants than in animals. First of all, the plant cells belonging to one individual do not necessarily have, as is usually the case in animals, the same genotype – the same number and kind of genes. On the contrary, there are tissues within the same plant which have different chromosome numbers, bear different mutant genes, and quite often have different copies of the same genes.
This process is called DNA amplification, and was discovered in 1973 by our group in Pisa in

plants. It allows the replication of specific DNA fragments when the remaining DNA is not replicating. This allows plants to modulate the expression of those sequences as more DNA copies often means more proteins coded. Then of course, plants also utilise methylation, conformational changes, differential activation of genes, mechanisms of ambiguity which allow the production of different proteins from the same genes etc.
In summary, plants have developed mechanisms by which they can be even more variable than animals – often much more variable. So they can choose the variability useful for survival and well being at each moment of their lives and in different environmental conditions out of a larger reservoir of variants than animals. This useful variation is the “benevolent disorder”, and the process of choosing it is an " exploratory strategy" in the sense that they explore the "landscape" of variation available to choose their life path which changes , according to needs, from moment to moment. Therefore, plants are like animals, but have to be more variable for simple reasons. In the first place plants move, but are less capable than animals of physically exploring different environments. Therefore, they have to change more in single environments. Moreover, plant individuals often have very long life spans and therefore will meet more environmental changes during their life cycle with which they can only cope with more plasticity.

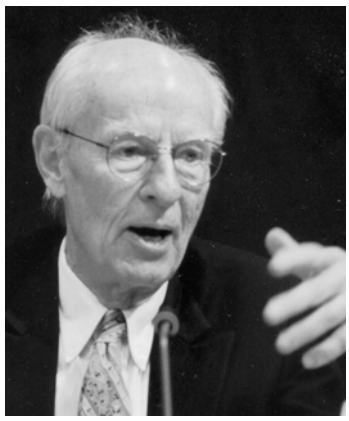
Transgenic Plants and Risk Assessment

FK: Plants have “benevolent disorder“ and they use “exploratory strategies”. What are the consequences for the production of transgenic plants?
MB: Evolution has developed finely tuned metabolic and gene networks where a plastic but coherent equilibrium has been established. Transgenes integrated through genetic engineering come from different species or kingdoms, code for entirely new functions and will be operating in a genetic network with which they had never been interacting. Moreover, they generally will not respond to the regulatory apparatus of the receiving organism, so a “construct” containing the “alien” gene and a regulatory sequence is needed. Inevitably then, genetic engineering is bound to introduce turbulence in the existing systems. A proof of this concept comes from experiments carried out by our group showing that the ancestral spontaneous introduction of bacterial genes by the natural “genetic engineer”, *Agrobacterium tumefaciens,* into plants of the genus *Nicotiana,* has significantly influenced the evolution of that taxonomic group of plant species. So I’m very sceptical – the impact of the induced “noise” by gene transfers is often a surprise and not very predictable.

FK: And regarding risk assessment?
MB: The effects of gene transfers may be morphological but also influence the physiology of the host, changing the relative concentrations of the metabolic components. Moreover, obviously, the low level of predictability of the outcome of a transformation experiment, is of primary relevance for risk evaluation before, and even after, the release of GMOs. In that respect, unpredictable risk levels are correlated with the variability in the number of copies inserted, their possible rearrangement, fragmentation and dispersion in the genome, their methylation and, as I said, the lack of coherence between the inserted gene and the receiving network.

3. Interview with Richard Strohman

Professor Emeritus, Department of Molecular and Cell Biology
University of California at Berkeley (USA)



FK: What was or is your research area at the University?
RS: I started as a cell biologist; I have my degree from Columbia University in New York in Biophysics – my field of interest became cell biology. I worked on human diseases like muscular dystrophy– a disease which was recognised to be caused by a single gene.

The Old Paradigm

FK: How many such monogenetic diseases are known today?
RS: Basically 2 to 3 % of all diseases in post industrial countries are

monogenetic. In general they are very rare. This mechanism: ONE GENE / ONE DISEASE could not have survived natural selection. What happened was that we transfer this simplistic paradigm of the gene to diseases which involve hundreds or perhaps even more genes. The pattern of health and disease is more due to interactions of many different 'players'. The complexity of these systems, and the interactions taking place within them and with the environment, are enormous and we do not understand this complexity. One should say that biology in general recognizes that that there is complexity, but that we are mostly ignorant about a proper approach to it. Genetic determinism is much too simple. We know there's something DIFFERENT going on but we don't yet understand it. In the absence of a new theory, the history of science shows that the mainstream scientific community will continue to pursue the old way of doing business – the old paradigm. This is also true for the agricultural domain, of course.

FK: The old paradigm and the necessity of a paradigm change is one of your main issues, as far as I can follow this up…
RS: Yes, we observe the failure of the major paradigm in biology called genetic determinism. The idea is, that the 'book of life' is programmed into the genome, and that development of an organism is simply encoded into DNA. Until recently no one – or very few people – were interested in complexity and acknowledged the complexity of living systems. So I'm writing a book about the history of the rise and fall of genetic determinism.

Epigenetics

FK: Let´s talk about epigenetics. I often use your description while talking about epigenetics: "DNA has been called the Book of Life by Human Genome Project scientists, but many other biologists consider DNA to be simply a random collection of words, from which a meaningful story of life may be assembled. In order to assemble that meaningful story, a living cell uses a second information system. It is "dynamic" because it regulates changes in products over time, and it is "epigenetic" because it is above genetics in its level of organization. And some of these changed products feed back to DNA to regulate gene expression. The key concept here is that these dynamic-epigenetic networks have a life of their own — they follow network rules not specified by DNA. And we do not fully understand these rules." Do you still agree with this definition?
RS: Yes absolutely… it is simplified but accurate. I think that Robin Holiday back in the 20th century gave a good explanation of epigenetics in philosophical terms : "Classical genetics has revealed the mechanisms for the transmission of the genes from generation to generation, but the strategy of the genes in unfolding the developmental programme remains obscure. Epigenetics comprises the study of the mechanisms that impart temporal and spatial control on the activity of all those genes required for the development of a complex organism from the zygote to the adult.
As such it establishes the basis for a level of organisational control above the genome; a level that is now well established in fact, but continues to evade decisive theoretical insight".

Epigenetic Systems are Sensory Systems between Genome and the Environment

FK: So it's not all in the genes. Genes of course are essential. We could not get along without the genome. But genes by themselves can do nothing.
RS: Exactly. Each week the journals are carrying research reports dealing with new examples of epigenetic control of gene expression. Nature, through evolution, has placed between the genome and the phenotype a number of complex systems. All of which are context dependant. And these systems together regulate not the gene sequences but the pattern of how genes are expressed. The expression of genes changes when the environment changes.[2]

FK: Epigenetics, then, is somewhere in between the environment and the genome?
RS: That's right. Epigenetics represents the totality of regulation mechanisms and there are many of them, we only knew of a few until now. So epigenetic systems are the bridge between the genome and the phenotype. These systems are really sensory systems in that they are open to the environment. They act to sense changes in the environment of the organism and then this information about environmental change is taken in by these systems. The information is then

[2] Richard Strohman, 2002, Science V 269, p. 701-704

translated into signals which feed back to the genome. It's a search for some kind of a genomic change in patterns of expression which might give the organism a way out of the environmental problem, or a way to react more efficiently to the environment so that their survival is enhanced. The final thing you have to say about epigenetic systems is that it's forming a bridge between the environment and the genome, it makes the organism-environmental relationship a singularity. Organisms and the environment are a single system. As Richard Lewontin has said: there are no organisms without an environment and there are virtually no environments without organisms. You can't separate them – they are utterly dependent on one another. So this is the remarkable finding also for the whole conservation movement, like Greenpeace or Friends of the Earth.
All these people who have been arguing for the necessity of environmental protection – they are now supported/verified by molecular biologists. It is true in medicine and it is true in corn fields. And yet the mainstream scientific community and related corporate biotechnology continues to invest billions of dollars in identification of causal genes.

FK: But how can the environment be encoded in the genome?
RS: We know today that this can be done through gene silencing and gene activation by epigenetic mechanisms. You're changing the expression patterns – you're methylating and silencing DNA or you're stimulating the chromosome with proteins, which regulates the accessibility of the DNA to transcription factors.
In the hard core molecular laboratories, it's only been two years since this encoding of the environment by epigenetic systems has been recognized. I think this complementary relationship is the stuff of a true scientific revolution.

Central Dogma and Agricultural Genetics

FK: Do the new findings in epigenetics also suggest that there should be a new risk discussion, that there are risks inherent to the technology itself?
RS: Yes, definitely. This whole idea, that you can go on like you were replacing some kind of a Lego brick…
The complexity is enormous. Poets say: when you face something as complex as the simplest living thing and you see how enormous it is, then you should become humble. You should have an air of caution about the work. You should broaden the base of the experimental questions you ask. And this is all, I think, becoming clear to a larger and larger audience.

