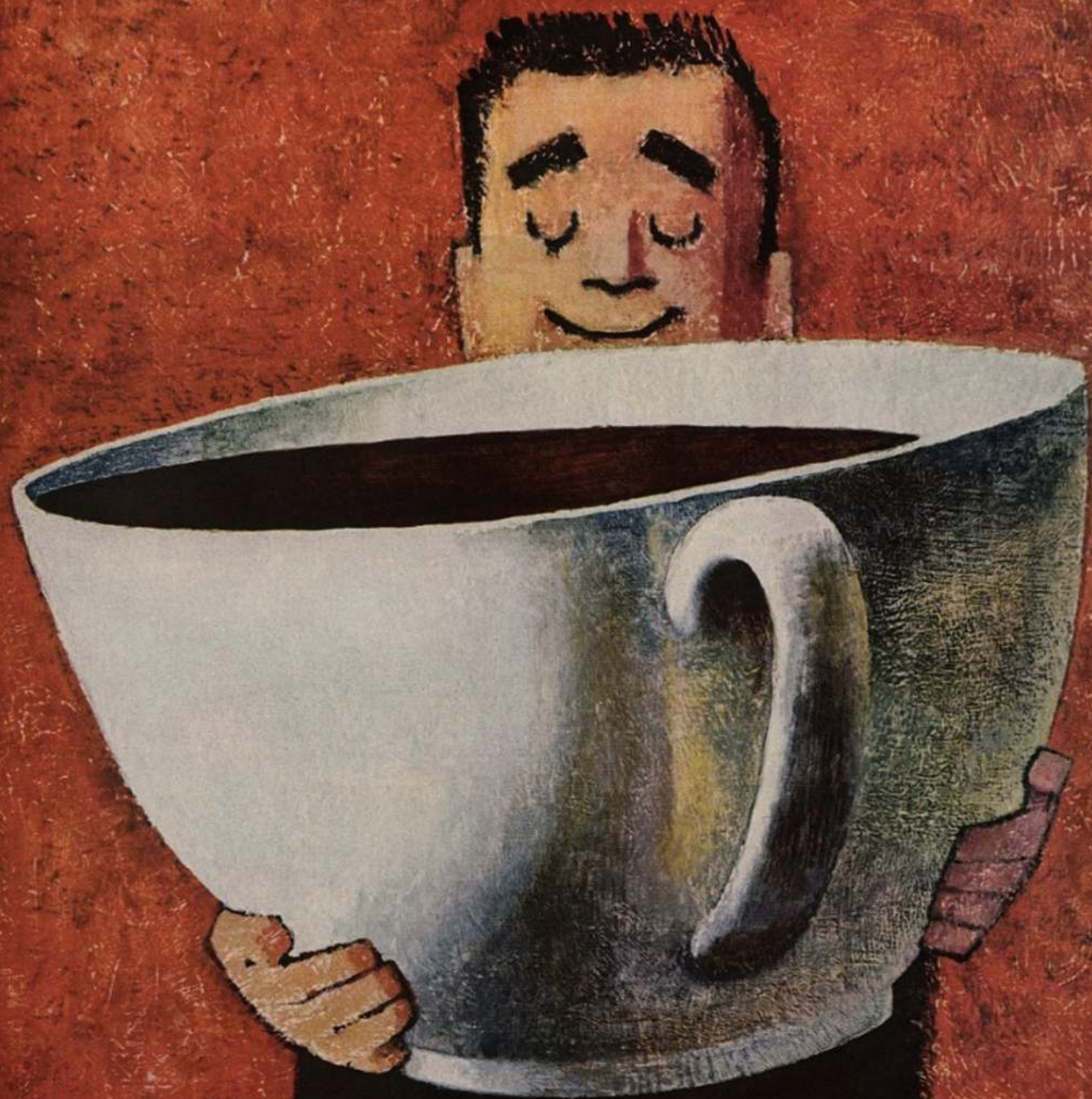


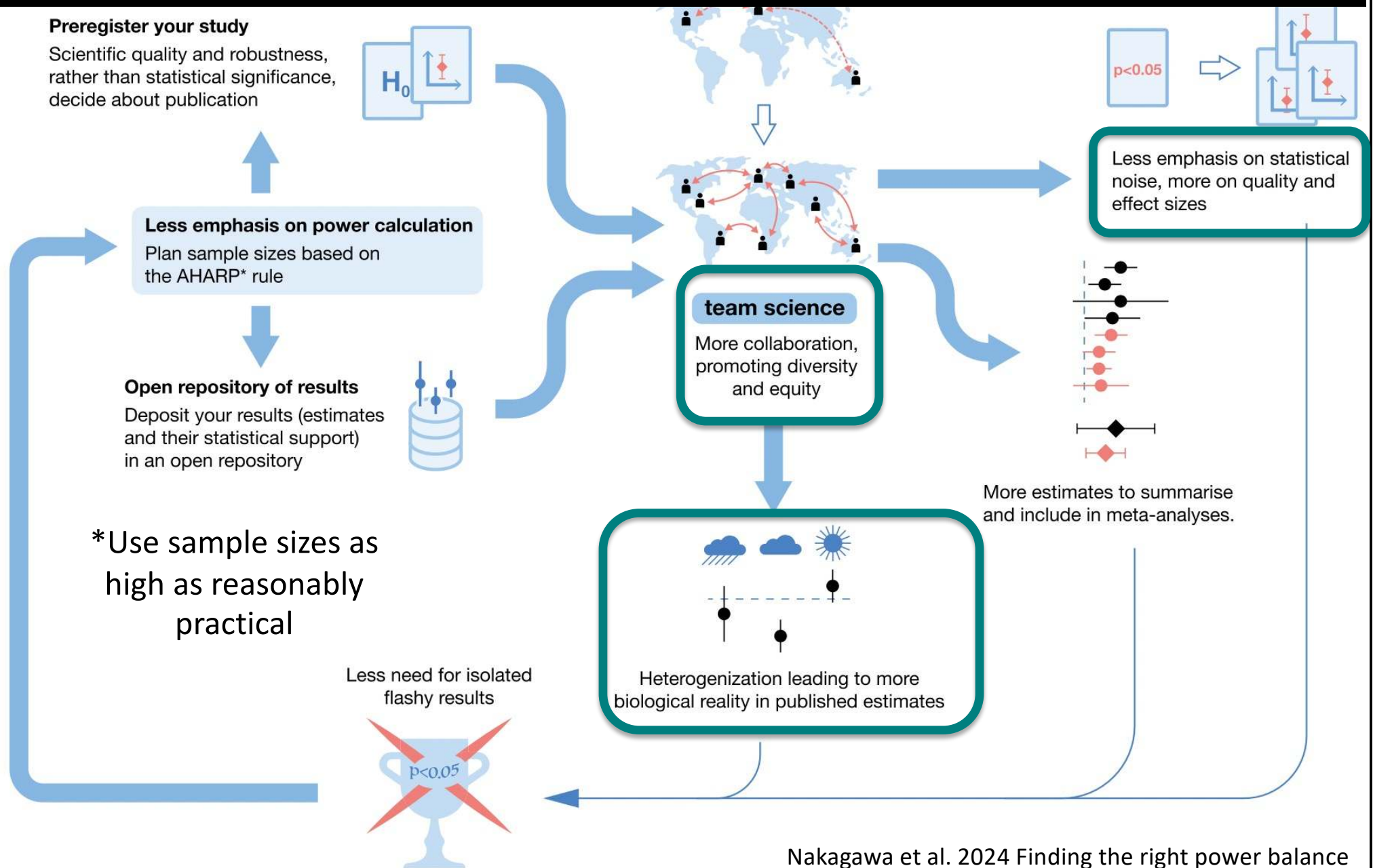
JOHN FALTER



So .. how do we avoid Apophenia?

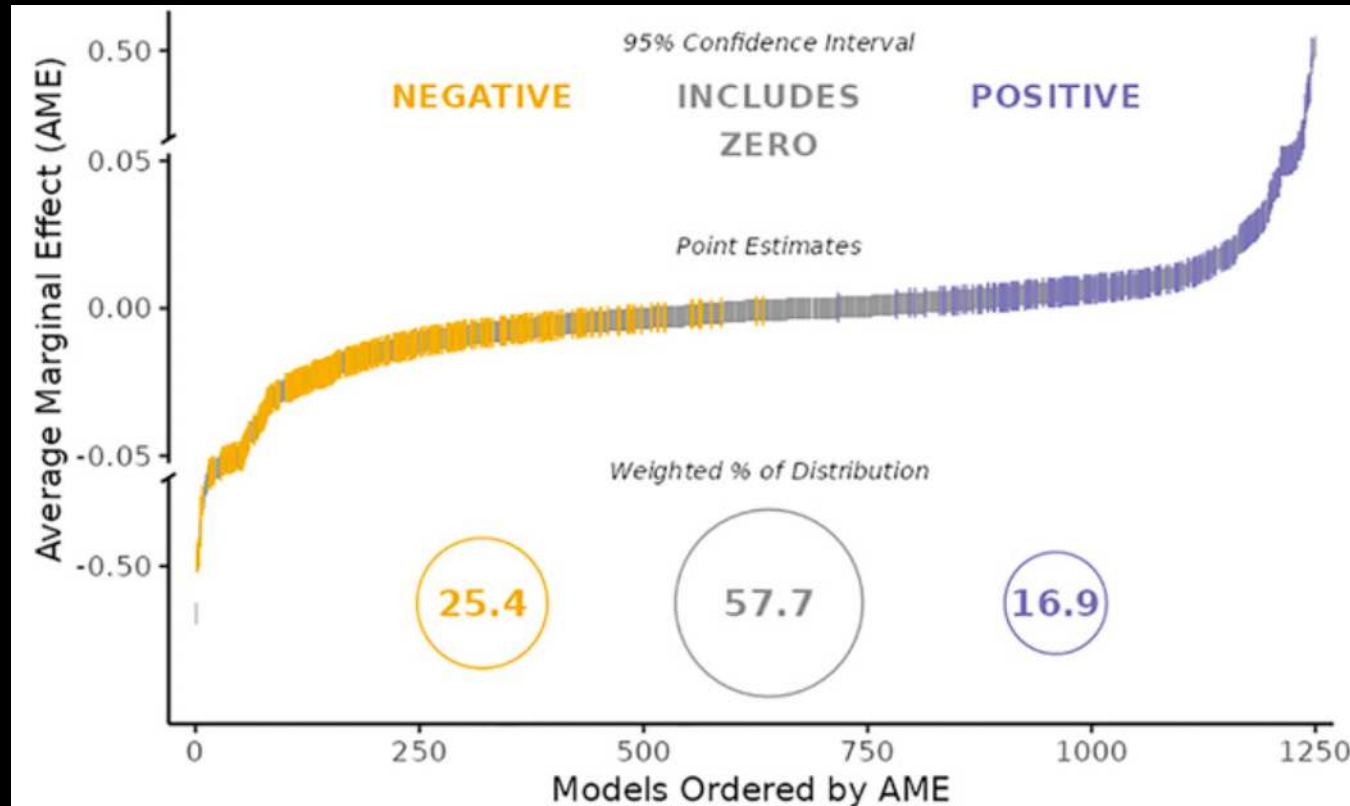
- Non-random patterns are abundant in genome scale data
 - We generally lack ability to calibrate our expectations
 - Null models, controls are very difficult to get “right”
- Double check your data and analyses
 - Plot your data, look at it, does it make sense on 1st principals?
- Test your hypotheses in independent ways
 - Genomics: independent datasets, independent analyses, across levels
 - Independent biological samples, GWAS vs. K-mer GWAS, mRNA vs. protein
 - Manipulation: functional validation via manipulation of genes, pathways
 - Experimental evolution, CRISPR KOs, environmental perturbations

One way out of the vicious cycle of power analysis and publication bias



Large scale replication study in social sciences

1 dataset analyzed by 161 researchers in 73 research teams



Do you expect the same variation in outcomes if this was repeated in your field of genomics?

