BINF 6350 ITSC 8350
Fall 2011
Biotechnology & Genomics Lab
Content 3

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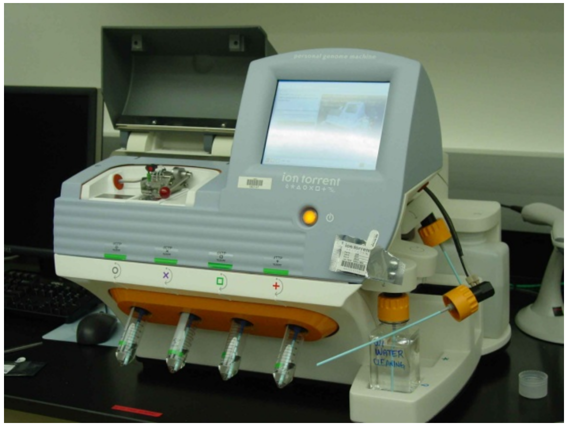
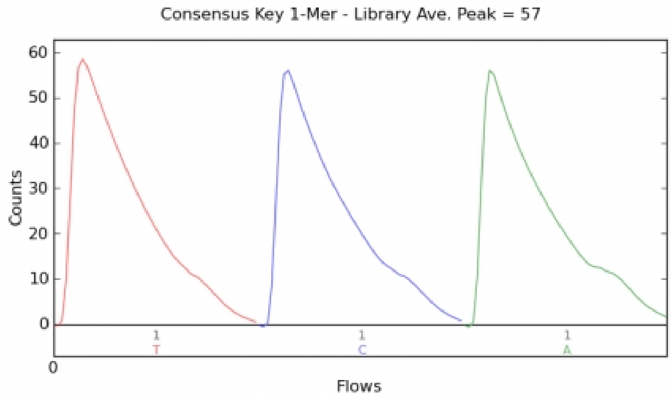
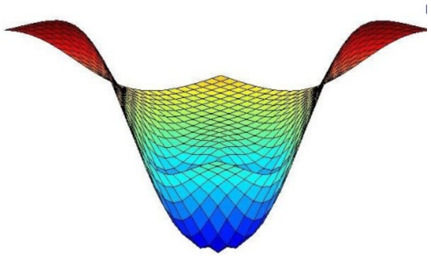
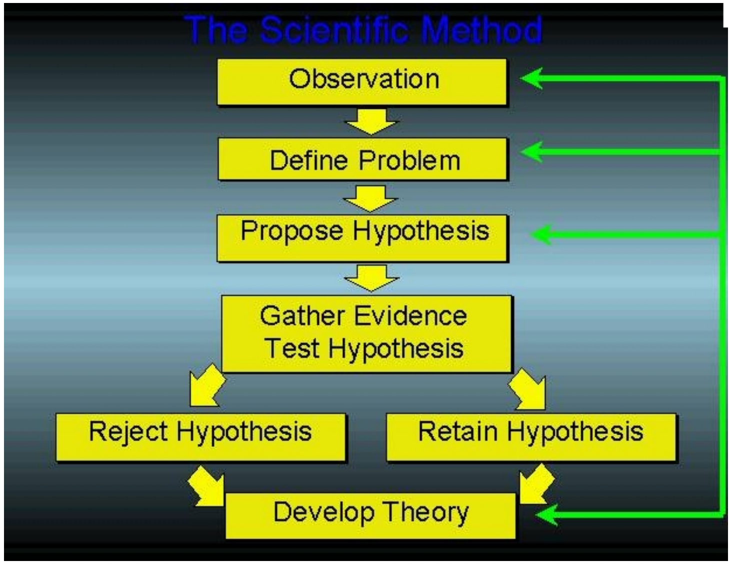






