

From Phenotype to Genotype

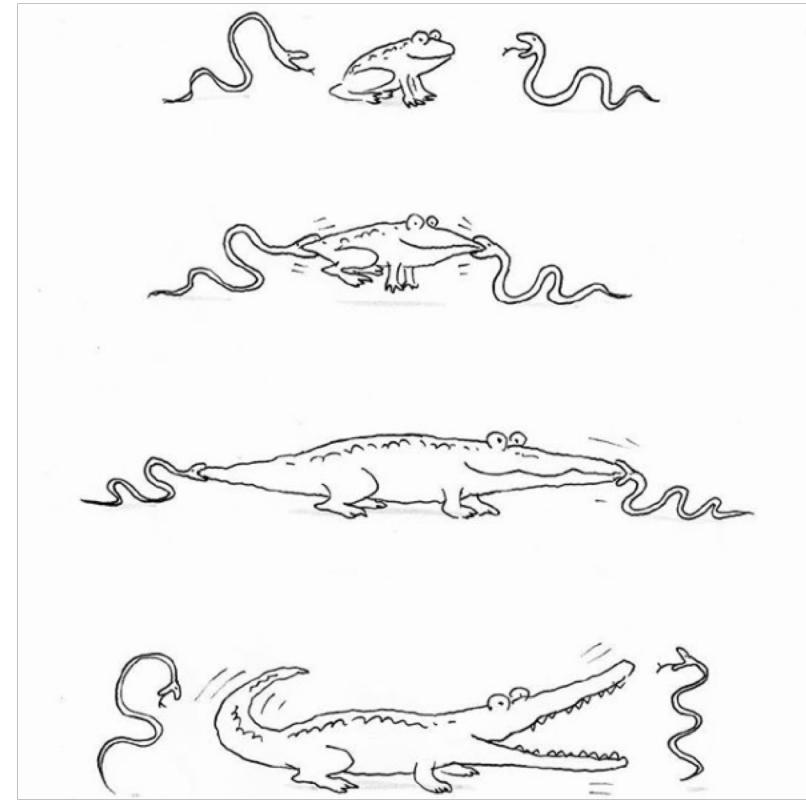
Maggie Schedl
BIO 594 Prada

Our Trajectory

- Evolutionary processes
 - Mutation
 - Gene flow
 - Drift
 - **Natural Selection**

How to do you know that selection is happening in a population?

How do you know what in the phenotype and what in the genotype selection is acting on?



<https://www.instagram.com/tangosleepless/?hl=en>

Overview

- Studying selection often begins with phenotypes
 - Ecological Example: Darwin's finches
- Associations of phenotype with genotype
 - Polygenic Traits
 - QTL Mapping
 - GWAS
- Testing the Association:
 - Expression studies, gene ontology
 - In-situ hybridization
 - Gene knock-outs
- Revisiting selection on genotype

The 3 Questions

1. What happened?
2. Why did it happen?
3. How did it happen?

Detecting Selection: Darwin's Finches

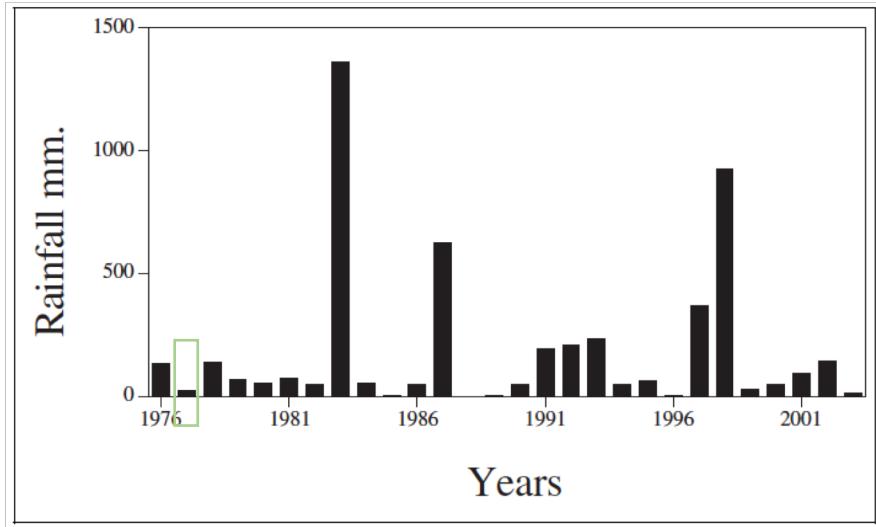
- Decades long study on finch species in the Galapagos
- All the Galapagos finches (14 sp.) diverged from 1 common ancestor
- One of the most well known examples of showing natural selection occurring in the wild



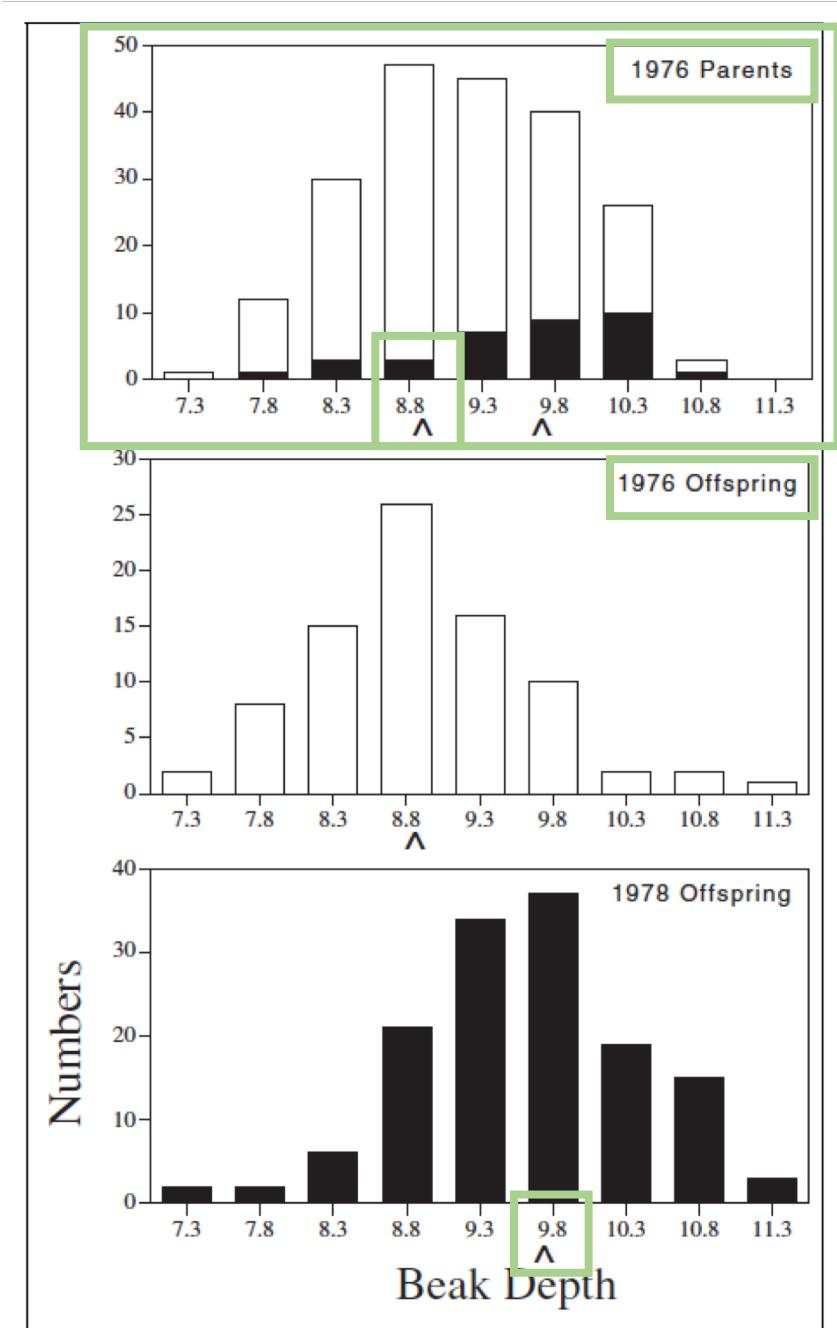
<https://www.charleyharperprints.com/shop/darwins-finches/>

Ingredients for adaptive evolution:

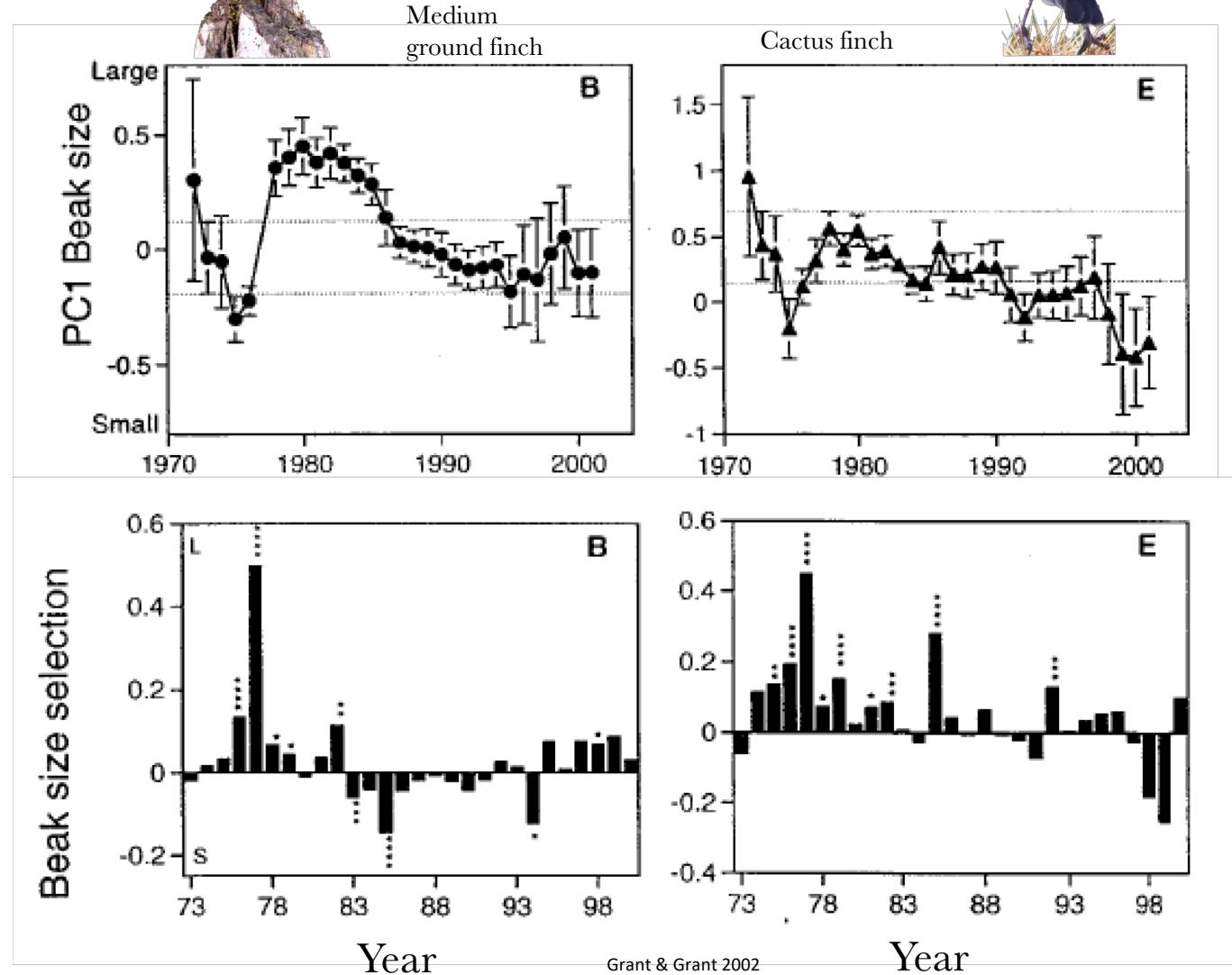
- The trait must vary
- The trait must be heritable
- The trait must be subject to natural selection



