The GMO pipeline – evolving biotechnologies but same old GM crops

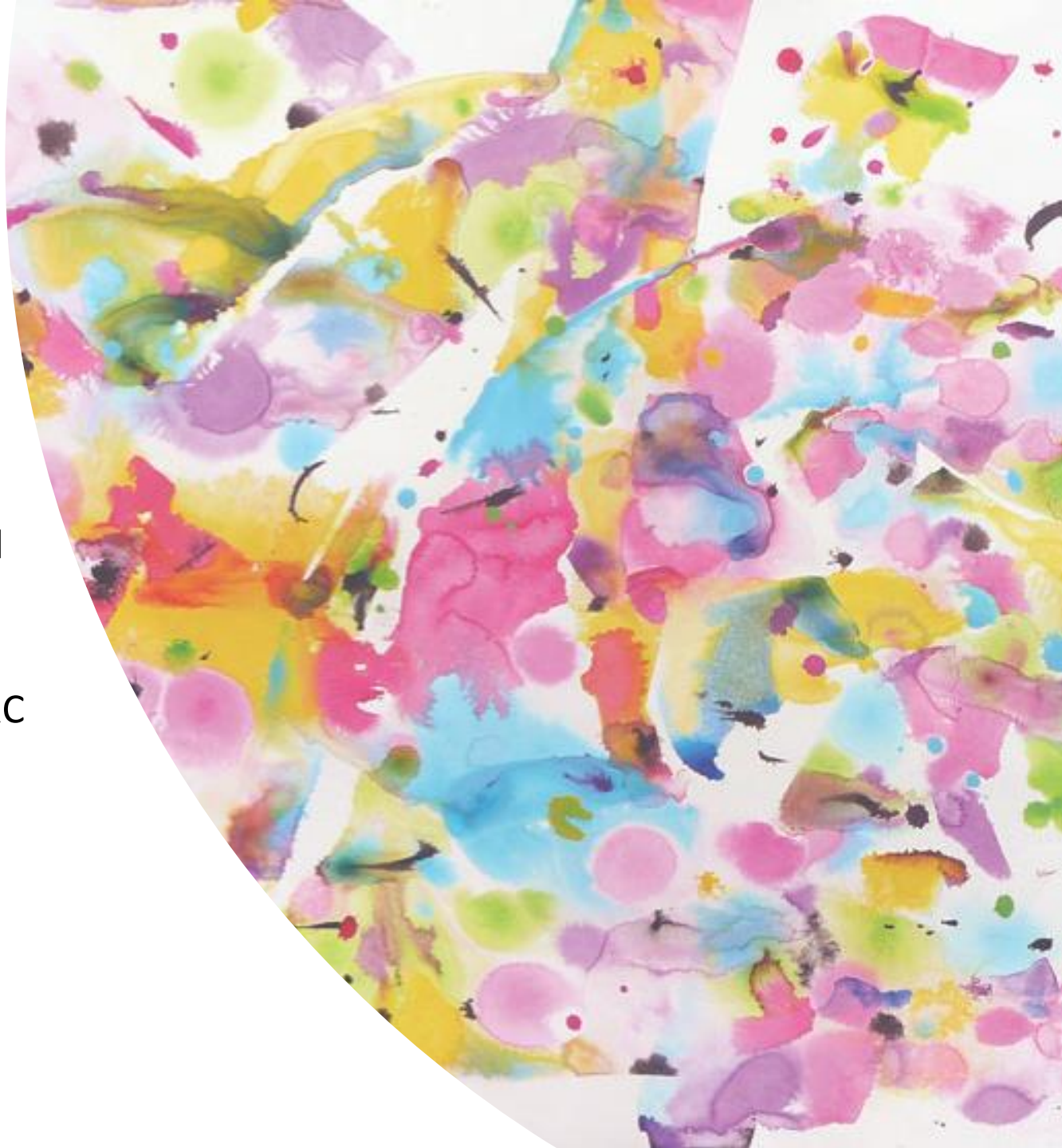
In Memory of Dr Mae-wan Ho

Overview of presentation

- Status of first generation GMOs after 20 years of commercialisation
  - failure to live up to grand claims of decreasing hunger, reducing chemical burden, improving agricultural traits in crops
  - Risks of GMOs largely materialised

- GMOs 2.0 – risks of crops developed with new biotech techniques to replace first generation GMOs

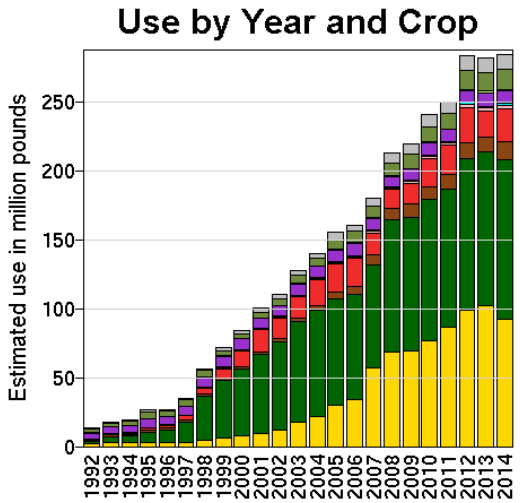
Two main types of GM crops currently grown