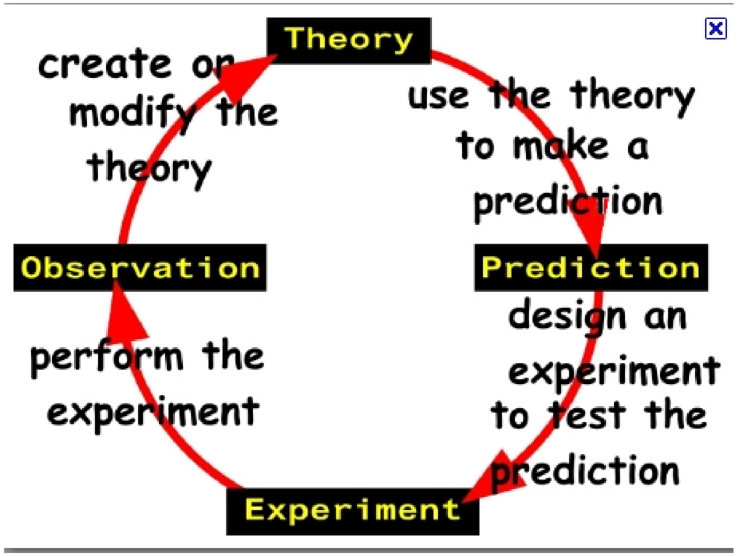
Topics

– The Scientific Method
– Experimental Design
  • For genomics



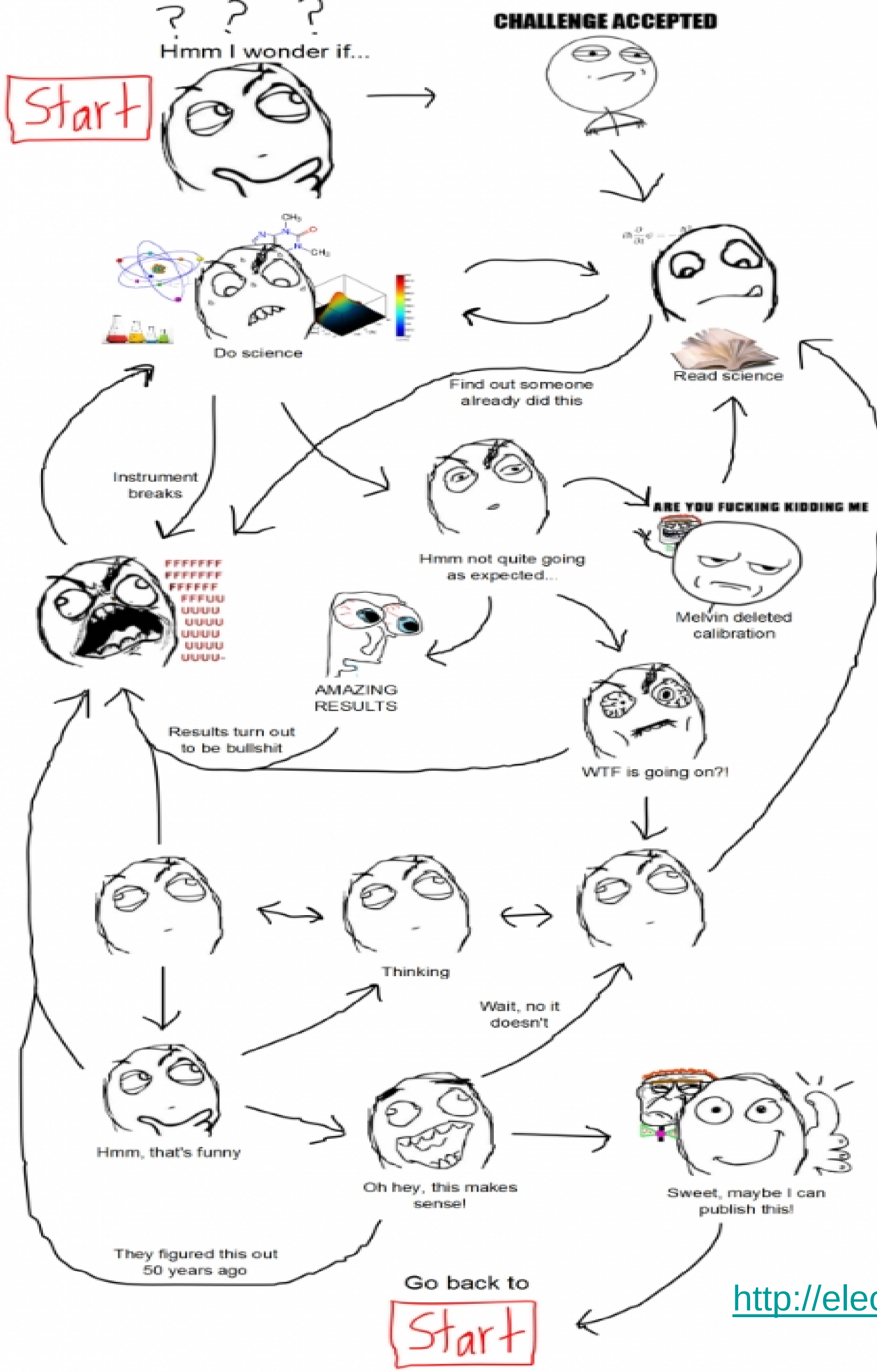
The scientific method formalizes a process of knowledge discovery.

- Observe
- Develop a ***testable hypothesis***
- Predict measurable outcomes
- Develop experiments to carry out tests
- Modify the hypothesis.
- Repeat until there are no discrepancies.



You have determined the conditions under which accurate predictions of outcomes will occur.

What really happens…..



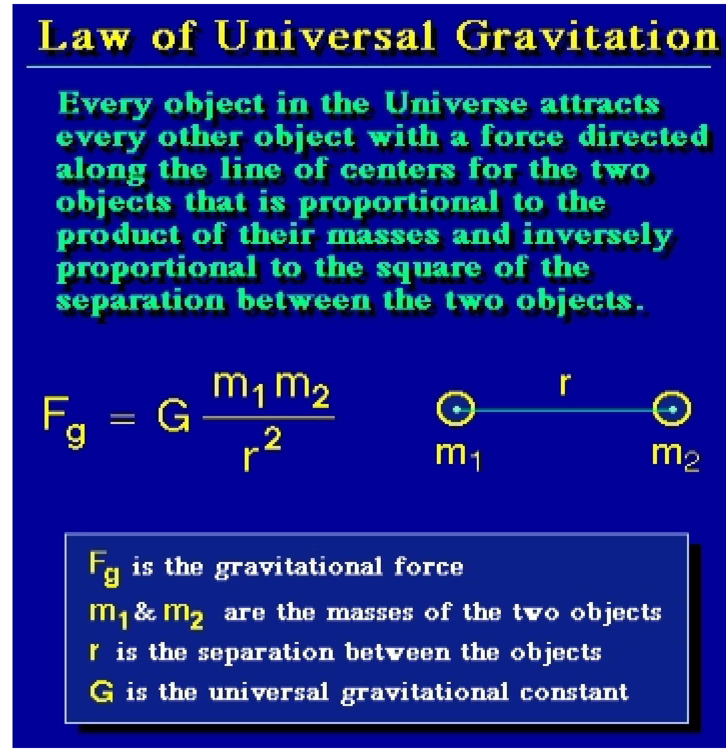
What if some of the evidence is contradictory?

1. Results are wrong → repeat, or redesign
2. Context is wrong, → important factors aren't controlled.

http://electroncafe.wordpress.com /

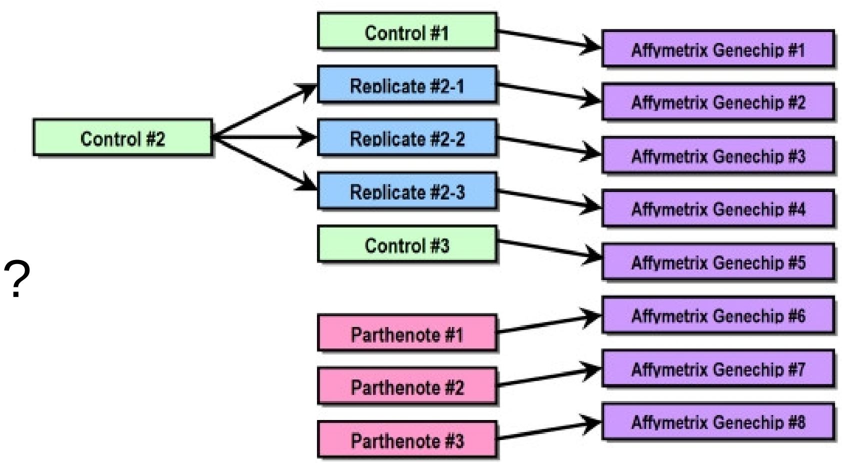
The obligatory reminder about what scientists mean by *laws* and *theories*.

- A Law tells you *what* happens: two objects having mass will accelerate towards one another.
  - A mathematically expressed relationship.
- A Theory tells you *why* it happens, the mechanism.
  - a framework for explaining a class of observations.
  - modifies over time as the range of applicable conditions is refined



Experiments should *answer* a question, although they usually lead to more questions.

