GENETICS, TREE BREEDING, AND THE AMERICAN CHESTNUT

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Artificial Selection

- Artificial selection
    - intentional breeding for certain traits, or combination of traits.

Vs.

- Natural selection
    - differential reproduction of organisms with certain traits is attributed to improved survival or reproductive ability

BREEDING A BLIGHT-RESISTANT CHESTNUT

TACF'S BREEDING PROGRAM

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Early Restoration Attempts

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- Cultural methods
  - Tree surgery, fungicide, forest gap barriers, eventually removal
- Identifying natural resistance among American chestnuts
  - 90% of existing trees determined to have escaped blight
  - Small number existing with a low level of natural blight resistance
- Replacement tree to fill niche
  - Expeditions to Asia, high hopes for Chinese chestnut
  - Ultimately failed, due to poor understanding of Chinese chestnut habitat requirements
  - Chinese chestnut since proven too small to compete in a mature forest setting

Early Breeding

- USDA Breeding Program
  - American chestnut x Chinese chestnut, Japanese chestnut or other
  - Only 50% American chestnut, or less if backcrossed to another Asian chestnut parent
  - Poor American characteristics and blight resistance – project abandoned in 1960's
- CAES Breeding Program
  - Started by Arthur Graves in 1920's and continued today
  - Wide variety of hybrids and backcrosses
  - Looking for blight-resistance and timber form
  - Working with the fungus as well

Genetics Refresher

- **Allele** – one possible state or form of a gene, distinguished from other alleles by phenotypic effects (Alleles for flower color: R = red, r = white)
- **Heterozygote** – An individual with different alleles for a particular trait (Rr)
- **Homozygote** – An individual with identical alleles for a particular trait (RR or rr)
- **Genotype** – the specific allelic or genetic constitution of an organism (genetic make-up)
- **Phenotype** – the observable properties of an organism that are genetically controlled (**expression** of genetic make-up)

Genetics Refresher

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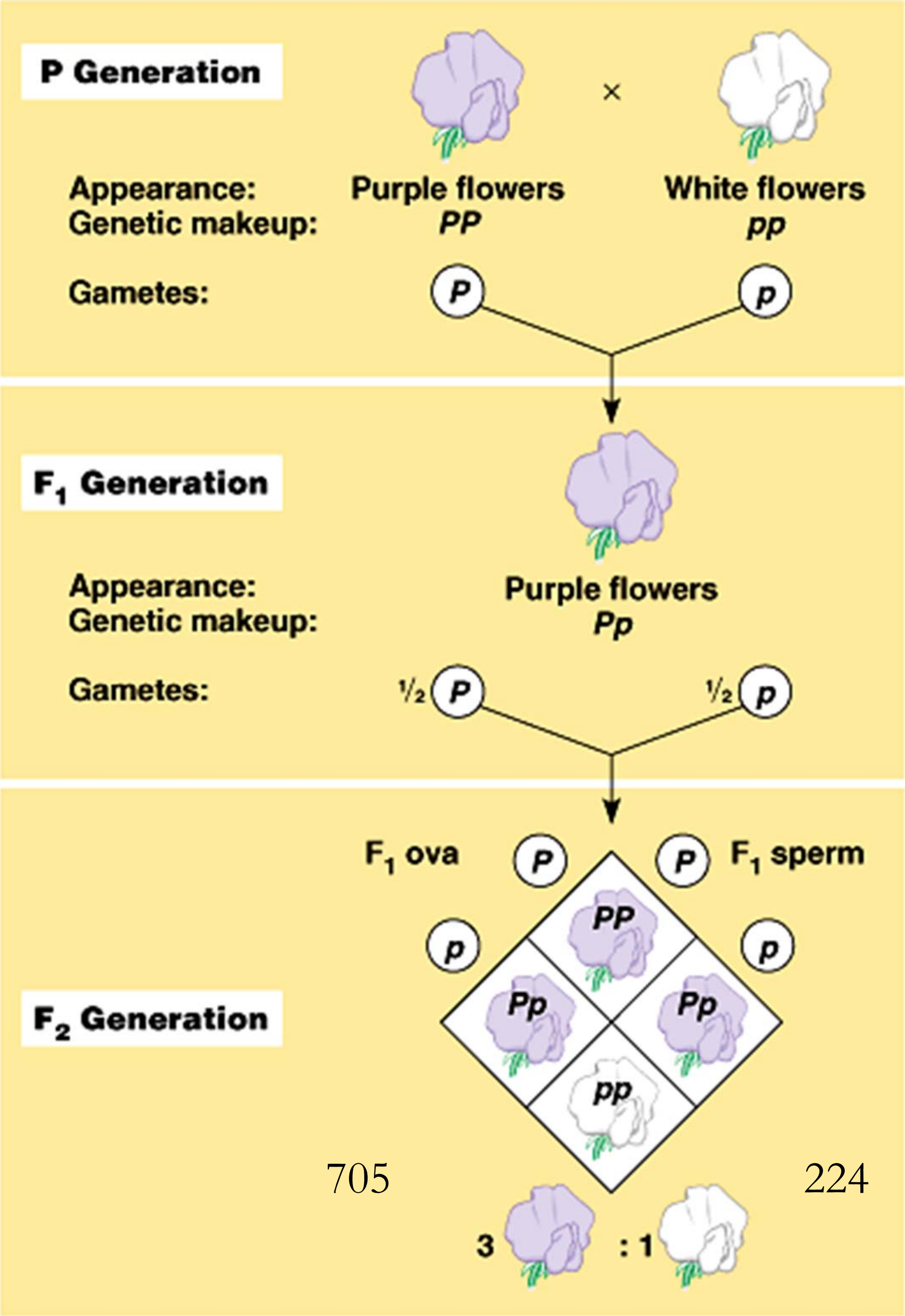
- **Dominant trait** – one that is expressed in a heterozygote
  - RR or Rr are both red because "R" is dominant
- **Recessive trait** – one that is not expressed in a heterozygote
  - Only rr is white because "r" is recessive
- **Incomplete dominance** – Expression of heterozygous phenotype that is distinct and often intermediate to that of either parent
  - RR is red, rr is white and Rr is pink
- **Segregating Population** - A population, generally the progeny of a cross, in which genetic differences are detectable, thus permitting identifcation of individuals having a desired trait and their selection for further breeding
- **Segregation** - The separation of alleles during meiosis so that each gamete contains only one member of each pair of alleles.


P Generation
×
Appearance:
Genetic makeup:
Purple flowers
PP
White flowers
pp
Gametes:
P
p
F1 Generation
Appearance:
Genetic makeup:
Purple flowers
Pp
Gametes:
1/2 P
1/2 p
F1 ova
F1 sperm
P
P
p
p
F2 Generation
PP
Pp
Pp
pp
705
224
3
: 1

VARIATIONS ON MENDEL'S PRINCIPLES

Incomplete dominance

- an offspring's phenotype is intermediate between the phenotypes of its parents

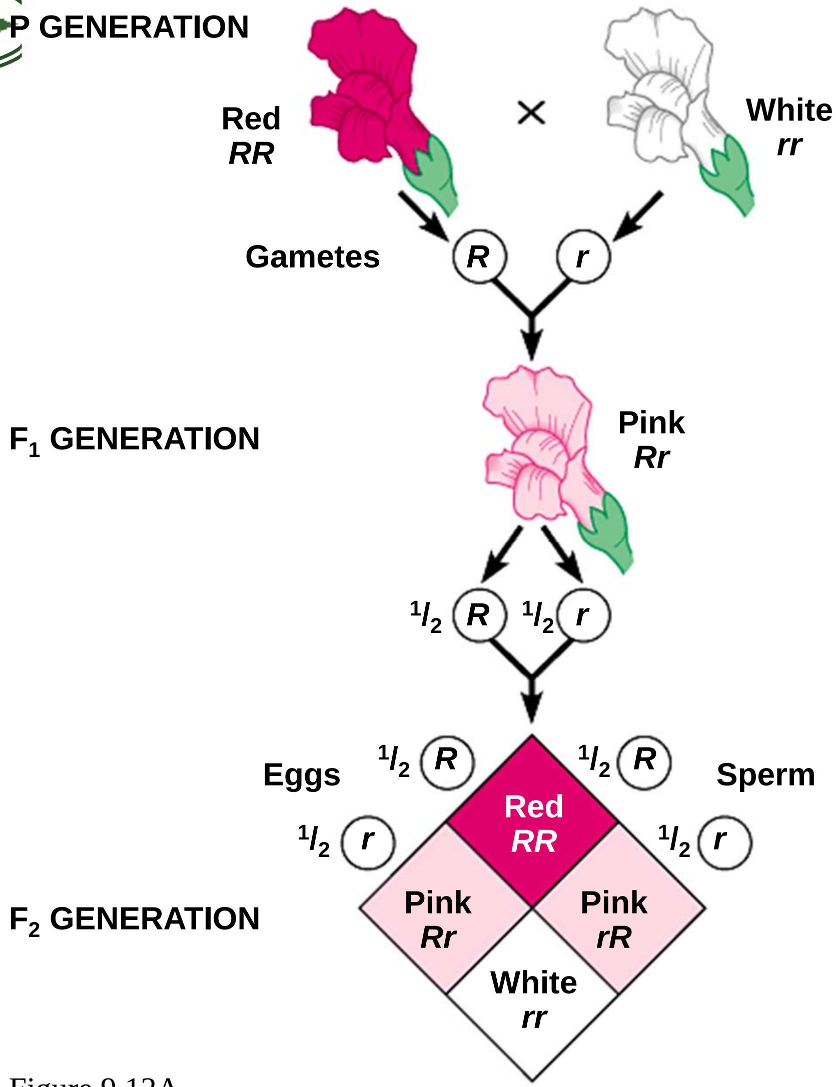
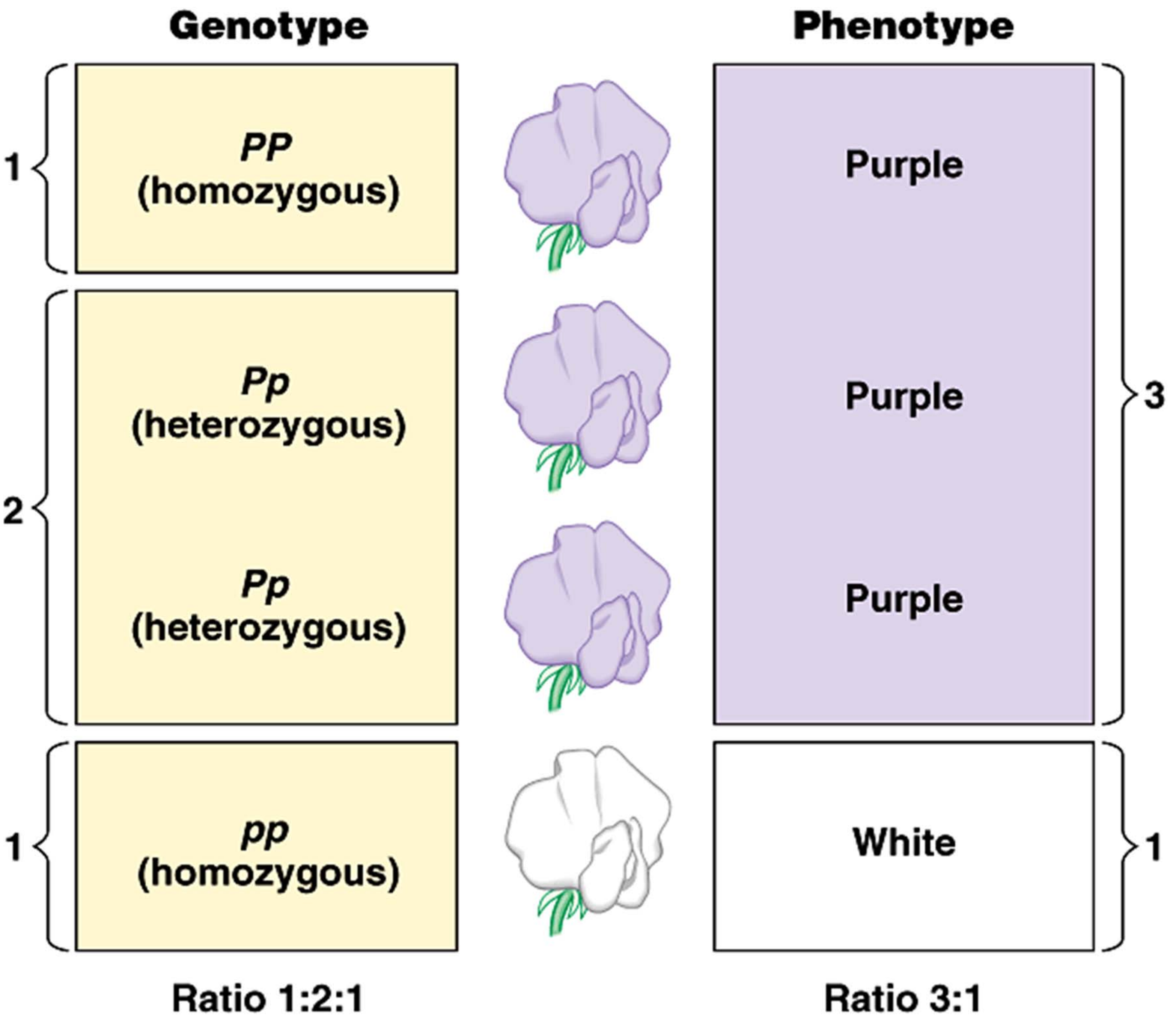