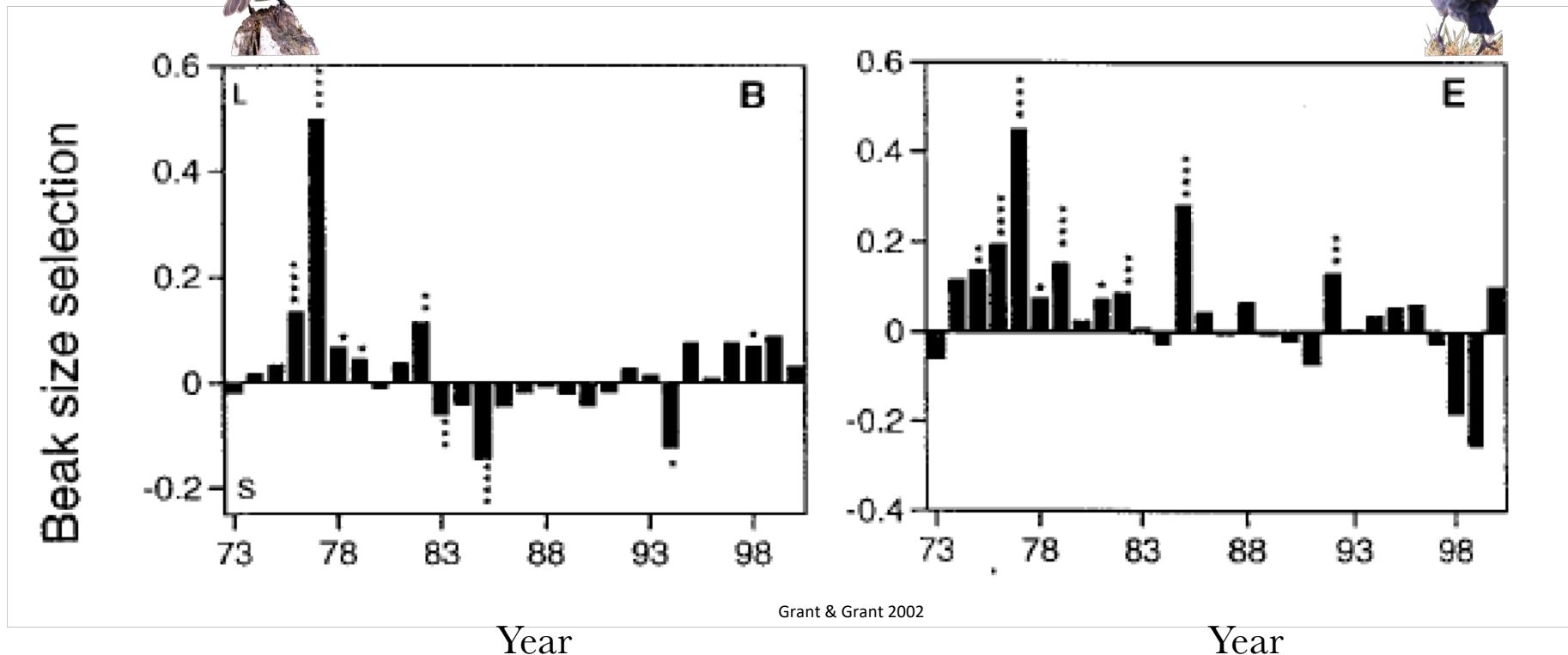
Selection and Phenotypes



Long Term Investigation of Selection



Measuring Selection

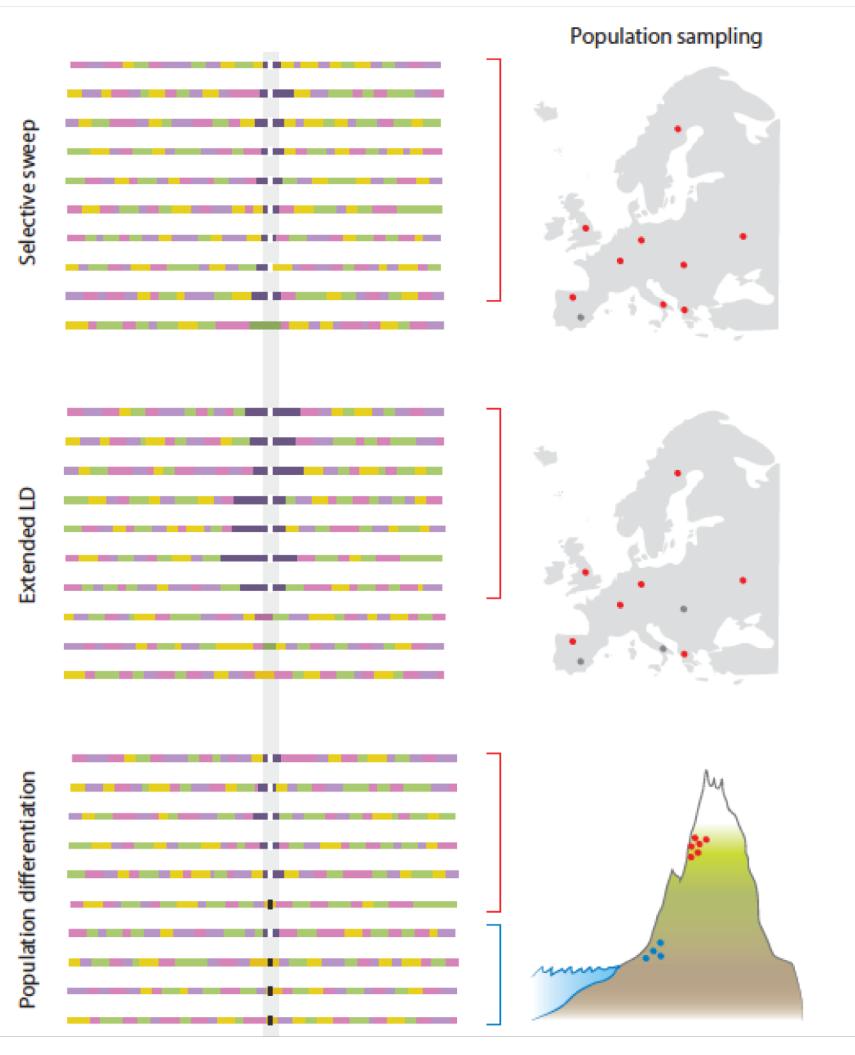


Selection Differentials: difference between trait mean of entire parental/breeding population one year to the next
Survival to the next year is considered a selection event

In our example we've seen from an ecological perspective what has happened to these finches and maybe why it has happened

We still want to know what has happened genetically to the populations and how physical variation happens

Genomic Detection of Selection

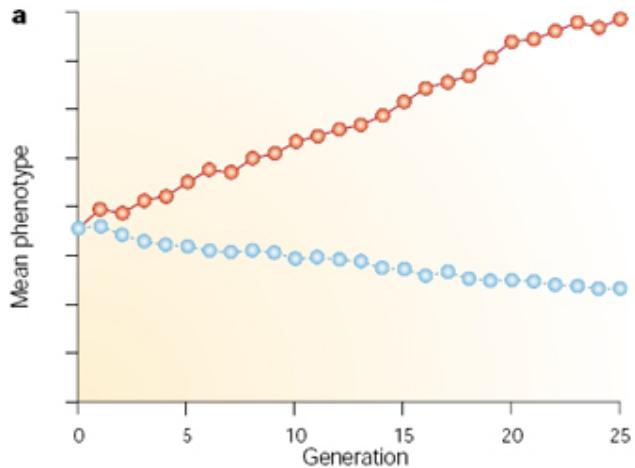


Frequency spectrum based methods

Linkage disequilibrium based methods

Large differences in frequencies of different populations indicating local adaptation

Quantitative Trait Loci (QTL Mapping)



Population or individuals that differ in a trait or phenotype that is under selection

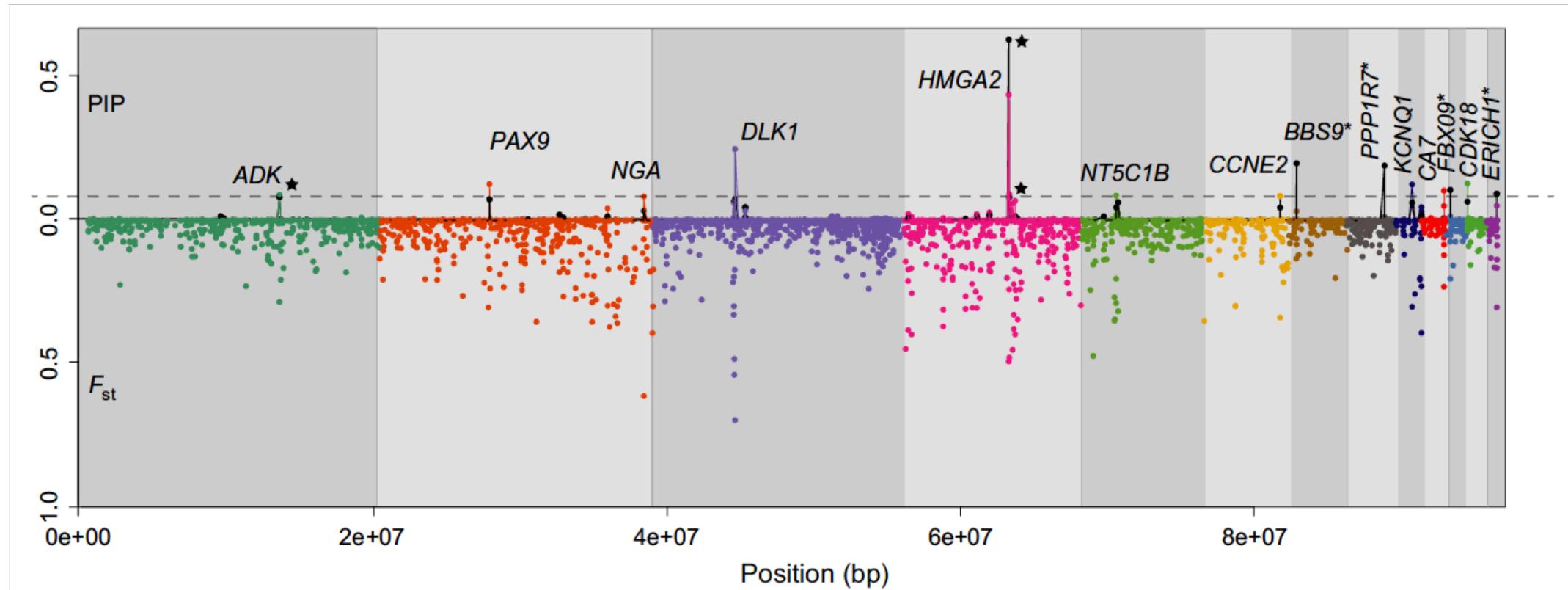
Cross two individuals with the differing traits and phenotype the offspring

Sequence offspring and associate blocks of chromosomes with differences in the traits you measured

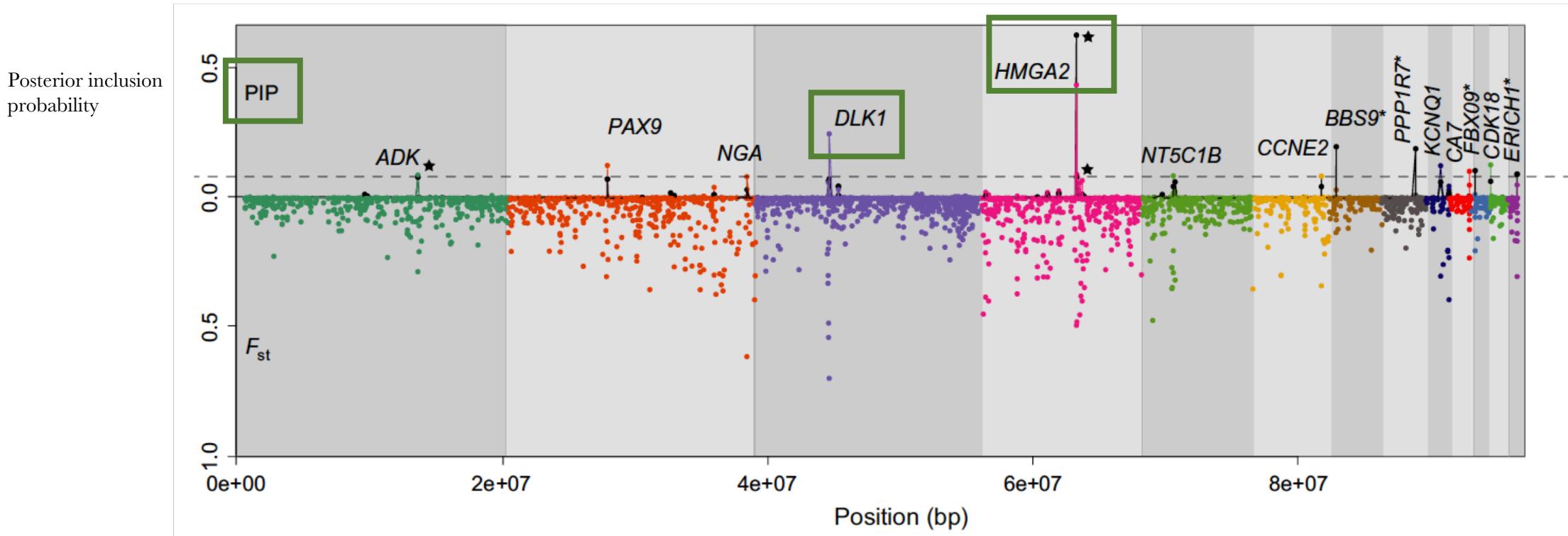
Limitations of QTL Mapping

- Needs the ability to cross individuals or sample many offspring in the field which may not be possible
- Resolution is only to areas of chromosomes, not individual SNPs