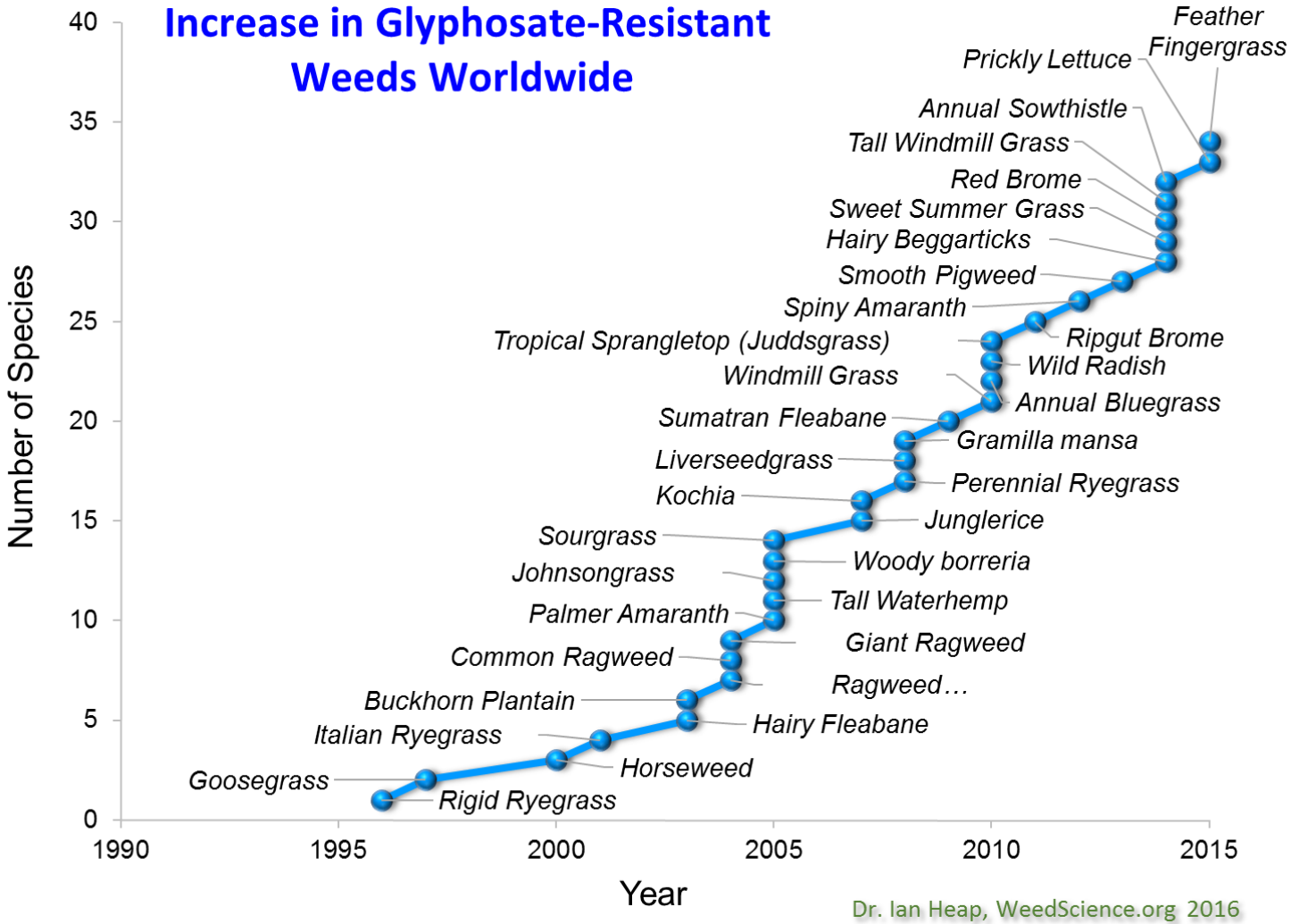
- **Herbicide-tolerant crops**:
  - Engineered to withstand herbicides
    - Make up ~ 80 % of all GM crops cultivated worldwide
    - Glyphosate tolerance most common
    - Glyphosate toxic to humans and environment. Recently re-classified as IARC probable human carcinogen.

- **Insecticidal crops**:
  - Crops engineered to produce insecticides that kill certain groups of insects e.g. Bt crops
    - Second most popular type of GM crop
    - Studies indicate toxicity of Cry toxins

GM crop traits failing – weed resistance spreading

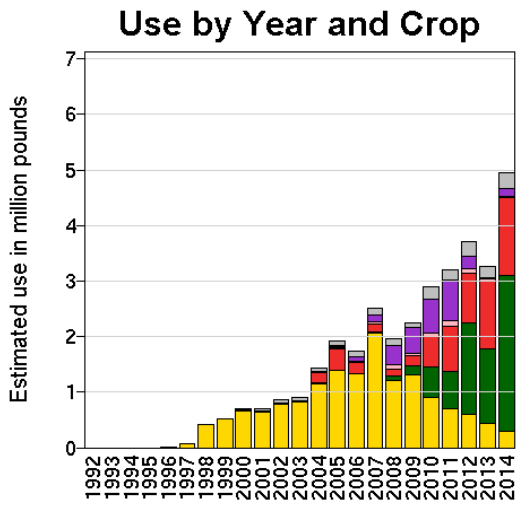


Glyphosate use on crops, US. *USGS data*

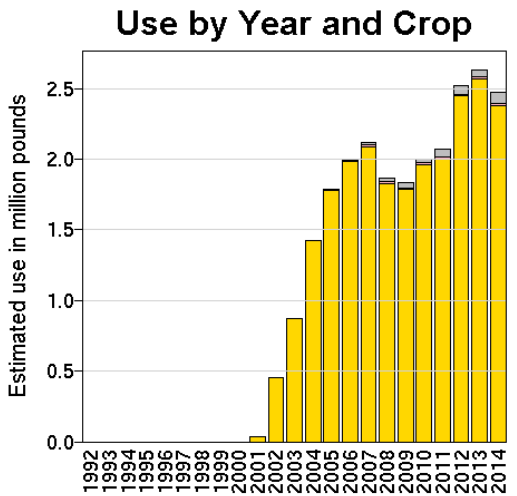


Rising chemical use due to failing GMOs

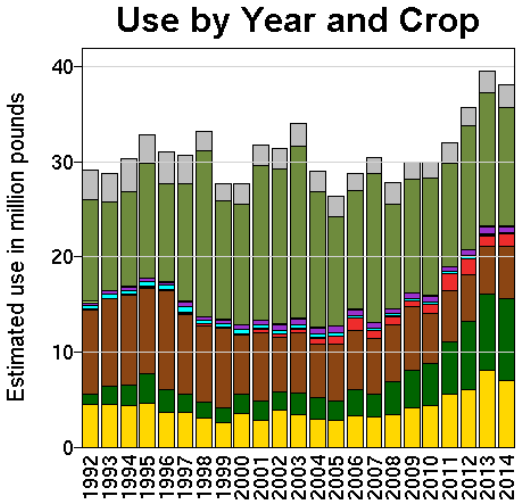
Pesticide use on crops in US *USGS data*