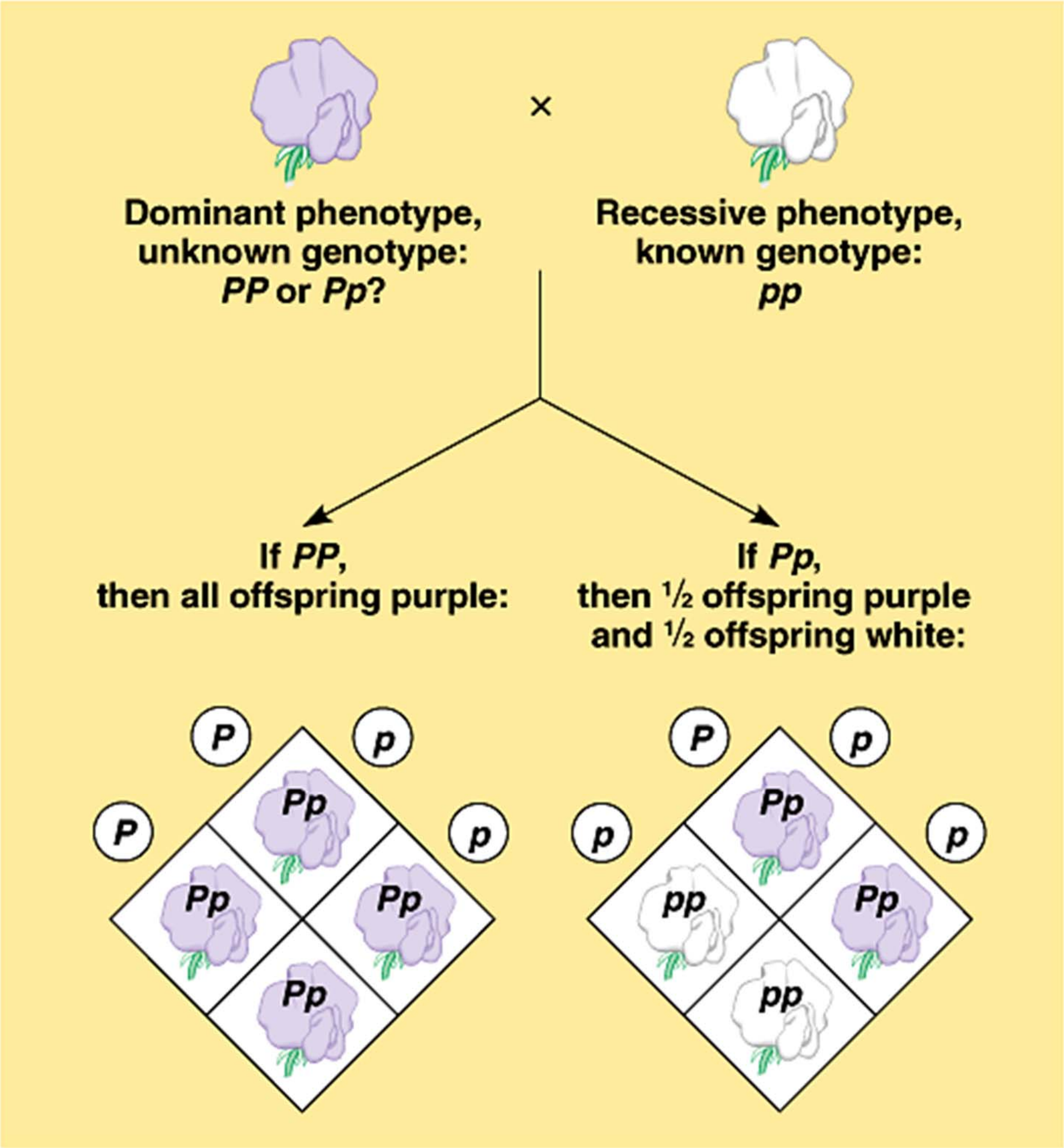
Figure 9.12A



	Genotype	Phenotype	
1	*PP* (homozygous)	Purple	3
2	*Pp* (heterozygous)	Purple	
	Pp (heterozygous)	Purple	
1	*pp* (homozygous)	White	1
	Ratio 1:2:1	Ratio 3:1	

Testcross / Backcross

- Punnett Squares can help reveal the genotype of organisms.
  - Use a testcross
  - Breed an individual of an unknown genotype w/ a HOMOZYGOUS, RECESSIVE individual
    - Use the ratio to determine genotype.



Dominant phenotype, unknown genotype: *PP* or *Pp*?

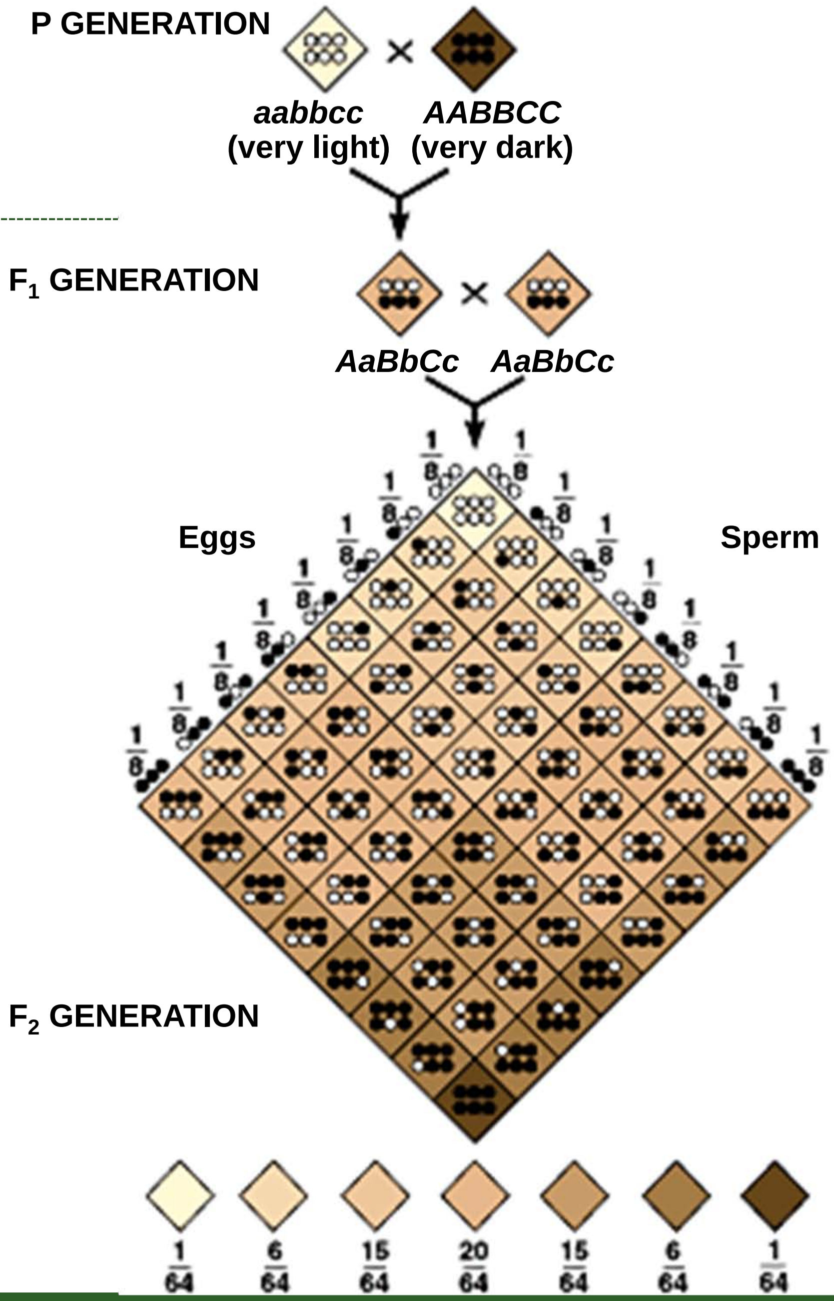
Recessive phenotype, known genotype: *pp*

If *PP*, then all offspring purple:

If *Pp*, then ½ offspring purple and ½ offspring white:

A single characteristic may be influenced by many genes

- This situation creates a continuum of phenotypes
- Quantitative traits
  - Example: skin color, height


P GENERATION
aabbcc
(very light)
AABBCC
(very dark)
F$_1$ GENERATION
AaBbCc
AaBbCc
Eggs
Sperm
1/8
F$_2$ GENERATION
1/64
6/64
15/64
20/64
15/64
6/64
1/64

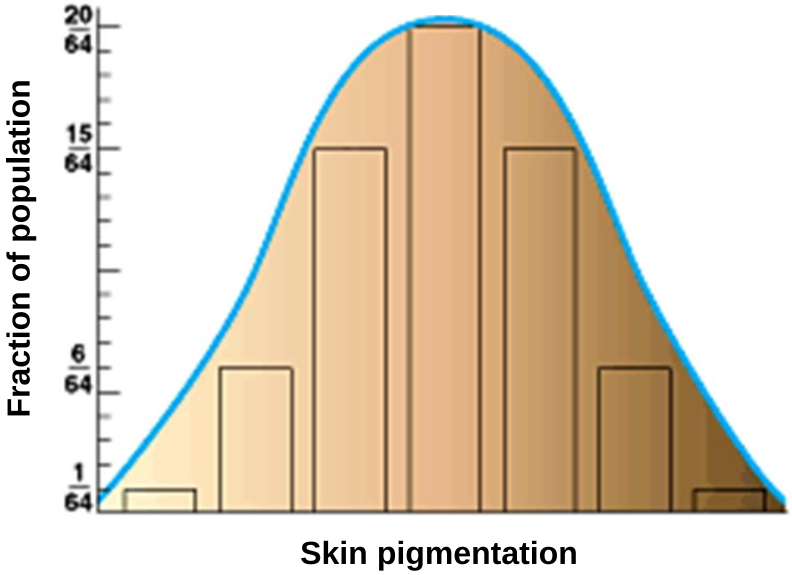

Fraction of population
20/64
15/64
6/64
1/64
Skin pigmentation

Figure 9.16

Connection: Many inherited disorders in humans are controlled by a single gene

- Most such disorders are caused by autosomal recessive alleles
  - Examples: cystic fibrosis, sickle-cell disease

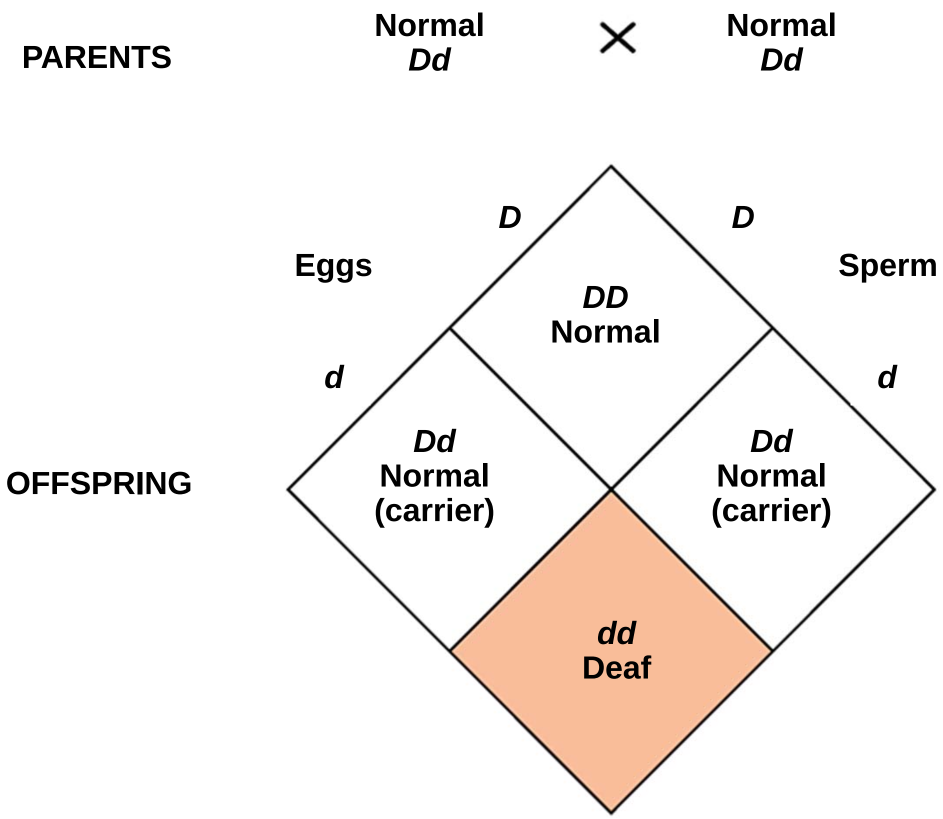
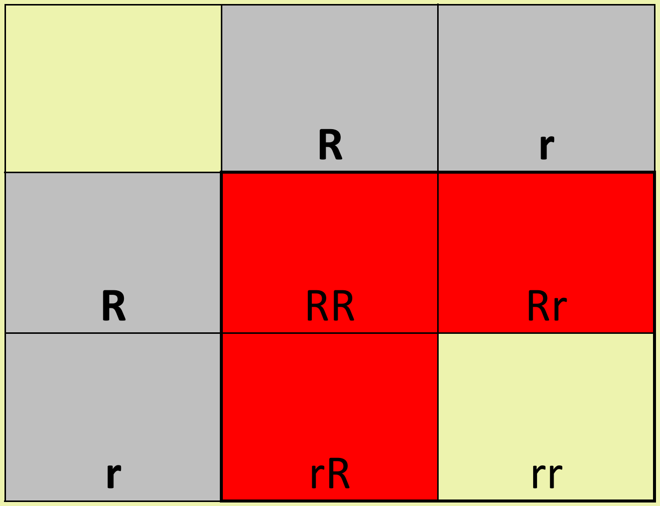


Figure 9.9A

Genetics Refresher

R = red
r = white

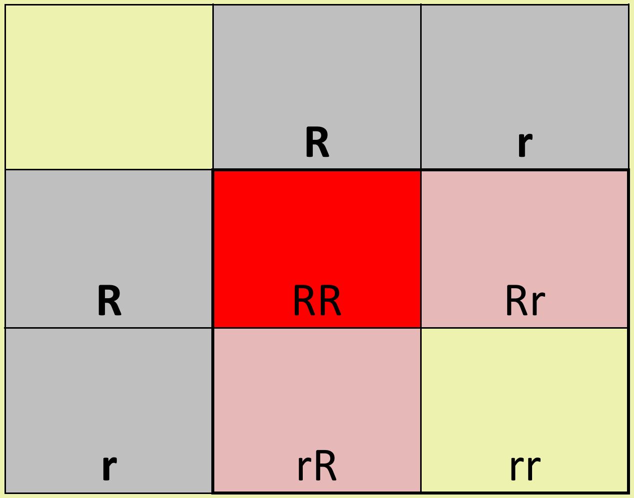
Dominant/Recessive Inheritance



	R	r
R	RR	Rr
r	rR	rr

Rr x Rr

Incomplete Dominance



	R	r
R	RR	Rr
r	rR	rr

Rr x Rr

Backcross Breeding

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- American chestnuts are fully susceptible to blight
- Some Chinese chestnuts are fully resistant to the blight, all have moderate resistance
- Blight-resistance is **incompletely dominant**
  - ○ Evidence from field tests
  - ○ Segregation of resistant vs. non-resistant offspring
  - ○ Ratios observed have led to:
    - 2 or 3 genes for blight resistance
    - Trees homozygous for blight resistance more resistant than heterozygous trees (based on observations and ratios)

American chestnut

Chinese chestnut

Backcross Breeding

- Combination of hybrid and backcross breeding
- Hybrid cross captures blight-resistance
  - American x Chinese
- Backcrosses breed out Chinese chestnut character and include more American chestnut character
  - Repeat enough times to re-capture desired amount of American character (3 or 4 times)
- Intercrosses (hybrid of two backcrosses) increase blight-resistance further
  - Each intercross increases chance of breeding trees homozygous for resistance (2 times)

American chestnut

Chinese chestnut

TACF Backcross Breeding Program

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- First generation hybrid to capture blight-resistance
  - American x Chinese = F1: ½ American, ½ Chinese. All are moderately resistant
- Offspring backcrossed with American chestnut over several generations to capture more American character
  - F1 x American = Backcross 1 (BC1): ¾ American, ¼ Chinese. 1 out of 8 are moderately resistant.
  - BC1 x American = BC2: 7/8 American, 1/8 Chinese. 1 out of 8 are moderately resistant.
  - BC2 x American = BC3: 15/16 American, 1/16 Chinese 1 out of 8 are moderately resistant.
- Intercross resistant offspring to increase blight-resistance
  - BC3F1 x BC3F1 = B3F2: 15/16 American. 1 out of 64 are highly resistant.
  - BC3F2 x BC3F2 = B3F3: 15/16 American. **All are highly resistant.**

