**Thomas Kono**

2017-08-02

RISS Interview Seminar

Slides: https://z.umn.edu/TK_RISS

# Who I Am

- Undergrad: UC Davis (Biochem. + Molecular Bio.)
- PhD: University of Minnesota (Applied Plant Sciences)
  - Advisors: Peter L. Morrell and Robert M. Stupar
- Postdoc: University of Minnesota (Agronomy/EEB)
  - Advisors: Candice N. Hirsch and Suzanne E. McGaugh

# Outline

**Can we predict the phenotypic effect of a mutation?**

**Tools:**

SNPMeta: Annotation of SNPs in non-model species

BAD_Mutations: Predicting deleterious SNPs

**Applications:**

Deleterious variant identification in two crop genomes

A different mutation type: genome content variation

# SNPMeta: Questions

- What information on SNPs is available through public data sources?
- How do annotations from GenBank entries compare to those from a reference genome?

# SNPMeta:
# A Photo Analogy

Purpose: to collect metadata on SNPs

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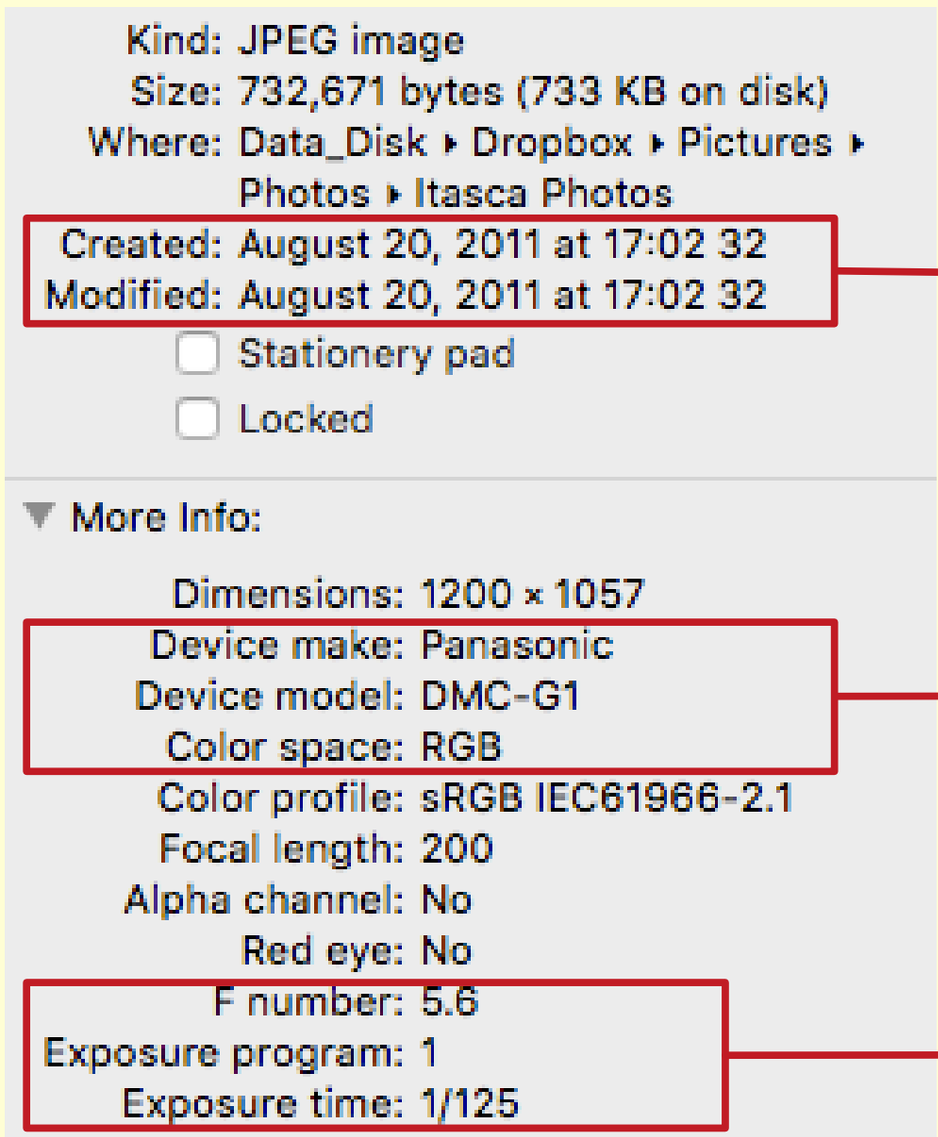
# A Photo Analogy

Purpose: to collect metadata on SNPs




Date info

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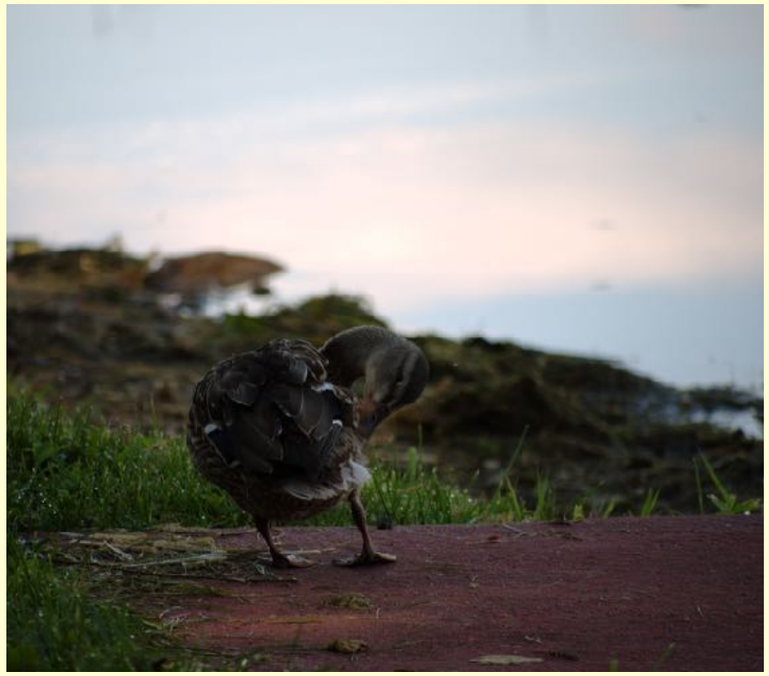
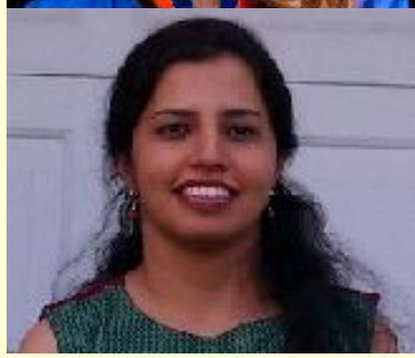
Exposure info



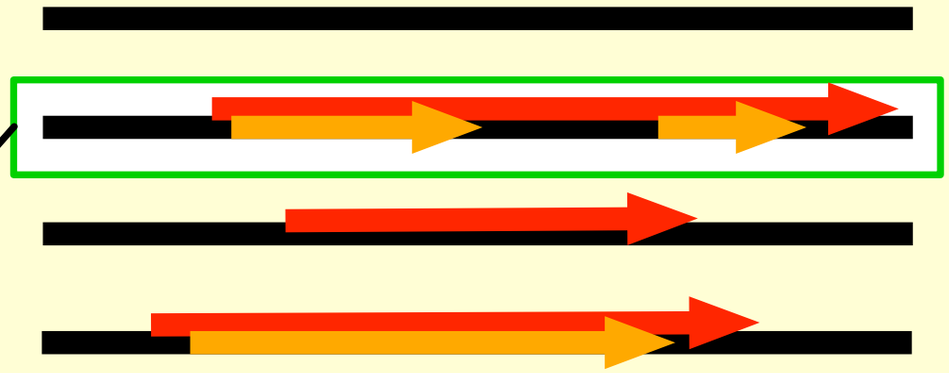
# SNPMeta: Pipeline Development

Amber Eule-Nashoba

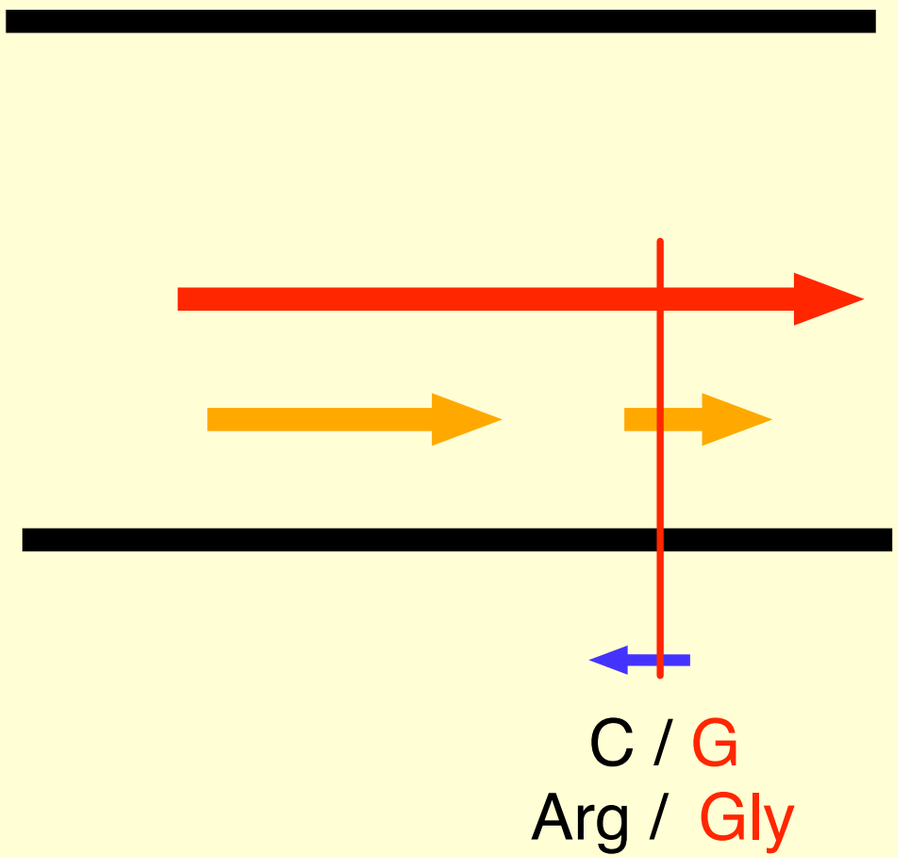


Kiran Seth

BLAST Hits



GenBank Record



# SNPMeta: Output to dbSNP

Gene Name

GenBank Number

Protein Impact

```
SNP: RIL01_12001_39
GENENAME: CG4629
ACCESSION: AE014134
COMMENT: nonsynonymous I S
SAMPLESIZE:
LENGTH: ?
5'_FLANK: ATTCACTCCATTTTCCAGAAGAGAGCGGCAGGGACAGCGACGCCGGCTGTAGCGGCGCCA
OBSERVED: T/G
3'_FLANK: TGGCAGCGGCAAAAAGCCCAGTGAGAGAGCCGTCATTTGAGAGGTCGCCGGCGGAGCGCC
||
```

