FRST302: Forest Genetics

Lecture 4.2 - Accelerating Tree Improvement II

Recap

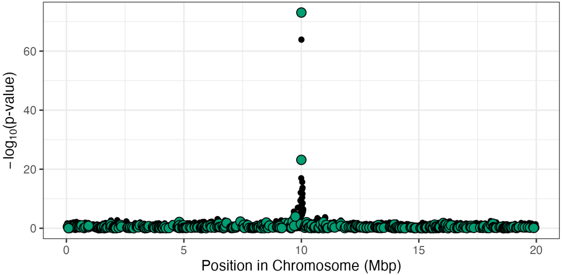
Association genetics is a powerful way to identify genes underlying important traits

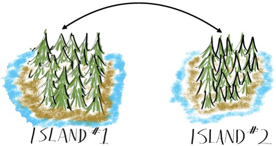
Association genetics **is not** a silver bullet as there are multiple statistical issues

Good at identifying large effect loci

Now What?

We identified a locus that increases a carrier's height by 3m!



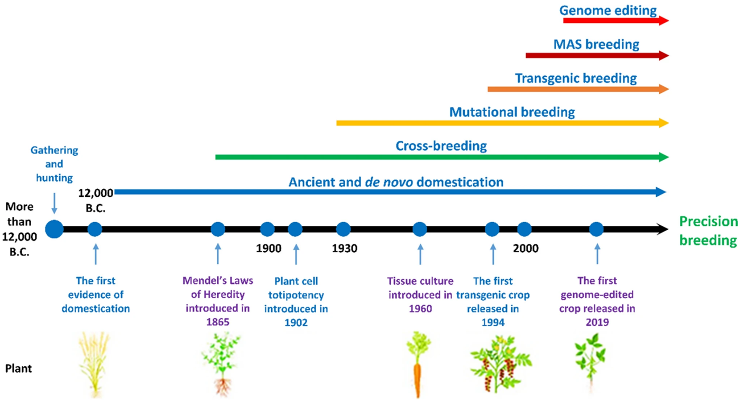


What are we going to do with that information?

Overview for Today

- Introduction to genetic modification and genome editing
- Methods of genetic modification and genome editing
- Application of these in forest biology

Major Milestones in Plant Breeding



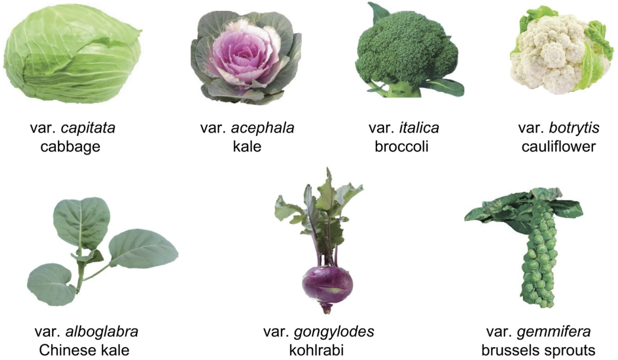
1. Domestication/traditional breeding
2. Genetics-informed breeding
3. Mutational breeding
4. Transgenic breeding
5. Genomic selection
6. Genome editing

Figure 1 from Van Vu et al. 2022 *Planta*

1. Domestication/Traditional Breeding

Humans have conciously (and unconciously) altered the traits of many species of plants and animals for time out of mind

Artificial selection alters allele frequencies, leading to populations with a desired pattern of trait variation



Modified from Cheng et al 2016 *Scientific Data*

Major Milestones in Plant Breeding

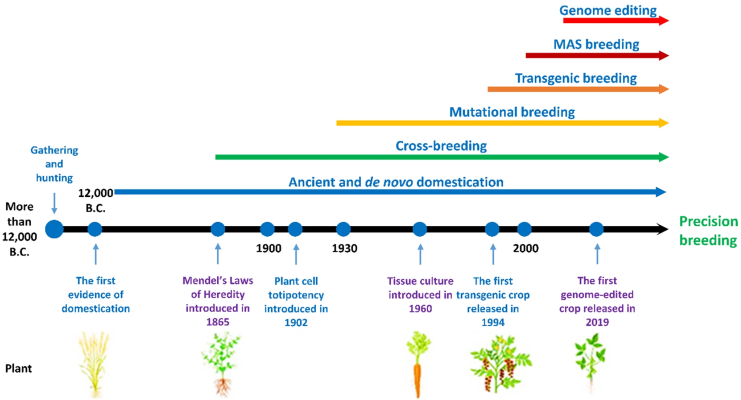


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Figure 1 from Van Vu et al. 2022 *Planta*

2. Genetics-informed breeding

This is what you learned about in Module 3
Each cycle takes from 20-30 years!