Genome Wide Association Studies (GWAS)



Genome Wide Association Studies (GWAS)



Each dot is a SNP or a haplotype

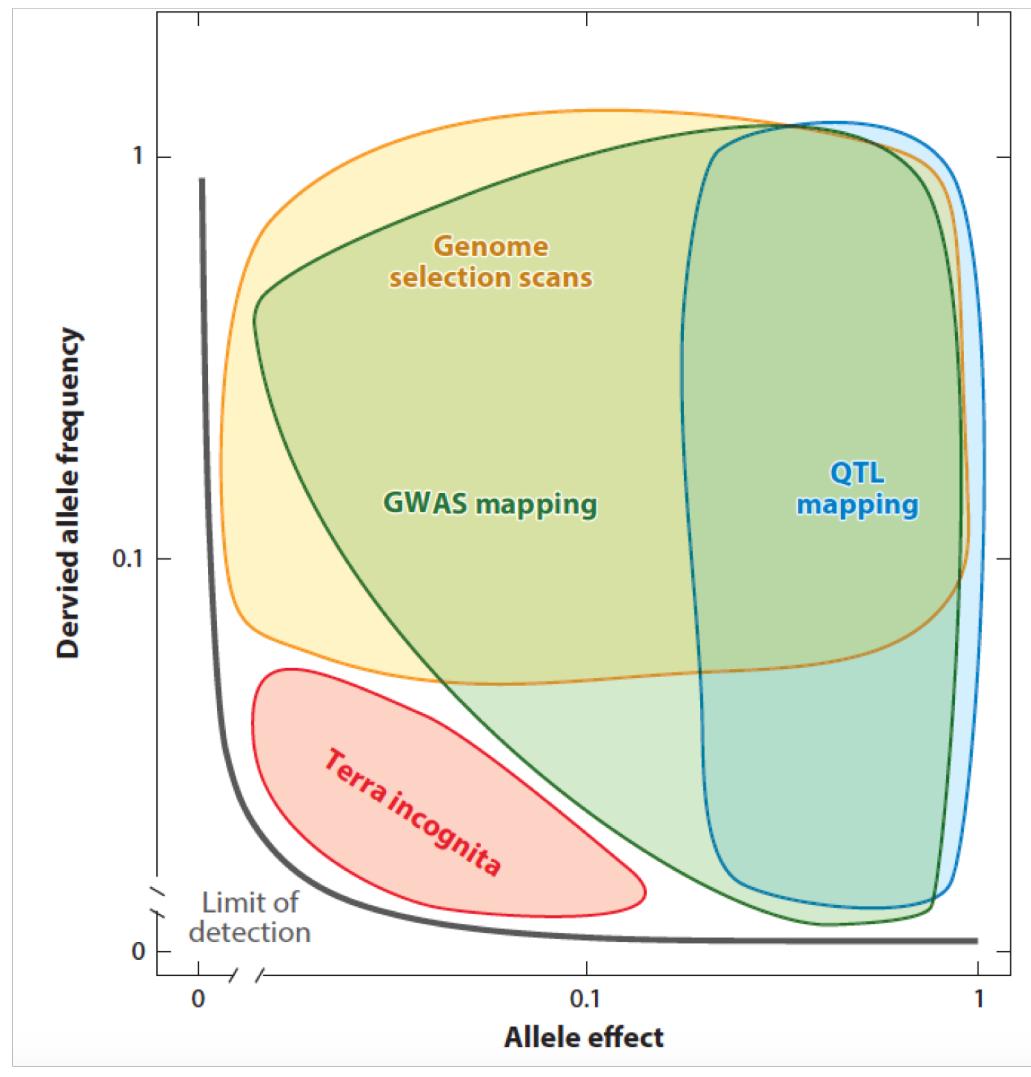
The x-axis is the chromosome or position on the genome assembly/scaffold

The y-axis is an association value with your trait/phenotype of interest

Limitations of GWAS

- You have to correct for population genetic structure
- Works best if you already have an assembled genome
- You can get results like NGA meaning no gene associated identified

