B3 Camp Lab Activities - and Results

B3 Summer Science Camp
at Olympic High School

June 2016

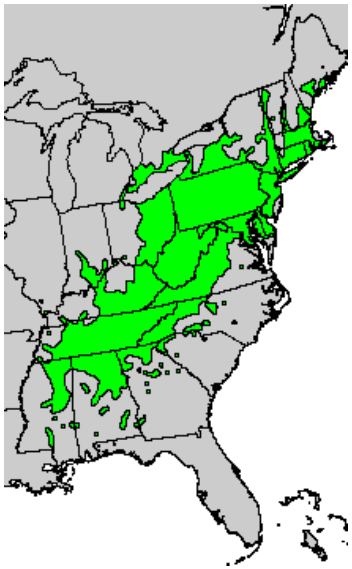
Dr. Jennifer Weller

Scientific process

- Identify an important and interesting problem.
- Experimental design/plan
- Decide on collecting and documenting techniques
- Processing samples – purify the {DNA}
- Transforming the DNA into signals
- Interpreting data and publishing the results

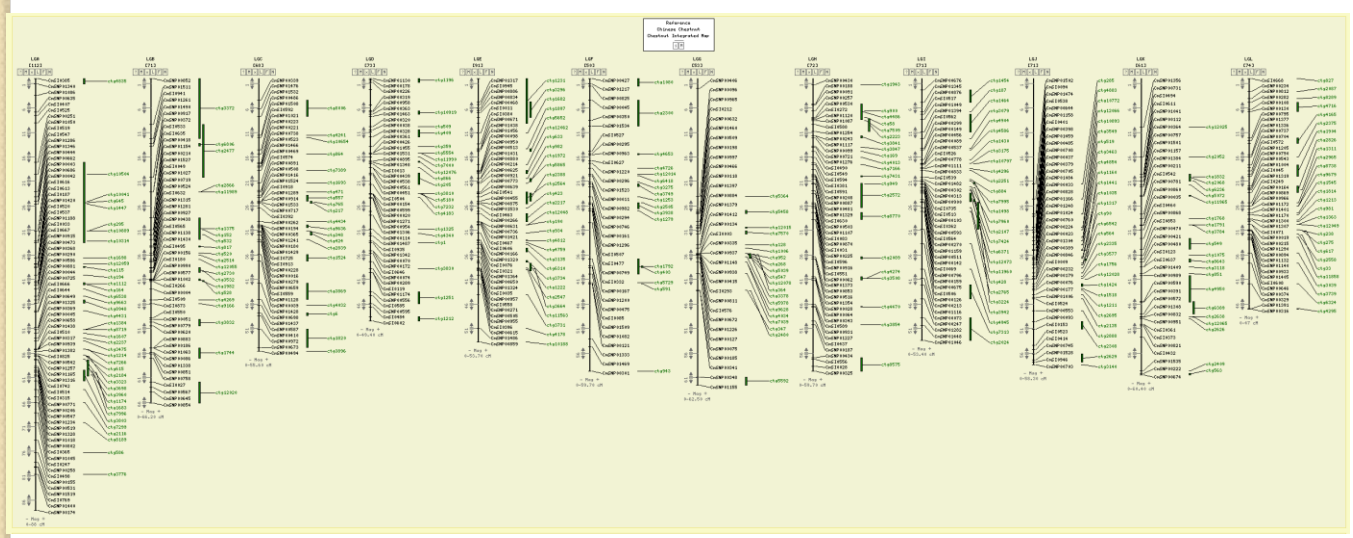
Identify an important problem

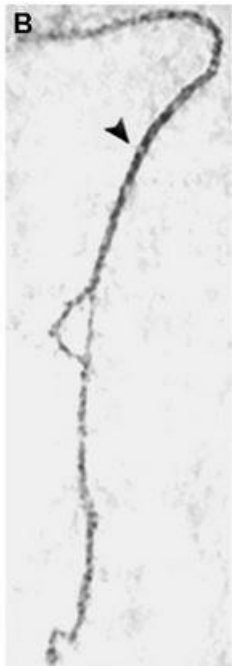
- Background
  - American Chestnuts have almost completely vanished - the disease-causing fungus has been identified and isolated
  - Chinese Chestnuts usually survive



Breeding Approaches

- Approach: Can you create crosses (hybrids of the two species)?