We already decided that this takes too long!

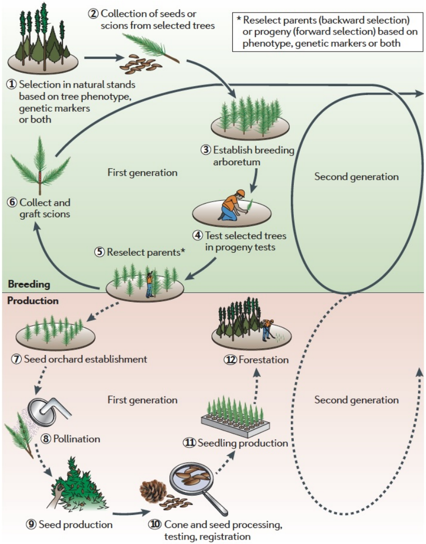

Image from Neale and Kremer 2011 *Nature Reviews Genetics*

Major Milestones in Plant Breeding

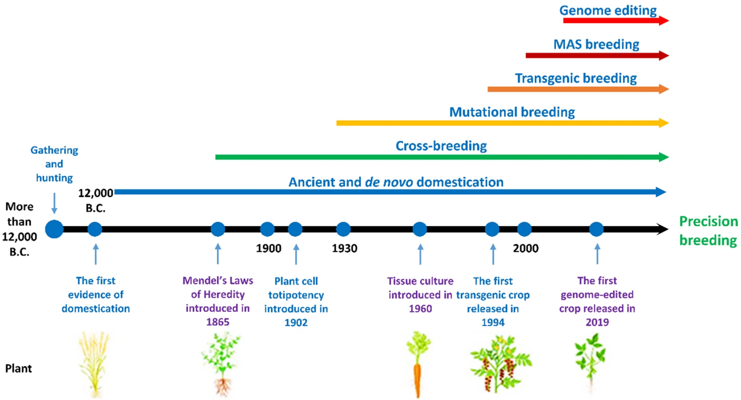

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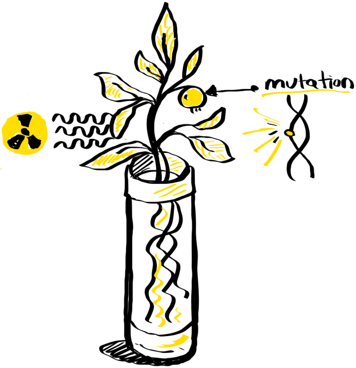
Figure 1 from Van Vu et al. 2022 *Planta*

3. Mutational Breeding

Applying mutagenic agents to give rise to desirable traits (e.g. chemicals, X-Rays, even *cosmic* rays!!)



- Speeds up the natural process of mutation
- Randomly introduces mutation to plant genomes
- Widely used in agriculture

L-R; HudsonAlpha Institute, Fantastic Four #1, 1964

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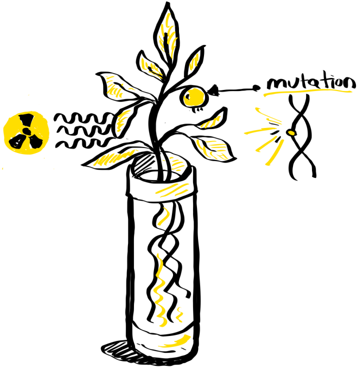

- Mutagenesis may give rise to useful mutations
- May lead to harmful ones in addition
- Useful mutants are backcrossed to utilize the new allele