Detection of Important Alleles

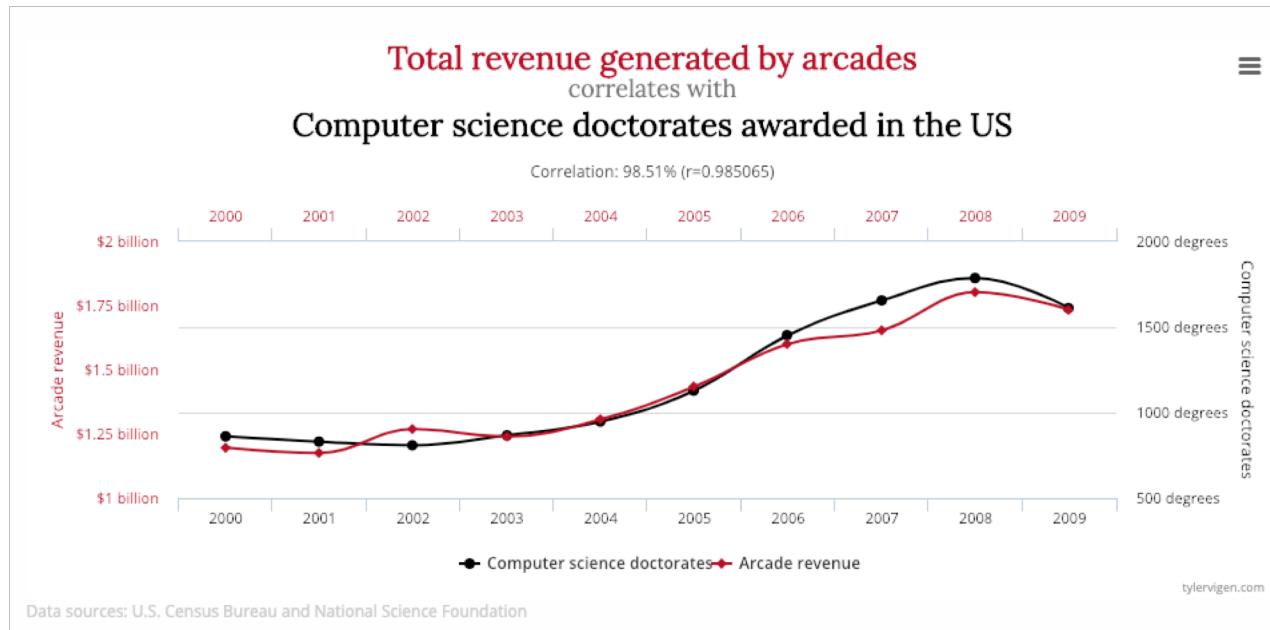




There is one major limitation to
Phenotype-Genotype Association studies

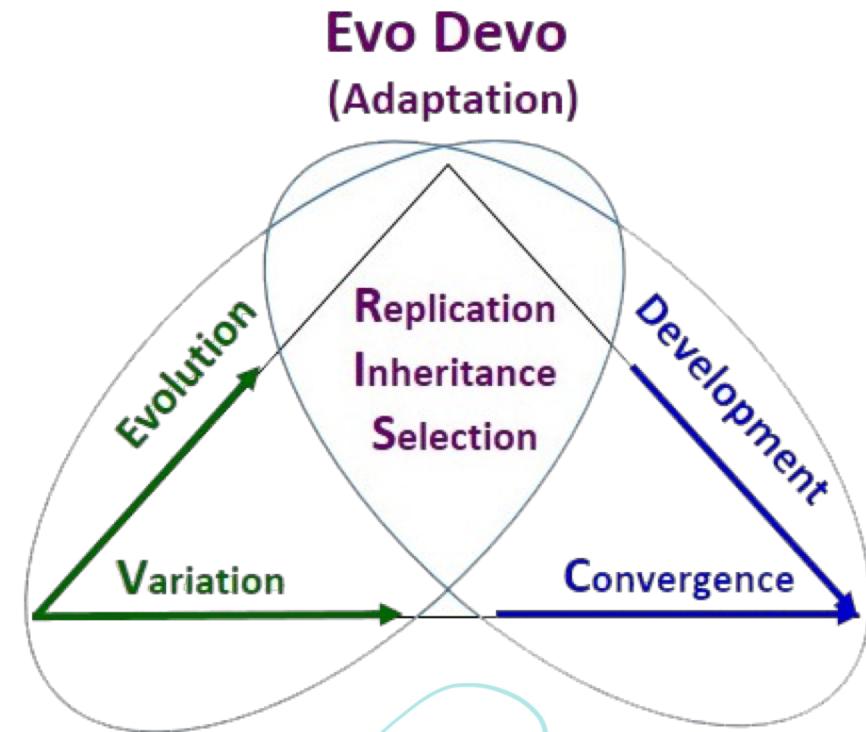


Correlation is not Causation



Testing Associations

- Expression Analysis
- In-situ Hybridization
- Knock-outs and other gene editing
- Many other ways!!



Expression Analysis

- Microarrays
- qRT-PCR
- RNA seq

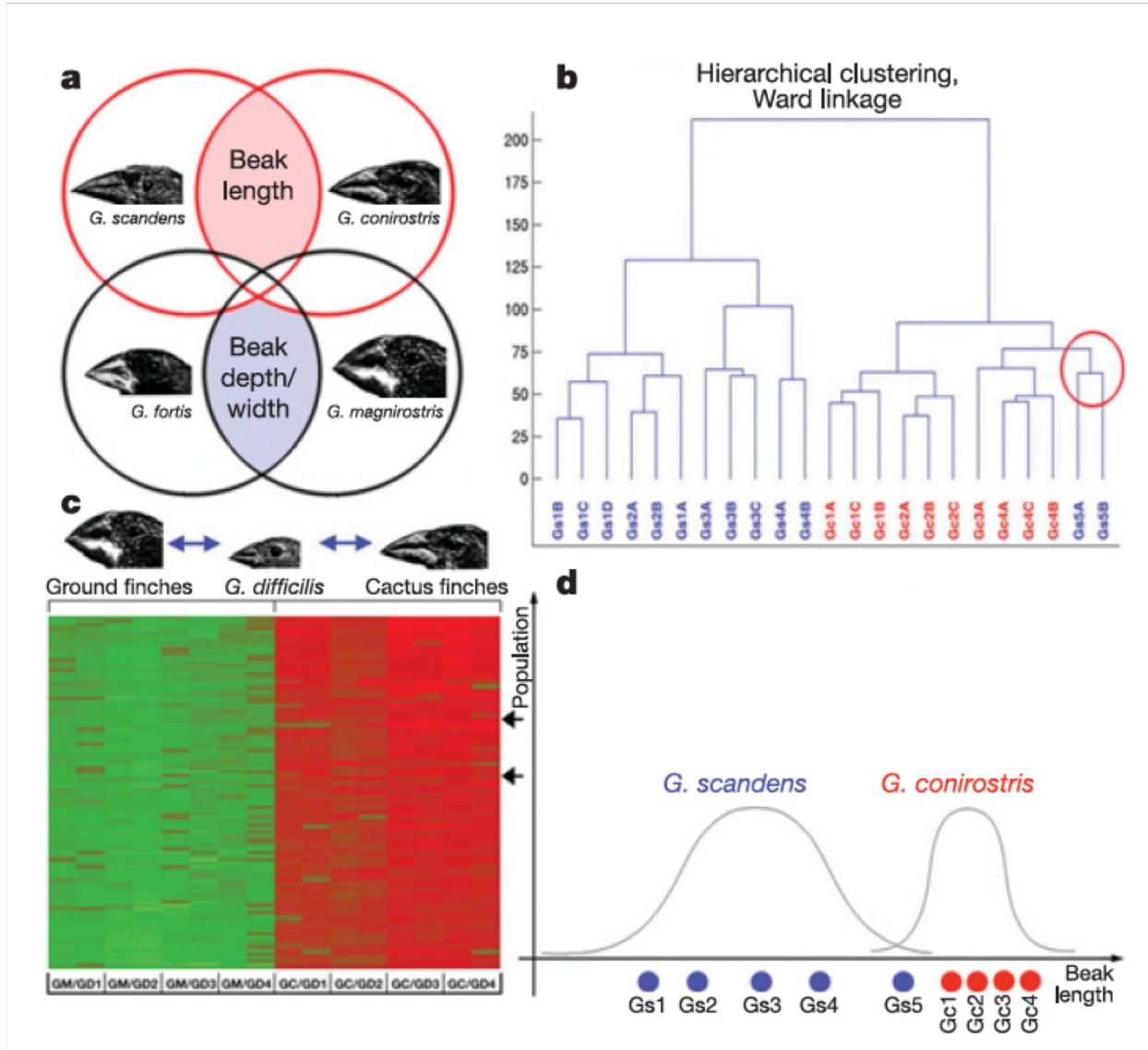
Microarrays

- Quantify amount of mRNA in an organism at a certain time
- Array or “chip” with DNA probes where cDNA or mRNA from the organism will bind
- Amount of binding measured and quantified



Strengthening Associations

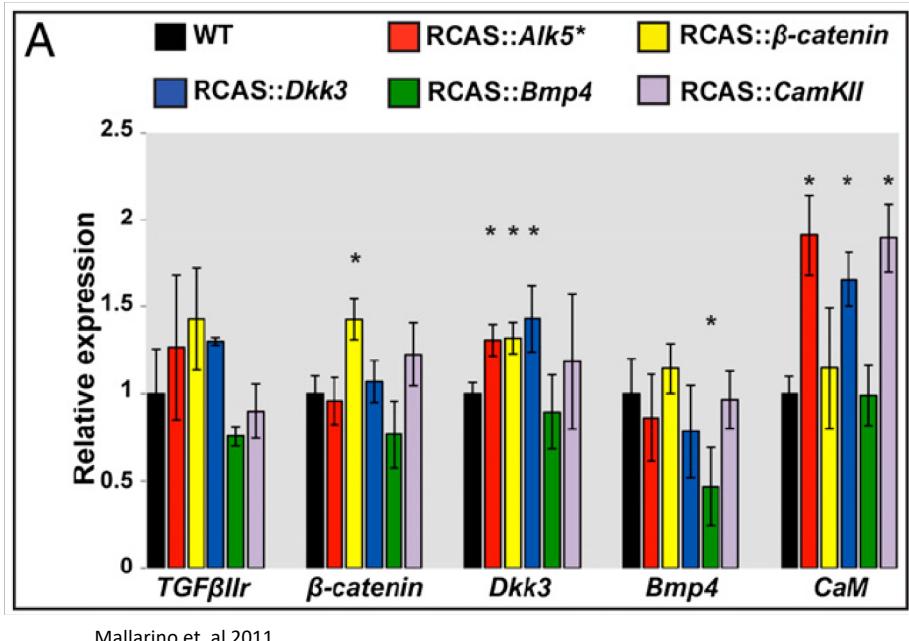
Comparison of gene expression profiles across beak morphology range in Darwin's finches