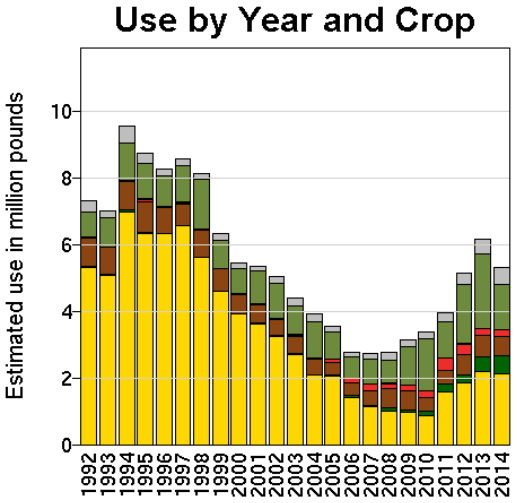
glufosinate



mesotrione



2,4-D



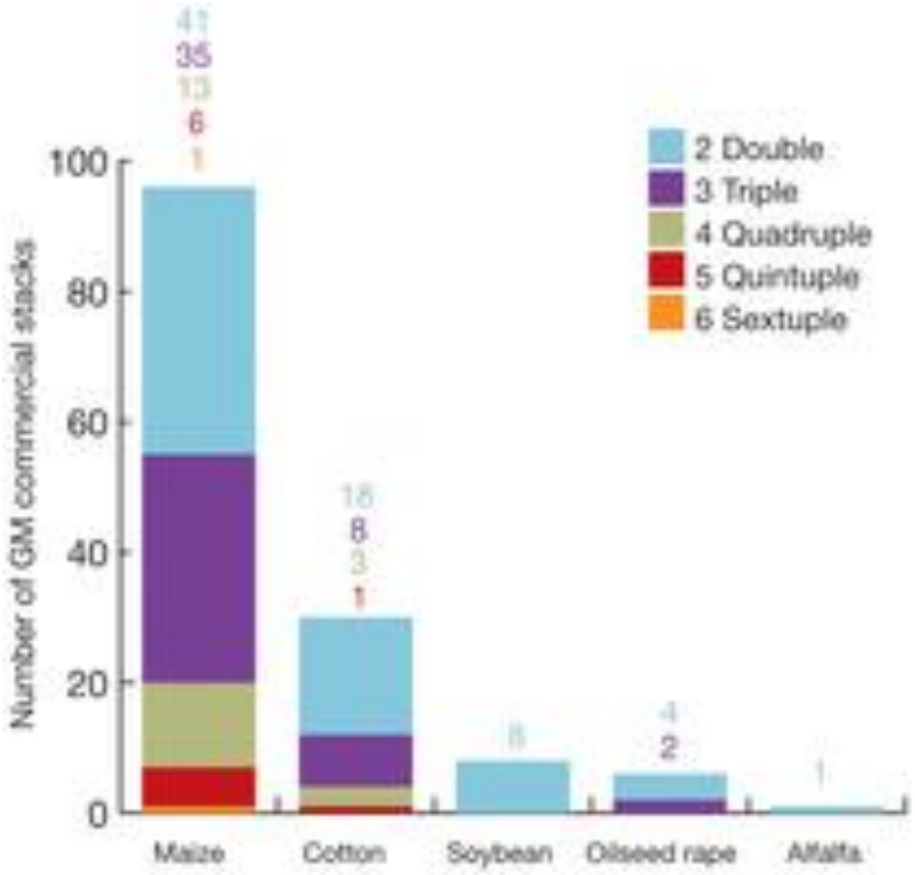
dicamba

Solution to herbicide resistance – more herbicide tolerant crops and more herbicide use!!

- GM crops tolerant to:
  - 2,4-D and glyphosate
  - Dicamba and glyphosate
  - Dicamba, glyphosate and glufosinate

  In development also crops tolerant to:
  Atrazine, isaflutole, meotrione, rimosolfuron, flumesulam, imazonox, nicosulfuran, imazethapyr, imazapic, bromoxynil, imazapyr,

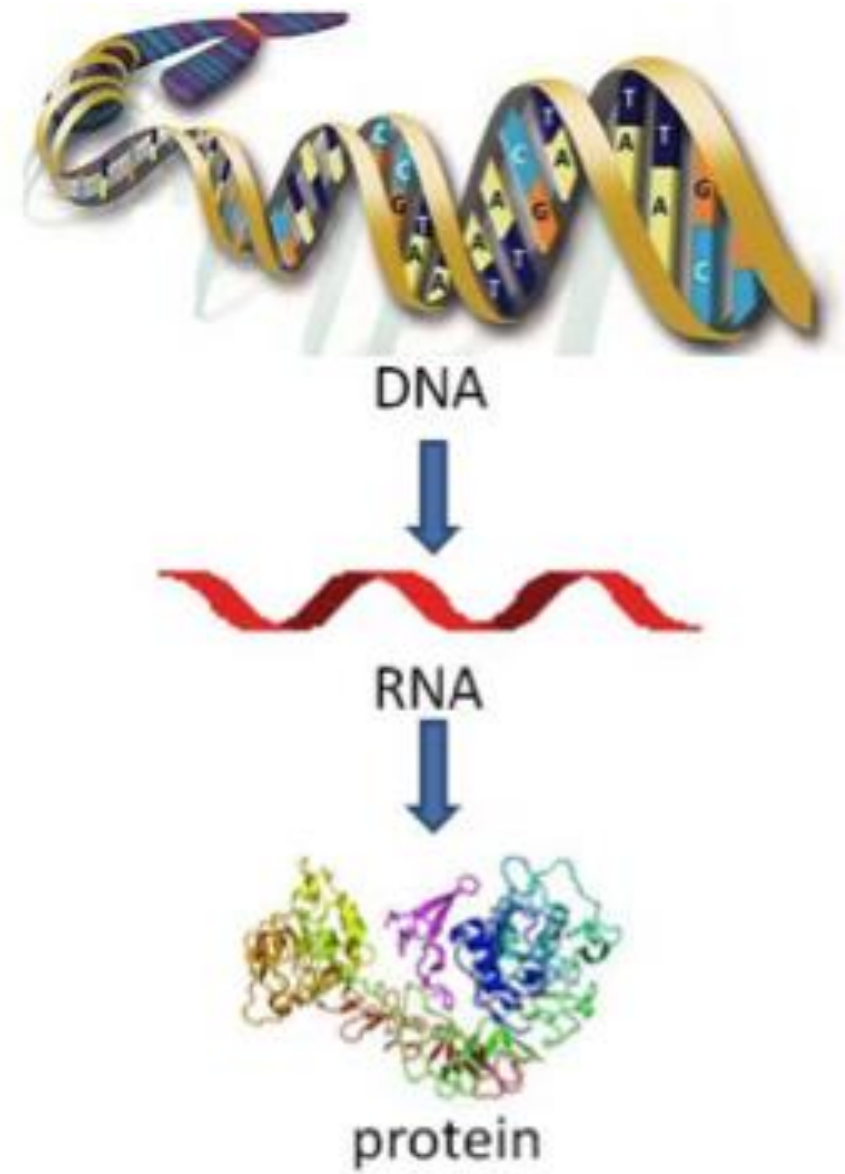
  - Stacked traits with multiple genes increasingly common – up to 8 transgenes in one crop