Mutational breeding is not used in forest genetics
It would be far too slow

**At least as far as I can tell!*

Major Milestones in Plant Breeding

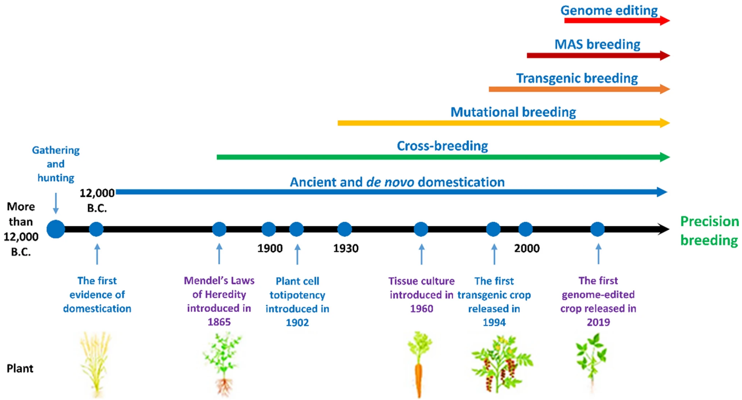

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Figure 1 from Van Vu et al. 2022 *Planta*

Transgenesis: using genetic engineering techniques to introduce genes from other lineages, species (or even kingdoms!)

Transgenic poplar plantation; Figure 2 Häggman et al 2013 *Plant Biotech*

Genetically Modified Organisms (GMOs) have been a hot topic for many years

- Food safety concerns
- Ethical concerns
- Ecological concerns

The definition of a GMO is still not clear to many people

E.g., does cloning and selective breeding lead to GMOs?

Image from American Scientist 2001

What is Genetic Modification?

Genetic modification (GM), or genetic engineering, is the direct manipulation of a organism's genome using biotechnology.

GM includes:

- **Transferring genes** within and across species
- **Adding new genes**
- **Knocking out genes**

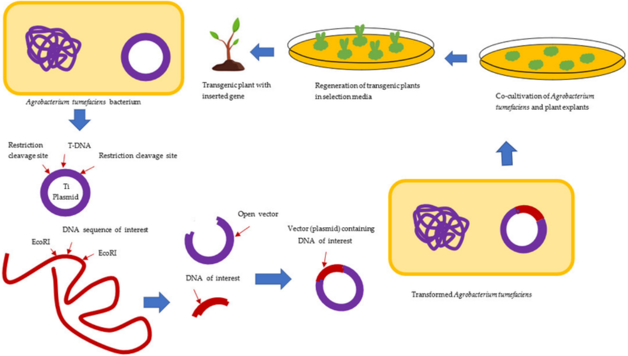
The world's first GM plant was produced in 1983

A gene from the bacterium *Agrobacterium tumefaciens* encoding an anitmicrobial resistance protein was transferred to tobacco

How is GM Acheived in Plants?

There are several GM methods that are used in plants:

Agrobacterium tumefaciens



L-R: A crown gall caused by *A. tumefaciens*; Schematic from Ghimire et al. 2023 *Sustainability*

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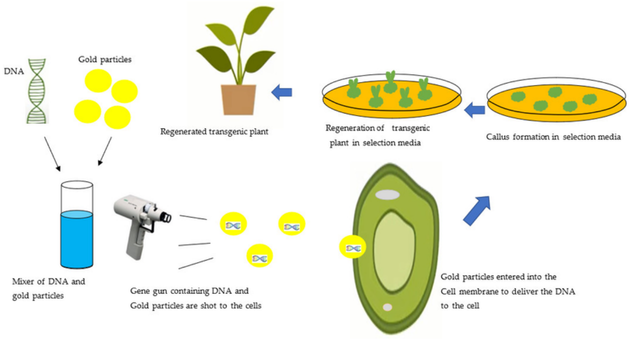
Gene Gun (a.k.a. BioListics)



L-R: Helios GeneGun; Schematic from Ghimire et al. 2023 *Sustainability*

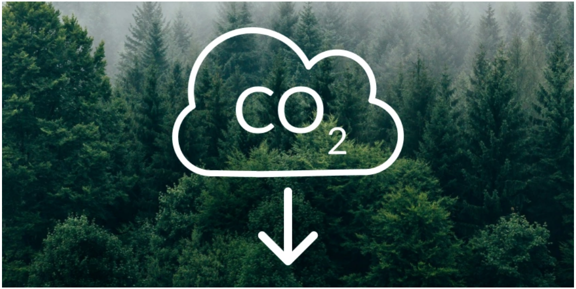
Put ethical, ecological and economic concerns aside for the moment...