Expression patterns cluster by species

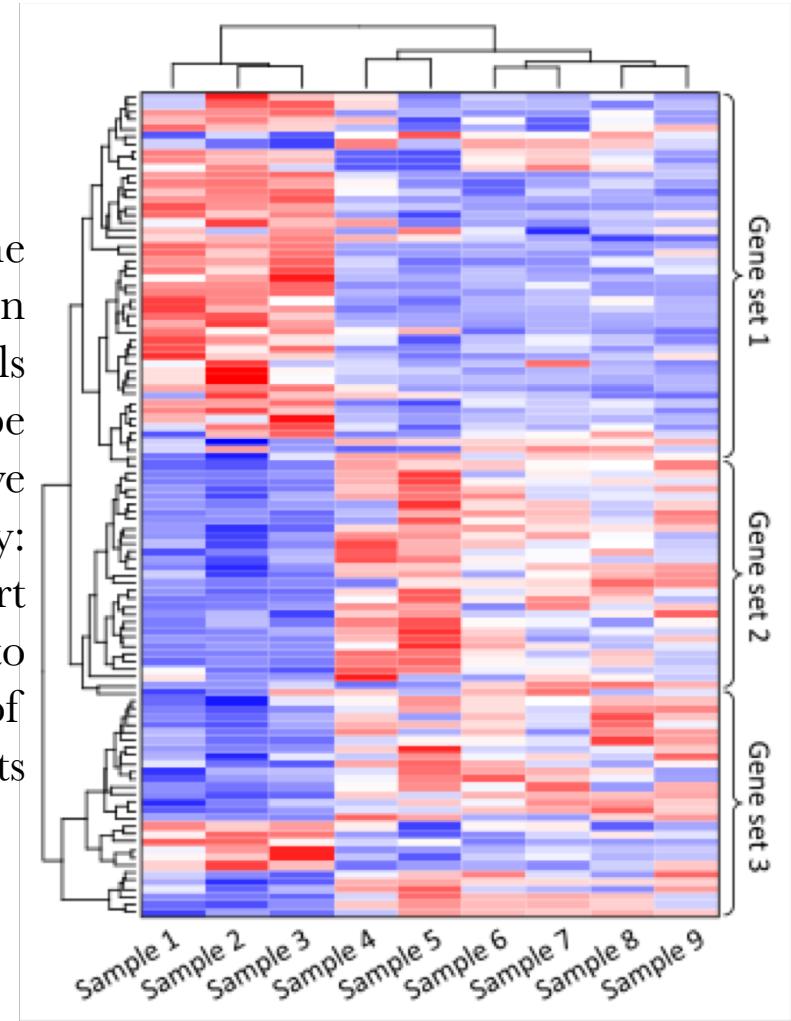
Compared upregulated or downregulated in comparison to *G. difficilis*
Focused on higher (5x) expressed genes in cactus finches

qRT-PCR and RNA Seq



Quantitative real-time PCR
Targeted quantification of how much of specific gene products are amplified (usually cDNA)

Sequence the mRNA in individuals
Can be quantitative
Gene ontology: coordinated effort across biology to identify function of gene products



<https://journals.plos.org/ploscompbiol/article/figure?id=10.1371/journal.pcbi.1005457.g006>

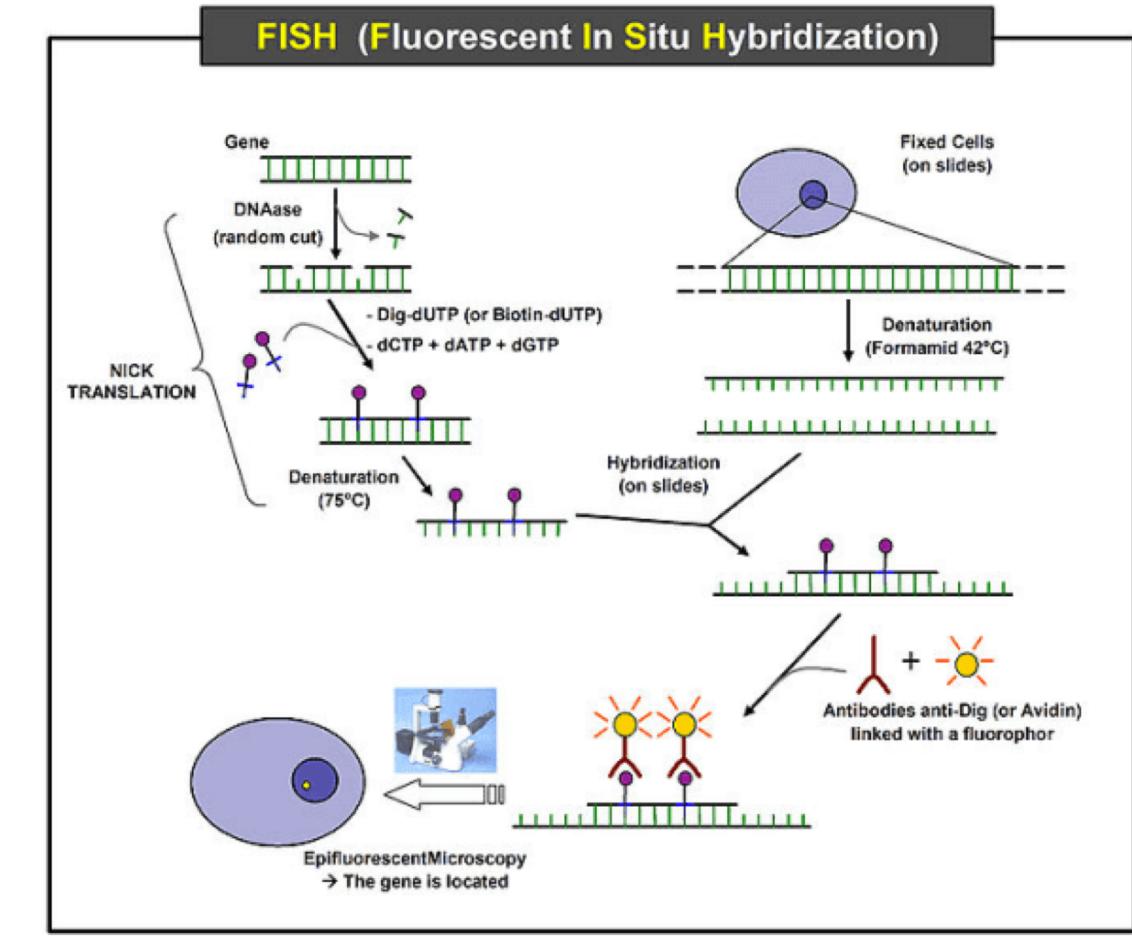


What can you now do if you have an allele that is associated with a trait under selection and is differentially expressed between groups?
(groups can be populations, species, or experimental selection groups)



In-situ Hybridization

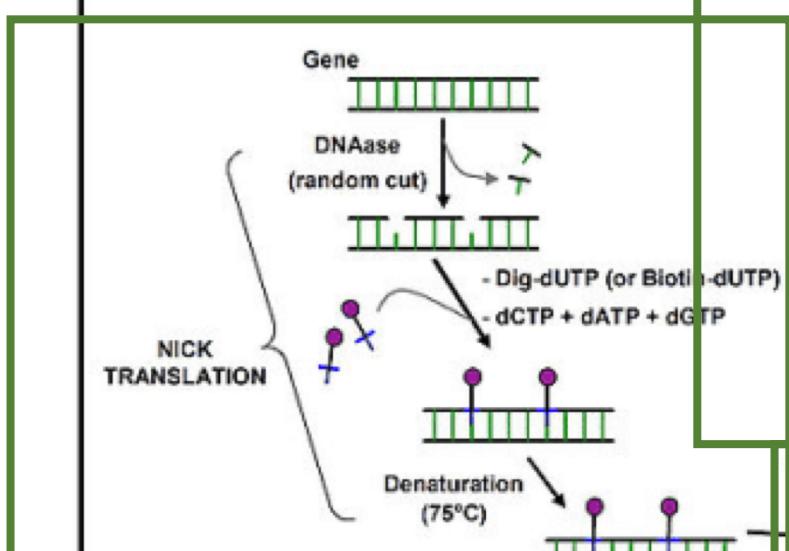
- Visualizes DNA or RNA (mRNA) *in-situ*
- Probes can be fluorescent, bind to a pigmented antibody, or produce a colored molecule once bound
- Tissue-specific expression, usually in development