SNP States



Kono et al. 2014

# SNPMeta: Availability

Software available in GitHub
https://github.com/MorrellLAB/SNPMeta

MOLECULAR ECOLOGY
RESOURCES

Molecular Ecology Resources (2014) **14**, 419–425 doi: 10.1111/1755-0998.12183

## SNPMeta: SNP annotation and SNP metadata collection without a reference genome

THOMAS J. Y. KONO,* KIRAN SETH,* JESSE A. POLAND†‡ and PETER L. MORRELL*

**Department of Agronomy & Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA, †Hard Winter Wheat Genetics Research Unit, USDA ARS, 2021 Claflin Road, 4008 Throckmorton Hall, Kansas State University, Manhattan, KS 66506, USA, ‡Department of Agronomy, 2021 Claflin Road, 2004 Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506, USA*

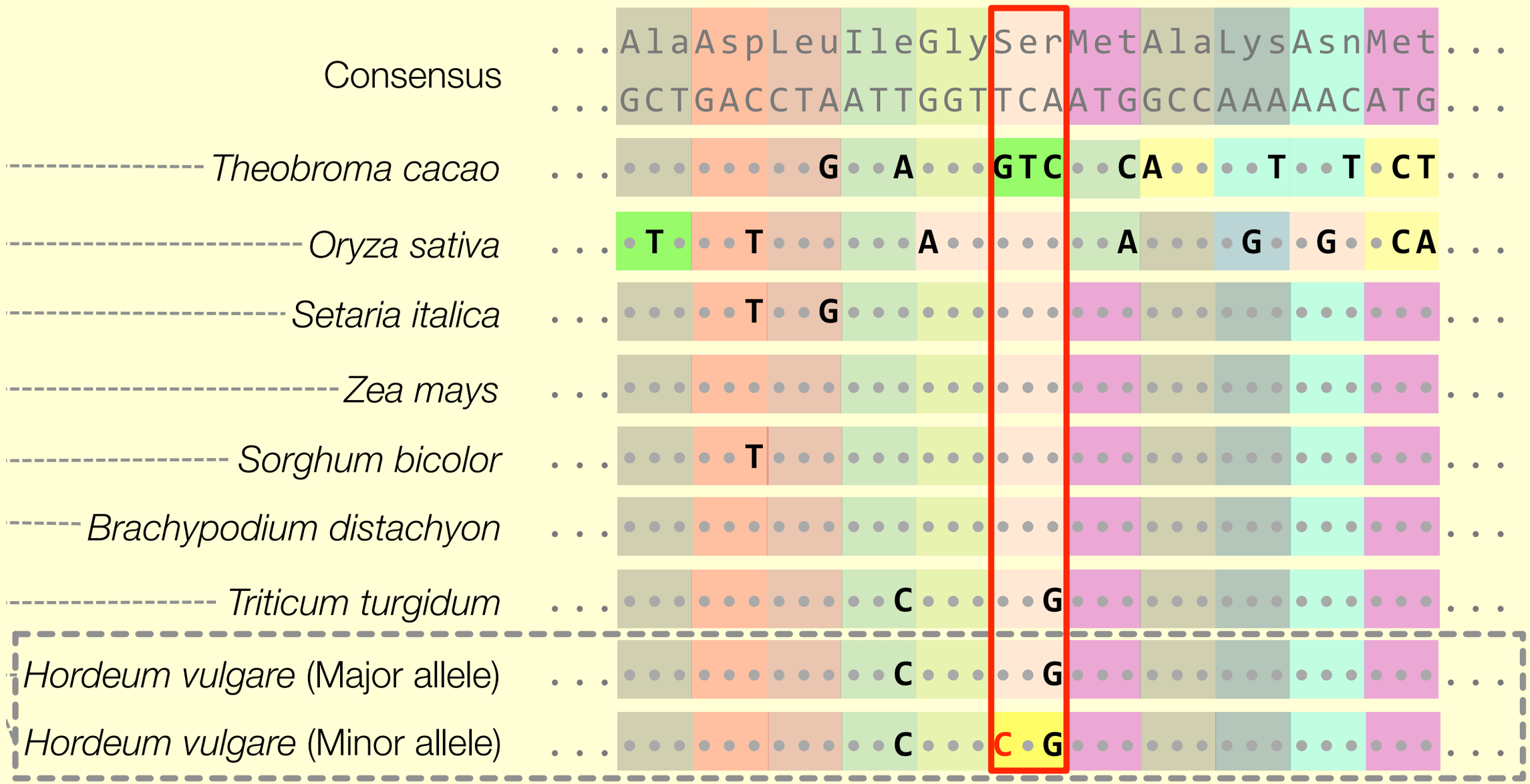
# BAD_Mutations: Yet Another Deleterious Prediction Program

- Why make another deleterious variant prediction program?
  - SIFT, PolyPhen2, PROVEAN, MAPP, GERP++, etc...
- Ideal program:
  - Is species-agnostic
  - Uses consistent high-quality data for each gene
  - Is hypothesis-driven
  - Corrects for reference bias[1]



1: Simons et al. 2014

# BAD_Mutations: What a Deleterious Variant Looks Like



# BAD_Mutations: Hypothesis Test

Based on a likelihood ratio test (LRT) of sequence constraint from Chun and Fay (2009)

$$LLR = \log \frac{L(D|T, \theta, d_N = Cd_S)}{L(D|T, \theta, d_N = d_S)}$$

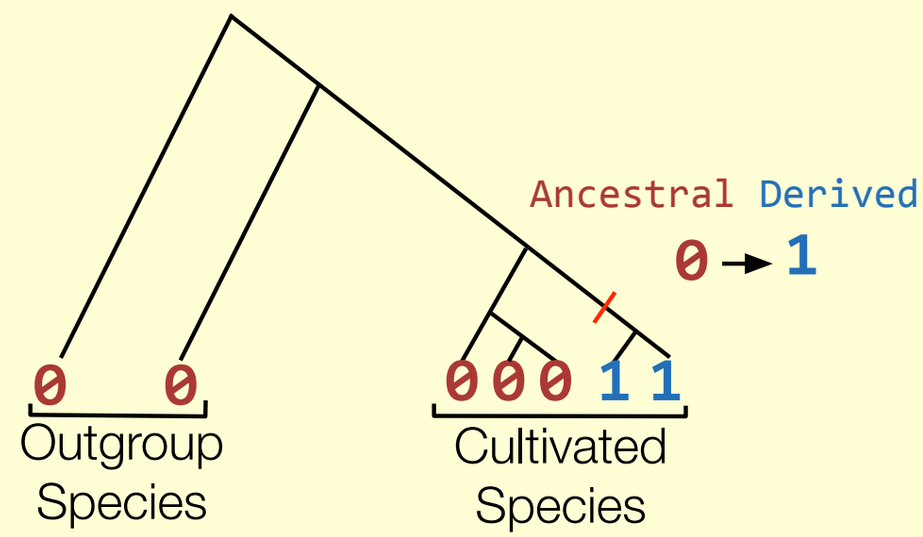
D = Codon alignment
T = Phylogeny
θ = Local substitution rate

# BAD_Mutations: Addressing Reference Bias

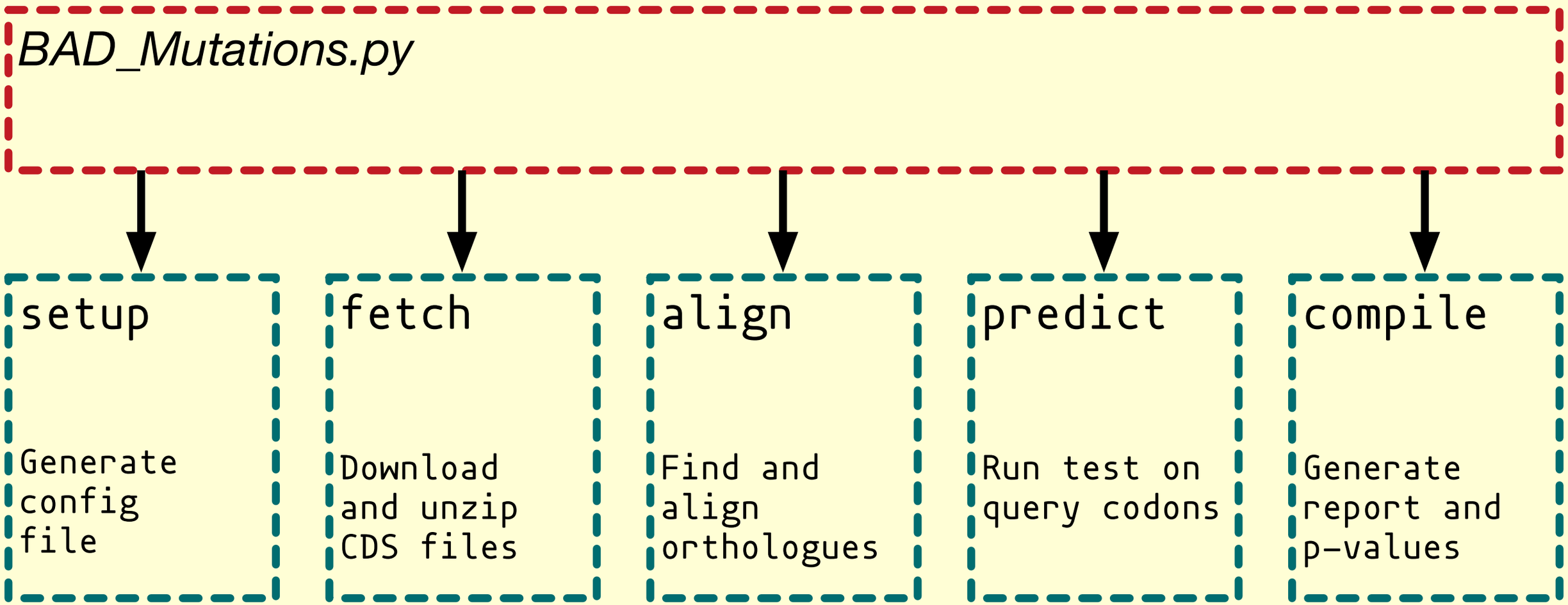
| | Morex (Reference) | Steptoe | Harrington | Kindred |
|---|---|---|---|---|
| SNP 1 | A | G | G | A |
| SNP 2 | T | C | T | T |
| SNP 3 | C | C | T | T |
| **Diff. From Reference** | **0** | **2** | **2** | **1** |