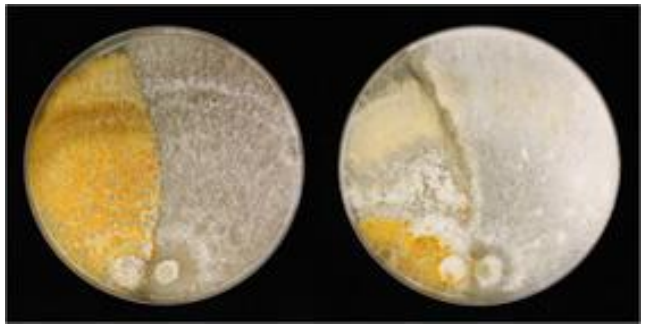


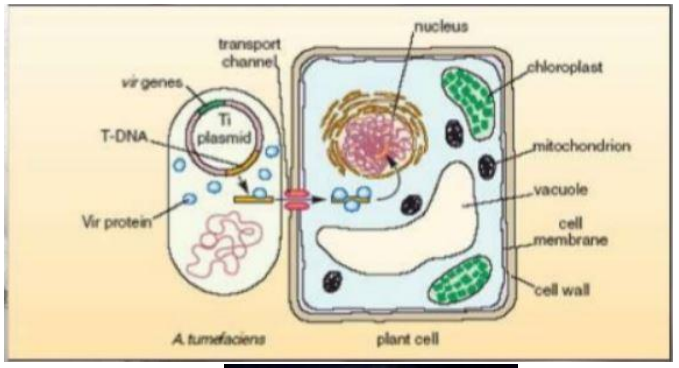


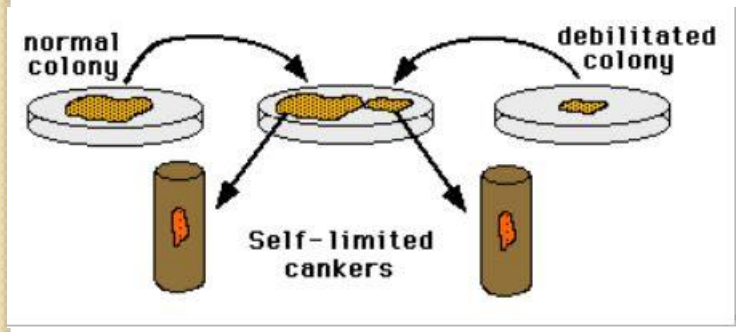
Polyploidy in plants; http://waynesword.palomar.edu/hybrids1.htm

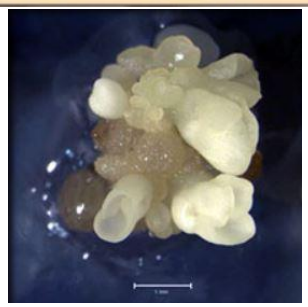
Biotech/biocontrol approaches

- Approach: Can you use biotechnology or biocontrol approaches?