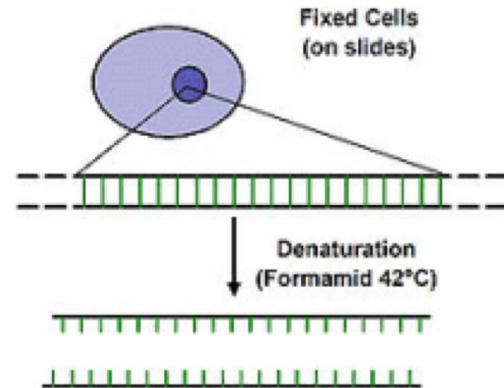
<https://www.creativebiomart.net/resource/principle-protocol-fluorescence-in-situ-hybridization-fish-protocol-342.htm>

FISH (Fluorescent In Situ Hybridization)

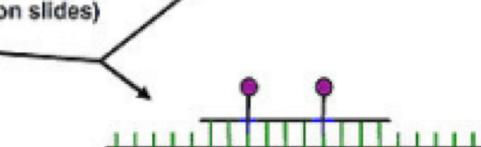
Probe Generation



Fixed Cells
(on slides)



hybridization
(on slides)

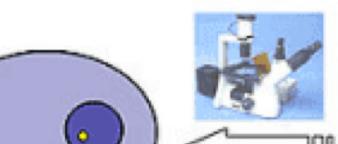


Tissue collection
and fixation

Probe and target
hybridization



Antibodies anti-Dig (or Avidin)
linked with a fluorophor



Epifluorescent Microscopy
→ The gene is located

Visualization

<https://www.creativebiomart.net/resource/principle-protocol-fluorescence-in-situ-hybridization-fish-protocol-342.htm>

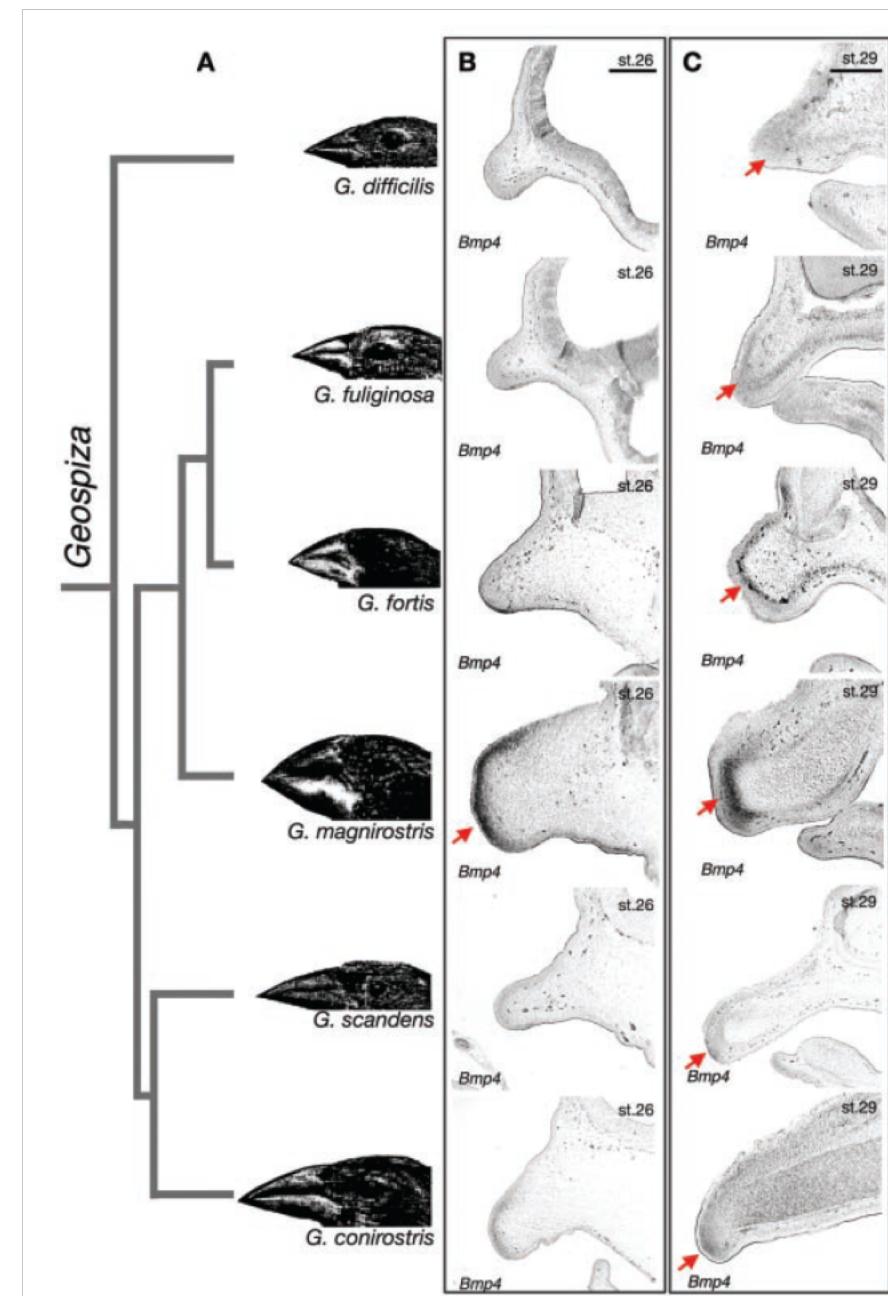
Example in Finches

What is the most obvious thing you see from this figure?

Developing mesenchyme (connective/skeletal tissue) of finch embryos

In-situ hybridization probes for bone morphogenic protein 4 (Bmp4)

Little or no Bmp4 present in smaller beaked finches during this stage of development



Abzhanov et. al 2004

Gene or Expression Editing Techniques

- Retrovirus
- Morpholinos
- Gene Editing
 - Knock-out
 - Targeted mutation

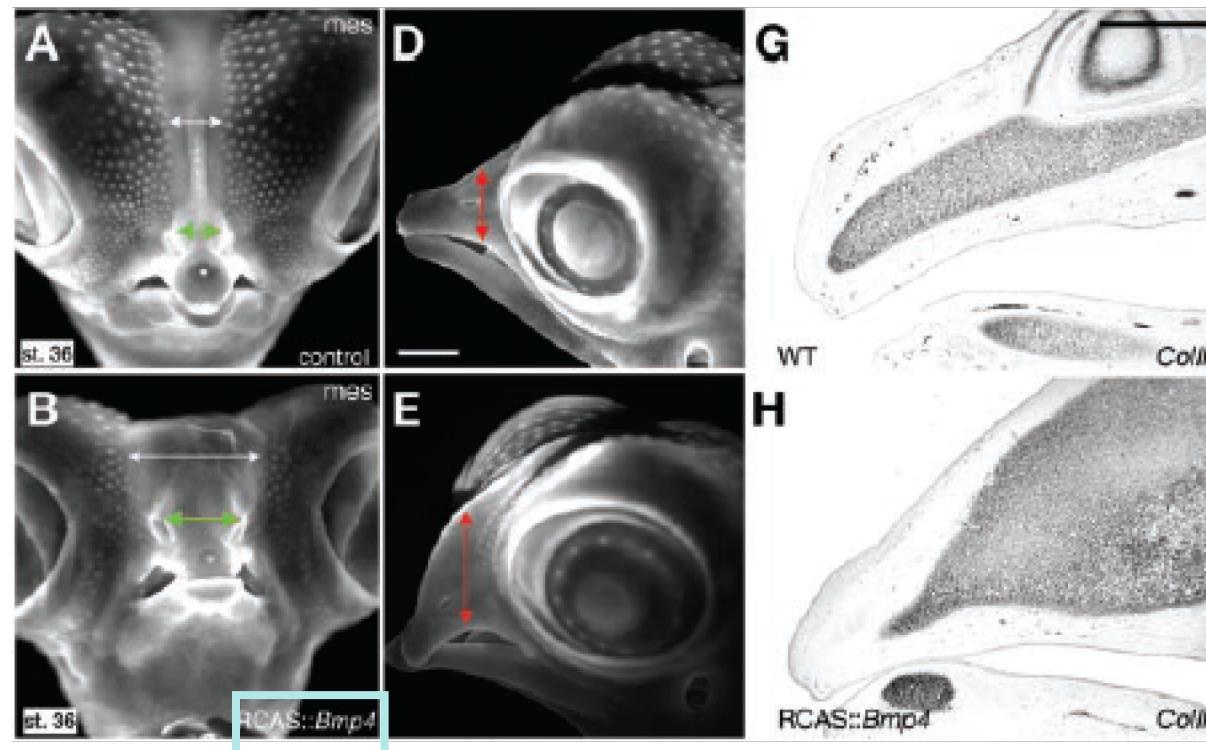
Experimentally altering gene expression

Experimentally deleting or mutating gene

Example: Retroviral addition of a gene

- RCAS: avian retrovirus used for molecular biology techniques
 - Retrovirus can acquire gene from “host”: add the gene you want to express
 - Transfect developing organism to replicate and express transcript