Nature, 2014

Central dogma of Molecular Biology – scientific premise of GMOs

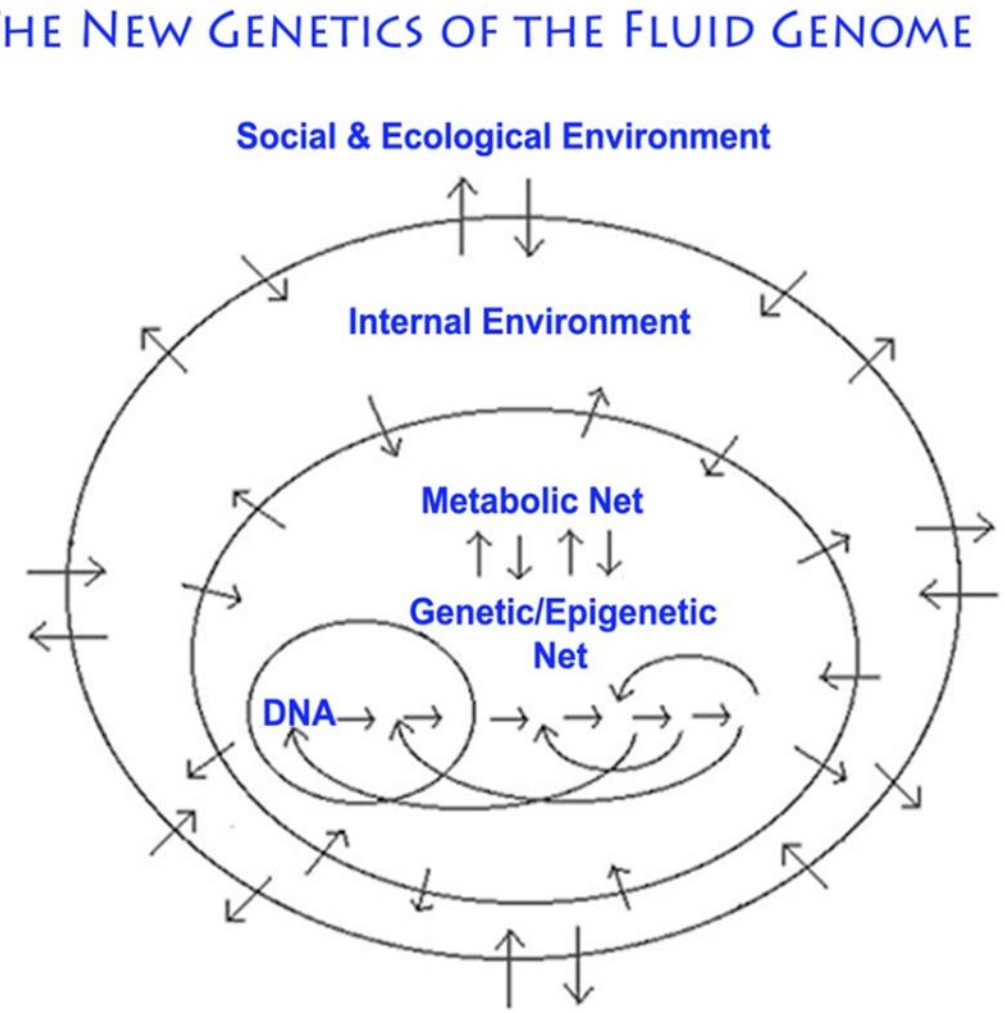
- Supposes that:
- An organism's genome - its total complement of genes - should fully account for its characteristic assemblage of inherited traits.
- individual “genetic messages” in DNA are faithfully copied or transcribed into RNA, which are then translated into proteins via a genetic code
- each protein determines a particular trait, such as herbicide tolerance, or insect resistance; one-gene-one-character.
- ***Maewan Ho: Theoretically, inserting a new genetic message into an organism will give it the desired character to serve our every need.***



New Genetics of Fluid Genome Disputes Central Dogma

- ***Outdated paradigm acknowledged by genetics field but not GMO producers***
- No simple one-to-one relationships between genes and characteristics
- No gene works in isolation
- Heredity is spread over web of organism-environment interrelationships

- *"an intricate cross-talk between the organism and its environment at all levels, with feed-forward and feed-back cycles in the epigenetic & metabolic networks of molecular interactions that mark and change genes as the organism goes about its business of living, with effects reverberating and amplified down the generations" Ho MW, 2013*



promoter	Gene of interest	terminator	Antibiotic resistance marker gene

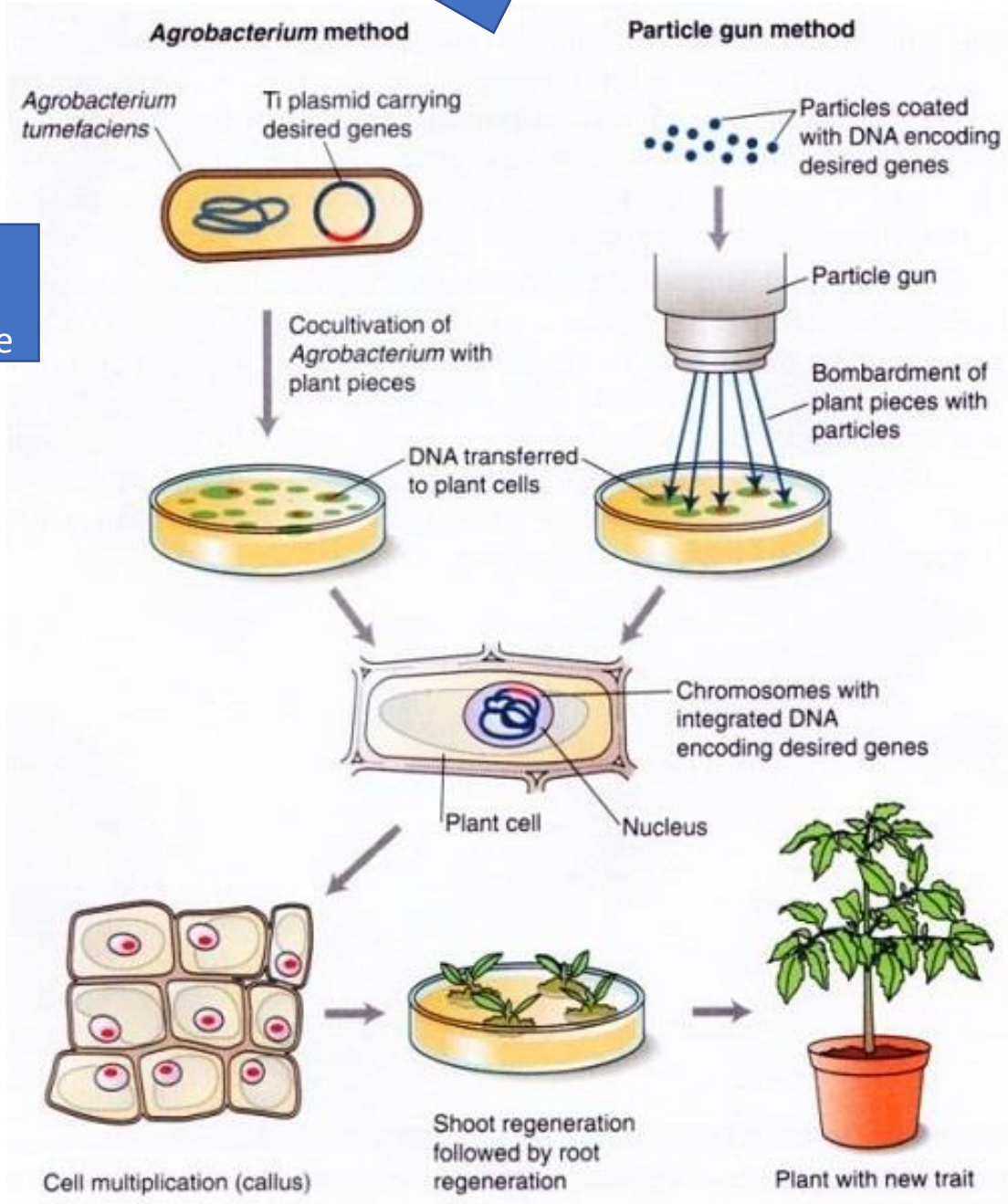
transgene

Process of genetic modification

UN Cartagena Biosafety Protocol definition:

A ‘genetically modified organism’ or ‘living modified organism’ is:

*Any living organism that possesses a **novel combination of genetic material** obtained through the use of **modern biotechnology.***



Unintended Effects of Genetic Modification Process

- *"Unintended effects can result from **the random insertion of DNA sequences** into the plant genome which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes."(Codex 2003)*

  - *Scrambling of host genome*
  - *Widespread mutations*
  - *Inactivation or activation of genes*
  - *Generation of novel RNA molecules including those that have regulatory function*

  - *Instability of transgenes*
  - *Horizontal gene transfer*

See Ho MW (2013). **The New Genetics and Natural *versus* Artificial Genetic Modification** *Entropy* **2013**, *15*(11), 4748-4781