TACF Backcross Breeding Program

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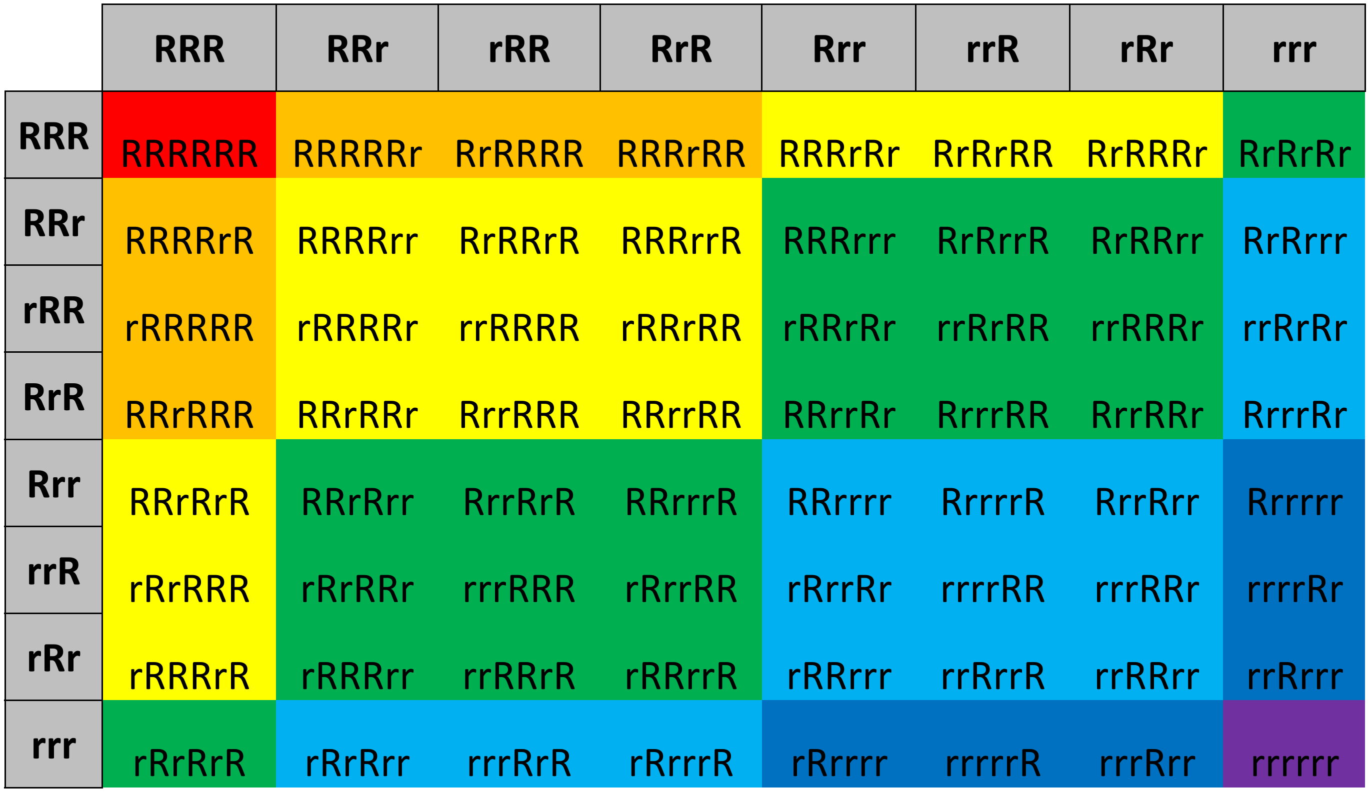
	rrr	
RRR	RrRrRr	3R
RRr	RrRrrr	2R
rRR	rrRrRr	2R
RrR	RrrrRr	2R
Rrr	Rrrrrr	1R
rrR	rrrrRr	1R
rRr	rrRrrr	1R
rrr	rrrrrr	0R

Resistance in backcross generations. Note: no offspring can be homozygous for resistance in these generations.

TACF Backcross Breeding Program



	RRR	RRr	rRR	RrR	Rrr	rrR	rRr	rrr
RRR	RRRRRR	RRRRRr	RrRRRR	RRRrRR	RRRrRr	RrRrRR	RrRRRr	RrRrRr
RRr	RRRRrR	RRRRrr	RrRRrR	RRRrrR	RRRrrr	RrRrrR	RrRRrr	RrRrrr
rRR	rRRRRR	rRRRRr	rrRRRR	rRRrRR	rRRrRr	rrRrRR	rrRRRr	rrRrRr
RrR	RRrRRR	RRrRRr	RrrRRR	RRrrRR	RRrrRr	RrrrRR	RrrRRr	RrrrRr
Rrr	RRrRrR	RRrRrr	RrrRrR	RRrrrR	RRrrrr	RrrrrR	RrrRrr	Rrrrrr
rrR	rRrRRR	rRrRRr	rrrRRR	rRrrRR	rRrrRr	rrrrRR	rrrRRr	rrrrRr
rRr	rRRRrR	rRRRrr	rrRRrR	rRRrrR	rRRrrr	rrRrrR	rrRRrr	rrRrrr
rrr	rRrRrR	rRrRrr	rrrRrR	rRrrrR	rRrrrr	rrrrrR	rrrRrr	rrrrrr

Resistance in intercross generations. Note: one cross should be homozygous for all resistance genes, and many homozygous for one or two resistance genes.

P = G x E

- Phenotype = Genetics x Environment
  - Describes the situation where there is a change in the performance ranking of given genotypes when grown in different environments.
    - Some traits are highly **ENVIRONMENTALLY PLASTIC**, prone to response to different environmental variables, while others are not.
    - Must be able to differentiate environmental causes, i.e good site vs. bad site, with the underlying genetics of any given situation.
- Best way to determine is to look at differential expression among clones.
  - So far, mostly anecdotal evidence.

How Do We Know it Will Work?

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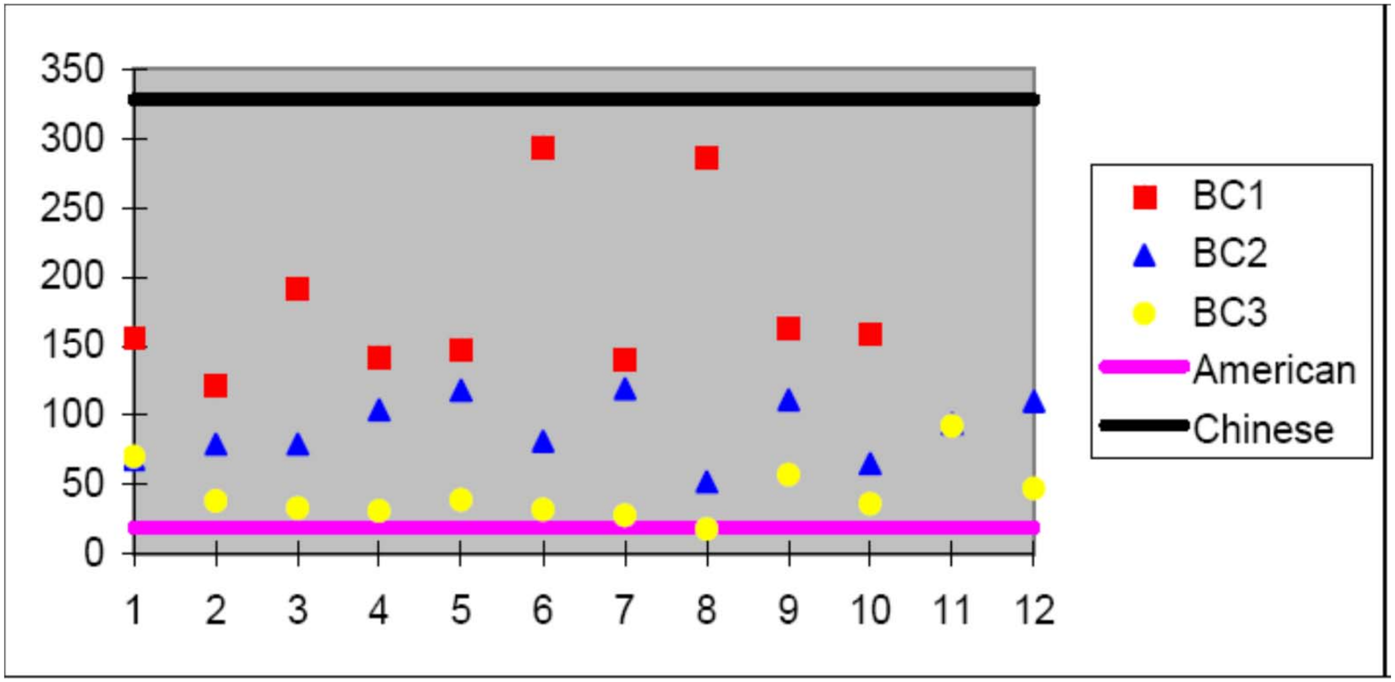
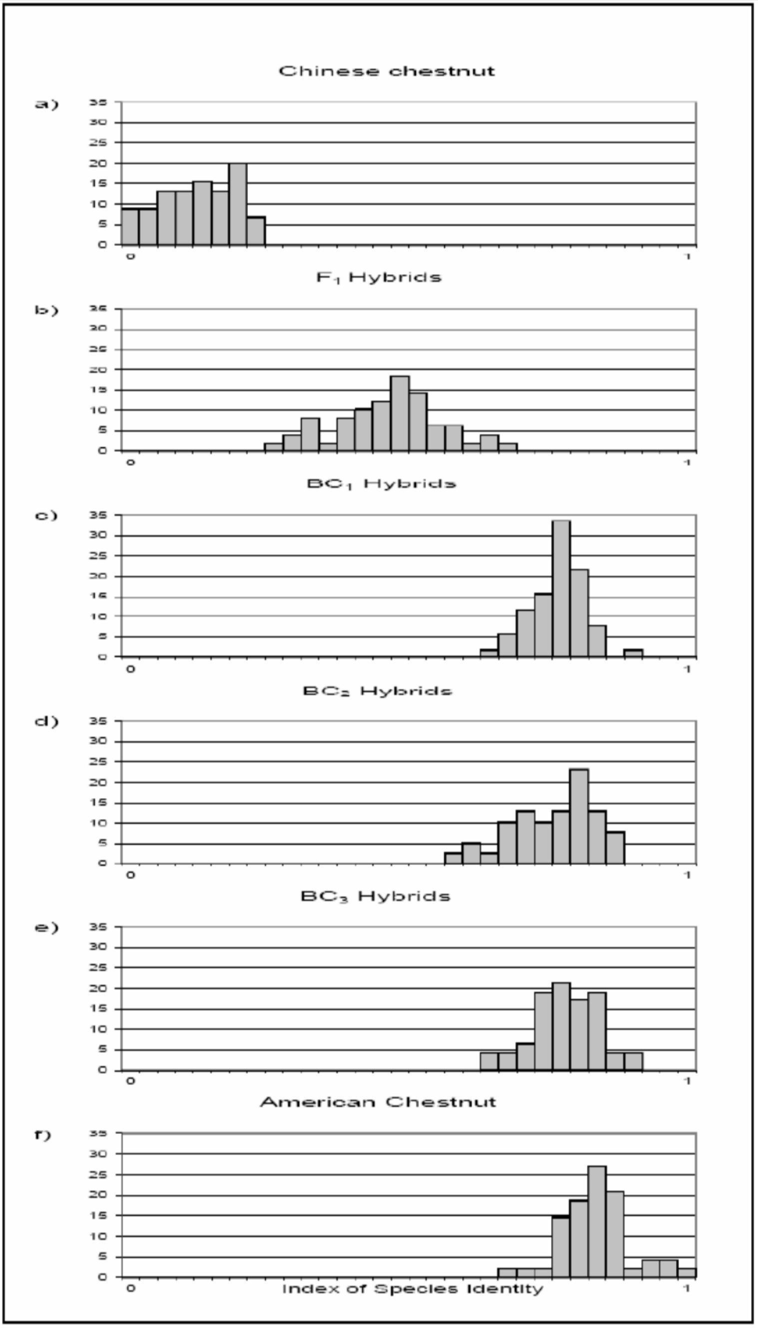
- We don't but . . .
- American character is being returned as expected
  - Molecular techniques to measure American character in various generations
  - Morphological analysis of those same generations

Dot Blot Analysis



Analysis of Morphological Characters

- 24 Variables Examined
- Created Index of Species Identity



What About Resistance

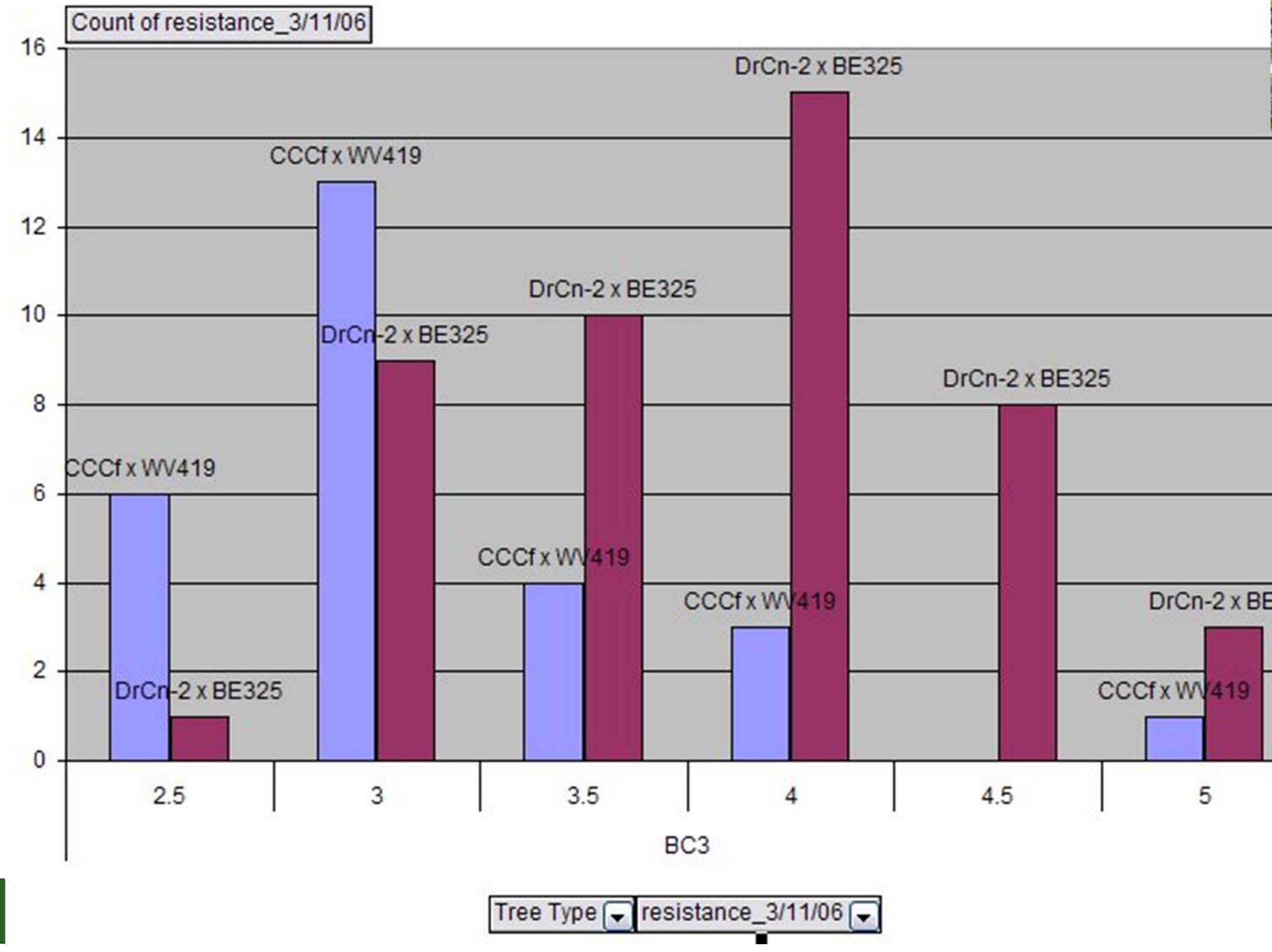
- F2 populations should have some members with high amounts of blight resistance
- B1F2 populations rated in 1990s shows high resistance

Actions of Resistance

1. Lignification of pre-existing cells
2. New wound tissue contains suberin

- Wound areas also become infused with
  - anti-microbial, host-derived secondary metabolites (phytoalexins) and
- proteins/enzymes
  - pathogenesis-related proteins (PRPs),
  - chitinase/ glucanase
  - others

Distribution of Riegelsville Ratings 4th Generation – BC3



BC3F2 Orchard Spacing and Resistance Screening

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Fixation of Resistance Alleles

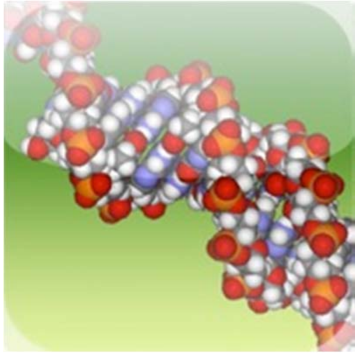
- Current selection of individuals at B3F2 generation have high resistance
  - But less than expected
    - First members inoc'ed in 2004 (VA) and 2005 (PA)
  - Science Audit 2006 = screening techniques of B3F2s = too harsh.