# BAD_Mutations: Addressing Reference Bias

| | Ancestral | Morex (Reference) | Steptoe | Harrington | Kindred |
|---|---|---|---|---|---|
| SNP 1 | A | A | G | G | A |
| SNP 2 | C | T | C | T | T |
| SNP 3 | T | C | C | T | T |
| **Diff. From Ancestral** | **0** | **2** | **2** | **2** | **1** |



# BAD_Mutations: Modular Design




Use config for controlling other subcommands

Download databases once, refresh when needed

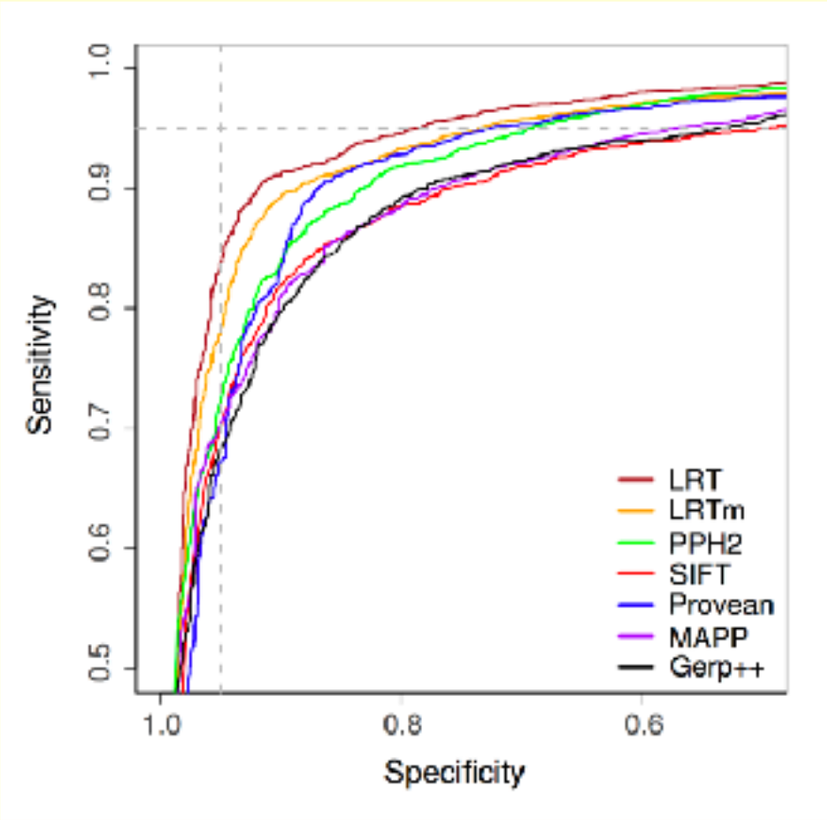
Parallelize on a gene-basis for a genome wide analysis

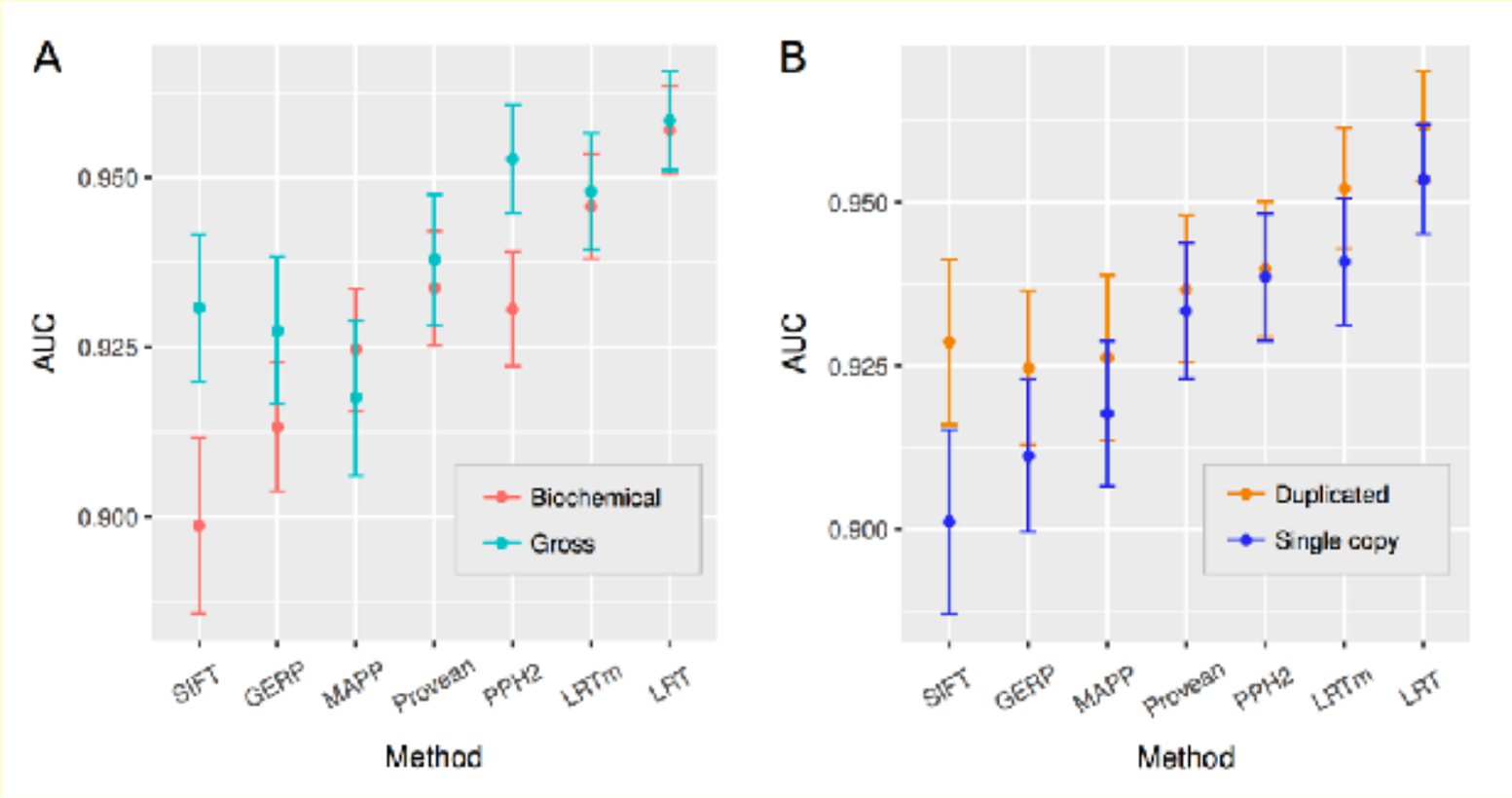
Parallelize on a gene-basis for a genome wide analysis. Slow.

Run locally, parses HYPHY text reports.

# BAD_Mutations: High Accuracy

Used a curated set of 2,910 SNPs in *A. thaliana* with mutant phenotypic effects and 1,583 SNPs at high frequency (neutral)






Kono et al. 2017 Preprint

# BAD_Mutations: Availability

- Manuscript in BioRxiv

New Results

**Comparative genomics approaches accurately predict deleterious variants in plants**

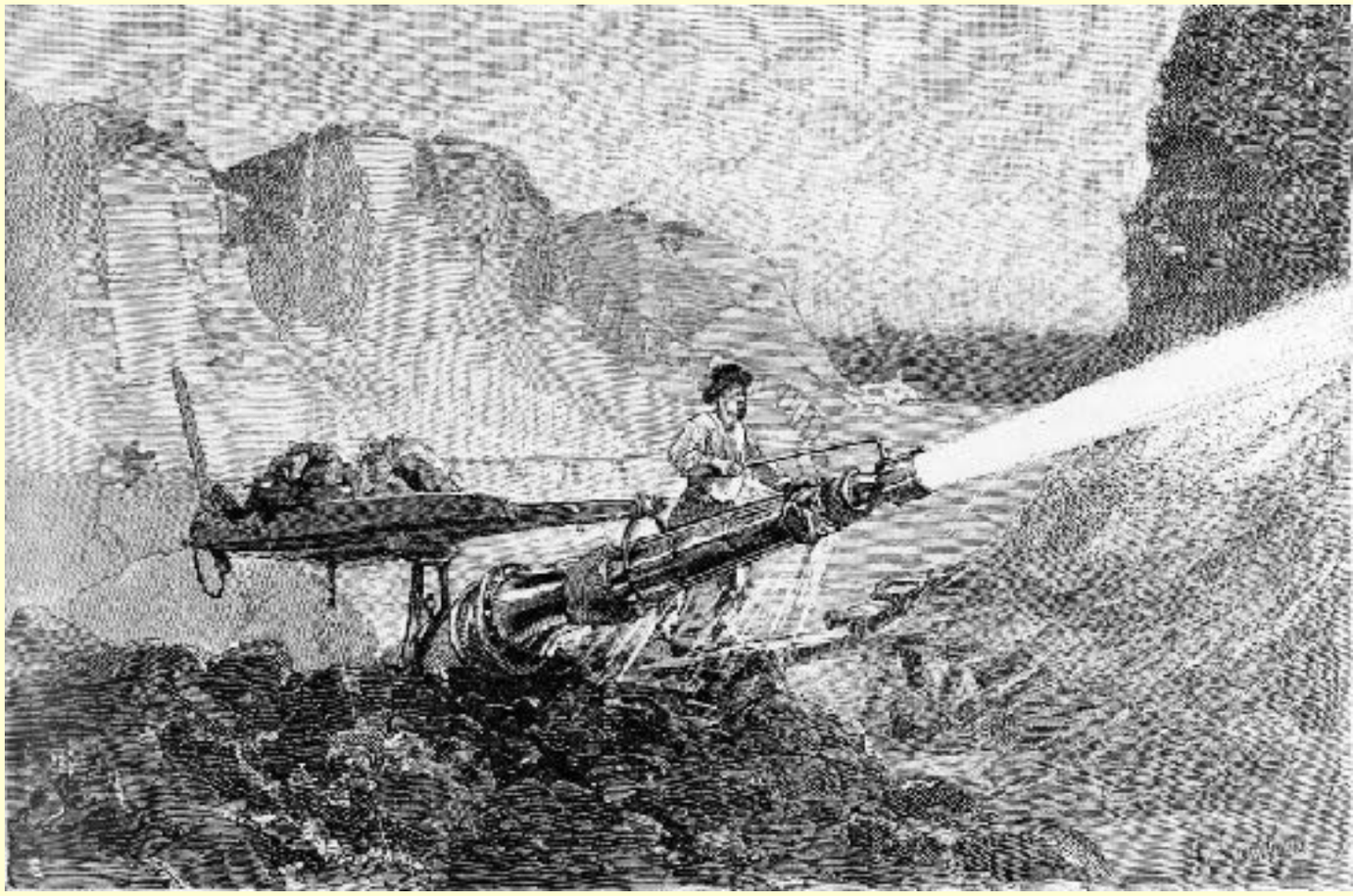
Thomas John Y Kono, Li Lei, Ching-Hua Shih, Paul J Hoffman, Peter L Morrell, Justin C. Fay