What are some ways that GM could be used in forest trees?

Carbon Sequestration

Carbon capture and sequestration by growing trees is a so-called "nature-based climate solution"

A core component of the carbon economy



Engineering trees to better capture and store CO_2 seems like a good goal

There is a *LOT* more that could be said about this topic

GM in Forest Trees: Example

Living Carbon is a company based in California that creates "*bespoke carbon sequestration solutions*"

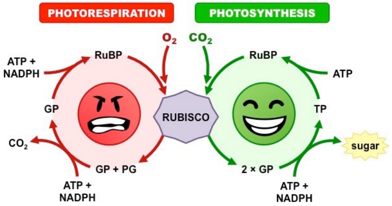
TRANSGENIC CONTROL

Living Carbon has used GM to develop trees to more efficiently capture carbon

https://www.theguardian.com/environment/2023/oct/15/superpower-plants-absorb-carbon-co2-climate-crisis

GM in Forest Trees: Example

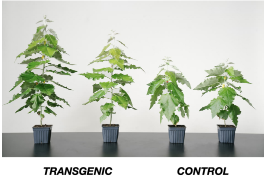
- In plant cells, chloroplasts carry out photosynthesis
- The enzyme at the heart of photosynthesis can also carry out photorespiration to recycle toxic by-products of photosynthesis
- **Photorespiration can reduce the efficiency of the conversion of light into biomass by 20-50%**

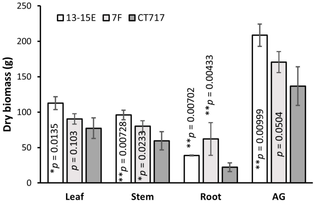


Don't memorize the molecules; Picture from BioNinja

GM in Forest Trees: Example

Living Carbon has produced transgenic hybrid poplar (*Populus tremula* × *Populus alba*)





- Introduced a gene encoding a small interfering RNA
- Silencing a gene involved in photorespiration
- Transgenic plants accumulated up to 53% more above-ground biomass than controls
- Results varied by transgenic line

Tao et al 2023 *Forests*

Limitations of GM

- The insertion of a new gene may not occur
- No control on targeting DNA locations
- One gene at a time
- Expensive and slow
- Under strict regulation

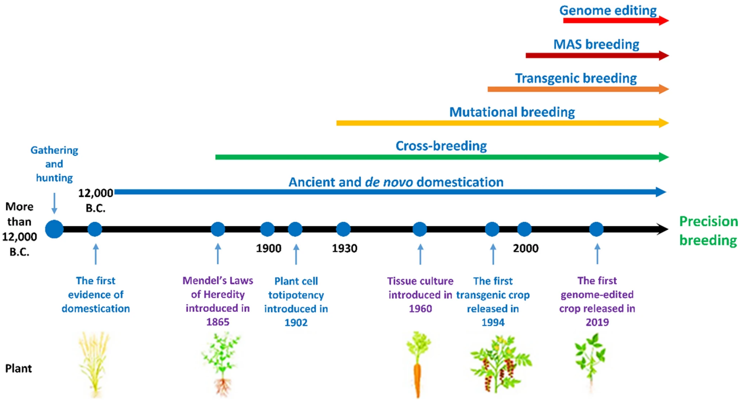
Homer introduced tobacco genes into tomato with a radiation-based procedure to produce his ToMacco

Questions?

Jennifer Doudna and Emmanuelle Charpentier

Do you know what they won the Nobel prize for?