ISIS

Scientists Discover
New Route for GM-gene "Escape"

genes can jump species via wounds, yes horizontal gene transfer happens, and at high the greatest, most underestimated hazard from GMOs released into the environment

Dr. Mae-Wan Ho

GM DNA *Does* Jump Species

Antibiotic Resistance *not* the Only Risk

ORIGINAL ARTICLE

Gene Technology and Gene Ecology of Infectious Diseases

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Consequences of unintended effects of GM on crops

Unintended effects on crop due to GM process

GM crop has altered compositional profile

Agronomic issues
Altered agronomic characteristics
e.g. stunted growth, reduced resistance to disease, variable, expression of transgene

Health Issues
Altered levels of toxins, allergens

Potential horizontal gene transfer to gut microbes

Environmental Issues
altered levels of toxins, allergens

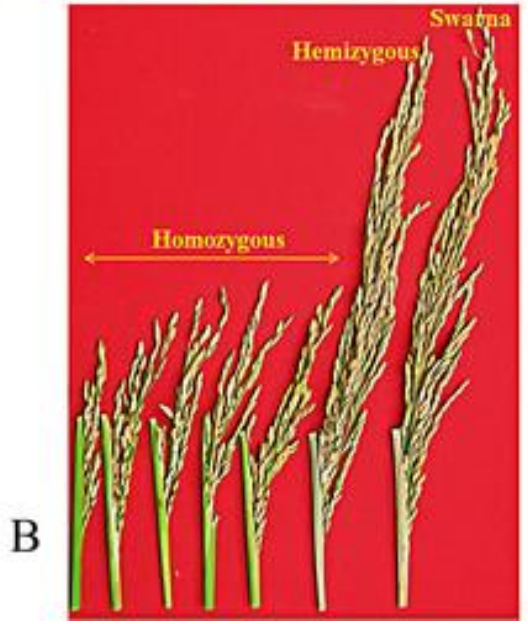
Potential for horizontal gene transfer to organisms e.g. soil microorganisms

Golden rice – 2017 study shows dwarfism and growth retardation

- Genetic modification process interrupted expression of genes involved in growth hormone production and photosynthesis.
- Unintended expression in leaves

- Effects were observed after crossing of GM line with a local Indian variety.

- The failure of commercialisation of Golden rice has not been the fault of anti-GM campaigners!



Burkina Faso phases out GM cotton due to reduced quality of cotton

- Burkina Faso – world renowned quality of cotton following 70 year breeding program
- Monsanto introduced Bt cotton in 2008 – introgressed the transgene into local varieties of high quality cotton. By 2013, 70% cotton was GM.
- Resulted in decline in cotton fibre length and ginning ratio, lost profits, trading arrangements
- Burkina Faso Cotton association seeking $80 million compensation from Monsanto

- **Insecticidal Bt Cotton**
  - increased susceptibility of root fungal disease caused by altered levels of sugars and amino acids (Li et al., 2009)
  - reduced levels of Bt toxins during flowering period and altered chemistry of mature plants reduced toxicity of Bt toxins to pests (Olsen et al., 2005)

- **Insecticidal MON810 maize** – a **Bt crop** carrying the **Cry1Ab toxin** (Singh et al., 2007, Rosatti et al., 2008)
  - Extra copy of the transgene insertion
  - Producing novel RNA nucleotide products due to the fusion of transgene with the maize genome

- **Herbicide-tolerant NK603 maize**
  - altered composition of nutrients in plant, including 28-fold rise in polyamines – can be toxic (Mesnage., 2016)
  - Used the latest in techniques to analyse 100-1000's of protein & metabolite levels in plants
  - Such global profiling 'omics' techniques are recommended by biosafety experts to be included in GM risk assessment

Reliable evidence obtained by scientists independent of the biotech industry fully corroborates real life experiences of farmers in the field GM feed and other exposures to GMOs invariably cause harm; regardless of the species of animal, GM crop, or the genes and constructs involved, and includes the most horrendous cases of excess deaths, birth defects, infertility, tumours, cancers

1. A ... lab ... ding trial reporte ... 2012 fo ... d rats ... bo ... ma ... e as controls and to de ... op large tumours, ... Be Ignor ... and Excess Cancers a ... Deaths with GM Feed: the Stats Stand Up, *SiS* 56) without the herbicide was ... Pituitary disease was up more than 2 fold in females and liver and kidney diseases up 1.5 to 2 fold in males on GM maize alone.
2. A Da ... his ... defec ... weak and s ... redu ... litter size. Thes ... were entir ... diet [13] (G ...
3. A meta-analysis pooling all available data on 19 feeding ... liver and kidney f ... 15] (GM Feed Toxic, New Meta-Analysis C ...
4. Professor emeri ... warned of "pathogen new to science" associated wi ... (Emergency: Pathogen New to Science Found in Roundup Ready GM Crops ... ist Defends Claim of Ne ... Pa ...
5. Be ... at the R ... ian Acade ... f Science ... ed th ... female rats f ... exce ... than half of the litter dying wi ... three weeks, while the surviving pups were completely ... Fed Rats: Stunted, Dead, or Ste ...
6. Between 2004 and 2005, hundre ... cotton handlers in Madhya Pradesh, India, reported allergy symptoms from exposure to Bt cotton containing Cry1Ac or both Cry1Ac and Cry1Ab proteins [20] (More Illnesses Linked to Bt Crops, *SiS* 30).

... transgenic pea containing ... normally harmless ... sitivities ... ther ... s in the diet [22, 23] (Trans- ... *SiS* 29).

... at the Universities of Urbino, Perugia and Pavia in Italy published reports indicating that GM-soya fed to young mice affected cells in the pancreas, liver and testes [24-28].

... ffered ... orid ... odies to the Cry1Ab protein w ... found in the villagers, there have ... ne remain ill ... 9).

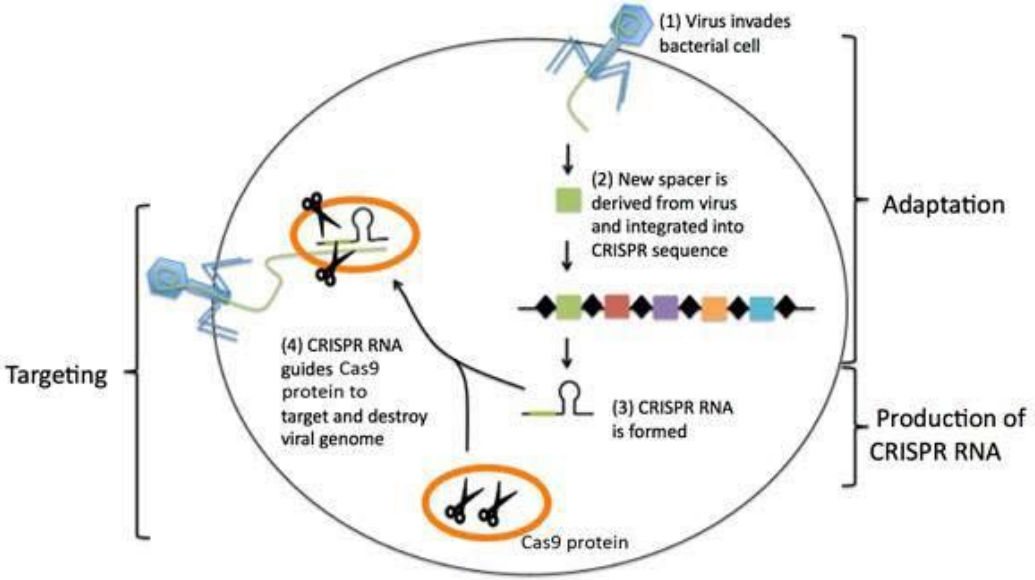
In 2004 Monsanto's secret research dos ... showed that ... between 2001 and 2002, a dozen cows died in Hesse ... many ... after eating Syngenta GM maiz ... 176 containing ... and more in the ... [31] (Cows Ate Gi ... aize & Died, *SiS* 21). In 2012, biotech giant Syngenta ... court case brought by the farmer that ended in 2007 [32] (Syngenta Charged fo ... ering up Livestock Deaths from ... s for- ... merly of the Rowett Institute in S ... reported dam- ... potatoes ... ning ... twice a ... uick as controls [33].

