Lies, damn lies, and .... genomics

you, your data, your perceptions and reality

Christopher West Wheat

Goal of this lecture

- Present a critical view of ecological genomics
- Make you uncomfortable by sharing my nightmares
- Encourage you to critically assess findings and your expectations in light of publication biases

Disclaimer

I'm a positive person

I love my job and the work we all do

I'm just sharing scrumptious food for thought

What if .....

50% of your favorite studies had conclusions that were just wrong?

How would that affect your expectations and work?

If the biomedical science has the most money and oversight, then ....

Their findings should be robust:

- Repeatable effect sizes
- The same across different labs
- The same across years

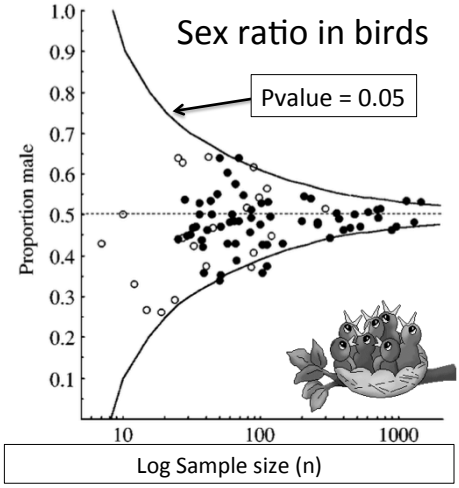
Publication replication failures

- Biomedical studies
  - Of 49 most cited clincal studies, 45 showed intervention was effective
  - Most were randomized control studies (robust design)

- Mouse cocaine effect study, replicated in three cities
  - Highly standardized study

Ioannidis 2005 *JAMA;* Lehrer 2010

Assessing reality using funnel plots



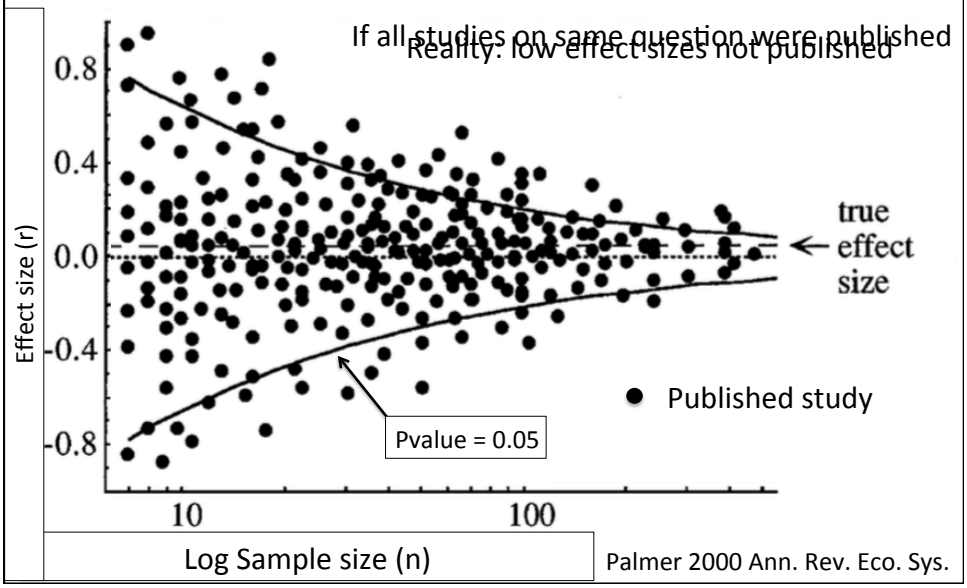
Small sample sizes affect measurement accuracy

Each dot = a study and has error

Study estimates are randomly distributed about the real value

Your study is just a random estimate of some idealized value

Publication bias increases effect size

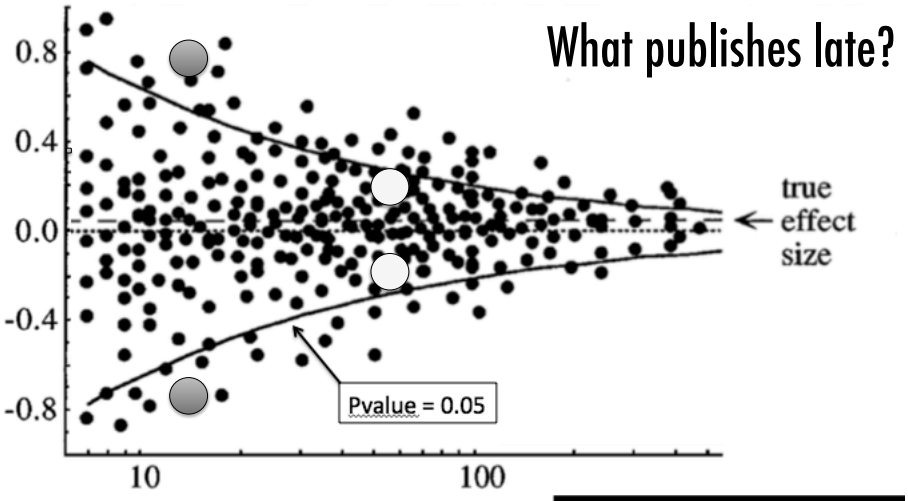


Palmer 2000 Ann. Rev. Eco. Sys.

What if there is no replication?

What is most likely to publish first & where?

What publishes late?



Palmer 2000 Ann. Rev. Eco. Sys.

Why Most Published Research Findings Are False

A research finding is less likely to be true when:

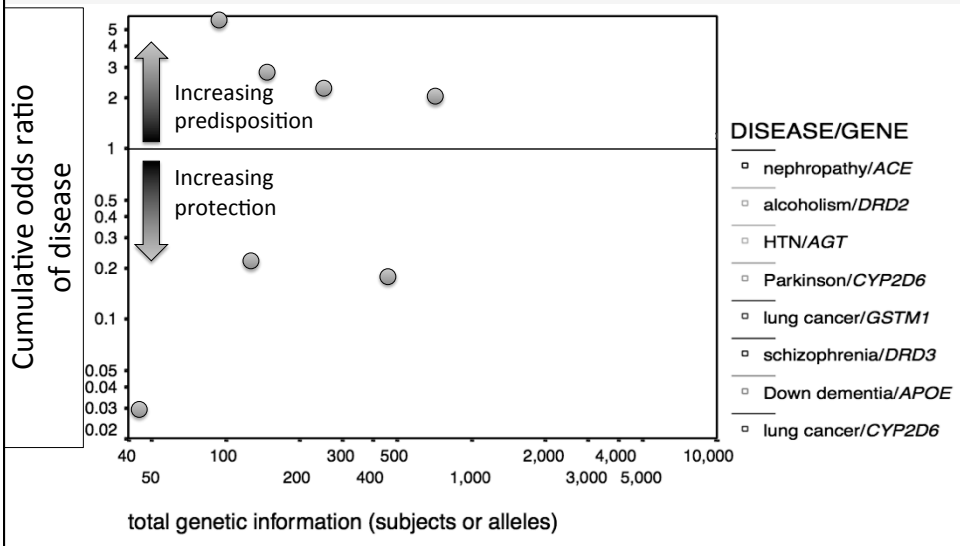
- the studies conducted in a field have a small sample size
- when effect sizes are small
- when there are many tested relationships using tests without *a priori* selection
- where there is greater flexibility in designs, definitions, outcomes, and analytical modes
- when there is greater financial and other interest and prejudice
- when more teams are involved in a scientific field, all chasing after statistical significance by using different tests

Ioannidis 2005 Plos Med.

But surely, this doesn't apply to genomics ....

Or does it?

8 topics first reported with P < 0.05



Ioannidis, J. P., E. E. Ntzani, T. A. Trikalinos, and D. G. Contopoulos-Ioannidis. 2001. Replication validity of genetic association studies. Nat Genet 29:306–309.

There are lies, damn lies, and ....

But wait, is that fair?

Are these really lies?

Where does this bias come from?

- Population heterogeneity
  –
- Publication bias
  –
    impact
  – Small & non-significant effects publish slow with low impact

Where does this bias come from?

YOU!!