Retroviral addition
of Bmp4 shows
increase in 3D beak
shape



Abzhanov et. al 2004

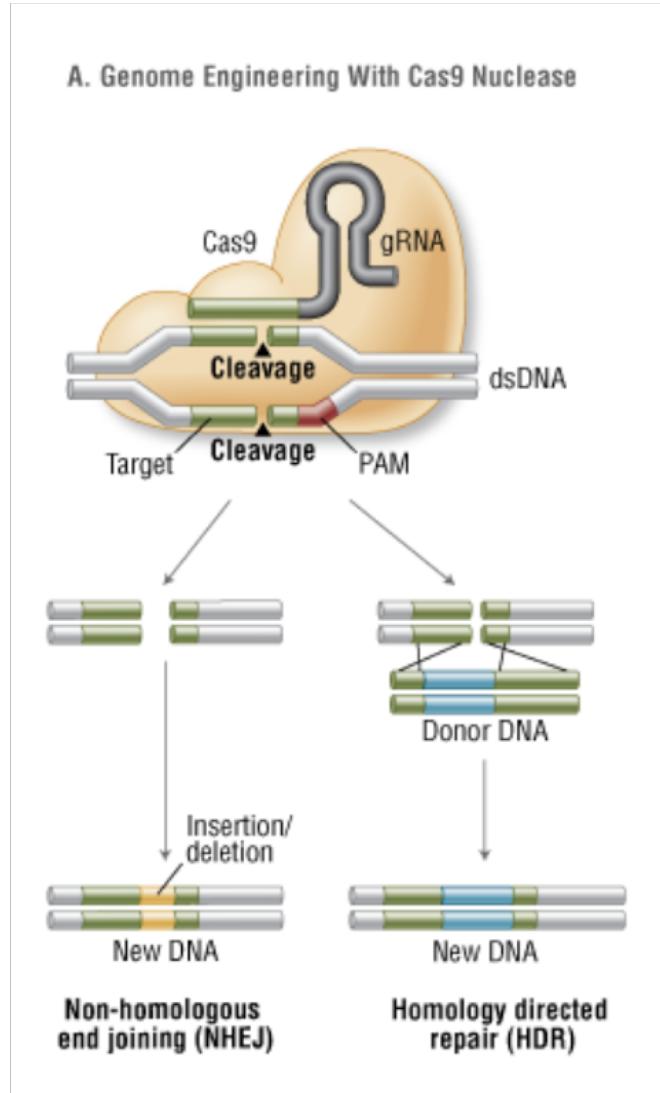
Morpholinos

- Typically an oligo
- Blocks maturation of RNA to mRNA
- Reduces expression
- Not really in use anymore in favor of “real” knock-outs



Strengthening Associations

Gene Editing Ex: CRISPR Cas9



- Clustered Regularly Interspaced Palindromic Repeats
- Cas9 enzyme uses guide RNA to base pair with targeted DNA
- Can either have incomplete repair of break and lose the gene (knock-out)
- Or insert a fragment (targeted mutation)

CRISPR not just for model systems?

Chapter Three - CRISPR/Cas9 as the Key to Unlocking the Secrets of Butterfly Wing Pattern Development and Its Evolution

Luca Livraghi ^{*}, Arnaud Martin [†], Melanie Gibbs [‡], Nora Braak ^{*}, Saad Arif ^{*}, Casper J. Breuker ^{*}

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<https://doi.org/10.1016/bs.aiip.2017.11.001>

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Abstract

With the exception of a few moth and [butterfly](#) species, gene-editing tools in [Lepidoptera](#) have been lagging behind other well-studied insects. In order to elucidate gene function across the order, it is necessary to establish tools that enable such gene manipulation. CRISPR/Cas9 is a promising technique and here we review the recent progress made in implementing the technique in butterflies; from broad patterning of the wing, to the development of specific colours in particular wing sections, to eyespot formation. The often species-specific responses to the CRISPR/Cas9-induced mutations in candidate genes, underscore the significance of these genes in the wide evolutionary diversification of butterfly wing patterns. We further discuss potential caveats in the interpretation of the resulting mutant phenotypes obtained through CRISPR/Cas9 gene editing. Finally, we discuss the possibilities CRISPR/Cas9 offers beyond mere knockout of candidate genes, including the potential for the generation of transgenics that will further elucidate the developmental genetic basis for wing pattern evolution.

CRISPR/Cas9-mediated genome editing in a reef-building coral



Phillip A. Cleves, Marie E. Strader, Line K. Bay, John R. Pringle, and Mikhail V. Matz

PNAS May 15, 2018 115 (20) 5235-5240; published ahead of print April 25, 2018

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Contributed by John R. Pringle, March 26, 2018 (sent for review December 20, 2017; reviewed by Denis Allemand and Ann M. Tarrant)

Article

Figures & SI

Info & Metrics

PDF

Significance

Coral reefs are biodiversity hotspots of great ecological, economic, and aesthetic importance. Their global decline due to climate change and other anthropogenic stressors has increased the urgency to understand the molecular bases of various aspects of coral biology, including the interactions with algal symbionts and responses to stress. Recent genomic and transcriptomic studies have yielded many hypotheses about genes that may be important in such processes, but rigorous testing of these hypotheses will require the generation of mutations affecting these genes. Here, we demonstrate the efficient production of mutations in three target genes using the recently developed CRISPR/Cas9 gene-editing technique. By clarifying aspects of basic coral biology, such genetic approaches should also provide a more solid foundation for coral-conservation efforts.

The 3 Questions Revisited

What happened: change in variable traits over time due to selection, understanding genes and expression

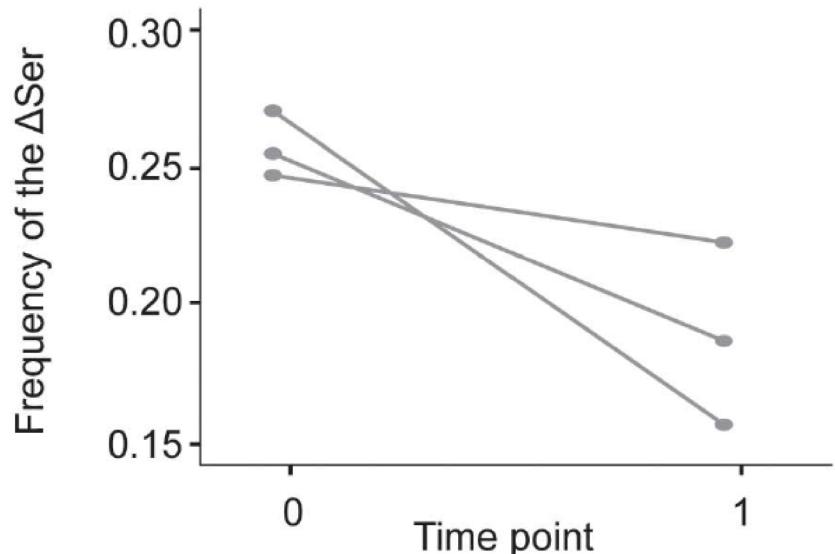
Why it happened: ecological or environmental processes

How it happened: developmental mechanisms underlying phenotypes

Can you detect selection on the variant you identified?

Conclusions

Revisiting Selection Differentials with Genotype instead of Phenotype



Barrett et al.

Calculated differences between wildtype Ser SNP *allele* frequency before and after selection event

$$\frac{\Delta \text{ SNP frequency}}{\Delta \text{ WT frequency}} - 1 = s$$

Detecting Selection
on Phenotypes

Detecting Selection
with Genomic
Sequencing

Associating
Phenotype with
Genotype (SNPs)

Associating Environment with
Genotype

Nebulous Associations

Expression Analysis

Evo-Devo Techniques

Biochemical Analysis

Functional Associations



Tuesday's Discussion Readings!

Barret et. al 2018

Bosse et. al 2016

Nadeau et. al 2016



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- <https://www.britannica.com/science/selection-coefficient>