... in Egypt found similar effects in the ... to containing a Cry1A protein [34]. ... ny (later Bayer Cropscience) sub- mitted data to UK regulators showing that chickens fed glufosinate-tolerant GM maize Chardon LL were twice as likely to die compared with controls [35] (Animals Avoid GM Food, for Good Reasons, *SiS* 21).

Next generation GMOs 2.0

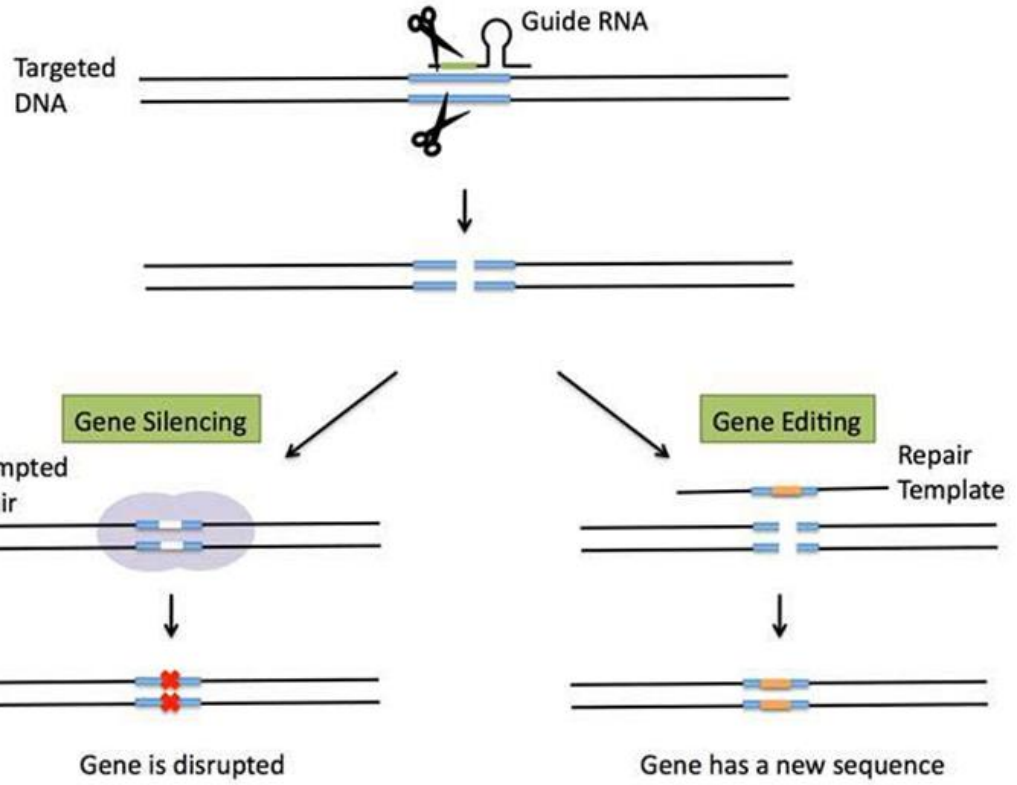
New techniques under discussion for possible exception from GMO legislation

- **Gene editing techniques** – targeted alteration of genomic sequences
- **Cisgenesis/Intragenesis** – identical to standard GMOs but DNA comes from genetically compatible species
- **RNA-dependent DNA methylation** – utilises epigenetic mechanism to silence genes of interest for few generations
- **Grafting** of non-GM stalks to GM rootstock
- **Agroinfiltration** – transient introduction of genetic material to part of plant, or cells
- **Reverse Breeding** –reconstituting hybrids from offspring by suppressing meiotic recombination in plants during breeding



CRISPR- a natural

immune defence in bacteria against viruses – targets and chops up viral DNA to kill them



Gene editing with CRISPR/Cas9
Similar process to standard GM

- Involves culturing plant cells
- Involves transformation of genetic material into cells

2017 *Nature* paper performed whole genome sequencing in mice (Schaefer et al., 2017)

- 1500 single nucleotide mutations,
- 100 larger deletions and insertions
- none of which were predicted by computer algorithms that are routinely used for predicting off-target effects

Off –target effects:

- Can edit other regions of the genome that have similar sequences to that of target sequence

2017 paper in rapeseed found the integration of 5 DNA vector backbone sequences in genome (Braatz et al., 2017)