And me …. All of us

Its arises from humans doing science

The way we think

The way our institutions work

Apophenia

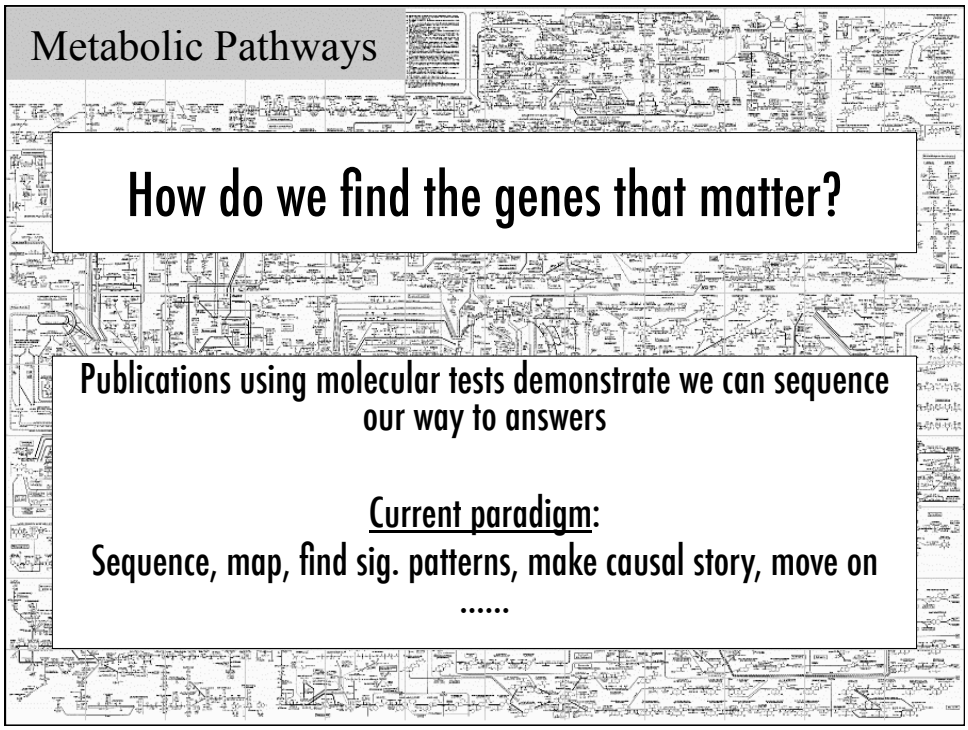
A universal human tendency to seek patterns in random information and view this as important

Story telling of Type 1 errors

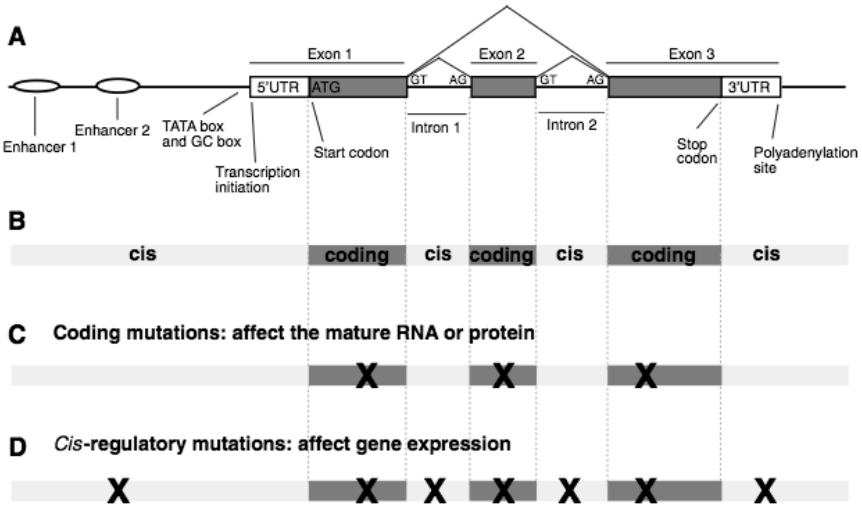
Celebration of the false positives

Outline

- Are there biases understanding the genomic architecture of adaptations?
- What is the power of molecular tests of selection?
- What does the dissection of some classic comparative genomics study reveal?



What is the architecture of a causal variant?



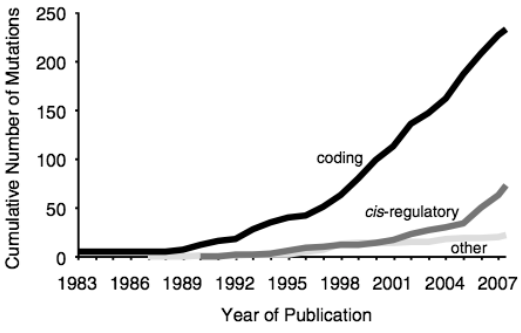
What type of variant?

– SNP, indel, TE, inversion, CNV?

Stern & Orgogozo 2008 Evolution

How predictable are adaptations?

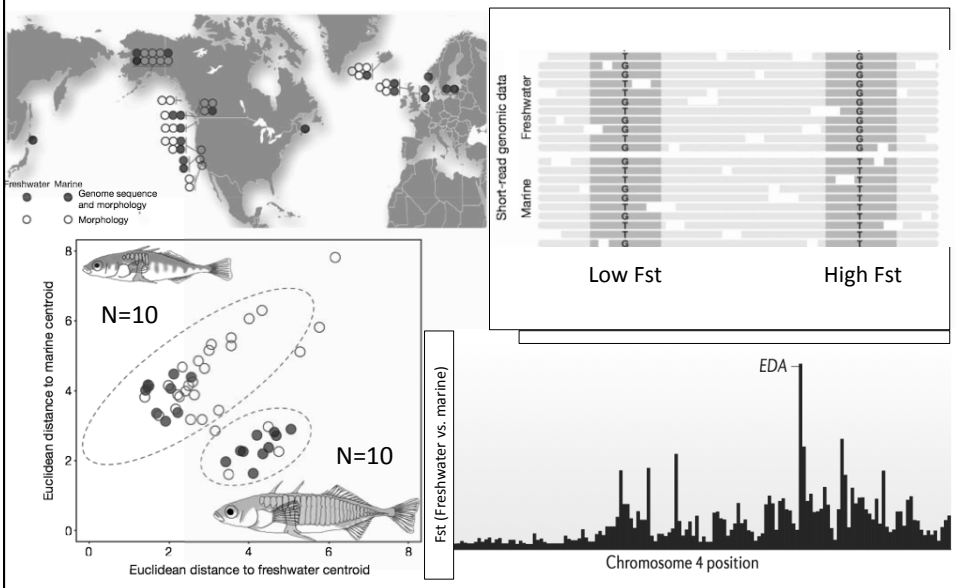
	Plants	Animals
Coding[1]	71	163
Cis-regulatory	26	48
Other[2]	16	7
Total	113	218
Null[3]	67	32



	Morphology	Physiology	Behavior
Coding[3]	62	170	2
Cis-regulatory	43	29	2
Other[4]	3	20	0
Total	108	219	4
Null[5]	41	58	0

Stern & Orgogozo 2008 Evolution

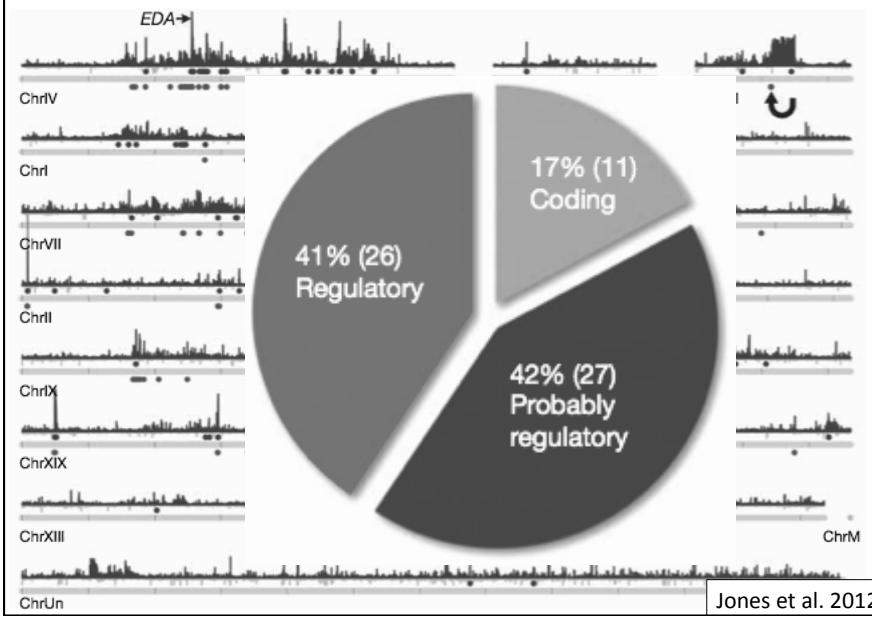
Individual genome sequencing: powerful insights



2-5 X per individual, sliding 2500 bp window, 500 bp step

Jones et al. 2012 Nature

Which regions are more important? Coding or expression?