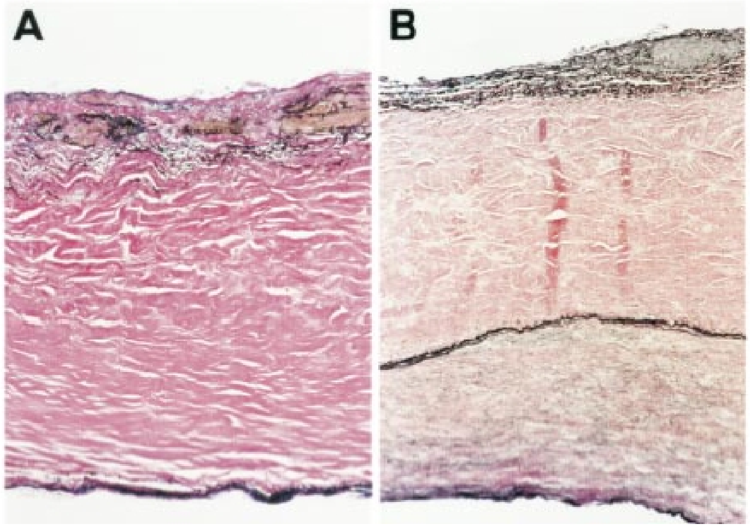
- Rigorous design and well-defined limits. Constraints:
  - Expense
  - Consequences of poor design?



- Common Questions
  - Boundaries: region where parameters influence the effect
  - Confidence level {in the observed effect} over some context.
  - Effective population: phenotype for subset of population that responds.
  - Interactions among factors

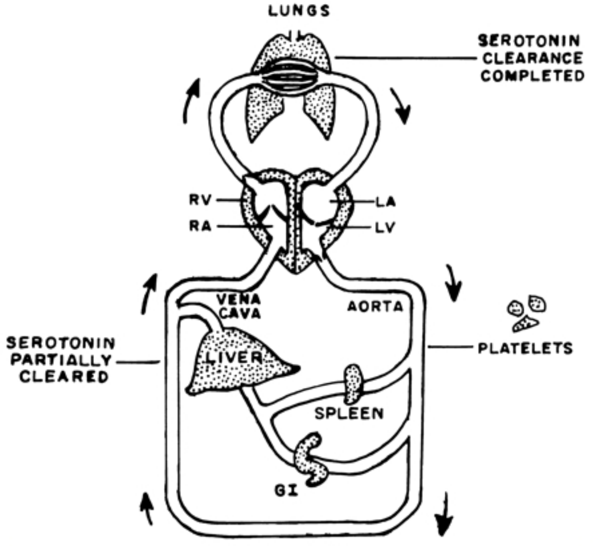
Improper sampling strategies and unexpected interactions frequently undermine conclusions.

- The Phen Fen debacle example
  - Sampling
    - Original study: 121 patients
    - Final application: 6 million
- Interaction: cocktail of 2 drugs: fenfluramine and phentermine
  - Each is safe alone and had been used for 10 years
  - Together a much larger proportion (from 0.0001% to 1%) developed damaged aortic valves.



Mechanisms of drug interactions and clearance explained the outcomes.

- Mode of Action
  - Epinephrine-like structures can cause hypertension (2%; not seen in animal models).
  - They increase serotonergic activity, including pulmonary vasoconstriction and vascular lesions.
  - Lungs clear most of serotonin, but these drugs inhibit that process.
    - As seen in point 2, high levels of blood serotonin cause pulmonary lesions.



Gene duplication is one mechanism for evolving the function of essential genes.

- Complicating factors:
  - Duplicated genes have varying levels of sequence similarity
  - Triggers for genes may be more significant than the sequences
  - Functional groups may have a very different context
- Sampling: genetic studies have to take into account the natural allelic diversity of an organism, and interactions between genes and between genes and the environment.
  - Control for genotype by using back-crossed lines.
  - Control for genotype by sampling a large number of lines
  - Control the environment so all lines experience the same conditions.
- So controls for biological variance
  - Minimize extraneous factors
  - Restrict range of variables of interest
  - Repeat treatments enough times to properly sample the distribution

Controlled crosses in plants

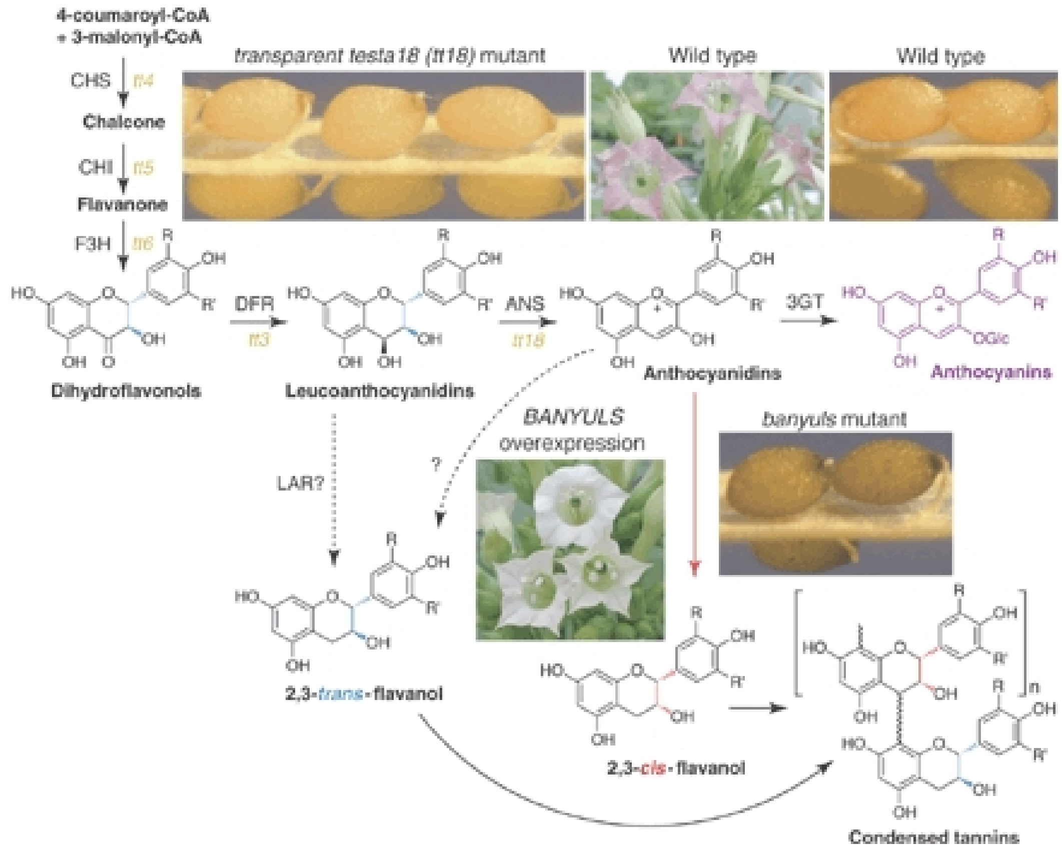
- Near isogenic lines
- Recombinant inbred population (RIL)
- P, F1, F2, etc.