**doi:** https://doi.org/10.1101/112318

Software available in GitHub

- https://github.com/MorrellLAB/BAD_Mutations
- Manual: https://github.com/MorrellLAB/BAD_Mutations/blob/master/Manual/Manual_v1.0.md

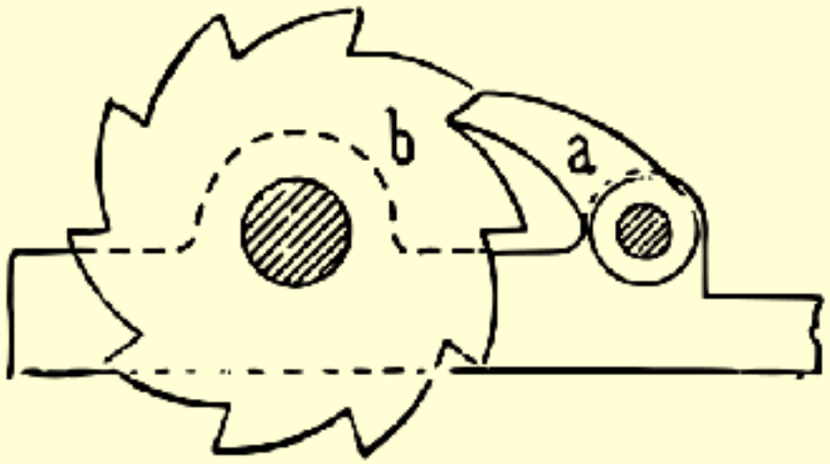
# Deleterious Variants: A “Gold Rush”



Lewontin 1974
Rockman 2012

# Deleterious Variants: Theoretical Basis

- Purging effects - Finite $N_e$ limits the effectiveness of purifying selection[1]
- "Muller's Ratchet"[2] - Deleterious mutations fix in low recombination regions
- Linked selection effects - genetic hitchhiking[3]




1:Takebayashi and Morrell 2001
2: Muller 1964
3: Hill and Robertson 1966

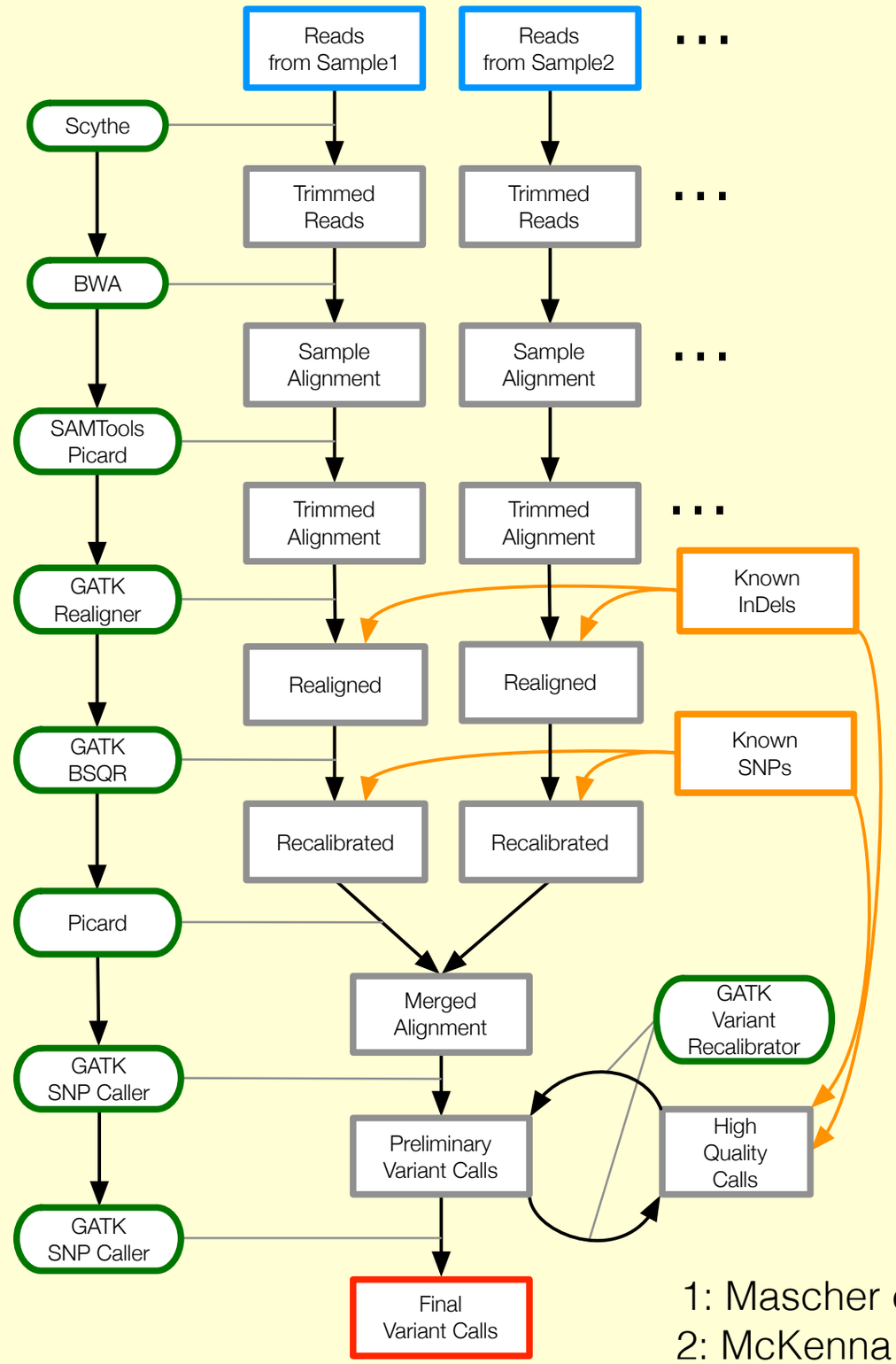
# Deleterious Variants: Questions

- How many putatively deleterious SNPs segregate in two crop species?

- Are SNPs that are causative for a phenotypic variant more likely to be annotated as deleterious, as compared to those without known phenotypic effects?

# Deleterious Variants: Resequencing Data Analysis Pipeline

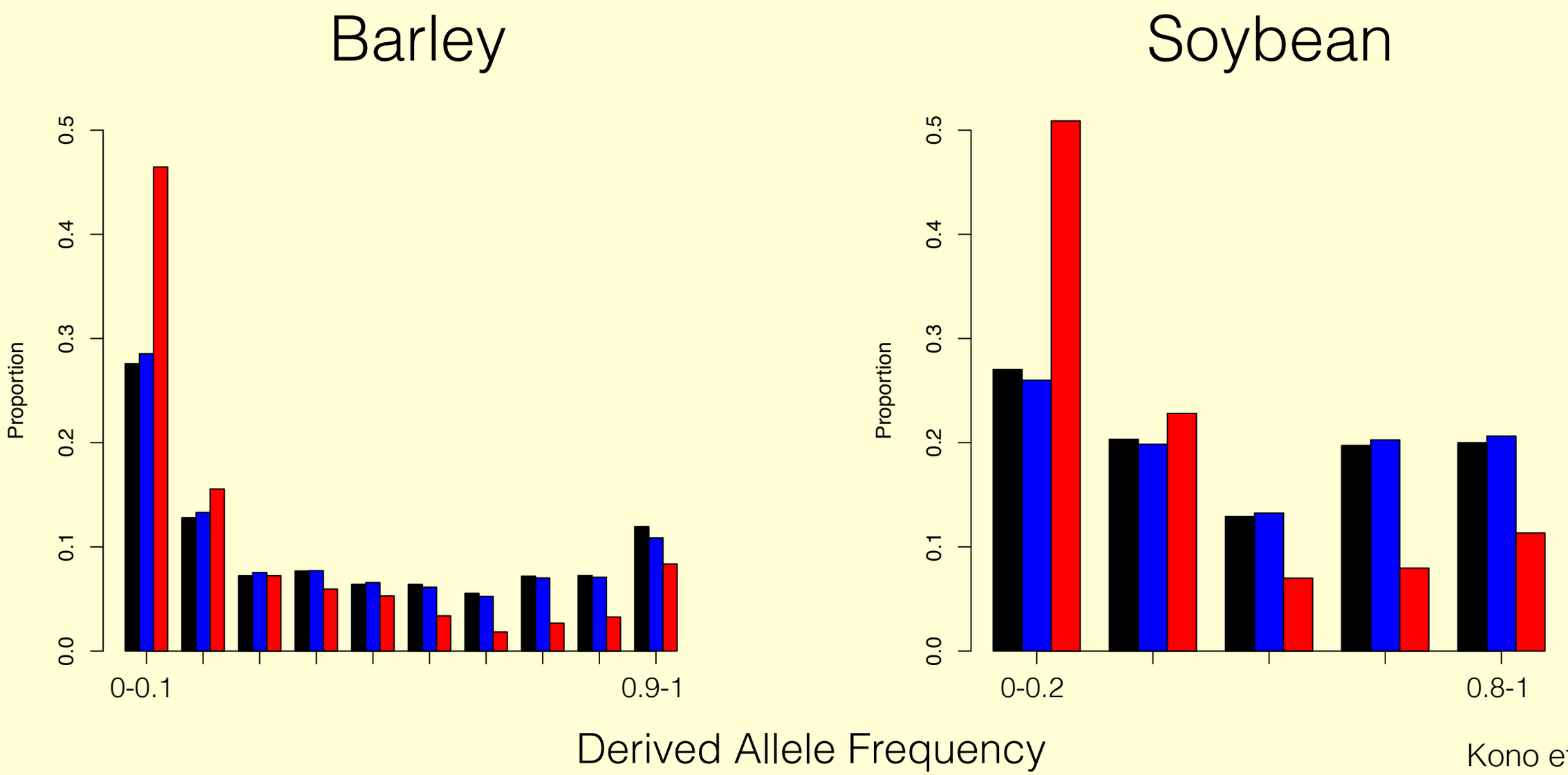
- Exome resequencing[1] of 15 barley accessions
- Whole genome resequencing of 8 soybean accessions
- Workflow based on GATK best practices[2,3]