Breznau et al 2022 Observing many researchers using the same data and hypothesis reveals ...

How many of you are trying to find genomic regions of importance for your phenotypes?

Are you using molecular tests of selection?

How do you decide what tools to use?

Does it matter?

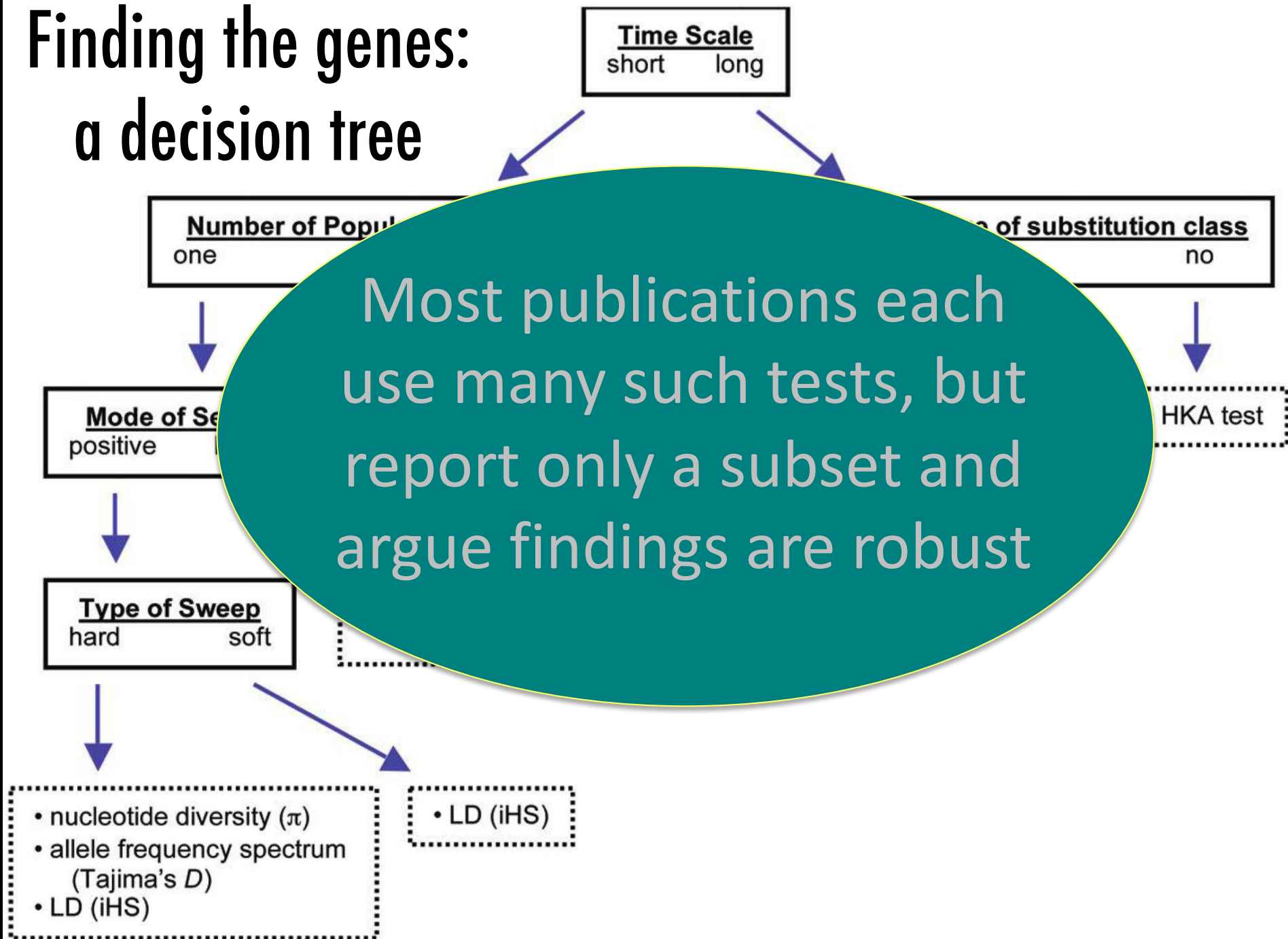
When do you stop running tests?

Which do you report?

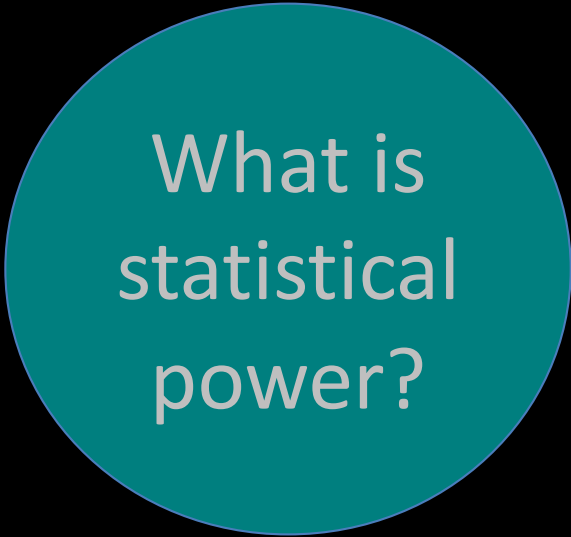
How do we identify the genes that matter?

- Molecular tests of selection are popular, but ...
 - What are their assumptions and statistical power?
- What are these tests detecting?
 - What is a footprint of selection?
 - How are they formed?
 - How large are they?, how long do they last?
 - How are they impacted by demographic history? Introgression? Aliens?
 - Should we even expect "footprints"?

Finding the genes: a decision tree



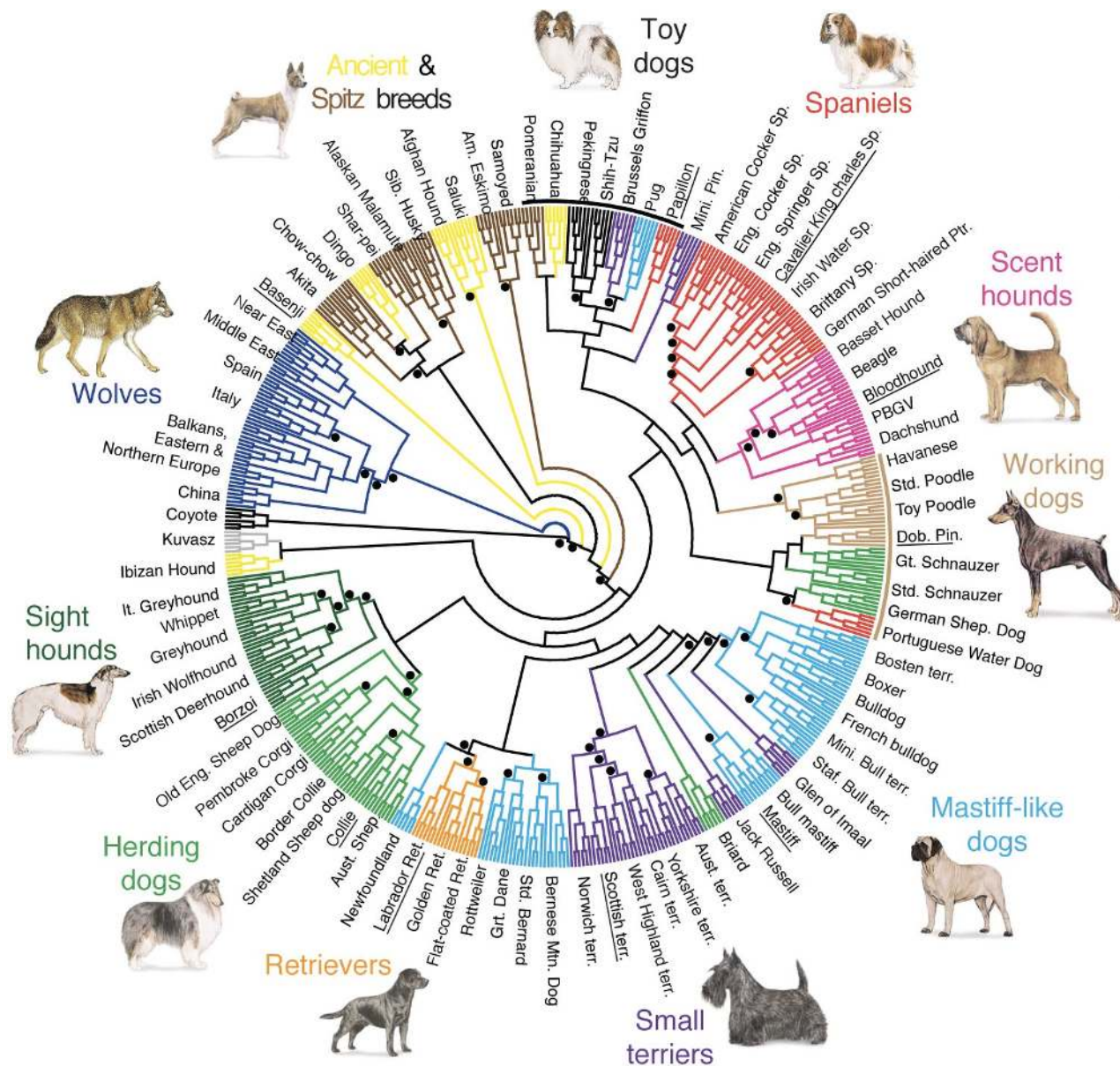
What power do we
have to detect
evolution by
natural selection?