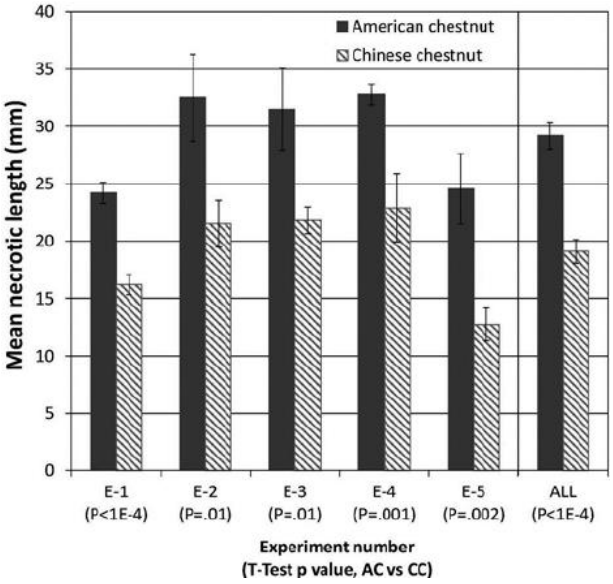


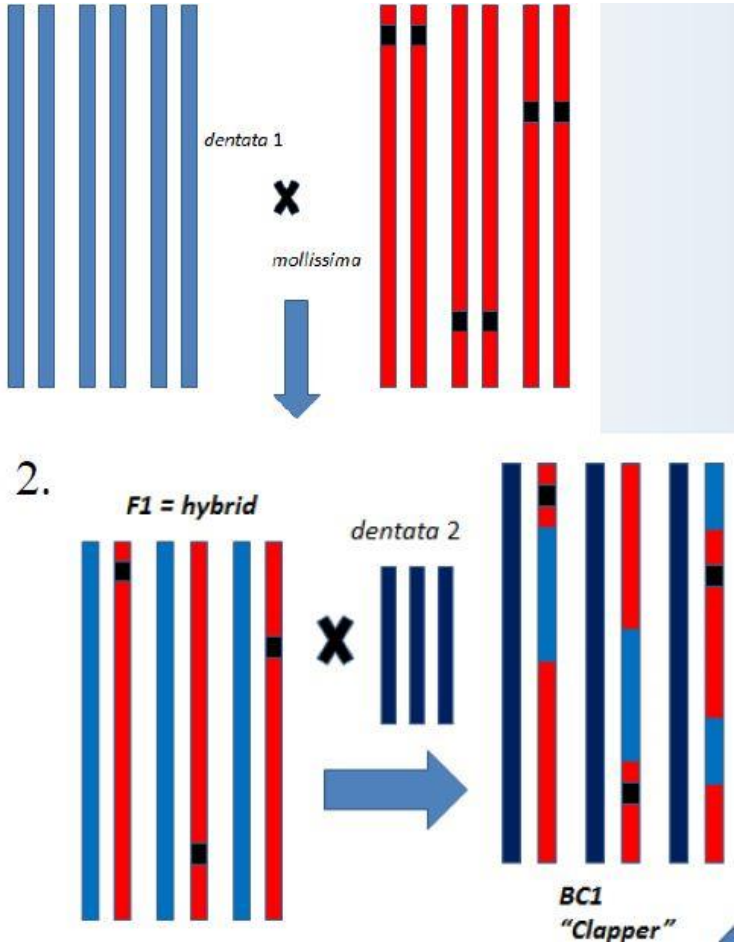


http://www.esf.edu/chestnut/tissue-culture.htm#

Conducting the experiments

- Sampling: For the breeding experiments
  - How many of the hybrids are disease resistant, and how much resistance is there (effect size)?
  - How many genes are we looking for?

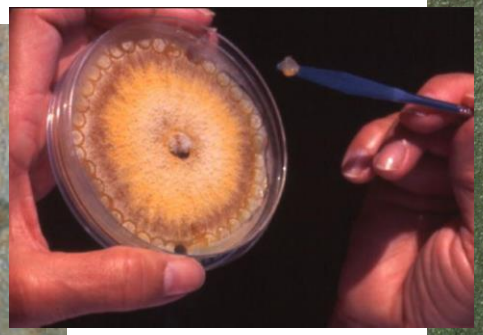




LaBonte & Woeste, PAG 2015

What type of data will help you find an answer?

- Test hybrid trees
  - Directly – a 'challenge' test.