In this case, there is indeed **permanent insertion of genetic material.**

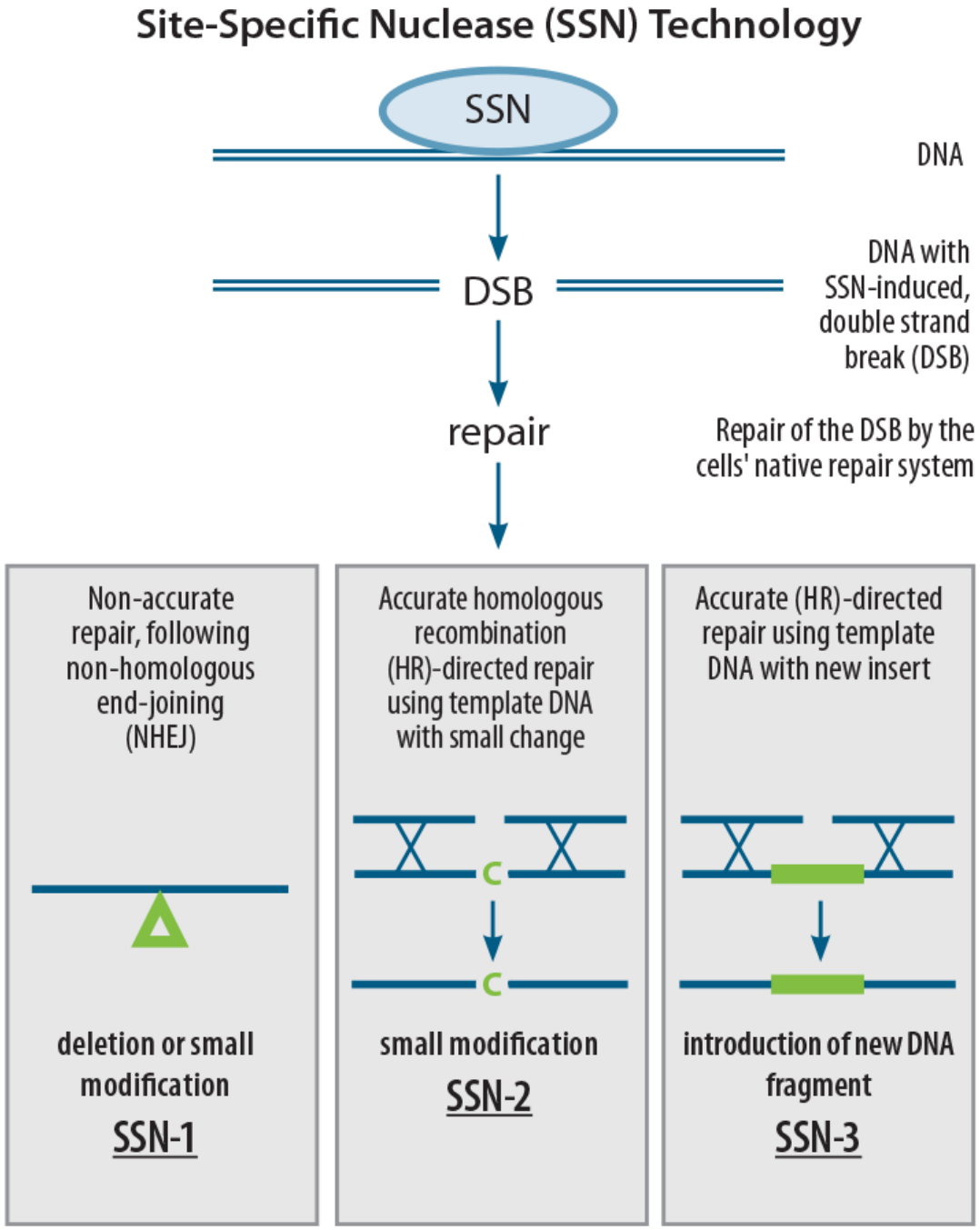
CRISPR

TOO FAST FOR COMFORT

A new gene-editing technique has taken the world by storm; it can disable or change specific genes in the genome of all animals including humans faster and more efficiently than ever before but it has raise unprecedented concern over safety and ethics ***Dr Mae-Wan Ho***

Unintended changes at target site:

Gene editing relies on endogenous repair mechanisms of cells to re-join the DNA after it has been cut by CRISPR



Unintended changes at target site:

Gene editing relies on endogenous repair mechanisms of cells to re-join the DNA after it has been cut by CRISPR

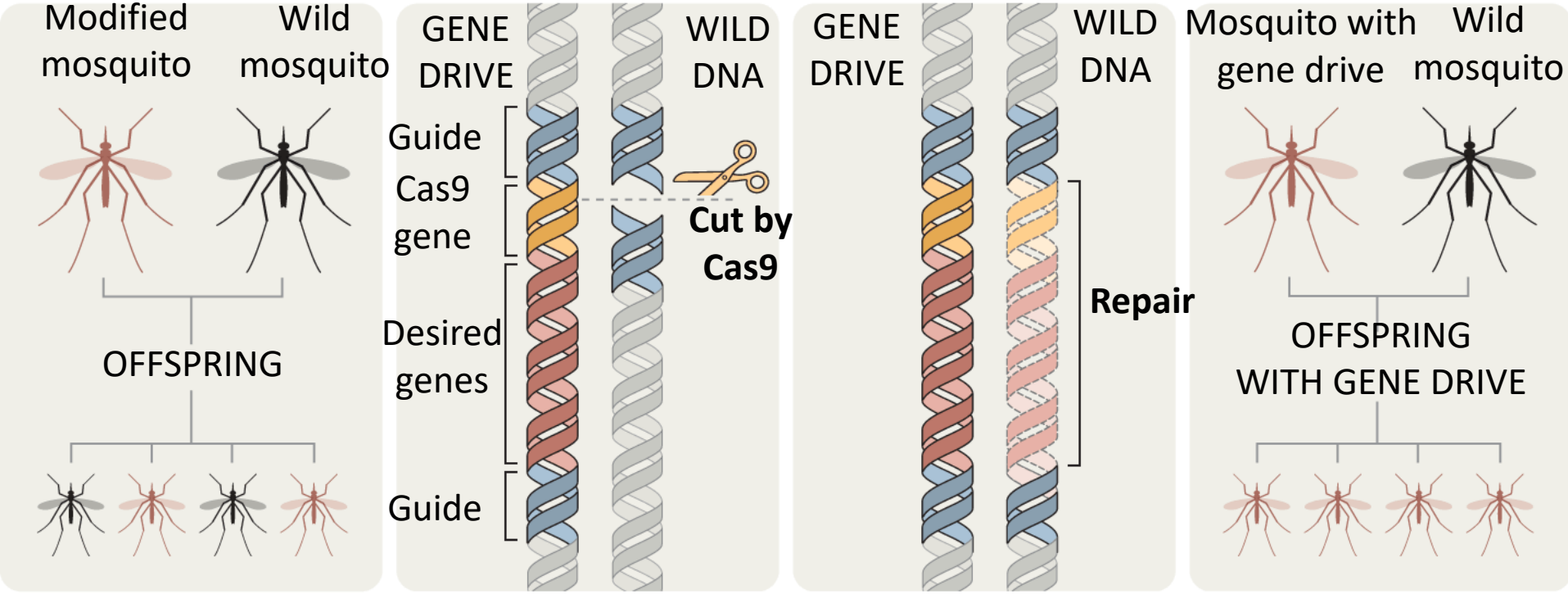
"Cellular repair of the double strand break [cut DNA] may result in mutagenic insertions or deletions (indels), or even in larger chromosomal rearrangements"

"For applications such as crop improvement, a means to track off-target mutations could assist in mutation removal by segregation during subsequent crosses."

- DuPont and Caribou Sciences

Woo et al. (2015) tested four plant species for CRISPR gene modification. The analysis of target sites alone showed a variation from -29 nt (or a deletion of 29 nucleotides) up to +33 nt (or the addition of 33 nucleotides). **Permanent introduction of novel DNA sequence**

Gene Drives – Mutagenic Chain reaction



INHERIT
A genetic change made to one parent usually has a roughly 50 percent chance of being passed down to offspring.