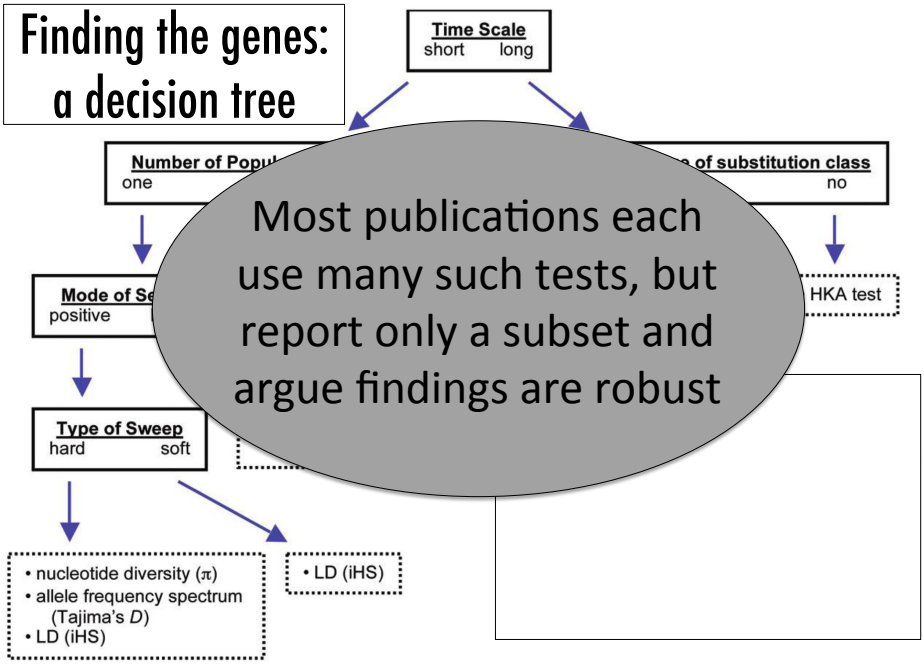


Jones et al. 2012 Nature

How do we identify the genes that matter?

- Molecular tests of selection are popular, but …
  - What are their assumptions and power?
- What are these tests detecting?
  - What is a footprint of selection?
    - How are they formed?
    - How large are they?
    - How long do the last?

Finding the genes: a decision tree



Most publications each use many such tests, but report only a subset and argue findings are robust

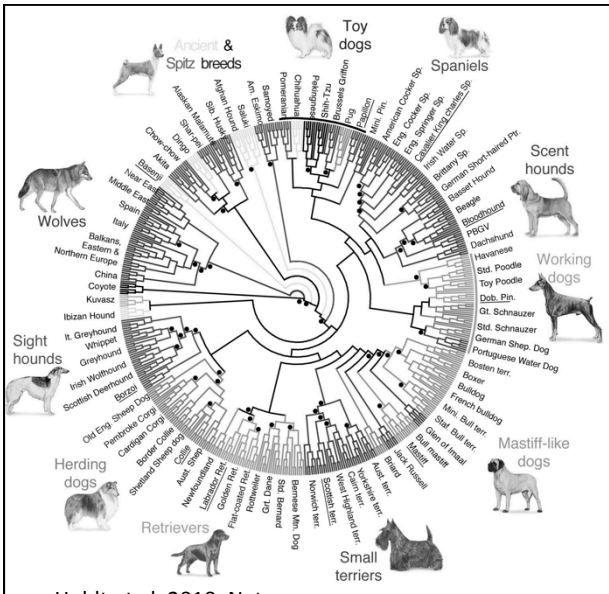
Hohenlohe et al. 2010 Int. J. Plant Science

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

Using a t-test, you would want power > 90% at reasonable sample size, right?



von Holdt et al. 2010. Nature

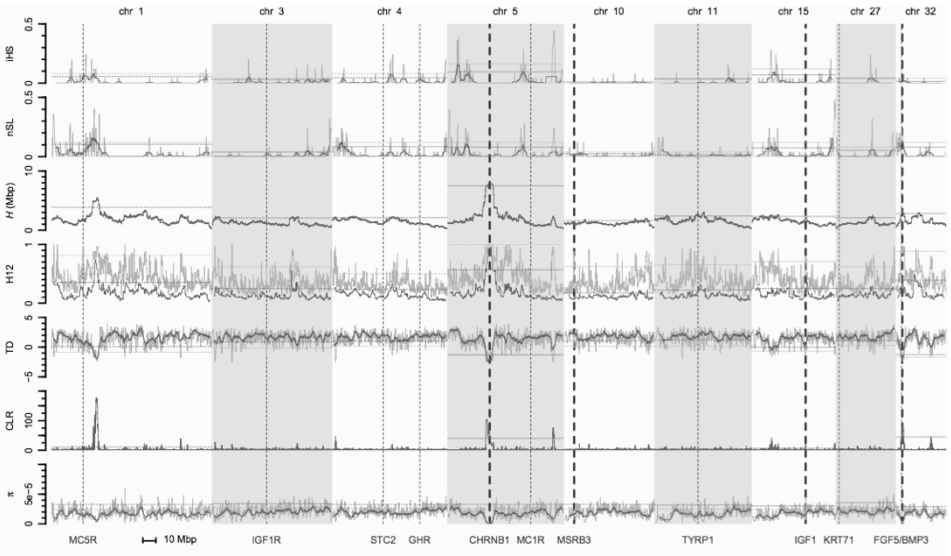
Breed specific morphologies

Test set of Schlamp et al. 2016:

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

How accurate are molecular tests of selection detect?

French Bulldog sample: low power (high type II error)

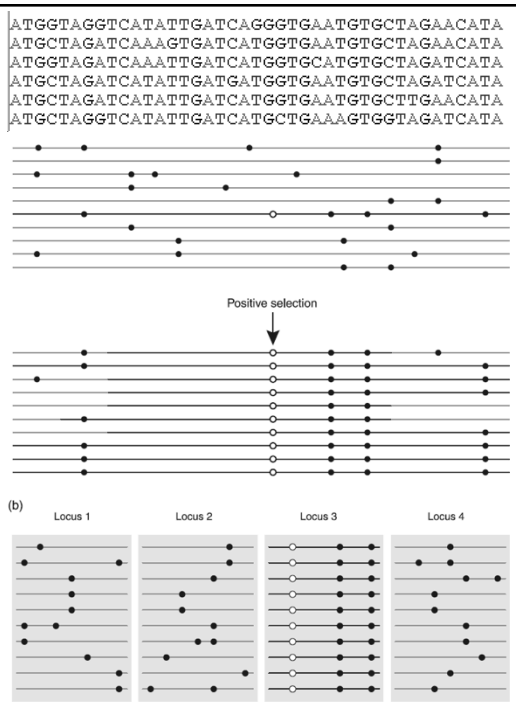


Schlamp et al. 2016. Evaluating the performance of selection scans to detect selective sweeps in domestic dogs. Molecular Ecology 25:342–356.

Why don't these these tests have much power?

Biological reality
vs.
theoretical population genetics?

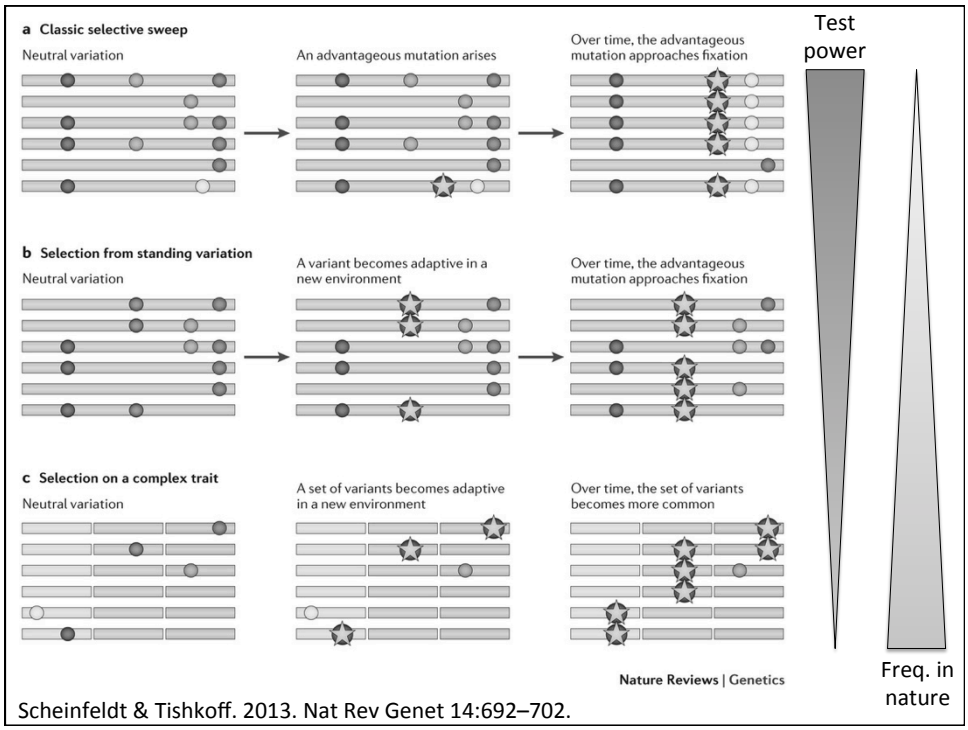
Directional selection:
an example of the expectations of hard selection