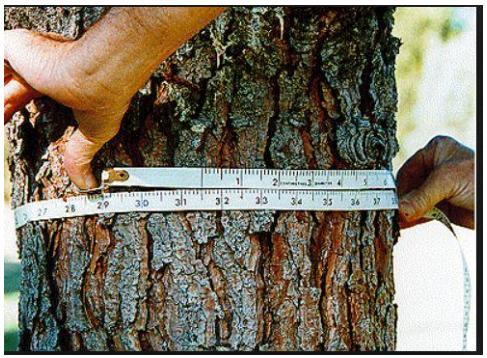
(6-18 months later)

- Indirectly – e.g. looking at the DNA
  - Molecular markers (patterns)
  - DNA sequence (all or part)
    - All: 12 chromosomes, 1mt, 1 cp (whole genome)
    - Targeted regions - specific resistance genes

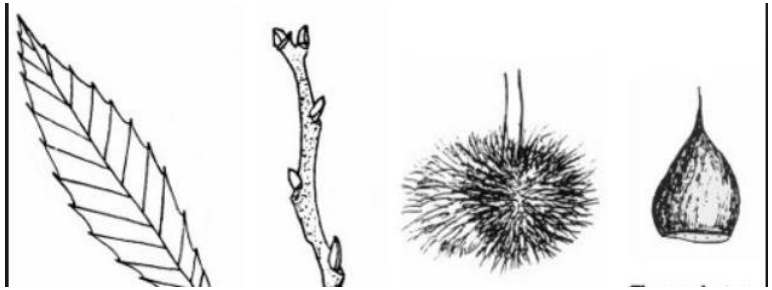
Collecting Considerations

- Environment
  - Controlled
  - Natural
- Level of variation expected
  - How many samples will you need?
- Phenotype measurements
  - Size, shape, mass, color
- Molecular measurements – requires tissue
  - Type of tissue
  - Proper Storage of the tissue

General Field tools



- Visual recorder (camera)
- Magnifying glass
- Rulers, measuring tape
- GPS locator
- Collecting bags, scrapers, scissors, vials or tubes
- Water-proof labeling pens
- Drawing pens
- Sealable transportable container
- Preservatives (ice for example)
- Protective gear (such as gloves)



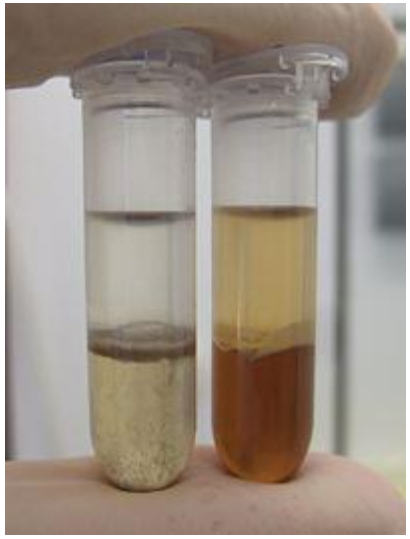
Recording Provenance

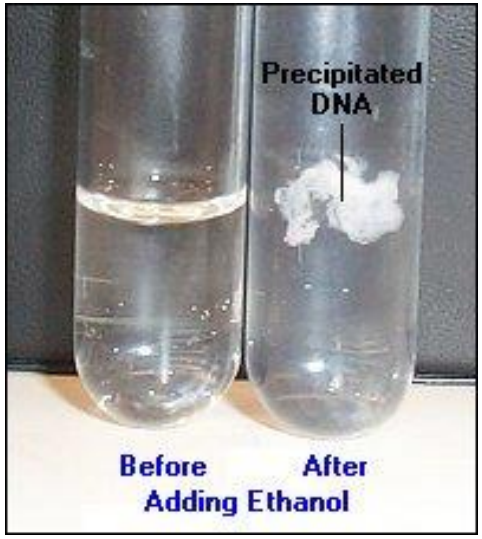
Provenance is a record of ownership of the sample, used as a guide to authenticity or quality. That is, capture what happened: the workflow, both intended and actual.