What is
statistical
power?

Power is the probability that the test will reject the
null hypothesis when the alternative hypothesis is
TRUE

Should independent molecular tests converge?



von Holdt et al. 2010. Nature

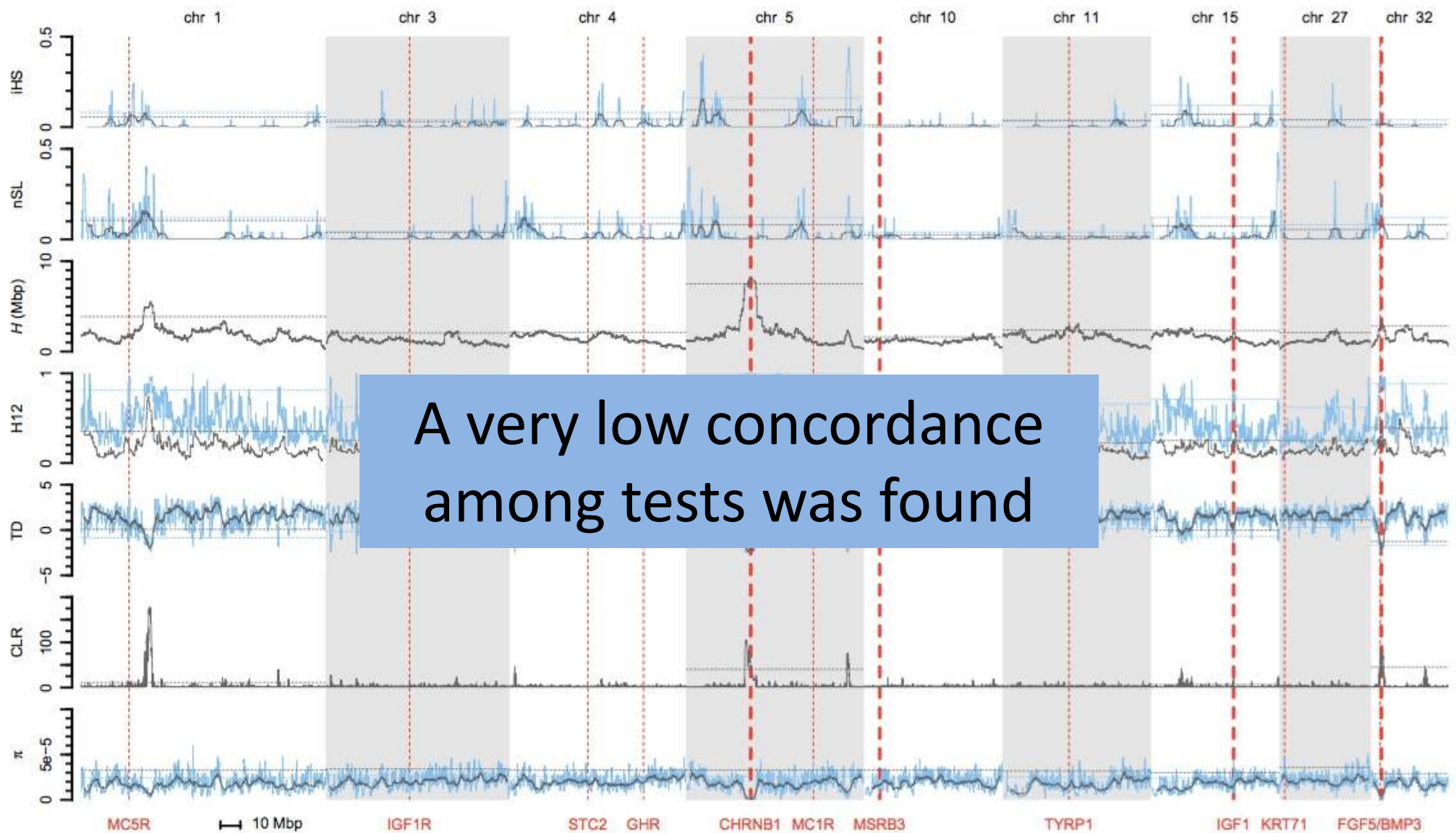
Breed specific morphologies

Test set of Schlamp et al. 2016:

- 25 breeds
- 12 causal loci identified by QTLs
- $N = 25$ / breed
- 7 tests of selection
 - $iHS, nSL, H, TajD$, etc.

How concordant are molecular tests of selection detect?

French Bulldog sample: red lines are causal QTL loci



Schlamp et al. 2016. Evaluating the performance of selection scans to detect selective sweeps in domestic dogs. Molecular Ecology 25:342–356.

Why don't these these tests agree?

biological reality
or
our expectations
or
theoretical population genetics

What if different
groups used
different tests?

Would that skew the
literature?

Is this common?
Should we worry?

Test your hypotheses in independent ways

- Genomic datasets:
 - These are really observational data where patterns we observe have been created by things we barely understand
 - This is similar to all studies using observational data
 - Very susceptible to false positives
 - Extremely large P-values can arise from extremely weak patterns, so ask yourself, does the effect and effect size have biological meaning?

Test your hypotheses in independent ways

- Derive hypotheses from your genomic results, then
- Test these hypotheses using relevant manipulations
 - functional validation via manipulation of genes, pathways, environments ... real hypothesis testing!!
 - Experimental evolution, CRISPR KOs, environmental perturbations
- If you can't manipulate, at least triangulate!

Triangulation



Robust research needs many lines of evidence
Replication is not enough

Triangulation — a checklist

- Use different approaches to address the same hypothesis, or extensions of hypothesis
- Sources of bias for each approach should be explicitly acknowledged, in opposite directions, and independent
- Results from more than two approaches are ideally compared

Functional genomic study of phenotypic plasticity

Identifying the genetic basis of plastic phenotypes is very challenging

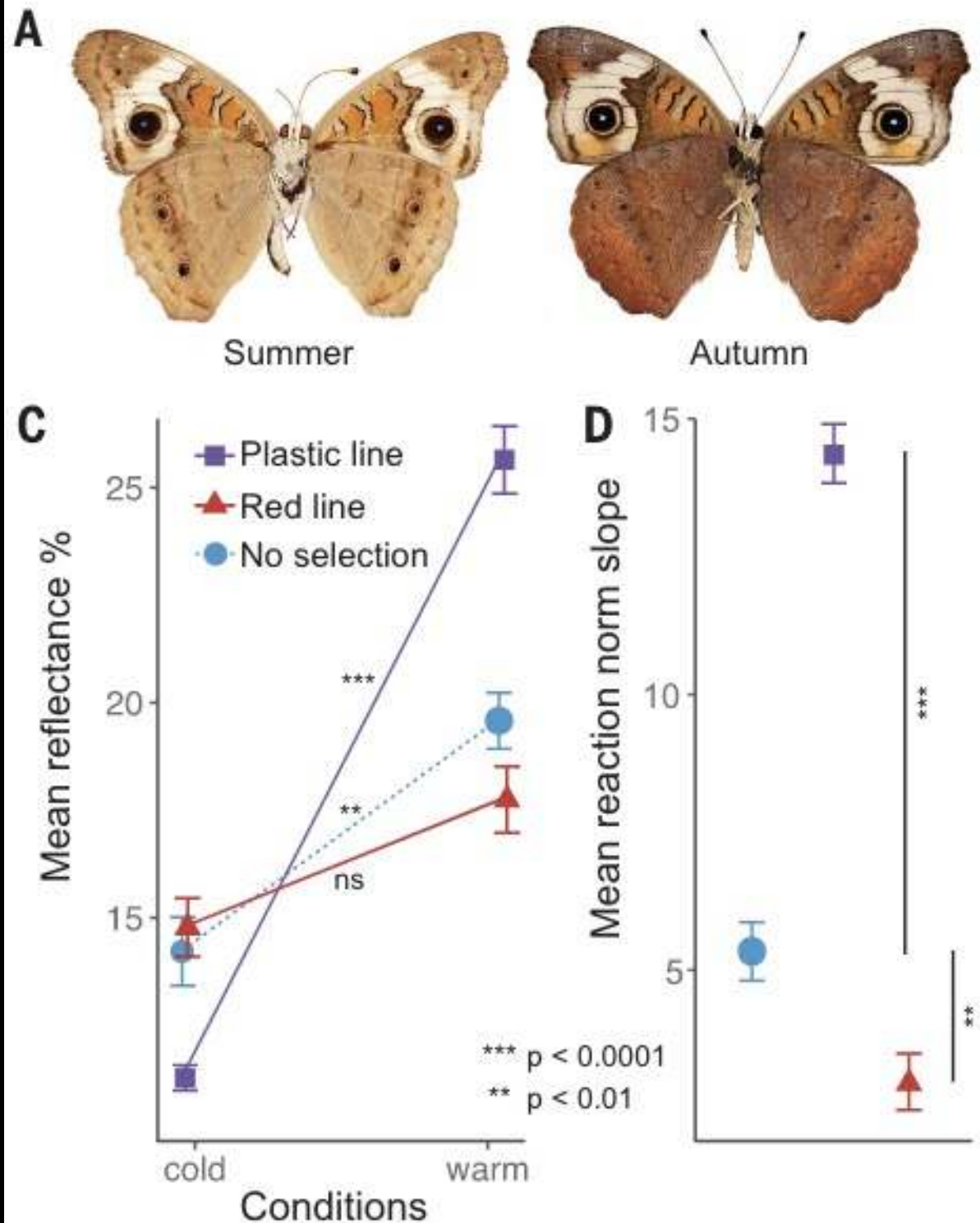
Here researchers used multiple experiments for triangulation