Major Milestones in Plant Breeding



1. ~~Domestication/traditional breeding~~
2. ~~Genetics-informed breeding~~
3. ~~Mutational breeding~~
4. ~~Transgenic breeding~~
5. ~~Genomic selection~~ (next lecture)
6. Genome editing

Figure 1 from Van Vu et al. 2022 *Planta*

Genome Editing

- Genome editing is a type of genetic engineering using "molecular scissors"
- These "scissors" create site-specific double-strand breaks **at desired locations** in the genome.
- The induced double-strand breaks are repaired with errors, resulting in targeted mutations ('edits').

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Limitations of GM not shared by genome editing

- ~~The insertion of a new gene may not occur~~
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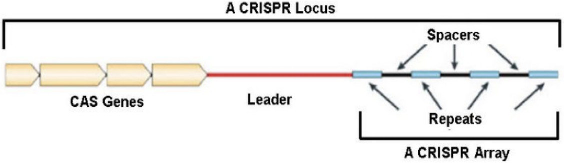
- ~~One gene at a time~~
- ~~Expensive and slow~~
- Under strict regulation

CRISPR-Cas9

Genome editing is typically achieved using the CRISPR/Cas system



Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

The CRISPR/Cas system is a bacterial immune system that confers resistance to foreign genetic elements and provides a form of acquired immunity

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Here's a simple video overview: `https://youtu.be/6tw_JVz_IEc?si=_H2TrKqrd5rSOpEL`

CRISPR-Cas9

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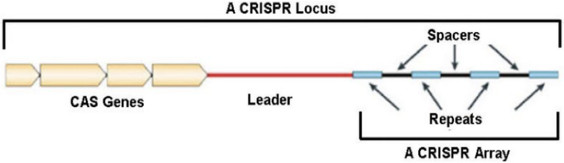


Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

A CRISPR locus:

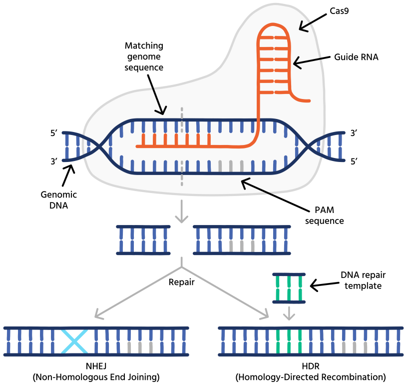
- Encodes the Cas9 protein (the "scissors")
- Transcribes a guide RNA to find the target

The two components form a complex to find and destroy the target DNA

Here's a simple video overview:
https://youtu.be/6tw_JVz_IEc?si=_H2TrKqrd5rSOpEL

CRISPR and Gene Editing

- CRISPR/Cas9 induces a double strand break at a specific DNA sequence
- Adding a synthetic "guide" can direct the system to make arbitrary cuts
- Add a custom exogenous DNA template for DNA repair to incorate into the host's genome

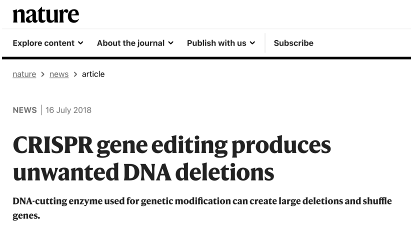


By *editing* DNA rather than inserting it, a wider range of changes are made possible

Unintended Side Effects

Genome editing with CRISPR/Cas9 can cause DNA deletions and rearrangements near the target site on the genome

A problem for clinical applications, maybe less so for in the context of plant breeding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NEWS | 16 July 2018

CRISPR gene editing produces unwanted DNA deletions

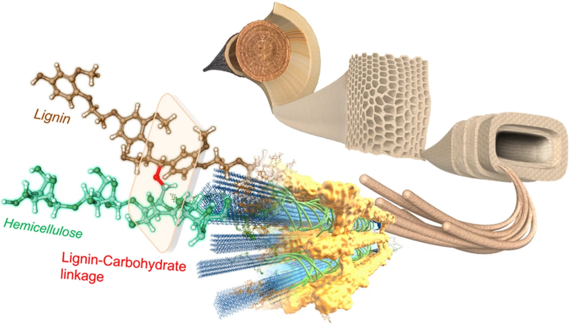
DNA-cutting enzyme used for genetic modification can create large deletions and shuffle genes.

Individuals with deleterious side effects from CRISPR could simply be removed from circulation

Put ethical, ecological and economic concerns aside for the moment...