Population genomics has been dominated by developing methods to detect hard sweeps for past two decades

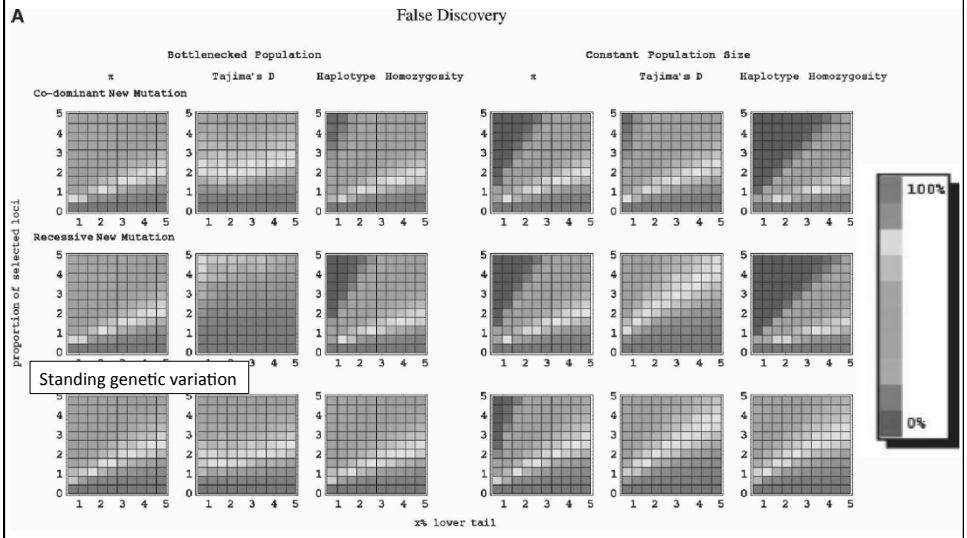
- But a proper 'null model' continues to be elusive, resulting in a high false positive rate since their inception

Storz 2005 Mol. Ecology



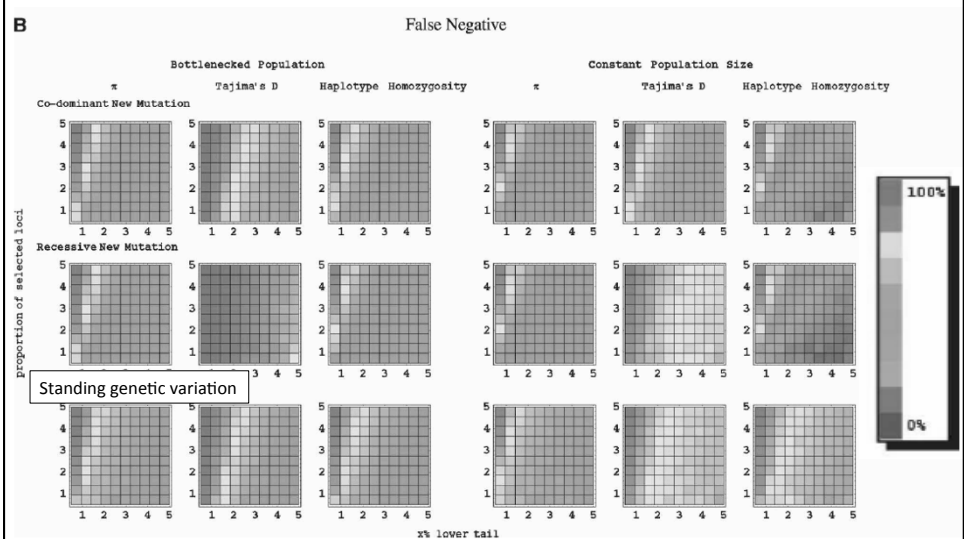
Scheinfeldt & Tishkoff. 2013. Nat Rev Genet 14:692–702.

Estimate of error rates using , Tajima's D, and haplotype homozygosity under the models for a human population



Teshima et al. 2006 Genome Research

Estimate of error rates using , Tajima's D, and haplotype homozygosity under the models for a human population



Teshima et al. 2006 Genome Research

Simulation conclusions

- Simulations suggest
  - empirical approaches will identify several interesting candidates
  - But will also miss many–in some cases, most–loci of interest
- False-discovery rate is higher when
  - directional selection involves a recessive rather than a co-dominant allele
  - when it acts on a previously neutral rather than a new allele
  - Demographic size changes rather than constant population size

Genomic scans yield an unrepresentative subset of loci that contribute to adaptations

Molecular tests ...

BASED ON 20 YEARS OF PUBLICATIONS

- Are still chasing an elusive null model ....
  - Each performs better than previous ones under a specific set of conditions, all have poor null model
- But ... under realistic biological conditions, they all
  - Have very low power (high type II error rates)
  - Have high false positive rates

How common are hard sweeps in nature?

- "we argue that soft sweeps might be the dominant mode of adaptation in many species" Messer and Petrov 2013 TREE

The lab?

- "Signatures of selection … [are] not associated with 'classic' sweeps … More parsimonious explanations include [selection on standing variation]" Burke et al. 2010 Nature

How common were hard sweeps in our history?

- "classic sweeps were not a dominant mode of human adaptation over the past 250,000 years"
- "much local adaptation has occurred by selection acting on existing variation rather than new mutation" 1000 Genomes PC 2010 Science
  Hernandez et al. 2011 Science

Certainly not everyone agrees ….

REVIEW

Received 24 Mar 2014 | Accepted 17 Sep 2014 | Published 27 Oct 2014

DOI: 10.1038/ncomms6281

On the unfounded enthusiasm for soft selective sweeps

Jeffrey D. Jensen[1,2]

- This is an important read, critical of
  - assumptions underlying soft sweep (selection on standing variation)
  - the low power of molecular tests to detect hard & soft sweeps

How likely does natural selection use standing variation in your species?

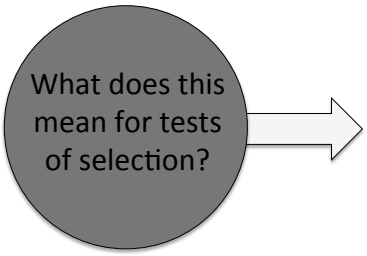
Thought experiment:

What fraction of species respond to selection in the lab? [redacted]

Why? [redacted]

If populations have variation, how likely is selection to use it? [redacted]

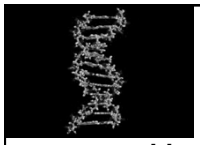
What's likelihood of selection on standing variation in wild? [redacted]



We have not been studying the dominant form of selection in the wild & cannot reliably detect it

Age and type of selection matters

- Novel mutation, large effect, hard sweep that goes to fixation
  - Probability of detection 20 – 90%, depending on demography, etc.
- Old mutation and / or polygenetic that does not sweep to fixation
  - Probability of detection close to 0
- Finding the causal mechanism
  - Coding > expression (but allele specific expression can be lightening rod for expression)
  - SNPs > more complex mutations (indel, TE, CNV)
  - Ongoing gene flow & grouping by phenotype across replicate populations helps a lot
- Demographic effects
  - Nearly all species have experienced a major demographic change in the past 10,000 generations
  - Demographic change significantly reduces power and increases false positive rates.
- What is the relative frequency of these?
  - What will be the architecture of your phenotype?
  - What does your method have the highest power to detect?