- How is this different from natural genetic variation?
- Phaseolus and Glycine flower color genetics

A common pathway controls seed coat color in Glycine max and Phaseolus sp.

- What do we mean by a pathway?
  - It uses the same starting material and chemicals
  - It has the same number of enzymes, and they

    operate on small molecules (chemical reactants) in the same order.



P. vulgaris

Common bean

Seed Coat Color Genes (*P. vulgaris)*

- T - totally colored seeds no effect on flower color
- t - white flower and required for partially colored seed coats (epistatic to V for violet flower)

- P - basic color gene - required to express pigment
  - P w/o color gene is colorless
- p - white seed coat and flower
- p^{mic} - white microphyle stripe on seed; violet flowers with no pattern
- p^{hbw} - stippled seed coat; violet flowers with lower <u>h</u>alf of the <u>b</u>anner petal <u>w</u>hite
- p^{stp} - stippled seed coat; white flowers with violet banner tip and pale violet periphery on wing petals

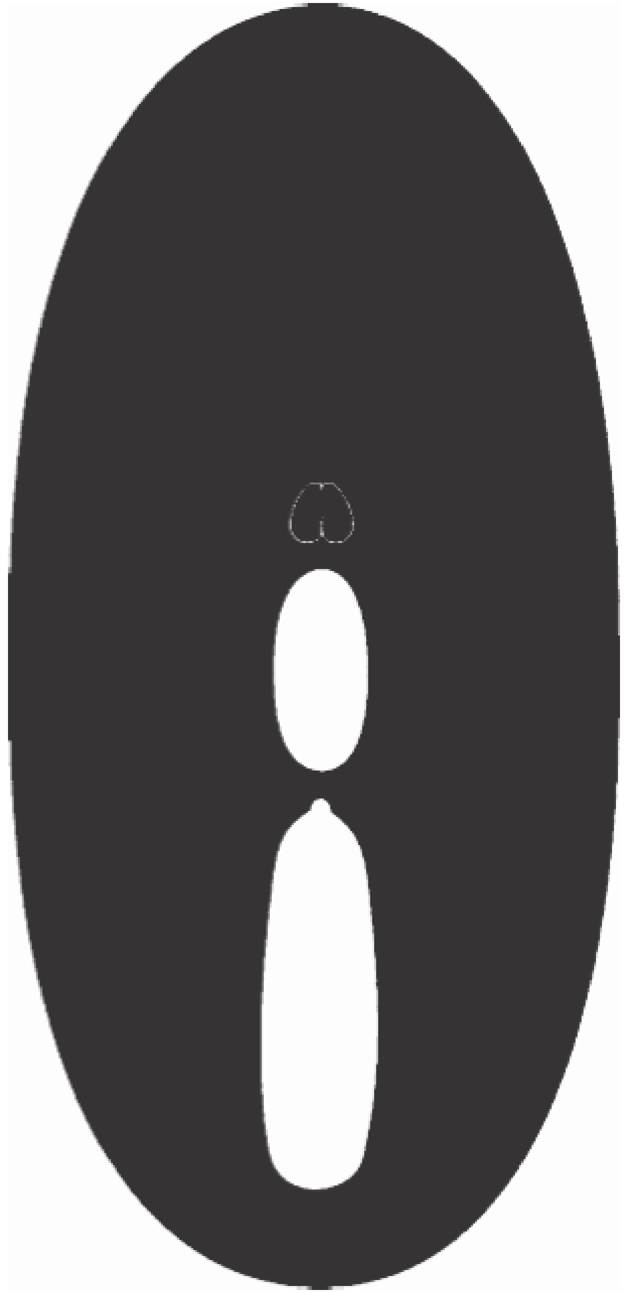


Fig. 1. Ventral view of the common bean seed coat pattern, white micropyle stripe, expressed by p^{mic}.

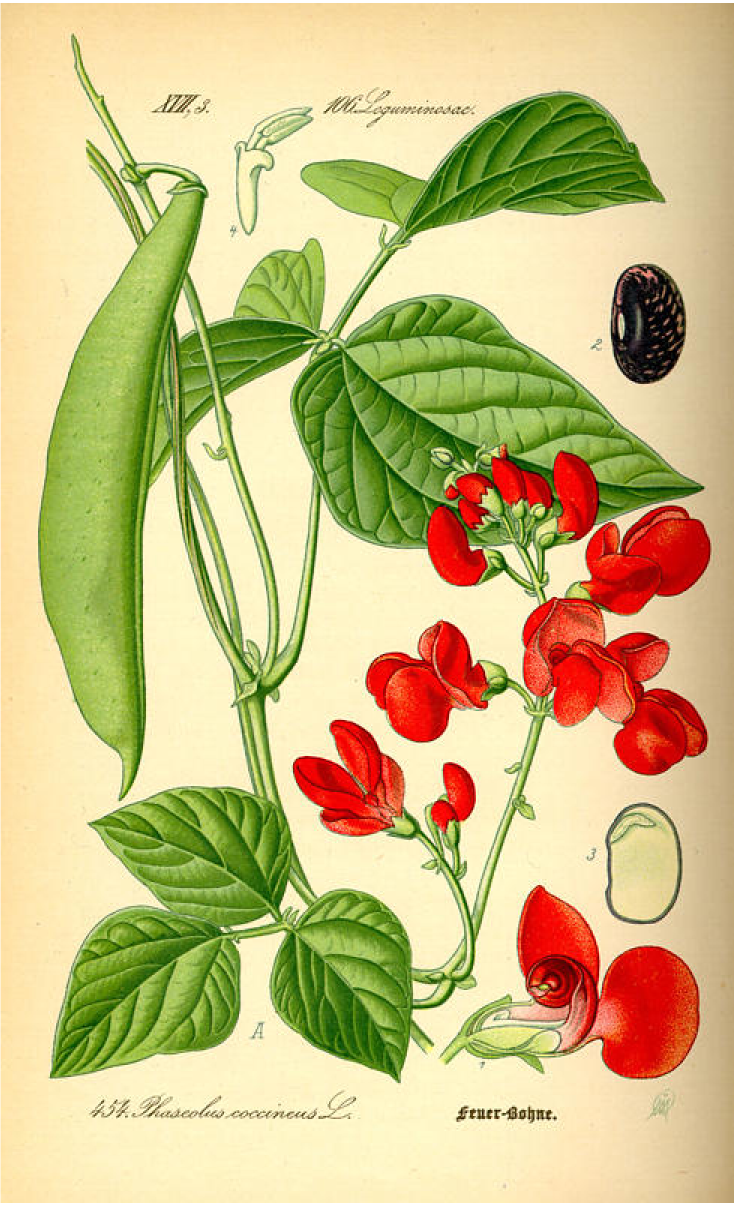
Seed Coat Color Genes (*P. vulgaris*)