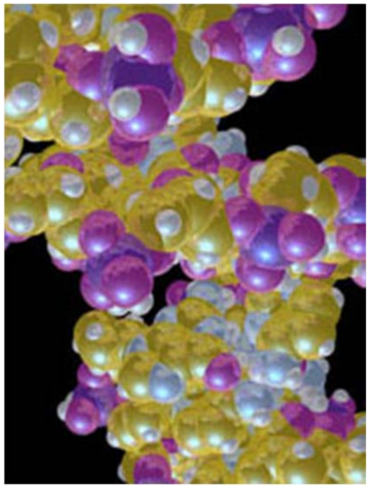
So what are the next steps?

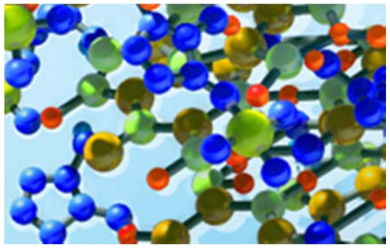
- Continue screening
  - Improve screening techniques
    - Leaf assays
- Molecular genetics!

MOLECULAR GENETICS

WHAT LIES BENEATH







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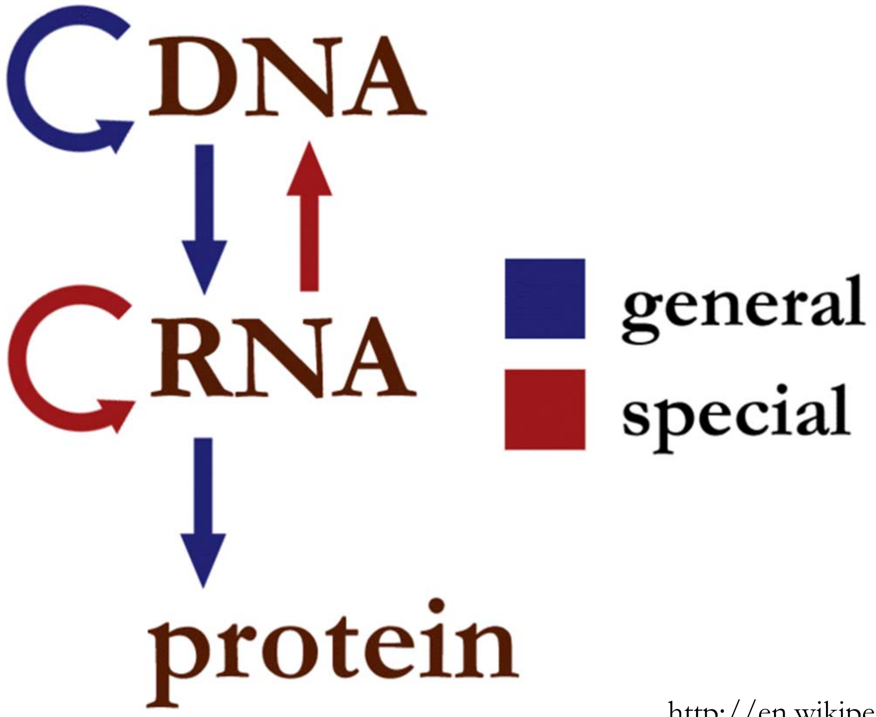
Presentation Author

Central Dogma of Molecular Biology

- Dogma
- DNA – RNA – amino acid - protein



DNA

RNA

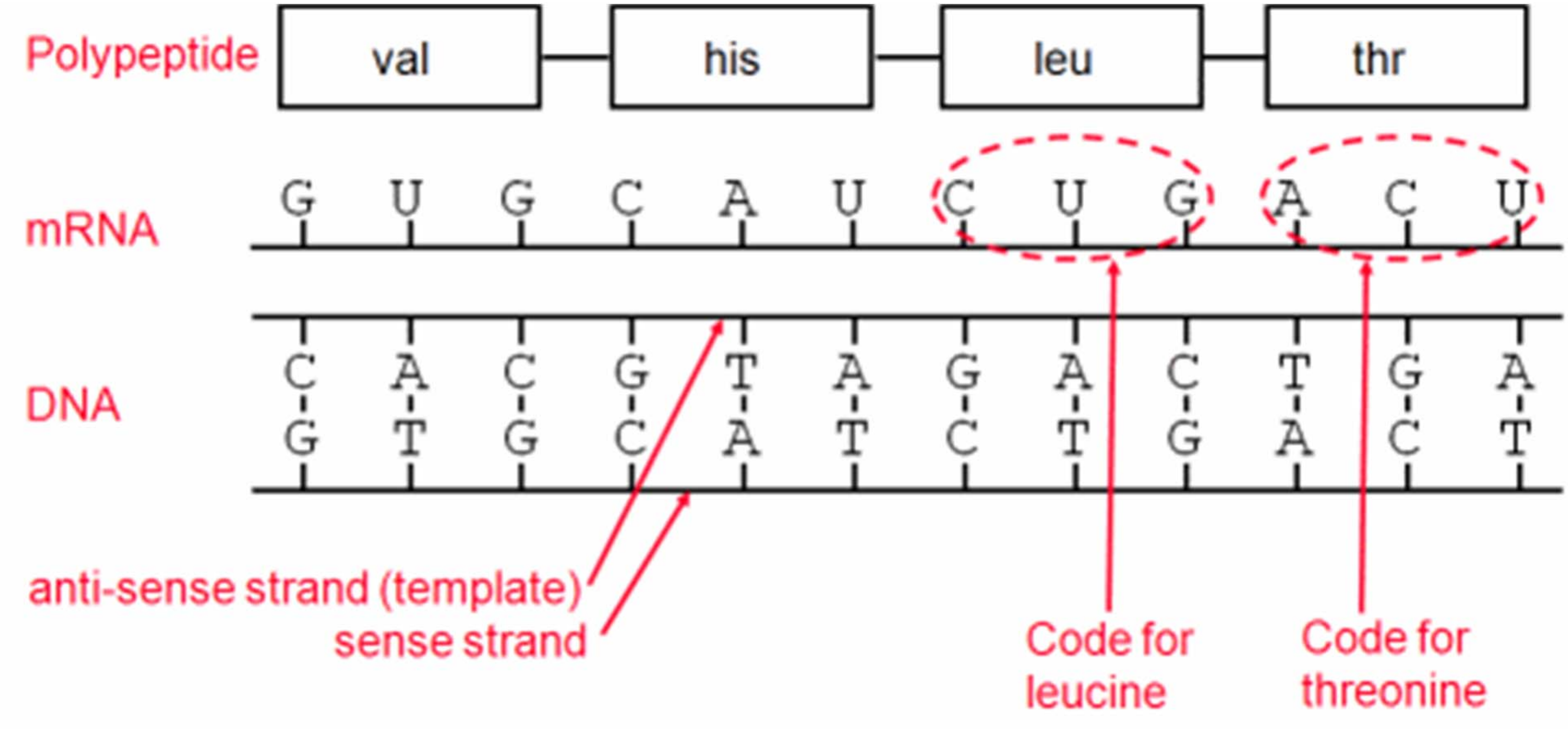
protein

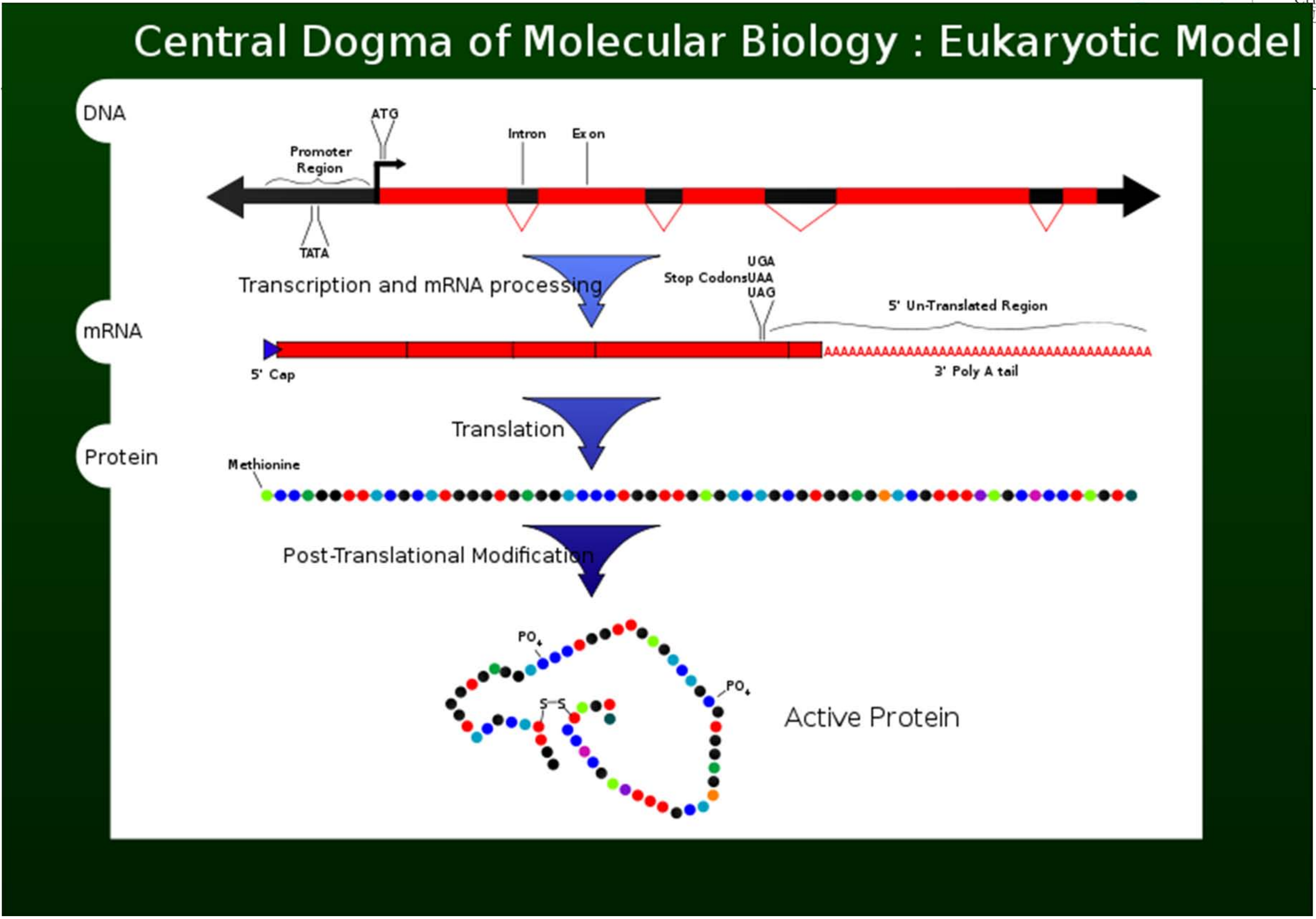
🟦 general

🟥 special

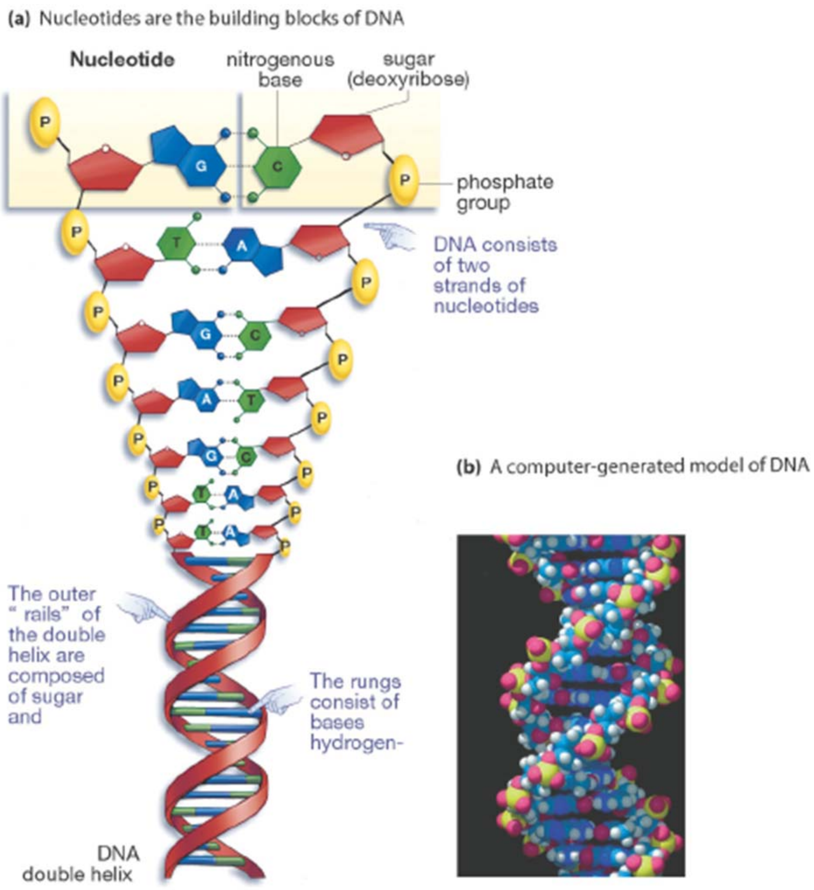
http://en.wikipedia.org/wiki/File:Centraldogma_nodetails.GIF