Get ready, here come the 1000^n genomes

- Roughly 20 arthropods sequenced to date
  - plans to sequen
- Many other larg

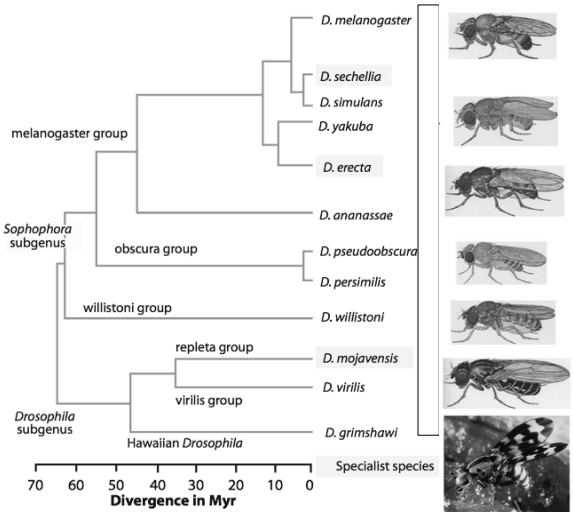
An unprecedented opportunity for large scale errors?

studying:

ationships

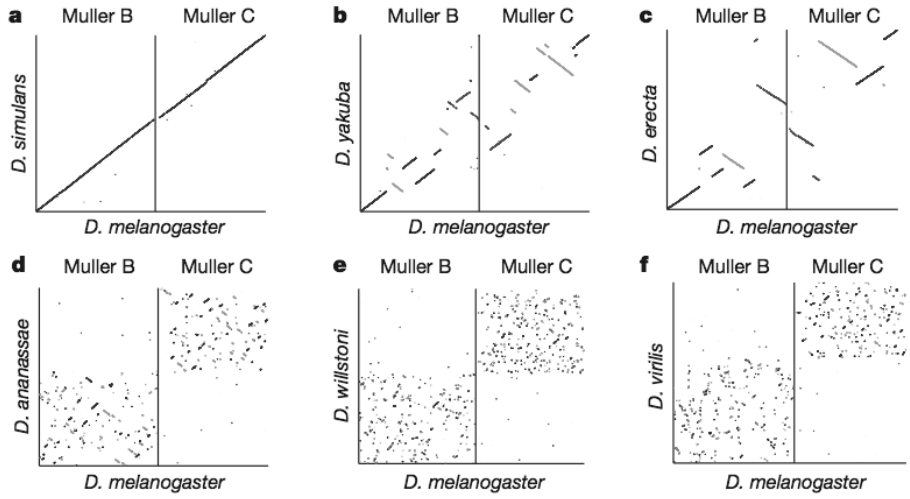
- Genome evolution
- Functional insights into genes and genomic features (e.g. regulation and inheritance)

Classic study: Evolution of genes and genomes on the *Drosophila* phylogeny



Drosophila 12 Genomes Consortium 2007 Nature

Tempo and mode of chromosome evolution



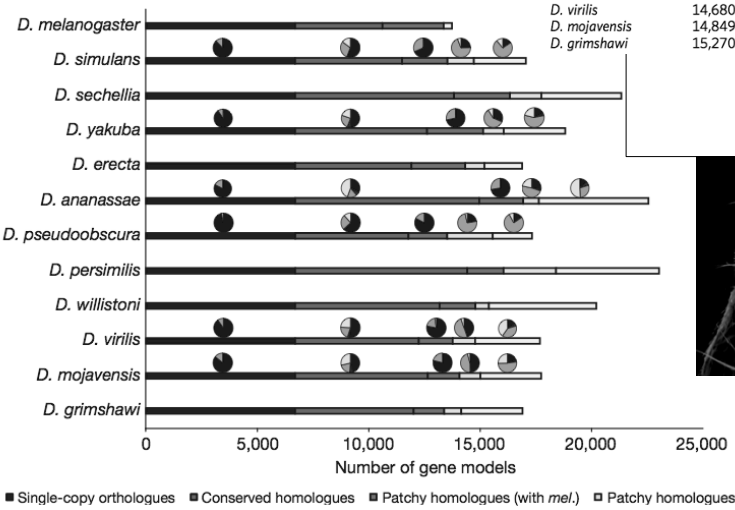
- > 20 My, chromosomal order completely reshuffled in Diptera

Drosophila 12 Genomes Consortium 2007 Nature

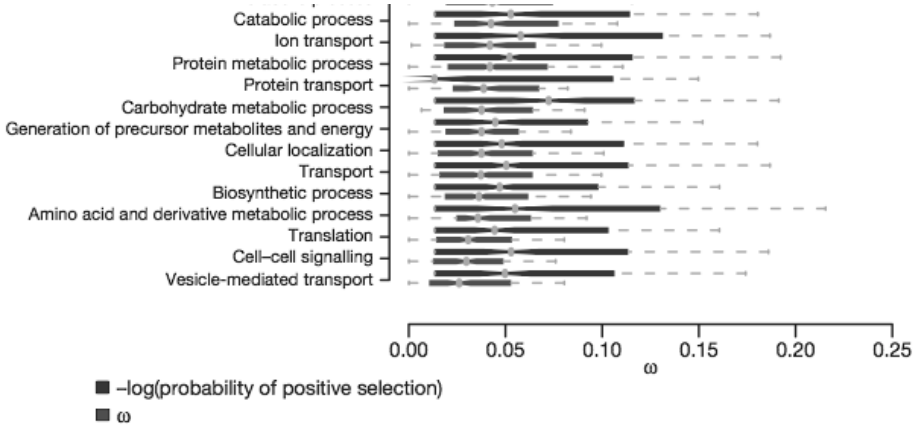
Genome evolution

Drosophila 12 Genomes Consortium 2007 Nature

	Total no. of protein- coding genes (per cent with *D. melanogaster* homologue)	Coding sequence/ intron (Mb)
D. melanogaster	13,733 (100%)	38.9/21.8
D. simulans	15,983 (80.0%)	45.8/19.6
D. sechellia	16,884 (81.2%)	47.9/21.9
D. yakuba	16,423 (82.5%)	50.8/22.9
D. erecta	15,324 (86.4%)	49.1/22.0
D. ananassae	15,276 (83.0%)	57.3/22.3
D. pseudoobscura	16,363 (78.2%)	49.7/24.0
D. persimilis	17,325 (72.6%)	54.0/21.9
D. willistoni	15,816 (78.8%)	65.4/23.5
D. virilis	14,680 (82.7%)	57.9/21.7
D. mojavensis	14,849 (80.8%)	57.8/21.9
D. grimshawi	15,270 (81.3%)	54.9/22.5



Selection dynamics across functional categories

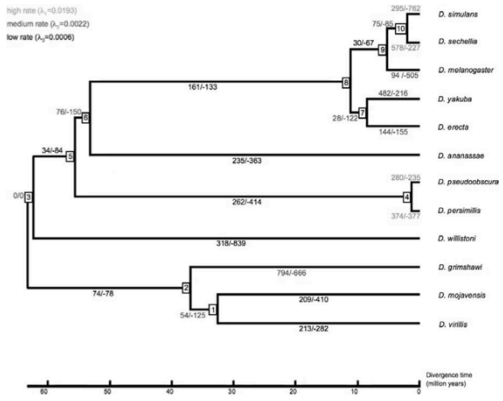


- 33.1% of single-copy orthologues have experienced positive selection on at least a subset of codons.

Drosophila 12 Genomes Consortium 2007 Nature

Gene Family Evolution across 12 Drosophila Genomes

- One fixed gene gain/ loss across the genome every 60,000 yr
- 17 genes are estimated to be duplicated and fixed in a genome every million years



Drosophila 12 Genomes Consortium 2007 Nature
Hahn et al. 2007 Plos Genetics

Comparative Genomics : a house of cards?

- Data scale is too large to thoroughly assess errors …
  - Perhaps the findings are just …. wrong

- All conclusions, at some stage, rest upon
  - Simple bioinformatics
  - Assumptions that get incorporated into seemingly unbiased methods

Lets exploring two pillars of these studies, their error and repercussions

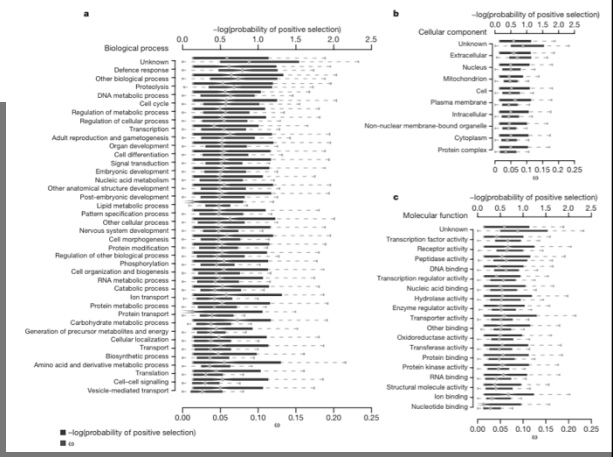
- Gene alignments in detecting positive selection
- Calibrations in temporal analysis

Published studies allow …

Follow up studies to reveal limitations

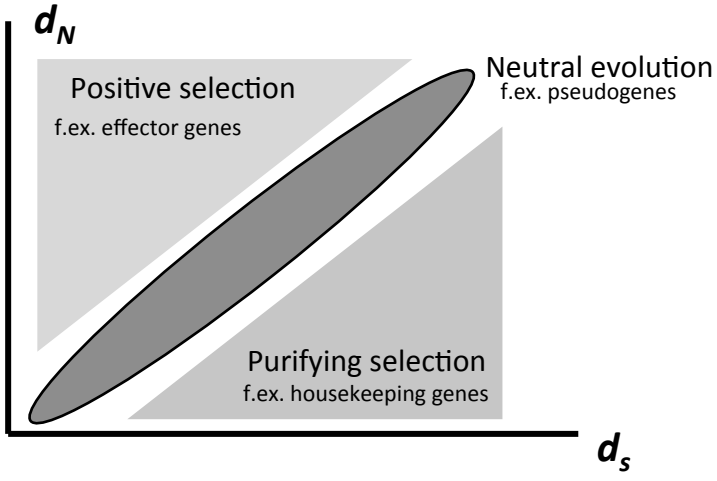
But, must have enough details to be repeatable

Genome-wide selection dynamics:



How robust are these conclusions?