FK: Could you comment on a thesis from the study "Das überholte Paradigma der Gentechnik": Epigentics is widely recognised in human and animal genetics. Probably also in plants. But when it comes to practical applications the out dated paradigm of the gene is still dominant.
RS: Yes, I agree completely. It's part of everything we've been talking about, in spite of the general awareness in the research community everywhere. The question is: how is it possible that such wrong-headed theories around genetic determinism could be tolerated for so long? Economic power and, of course, the mixture of the university with the corporations when you can not tell the difference between the university scientist or somebody working for Monsanto anymore. They are all trained the same way. They all believe – I mean you have to actually extend to them some sympathy because in some way they actually believe at some level that what they do is correct.

FK: They want to save the world.
RS: Yes! What could be wrong with that. So here we are, going into the 21st century; we know that the deterministic model is incomplete.

Epigenetics and Genetics – a new Paradigm?

FK: Is there a new paradigm in sight? Are we witnessing a paradigm change? What about system biology?
RS: The beauty of the epigenetic model is that it does not throw out genetics. It's complementary. It's an evolutionary idea in a certain sense but it maintains genetics as not only a useful but a necessary aspect of the new paradigm. It gives the geneticists something to do, and it gives them some security, because as this paradigm goes forward we will certainly need the genetic databases. We have a novel kind of reflection of the revolution that went on in quantum mechanics.

In quantum mechanics you needed two different systems to explain the same observation, that is to say that light could be both a particle and a wave. Now we see that genetics does not explain anything. But when complemented by these dynamical epigenetic systems, the two together make the picture completely meaningful. And for the first time in some years we are able to march forward with a complementarity between these dynamical epigenetic systems, these informational systems on the one hand, and the genome on the other. There's a very important historical loop here. It's what Waddington proposed a long time ago.There is no way to go forward without a marriage of genetic with epigenetic thinking and experiment.
So I think from that very practical point of view, there is every good reason to rearrange our policies and to rearrange our funding policy so that it reflects a need to begin to recruit people who understand systems biology. Most of the genetic or molecular community does not.
There are a few bastions of expertise in Europe, in the United States and elsewhere, but basically we need to put money into the system so we begin to train people who understand the mathematical aspects, who are at home with complexity theories, who know about systems. And especially we need to find or train students in organismal biology. So this will take some time.

FK: The paradigm change has not happened yet?
RS: We're not there yet. It's a whole new way of looking at life and yes, it's in its infancy so it's not complete.

4. Interview with Dr. Cesare Gessler

Area of Research: Marker-Assisted Breeding
ETH Zürich (Switzerland)

FK: Cesare Gessler, your area of research is breeding apples. What is being researched?
CG: At ETH Zürich I have a research group which for years now has been concerned with the apple genome and diseases that affect apples, such as scab and mildew. Together with others we are trying to decode the apple genome and find out where genes resistant to scab and mildew are. The next thing we are attempting is to make markers from these genes. The genetic markers will then make it possible to select for varieties of apples resistant to scab and mildew in the traditional way, entirely without genetic engineering. We are working closely with the research institute at Wädenswil, which is carrying out the actual work of cultivation. This approach is highly promising. Breeders can see even if very small cuttings contain various resistance genes; it isn't necessary to wait until the trees have grown. And we can always look for several resistance genes at once. This is almost impossible in breeding without marker selection.

FK: How are scab and mildew combatted then today? How many times does spraying have to be done?
CG: Scab needs the most spraying. In fact, crops are only sprayed to combat scab, since all other diseases are included. An organic farmer sprays 15 to 20 times with copper compounds, a conventional or IP [integrated pest management] farmer 10 to 12 times – with strobilurin for example.

FK: Turning to your work: how many resistance genes does an apple have, is this known?
CG: There are very many. We're far from knowing them all. But today we do know that each variety of apple has different combinations of resistance genes. We now know five resistance genes which are effective in an apple. We have only sequenced one of these. In the case of the others we can only find out if they are in an apple variety or not. We have made Vf markers, as they are called, to enable us to select well. Researchers are working with these markers at Wädenswil. We have also begun to make markers for other resistance genes.

FK: And with these markers you can see if a new variety of apple has enough broad resistance, that is, one based on several genes?
CG: Yes and no. Sometimes just one gene is effective. With Golden Delicious we have identified a resistance gene and know exactly where it's located.

FK: Only one?
CG: There are probably many more. What is interesting is that this one resistance gene we have located is no longer effective in Europe. The pathogen has already adapted to it. In other areas of the world, however, the resistance gene functions.

FK: That's a really narrow genetic base.
CG: It's a problem, because most of an apple's characteristics are multifactored and multi-genetic, with interplay between many genes and many other factors. The worldwide monoculture of one single variety of apple is astonishing too. When we think of apples we think of Golden Delicious.

FK: What other research projects on marker-assisted breeding do you know of in Europe?
CG: With apples there is research in the Netherlands, France, Germany and England. There is the EU Hidras project, which we also work on. Then there is the prunus initiative, a marker assisted breeding programme for damsons, peaches and nectarines – so a lot of different varieties of fruit. In Germany, grape vines are also being researched to see if genetic markers might improve breeding. In the US there is a lot of research on tomatoes using marker assisted breeding. Research in Switzerland is only with apples.

FK: What are the prospects for marker assisted breeding?
CG: I'm convinced there is a great future for breeding with molecular markers. The system isn't self-supporting today, not even with apples. Developing it is very expensive. We don't yet have testing systems which would enable us to analyse next-generation produce cheaply and rationally. Prices have come down enormously but they are still too high. We are working on developing robots, extraction robots, which can scan hundreds of plants as a matter of routine. Then it would be economic.

FK: Could molecular markers be used in all plant breeding?
CG. One can't generalise. Molecular markers are good particularly with crop plants with a long life, but perhaps require too much of an outlay for plants where new varieties come onto the market every couple of years. A wheat variety is long-lived, so it could pay there. With tomatoes too, since tomatoes have to be resistant to almost all diseases. It is very complex, and a lot of genes are involved; marker assisted breeding can then be important.
One day we will probably only do molecular marker assisted breeding, to a very advanced stage. In addition degustative tests will be made, because we know very little about how tastes are formed.

Transgenic Plants

FK: Numerous plant geneticists regard genetic engineering as showing the way forward for the future. What do you think?
CG: Genetic engineering is used with apples too, particularly in the US. There they're attempting to integrate a chitinase gene into the apple genome, for example, to make it resistant to fire.
We don't like this. The approach alone I find problematic. Some genes are taken – from bacteria or fungi, for example – and incorporated into the genome. With apples, especially, this is not the right approach. After all, I eat an apple as something fresh and unprocessed. European consumers will never consent to buying such genetically modified apples.
Personally, I can't just easily accept a fish gene in an apple. Nor would I like a 35S promoter, which comes from a virus, in my apple. And I don't find antibiotic resistance genes entirely acceptable either as this can produce problems with horizontal gene transfer for example. I don't necessarily believe there could be really big problems, but I simply don't feel comfortable with the idea.
Genetic engineering has not been fully developed. The products of genetic engineering today are still at the level of dinosaur technology. We use genes which are foreign to a species, not knowing where they are inserted, and knowing nothing or virtually nothing about the subtle consequences there may be for genes or their environment. Over 95 per cent of today's transgenic plants, by the

way, have just only two characteristics – they are resistant to herbicides and have a Bt toxin gene, and sometimes both – I don't think this is very sensible.
But I see positive aspects for genetic engineering in the future too. I don't see genetic engineering as good or bad, it's the product. A product can be pointless, dangerous, socially threatening, etc., or it may have clear benefits for society and the environment.
To go back to apples as an example. Should we manage to insert genes absolutely accurately, and not need any foreign promoters or antibiotic-resistant genes, I could then imagine that transferring genes specific to the species – that is, only apple genes with apples – would be purposeful. We could then, for example, exchange apple-specific resistance genes that are no longer functioning for genes which are effective. In other words, directly and purposefully insert apple genes. And there would be no risk. These are only the species' own genes. There could be a future for such genetic therapy. But we are miles from this today. It will be a long time before then, should it be possible. Five, ten, twenty years; I don't know.

Epigenetics and Risks

FK: What risks do you see in producing transgenic plants?
CG: The risks of genetic engineering are what I call phantom risks – we can't define them. How big a phantom risk is and what course it may take cannot be predicted. The 35S promoter, for example, is a phantom risk. It could be that a new super virus will be created through recombination of 35S and other viruses. This is quite plausible.