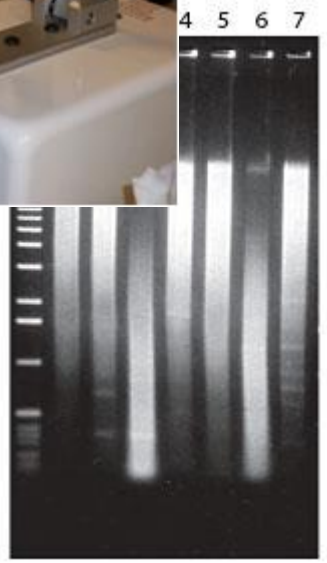
- Use waterproof ink
- YOUR name and date on the notebook
- Include a table of contents (leave some pages at the beginning or end)
- If not pre-printed, number pages as you go.
- Track what the plan was and how you carried it out
  - The plan explains preparations and steps
  - How you carried it out explains the variables and true measurements (also mistakes), images, explanations made *as the process occurs*
  - At the end of the day, make time at the end of the day to summarize results and observations, recommendations for the next lab, then initial the page.
- Frequently used methods, recipes for solutions and calculations can go in an appendix

Processing Samples

- Break open cells
- Purify the DNA
- Concentrate the DNA
- Quantify the DNA



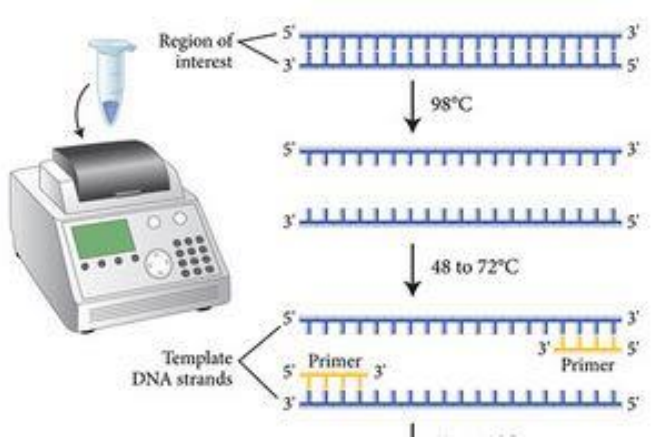


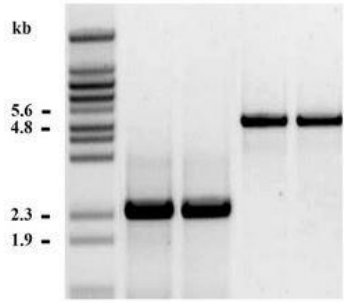


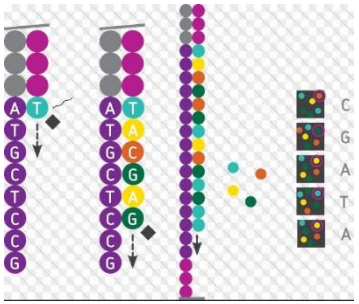
Transforming samples creates data

Use PCR to amplify (part of) a gene

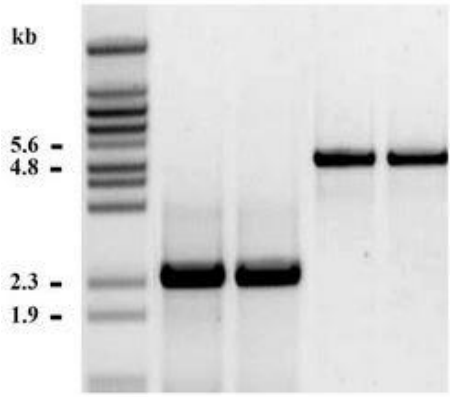
- If the lengths are different for parents, run the fragments on a gel
- If the lengths are the same, sequence the PCR fragments