1: Mascher et al. 2013
2: McKenna et al 2010
3: DePristo et al. 2011

# Deleterious Variants: Enrichment for Low Frequency

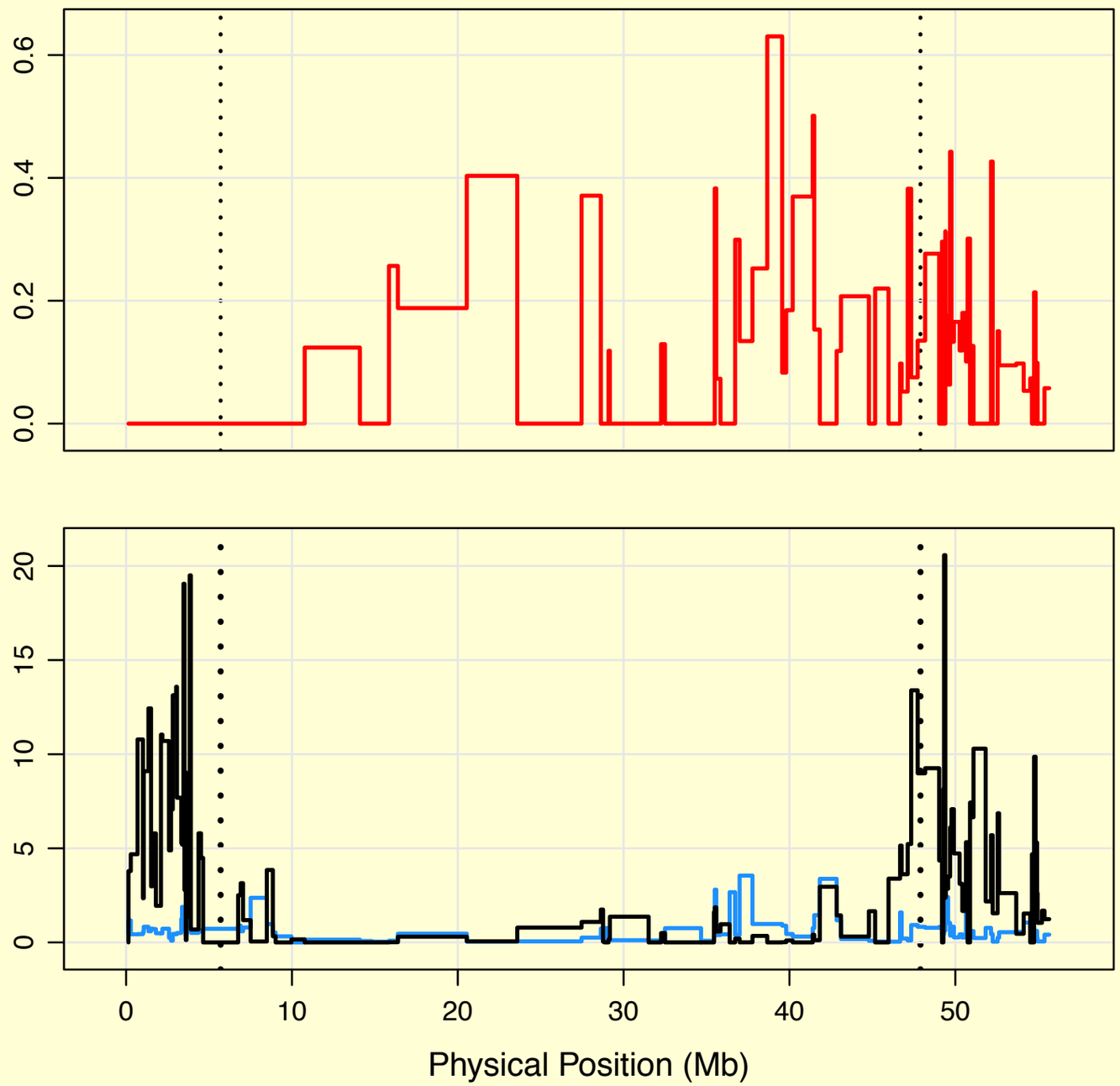
- Deleterious variants tend to be at lower derived frequency than “tolerated” variants




Kono et al. 2016

# Deleterious Variants: Enrichment in Low Recombination Regions

- Tend to occur in low recombination regions




Pericentromere boundary
Prop. Deleterious SNPs
Recom. rate (cM/Mb)
Nucleotide diversity



Kono et al. 2016

# Deleterious Variants: Causative SNPs are "Deleterious"

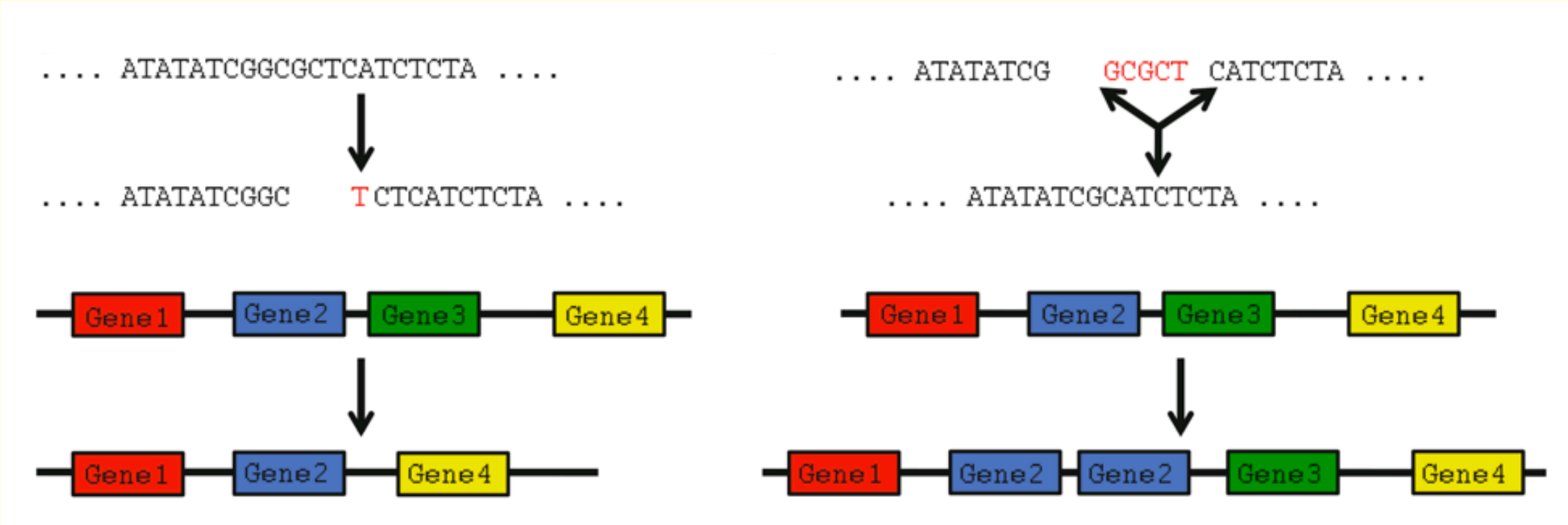
- Causative variants tend to be called deleterious more frequently than those without *a priori* known phenotypic impacts

| | Tolerated | Deleterious | Total |
|---|---|---|---|
| Causative | 23 (**67.6%**) | 11 (**32.4%**) | 34 |
| No Known Phenotype | 29,259 (**94.2%**) | 1,790 (**5.8%**) | 31,046 |



Kono et al. 2016

# A Different Mutation Type: Genome Content Variation






Hirsch 2014

# Tandem Duplicate Evolution: Background

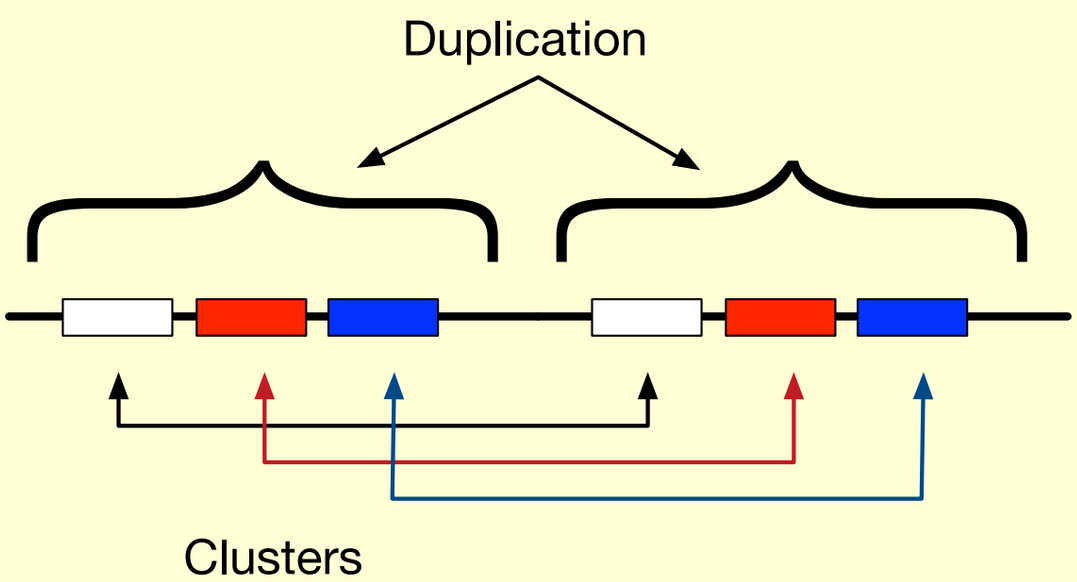
- Tandem duplicates may be particularly compelling because they are segmental duplications of genes or gene fragments
- May have interesting evolutionary outcomes because they are duplications in the same genomic "neighborhood."
- New long read assembly in maize allows for better resolution of tandem duplicate sequences