FK: Can we say epigenetics shows us that phantom risks are inherent to the system? Meaning that generally speaking unpredictable risks arise when there is a genetic transfer?
CG: Yes, of course.

FK: Risks we can neither calculate nor control?
CG: Well, not at the moment at least. What the situation will be like in five years we shall see. It can't be very long now.
Epigenetics is a reality, giving rise to phantom risks which hardly anyone can deny. Often I don't see these, and there are often studies which deny a risk and studies which draw attention to the self-same risk – this is typical of phantom risks. It is often difficult to prove something or other in such complex regulatory systems.
To give you one example. If I insert some piece of DNA in a plant genome today, I don't know where it goes, or what I will alter in addition along the whole chain from gene to protein. I don't know which regulatory relationships I'm intervening in.
And another point, today we know a lot of genes which, for the moment, are not functional. We know, for example, whole clusters of resistance genes exist. For the time being they are not functional; they have been shut down. But when a pathogen alters, and changes its identifying proteins, it can happen that the plant, thanks to a resistance gene which until then has not been functional, nonetheless recognises the pathogen. This gene then has a function even if it hadn't been active for a long time.
Added to this are all the regulatory activities of genes. Genes don't only code proteins, they are themselves active in regulating genes. This was not realised for a long time. At the moment we still understand very little about this. But we do know that many of the earlier theories are simply not correct. Today we know, for example, that there is interaction which goes forwards and backwards. The simple rules we learned at school are not right, as any seriously-minded scientist today knows. All this shows that phantom risks are inherent to genetic engineering as it is practised today. Today's transgenic plants ought not to be released into the open.

Releases

FK: Could they be tested in greenhouses?
CG: Certainly. We don't need outdoors at all. There is still a lot to research in the phytotron. This is a chamber where conditions are under absolute control, where light, humidity and temperature are precisely regulated. Not until everything functions and we have really tested it can we take something out onto the field. We aren't nearly that far along. As long as we still need this 35S promoter, for example, I just don't see why we should go from there onto the field.

Moratorium

FK: What do you think of a moratorium?
CG: I think it's a good idea. Let's have a moratorium. We will talk about it again in five years. Perhaps we'll know more then, perhaps not. But one point I don't agree with the opponents of genetic engineering about. We don't want a tactic of prevention; we want to make a serious assessment of the risks. What emerges from this is an open question.

FK: But it's my impression that commercial pressure is so great that there isn't enough time left for a careful assessment of the risks. Partly because of patents. Claims are marked out – the first there takes it all. We need a stop to that, a moratorium.
CG. Right! I completely agree! We need a moratorium, in order to research the risks well too. For this reason I support a new public peoples' initiative in Switzerland calling for a five-year moratorium on commercial releases. I think that's good.
We need a moratorium to finally silence discussions on releasing or not releasing. So that we researchers really will have the freedom to look closely at the problems of the phantom risks. Then we can talk about this again in five years' time. We need a moratorium so we can lead the way for the future. We don't need obsolete technologies and their products; we want technologies and products that very clearly do something for the public good, the environment and nature. So we need a moratorium with an eye to the future like this. We should work towards this end.
On the other hand a moratorium in the sense of a standstill is not enough. The time must be used intensively so as to devise the technologies I referred to. And society must start to discuss what products we want.

5. Interview with Matthias Fladung

Federal Research Centre for Forestry and Forest Products (BFH) Grosshansdorf (Germany)

FK: Mr. Fladung, you work at the Institute for Forest Genetics and Plant Breeding [Institut für Forstgenetik und Forst-Pflanzenzüchtung] in Grosshansdorf. What is your area of research?
MF: We carry out biosafety research on transgenic poplars. We study the genome and its stability, and epigenetics is also on our research agenda. The second major area we work in concerns ecological questions. We look, for example, at pleiotropic effects in transgenic poplars, or investigate possible genetic transfers.

FK: Pleiotropic effects and epigenetics are on your research agenda?
MF: Most definitely. There are regulatory mechanisms above the level of the genome – epigenetics. Here we are interested in what impacts epigenetic regulations have on biosafety research.
We have got to know the genome of trees better and better in the last few years, not least through the poplar genome project, which has given us the complete sequence of the poplar genome, the third species of plant altogether. Not until now have we known the ways in which DNA sequences function, and the regulatory mechanisms in the genome. We are discovering interdependencies between the function of genes and epigenetic regulating mechanisms.

FK: Have you investigated such effects with transgenic plants in particular?
MF: We are at the very beginning of this, and starting the work with an entirely open mind. We investigate transgenic plants, cultivated plants and wild plants and compare them. To start with, all plants are equally harmful or harmless – we don't say at the outset that transgenic plants are harmful.

FK: What have your investigations revealed?
MF: First of all, a surprise. Using AFLP [Amplified Fragment Length Polymorphism] Analysis, we analysed the genome of four independent transgenic strains and compared them

with a non-transgenic control strain. We were very astonished at how great the similarities were and how stable the band patterns were. There were only three bands in which the transgenic poplars differed, three out of almost a thousand. That is just astonishing.
But if we had crossed the plants with one another, the crossed F1 plants would have shown much greater differences from their parents than we would have found between the transgenic strains. This means the genome was shaken up a great deal by crossing. The differences emerging there are much greater than between transgenic and non-transgenic plants. Genome stability or instability is also influenced by a great many factors, by recombinations, by regulatory mechanisms – there is a need for more research here.

FK: So you don't believe introducing genetic constructs presents a special risk?
MF: The indications at least suggest for the moment they don't, as far as genome stability is concerned. We also plan to investigate the effects of transposons and viruses in the plant genome. Transposons are potentially mobile elements within the plant gene; they are either integrated in one place in the gene, or they jump about in the genome and reintegrate themselves at another place. As a result, they cause a whole lot of genomic displacements, instability and recombination to occur. These effects also ought to be taken into consideration in discussing instability in the genome possibly induced by genetic engineering.
But it may still be that genetic changes produce new kinds of risk which may not be caused by viruses and transposons. We are studying this too at the moment.

FK: If I understand you correctly, you are very much at the beginning of this research.
MF: Indeed, yes. But we do know that the plant genome is very flexible. There are processes in nature which create enormous confusion for genomes. Besides the processes already mentioned recombination, transposons and crossing – look at polyploidisations in the plant kingdom. When everything is taken into consideration changes possibly induced by genetic engineering appear in a quite different light.

FK: A question about risk assessment. As you said, epigenetic effects can play a role in assessing the biosafety of transgenic plants. But today's safety requirements are based largely on the old paradigm of genetic engineering. Doesn't the whole risk assessment procedure have to be revised?
MF: The role of epigenetics in biosafety should in all events be more closely investigated. There is certainly a need for research into this. But it isn't as if epigenetic effects have gone entirely unheeded until now. Take, for example, genetically modified Bt corn. This is the best studied variety of corn ever. A relatively great deal is known about it. Were an epigenetic change indeed to have occurred induced by genetic transfer, if, for example, epigenetics played a role in some regulating mechanism, this would have been discovered.

FK: You work with transgenic trees. Do you think these might be released in the next few years?
MF: This question is easy to answer. Commercial transgenic poplars are already cultivated on a large scale in China already – Bt poplars.

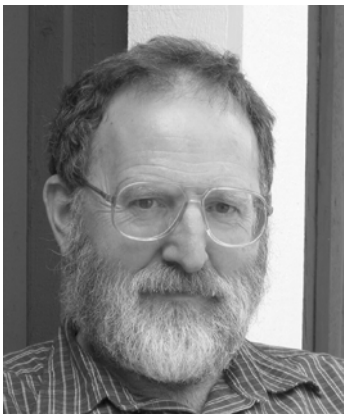
FK: But this doesn't mean they ought to be grown in the rest of the world.
MF: Only in China are there transgenic poplar plantations in commercial use to date. When is safety guaranteed? But it is difficult to guarantee it unreservedly at present.

FK: That's just one of the problems. But what do you think about the situation in Europe?
MF: Transgenic poplars have not yet been commercially released in Europe. First the question of their safety must be cleared up. Once this is assured, I could imagine that transgenic poplars and trees will be cultivated over here too. If, for example, transgenic lignin-reduced trees really do live up to their promise – of less use of chemicals and more environmentally-friendly processes for getting hold of their pulp – then we ought to at least discuss using transgenic trees. And if there are non-transgenic trees that have the same characteristics as transgenic trees, the former should be preferred. Transgenic trees could possibly also be used for phytosanitation, that is, for cleaning up soils contaminated with heavy metals. There are still a lot of ifs and buts, of course. When you ask me if I can imagine the commercial release of transgenic trees tomorrow, my answer would be: probably not. The day after tomorrow? Yes, perhaps.