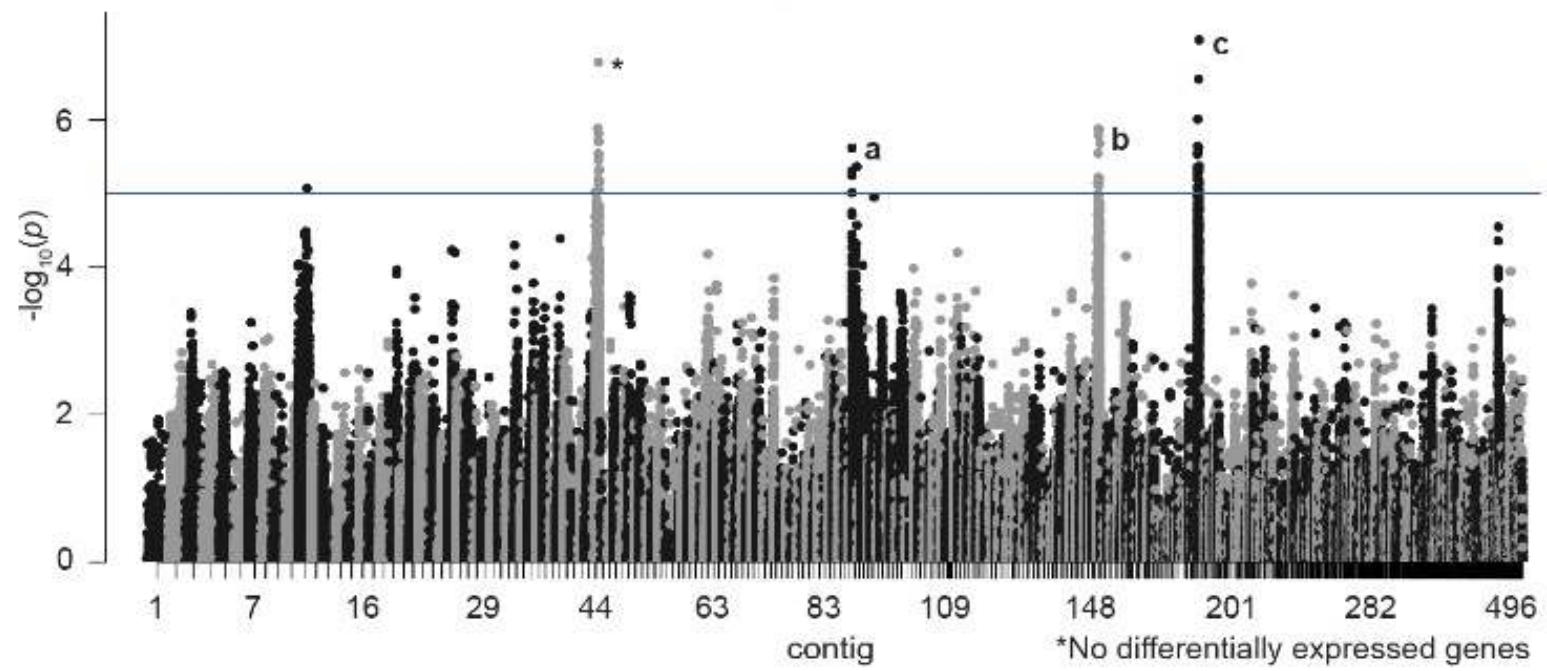
- Experimental evolution to fix trait so they could QTL map it
- GWAS between the alternative lines of high vs. low trait
- RNAseq between the alternative lines of high vs. low trait
- CRISPR-Cas gene KO to test candidate genes

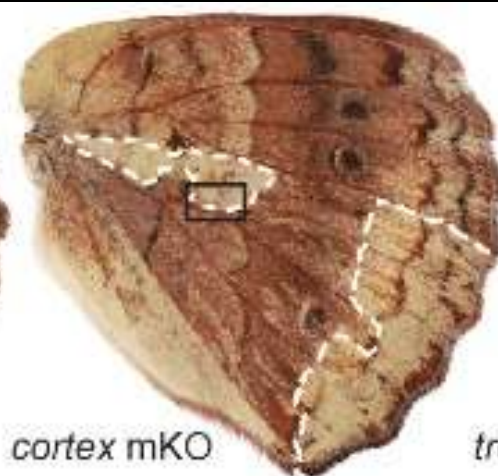
Genomic architecture of a genetically assimilated seasonal color pattern

- Made selection line having no plastic response
- Crossed back to plastic line
- GWAS on offspring for plastic response

Burg et al. 2020. Science.







Functional genomic study of phenotypic plasticity

- An integrated study identified several genes underlying a plastic phenotype
- Triangulation involved
 - Manipulation of trait using experimental evolution
 - Intersecting GWAS and RNAseq results
 - Functional validation using gene KOs of candidates
- Important additional step we should all do
 - Investigated gene without annotation, found functional association, increased knowledge of phenotype for future studies (got to name it)

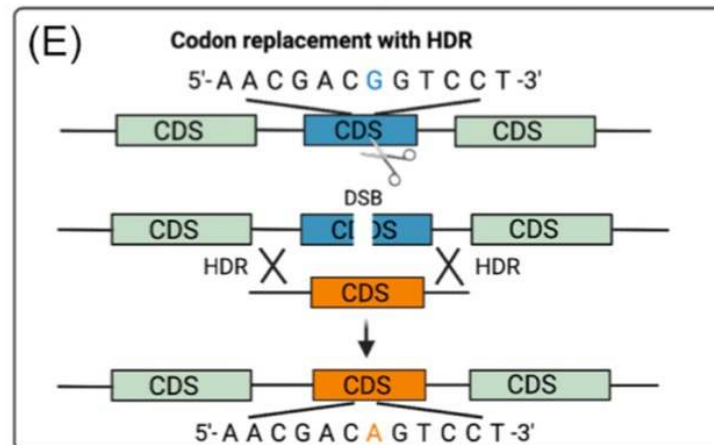
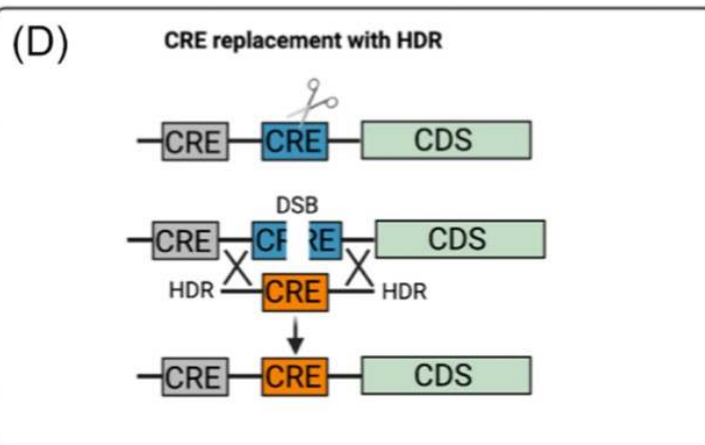
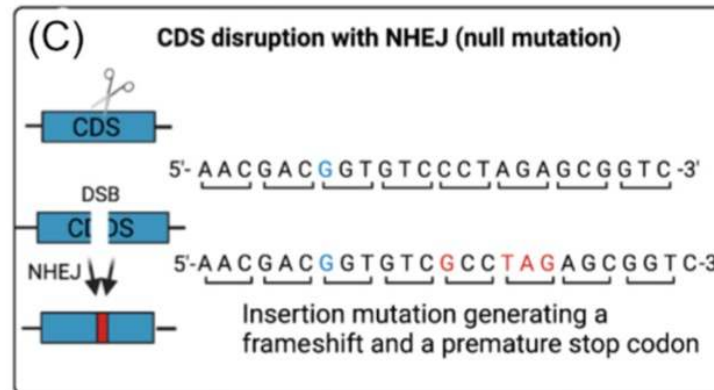
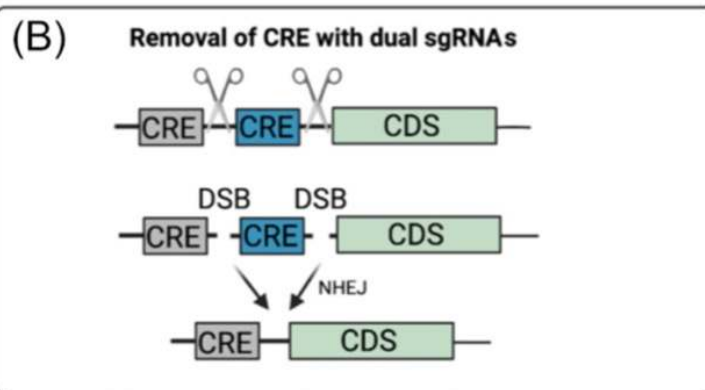
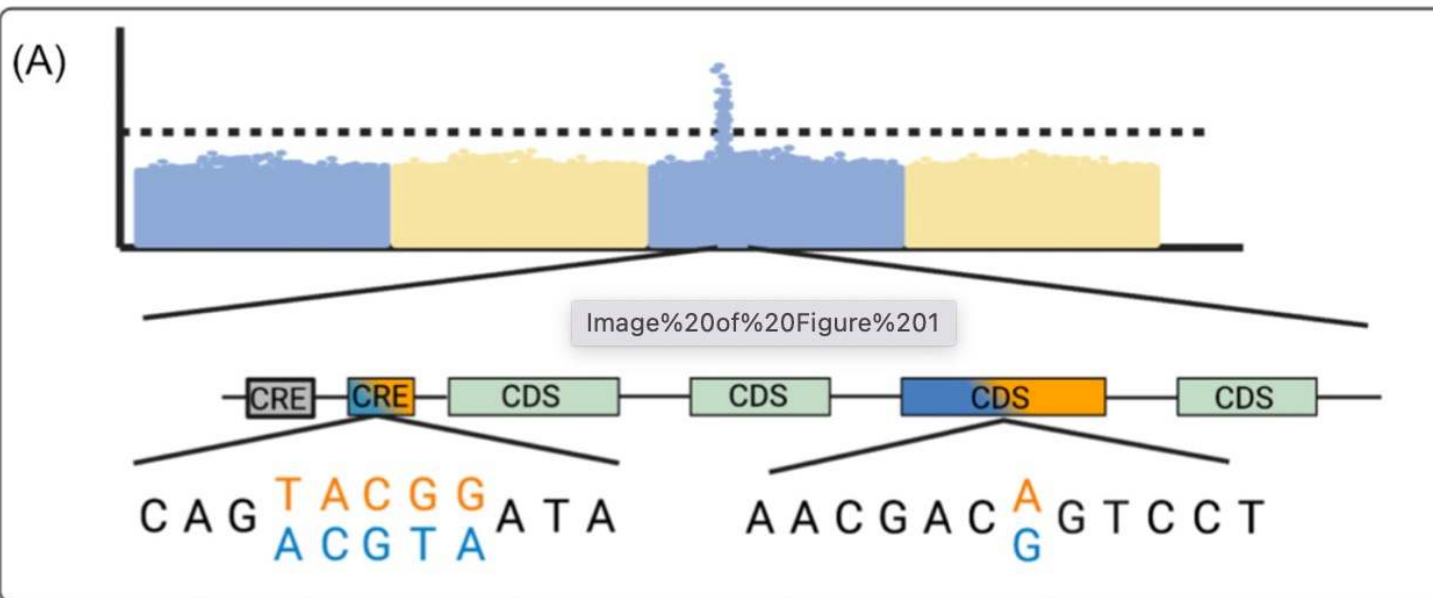
Review

Functional genomic tools for emerging model species

Erik Gudmunds,^{1,*} Christopher W. Wheat,² Abderrahman Khila,^{1,3} and Arild Husby ^{1,*}

**Recent review covering diverse means of validation across
diverse taxa**

**As genomics gets cheaper, invest more in validation instead of
just more sequencing!!!!**



Bioinformatic wisdom, pt. 1

- Expect errors and noise
 - Analysis results need many rounds of refinement
 - Invoke biological causes of results last
- 70% of your time will be troubleshooting
 - This is normal, keep a notebook, intermediate files
- Fear the new and shiny programs that will simplify your life
 - 80% of all new software will not be usable
 - Un-installable, no manual, no test examples, not repeatable
 - Beware of these red flags, as many authors only seek a publication and won't help

Cookbooking ...