Central Dogma of Molecular Biology

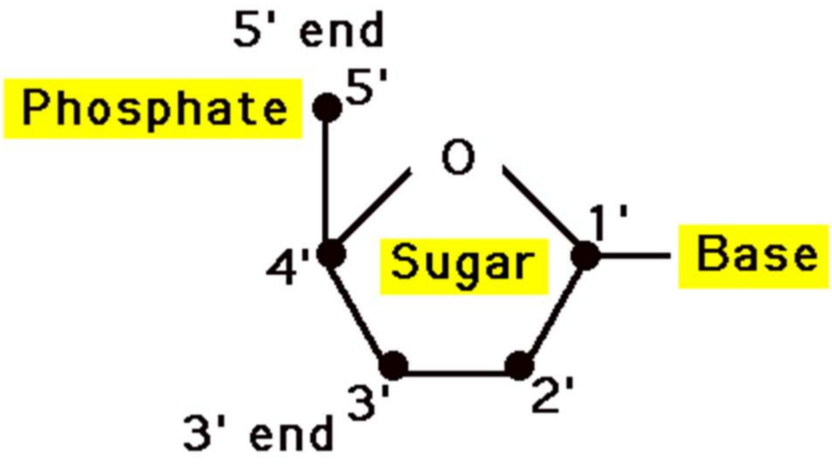




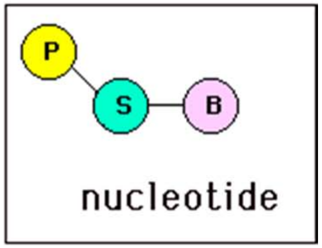
http://en.wikipedia.org/wiki/File:Cdmb.svg



- Composed of 2 polymers of nucleotides
- Polymers are oriented in antiparallel
- Molecule resembles a spiral staircase of complementary base pairs

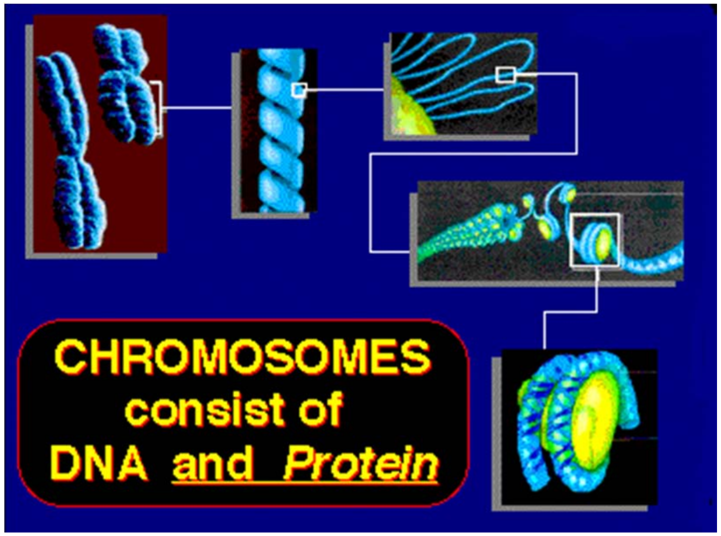


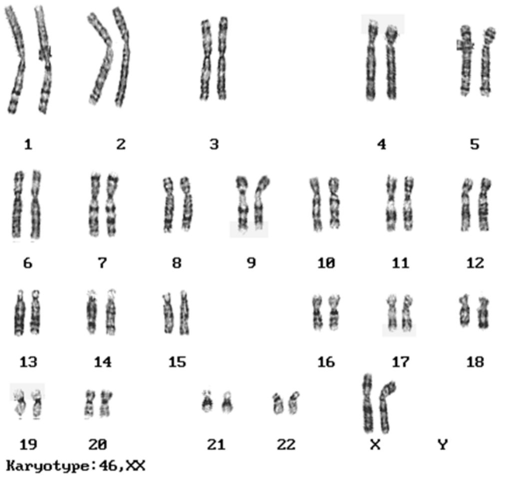
nucleotide



- Each nucleotide of DNA contains:
  - Deoxyribose
  - Phosphate
  - Nitrogen base (either A, G, C, T)
  - * RNA has Ribose and U instead of T*

Chromosomes



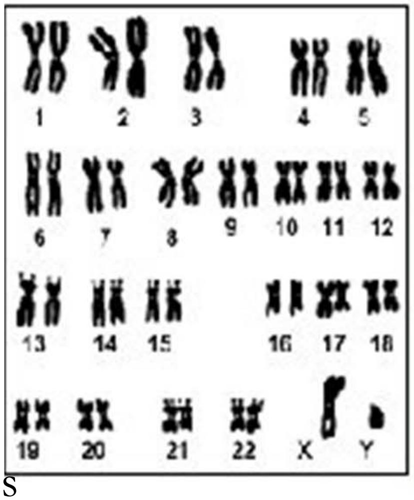


- A **chromosome** constitutes an entire DNA molecule + protein
  - Protein = histones
  - Supercoiled DNA in nucleosomes
  - Humans contain 46 such molecules (23 pairs)
    - 44 somatic chromosomes
    - 2 sex chromosomes (X +Y)
  - Chestnuts have 24 chromosomes (12 pairs)

We each have genes

Found on every chromosome and

They code for traits

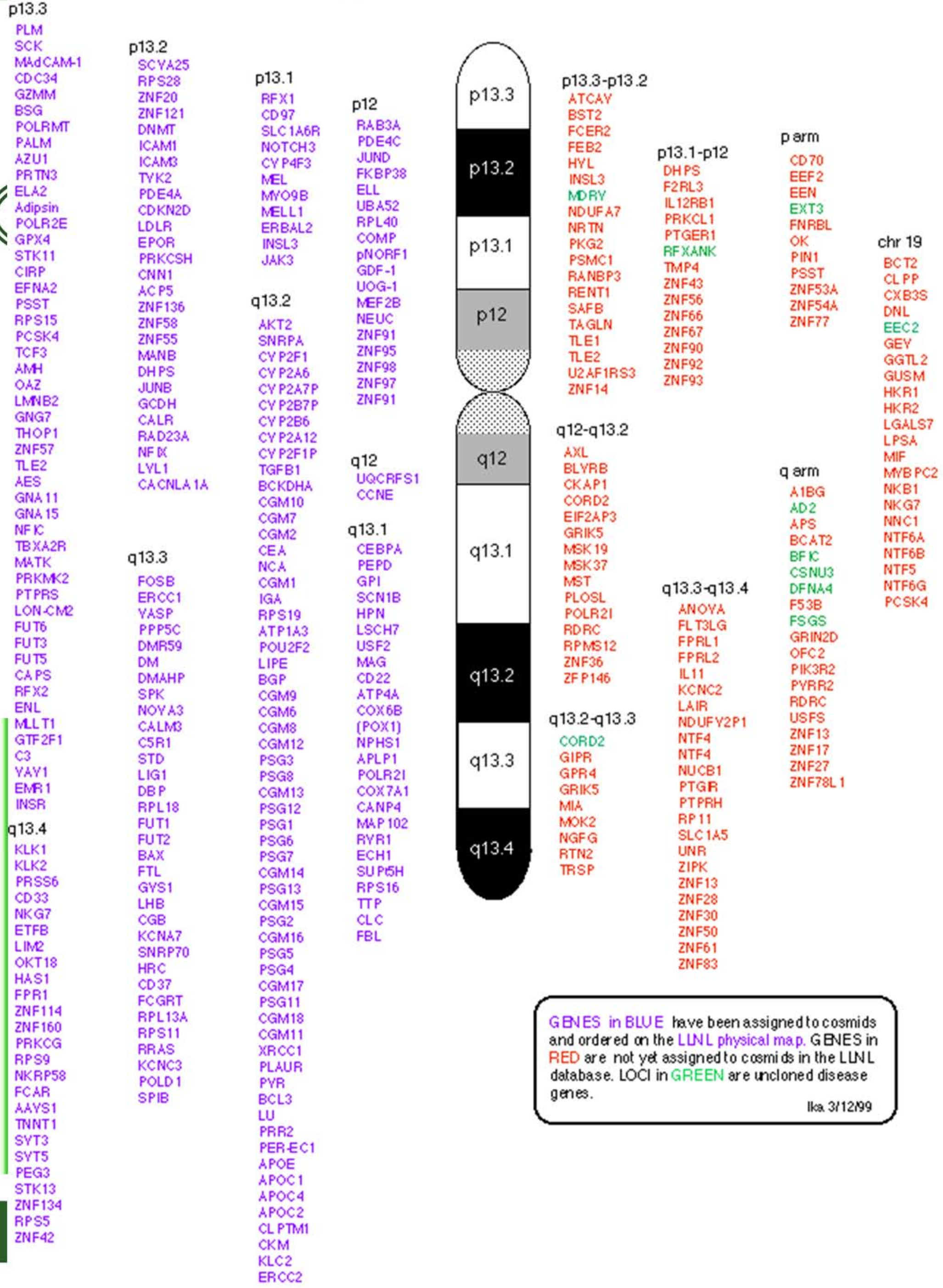


Rollercoaster of Genes

by *Dr. Annette M. Parrott*

http://www.nclark.net/rollercoasterofgenes.ppt

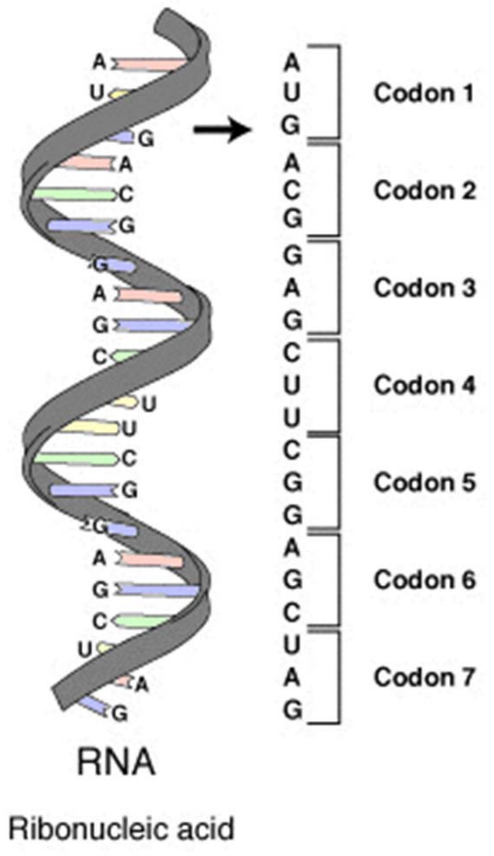
Genes Mapped To Chromosome 19



GENES in BLUE have been assigned to cosmids and ordered on the LLNL physical map. GENES in RED are not yet assigned to cosmids in the LLNL database. LOCI in GREEN are uncloned disease genes.

lka 3/12/99

DNA – RNA - Protein



		2nd base							
		U		**C**		**A**		**G**	
1st base	**U**	**UUU**	(Phe/F) Phenylalanine	**UCU**	(Ser/S) Serine	**UAU**	(Tyr/Y) Tyrosine	**UGU**	(Cys/C) Cysteine
		UUC	(Phe/F) Phenylalanine	**UCC**	(Ser/S) Serine	**UAC**	(Tyr/Y) Tyrosine	**UGC**	(Cys/C) Cysteine
		UUA	(Leu/L) Leucine	**UCA**	(Ser/S) Serine	**UAA**	Stop (Ochre)	**UGA**	Stop (*Opal*)
		UUG	(Leu/L) Leucine	**UCG**	(Ser/S) Serine	**UAG**	Stop (*Amber*)	**UGG**	(Trp/W) Tryptophan
	C	**CUU**	(Leu/L) Leucine	**CCU**	(Pro/P) Proline	**CAU**	(His/H) Histidine	**CGU**	(Arg/R) Arginine
		CUC	(Leu/L) Leucine	**CCC**	(Pro/P) Proline	**CAC**	(His/H) Histidine	**CGC**	(Arg/R) Arginine
		CUA	(Leu/L) Leucine	**CCA**	(Pro/P) Proline	**CAA**	(Gln/Q) Glutamine	**CGA**	(Arg/R) Arginine
		CUG	(Leu/L) Leucine	**CCG**	(Pro/P) Proline	**CAG**	(Gln/Q) Glutamine	**CGG**	(Arg/R) Arginine
	A	**AUU**	(Ile/I) Isoleucine	**ACU**	(Thr/T) Threonine	**AAU**	(Asn/N) Asparagine	**AGU**	(Ser/S) Serine
		AUC	(Ile/I) Isoleucine	**ACC**	(Thr/T) Threonine	**AAC**	(Asn/N) Asparagine	**AGC**	(Ser/S) Serine
		AUA	(Ile/I) Isoleucine	**ACA**	(Thr/T) Threonine	**AAA**	(Lys/K) Lysine	**AGA**	(Arg/R) Arginine
		AUG[A]	(Met/M) Methionine	**ACG**	(Thr/T) Threonine	**AAG**	(Lys/K) Lysine	**AGG**	(Arg/R) Arginine
	G	**GUU**	(Val/V) Valine	**GCU**	(Ala/A) Alanine	**GAU**	(Asp/D) Aspartic acid	**GGU**	(Gly/G) Glycine
		GUC	(Val/V) Valine	**GCC**	(Ala/A) Alanine	**GAC**	(Asp/D) Aspartic acid	**GGC**	(Gly/G) Glycine
		GUA	(Val/V) Valine	**GCA**	(Ala/A) Alanine	**GAA**	(Glu/E) Glutamic acid	**GGA**	(Gly/G) Glycine
		GUG	(Val/V) Valine	**GCG**	(Ala/A) Alanine	**GAG**	(Glu/E) Glutamic acid	**GGG**	(Gly/G) Glycine

2nd base

se

	U		C		A		G	
U	**UUU**	(Phe/F) Phenylalanine	**UCU**	(Ser/S) Serine	**UAU**	(Tyr/Y) Tyrosine	**UGU**	(Cys/C) Cysteine
	UUC	(Phe/F) Phenylalanine	**UCC**	(Ser/S) Serine	**UAC**	(Tyr/Y) Tyrosine	**UGC**	(Cys/C) Cysteine
	UUA	(Leu/L) Leucine	**UCA**	(Ser/S) Serine	**UAA**	Stop (Ochre)	**UGA**	Stop (*Opal*)
	UUG	(Leu/L) Leucine	**UCG**	(Ser/S) Serine	**UAG**	Stop (*Amber*)	**UGG**	(Trp/W) Tryptophan
C	**CUU**	(Leu/L) Leucine	**CCU**	(Pro/P) Proline	**CAU**	(His/H) Histidine	**CGU**	(Arg/R) Arginine
	CUC	(Leu/L) Leucine	**CCC**	(Pro/P) Proline	**CAC**	(His/H) Histidine	**CGC**	(Arg/R) Arginine
	CUA	(Leu/L) Leucine	**CCA**	(Pro/P) Proline	**CAA**	(Gln/Q) Glutamine	**CGA**	(Arg/R) Arginine
	CUG	(Leu/L) Leucine	**CCG**	(Pro/P) Proline	**CAG**	(Gln/Q) Glutamine	**CGG**	(Arg/R) Arginine
A	**AUU**	(Ile/I) Isoleucine	**ACU**	(Thr/T) Threonine	**AAU**	(Asn/N) Asparagine	**AGU**	(Ser/S) Serine
	AUC	(Ile/I) Isoleucine	**ACC**	(Thr/T) Threonine	**AAC**	(Asn/N) Asparagine	**AGC**	(Ser/S) Serine
	AUA	(Ile/I) Isoleucine	**ACA**	(Thr/T) Threonine	**AAA**	(Lys/K) Lysine	**AGA**	(Arg/R) Arginine
	AUG[A]	(Met/M) Methionine	**ACG**	(Thr/T) Threonine	**AAG**	(Lys/K) Lysine	**AGG**	(Arg/R) Arginine
G	**GUU**	(Val/V) Valine	**GCU**	(Ala/A) Alanine	**GAU**	(Asp/D) Aspartic acid	**GGU**	(Gly/G) Glycine
	GUC	(Val/V) Valine	**GCC**	(Ala/A) Alanine	**GAC**	(Asp/D) Aspartic acid	**GGC**	(Gly/G) Glycine
	GUA	(Val/V) Valine	**GCA**	(Ala/A) Alanine	**GAA**	(Glu/E) Glutamic acid	**GGA**	(Gly/G) Glycine
	GUG	(Val/V) Valine	**GCG**	(Ala/A) Alanine	**GAG**	(Glu/E) Glutamic acid	**GGG**	(Gly/G) Glycine