FK: Not tomorrow because there is still a great need to research?
MF: Exactly. Trees have special characteristics, for example the fact that they have a long life. The generative and vegetative distribution of the genome is more difficult to control. This is something we are researching into too. We conducted a five-year release experiment with transgenic miniature aspens. We put a fence around them and monitored them regularly – the root system in the ground, the whole of the plants, their blossom – everything. Because, of course, no vegetative or generative reproduction of any kind should be allowed to occur. And what did we find after five years? The trees had tried to reproduce via their root suckers. Had we not controlled the release area carefully, the trees could have spread without control. But how is the situation to be assessed after ten or twenty years?

6. Interview with Dr. Richard Firn

Department of Biology
University of York (United Kingdom)



FK: Richard Firn, you are a member of the Biology Department at the University of York (UK). What is your research area?

"The Screening Hypothesis"

RF: I work on two different projects. One is the way in which plants respond to light and gravity. The second project is working on what I call the "screening hypothesis", which I developed together with Clive Jones from the Institute of Ecosystem Studies in Millbrook New York. Our question was: why do plants and microbes make maybe half a million different chemical substances? What evolutionary processes have driven this massive proliferation of chemical diversity? We rejected the existing evolutionary models and devised a new model using the analogy of the human search for biologically active chemicals – screening trials using large chemical libraries. Having formulated the "screening hypothesis", we extended the thinking to a more general model for the evolution of biochemistry. Rather than thinking mainly about the properties of enzymes, we contend that it is the properties of the new chemicals made by enzymes that are the focus for selection.
What is really quite surprising is that biochemists have never been very interested in the evolution of biochemistry as an entity. They looked maybe at the evolution of a single enzyme or a part of a pathway. Clive and I were interested in the question why biochemistry is like it is. If you look at biology before Darwin, people were very good at identifying organisms, describing them and trying to classify them, but without an evolutionary perspective there was no understanding of why organisms were so diverse.

I was trained originally as an organic chemist. As a student I tried to make herbicides which kill plants. That experience told me that it's very hard to predict which chemical would kill a plant. Furthermore, most synthetic chemicals I made had no effect on a plant at all. I learned that the chance of any man made chemical having a biological activity was incredibly low. This is true for all types of pesticides, for plant hormones, for pharmaceutical drugs or any other form of biological activity.
So if humans with intelligence can't make biologically active chemicals on demand, how could a fungus or a plant be so remarkable that every secondary metabolite they made was biologically active – the prevailing dogma when Clive and I came to the subject. This simple paradox led to the "screening hypothesis".

Instead of building en evolutionary model on the supposition that biological activity was a common property for secondary metabolites, we built an evolutionary model on the evidence that biological activity is a rare property of any molecule to possess. Suppose that one out of a thousand chemicals might have the properties to bring benefits – that is the rough ratio of our experience. Then plants and microbes must have evolved to overcome that constraint. The only way is to make as many diverse chemicals as possible and evolution will sort out the ones which work, but it won't do that quickly. Therefore, most secondary metabolites that a plant or a fungus makes will

have no role in the plant whatsoever – the secondary metabolites we find in plants or microbes are the current screening programme of the organism.

FK: This reminds me of the way our immune system is working: By creating a huge redundancy of molecules and antibodies which then are filtered out later on…
RF: Absolutely right. The human immune system and plant and microbe natural product metabolism are different ways of addressing the same problem – a low probability of any one molecule possessing the appropriate molecular structure to bind reversibly with another molecule at low concentrations. So the "screening hypothesis" suggests that, because potent biological activity is an extremely rare property for any molecule to possess, that the synthesis of many compounds which bring no short term benefit to the producer is a necessary part of the overall mechanisms employed by plants to produce the occasional chemical which possesses useful biological activity. Most secondary products are only redundant in the way that most antibodies are redundant. The production of the majority of these substances results in no short-term benefit but short term costs are compensated for by the longer term benefits that result when the rare biologically active compound is made. The majority of secondary products and antibodies are not redundant in any commonly accepted use of the word because they are a necessary consequence of the need to generate chemical diversity.

FK: Does this explain the huge variety in plant metabolites?
RF: Yes, That's why sunflower oil is different from rapeseed oil or coconut oil. A cell needs an appropriate mix of fatty substances to serve in membranes but many lipid molecules possess similar physicochemical properties and exact mix is unimportant, hence evolution has come up with many different lipid mixes that work. Finally we come to what is called primary metabolism – the biochemical pathways that are common to most plants. In primary metabolism, the selected property in evolution is the ability to fit within the overall metabolic grids and pathways. The evolutionary opportunities for variation were quickly constrained as the viable organisms intermeshed their pathways in totally dependent ways. Thus, while at one extreme evolution seeks to generate chemical diversity to optimise the chances of finding the rare biologically active molecule, at the other extreme primary metabolism seeks to avoid any chemical experimentation and rather hones an already well adapted interdependent system.
We have to understand that the rules for primary and secondary metabolism will be very different. The enzymes of the primary metabolism must be substrate specific; there is little space for diversity. But the enzymes of the secondary metabolism are not substrate specific because it is the lack of substrate specificity that gives to opportunity for generating and retaining chemical diversity. Primary metabolism has evolved to be fairly predictable while secondary metabolism has evolved to be unpredictable.

Genetic Engineering

FK: Do you have an example? I learned at the University that enzymes are always substrate specific, e.g. catalysing a specific reaction.
RF: In the Grand Fir (*Abies grandis*) two enzymes can make multiple products from a single substrate. One enzyme can produce 52 and the other 34 different products. Up to now biochemists usually took the rules for the primary metabolism (an enzyme is always substrate specific) as universal rules for all enzymes, which we can now see is wrong. If you consider supplementing the genetic capacity of an organism by introducing a gene coding for an enzyme you need to known how substrate specific the introduced enzyme will be.

FK: So if I understand correctly, the predictability of a gene transfer depends very much on the gene and the coded enzymes?
RF: That's correct. Basically enzymes of the primary metabolism are most predictable. But they are hardest to manipulate because essentially they have evolved to be very rigid and predictable. Whereas when you go into natural products or secondary metabolism, the whole point of it is that it's unpredictable. Evolution has selected mechanisms that are inherently unpredictable. And if you imagine that you can predict the unpredictable, that is an illusion.
So when you introduce a gene affecting the secondary metabolism, it can have quite unpredictable outcomes, as in the following examples:
First, the introduction of an enzyme expected to produce a single new chemical could also

produce other new compounds owing to the substrate tolerance of existing enzymes. Second, the introduction into an new organism of a gene encoding an enzyme involved in secondary metabolism could produce more than one product owing to the substrate tolerance of the introduced enzyme.
Third, the introduction of a gene into an organisms could disturb secondary metabolite production simply as a consequence of the random gene insertion, with unplanned and unexpected increases in the content of some compounds, owing to changes in the metabolic flux through matrix pathways.
Of major concern is the fact that the secondary metabolite profiles of individuals in a population can vary considerably and can vary depending on the prevailing conditions. So the effect of the introduction of a gene into a plant might be predictable only under defined conditions that may not be achievable in the field. The secondary metabolite profile is complex, and extremely small amounts of highly potent compounds could have profound biological consequences. However, the Screening Hypothesis also tells us that most chemicals made will have no potent biological activity so that offers a little comfort.

FK: What always strikes me is the fact that there are only two traits of transgenic plants grown worldwide – herbicide resistant plants and insect resistant Bt-plants. Industry surely would like more traits, not least because of the growing resistance in Europe and elsewhere.
The success of these two traits and pathways could be that they are pretty isolated from others, that they are not intertwined with other pathways. But that is not true for most other traits and pathways. Therefore all the failures – what may work in the lab fails in the field. Would you agree with this thesis?
RF: Yes. If you put a gene-coding for Bt protein into a plant all you have to worry about is: will this gene being expressed and will the protein have the properties you want? The properties of the new protein are essentially irrelevant to the plant. As far as the plant is concerned, it's just another protein, which is not affecting the plant, it will just tolerate it. That is fairly predictable.
Likewise herbicide tolerance: the current ones are the type where they essentially substitute a pathway. They tend to be coming from what looks more like a primary metabolism type of pathway. But that might not be true for all ways of achieving herbicide tolerance. Every case has to be judged on the basis of what we know about the biochemical rules applying to the type of enzyme being introduced.

Blind Spots

FK: What you're telling gives us a completely new way to look at metabolic pathways. Like as if you looked at a box from the outside and from above, and suddenly you see the inside from below. How come this has not been known for some time now?
RF: The problem is that biochemists never thought about evolution. So I try to explain to my students why our thinking was so constrained, why we worked in this area for decades and had not really thought about these simple concepts. I think it's quite important to realize that scientists can have a completely blinkered view of the world sometimes. They can ignore important things quite readily because of history. Biochemistry was a subject that split from chemistry departments in the early 20th C and as such it was uninfluenced by concepts of Darwinian evolution.