- C, D, J - color genes expressed in presence of P
- G, B, V, Rk - modifying genes (intensifying effect)

- B - interacts with nearly all combinations of genes for seed coat color; regulates the production of precursors of anthocyanins in seed coat color pathway

- V (T P B) - expressed bishops violet flower and black seed coat
- v (T P B) - white flower mineral brown seed coat
- v^{lae} (T P) - gives pink flowers and rose stem
- V^{wf} - derived from *P. coccineus* with the properties of V, but pleiotropic effect of white flower color, no effect on black seeds

- Wb (T P B) - derived from *P. coccineus,* white banner petal and wings of pale violet



P. coccineus Painted Lady

Seed Coat Color Genes (*P. coccineus)*

- Painted Lady - bicolor flowers with a vermilion banner petal and pure white winges
- *Sal* - salmon red flower (required for vermilion)
- *Am* - probably required for vermilion

- Two colored flowers
  - Darker standard than wings (Painted Lady)
  - White banner type (Wb)

- Proposed inheritance of Painted Lady flowers
  - *bic* - recessive trait (bicolorata)

- What was their original hypothesis?
  - Test of *bic* is actually an allele of *Wb*

Table 1. Genetic tester stocks in common bean used in this investigation with Plant Introduction (PI) accession numbers and phenotype description.

Code	Genetic stock	PI number	Seed coat and flower description
P_1	5-593	608674	Black seed, purple flower
P_2	*v* BC_3 5-593	608679	Mineral brown seed, white flower
P_3	V^{wf} BC_3 5-593	608710	Black seed, white flower
P_4	*wb* BC_3 5-593	635121	Black seed, *white banner* petal
P_5	*t Z Fib* BC_3 5-593[z]	608701	Expansa with *fibula* arcs seed, white flower
P_6	p^{mic} BC_3 5-593	608709	Black seed with white micropyle stripe, purple flower

[z]This stock expresses the partly colored pattern expansa with fibula arcs, where *Fib* changes self-colored to expansa and suppresses color in the corona zone.

- 5-593
  - Homozygous dominant for all color determing loci
    - T P [C r] Z J G B V Rk Asp
  - Black seed coats
    - J Asp
  - Bishop violet flowers

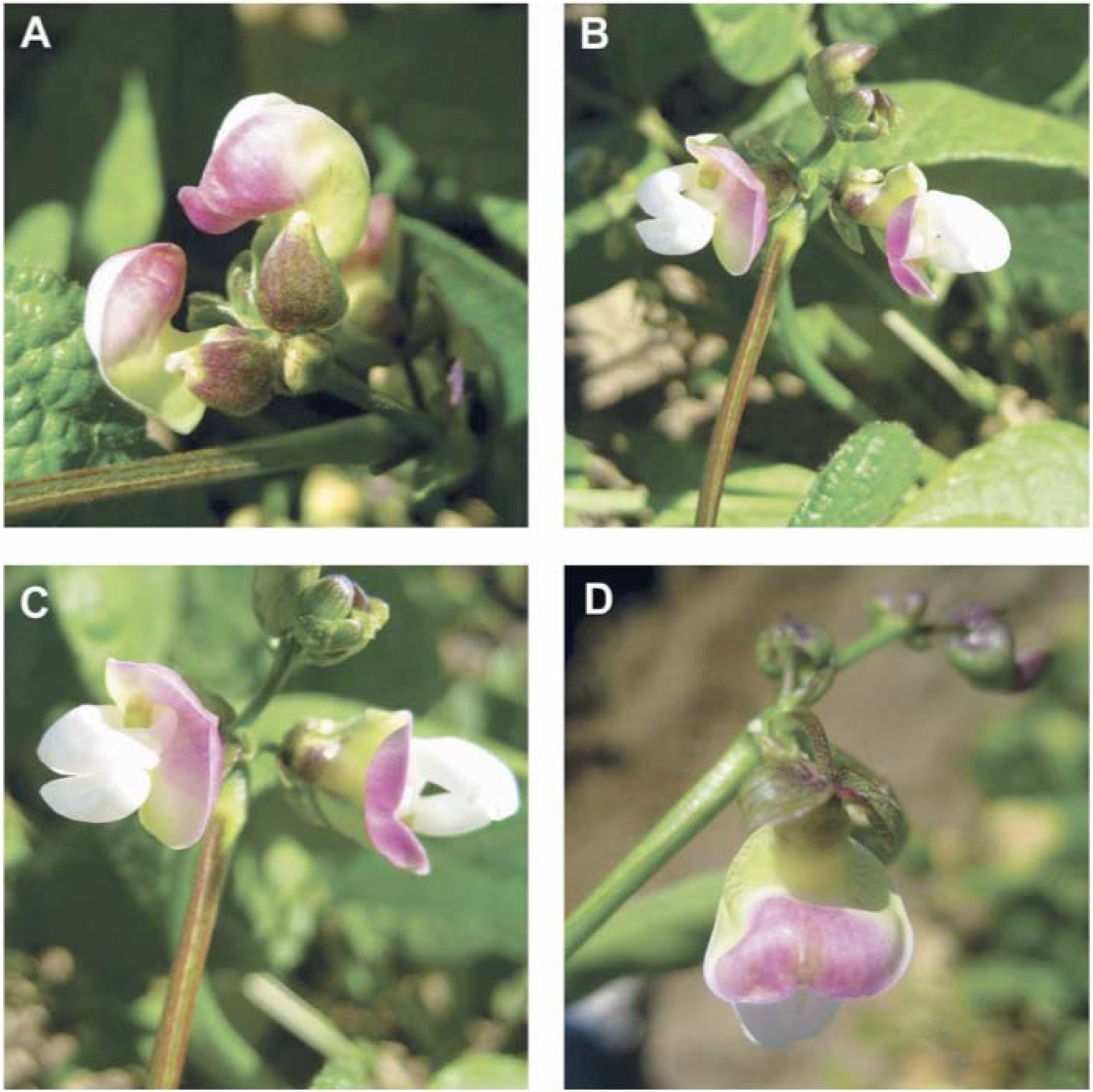

A
B
C
D

Table 2. Segregation in common bean for bicolor flower color pattern and seed coat color in the F_2 and F_3 (from 35 randomly selected F_2 parents) from the cross of bicolor flower selection line 33 (*bic V*) × purple flower 5-593 (*Bic V*).