- Google and AI are your friends
- Use them, but don't trust them ..
- Test what you use, learn from it, build your own toolbox



Keep good bioinformatic notes

- I keep a special file with commands I learned, like and validate
 - use it to quickly find commands, refresh memory
- Use positive and negative controls to test the output of the commands you run (like all experimental biology)
 - I call these sanity checks
 - Always test to make code is working correctly
 - Great reason to use > 1 method, right?
- Read up on good file structure, version control, and how to parallelize your commands

Publish your code, no matter how messy

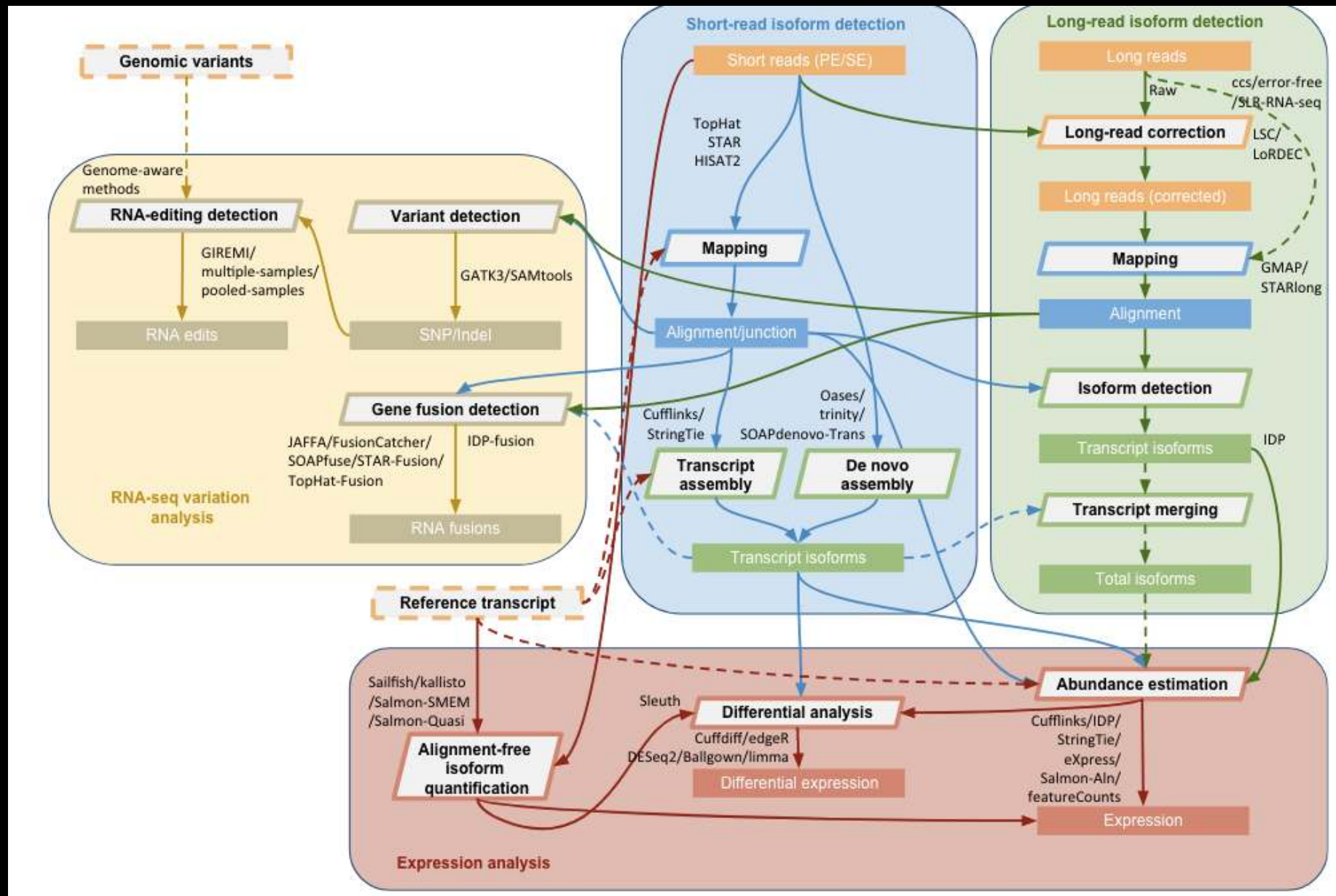


Yours is without a doubt the worst code I've ever run



But it runs

Many different ways to make a pipeline

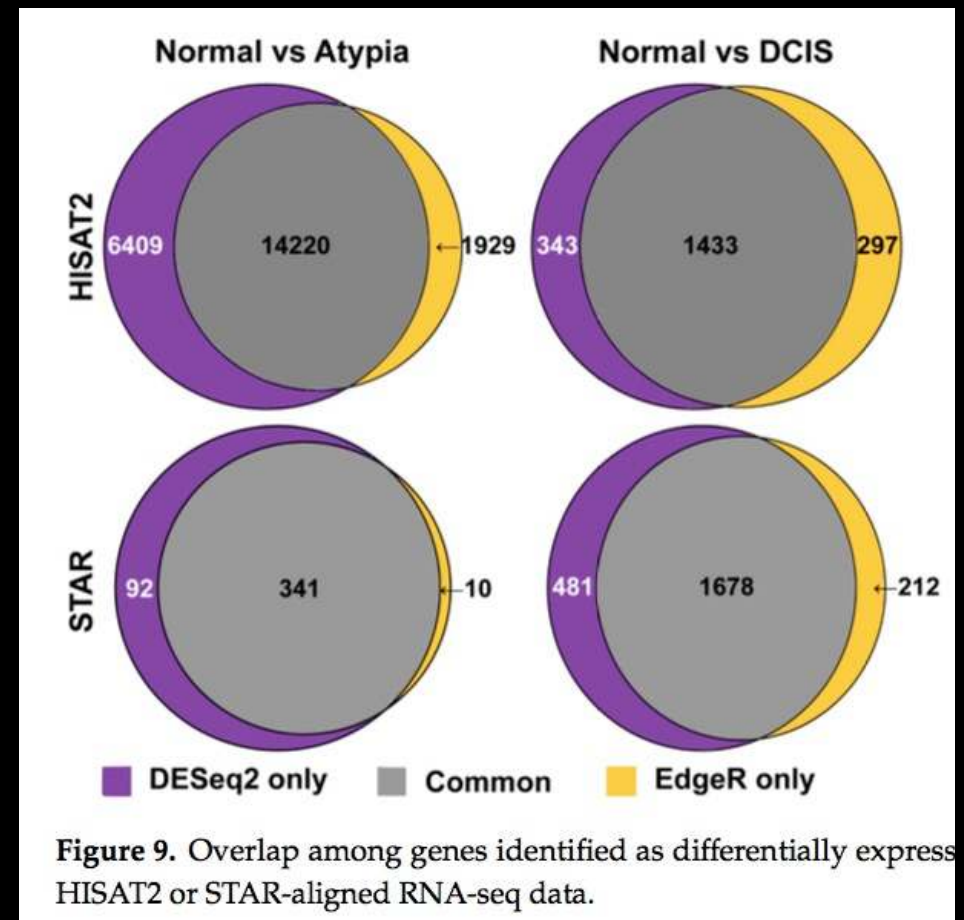


Many tools, performance varies across species, samples.

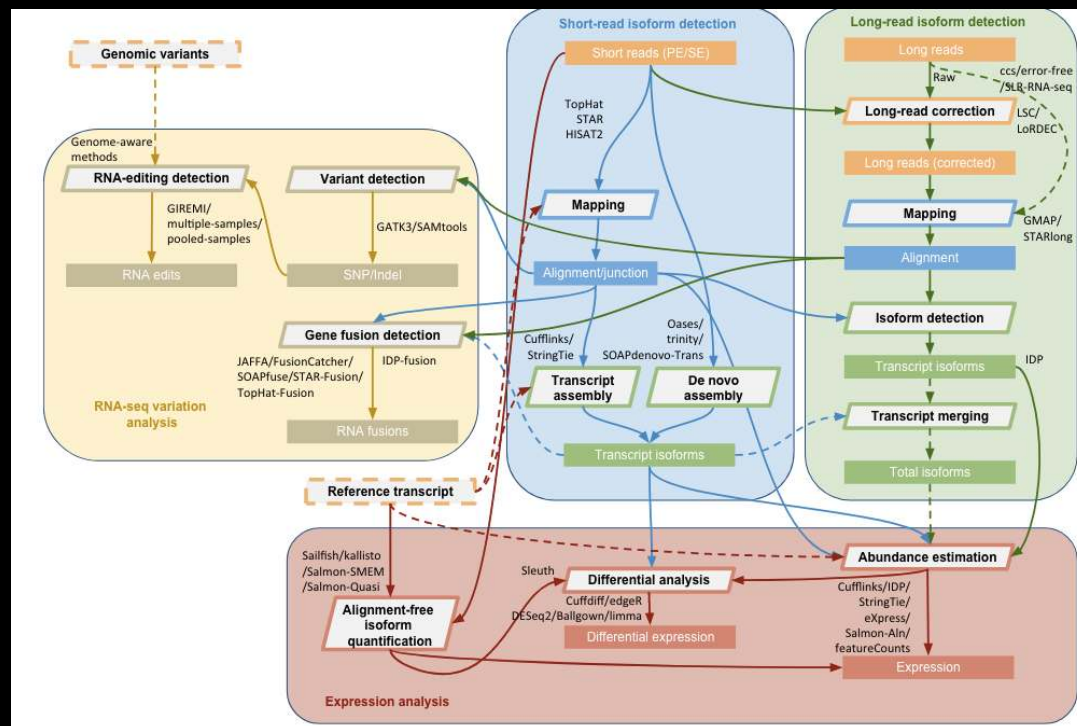
This is no BEST tool or setting across species

Differential expression detection can vary by:

- Mapper
- Analysis software
- Reference genome
- Species



Doing many analyses ? analysis paralysis is common



Which is the right way?