FK: How did you get involved in these issues?
RF: I originally came from an agricultural chemistry background and came by accident into a biology department. And that was an advantage. I could look at phenomena without being too tainted by past history – I had not been taught the dogmas as a student.

Epigenetics and Transgenic Plants

FK: A similar paradigm change seems to be happening in genetics. The central dogma of the gene proofs to be incorrect if taken alone; epigenetics is gaining importance.
RF: There's lots of evidence for epigenetic effects, but for decades the findings didn't fit into the accepted paradigm. They were something which people would rather ignore and scientists are very good at ignoring things when it suits them.
I often feel that some terms used to describe genetic manipulation have helped to hide the very nature of cells. Cells do not have "blueprints". Cells can only be "engineered" to a limited extent. I sometimes feel that cells are more akin to software than to machines.

FK: So what are the consequences, in your view, for risk assessment in plant biotechnology?
RF: Risk assessments are all about using knowledge and understanding to predict outcomes. The growing understanding of the mechanisms underlying epigenetic effects reveals a complexity that must inevitably mean that risk assessments being made of GM plants will carry greater uncertainty than one might have liked.

FK: And what would you suggest as consequence for deliberate releases?
RF: I don't feel there is a single answer, beyond a caution that the simple views that have predominated for the last two decades may need some revision. It would be nice if there was a greater humility and more experts would admit the limits of their knowledge.

7. Interview with Gilles-Eric Seralini

Professor in Molecular Biology
University of Caen (France)

FK: Gilles-Eric Seralini, you are professor for molecular biology at the University of Caen. You also sit on different committees dealing with risk assessment of GMOs. Could you describe them?
GS: Since 1998 I have been a member of two commissions for GMO evaluation before and after commercial release – commissions of the French Ministry of Agriculture and Ministry of Ecology. I was an expert for the European authorities on the first panel in the WTO conflict with the United States, concerning the GMO moratorium. And last but not least, I'm president of the scientific council of CRIIGEN, the Committee for Independent Research and Information on Genetic Engineering. The evaluation procedures of GMOs appeared to a number of us quite insufficient, so we founded this association.I have written several books in french on these questions in particular "Génétiquement Incorrect" and "Ces OGM qui changent le monde" (Flammarion Ed.).

Pesticides associated with GMOs and Health Questions

FK: What is your research subject?
GS: We are looking at the effect of pesticides on health – particularly those pesticides associated with GMOs. You may know that nearly 100% of all GMOs are either tolerating or producing pesticides. So virtually all GMOs commercialized in agriculture have been designed to contain pesticides which they absorb or produce, or absorb and produce. And it's one herbicide – the herbicide Roundup from Monsanto – to which 75 or 80 % of all GMOs have been made tolerant. They have been modified to be able to accept it and put it in a cell without dying. But these pesticides – including Roundup – are not harmless.

FK: What did your research show?
GS: We showed that human placental cells are very sensitive to Roundup, to concentrations lower than the agricultural use. This could explain misscarriages and premature births in North America in farmers. We noticed some other effects too. The longest tests with animals directly eating GMOs, were done by Manuela Malatesta and her team. She fed GMOs to mice for 8 months and published 4 papers in peer-reviewed international papers. So I invited her to my lab to give a lecture. She has shown deletorious effects of GMOs on various organs.

FK: A few weeks ago Greenpeace won a legal case against Monsanto in Germany. Monsanto had to publish their confidential experiments with rats fed with their transgenic MON863 maize. You had a close look at them and said that these experiments showed statistically significant differences between rats fed on transgenic maize and those on normal feed, after a time of 90 days.
GS: Yes. Numerous parameters, including blood composition and detoxification organs, such as kidneys, were different. For me, this is one of the main concerns regarding GMOs. So if one wants to increase or improve GMO regulations, it would be very simple: you just have to assess GMOs like pesticides.

First Request: Assess GMOs like Pesticides

FK: Very simple? But what is the difference between GMO and pesticide regulations?
GS: The pesticide directive, CEE/91/414, requires a much more thorough assessment. When you assess a new pesticide, you have to give the new pesticide to three species – generally rats, mice and dogs – for three months. It further prescribes that the new pesticide is given in food for one year to one species – generally dog – and for two years to another one – generally rat. There is absolutely no scientific reason to avoid these kind of experiments for actual GMOs.
The Monsanto study on the MON 863 maize we just talked of appears to be the best and the longest one that has been performed with mammals. And already this study shows significant effects in comparison to control laboratory animals. So we should force industry to publish their results and we should enforce such long term tests.

FK: What are the actual requirements for GMOs regarding toxicity tests?
GS: There is no mandatory test. The tests that Monsanto did are not required yet. I think it is stupid to give GMOs to people for an entire life time when at the same time there is no requirement to undertake toxicity tests even for three months.

Epigenetics and Risk Assessment

FK: Could long term toxicity tests also be a step further in evaluation of unintended effects and epigenetic changes?
GS: Absolutely. Of course today all regulations also include unintended effects. But what the proponents of GMOs say is that epigenetics and unintended effects are *a priori* not different for a transgene and a regular gene. These risks, they say, such as transposition or insertional mutagenesis or methylation of new genes, are common for all genes. They claim that a difference has not been demonstrated so far.

FK: What is your opinion? Our argument is that the transgene insertion is random, that we don't know where the genes are going, in how many copies, and what deletions or recombinations or other disturbances there will be. There is quite a difference between transgenic maize and conventionally bred maize.
GS: I personally think that if we take an evolutionary point of view, there is a whole bunch of data that can be analyzed. We can consider that artificial genes inserted by chance in the genome could have more chances of being supressed by methylation, for example, or be subject to more gene rearrangement than other genes.
But there is not much evidence, and this also has a reason. When you study the role and regulation of genes, you undertake a lot of trials and you only select the GMOs that do not have these kind of problems. That means that 98% of all the GMOs that you produce do not function just because the gene might be methylated or the gene might be present but is not used normally by the organism which received it. All the organisms that are modified but do not express the gene are put in the garbage.
So you find very few studies on these issues. There are some papers about insertional mutagenesis or transgenesis in plants showing that artificial gene constructs may be more unstable than others. This has led to the discovery of antisense RNA or RNAi.
So I think that we should bear in mind that the study of the composition and the analysis of the substantial equivalence is far below the level of sufficiency to be able to predict any toxicity or any unintended effect of a plant. At least we should introduce mandatory scientific toxicity tests, which is not the case today.

Second Request: Resequence the Transgene after Insertion

We should also make it obligatory that a transgene is sequenced after the insertion, not just before the insertion. This could be a good control in order to avoid the first level of epigenetic effects, to see if there is any instability.
Some researchers, like Ives Bertheau in France, showed that in almost all GMOs the transgenes are no longer the ones that are published by the companies. A network of labs was involved in doing these studies.One reason for these changes could be that the transgenes or the transgenic plants are not stable. Another reason could be that the initially published patent was not correct anymore because there was an evolution of the transgene not mentioned in the patent.

Insufficient Risk Assessment

FK: You do not think that commercial releases are safe?

GS: Of course not. I'm certain of the fact that risk assessments are insufficient today, not only because of epigenetics, but also because a lot of the GM plants are supposed to make pesticides that are not tested on health. And then the transgenes are all artificial gene-constructs. Often it is said that they are natural ones, but in fact some of them are 44 % different, for instance in the case of Bt maize 176.

FK: Why 44%?

GS: Either because they have been redesigned with mutations in order to make the toxin more stable, or because the toxin has been truncated by 44% in order to give it a wider spectrum and to make it more soluble in cells. It is an artificial toxin that doesn't exist in the natural surroundings. But I think it would be sufficient to ask first for toxicity tests for GMOs according to the pesticide directive, CEE/91/414, and second for a resequencing of the transgene after insertion. These requirements could encompass the problem of epigenetics as well. We don't have to go so deep in the scientific explanation, and to call for epigenetics in order to show that the actual evaluation, assessment of GMOs is by far insufficient. And with these two requirements we would not have any commercial releases yet.

FK: I still think it is worthwhile to show that the central dogma of the gene is outdated, that there is a new paradigm where epigenetics plays a mayor role, together with other mechanisms, some of them not known yet, and that these new findings require a reevaluation of our risk assessment procedures.

GS: I agree. We discovered that there is a real living ecology in genes. There is a big fluidity in the genome, and the behaviour of one transgene is not predictable. I call this ecogenetics. But I heard this argument also taken by proponents of GMOs. They say: “Genome fluidity has already been demonstrated in plants, in all plants, so when you eat maize you encounter unintended effects all the time. There is no difference for GM maize“. So I tend to take a pragmatic point of view. Make toxicity tests for GMOs according to CEE/91/414 mandatory and enforce a resequencing of the transgene after insertion.