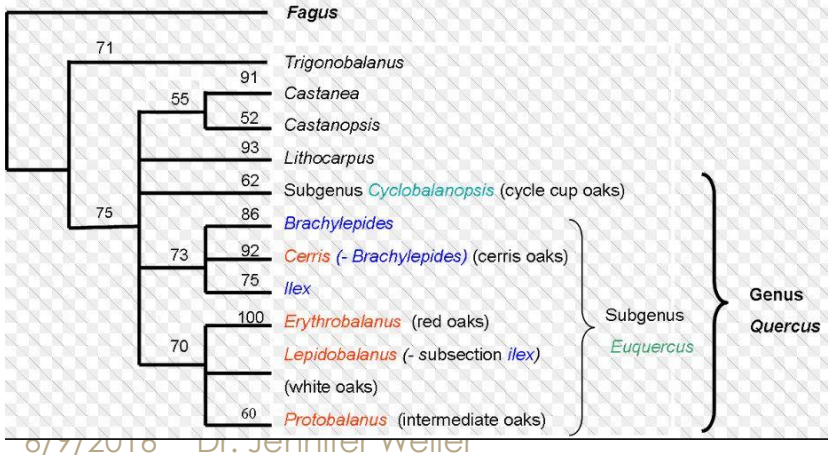
Data Interpretation to new Knowledge



Name/Length	kbp	kbp
Sample 1	2.3	
Sample 2	2.3	
Sample 3		5.1

GTTGGATTTAAAGCTGGTGTTAAAGATTATAAATTGACTTACTACACCCCAGAGTATGAAACTAAGGATA

GTTGGATTCAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATA



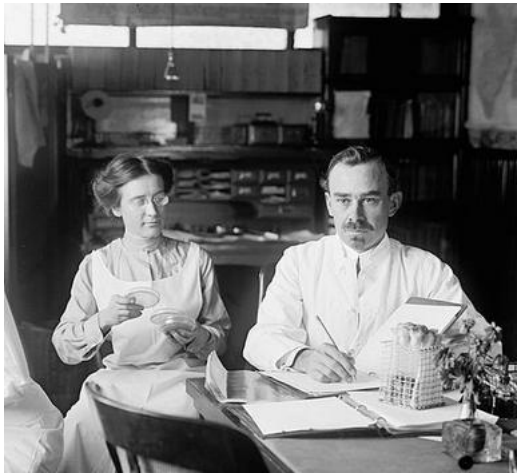
http://www.slideshare.net/raunakms1/raunak-2-barcodebronx
http://w3.pierroton.inra.fr

First Steps

- Prepare your notebook - set it up
- Put any information in it you will want in the field
  - Examples of leaves
  - Directions for collecting and labeling samples
  - Directions for Pryor Farm activities
    - Pollination
    - Fungus challenge

Preparing your notebook

- For the field days: working as a team, set up one notebook ahead of time to share (one can collect while the other records)
- For the field days: work as a class to set up labeling codes unique for each team (the tubes are pretty small).
  - Name, date, location code, details in notebook
- When you collect samples, write in your notebook exactly what the label is for every sample.
  - The samples go on dry ice (carried by Dr. Weller) and will be stored in a -80 Freezer until June 21st when we will process them.



Team 1, Maria Echeverria could be → T1_me
Team2, Tripp Stender could be → T2_ts
Location could be L1, L2 etc, and if there are replicates you could use a, b.
Leaf tissue could be L, bark scrapings B and soils samples S:

T1_me_L
6/15/15
L1a

Sample Notes

- Collected leaf sample in a Baggie labeled T1-me_L, 6/15/15, L1a which was at the first collection point, GPS coordinates are: 35.213316, -81.293555.
- Bark scrapings were collected into a tube that has the label: T1_me_B, 6/15/15, L1a.
- There is a duplicate of each sample, the labels are T1-me_L, 6/15/15, L1b and T1_me_B, 6/15/15, L1b.

Comment 1: Maria took a picture of the tree – the printed image will be placed here: (indicate a spot)
Comment 2: We were only able to find shoots coming from the stump, the tree seems to have died.
Comment 3: The bark has speckles of bright orange on it (see picture) – this is what we scraped into the tube.