# Tandem Duplicate Evolution: Questions

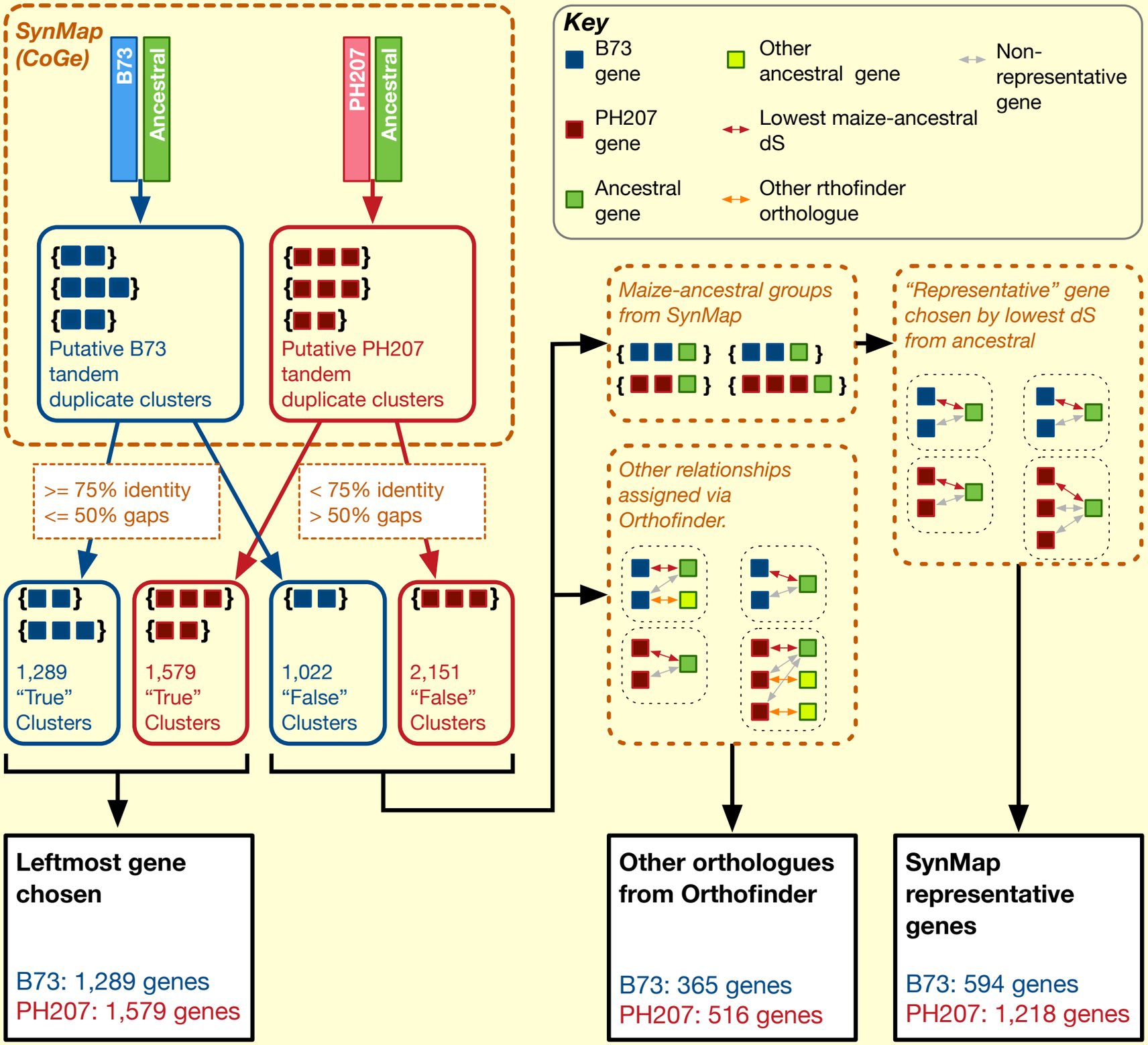
- Where are tandem duplicate genes in maize? Is there a genomic feature that explains their distribution?

- How old are tandem duplications in maize? Do they arise continuously, or happen in “bursts?”

- What are the estimated functional outcomes of tandem duplicate genes in maize? Is there a relationship between the age of a duplication and its outcome?



# Tandem Duplicate Evolution: Identifying Tandem Duplicates

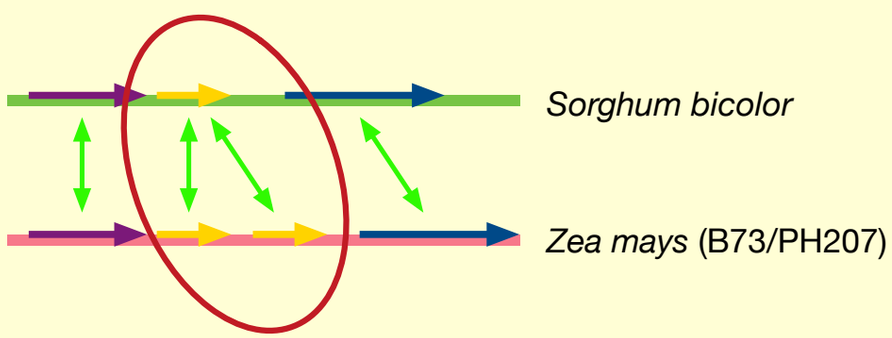




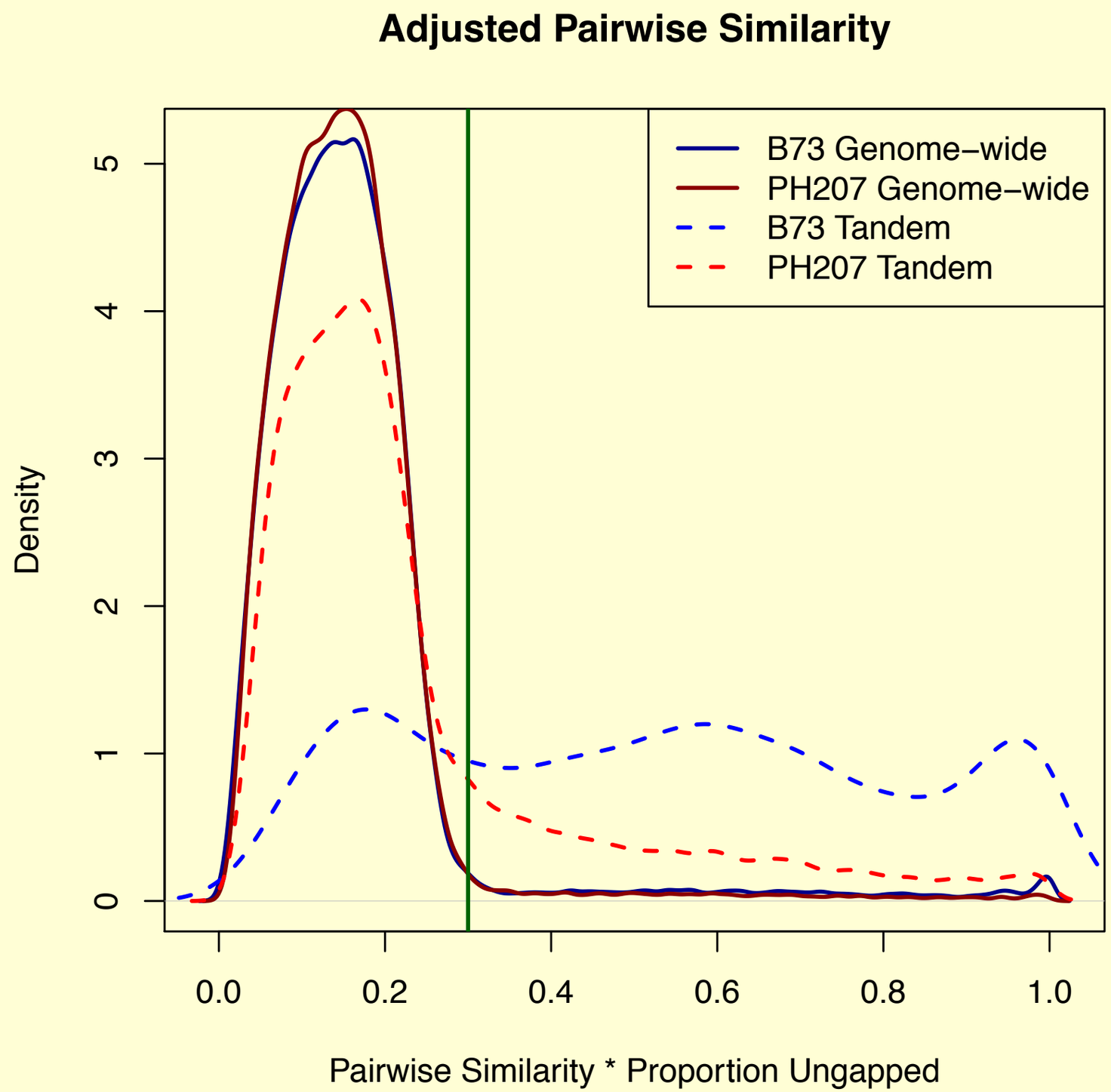

Brohammer et al. *In prep*

# Tandem Duplicate Evolution: Identifying Tandem Duplicates

- Had to filter tandem duplicates - CoGe identification procedure was over-calling




- "Adjusted pairwise similarity" = pairwise similarity, down-weighted for gapping



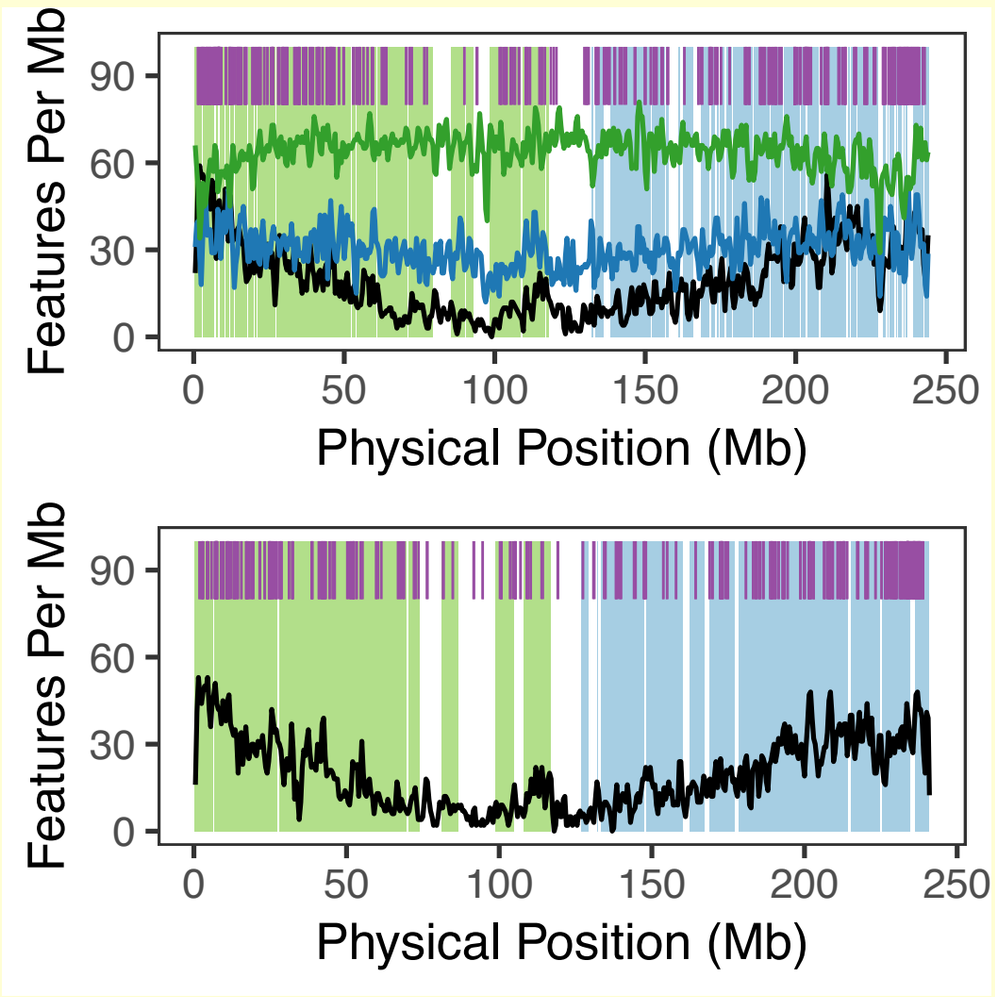
# Tandem Duplicate Evolution: Most Duplicates are Shared