8. Interview with Manuela Malatesta

Professor at the Institute of Histology and Laboratory Analyses
University of Urbino (Italy)

FK: Manuela Malatesta, you’re a professor at the University of Urbino (Italy). What is your research area?

Effects of Transgenic Soya on Liver Cells

MM: I’m an electron microscopist. I use the electron microscope with several techniques such as cytochemistry, immunocytochemistry or *in situ* hybridization. With these techniques we can localize several molecules on tissues and cells, a liver cell for example. In this way we can analyze not only the morphology but also the molecular composition of a given cell. My main interest in the last few years were the possible effects of a diet containing GM-food on mice. We analyzed tissues and cells after mice have been fed with transgenic soya.

FK: What experiments did you do?

MM: We fed two groups of mice. One group was fed on a standard diet, containing 14% of genetically modified soybean – it was soya with a Roundup Ready resistance. The other group was fed on the same diet but the soybean was natural. The mice were analyzed at different ages: at 1 month, 2 months, 5, 8, 12, 18 and we are waiting for the 24th month old mice to be analyzed. We look at liver cells and, in particular, their nuclei.

FK: This means you also analyze old mice?
MM: That's right. A 24 month old mouse is already an old mouse. It's important to look at long term effects, and we're also interested in the effects of a diet containing genetically modified soybean on the ageing process.

FK: And how did you analyze the liver cells?
MM: We carried out ultrastructural morphometrical studies on liver cells of these mice: we measured the cellular structural components by an image analysis system in order to obtain quantitative data for statistical comparisons. Then we did immunocytochemical studies. This technique allows us to localise specific molecules on the cellular structures by using antibodies which recognize and bind to the molecules under investigation. We wanted to look at modifications of nuclear components of these cells which are involved in multiple metabolic pathways related to food processing.

FK: What were the results?
MM: We found no difference in the cytoplasmic organelles (such as the mitochondria, where the energetic molecules needed for any cellular activity are produced, or the rough endoplasmic reticulum and the Golgi complex, where proteins are synthesised and processed) between the 2 groups. But we found significant modifications of some nuclear features in GM-fed mice. In particular, GM-fed mice showed irregularly shaped nuclei – the nuclei looked like they were corrugated – whereas the nuclei of the control animals had a smooth and roundish shape. An irregular shape generally represents an index of high metabolic rate, and a higher number of nuclear pores suggests an intense molecular trafficking. Moreover, the irregular nuclei of the GM-fed mice had nucleoli with numerous small fibrillar centres and abundant dense fibrillar components – and these modifications are again typical of an increased metabolic rate. So our conclusion is that the GM-diet can influence nuclear features of liver cells in young and adult mice. The liver of a mouse fed on genetically modified soybean seems to have cells working harder than a liver of a mouse fed on natural soybean.

FK: Do you know if these effects are due to the new protein of the foreign gene construct or if it's the transgenic soya as a whole being responsible for these changes. In other words did you also make trials with the new protein alone?
MM: Monsanto writes that a Roundup Ready soybean produces only a few proteins typical of this kind of soybean. We sent the two soybeans – the transgenic and the 'normal' one – for analysis to the Istituto Zooprofilattico Sperimentale, an institution with which we collaborate. They found that they are substantially equivalent, they have the same nutritional value. But this result can not rule out the possibility that the genetically modified soybean produces some different proteins and that this could explain the differences found between the two animal groups. That's one of our hypothesis.

FK: And the others? What possible explanations could there be for these significant differences between the nuclei of GM-fed and normal fed mice?
MM: At the moment we have three hypotheses:
The first one is that there is a new protein in the genetically modified soybean – or maybe several new proteins – which could affect the liver cells. It is known that the intake of different amino acids or nucleotides (the molecules constituting RNA and DNA) can modulate RNA and protein synthesis in the liver.
The second hypothesis is that maybe there were some traces of glyphosate – the chemical of the herbicide, Roundup – in our genetically modified soybean. Two years ago there was a Danish study that demonstrated traces of glyphosate in the soybean millings and bread. So we hypothesized that maybe in our mouse feed we have some traces of glyphosate. But we could not find anyone who could analyze our feed.
The third hypothesis is based on a study which demonstrated that this genetically modified soybean contains lower amounts of phytoestrogens. These compounds are able to act on the liver cells to different levels affecting a lot of metabolic pathways so maybe a different intake of these phytoestrogens can lead to the differences found in the liver nuclei.
These are our three hypotheses. Unfortunately, for the moment we cannot prove any of them.

FK: What other experiments did you and your group do?
MM: Well, after demonstrating the differences in liver cells we wondered if they are reversible. This is the last experiment we did. We fed mice which had been fed on genetically modified soybean from weaning, with natural soybean. So we reversed their diet. In parallel, we fed control mice for one month with genetically modified soybean. Then we looked at the nuclei of their liver cells. We found that a one month diet of GM-free feed could reverse some of the nuclear features in adult mice who were fed on GM-feed. So this suggests that the modifications related to GM soybean are potentially reversible. At the same time, we found that some modifications are inducible in adult mice in a short time, in fact, mice raised with natural soya showed these effects in one month when fed on GM-soya.

Further Research – Lacking Funds

FK: Apart from the liver – did you analyze other organs and cells?
MM: We analyzed the effects of a GM-soya diet on pancreas and testis. And we also found significant differences, which disappear in the pancreas when the diet is changed, while in the testis the differences disappear with the aging of the mice. We found that in the testis there was a decrease in transcription and splicing activity in mice fed on genetically modified soybean at 2 and 5 months of age. But at 8 months of age all differences disappeared. So it's possible that the testis compensates for this problem. And again we hypothesized that maybe the Roundup, the glyphosate, induces these differences, because it is known that glyphosate interferes with RNA transcription and splicing. So these are our results, but again we do not know for sure why they occur.

FK: What are your next steps?
MM: Looking for funds, plainly. We definitely want to continue these studies, but we have no money. This month is the last month I can work because I will pay the last salary to my last collaborator, and after my holidays in September when I come back to Urbino, I will be able to teach, that's all.

FK: No research anymore? That's a shame!
MM: We tried everything, we applied for funds to foundations, at the Ministry, everywhere. But it seems impossible to find any money in this period.

Transgenic Plants and Authorisation Procedures

FK: I do hope you'll find some funds and will keep my fingers crossed. Another question, Manuela, what are your recommendations for further handling of GM food and deliberate releases?
MM: I would like more information, more scientific tests before an introduction of such compounds

FK: What kind of scientific tests?
MM: It needs long-term trials with GM-fed animals. The ones required now are too short-term. Then it needs multidisciplinary studies, combining biochemistry, electron microscopic histology and also microbiology. In fact, we should also think about the intestinal bacteria. They could change due to diet changes, and we do not know very much about them. And only after these studies could we accept transgenic food in our markets. Now, I'm not sure.

FK: These studies aren't done now, obviously. And furthermore, industry studies are often kept secret. Monsanto just had to release such a study on the transgenic maize MON 863, because a legal intervention of Greenpeace.
MM: I know. But this is not enough. This is one case, but we need laws, European laws, in my opinion, that regulate these controls. The controls must be made by public institutions, not by the producer.

FK: Not by the producer?
MM: The first one, the first control, obviously, must be made by the producer. But before setting the transgenic plants in the market, in my opinion, the controls must be made by public, independent institutions. But that's an opinion as a citizen.

9. Interview with Dr. Beatrix Tappeser

Head of Genetics Division
Federal Agency for Nature Conservation (BfN), Bonn (Germany)

FK: Beatrix Tappeser, you are a specialist department head responsible for the implementation of the law on genetic engineering at the BfN [Bundesamt für Naturschutz, Federal Agency for Nature Conservation]. Can you describe your work?
BT: The BfN in Germany is integrated in the approval procedures for releasing and marketing transgenic plants. Two special areas with different foci work together here at the BfN. Department II 2.3, of which I am the head, is concerned with environmental risk and environmental impact assessment. Its "neighbour" department, I 1.3, is at the same time involved with approaches to monitoring transgenic organisms, scrutinising monitoring plans submitted to it to see if they are in accord with legal requirements. Beyond this, I am in charge of the skills centre for genetic engineering, the purpose of which is to bring together and integrate the entire expert knowledge the BfN has on protection of species and biotopes, impacts on biodiversity and protection of nature and the countryside, in order to assess the potential effects of transgenic plants on nature and the environment.