Codon based tests of selection

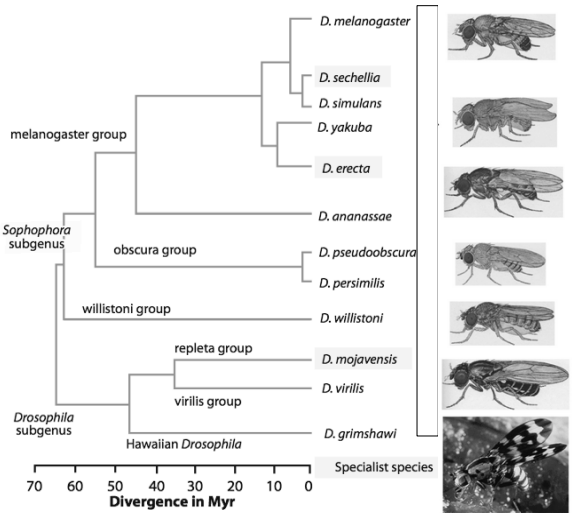


d_N / d_s ratio

```
> 1 positive sel.
= 1 neutral
< 1 purifying sel.
```

IMPRS workshop, Comparative Genomics

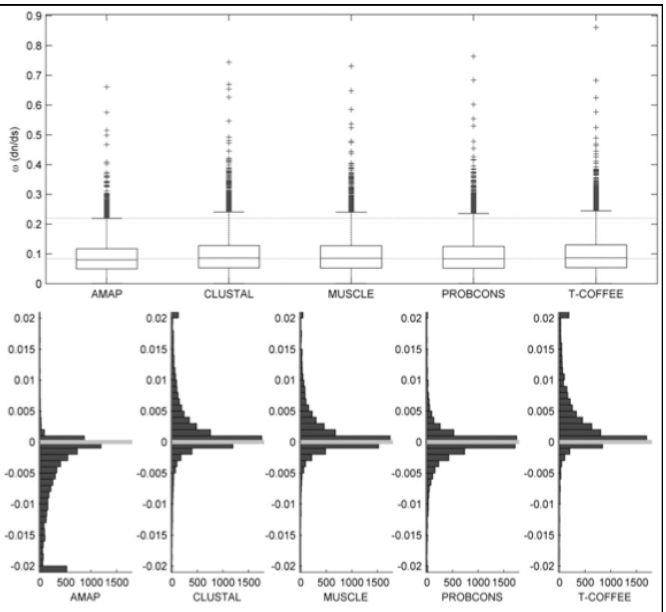
Evolution of genes and genomes on the *Drosophila* phylogeny



Drosophila 12 Genomes Consortium 2007 Nature

dN/dS estimates by aligner

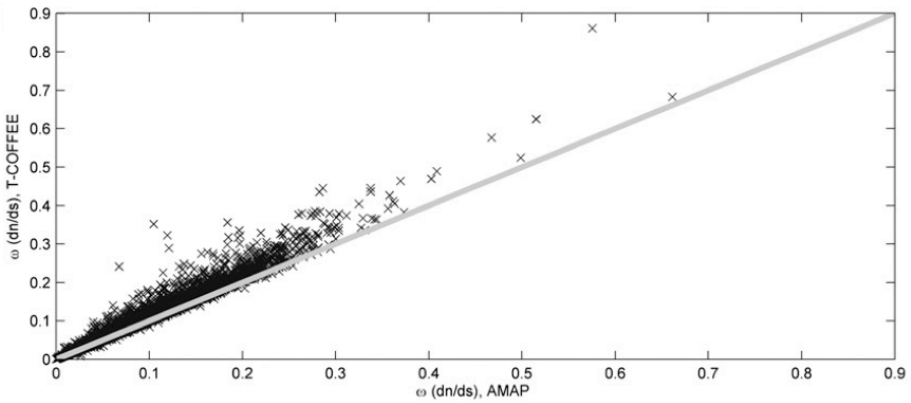
- 6690 orthologs
- 5 alignment methods
- Alignment methods affect dN/dS estimates



Markova-Raina & Petrov 2011 Genome Biology

Comparing results across methods is responsible bioinformatics!!!!!

Since we can't look at our data, we need approaches that allow 1st principal assessments

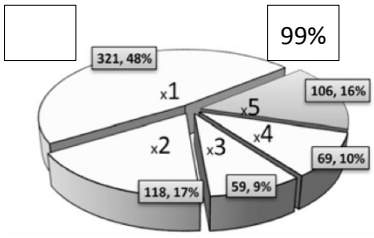


Markova-Raina & Petrov 2011 Genome Biology

Aligner tool has a larger effect than biology

	12 genomes, M7/8		12 genomes, M1a/2a		12 genomes, M7/8, with removed gaps		*Melanogaster* group, M7/8	
Aligner	**95% (a)**	**99% (b)**	**95% (c)**	**99% (d)**	**95% (e)**	**99% (f)**	**95% (g)**	**99% (h)**
AMAP	817	213	256	110	558	104	973	257
MUSCLE	1043	306	379	192	764	155	1134	366
ProbCons	1013	281	346	180	801	182	1128	371
T-Coffee	1290	479	612	353	824	173	1248 (909)	463 (218)
ClustalW	902	261	244	117	666	112	1269	453
Total in 5	1902	673	799	441	1562	384	1737 (1723)	652 (620)
PRANK	468	49	49	16	258	42	581	70

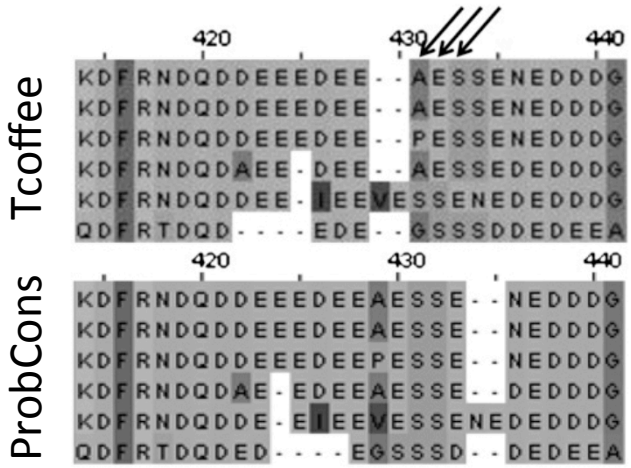
Number of significant genes in common across 1, 2, 3, 4, or all 5 of the alignment methods



Markova-Raina & Petrov 2011 Genome Biology

Alignment results highlight importance of alignment score!

- Tcoffee finds 3 selected sites indicated by arrows
- ProbCons identifies region with low alignment score, not used



Markova-Raina & Petrov 2011 Genome Biology

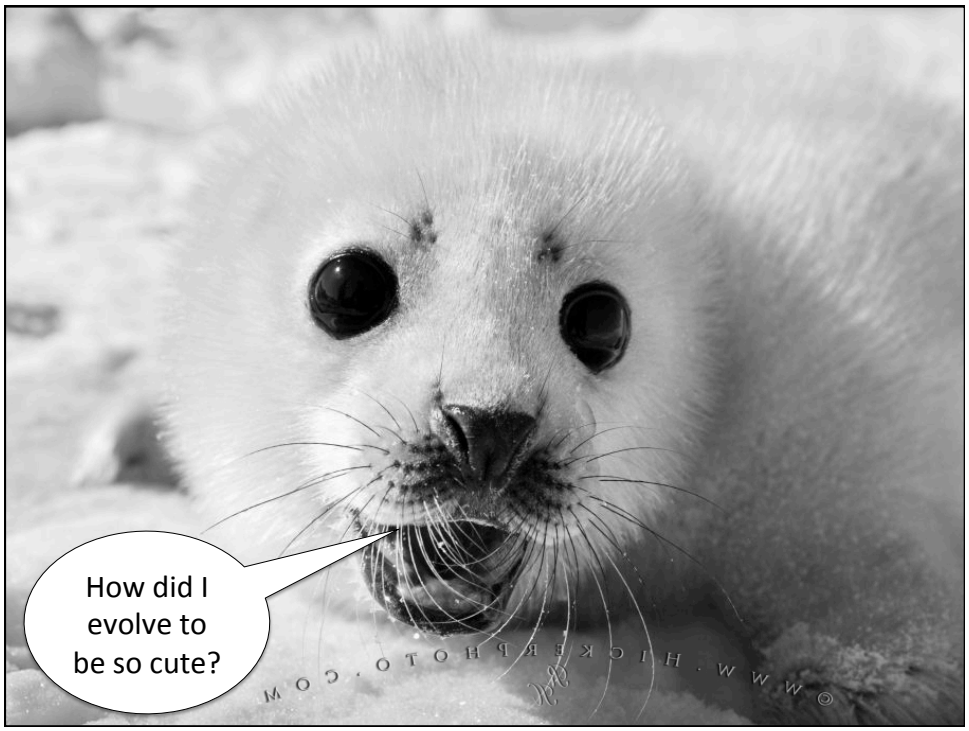
What about recent genomes?