- Just start by get through a single pipeline, start to end
 - Then try different approach to assess your first results
- Used published data & code, then try additional approaches

Bioinformatic wisdom, pt. 2

If all publications provided all their code, science would advance faster, with more accuracy

Provide your code with all your publications, along with all your data. Be part of the solution.

Look at others other peoples online, open access code & pipelines:

- Discover new ways of coding, reporting, working
- Gain confidence in your skills
- Become frustrated that other published work is not repeatable

If bioinfo work is not reproducible, how much can we trust it?

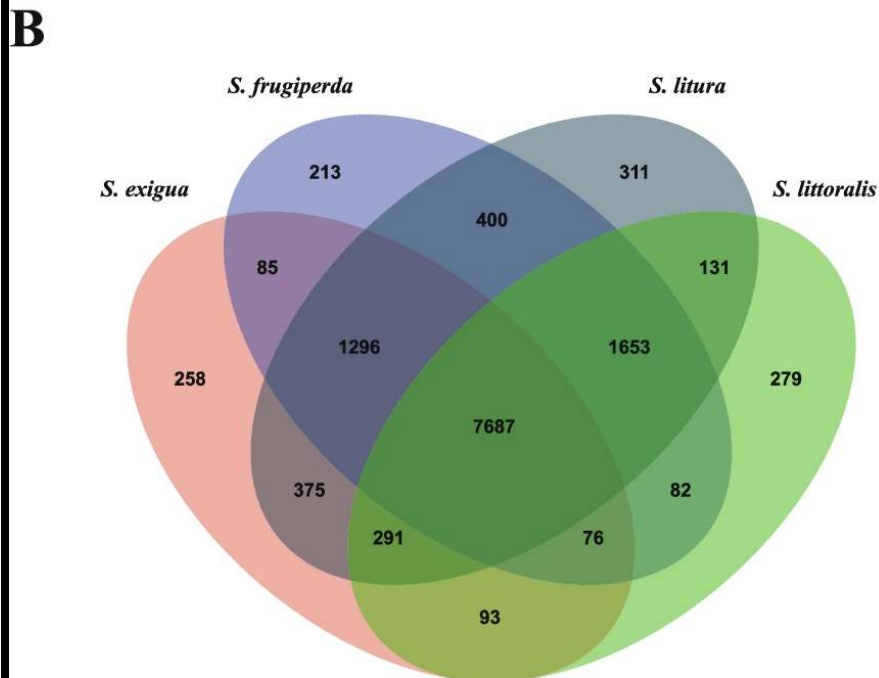
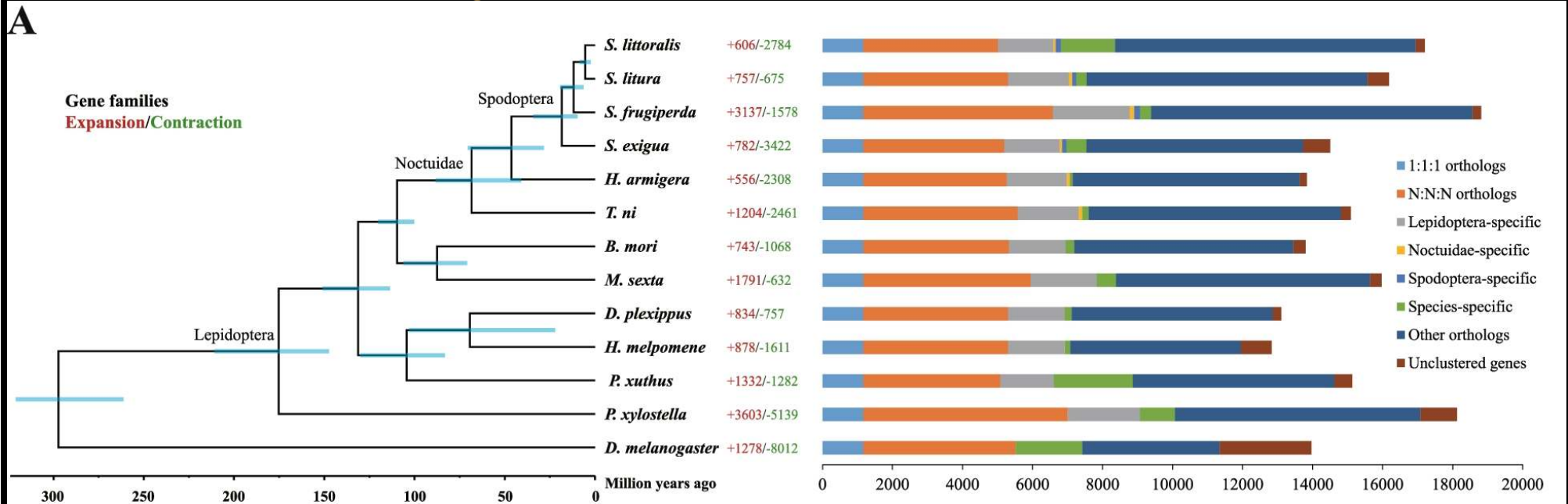
Bioinformatic wisdom, pt. 3

- Data management
 - Get your raw data uploaded to ENA as soon as possible.
 - Its a free backup and you can set embargo date
 - keep pushing the date on the embargo
- Reproducibility is super important
 - Know about Snakemake or Nextflow ... but
 - Be careful of how you invest your time, as some people will try to convince you to learn their pipeline ... that you use once ...
- Is the pipeline you want to invest months in ... for
 - you, or others?
 - A few, or many samples?
 - A way to help you advance your science and career?

So ... how many of you are sequencing a genome?

- What does that mean? Have you told your mom?
- What kind of genome are you generating?
- How accurate do you need your genome to be?
 - Short term vs. long term goals?
 - Are these in conflict?

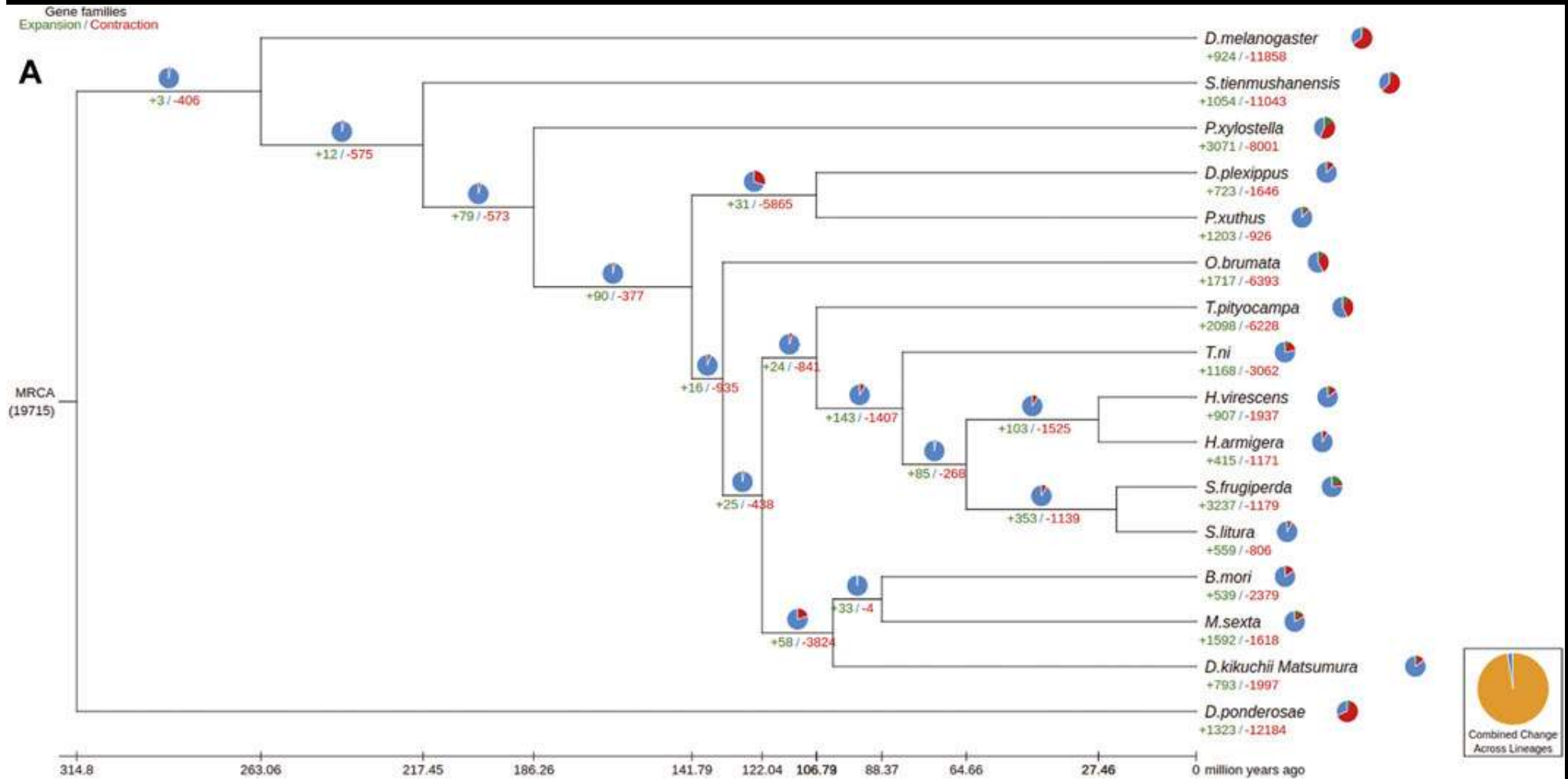
Comparative genomics commonly use annotations