DNA Sequencing

```
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```

http://www.genome.gov/19519278

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Sequencing

How to know what genes to what?

- Mutations
    - Deletions or insertions
        - Knockouts
        - Generally performed on “lower” life forms.
    - Look at vastly different phenotypes and compare their sequences
- BLAST
    - Use computer software to compare sequences of unknown function to those of known function.
- Continue to build library of more and more complex organisms.

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GENETIC ENGINEERING AND TRANSFORMATION

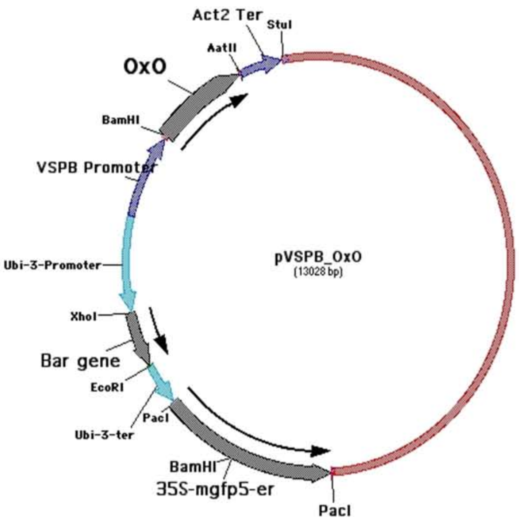
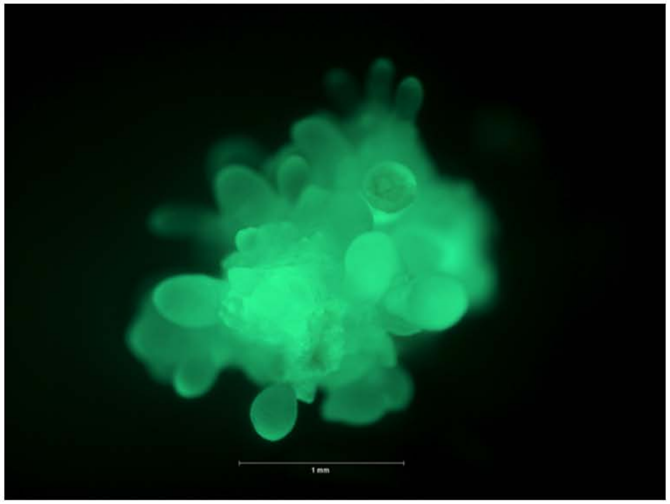
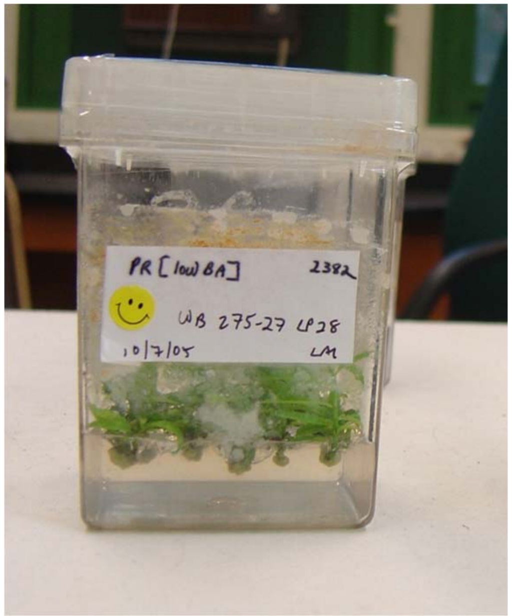


Figure 1. Plasmid map of the binary vector pVSPB-OxO.





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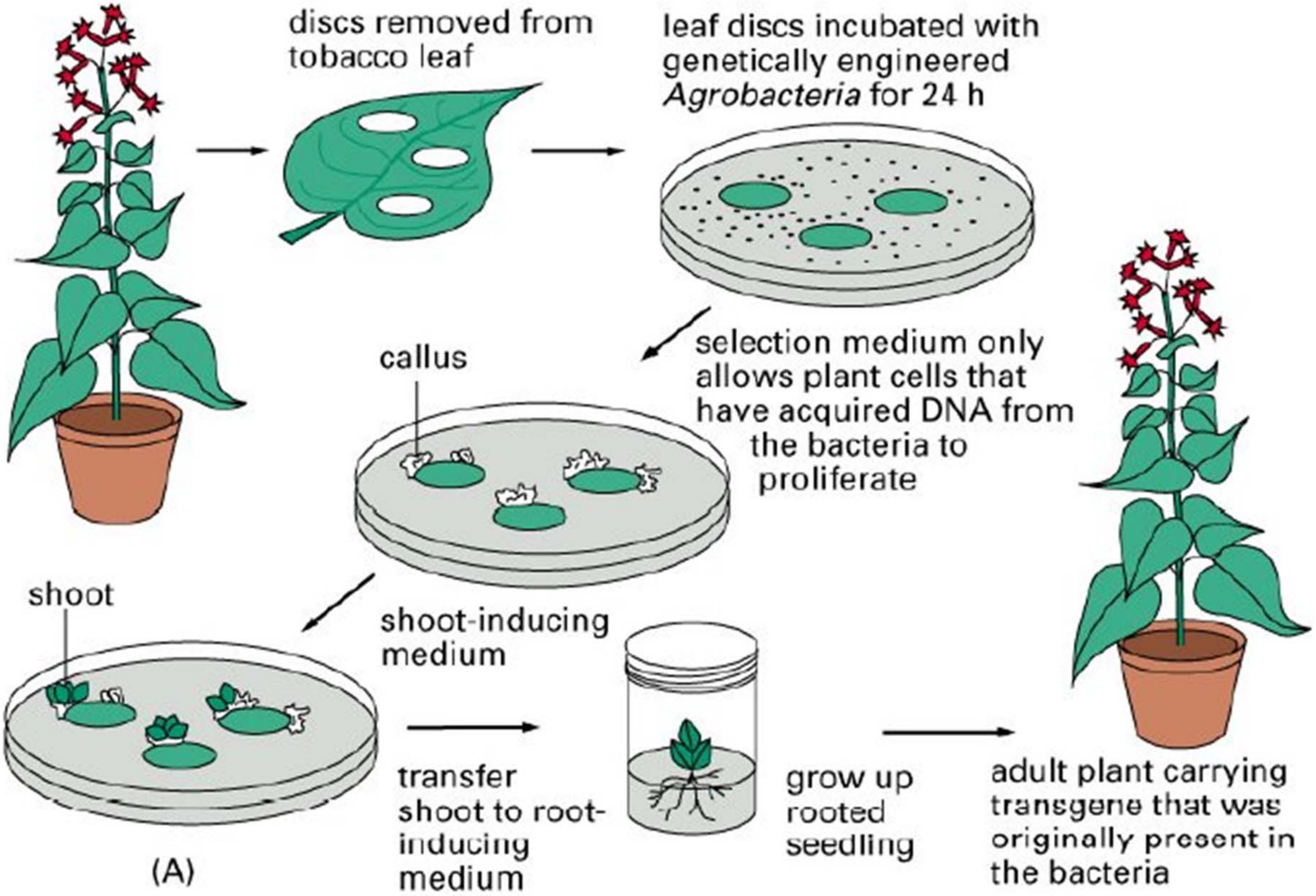
Genetic Engineering

- Gene guns
  - Pollen
  - Embryos
- Agrobacterium mediated transformation

Crown gall is an overgrowth of plant tissue (usually the roots or stem) caused by *Agrobacterium tumefaciens*

- Crown gall has a very <u>wide host range</u> (640 spp. 93 families)!
- ornamentals such as rose, Chrysanthemum and daisies
- fruits such as raspberries and grape
- tree crops such as apple, pear and cherry
- field crops such as cotton, sugar beets, tomatoes, and beans



discs removed from tobacco leaf

leaf discs incubated with genetically engineered *Agrobacteria* for 24 h

selection medium only allows plant cells that have acquired DNA from the bacteria to proliferate

callus

shoot-inducing medium

shoot

transfer shoot to root-inducing medium

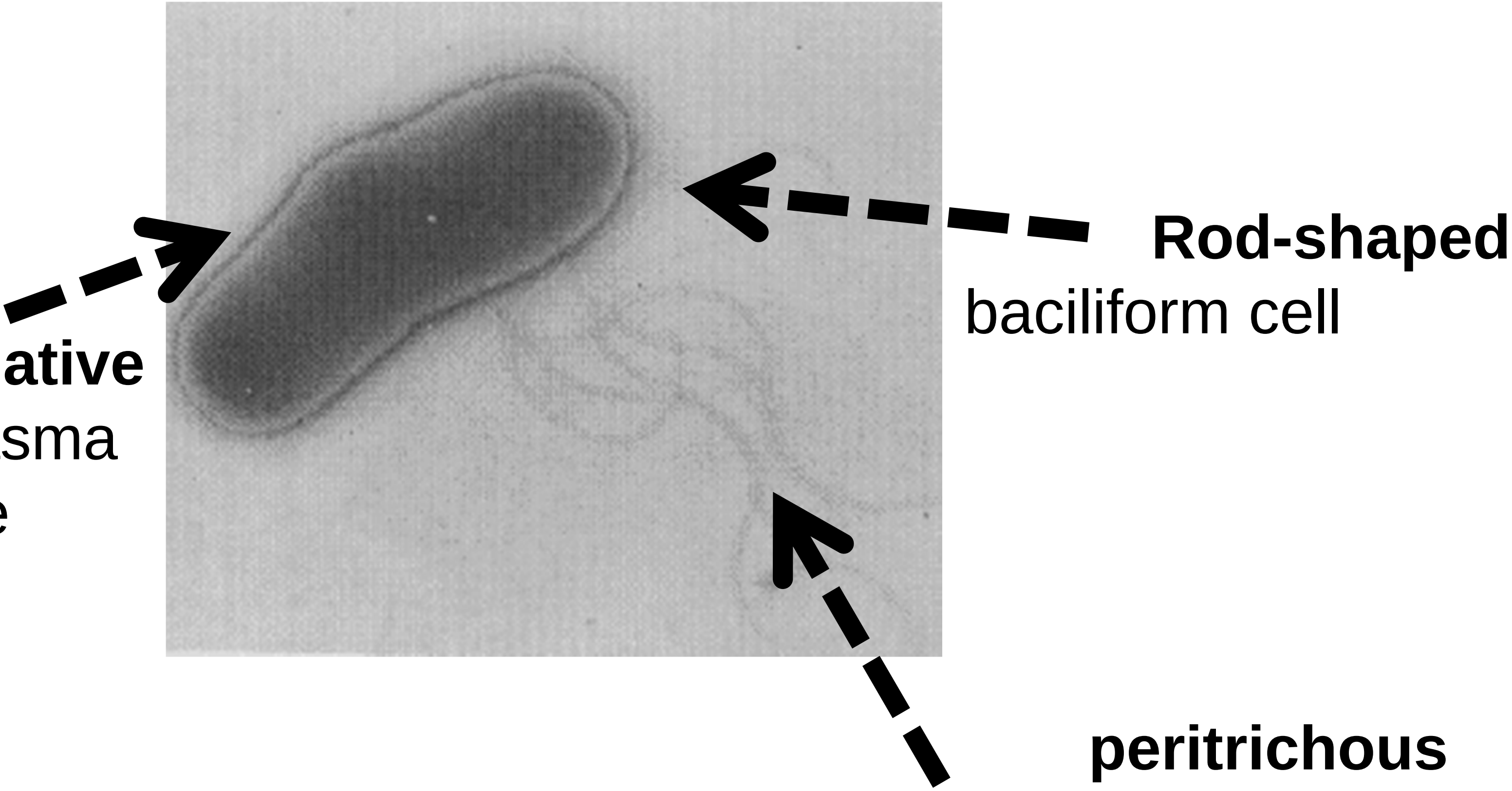
grow up rooted seedling

adult plant carrying transgene that was originally present in the bacteria

(A)

Alberts et al. (2002) Molecular Biology of the Cell 4/e

Distinctive physical features of *Agrobacterium tumefaciens*



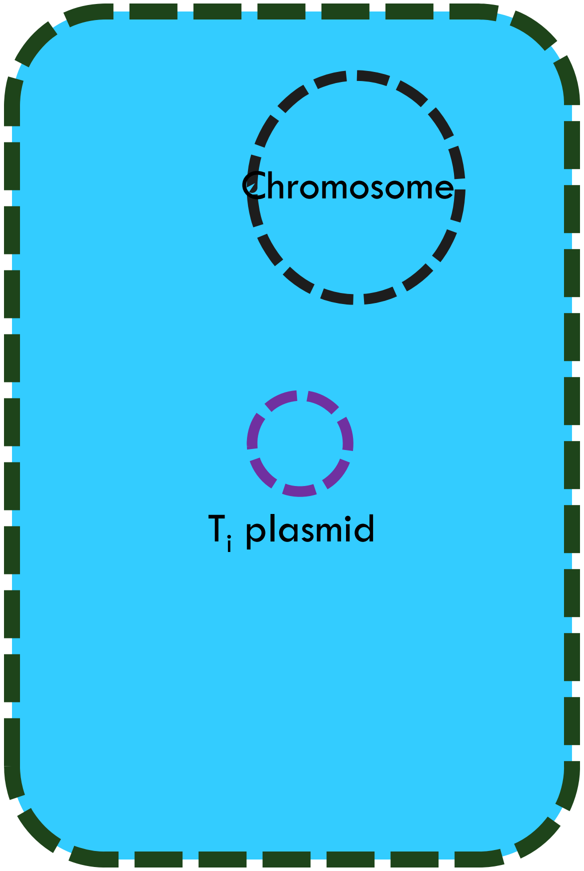
Rod-shaped
baciliform cell

Gram negative
double plasma
membrane

peritrichous
(arranged around cell)
flagella