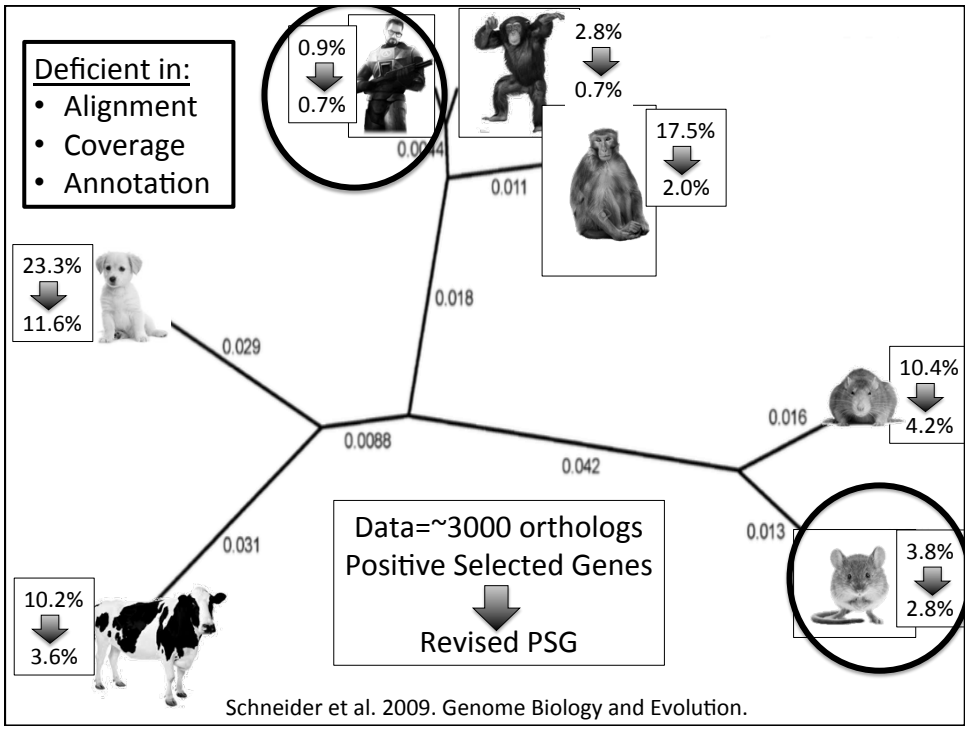
Surely they are better?

and mammals ... they have good genomes

and alignment problems rarely happen

... right?



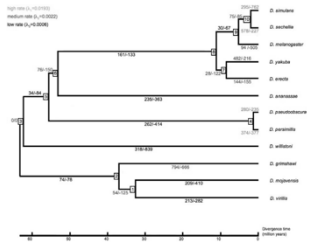


Schneider et al. 2009. Genome Biology and Evolution.

Temporal inference:

fact or fiction?

Timing of divergence

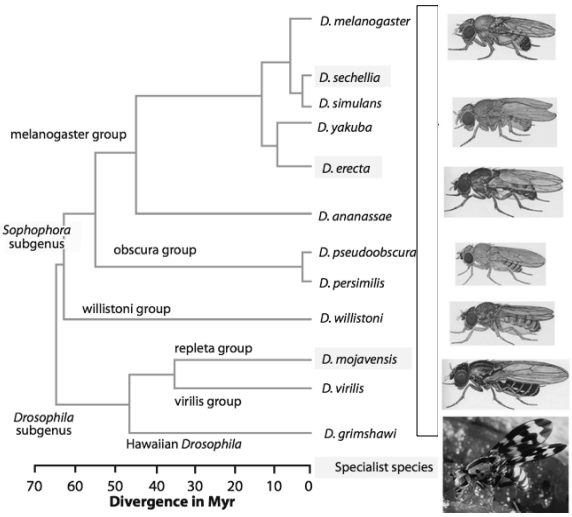


- Directly affects rate estimates
- Deriving unbiased dates from molecular data
  - Large field of software development

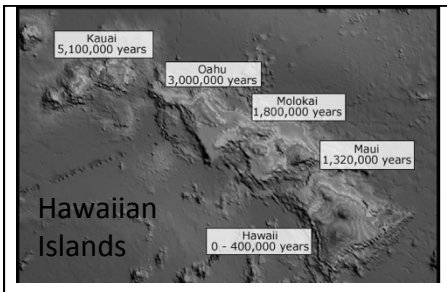
- Bayesian methods, while potentially informative and unbiased
  - Can be easily, and are routinely, abused

Wheat and Wahlberg 2013 TREE

Evolution of genes and genomes on the *Drosophila* phylogeny



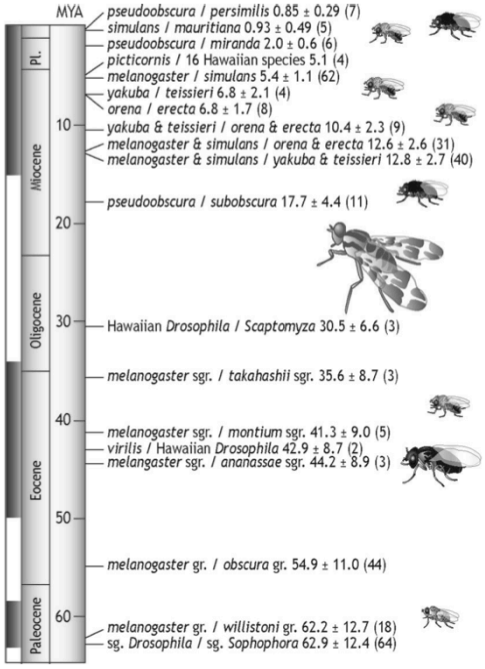
Drosophila 12 Genomes Consortium 2007 Nature



Calibration: Kauai age of 5.1 my for divergence of two Hawaiian species

1. No phylogeny
2. Fixed clock rate
3. Between 3 – 64 genes in pairwise comparisons

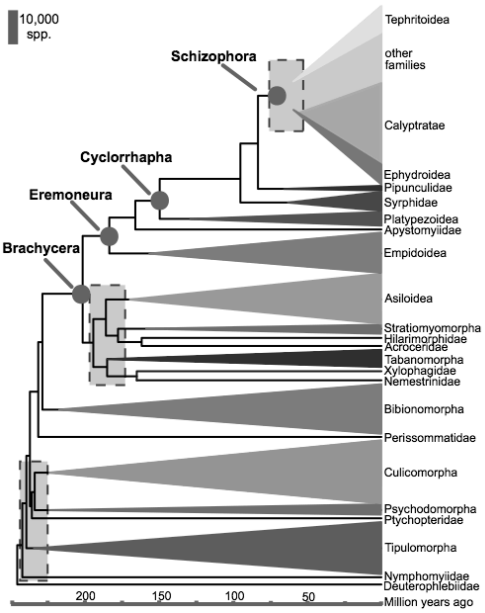
Temporal patterns in fruitflies (Tamura et al. 2004 MBE)



Drosophila clade:

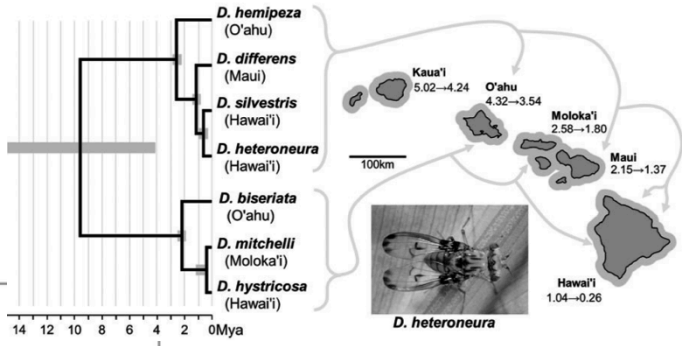
- Schizophora constrained to maximum of 70 Ma
- Without constraint, goes to 115 Ma

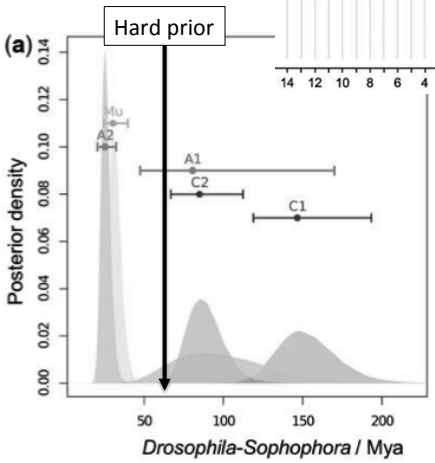
What is reality?