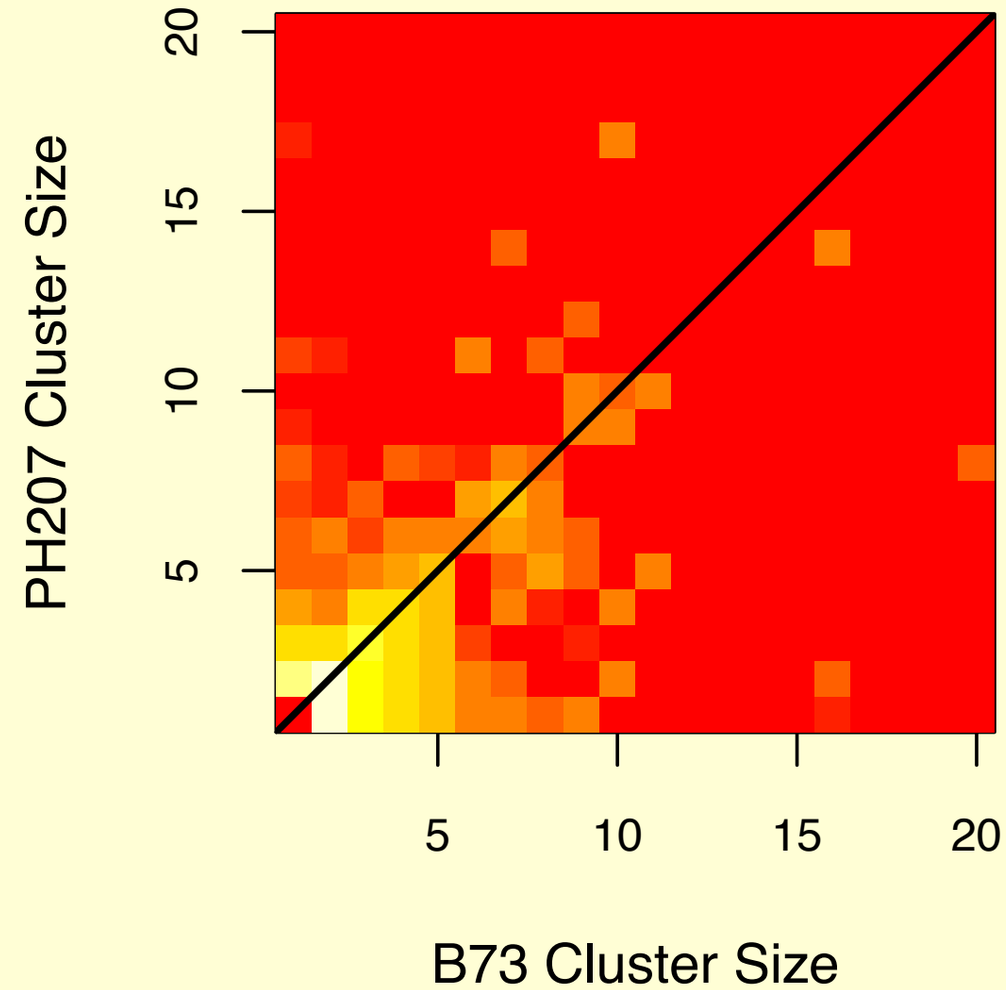
Tandem duplications happen where there are genes