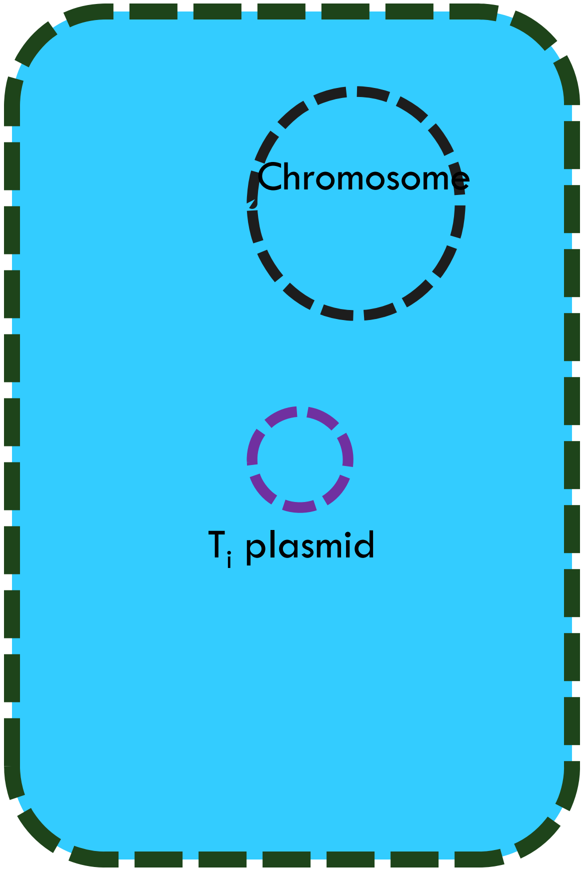
Slides courtesy Steve Conway, PPATH PSU

Agrobacterium tumefaciens



- **Chromosome**
  - Functional genes of bacteria
  - Genes for reproduction, metabolism, etc.
- **Plasmid:**
  - Extra-chromosomal genes
  - T_i - codes for galls

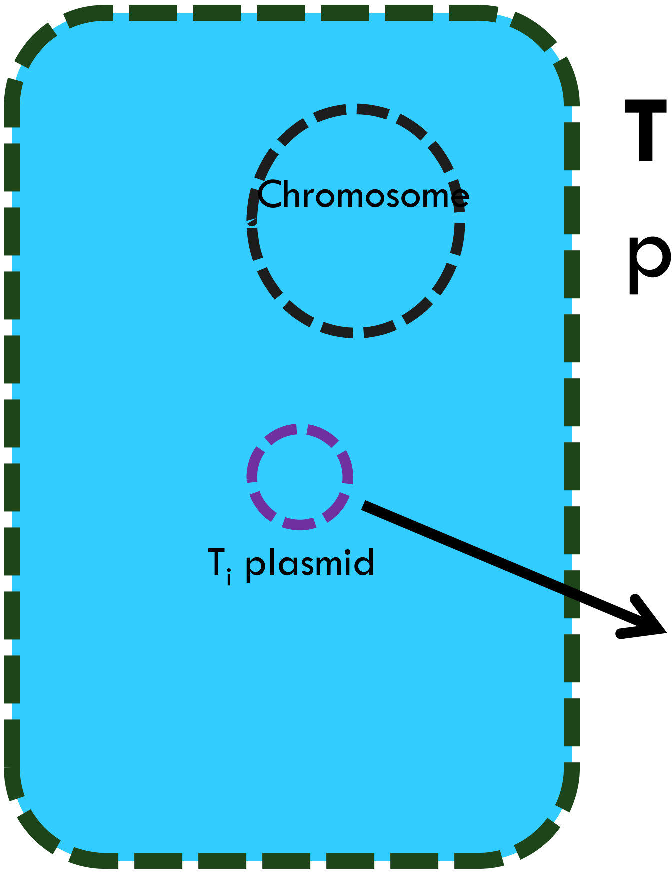
Agrobacterium tumefaciens



A very unusual form of pathogenicity: *A. tumefaciens* integrates DNA from Ti plasmid into host DNA!

Slides courtesy Steve Conway, PPATH PSU

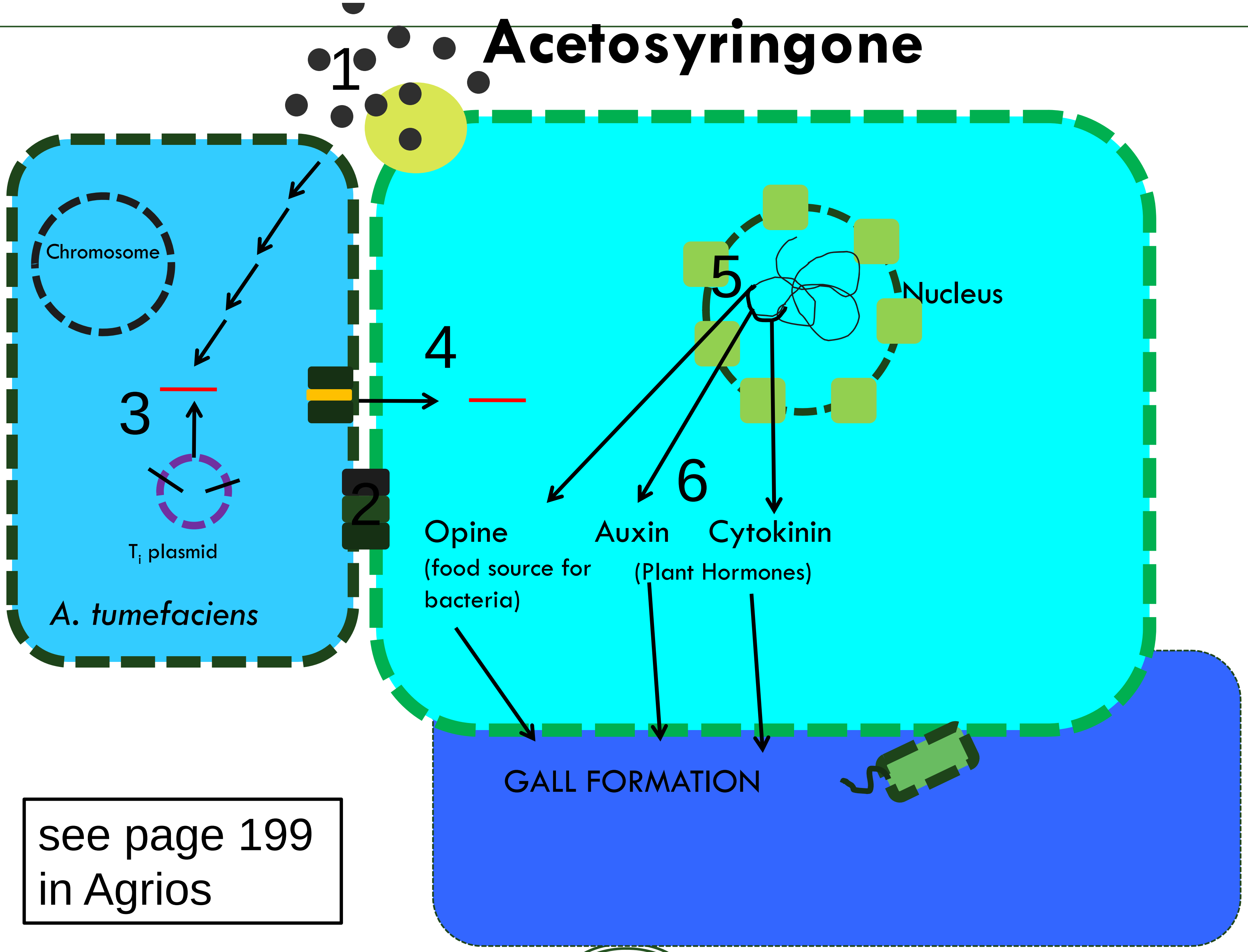
Agrobacterium tumefaciens



T_i plasmid: Tumor-inducing plasmid

Many specific genes

2. bacterial cell **binds** to plant cell using gene products from the Ti plasmid



Acetosyringone

1

Chromosome

3

T_i plasmid

2

4

A. tumefaciens

5

Nucleus

6

Opine

(food source for bacteria)

Auxin

Cytokinin

(Plant Hormones)

GALL FORMATION

see page 199
in Agrios

Slides courtesy Steve Conway, PPATH PSU

Genetic Transformation

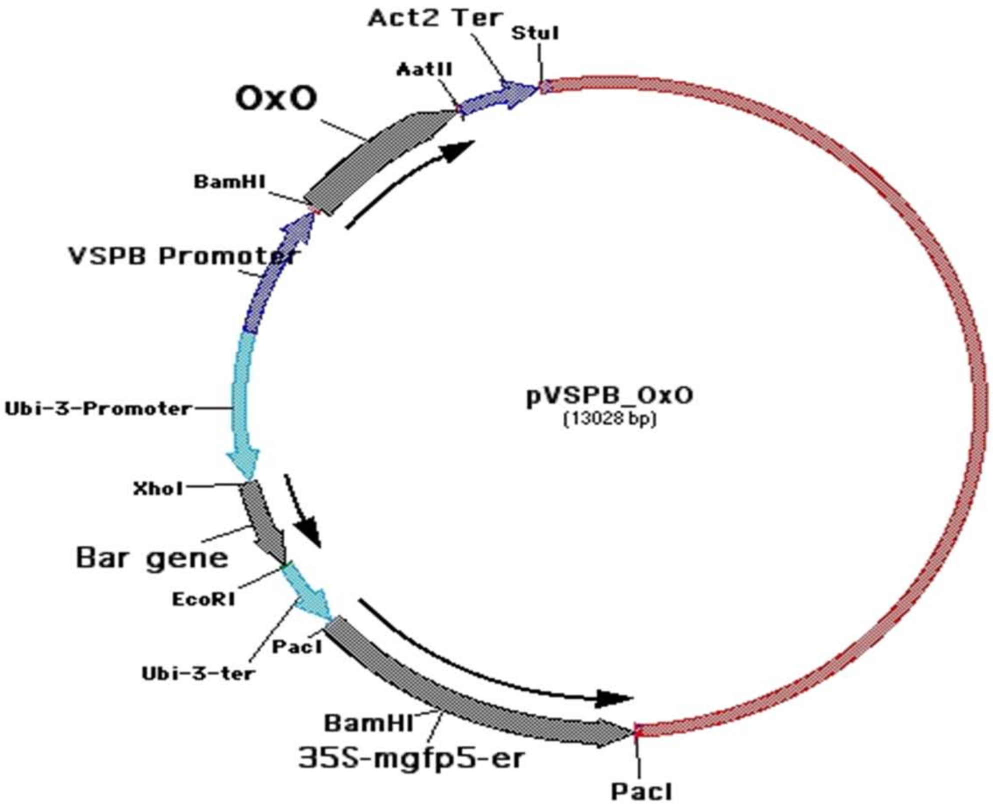


Figure 1. Plasmid map of the binary vector pVSPB-OxO.

- *OxO*-gene construct plus many others
- Oxalic Oxidase
- 25 other transformation events.

Genes & Vectors

1. Oxalate oxidase (OxO) gene from wheat
   - A. Detoxifies oxalic acid produced by the blight fungus & protects the lignin produced by the tree

pΔVspB-OxO, pTACF3, pTACF7, p35S-CNO, p35S-OxO

2. ESF39 or ESF12 antimicrobial peptide
   - A. Kills the blight fungus, *Cryphonectria parasitica*
   - B. Might also be useful against *Phytophthora* cankers

Steven N. Jeffers
Clemson University

pTACF6, pTACF7, pCWEA1

3. Chitinase from *Trichoderma*
   - A. Degrades the cell wall of *C. parasitica*

p35S-CNO

4. Ac-AMP1.2 antimicrobial peptide from Amaranth

pCWEA1, pCA1

Control vectors: pGFP & pWVK147

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King

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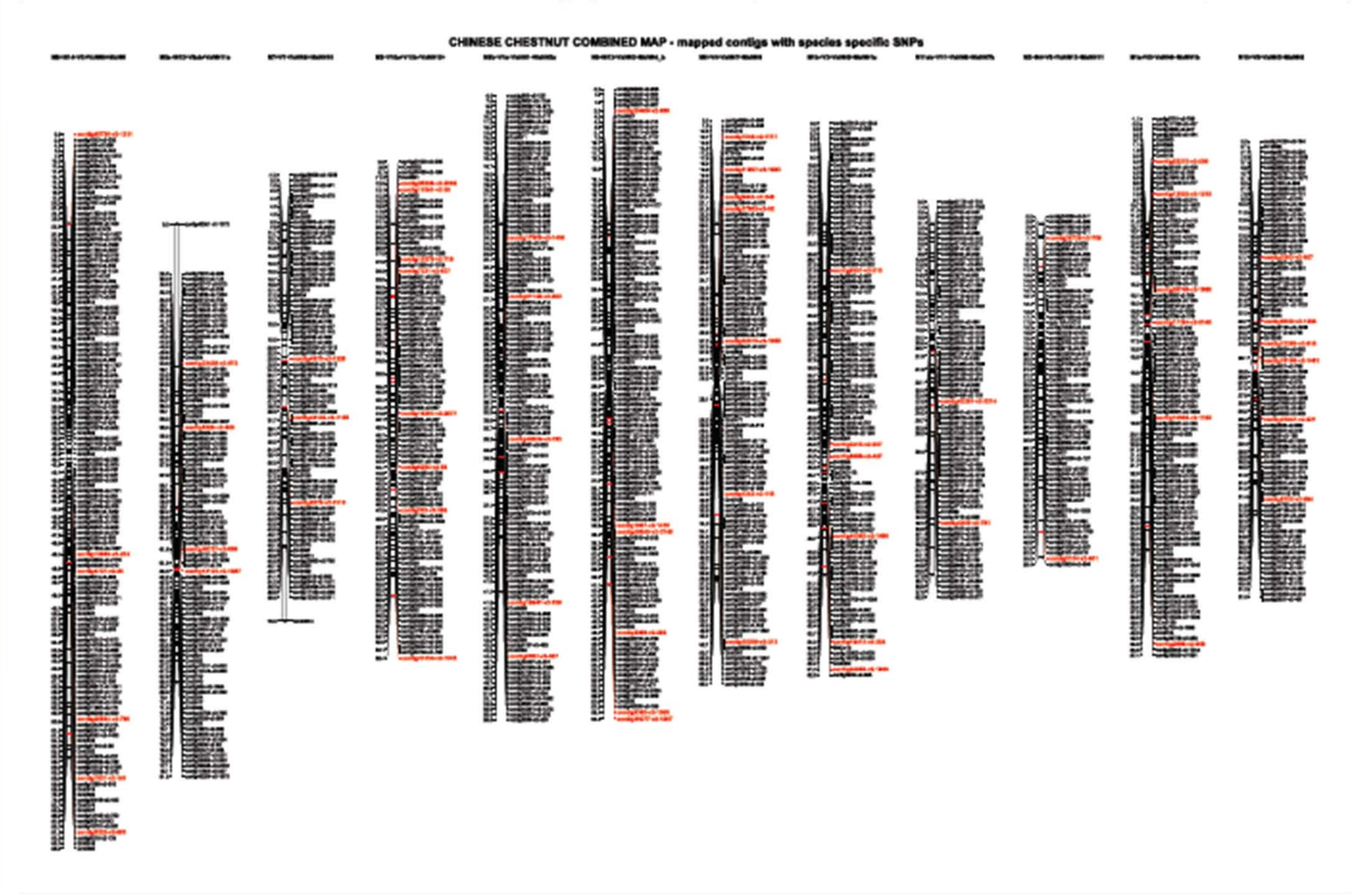
CHINESE CHESTNUT COMBINED MAP - mapped contigs with species specific SNPs

Agrobacterium-Mediated Transformation

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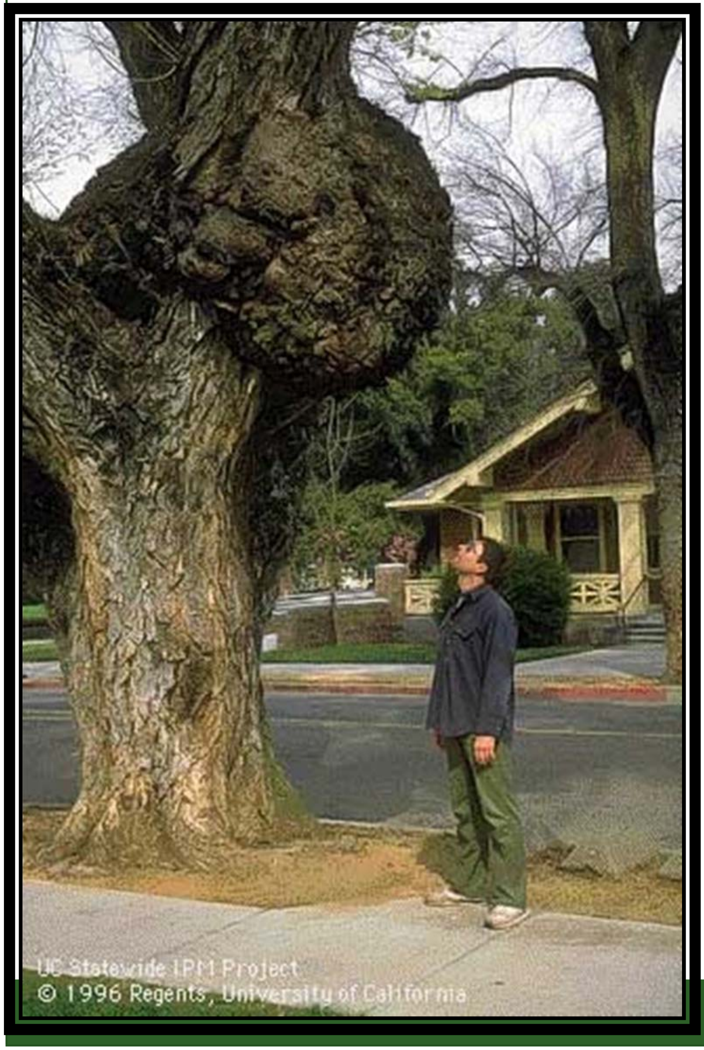