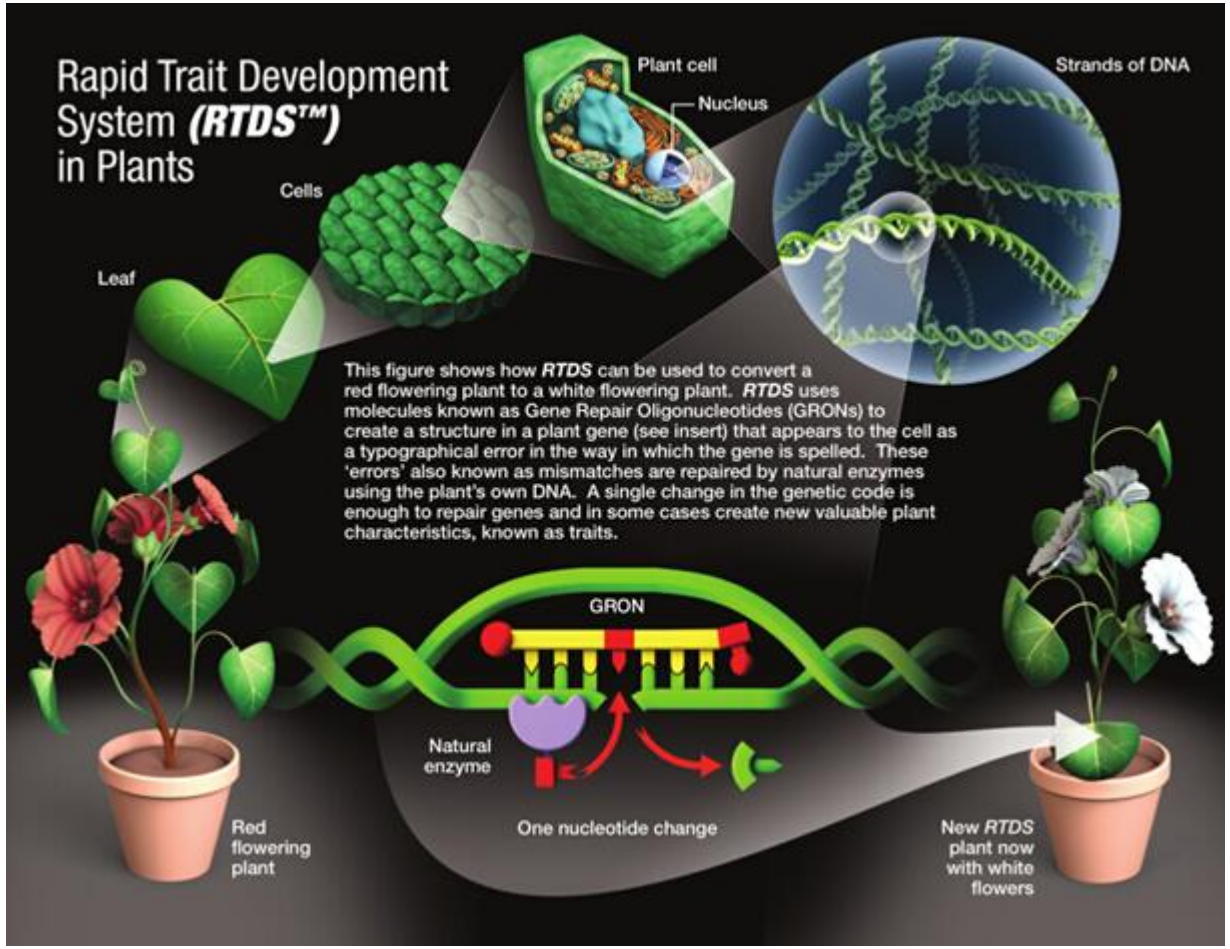
Gene drive system may be able to increase the odds of spreading a genetic change to all offspring, and eventually through an entire population.

MATCH AND CUT
A gene drive is a segment of engineered DNA that typically contains a guide sequence, a gene for an enzyme called Cas9 and any desired genes that researchers want to spread in the population.
If the guide sequence matches a stretch of DNA inherited from the wild parent, the wild DNA will be cut by the Cas9 enzyme.

REPAIR AND COPY
The cell repairs the cut in the wild DNA, using the matching strand of DNA from the genetically modified parent as a template.
Once repaired, the wild DNA will contain both the Cas9 gene and the desired genes.

SPREAD
Because the gene drive effectively inserts itself into any wild DNA it is paired with, a single copy from one parent is enough to spread the gene drive and its desired genes to all offspring.
The technique has worked in the lab, but researchers are exploring the ethics and risks of releasing a gene drive into the wild.

Gene editing via oligonucleotide-directed mutagenesis



Introduce short DNA sequences into the cell

- Same technique as classic GM.
- Involves introduction of DNA

- Short DNA sequences are put into plant cells that are identical to the gene that they are trying to edit, except for the desired mutation

- Relies on hijacking natural DNA repair mechanisms in the cell that use identical DNA sequences as a template to correct a mutation when one arises.

- Off-target effects: Potential to alter other genes that have a similar sequence to the gene of interest

Cisgenesis/Intragenesis

- Identical process to standard GM procedures
- Cisgenesis = genetic material introduced is not recombinant, and are introduced into a sexually compatible species
- Intragenesis – genetic material introduced is recombinant, but derives from sexually compatible species.

Transgenic Pea that Made Mice Ill

Raises serious safety concerns on transgenic proteins in general that must be addressed while a ban on all GM food and feed is imposed. Dr. Mae-Wan Ho

Examples of GMOs 2.0

- CIBUS have made GM herbicide tolerant (inc. glyphosate) canola, rice, potatoes, flaxseed. Trademarked as Rapid Trait Development System (RTDS)

  Marketed as NON-GM on their website.
  - Canola – approved in US
  - Expected approval in Canada for 2017

- Non-browning mushroom made by gene editing (CRISPR/Cas9)
- Sulfonylurea and imidazolinone herbicide tolerant rice made by gene editing (meganucleases)
- Limited useful traits shows that crude genetic reductionist principles are outdated, and not the answer to addressing the complexity of plant traits, nutrition, agriculture and health.

GMOs 2.0 similar and additional risks to current GMOs

- Involve the use of biotechnological techniques
- Involve the introduction of novel genetic genetic material
- Involve cell culture techniques

Additional risks:

- Gene editing techniques can have off-target effects by altering the genome in unintended places

GMOs 2.0 should not be excluded from legislation

- Status of EU – expected a decision in 2018
  - UK
  - Austria
  - Germany
  - Sweden
- Status of US and Canada – crops already approved
- SA: Push for inclusion above “threshold” of natural variation beyond natural breeding and mutagenesis techniques
- Legislation should be updated to incorporate latest global profiling techniques to assess unintended effects

I take science to be *reliable knowledge of nature that enables us to live sustainably with her*.

We must always finish our thoughts and follow them to the end

Thank you all and be GMO Free!

THANK YOU MAE-WAN

Thank you also to Peter, Jules, Ching

GM insecticidal crops failing

- **Secondary Pests**:

  China – infestation of Bt cotton with mirid bugs and leaf hoppers resulting in 'pest status' associated with Bt crop cultivation (*Wu et al., 2002; Lu et al., 2010*)

  India – whitefly secondary pest attacks leading farmers to return to Indian varieties
  - 15 % drop in Monsanto Bt cotton sales in 2016.
  - epidemic of farmer suicides linked to Bt cotton cultivation in rain-fed areas *(Gutierrez A et al. 2015)*

- **Pest resistance** to Bt toxins
  - Stem borer resistance to Cry1Ab Bt toxins in S. Africa (Van de Berg et al., 2007)
  - pink bollworm resistance to Cry1Ac Bt toxin in US (Monsanto, 2010), India, China (Zhang et al., 2011)