Most tandem duplicates are shared between B73 and PH207

B73

PH207

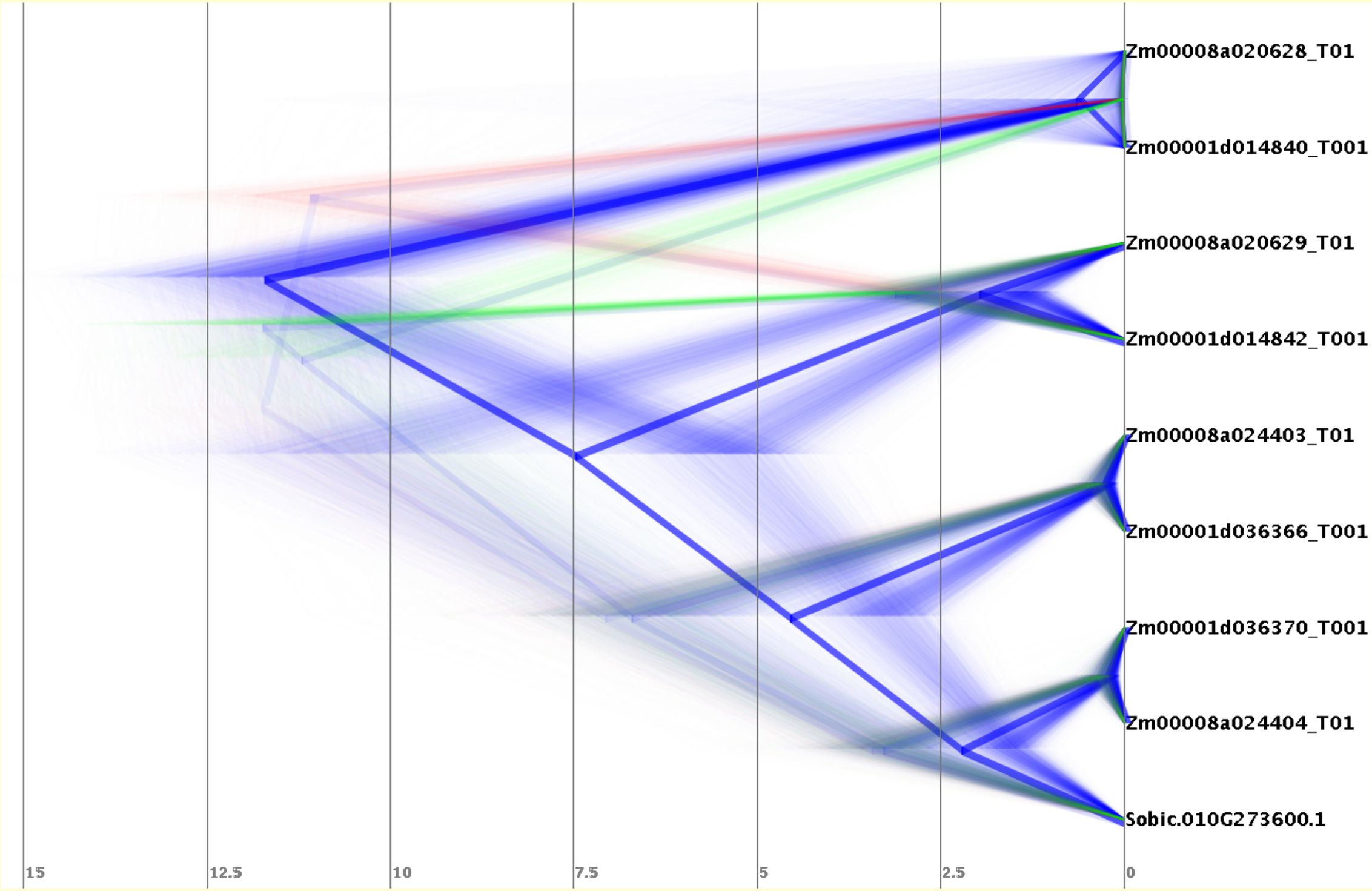





Kono et al. *In prep*

# Tandem Duplicate Evolution: Diverge Date Estimation



# Genome Content Variation: Background

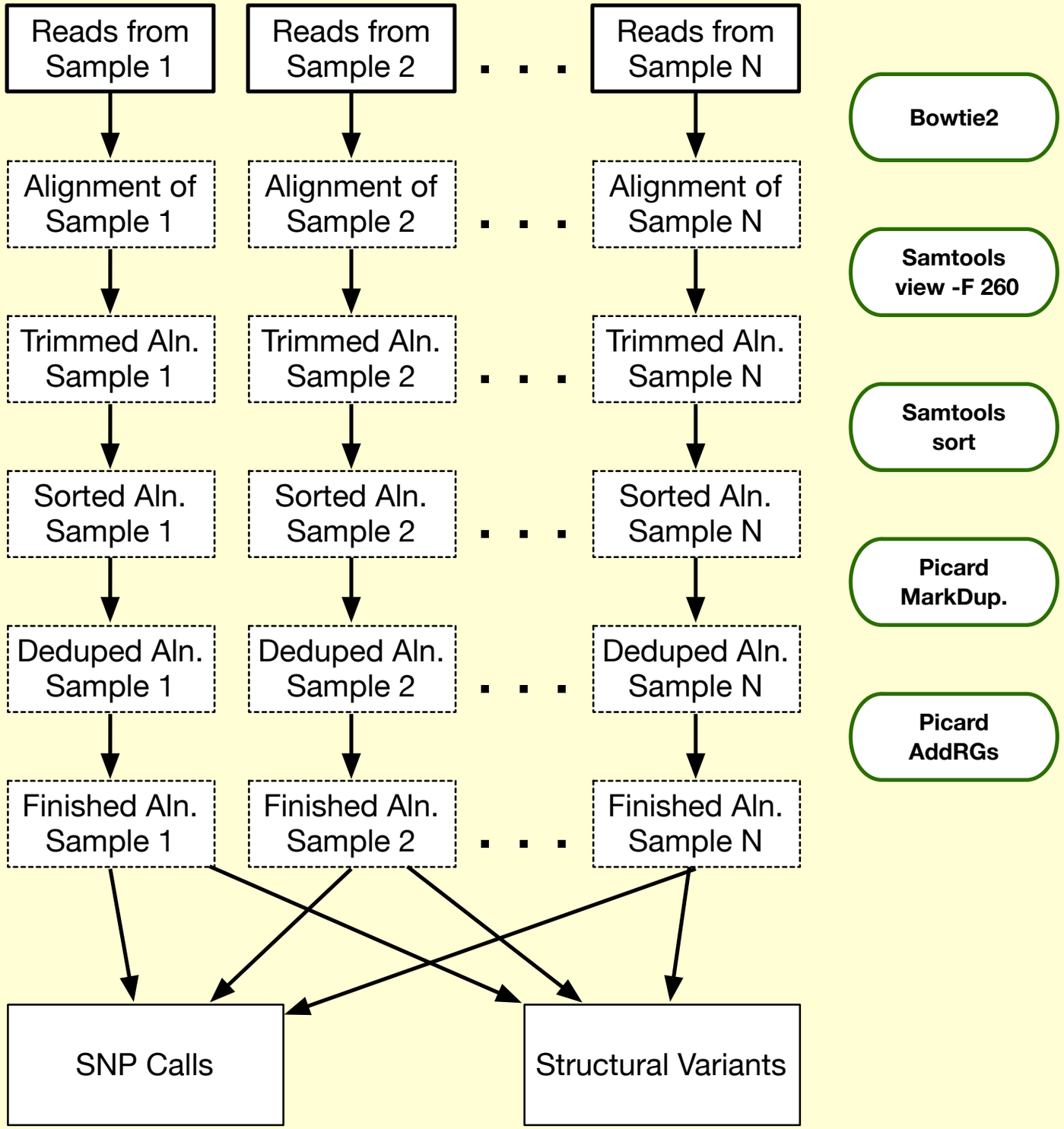
- Presence-absence variation and copy number variation may play a large role in phenotypic variation

- SNPs can only explain so much variation in GWAS, and most SNPs are thought to have neutral or very small effects on phenotypic variation

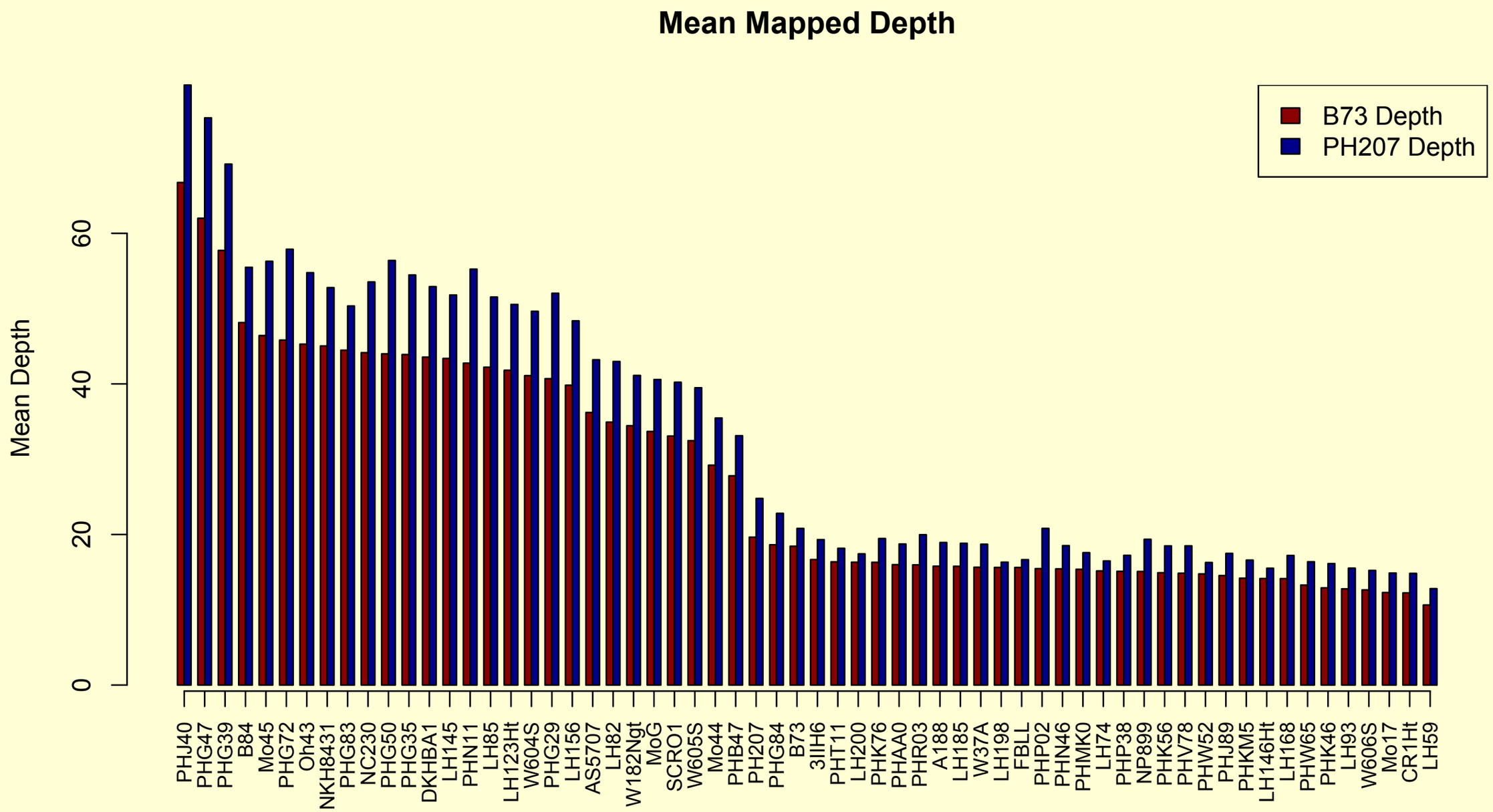
- Gene deletions/duplications, on the other hand, may have much greater impact, as they more drastically alter protein function and regulation

# Genome Content Variation: Sequence Analysis Pipeline

- Whole genome resequencing of 62 maize inbred lines
- Currently have short read resequencing, but will collect long read data
- Mapped against two reference genomes

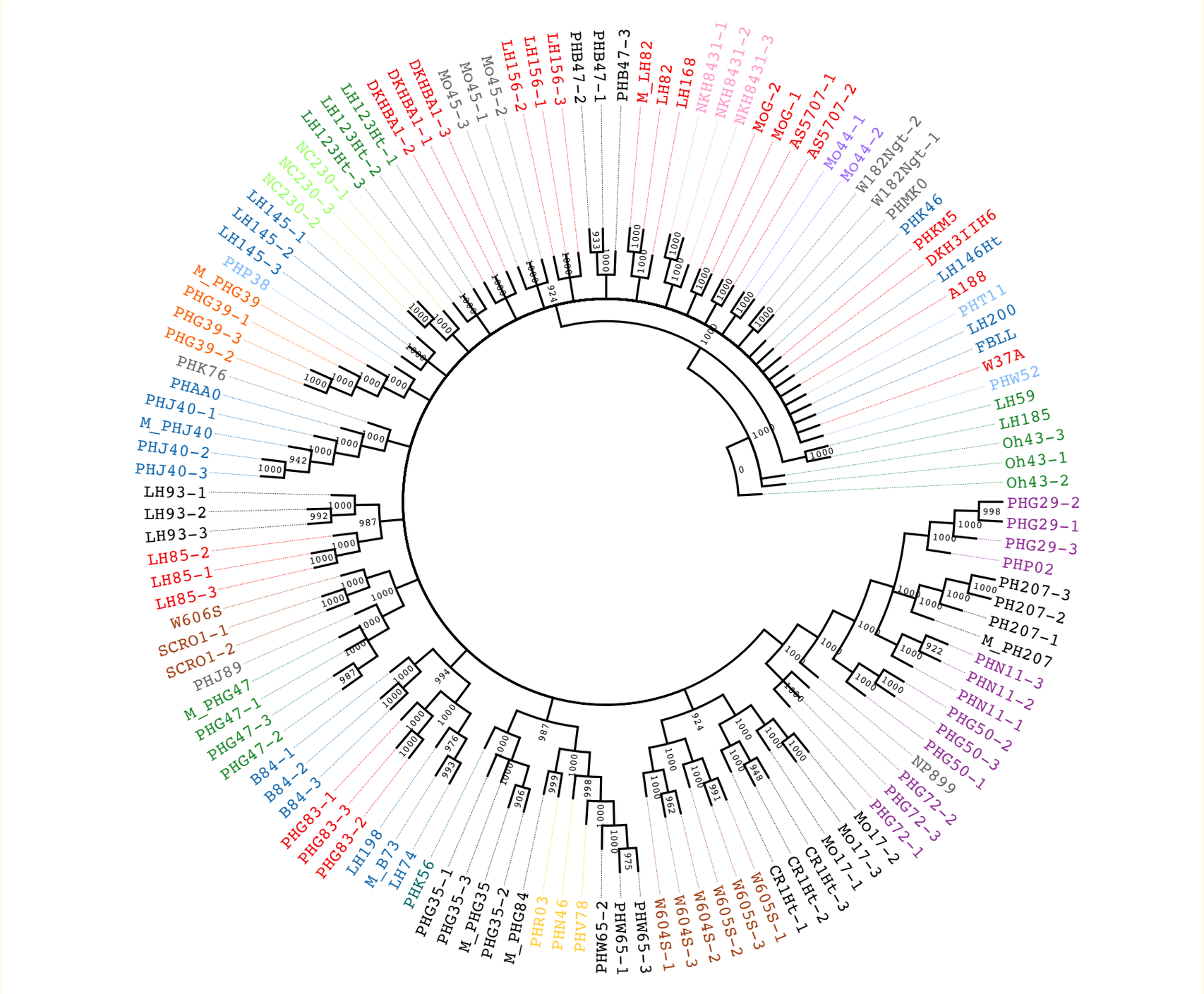


# Genome Content Variation: Varying Depths of Sequencing



# Genome Content Variation: A "Biological QC"

- Bootstrapped NJ tree for sample verification
- Samples from multiple libraries and data sources cluster by genotype



# Genome Content Variation: Next Steps

- First: downsampling analysis with the high-coverage samples
  - Identify the nature of the tradeoff between sequencing depth and variant identification sensitivity
  - What depth gives a good balance between variant discovery and cost?
- Later: Call genome content variants, and estimate the frequencies of gene loss and duplication

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Kiran Seth
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