Epigenetics and Approval Procedures

FK: Knowledge that epigenetics also plays a great role in plants and that, as a result, introducing foreign genes is basically fraught with uncertainties, has grown rapidly in the last few years. But the outdated genetic dogma was for a long time the basis of an entire "safety philosophy". But if the basis proves itself inadequate, this also affects the whole building above – risk assessment, application and approval procedures, (long-term) monitoring, etc. Has anything changed in the last few years? Have findings in epigenetics made inroads into this "safety philosophy"?
BT: On the European level there is Directive 2001/18 on releases and the "Food and Feed" regulation 1829/2003. Applications for import, cultivation and marketing have to be made in accordance with one of these two sets of regulations. The basic principles for environmental impact assessment are laid down in the appendix to the releases directive, and these also apply for the food and feed regulation. Direct and indirect, immediate and delayed effects are supposed to be assessed, of course in accordance with state-of-the-art science and technology. It is laid down in both the appendix and the law itself that new information has to be passed on by the applicant and incorporated into the assessment made by the authorities. This stipulation is broadly speaking aimed at findings made in monitoring, for example, but it also of course applies to the scientific basis of risk assessments. With old applications this can in theory lead to an existing market authorisation being altered or withdrawn.

EU and EFSA

FK: So there is a certain room for interpretation for an application to be checked?
BT: Yes, of course. What this means in practice is that the European Food Safety Authority, the EFSA, is increasingly taking the lead here, since applications are increasingly being made under the food and feed regulation.

FK: Does this only apply to food and feed or to the cultivation of transgenic plants?
BT: To both. If transgenic plants are used as food and feed and have been grown in Europe and not just imported, the EFSA remains responsible. The principle of "one door, one key" applies. Applications should only have to be made at one point. Member countries are involved and can make comments. The EFSA then makes a statement of its position taking these comments into account.

FK: The comments by member countries are not binding then, they are just advisory.
BT: Exactly. The EFSA in 2004 published the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. In our view the environmental impact assessment is depicted very weakly there. This may have something to do with the fact that there are hardly any ecologists in the EFSA.

FK: Can you give an example of how the approval procedure is gone through in the EU?
BT: One recent example is Bt corn 1507. The application is for both importing and processing, and for growing it in Europe too. There have been a whole series of objections from many member states including Germany, the UK, Denmark, France and Poland. The EFSA, having been called on to examine these by the EU Commission, saw the objections as groundless or without substance, and made a positive appraisal. The Commission thereupon applied for the corn to be authorised. This application then failed to get the necessary support in the regulating committee, where the member countries are called on to agree to the Commission's application. The Council of Ministers, too, which has to decide when no agreement has been reached at the lower level, did not vote with the majority necessary for approval; nor, however was the majority needed for a rejection attained. This means that now the Commission decides. Experience with this procedure suggests the Commission will probably issue authorisation.

FK: How does this work in practice? A US company makes an application for the cultivation of a new variety of transgenic corn. What happens then?
BT: The application goes to the EFSA, say. All the national authorities can then download it from the internet and examine it within a given deadline. We at the BfN look particularly at the data and statements about impacts on biodiversity and the environment, and collate our notes in a commentary. But mostly the data is not very satisfactory. This makes careful planning difficult.

Risk Assessment Criteria

FK: What then are the criteria used in examining an application?
BT: We have three aspects which we in all events incorporate into our examination:

1. The molecular characterisation of the introduced genetic sequences including the integration points and possible reorganisation both at the integration points and in the introduced genetic construct. Also included here are expression data on the genetic sequences introduced taken from as many years as possible and under the most diverse climatic conditions.
2. The characterisation of whole plants, i.e. determining whether there have been changes in composition with respect to their metabolism, and what these changes are, and characterisation of the phenotype. Here it is equally important to have data from several years and regions of cultivation.
3. The third level is concerned mainly with systems. Here we would like to see data on effects on non-targeted organisms, food chains, soil organisms; and feeding studies. While these are usually only collected under the assessment of impacts on human and animal health, they nonetheless make it possible to infer, at least as indicators, possible effects on mammals living in the wild.

Seldom, however, do most of the documents submitted contain adequate and sound data or statements on all the areas under examination I have referred to. The EU member countries often have much higher requirements on the extent and standards of data than the EFSA's GMO panel.

FK: Can you sum up what has changed in the last few years?
BT: In a certain way something has changed. It has become recognised that there are inherent uncertainties in transgenics with respect to genetic change in plants. Epigenetics is not addressed directly, but the requirements for documents submitted have become higher (even if they are certainly not enough) and this can be interpreted as a reaction to a change in the state of science. But this has yet to percolate down to where it is reflected in the practice of risk assessment or to there being a common attitude on the part of European institutions or authorities in the member states.

III. Life is Complexity

Christoph Then
Greenpeace Germany

The End of the Genetic Building Blocks

In 2001, the first map of the human genome was produced. One of its biggest surprises was that humans only have about 25,000 genes, not the 100,000 that had been predicted. This number of genes still has to code for the several hundreds of thousands of proteins produced by cells. Therefore, the 'one gene, one protein' hypothesis, which had formed the basis of our understanding of gene function, has had to be abandoned. Far fewer genes than had been assumed take on much more complex tasks than imagined, and it is now known that 40-60 per cent of all human genes have not just one function but several. Craig Venter, one of the driving forces behind the decoding of the human genome, has called for the function and effect of genes to be redefined. "The modest number of human genes means that we must look elsewhere for the mechanisms that generate the complexities inherent in human development ..." (Venter, 2001).

Therefore, instead of viewing a gene as a deterministic building block, it now has to be seen as a part of a systemic unit which has many factors influencing the outcome. In fact, the full role of a gene can only be explained by looking at its context as a whole. As a study by the German Department of the Environment puts it: "Genes never have an effect in isolation. Their effect is (co-)determined by the genetic background and the environment." (Pickardt, 2002)

To explain the regulation of the genome, the science of "epigenetics" is being turned to. Literally, epigenetics means 'above' or 'in addition to' genetics. It is defined as *heritable* changes in gene function that occur without a change in the sequence of nuclear DNA. Epigenetic mechanisms are mediated by the secondary architecture of the DNA which is influenced by environmental signals from elsewhere in the cell and beyond. So epigenesis is subject to dynamic, non-linear processes, interaction with the environment and various controlling processes in cells (see also account by Katja Moch and interviews with Buiatti and Strohman).

What does this new understanding of how genes and organisms function mean for the genetic engineering of plants and predicting the impact of introducing new genes? One implication is that because the DNA sequence alone does not define the function of a gene, predicting the effect of moving one gene to another organism is not as straight forward as has been assumed. The network of epigenetics is too complex to be able to make reliable statements on the result of gene transfer as Maleszka (1998) has shown in comparative studies of the genes of fruit flies, yeast and humans.

Even if a certain function is transferred, the function of a gene may be affected by the environment (such as in conditions of drought, disease, or salinity) in ways which are too complex to be predictable from our current knowledge. Even apparently very successful and stable genetic modifications, such as in Roundup Ready soybean, have been affected by environmental conditions, where high temperatures have been associated with stem splitting (Coghlan, 1999). Another example is poor performance of Bt cotton under high temperature. A investigation from China (Chen, 2005) concludes that high temperatures are most probably responsible for causing a drop in Bt (Cry1A) concentrations in leaves.

Effects on gene function are manifested by changes in the proteins they code for and even small changes in the structure of proteins can have dramatic consequences. As Craig Venter described the human genome, "At the protein level, minor alterations in the nature of protein-protein interactions, protein modifications, and localization can have dramatic effects on cellular physiology" (Venter, 2001).

Mechanisms of Evolution

Bacteria commonly transfer genes between species and this is one important mechanism of acquiring genetic variation in their process of asexual reproduction. In contrast, in higher life forms

– such as plants – sexual reproduction is an important source of genetic variation. Heredity is thus affected in systems by which alterations are allowed, while the order of the genome as a whole, including its epigenetic mechanisms, is preserved. Heredity with higher forms of life comes about by "orderly genomic distribution" (Vogel, 1998).

Plants are constantly exposed to one common cause of mutations in genes – ultraviolet light. To protect their genes from unwanted alterations and pass on their complex genetic regulation so that it is stable and able to function, plants have developed diverse mechanisms to limit the occurrence of mutations. In addition, genetic variations and mutations in an individual plant, if not immediately corrected, are tested in interaction with the ecosystem over long periods of time before they become established in a species. In this way, the genome of life forms is "optimised" by evolution over long periods of time (Mayr, 2003).

As a result, although the genome is permanently subject to a kind of "background noise" of mutations, over long periods of time the basic structure of the genome does not necessarily alter. For example, grasses still display great similarities in the structure of their genes after 60 million years of evolution. "Genetic mapping of wheat, maize and rice and other grass species with common DNA probes has revealed remarkable conservation of gene content and gene order over the 60 million years of radiation [UV light] of Poaceae“ (Gale,1998). There is great conservation not only of genetic information, but the order of the genes on chromosomes also follows a particular arrangement (syntheny).