Typical genome report
comparing gene content
among species

- Rates of birth, death
- Lineage specific genes

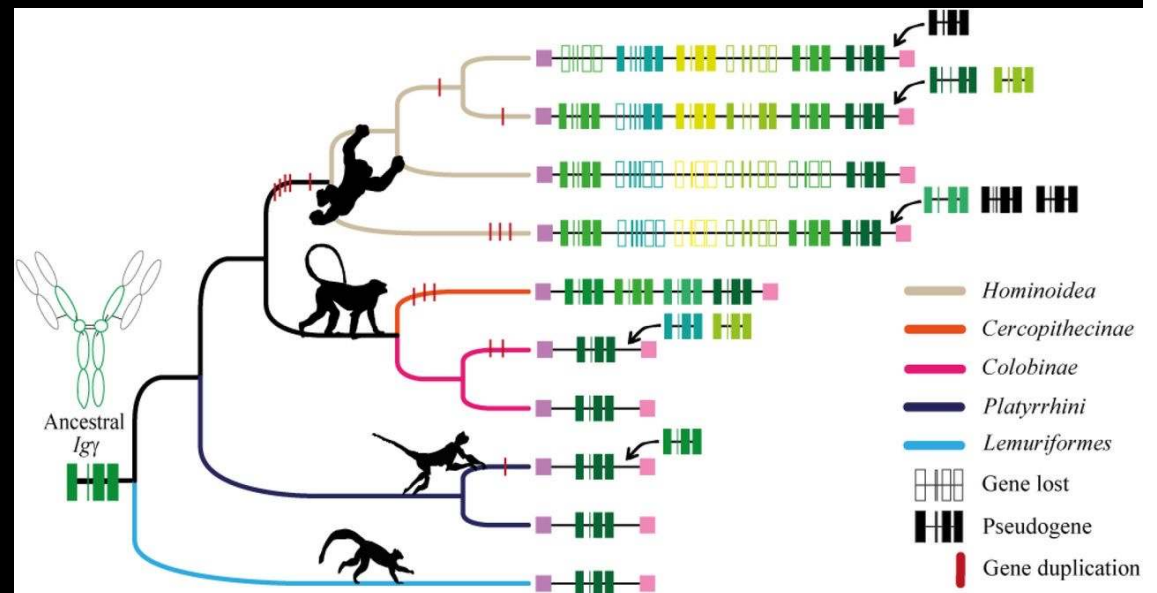
Gene birth-death dynamics



Gene birth-death dynamics: biased, artifacts, or meaningful results?

- Are changes in gene numbers across species meaningful?
- Fundamental and important evolutionary question
- Very difficult to assess accurately
 - Need good genomes, annotations
 - Then good analyses

Immunoglobulin heavy constant gamma gene evolution



Are all annotations equal among species?

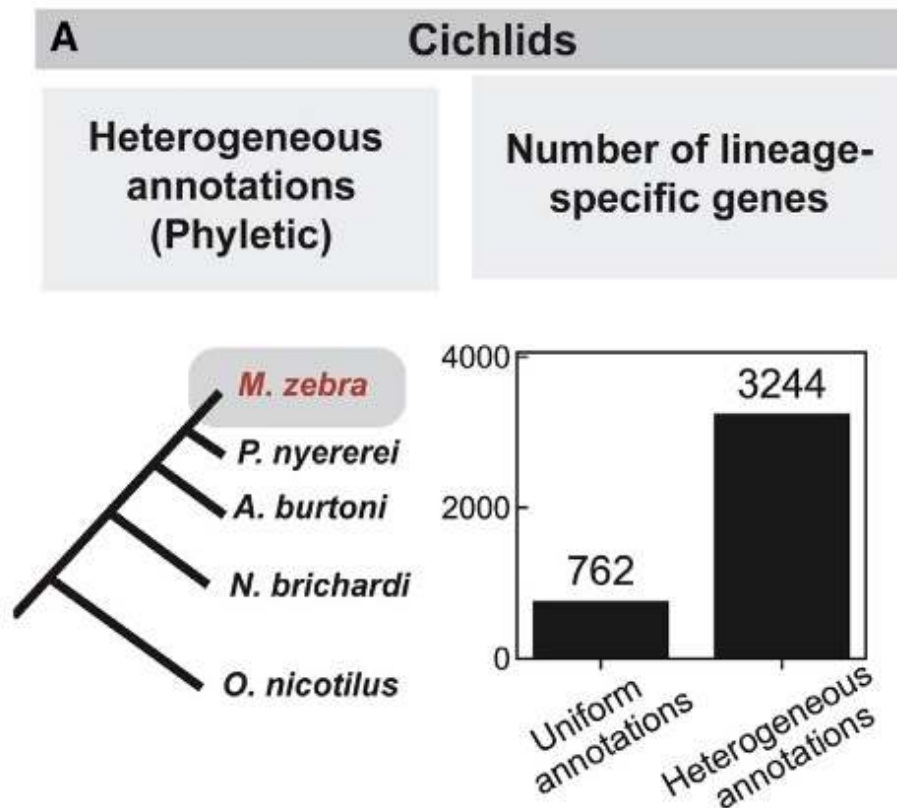
- Do species genomes differ in:
 - When they were sequenced, thus technology?
 - The quality of their assembly (e.g. N50, haploid state)?
 - How they did their annotation (proteins only vs. lots of RNAseq)?

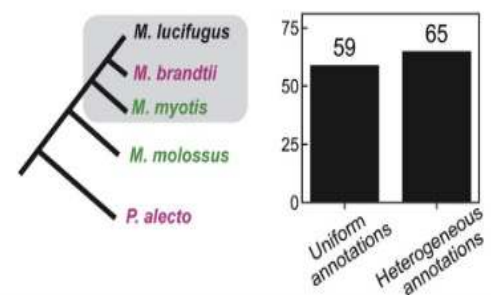
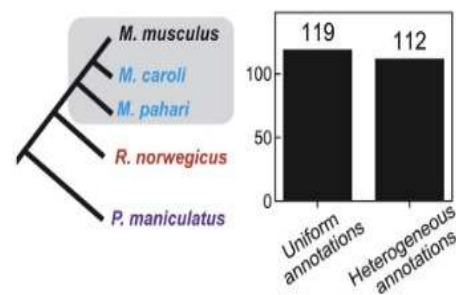
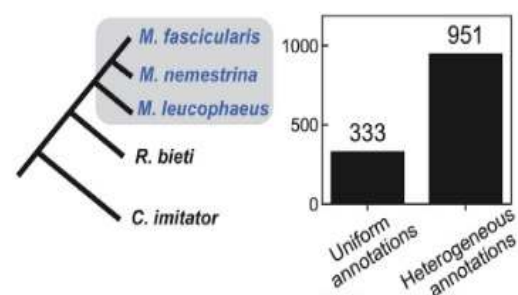
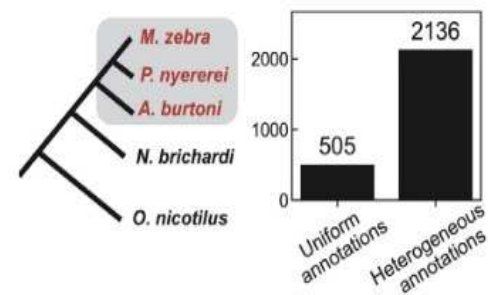
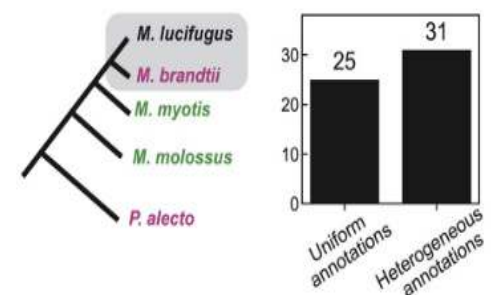
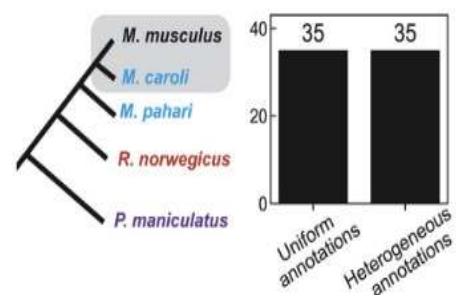
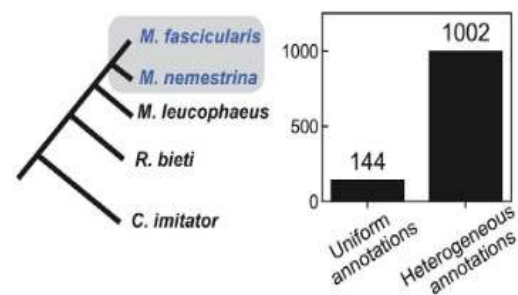
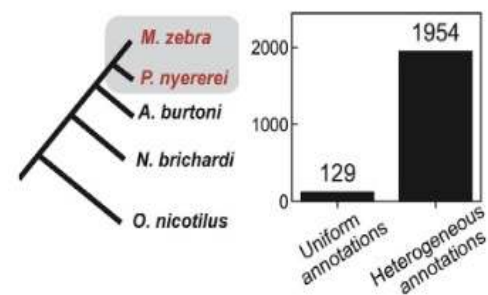
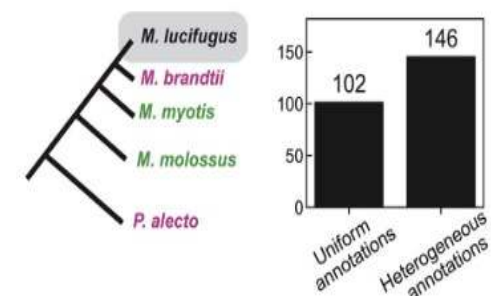
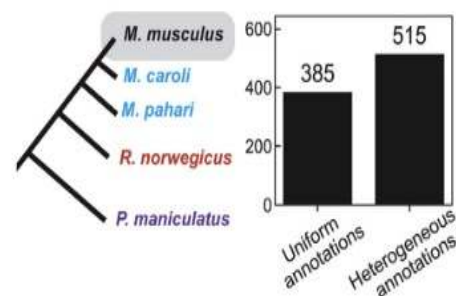
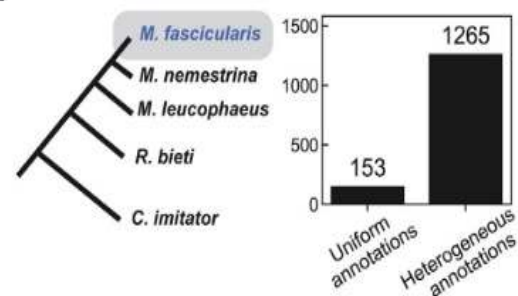
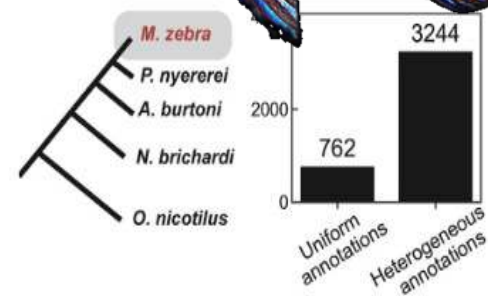
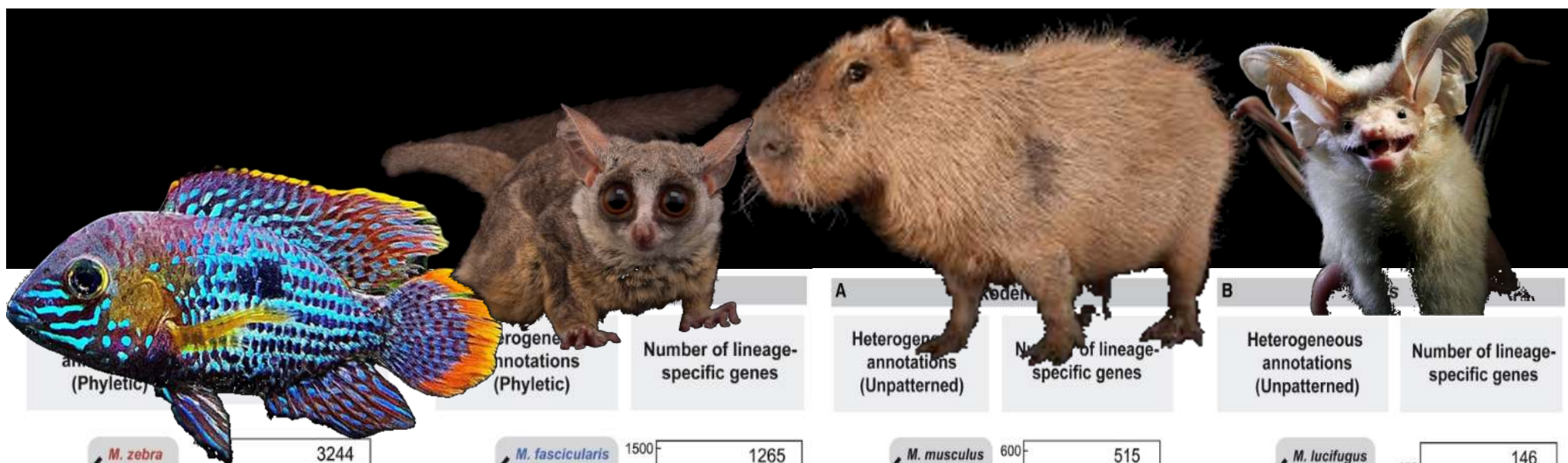
Then resulting annotation protein sets likely differ due to
technology, not biology