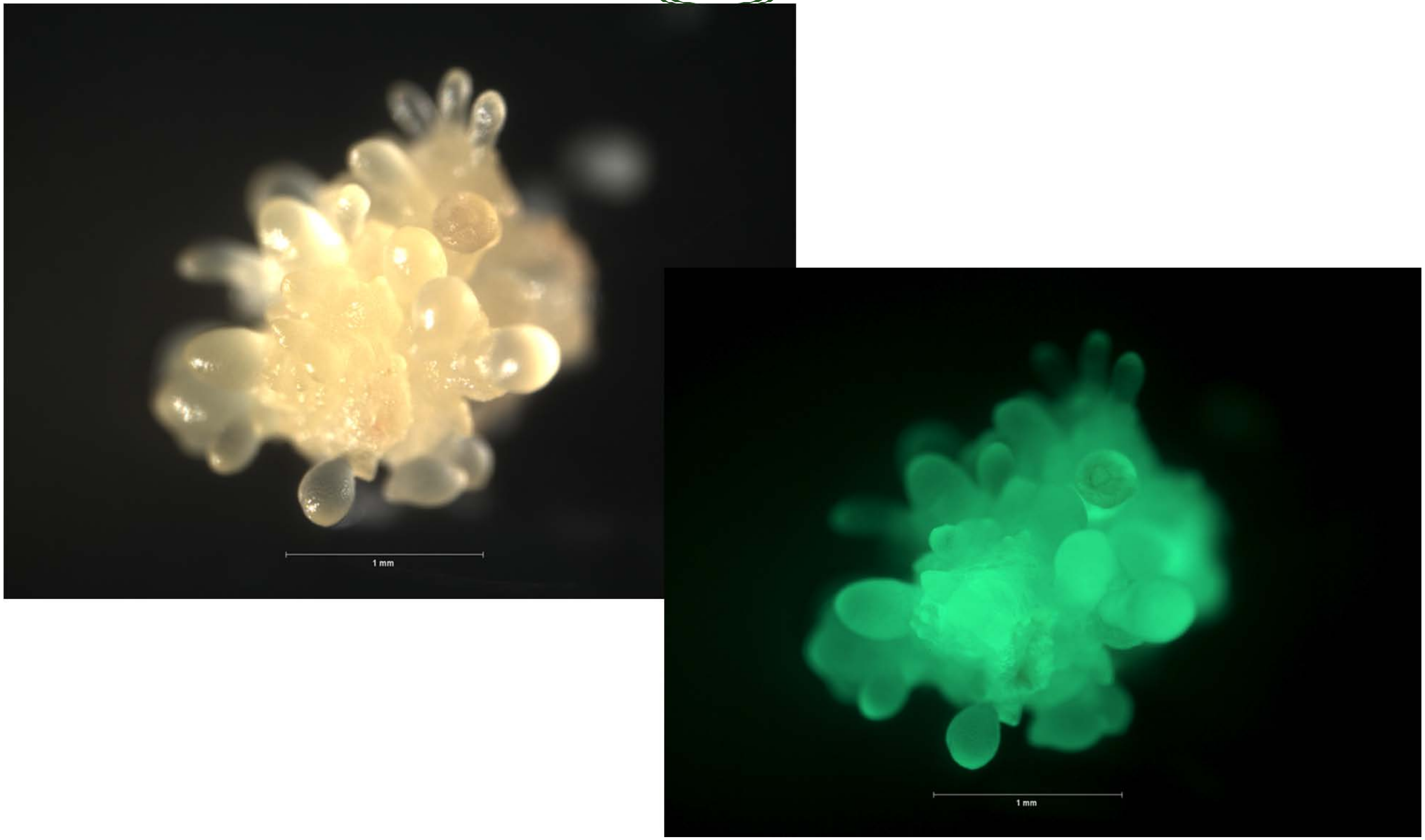

UC Statewide IPM Project
© 1996 Regents, University of California

Was Transformation Successful?




PR [low BA]
2382
WB 275-27 LP28
10/7/05
LM
22 4:33PM

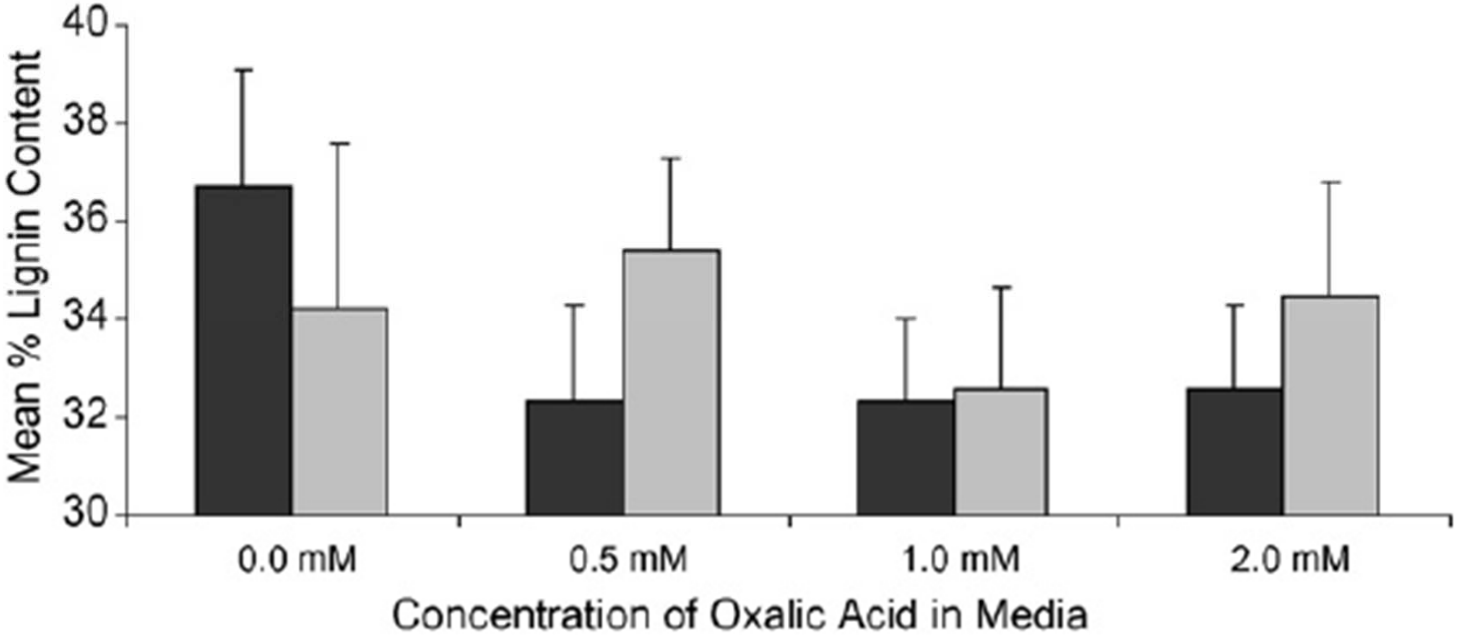


Fig. 4. Percent lignin content of untransformed (dark grey) and transformed (light grey) callus samples cultured on media with various oxalic acid concentrations. Each bar represents the mean of three tissue lines. Each bracket represents one standard error of that mean. An independent contrast revealed that untransformed callus tissue grown on media containing oxalic acid showed a significant decrease in lignin content ($p = 0.0024$) compared to samples grown in the absence of oxalic acid. Transformed tissues, however, did not exhibit a corresponding decrease in lignin content ($p = 0.9710$).

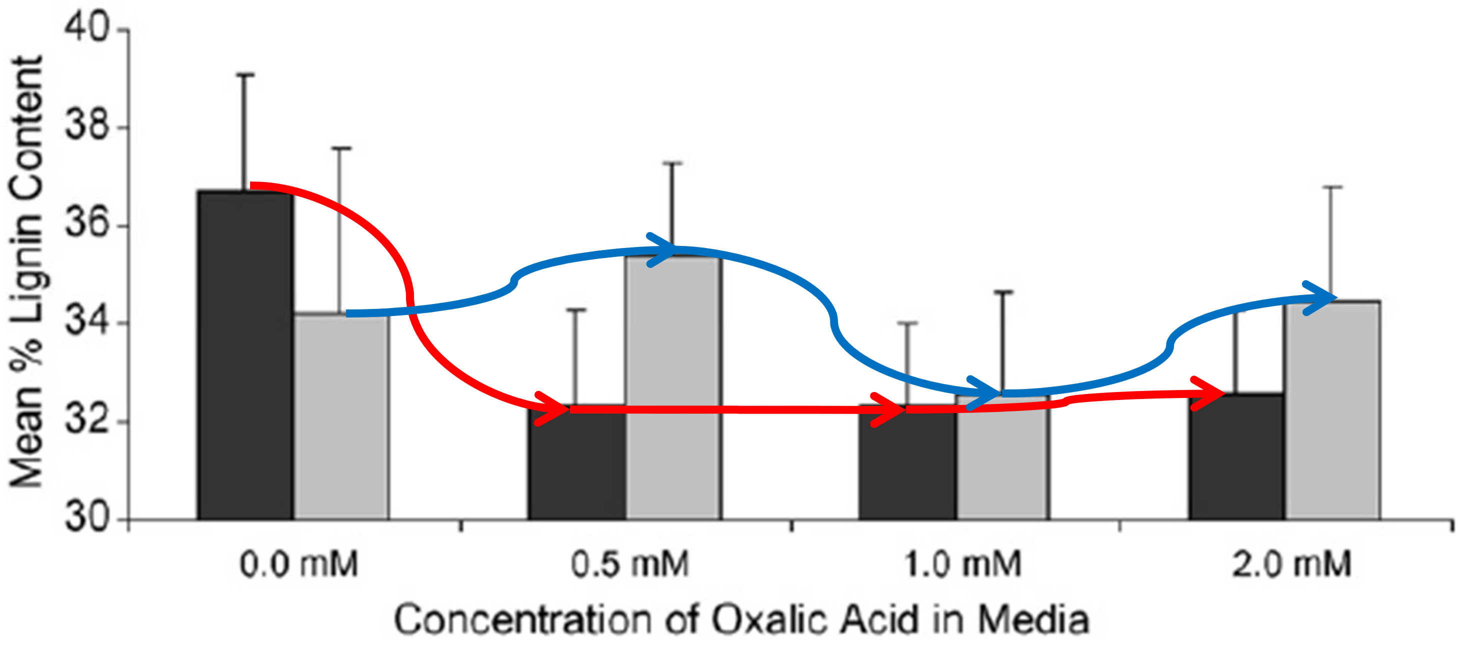


Fig. 4. Percent lignin content of untransformed (dark grey) and transformed (light grey) callus samples cultured on media with various oxalic acid concentrations. Each bar represents the mean of three tissue lines. Each bracket represents one standard error of that mean. An independent contrast revealed that untransformed callus tissue grown on media containing oxalic acid showed a significant decrease in lignin content ($p = 0.0024$) compared to samples grown in the absence of oxalic acid. Transformed tissues, however, did not exhibit a corresponding decrease in lignin content ($p = 0.9710$).

Tissue Culture → Pots

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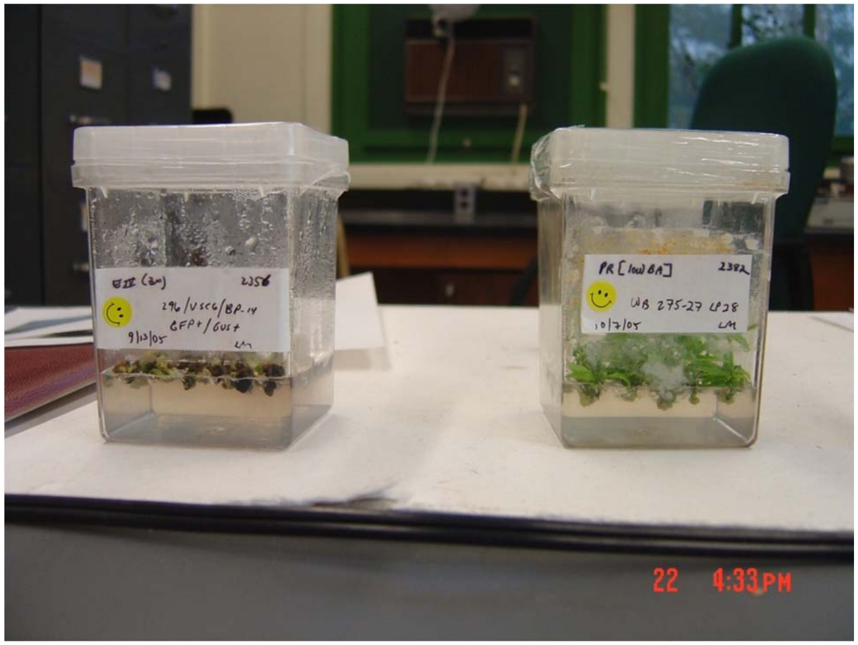

Pots → Field

- 400 -> 15


22 3:26 PM

Transgenic Outplantings

- Highly regulated
    - Cannot be allowed to release pollen
- Locations:
    - Lasdon Arboretum, Somers, NY
    - Lafayette Experiment Station, Syracuse, NY
    - Zoar Valley WMA, Zoar Valley, NY

TACF Annual Meeting - NY

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The American Chestnut Foundation

28th Annual Meeting

October 21-23, 2011

Beaver Hollow Conference Center

Java Center, New York

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