What are some ways that genome editing be used in forest trees?

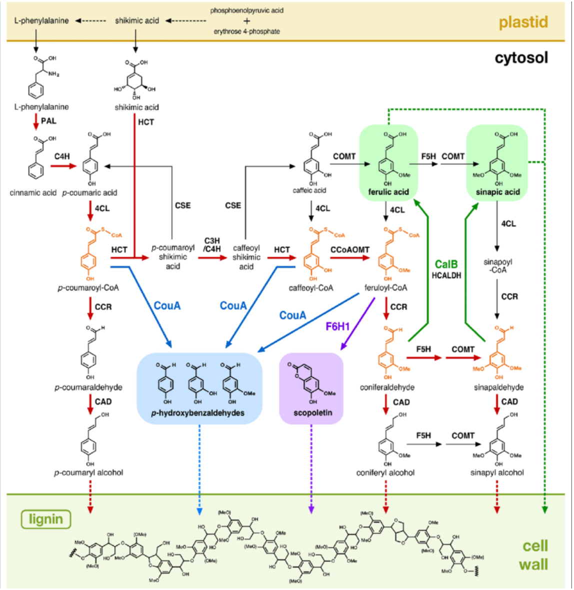
Lignin Removal



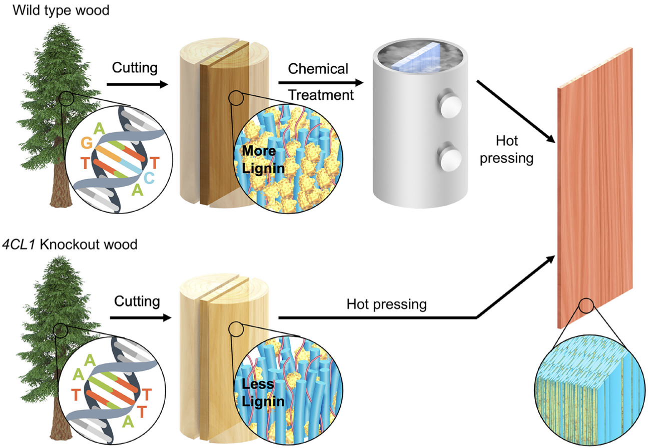
- Lignin is one of the most abundant polymers on earth
- Removal of lignin from pulp is a key part of paper manufacture
- High density engineered wood can be made from wood that has been treated for reduced lignin
- The process of delignification consumes energy and generates toxic byproducs

Nishimura et al. 2018 *Sci. Reports*

Lignin Biosynthesis

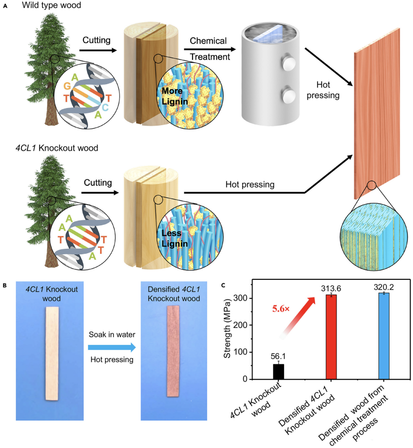


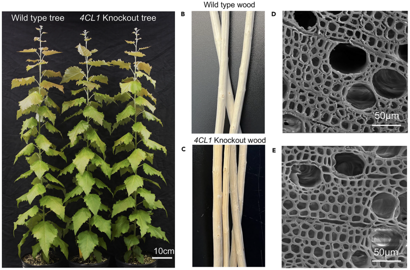
Genome Editing to Reduce Lignin Content



Liu et al 2024 Matter

Genome Editing to Reduce Lignin Content





Figures 1 and 2 (modified) from Liu et al 2024 Matter

Learning Outcomes

Association genetics

Statistical issues in association genetics:

- Population structure can lead to false positives
- Multiple comparisons makes tests more stringent
- Works well for large effect loci, not so much for small effects
- Effect sizes may be upwardly biased (i.e. Beavis effect)

A Question to Think About...

Traditional Breeding | Genetics-informed Breeding | Mutational Breeding | Transgenic Br