Generation	Progenies (no.)[z]	Parental phenotype		Segregation classes			
		Seed	Flower	Black seed, purple flower *Bic*/– (no. observed)	Brown seed,[y] bicolor flower[x] *bic*/*bic* (no. observed)	χ^2(3:1)	*P*
F_2	1	Black	Purple	55	13	1.255	0.26
F_3	6	Black	Purple	158			
F_3	17	Black	Purple	309	104	0.007	0.93
F_3	12	Brown[y]	Bicolor[x]		254		

[z]For the data 6, 17, and 12, χ^2 (1:2:1) = 2.086, *P* = 0.35.
[y]This brown color is darker and more olive than mineral brown, which is expressed by *P C J G B v*, and this seed coat color was never reported previously.
[x]Bicolor flowers have purple (bishops violet) banner petal and white wings (see Fig. 3).

Table 3. A third backcross of the bicolor (tentatively *bic*) stock 179c to 5-593, allelism test crosses between the *bic* flower stock 179c and five genetic testers, and two additional test crosses between *wb* BC_3 5-593 and two genetic testers in common bean.

Test cross[z]	Phenotypic segregation classes with genetic hypothesis (no. observed)				Ratio tested	χ^2	*P*
$P_1 \times$ 179c (*Bic* × *bic*)	Black seed, purple flower *Bic*/– (342)	Brown seed, bicolor flower *bic*/*bic* (98)			3:1	1.746	0.19
179c × P_2 (*bic* × *v*)	Black seed, purple flower *Bic*/– *V*/– (184)	Brown seed, bicolor flower *bic*/*bic* *V*/– (96)	M.B. seed,[y] white flower –/– *v*/*v* (96)		9:3:4	12.842	0.002
179c × P_3 (*bic* × V^{wf}) [expected]	Black seed, purple flower *Bic*/– *V*/– (127) [102.375]	Black seed, white flower *Bic*/– V^{wf}/V^{wf} (23) [34.125]	Brown seed, bicolor flower *bic*/*bic* *V*/– (27) [34.125]	Brown seed, white flower *bic*/*bic* V^{wf}/V^{wf} (5) [11.375]	9:3:3:1[x]	14.611	0.002
$P_4 \times$ 179c (*wb* × *bic*)	Black seed, purple flower *Bic*/– *Wb*/– (191)	Black seed, white banner *Bic*/– *wb*/*wb* (53)	Brown seed, bicolor flower *bic*/*bic* *Wb*/– (59)	Brown seed, white flower *bic*/*bic* *wb*/*wb* (27)	9:3:3:1	3.533	0.32
$P_5 \times$ 179c (*t* × *bic*)	Black seed, purple flower *Bic*/– *T*/– (164)	Black seed, white flower *Bic*/– *t*/*t* (70)	Brown seed, bicolor flower *bic*/*bic* *T*/– (40)	Brown seed, white flower *bic*/*bic* *t*/*t* (20)	9:3:3:1[w]	8.319	0.04
179c × P_6 (*bic* × p^{mic})	Black seed, purple flower *Bic*/– *P*/– (172)	Black seed with white micropyle stripe, purple flower *Bic*/– p^{mic}/p^{mic} (51)	Brown seed, bicolor flower *bic*/*bic* *P*/– (60)	Brown seed with white micropyle stripe, bicolor flower *bic*/*bic* p^{mic}/p^{mic} (13)	9:3:3:1	2.547	0.47
$P_4 \times P_5$ (*wb* × *t*)	Black seed, purple flower *Wb*/– *T*/– (74)	Black seed, white banner *wb*/*wb* *T*/– (26)	Expansa seed,[v] white flower *wb*/*wb* *t*/*t* (32)		9:3:4	0.094	0.95
$P_4 \times P_6$ (*wb* × p^{mic})	Black seed, purple flower *Wb*/– *P*/– (72)	Black seed with white micropyle stripe, purple flower *Wb*/– p^{mic}/p^{mic} (33)	Black seed, white banner *wb*/*wb* *P*/– (30)	Black seed with white micropyle stripe, white banner *wb*/*wb* p^{mic}/p^{mic} (8)	9:3:3:1	2.790	0.43

[z]The genetic tester stocks referenced by P with subscript numbers are listed in Table 1.
[y]M.B. = mineral brown seed coat color with genotype *T P C J G B v*.
[x]For the test cross $P_3 \times$ 179c, the orthogonal contrasts are as follows: $\chi^2_{Bic} = 5.341$, $P = 0.021$, $\chi^2_V = 8.974$, $P = 0.005$; $\chi^2_L = 0.296$, $P = 0.59$.
[w]For the test cross $P_5 \times$ 179c, the orthogonal contrasts are: $\chi^2_T = 4.939$, $P = 0.03$; $\chi^2_{Bic} = 3.306$, $P = 0.07$; $\chi^2_L = 0.074$, $P = 0.79$.
[v]Expansa = expansa with fibula arcs (Fig. 2).