Will this impact analyses that rely upon accurate protein sets?

Non-standard annotations introduce major artifacts

- Lineage specific genes inflated by
 - 10 to 1000's of genes, with increases up to 15 fold



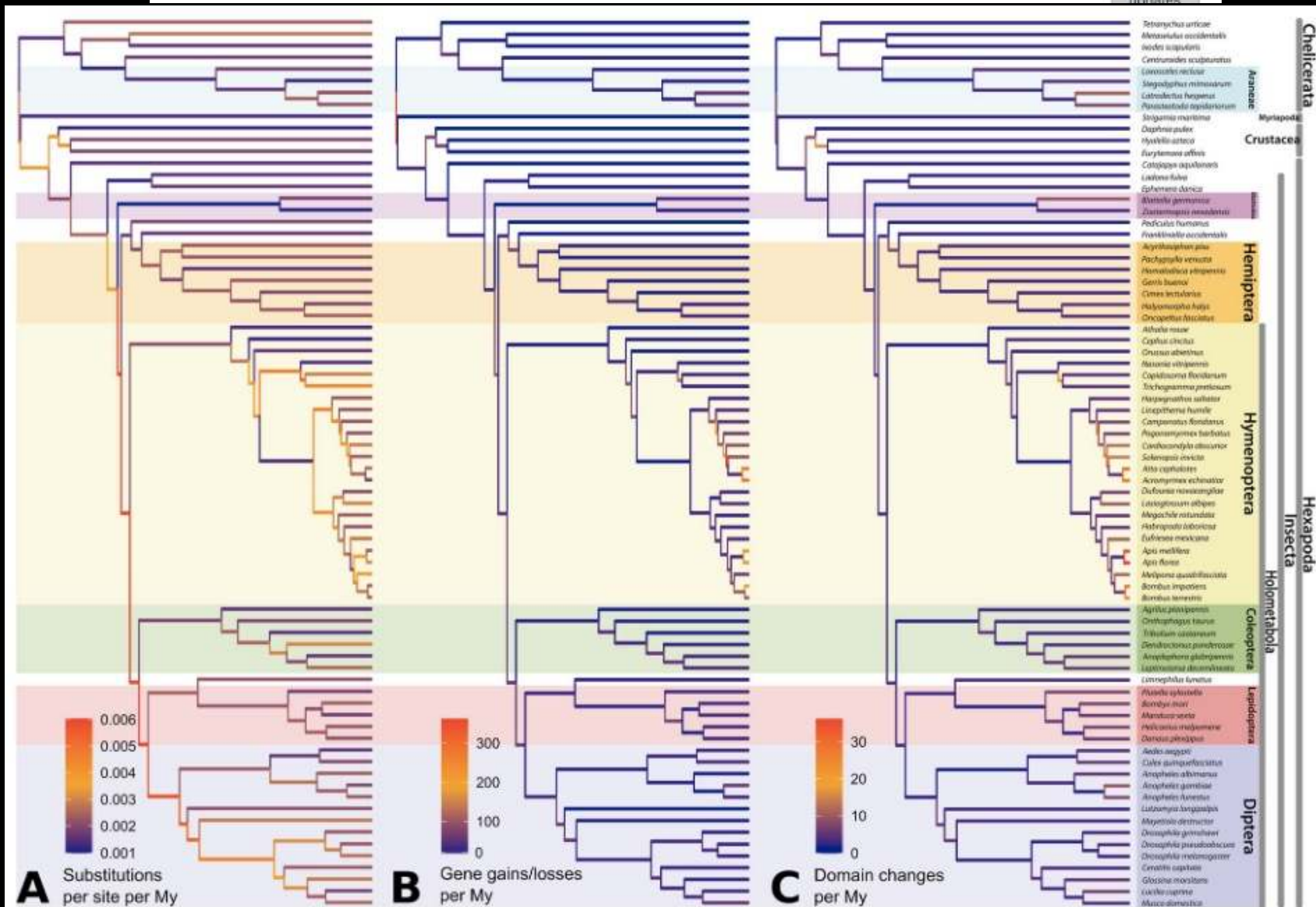


What are the ramifications?

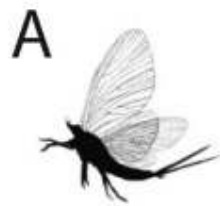


Open Access

Gene content evolution in the arthropods

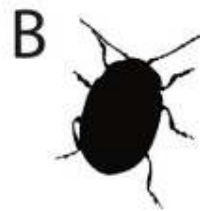


Some major conclusions of the paper



A Last Insect
Common Ancestor:
147 emergent gene
families

Function	Emergent families
Wing morphogenesis	EOG86HJQQ
	EOG8TMTG9
	EOG80ZTDS
Exoskeleton development and pigmentation	EOG8Q2GZG
	EOG8RZ1DS
	EOG8VDSCK
	EOG8WHC14
	EOG8XPT03
Adaptation to terrestrial environment	EOG83XXJ1
	EOG82VBZ4
	EOG8PVRGC
	EOG8HTC7X
Larval behavior	EOG81K1SK



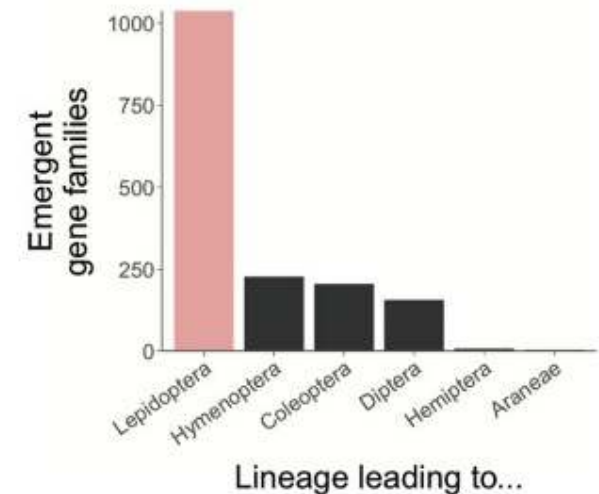
B Last Holometabolous
Common Ancestor:
10 emergent gene
families

Function	Emergent families
Anterior head segmentation	EOG8HDW8X
Nucleosome assembly	EOG8G1PZD
Transporter activity	EOG847J8K
Transferase activity	EOG8ZPH98
Serine-type endopeptidase	EOG8QJV3F

+ 5 families with no known function



C Last Lepidopteran
common ancestor:
1,038 emergent
gene families



“Although the majority of these gene sets were built using MAKER, variation in annotation pipelines and supporting data, introduce a potential source of technical gene content error in our analysis.”

Proteins sets:

a mixed bag of isoforms and pseudo-duplicates

- Unfortunately, many studies are not isoform filtering their protein sets prior to analysis
 - Using raw protein sets from genome projects must always be filtered down to one protein per locus
 - This will have ramifications at all levels
 - Will severely impact ortholog assessments, gene birth death analysis
- Many genomes are not properly haploidified
 - Causes a pseudo-inflation of predicted genes
 - Creates artifacts in analyses



Post-genomics challenge

“What we can measure is by definition uninteresting and what we are interested in is by definition immeasurable”

- Lewontin 1974

“What we understand of the genome is by definition uninteresting and what we are interested in is by definition very damn difficult to sequence and assemble and annotate and analyze at the genomic scale”

- Wheat 2015

Interrogate your results

- “you need to be in charge of the analysis”
 - The more you analyze your data, your confidence will grow
 - Let your findings talk to you in different ways
 - Graph your results – visualize the patterns, assess 1st principals
 - Always start with PCA or MDS plot (how do your samples cluster?)
 - Compare with your different analysis results
 - If you find interesting genes or patterns, can you test this hypothesis?
 - Using independent samples?
 - At a higher level of biological organization?
 - In some manipulative, functional way?
-

Molecular spandrels:

Story telling
vs.
Causal understanding

Genomics is full of adaptive stories

Treat your findings as hypotheses

How can you test these?

Never forget your origins and biases



**Find ways to test your genomic hypotheses,
cause they are easy to get and believe**

Come say hi if you're in town!



Stockholm
University