Episodic radiations in the fly tree of life (Wiegmann et al. 2011 PNAS)

Determining objective priors is challenging





Priors in Bayesian rel. clock analysis:

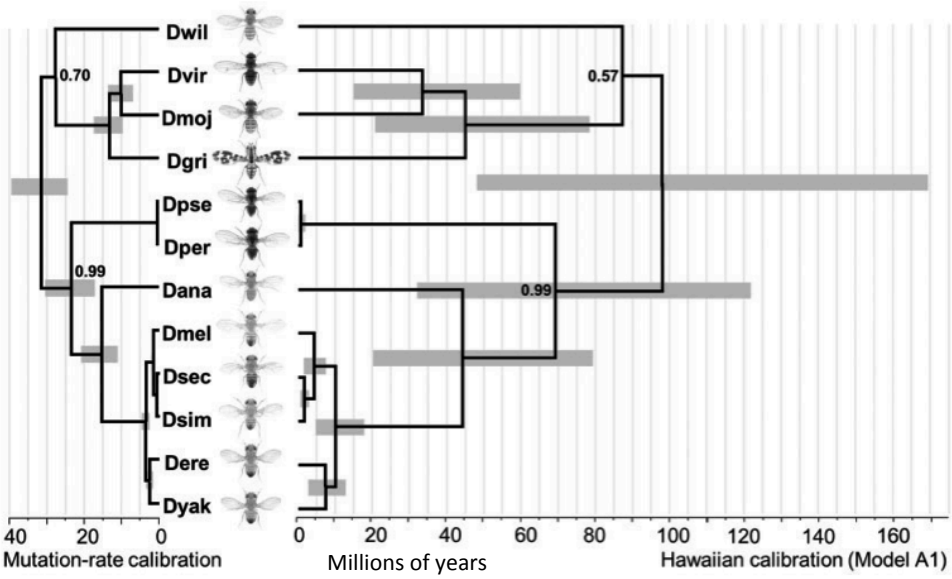
Mu = lab observed mutation rate

A1,2 = geological calibration, small Ne

C1,2 = geological calibration, large Ne

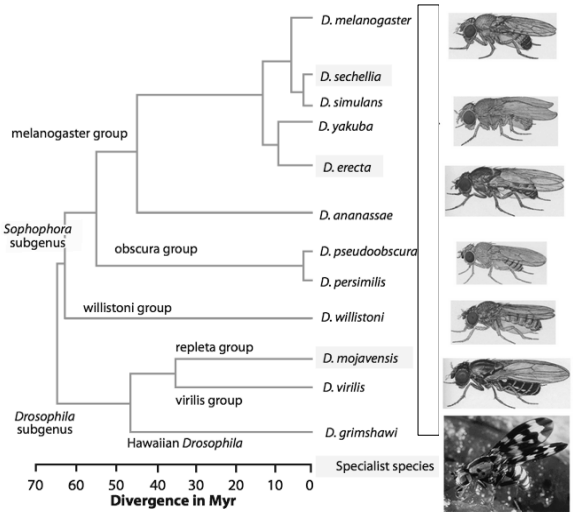
Obbard et al. 2012 Mol. Biol. Evol.

Priors directly influence posteriors



Obbard et al. 2012 Mol. Biol. Evol.

Thus, the age of this clade is fiction



Drosophila 12 Genomes Consortium 2007 Nature

Post-genomics challenge

"What we can measure is by definition uninteresting and what we are interested in is by definition unmeasureable"

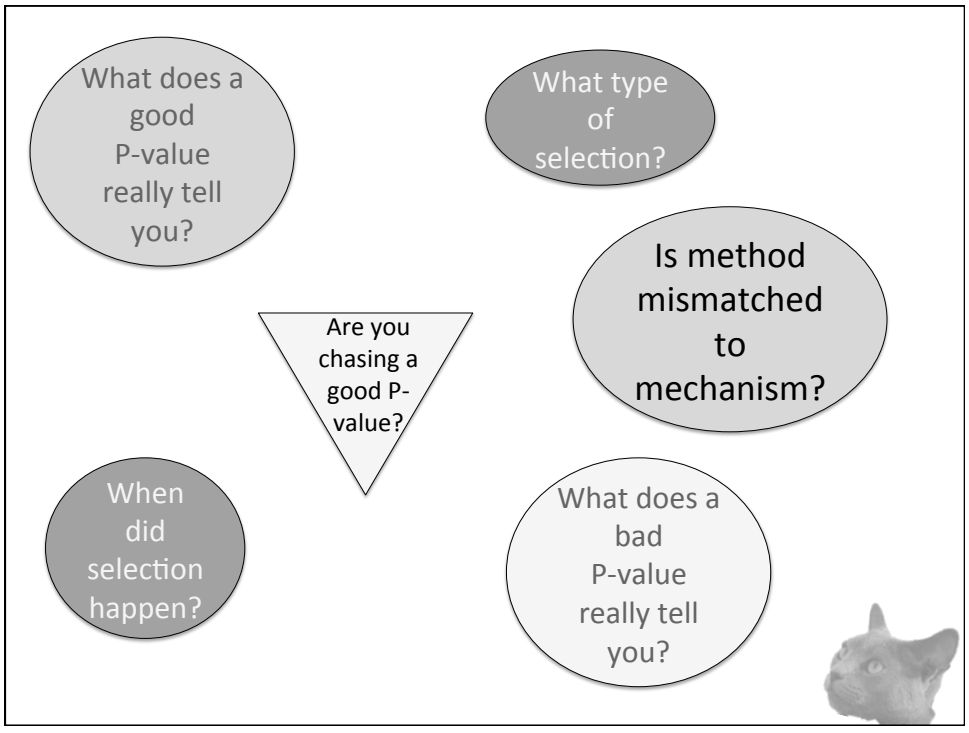
- Lewontin 1974

"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 genomic scale"

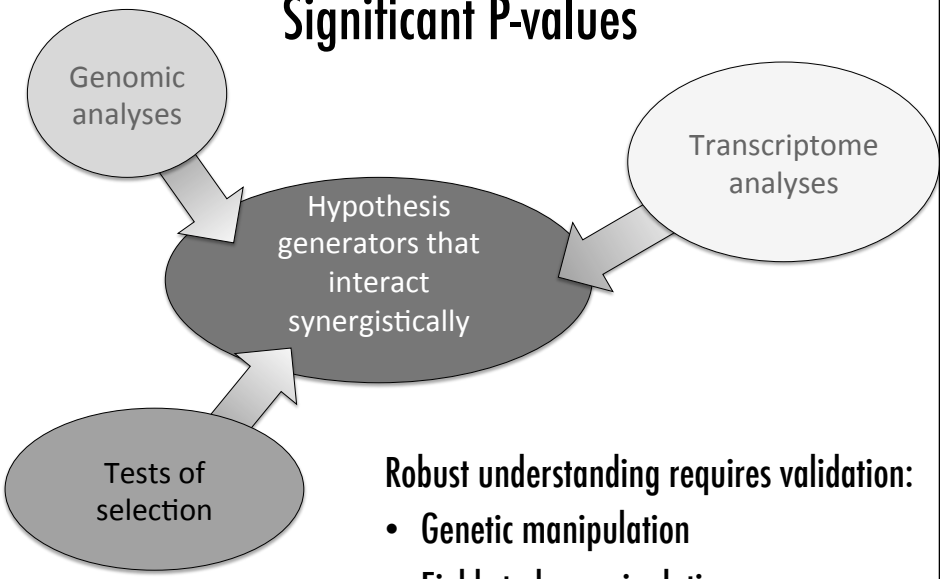
- Wheat 2015

For example:

- indels & inversions
- gene family dynamics
- evolutionary dynamics



Significant P-values



Robust understanding requires validation:

- Genetic manipulation
- Field study manipulations

Goal of this lecture

- Present a non-typical view of ecological genomics
  - So you have a more complete view of the field
- Make you uncomfortable
  - Provide a context for understanding your results
- Encourage you to rethink the reality presented by publication biases
  - Overcoming this bias is a continual challenge


sallyedelsteincollage.com