Flower and seed color in soybean

- T, W1, I, R O – control seed coat color
- W1, W3, W4, Wm, Wp – control flower color
- T, Td – control pubescence color

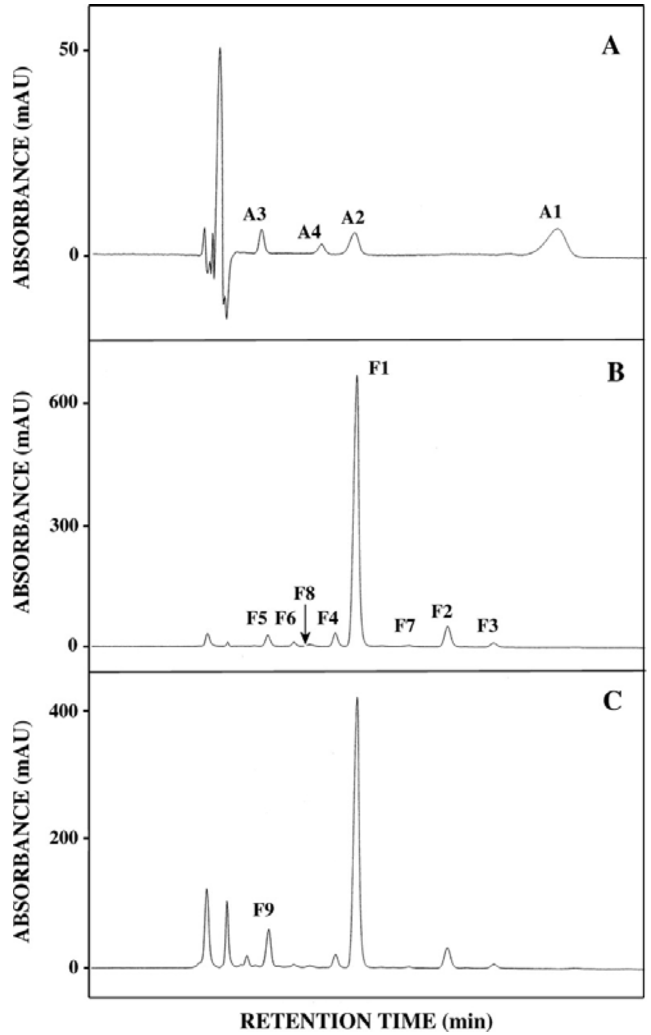
- T = flavonoid 3’-hydroxylase (presumably)
- W1 = flavonoid 3’5’-hydroxylase (presumably)
  - Pleiotropic effect on flower and hypocotyl color
- W3 & W4 – epistatic under W1 genotype
  - W3W4 dark purple
  - W3w4 dilute purple (purple throat)
  - w3W4 purple
  - W3w4 near-white
- W4 encode DFR?
- I = chalcone synthase

Analysis of Flavonoids in Flower Petals of Soybean Near-isogenic Lines for Flower and Pubescence Color Genes

Table 1
Soybean NILs for flower or pubescence color genes used in this study

Line name	Line designation	Flower color	Pubescence color	Donor	Genotype
Clark	—	Purple	Tawny	—	*W1W1 w3w3 W4W4 WmWm TT TdTd*
Clark-*w1*	L63-2373	White	Tawny	T139	*w1w1 w3w3 W4W4 WmWm TT TdTd*
Clark-*w4*	L68-1774	Near-white	Tawny	Laredo	*W1W1 w3w3 w4w4 WmWm TT TdTd*
Clark-*W3w4*	L70-4422	Dilute purple	Tawny	Laredo	*W1W1 W3W3 w4w4 WmWm TT TdTd*
Clark-*t*	L67-483	Purple	Gray	Higan	*W1W1 w3w3 W4W4 WmWm tt TdTd*
Clark-*td*	L66-260	Purple	Near-gray	PI 91.160	*W1W1 w3w3 W4W4 WmWm TT tdtd*
Harosoy	—	Purple	Gray	—	*W1W1 w3w3 W4W4 WmWm tt TdTd*
Harosoy-*wm*	T235	Magenta	Gray	—	*W1W1 w3w3 W4W4 wmwm tt TdTd*
Harosoy-*T*	L66-707	Purple	Tawny	Clark	*W1W1 w3w3 W4W4 WmWm TT TdTd*

HPLC chromatogram of anthocyanins (A), flavonols (B), and dihydroflavonol (C) extracted from flower petals of Clark.



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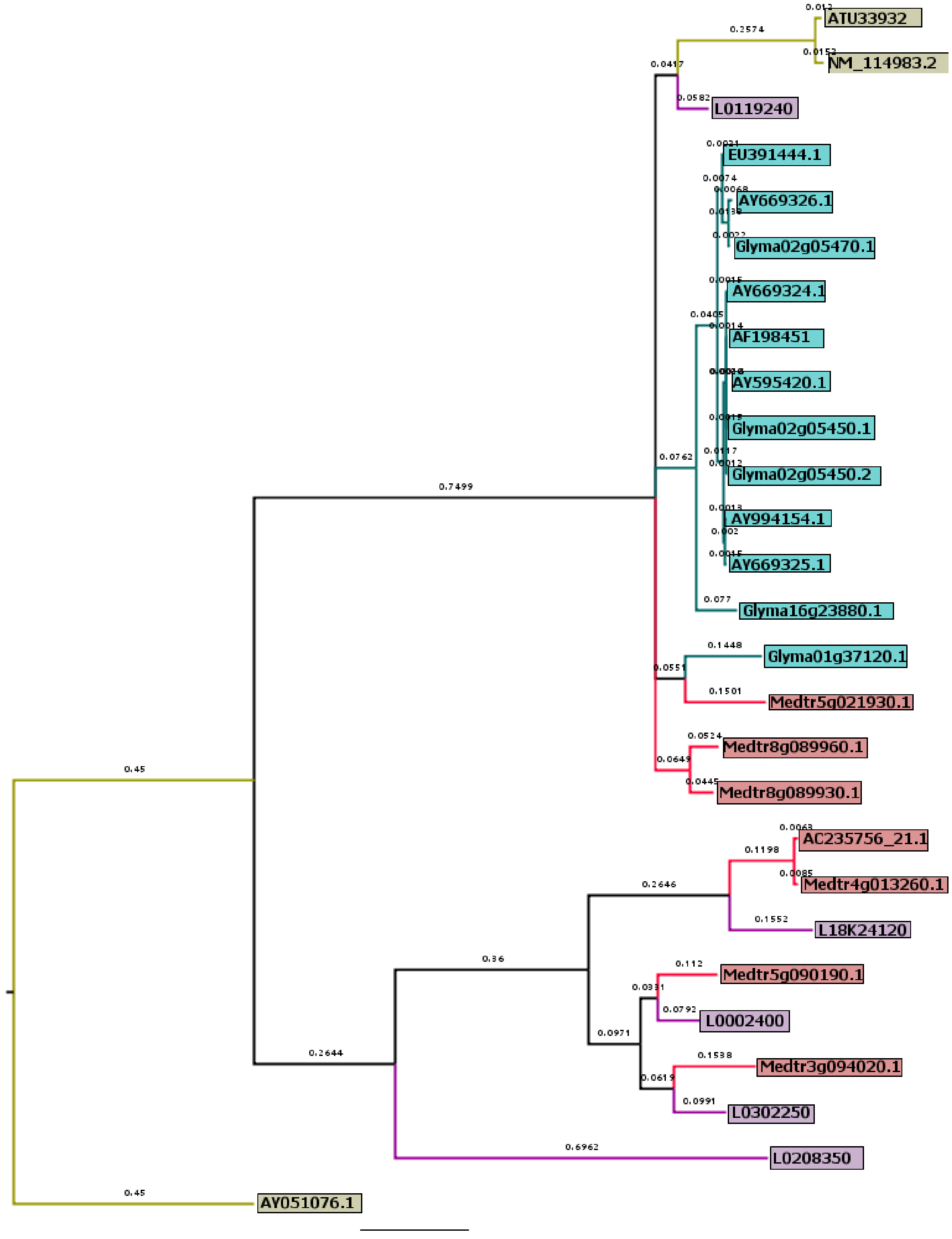