Conservation of order in the genome plays a crucial role in evolution. The organisation of the genome in higher forms of life, particular that of plants, constitutes a balancing act between chaos and order, diversity, change and stabilisation. Interfering in this complex process by transferring single genetic building blocks without sufficiently understanding how it all works seems irresponsible, but it is even worse for altered organisms to be released straight into the environment.

Shotgun Genetic Engineering

Genetic engineering (GE) of plants breaks up of the regulation of genes by crudely inserting new genes into plants and then rapidly multiplying these up. Inevitably, genetic information and activity, and the regulation of cells are altered.

"A genetically transferred gene sequence must then be understood as genetic information the context of which has been altered in an uncontrolled way," says a German Department of the Environment study (Pickardt 2002). The borders between species and cells are overcome with the help of certain bacteria (*Agrobacterium tumefaciens*), gene guns (bombarding cells with DNA coated particles of gold) and other crude techniques. It is not possible to control where in the genome a gene becomes located, the number of copies introduced or its interaction with other genes.

In contrast, normal cultivation and breeding maintains the orderly system of heredity (gene structure and epigenetics), as developed, tried and tested by evolution. It uses the great natural range and flexibility designed in biodiversity. The genomes of different species of plant can sometimes be re-combined, as with triticales (a cross between species of wheat and rye), but such re-combination is limited to a few exceptions where the genetic regulation of plants does not make such exceptional steps in breeding impossible.

Even when the somewhat dubious methods of mutation breeding are used (using mutagenic radiation or chemicals), borders between species are not crossed, nor are plants directly forced into some metabolic path or other. The system of chromosome-linked heredity, coupled with the regulatory framework of epigenetics, also seems to be crucial to the success of mutation breeding. For example, in a large-scale research programme to breed sunflowers with a special oil quality, 30,000 seeds were mutated without achieving the desired results. A new quality in oil was only achieved by mutation of one line which already had a suitable genetic background. "The isolation of similar mutants in isogenic parental lines illustrates the importance of the genetic background in the development of specific mutants with an altered seed oil fatty acid composition“ (Fernandez-Martinez, 1997).

In genetic engineering, several thousand experiments are necessary before plants with the desired GE trait able to survive and appear “normal” finally come into existence. In contrast to the trials with sunflowers described here, what decides if genetic engineering is successful is not whether new genetic information is usefully integrated into the genome as a whole, but whether genetic regulation can be so disabled that a new metabolism can be forced onto the plants. For example, in GE plants to be insect resistant, the production of the Bt insecticide within the plant should, according to the logic of genetic technology, function in simply all maize varieties and cotton species, regardless of their special genetic background.

Special Risks with Plants

Plants possess a complex chemistry arising from their secondary metabolism which also makes interfering with their genes especially problematic. While primary metabolism regulates all the plant's basic functions, such as feeding, growth and reproduction, substances arising from secondary metabolism may have no recognisable significance for the immediate survival of individual cells or the plant as a whole. Groups of substances in this category include alkaloids, terpens and phenol-like substances. Many of these substances are highly toxic; others can be used pharmacologically. There are thousands of secondary substances, and their concentration can fluctuate greatly in the course of a plant's life.

Interactions between plants and their environment are much more complex than had until recently been assumed. Secondary substances accordingly have multifarious functions; many, like plant hormones, for example, have a signalling function. They influence the activities of other cells, control metabolic activities, or coordinate courses of development in the whole plant. Such substances are particularly important for plants' communication and defence against pests. It is known, for example, that the scent spectrum of tobacco, cotton and maize varies according to the pest attacking (see interview with Firn).

So plants are not self-contained programmes, but have open systems which react to environmental influences. Stress or interaction with the environment can affect their secondary metabolism, growth or reproduction. This variability introduces a major uncertainty in predicting the behaviour and performance of genetically engineered organisms when grown not just under greenhouse conditions but in nature.

The Underestimated Risks from Genetically Engineered Plants

In the face of the latest scientific findings about epigenetics and the complex interaction between plants and the environment, our ability to predict confidently the risks of genetically engineered organisms must be treated skeptically. In particular, examining such products primarily for the sake of seeing if they are safe for release in commercial use in agriculture and food manufacture seems an extremely questionable undertaking.

The shortcomings of genetically engineered organisms risk assessment becomes clear when it is compared to test procedures used in authorising drugs. In the latter, only a defined substance is usually tested – in accordance with a precisely described procedure to the point where clinical tests are carried out on human beings. For several reasons, this test procedure cannot be transferred to genetic plants. Plants are living systems whose characteristics change constantly as a result of processes such as growth, flowering, seed formation and environmental influences, whereas the quality of drugs must be stable. Interaction with the environment – outcrossing, spreading and impacts on complex ecosystems and so forth – does not have to be taken into account in testing drugs.

Companies like Monsanto, Bayer and Syngenta tend to deny the scientific reality for business reasons. Given the complex regulatory processes and many unsettled questions, molecular genetics is unduly simplified and reduced to commercially utilisable units. It is unacceptable that, in an attempt to make profits with this technology, an incalculable risk to large parts of the biosphere is being run. "What the public fears is not the experimental science but the fundamentally irrational decision to let it out of the laboratory into the real world before we truly understand it“ (Commoner, 2002).

Breeding based on natural biological diversity and using modern methods like genetic diagnosis (marker assisted breeding) seems to have the potential to be more efficient and reliable. In the

face of success in normal breeding, where such diverse forms of growth as broccoli and cauliflower have been developed by selection from *Brassica oleracea*, the progress made by genetic breeders looks rather limited. The example of cauliflower and broccoli illustrates how variability exists for use in normal breeding and marker assisted breeding, without plants having to be genetically engineered.

Syngenta's research director David Lawrence gave a surprisingly frank account of the situation in an article in the Die Welt newspaper on 29 November 2004 WELT ("Syngenta stoppt Gentechnik-Projekte in Europa", written by H.Crolly). Traditional methods, he said, had proven themselves to his company as often being more effective than biotechnology. "We have experimented a lot with genetic engineering with seeds and plant protection, and have often failed." In contrast, he pointed to the exceptional success that had been achieved by traditional approach, the best example of this being the "Pure Heart" watermelon. "This picnic-sized product of Syngenta's breeding is not only more apt for single households than the conventional big-family monstrosity, it has a thinner outer skin, is seedless and tastes just as sweet at its extremities as it does in the middle. It is due to go onto the market in Europe in 2005, and is already being sold in the US."

Conclusions

Recent scientific research and the interviews published in this reader have importance for the creation and assessment of genetically engineered plants. The new information emerging from studies of epigenetics shows that gene sequences alone do not determine how a gene or organism functions. The architecture of the DNA and how it is influenced by the environment also influences when and where a gene functions. This introduces a complexity that means that we do not have sufficient knowledge to predict confidently how a genetically engineered organism will behave in all environments. Disturbingly, official risk assessments of GE plants have failed to take this dimension of genetics into account.

However, as the new paradigm on epigenetics emerges and takes shapes, scientists and people are likely to realise that GE is "dinosaur technology" and that there are much better solutions around for future plant breeding than producing GE crops.

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Interviews with nine scientists about the risk from genetically manipulated plants - for example:

“The human genome has approximately 30,000 so called “coding genes” and these can code for more than 500,000 different proteins. Therefore, gene “ambiguity” - more proteins coded by a single gene - is very high.”
(Professor Marcello Buiatti, Italy)

“The products of genetic engineering today are still at the level of dinosaur technology. We use genes which are foreign to a species, not knowing where they are inserted or what else will change in the whole chain from gene to protein.”
(Dr Cesare Gessler, Switzerland)

“So I think that we should bear in mind that the study... is far below the level of sufficiency to be able to predict any toxicity or any unintended effect of a plant.” (Professor Gilles-Eric Seralini, France)

“It would be nice if there was a greater humility and more experts would admit the limits of their knowledge.” (Dr Richard Firn, UK)

Greenpeace Austria / Central and Eastern Europe, Siebenbrunnengasse 44, 1050 Vienna, Austria.
E-mail: office@greenpeace.at

Greenpeace Switzerland, Heinrichstraße 147, 8005 Zurich, Switzerland.
E-mail: gp@greenpeace.ch

Greenpeace Luxembourg, 34 Avenue de la Gare, 4130 Esch/Alzette, Luxembourg.
E-mail: greenpeace@pt.lu

Greenpeace e.V., 22745 Hamburg, Germany. Tel. ++49 (0)40 30618 0. Fax ++49 (0)40 30618 100.
E-mail: mail@greenpeace.de. Berlin political unit, Marienstraße 19-20, 10117 Berlin.
Tel. ++49 (0)30 88 99 0. Fax. ++49 (0)30 88 99 30. Internet: www.greenpeace.de