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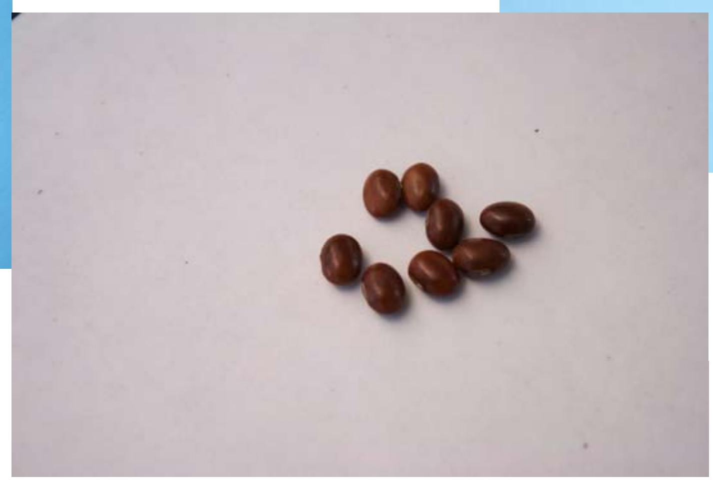

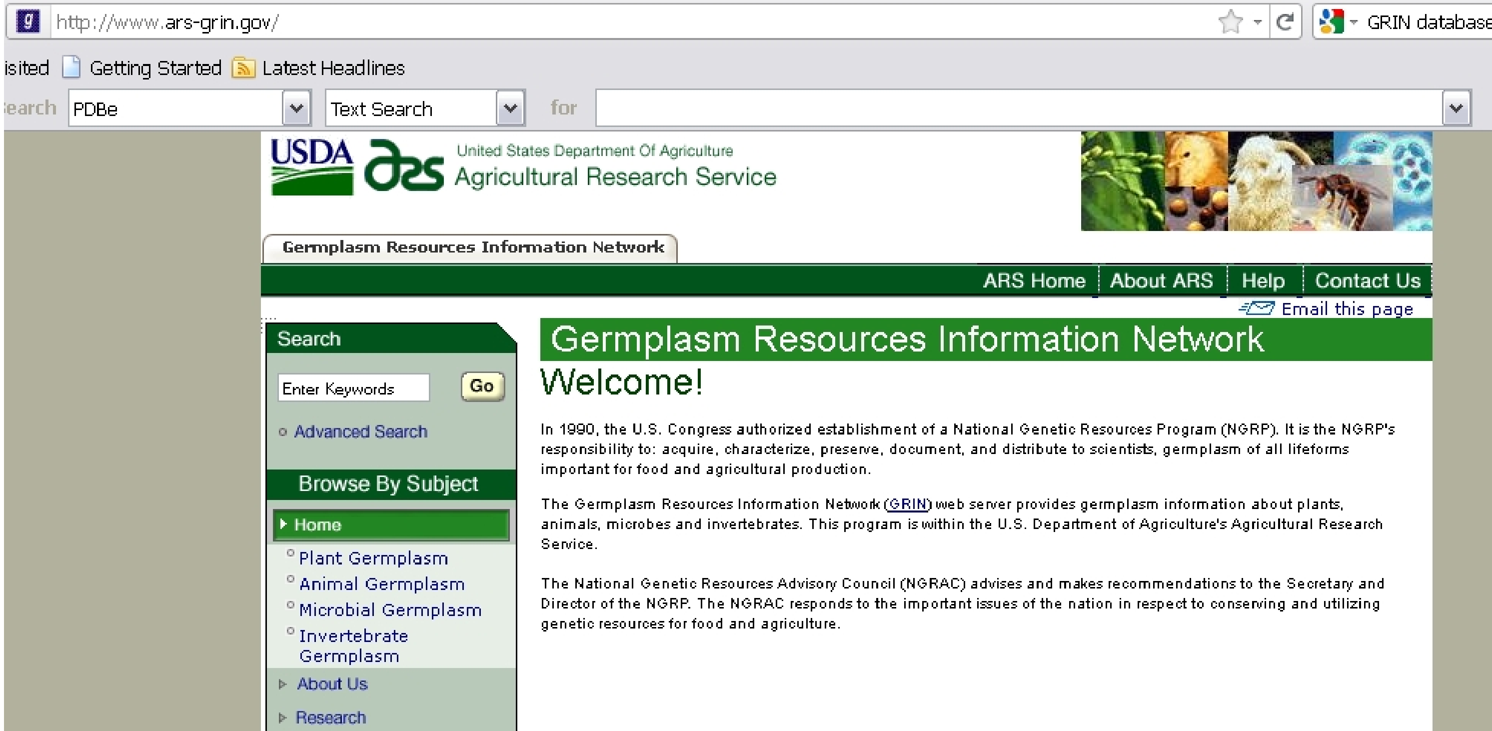

1. "A New Gene, *bic, with Pleiotropic Effects* (with *T P V) for Bicolor Flowers and Dark Olive Brown Seed Coat in Common Bean"* Mark J. Bassett, Phillip N. Miklas, Amer. Soc. Hort. Sci. 132(3):352-356.2007.

2. "*Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology*", Brenda Winkel-Shirley, Plant Phys 2001. 126: 485-493

3. "*Flower color variation: A model for the experimental study of evolution*", Michael T. Clegg and Mary L. Durbin, PNAS 97(13) 7016-7023 (2000).

Image: GRIN database http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl