Hypothesis Testing

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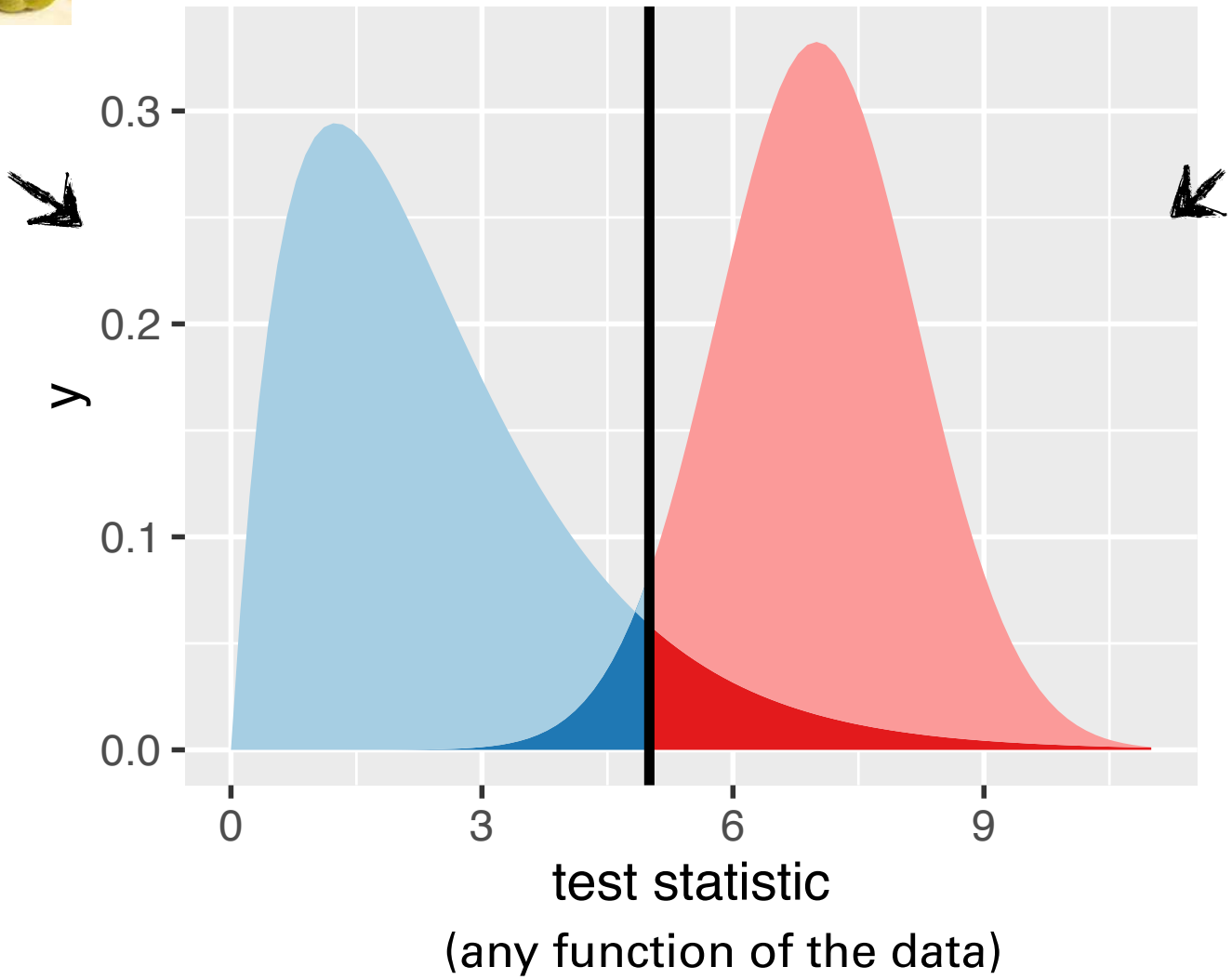
Aims for this Lecture

Understand the basic principles of hypothesis testing, its pitfalls, strengths, use cases and limitations

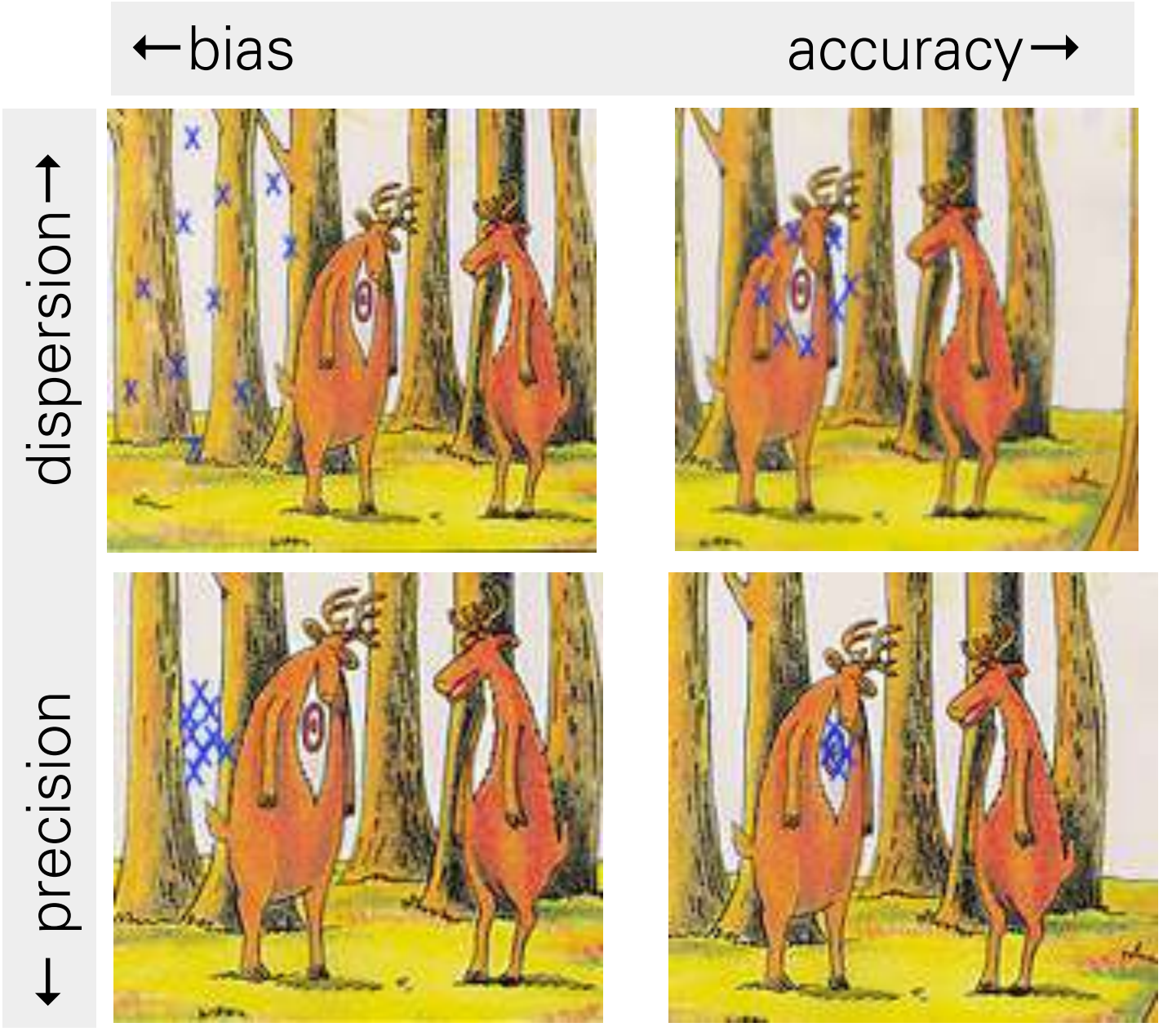
What changes when we go from single to multiple testing?

False discovery rates, p-value ‘adjustments’, filtering and weighting

Testing vs Classification



Accuracy vs Precision - Bias vs Variance



Karl Popper (1902-1994)

Logical asymmetry between verification and falsifiability.

No number of positive outcomes at the level of experimental testing can confirm a scientific theory, but a single counterexample is logically decisive: it shows the theory is false.

Example

Toss a coin a number of times ⇒

If the coin is fair, then heads should appear half of the time (roughly).

But what is "roughly"? We use combinatorics / probability theory to quantify this.

Suppose we flipped the coin 100 times and got 59 heads. Is this 'significant'?

Binomial Distribution

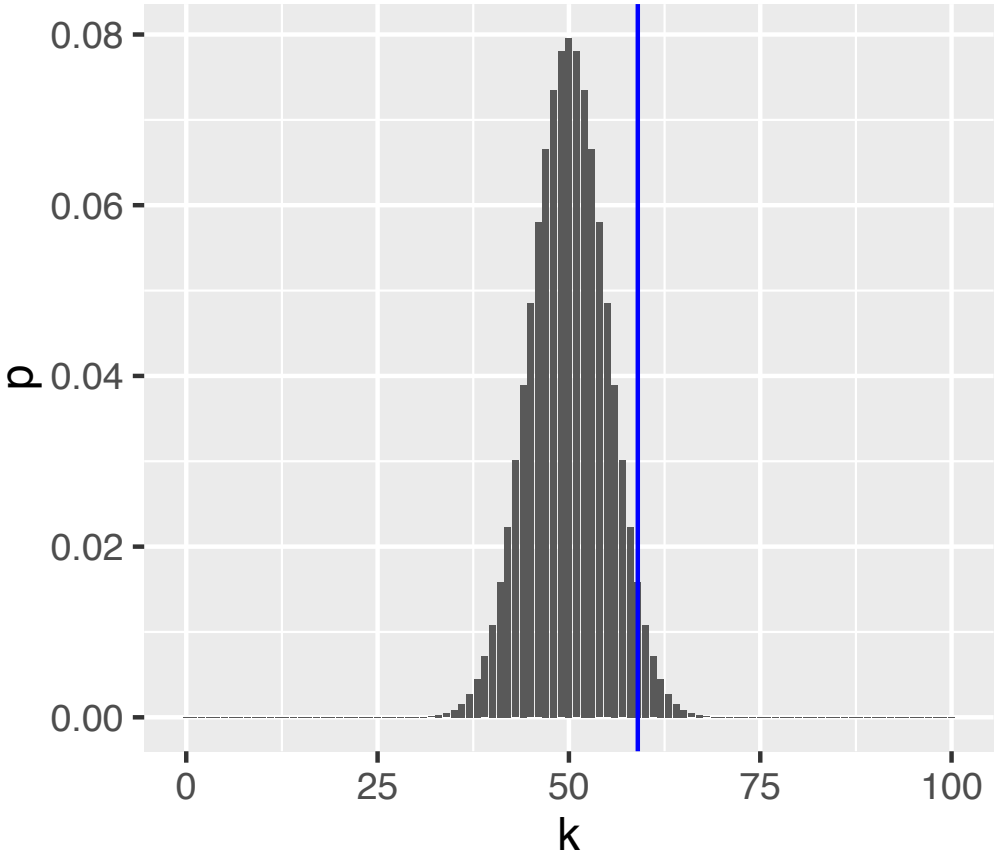


Figure 6.3: The binomial distribution for the parameters $n = 100$ and $p = 0.5$,

$$P(K = k \mid n, p) = \left(\begin{array}{c} n \\ k \end{array} \right) p^k \, (1-p)^{n-k}$$

Rejection Region

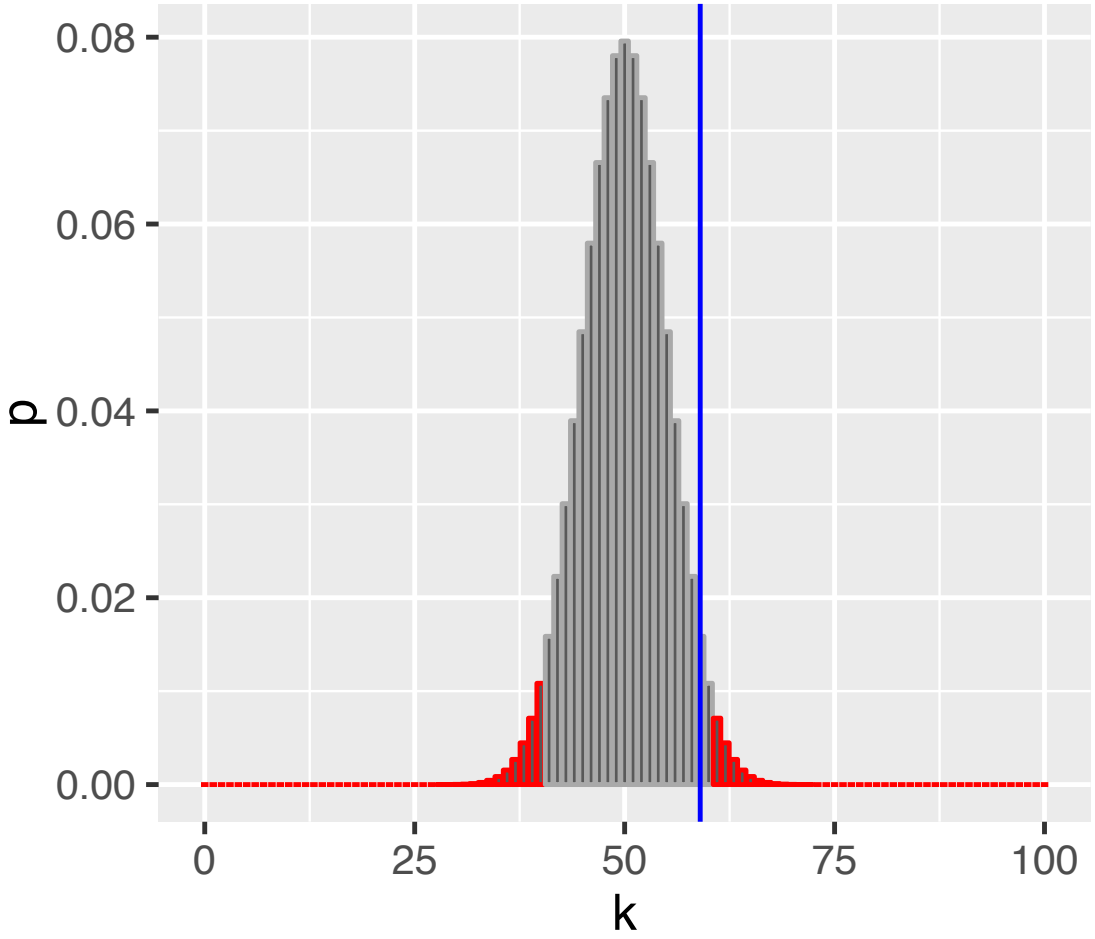


Figure 6.5: As Figure 6.3, with rejection region (red) whose total area is $\alpha = 0.05$.

Questions

- Does the fact that we don't reject the null hypothesis mean that the coin is fair?
- Would we have a better chance of detecting that the coin is not fair if we did more coin tosses? How many?
- If we repeated the whole procedure and again tossed the coin 100 times, might we **then** reject the null hypothesis?
- Our rejection region is asymmetric - its left part ends with 40, while its right part starts with 61. Why is that? Which other ways of defining the rejection region might be useful?

The Five Steps of Hypothesis Testing

Choose an experimental design and a data summary function for the effect that you are interested in: the **test statistic**

Set up a **null hypothesis**: a simple, computationally tractable model of reality that lets you compute the null distribution of the test statistic, i.e. the possible outcomes and each of their probabilities.

Decide on the **rejection region**, i.e., a subset of possible outcomes whose total probability is small (<= **significance level**).

Do the experiment, collect data, compute the test statistic.

Make a **decision**: reject null hypothesis if the test statistic is in the rejection region.

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This is the idealised scenario, "orthodoxy".
Reality, esp. in retrospective 'data-mining' is typically different.

Do the experiment, collect data,
compute the test statistic.

Make a **decision**: reject null hypothesis
if the test statistic is in the rejection region.

Examples of Null Hypotheses:

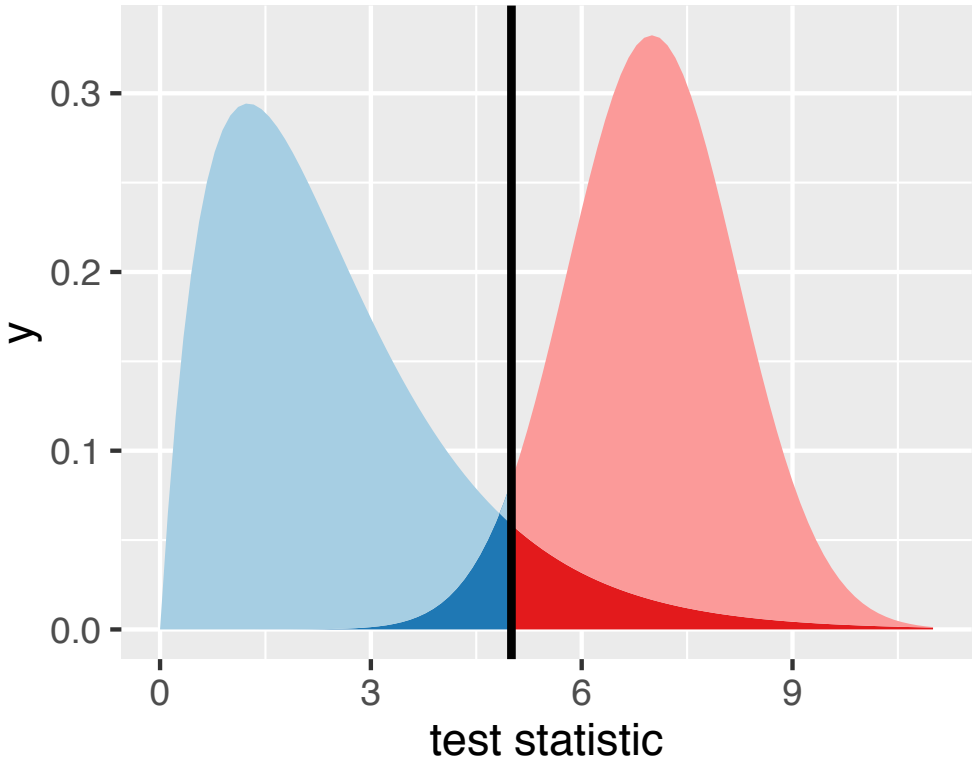
- The coin is fair
- The new drug is no better or worse than a placebo
- The effect of that RNAi-treatment on my cells is no different than that of a negative control treatment

These are not Null Hypotheses:

- The number of heads and tails were the same
- The coin is not fair
- The drug is worth its money

Types of Error in Testing

Test vs reality	**Null hypothesis is true**	**. . . is false**
Reject null hypothesis	Type I error (false positive)	True positive
Do not reject	True negative	Type II error (false negative)



Parametric Theory vs Simulation

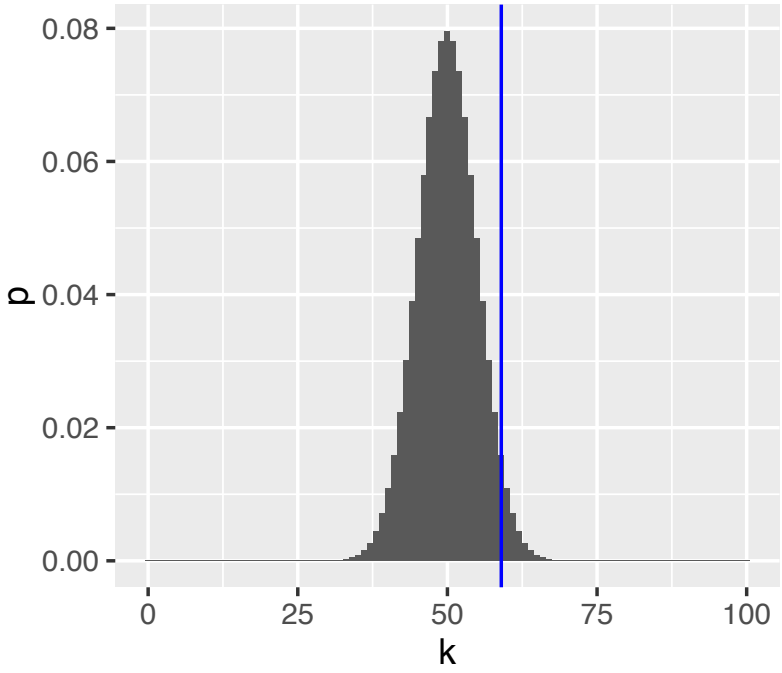


Figure 6.3: The binomial distribution for the parameters $n = 100$ and $p = 0.5$, according to Equation (6.1).

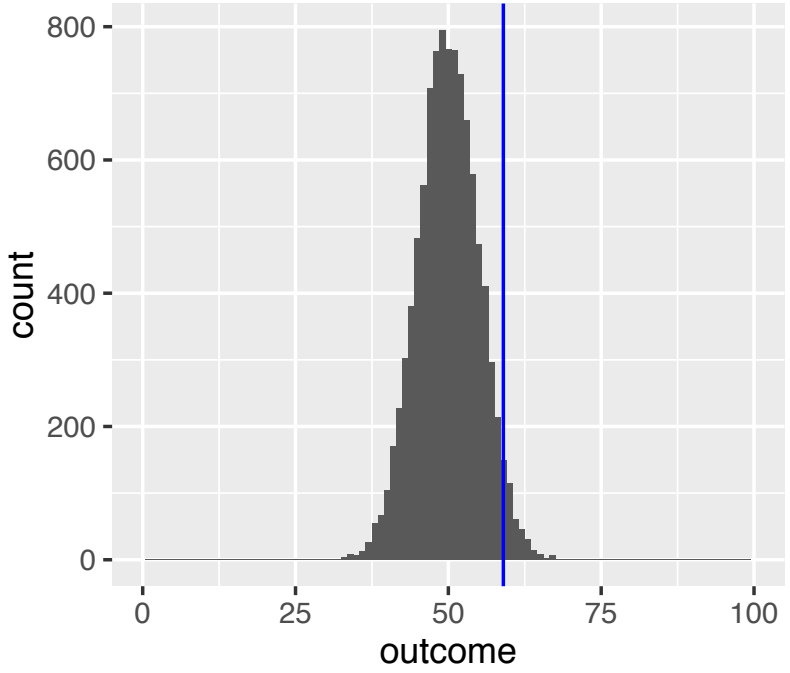
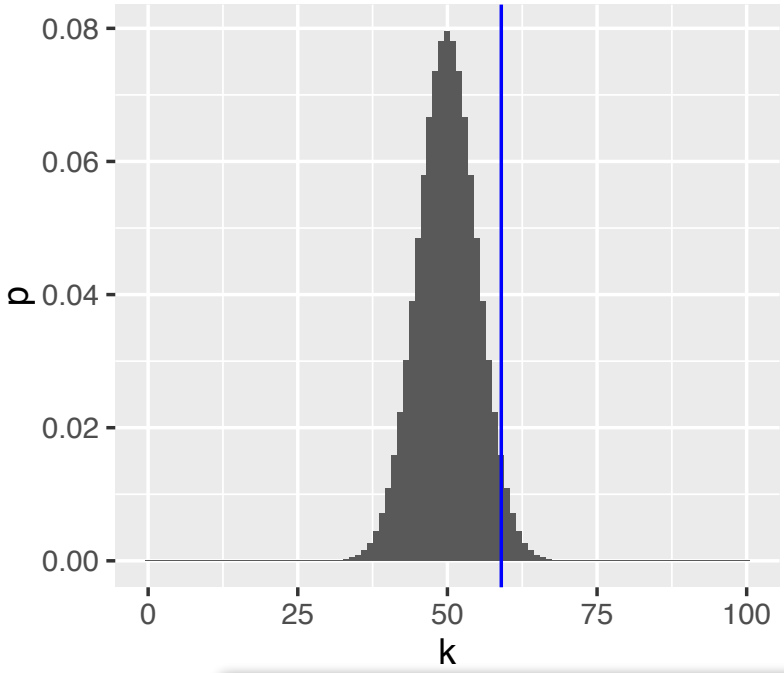


Figure 6.4: An approximation of the binomial distribution from 10^4 simulations (same parameters as Figure 6.3).

Parametric Theory vs Simulation



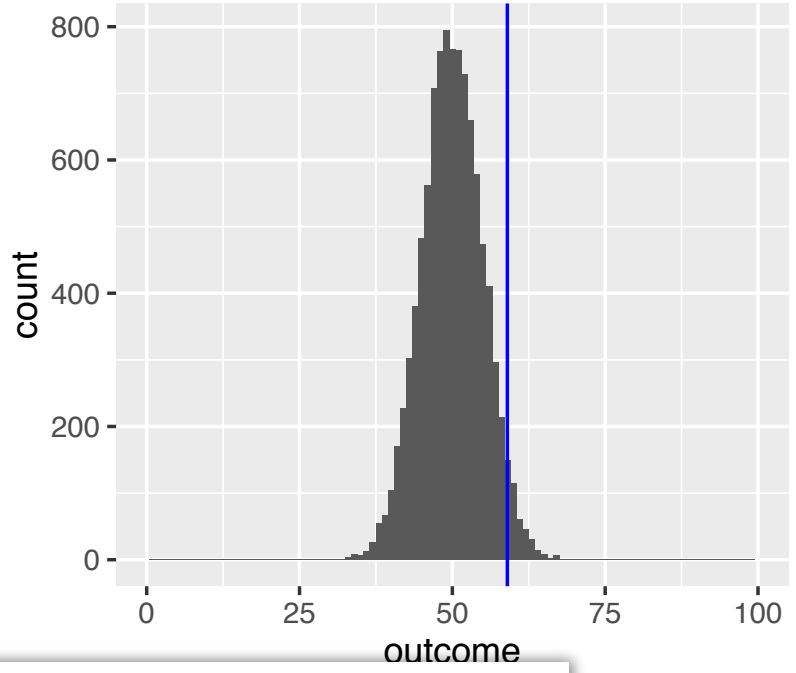


Figure 6.3: T
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according to

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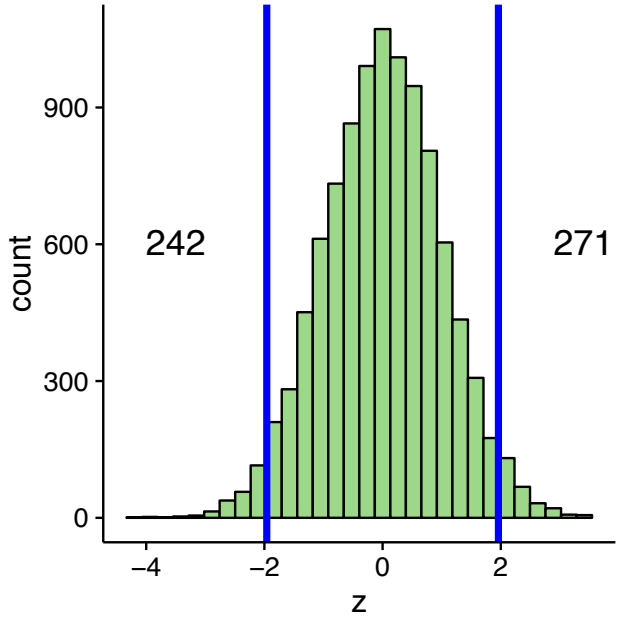
simulations
).

Q:
Discuss pros and contras for each

p-Values as Random Variables

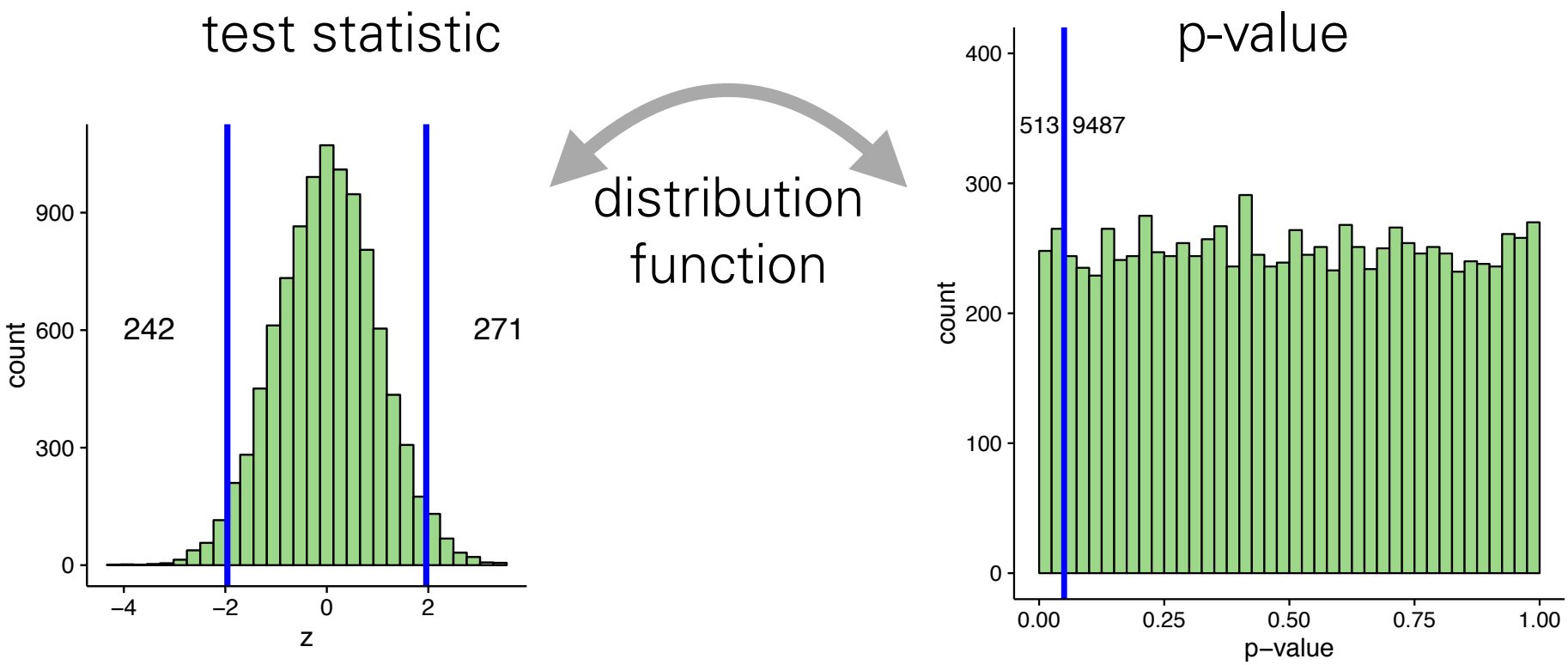
test statistic



distribution function

p-value

p-Values as Random Variables



The Test Statistic

Suppose we observed 50 tails in a row, and then 50 heads in a row. Is this a perfectly fair coin?

We could use a different test statistic: number of times we see two tails in a row

Is this statistic generally and always preferable?

Power

There can be several test statistics, with different power, for different types of alternative

Continuous Data: the t-Statistic

$$t = c\,\frac{m_1 - m_2}{s}$$

- Can also be adapted to one group only
- Relation to z-score

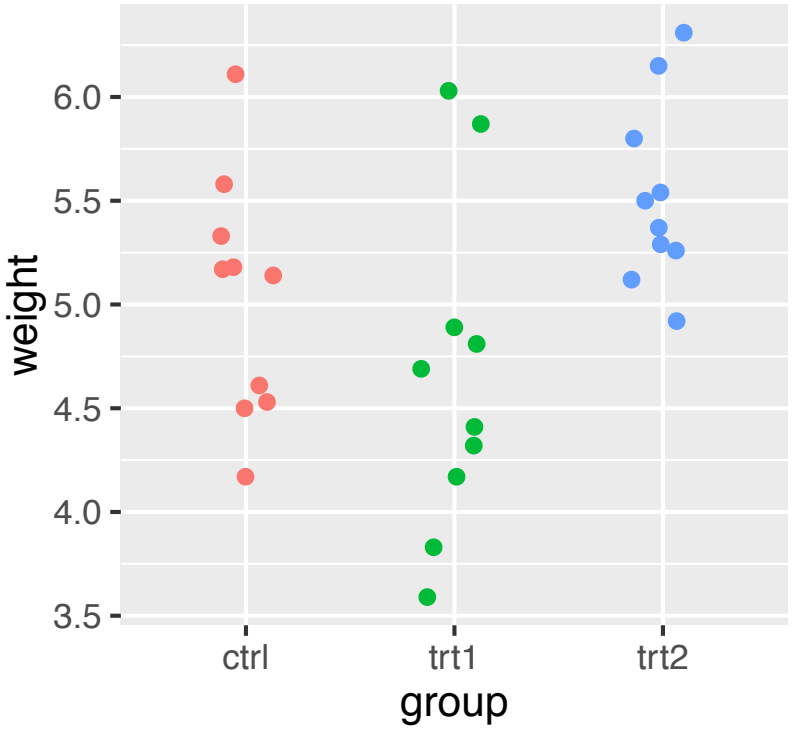


Figure 6.7: The `PlantGrowth` data.

$$\begin{aligned}
m_g &= \frac{1}{n_g}\sum_{i=1}^{n_g} x_{g,i} \qquad g = 1,2 \\
s^2 &= \frac{1}{n_1 + n_2 - 2}\left(\sum_{i=1}^{n_1}(x_{1,i} - m_1)^2 + \sum_{j=1}^{n_2}(x_{2,j} - m_2)^2\right) \\
c &= \sqrt{\frac{n_1 n_2}{n_1 + n_2}}.
\end{aligned}$$

t- (and |t|-) Distribution

If the data are identically normal distributed and independent, then under H_0, t follows a 't-distribution' with parameter n_1+n_2 (a.k.a. degrees of freedom)

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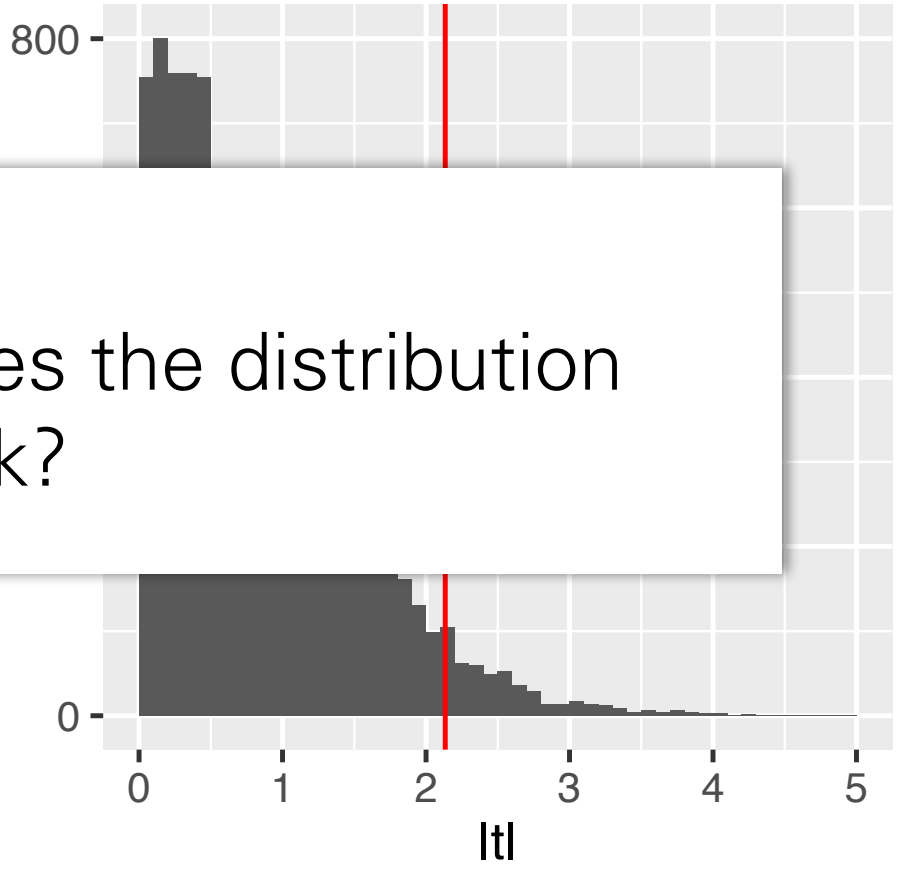

Figure 6.8: The null distribution of the (absolute) t-statistic determined by simulations – namely, by random permutations of the group labels.

Comments and Pitfalls

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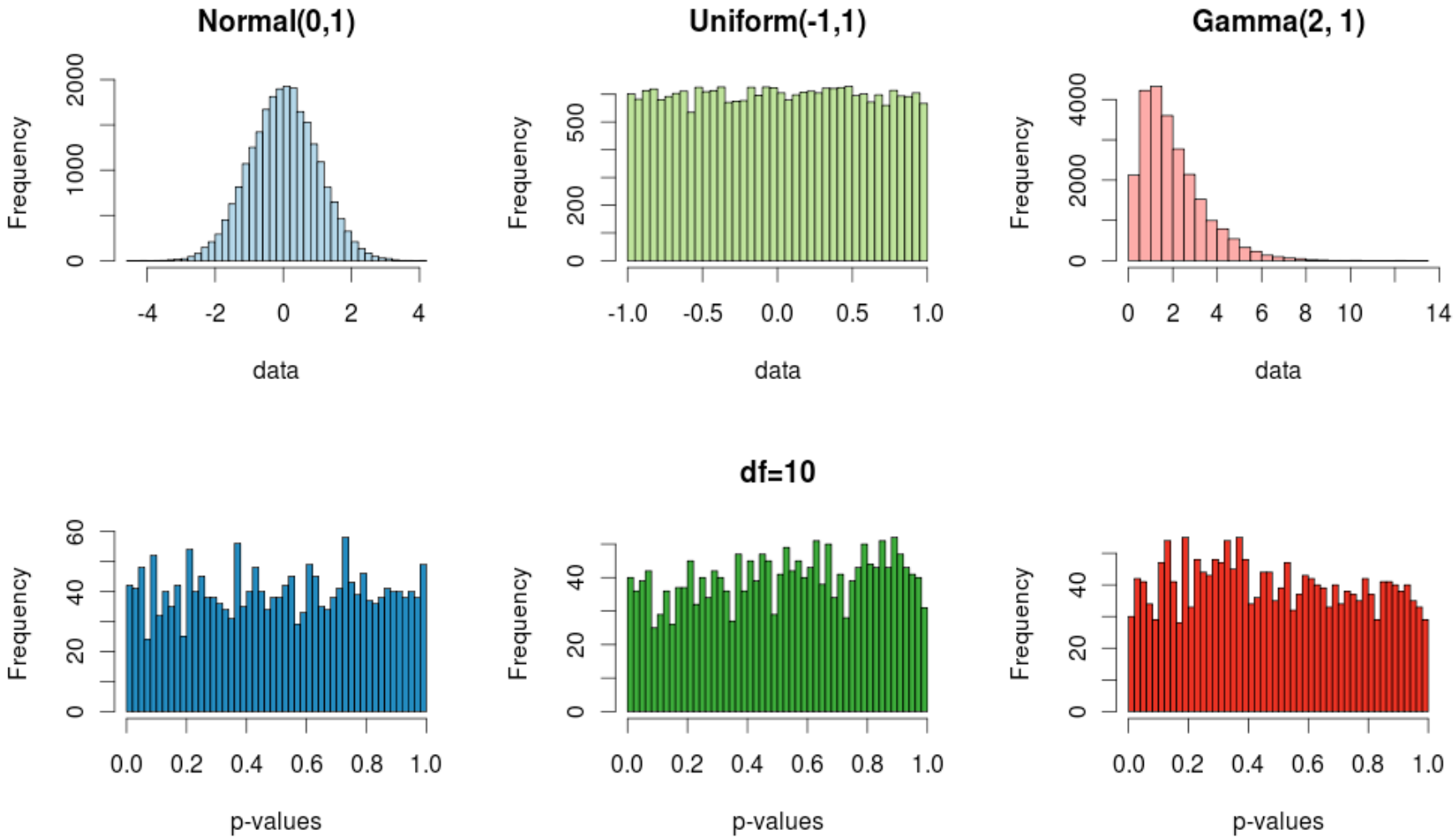
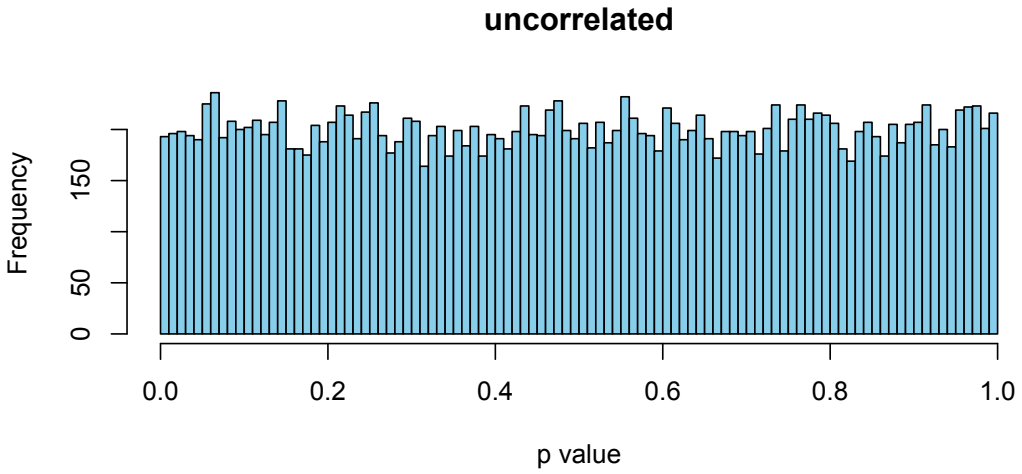
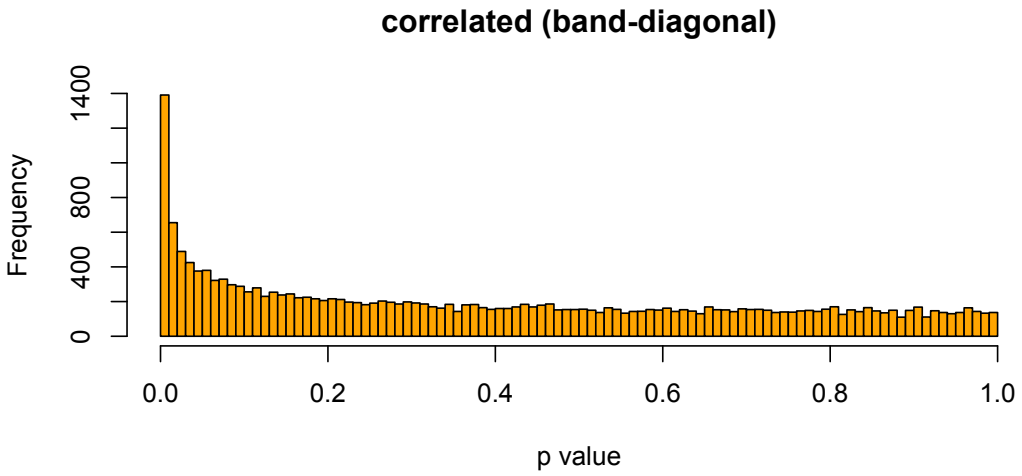
If the data are dependent, then p-values will likely be totally wrong (e.g., for positive correlation, too optimistic).

Different Data Distributions – Independent Case



t-Test Looses Error Control if Independence Assumption Does not Hold






```r
library("mvtnorm")
library("genefilter")

p = 30     ## number of samples
n = 20000  ## number of genes

mu = rep(0, p)
dp = diag(p)

sigma = list(
 'uncorrelated' = dp,              ## unity matrix
 'correlated (band-diagonal)' =    ## band diagonal
      (row(dp)==col(dp)) + 0.5 * (abs(row(dp)-col(dp))==1))

lapply(sigma, print)

## generate data
x  = lapply(sigma, function(s) rmvnorm(n = n, mean = mu, sigma = s))

## tests
tt = lapply(x, rowttests)

par(mfrow=c(length(tt), 1))
for(i in seq(along=tt))
    hist(tt[[i]]$p.value, breaks=100, col=c("skyblue", "orange")[i],
         main=names(tt)[i], xlab="p value")
```

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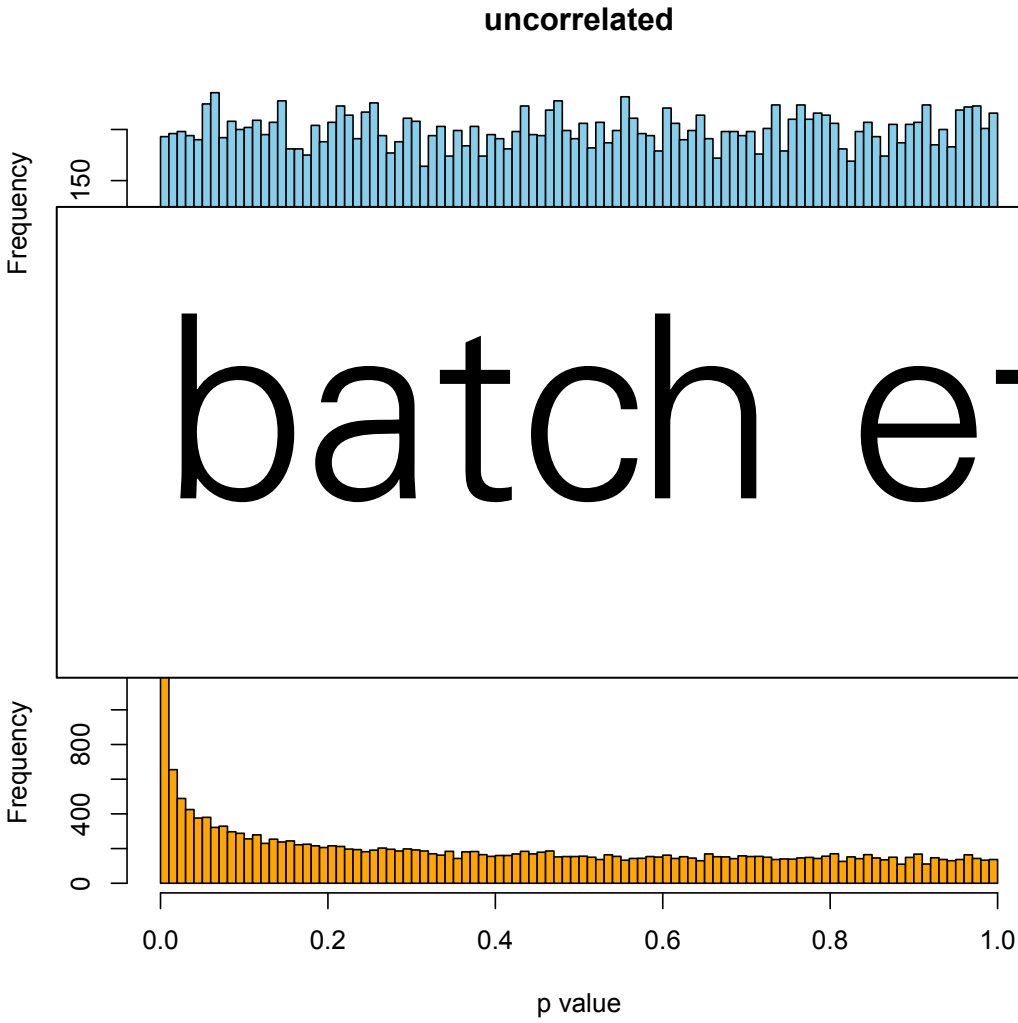

batch effects!

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```
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Avoid Fallacy

The p-value is the probability that the data could happen, under the condition that the null hypothesis is true.

It is not the probability that the null hypothesis is true.

Absence of evidence ≠ evidence of absence

Recap: Single Hypothesis Testing

p-values are random variables: uniformly distributed if the null hypothesis is true - and should be close to zero if the alternative holds.

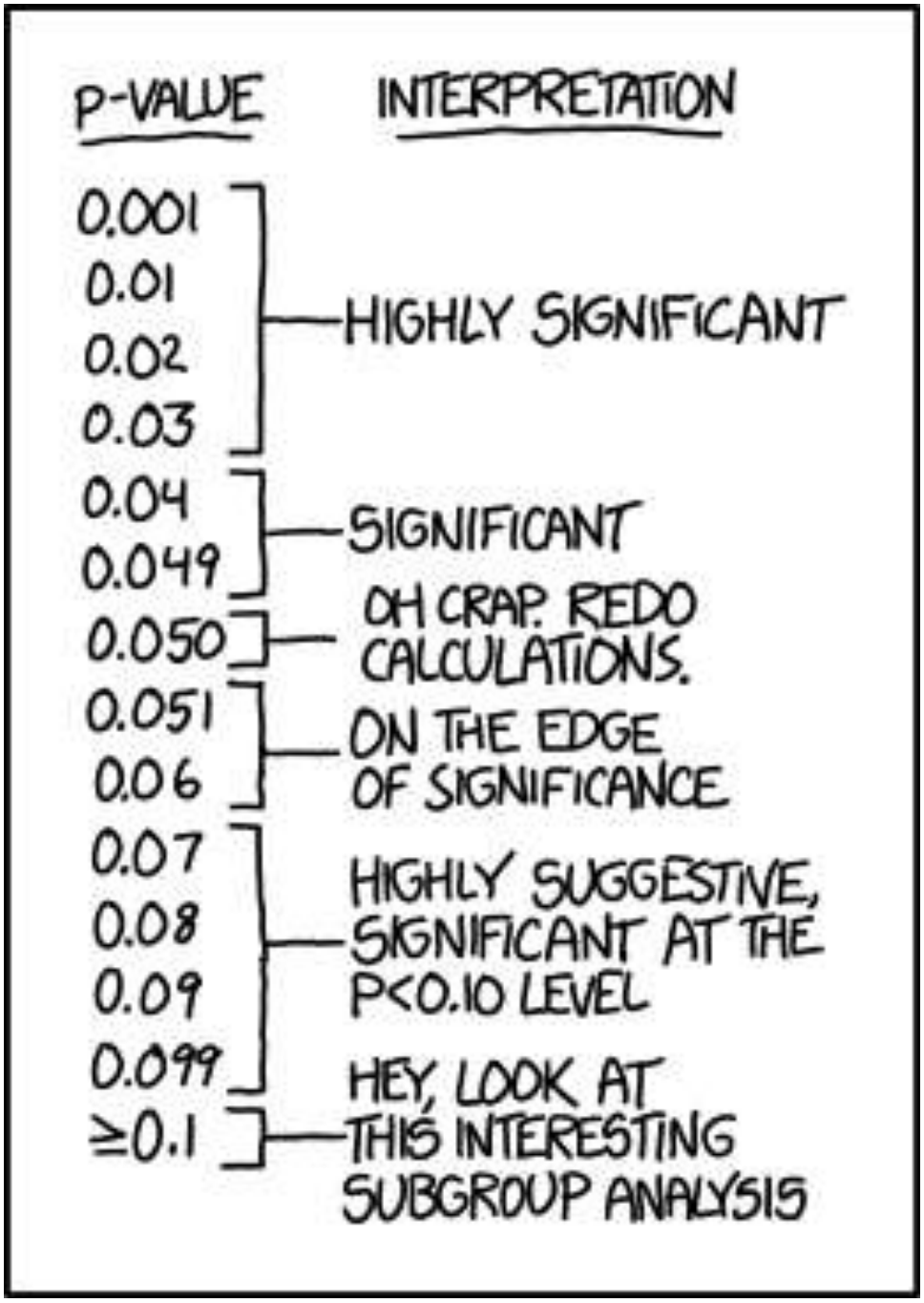
Note: We only observe one draw.

We prove something by disproving ('rejecting') the opposite (the null hypothesis). Reject = Discover.

Not rejecting does not prove the null hypothesis

Repeating the experiment (under the null): Around 5% of the times the p-value will be less than 0.05 by chance

All this reasoning is probabilistic. Testing & p-values are for rational decision making in uncertain contexts.


P-VALUE
INTERPRETATION
0.001
0.01
0.02
0.03
HIGHLY SIGNIFICANT
0.04
0.049
SIGNIFICANT
0.050
OH CRAP. REDO CALCULATIONS.
0.051
0.06
ON THE EDGE OF SIGNIFICANCE
0.07
0.08
0.09
HIGHLY SUGGESTIVE, SIGNIFICANT AT THE P<0.10 LEVEL
0.099
≥0.1
HEY, LOOK AT THIS INTERESTING SUBGROUP ANALYSIS

What is p-Value Hacking ?

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'outlier' removal
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The ASA's Statement on p-Values: Context, Process, and Purpose
Ronald L. Wasserstein & Nicole A. Lazara DOI: 10.1080/00031305.2016.1154108

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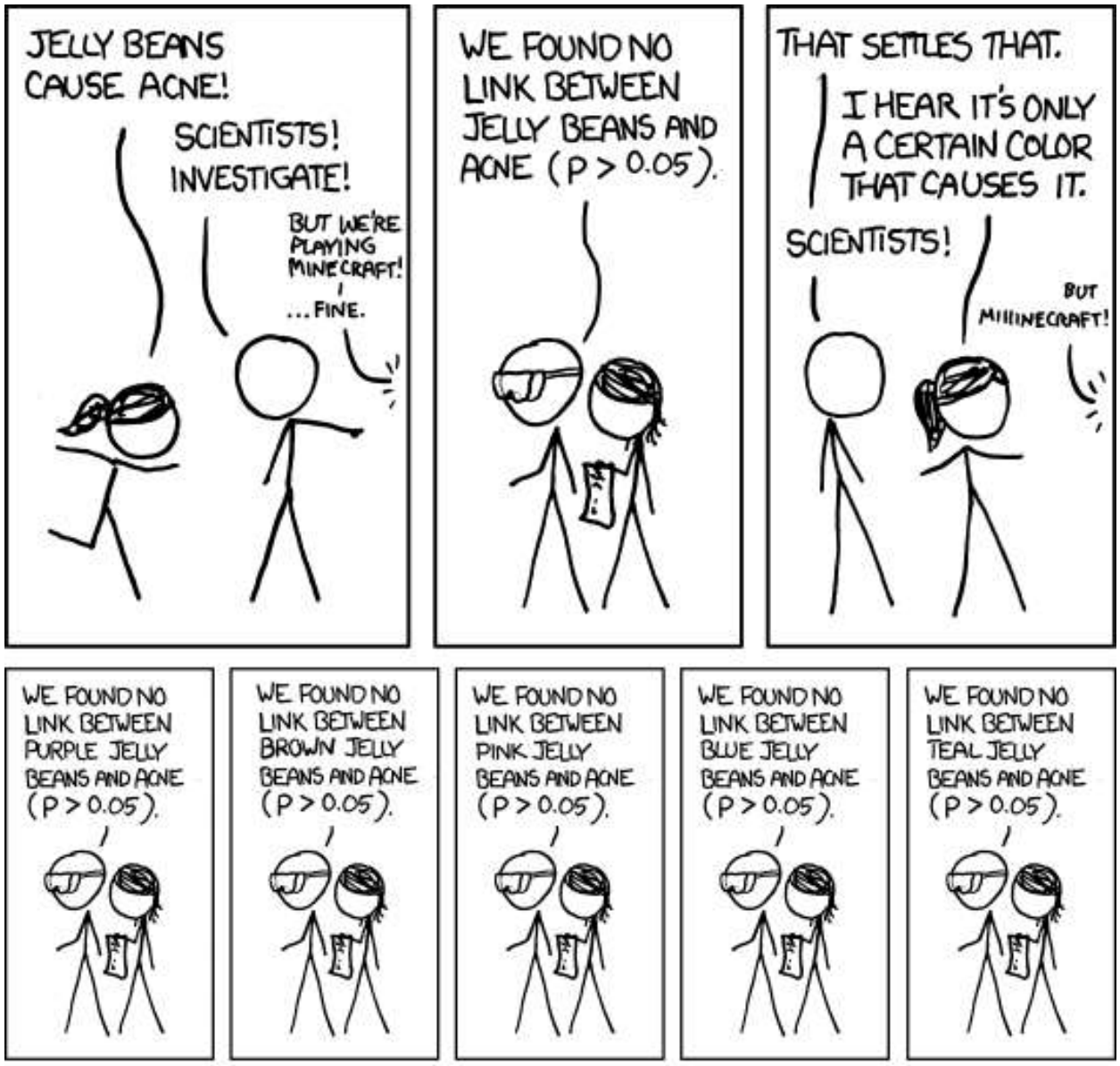
What can we do about this?

Multiple Testing



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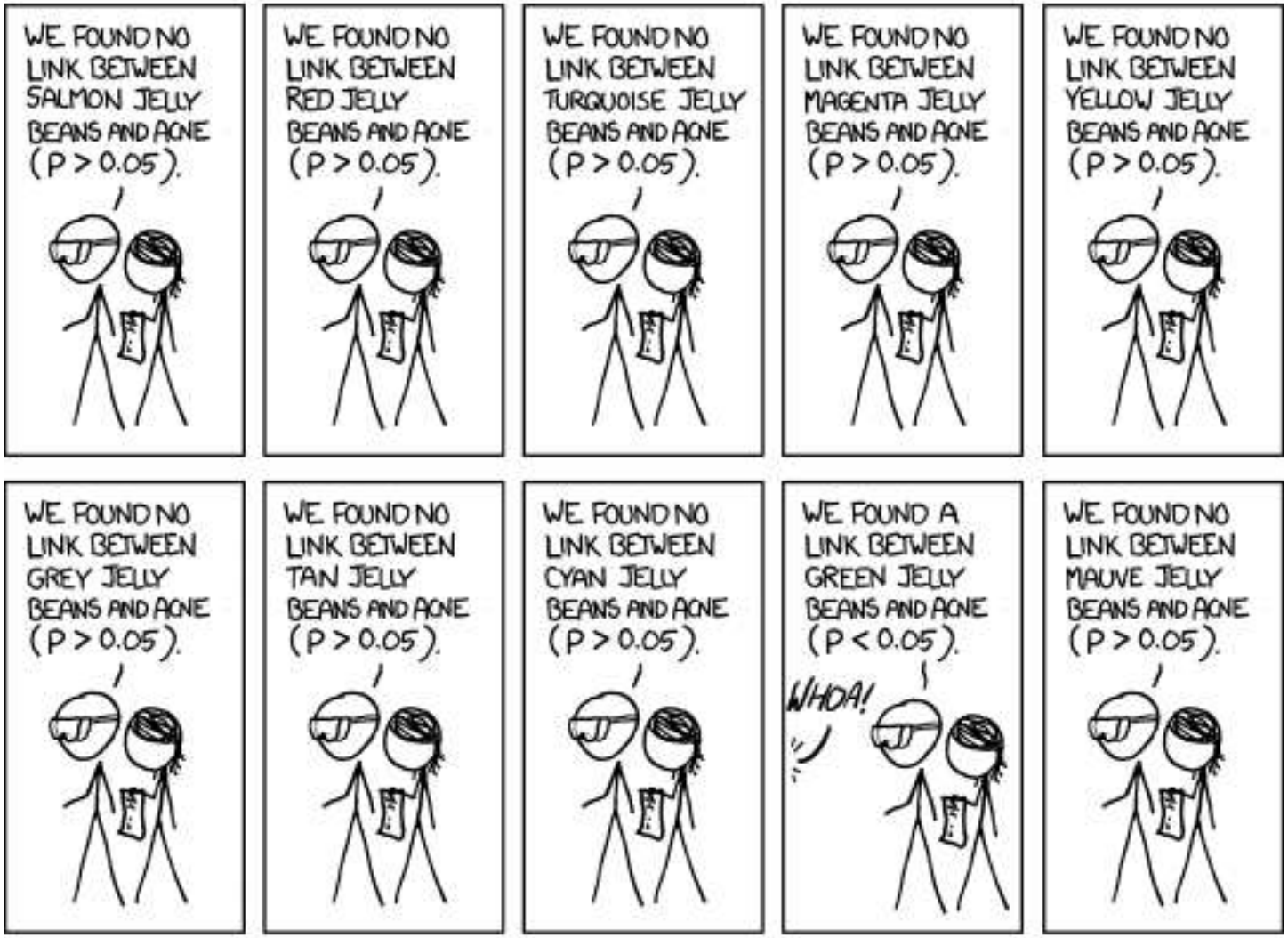

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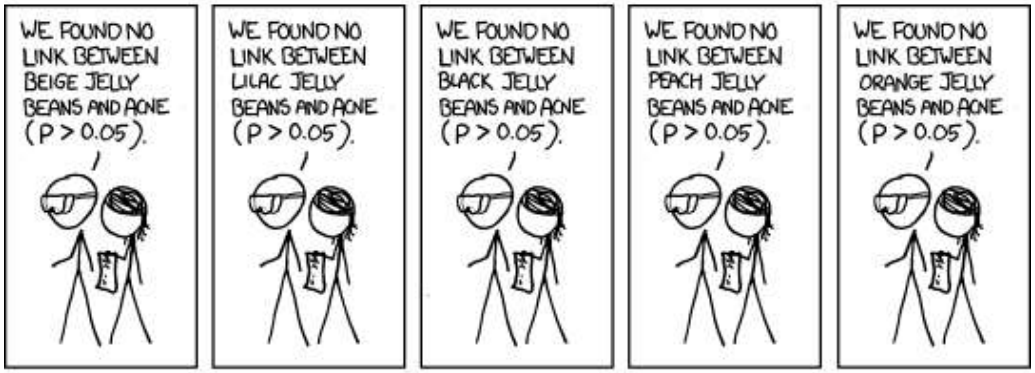
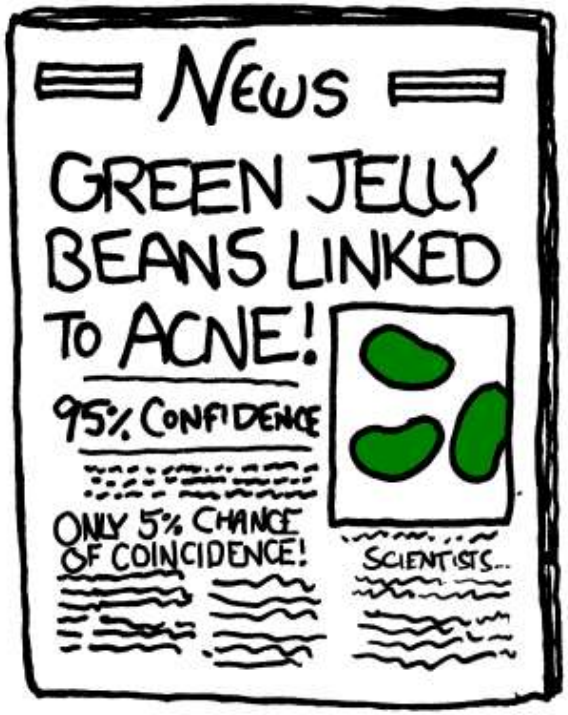
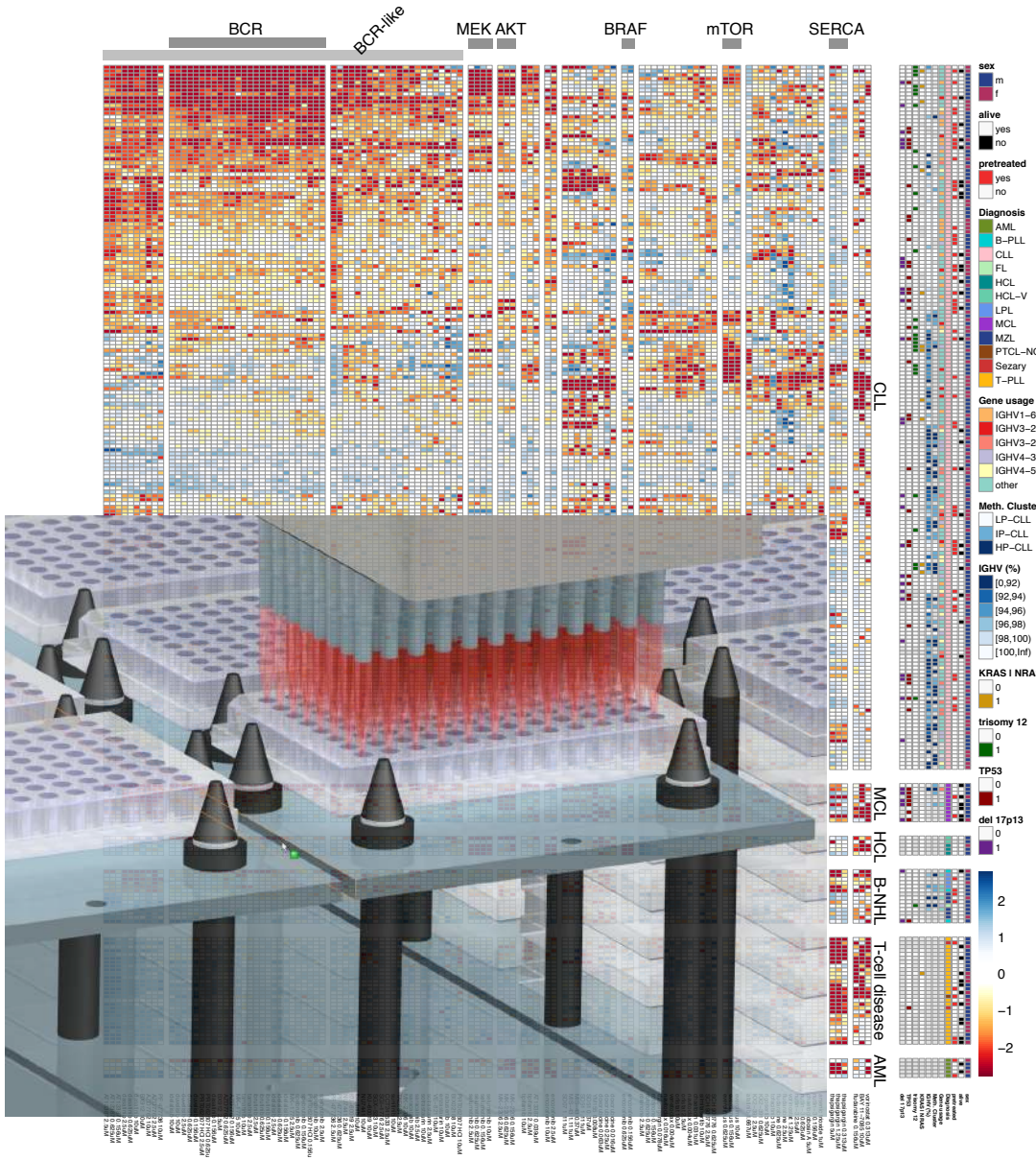




Multiple Testing

Many data analysis approaches in genomics employ item-by-item testing:

- Expression profiling
- Differential microbiome analysis
- Genetic or chemical compound screens
- Genome-wide association studies
- Proteomics
- Variant calling
- …



The Multiple Testing Burden

When performing several tests, type I error goes up: for

$\alpha = 0.05$ and n indep. tests, probability of no false positive result is

$$\underbrace{0.95 \cdot 0.95 \cdot \ldots \cdot 0.95}_{\text{n–times}} \lll 0.95$$

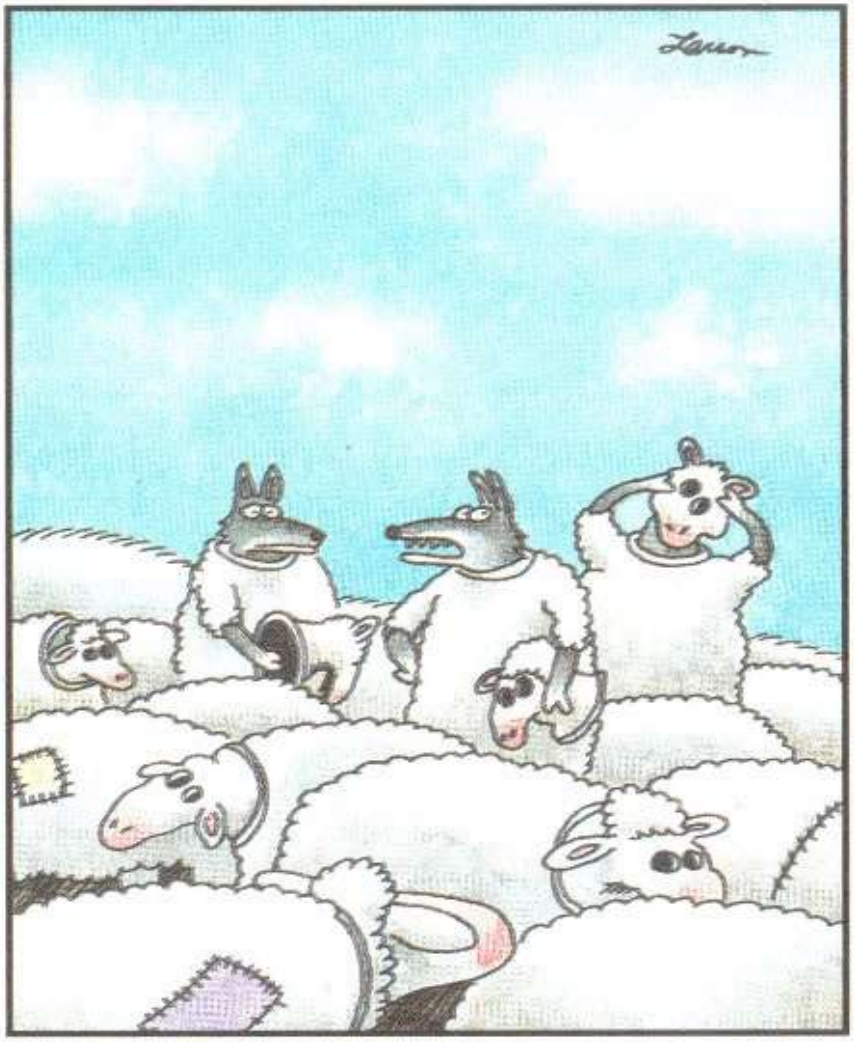
False Positive Rate and False Discovery Rate

FPR: fraction of FP among all true negatives

FDR: fraction of FP among hits called

Example:
20,000 genes, 500 are d.e.,
100 hits called, 10 of them wrong.

FPR: 10/19,500 ≈ 0.05%
FDR: 10/100 = 10%



"Wait a minute! Isn't anyone here a real sheep?"

Experiment-Wide Type I Error Rates

Test vs Reality	**Null Hypothesis is true**	**. . . is false**	**Total**
Rejected	V	S	R
Not rejected	U	T	$m - R$
Total	m_0	$m - m_0$	m

- m: total number of hypotheses
- m_0: number of null hypotheses
- V: number of false positives (a measure of type I error)

Family-wise error rate (FWER): The probability of one or more false positives, P($V > 0$). For large m_0, this is difficult to keep small.

False discovery rate (FDR): The expected fraction of false positives among all discoveries, E[$V / \max\{R, 1\}$].

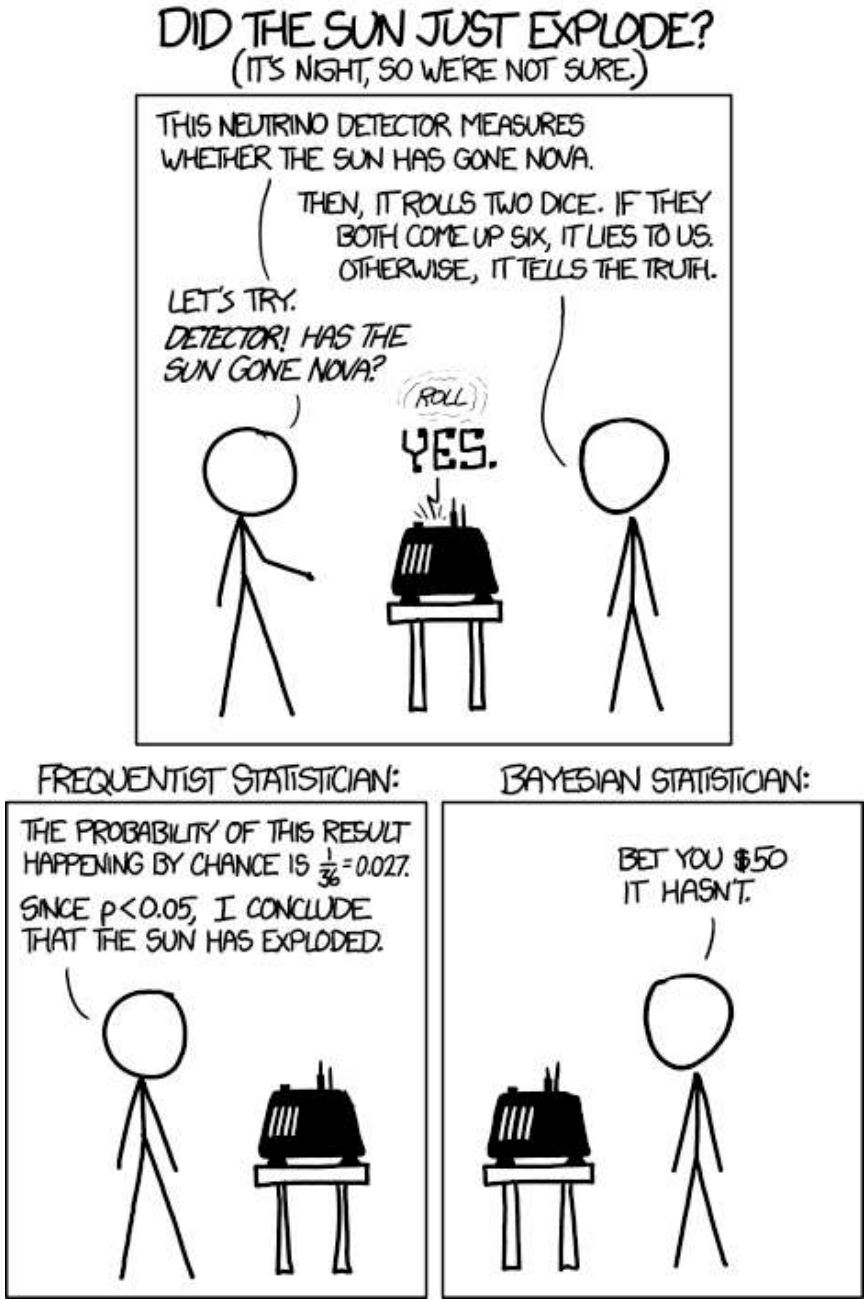
NB: if m_0=m, then FDR=FWER

Bonferroni Correction

For *m* tests, multiply each *p*-value with *m*.

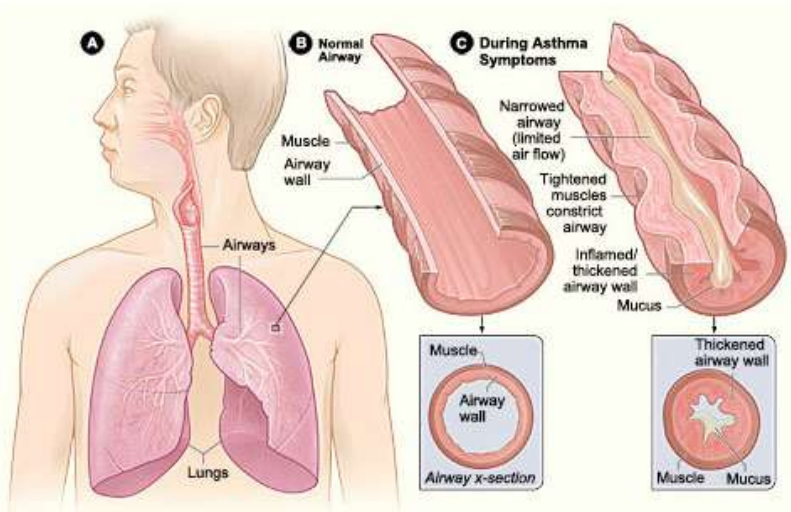
Then see if anyone still remains below α.

The Multiple Testing Opportunity



Data set 1: RNA-Seq

Transcriptome changes in four samples of primary human airway smooth muscle cells treated with dexamethasone, a synthetic glucocorticoid. 1 μM for 18 h.



cellline	dexamethasone
N61311	untrt
N61311	trt
N052611	untrt
N052611	trt
N080611	untrt
N080611	trt
N061011	untrt
N061011	trt

DESeq2 differential expression analysis:

gene i, sample j:

$$K_{ij} \sim \text{NB}(\text{mean} = \mu_{ij}, \text{dispersion} = \alpha_j)$$

$$\mu_{ij} = s_j q_{ij}$$

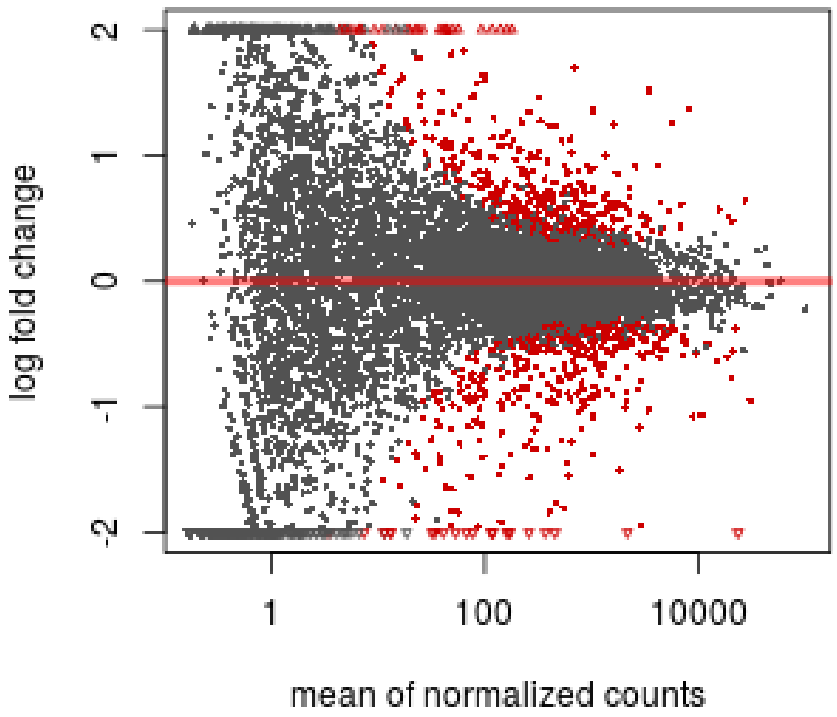
$$\log q_{ij} = \sum_r x_{jr} \beta_{rj}$$

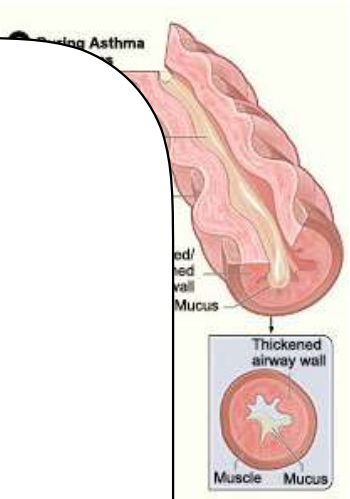
```
design <- ~ cellline + dexamethasone
```

Himes et al. "RNA-Seq Transcriptome Profiling Identifies CRISPLD2 as a Glucocorticoid Responsive Gene that Modulates Cytokine Function in Airway Smooth Muscle Cells." PLoS One. 2014 GEO: GSE52778.

Data set 1: RNA-Seq

Transcrip
sample
smooth
dexame
glucocc





cell	
N61	
N61	
N05	
N05	
N08	
N08	
N0610	
N061011	trt

analysis:

persion $= \alpha_j$)

design <- ~ cellline + dexamethasone

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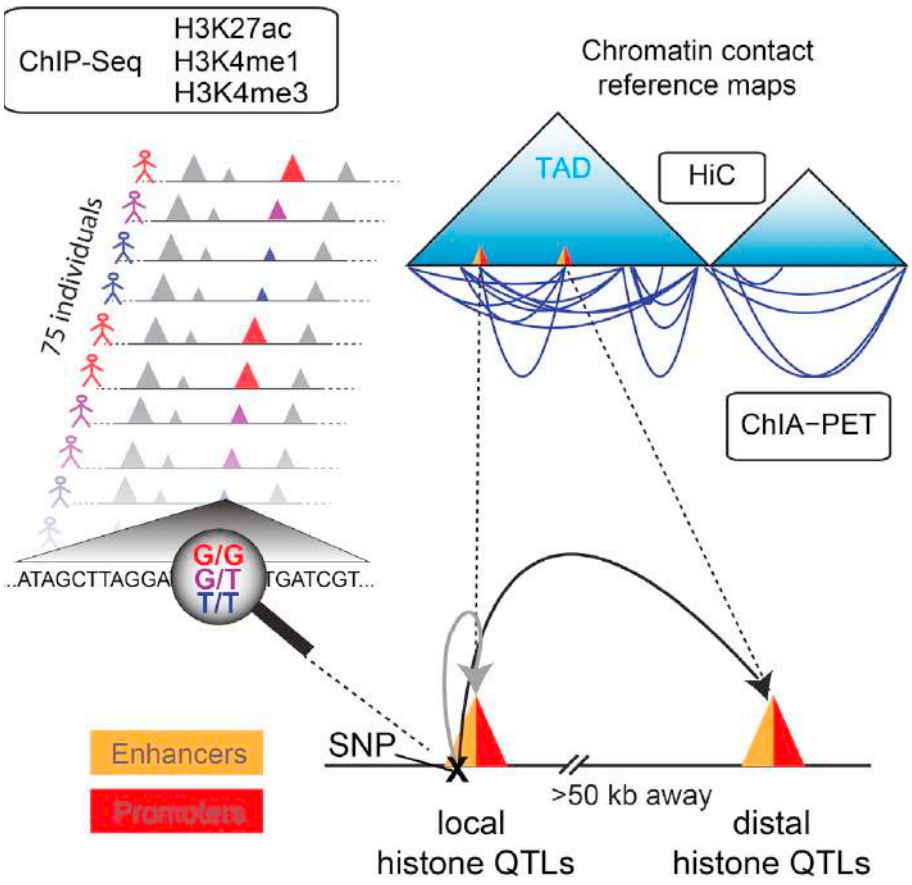
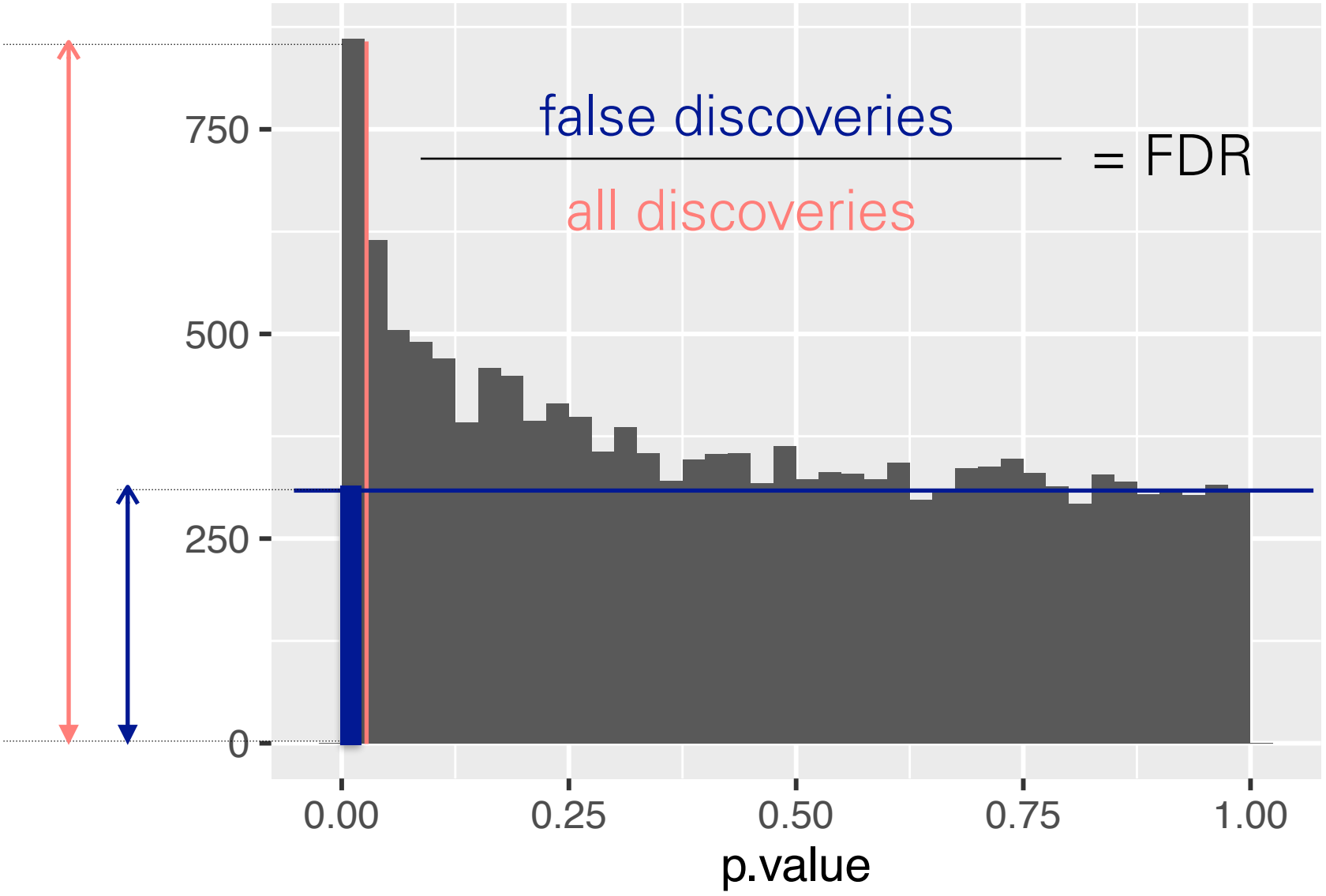
Data set 2: hQTL

ChIP-seq for histone marks in lymphoblastoid cell lines from 75 sequenced individuals.
Local QTLs: find best-correlated SNP within 2kb of peak boundaries: 14,142 hQTLs, involving ~10% of all H3K27ac peaks (FDR=0.1, permutations)
Distal: distance cutoffs from 50 to 300 kb; also HiC



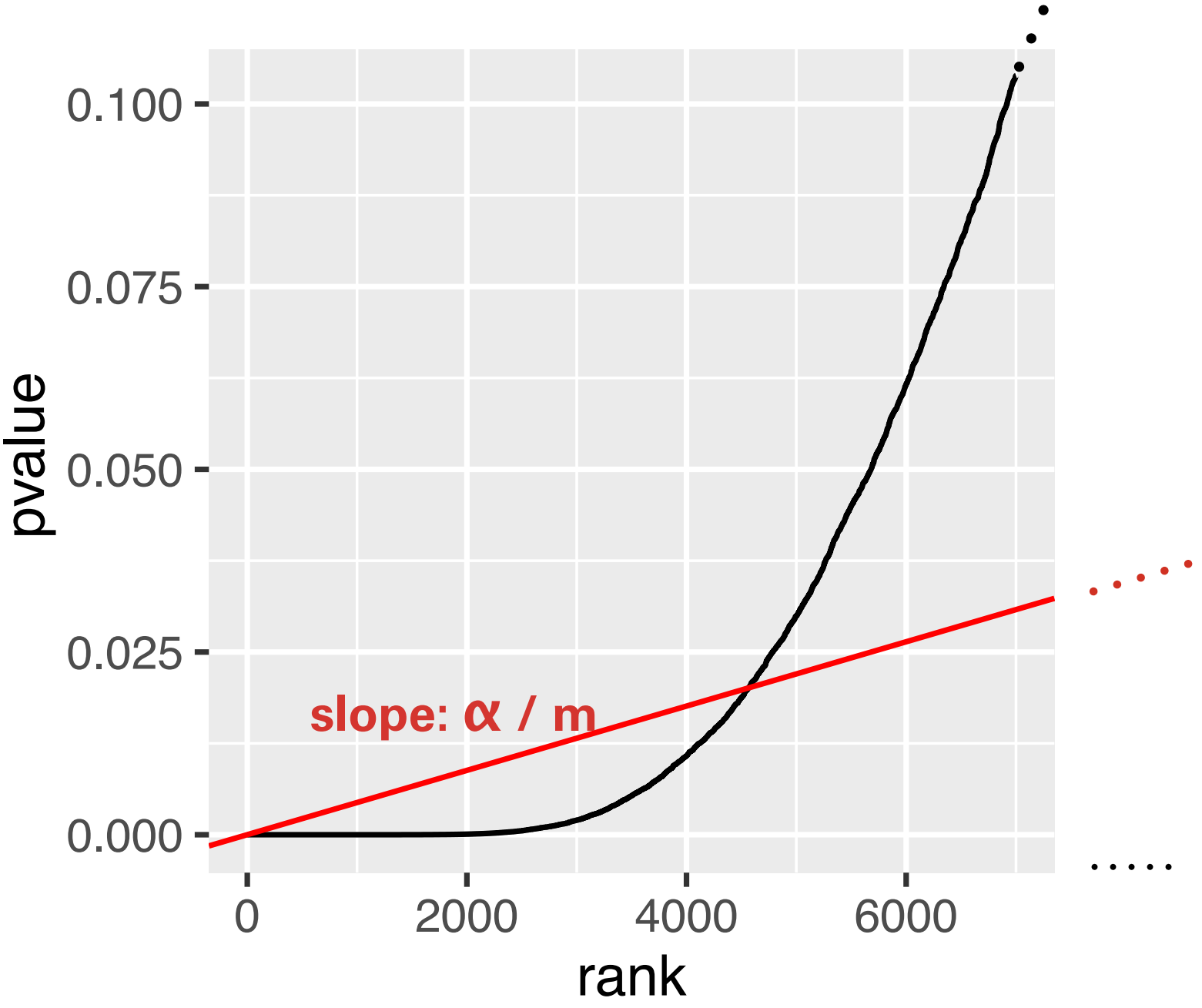
Grubert, Zaugg, Kasowski, et al. Genetic control of chromatin states in humans involves local and distal chromosomal interactions. Cell (2015).

False Discovery Rate

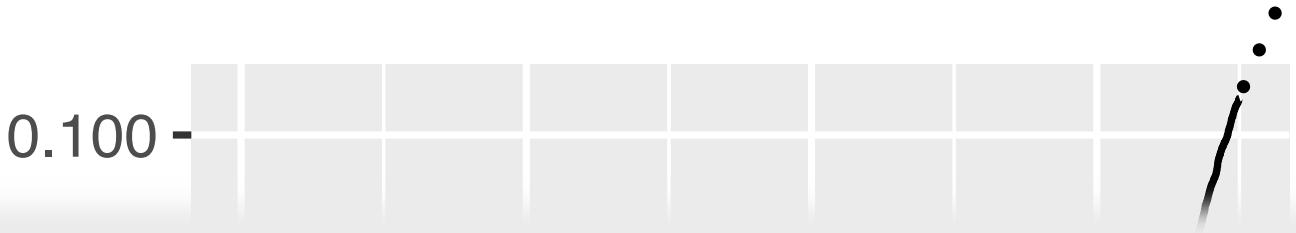


Method of Benjamini & Hochberg (1995)

Method of Benjamini & Hochberg

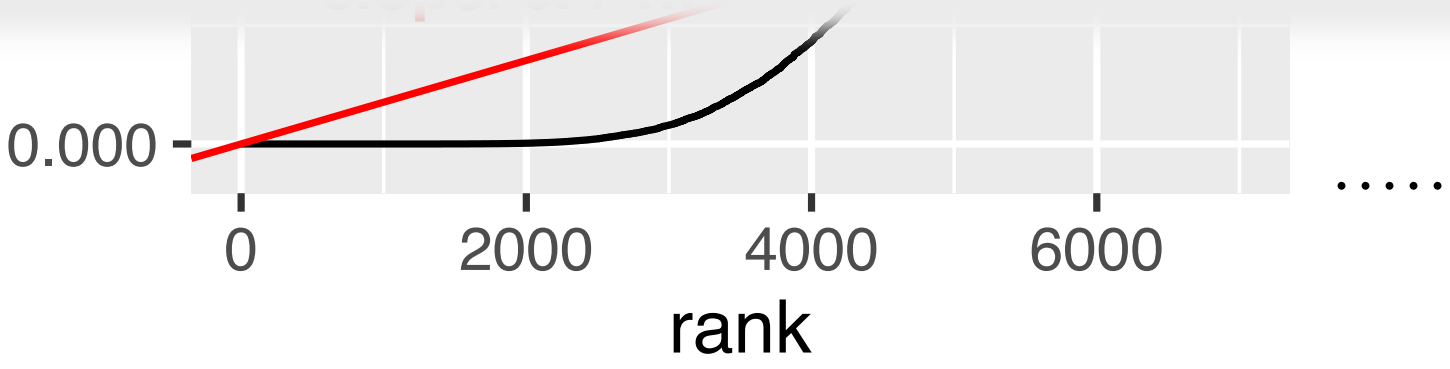


Method of Benjamini & Hochberg

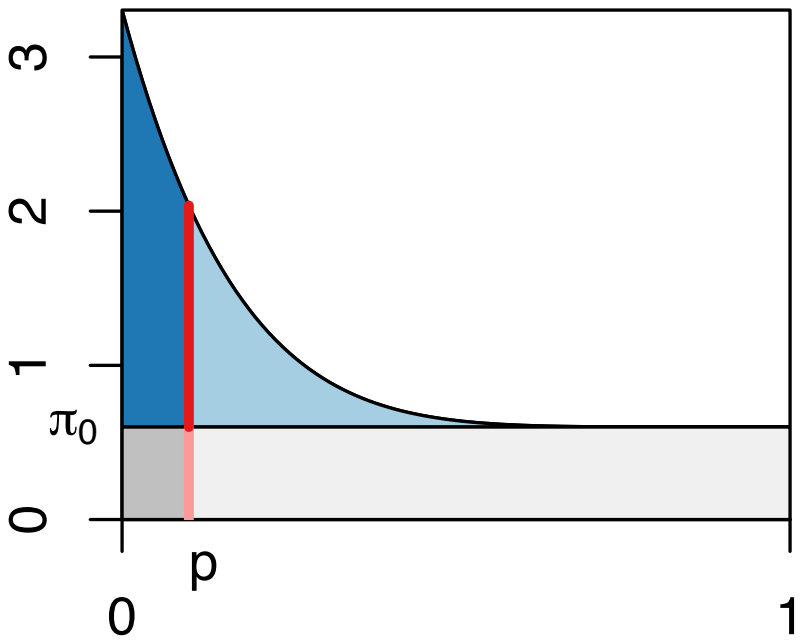



```
BH = {
        i <- length(p):1
        o <- order(p, decreasing = TRUE)
        ro <- order(o)
        pmin(1, cummin(n/i * p[o]))[ro]
    }
```

takes a list of p-values as input and returns a matched list of ‘adjusted’ p-values.



The Two-Groups Model and the Local False Discovery Rate



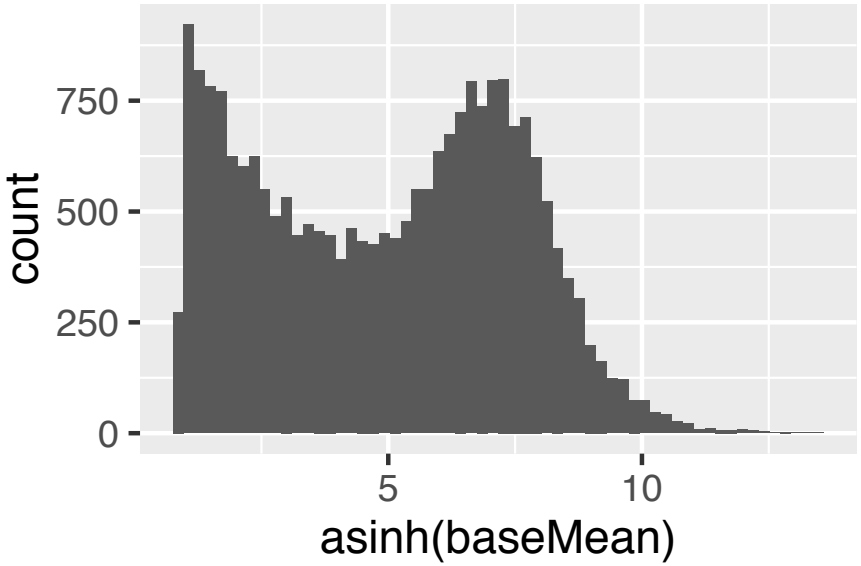
$$f(p) = \pi_0 + (1 - \pi_0) f_{\text{alt}}(p)$$

$$\text{fdr}(p) = \frac{\pi_0}{f(p)}$$

FDR: a set property. A single number that applies to a whole set of discoveries.

fdr: a local property. It applies to individual hypotheses.

Not all Hypothesis Tests are Created Equal



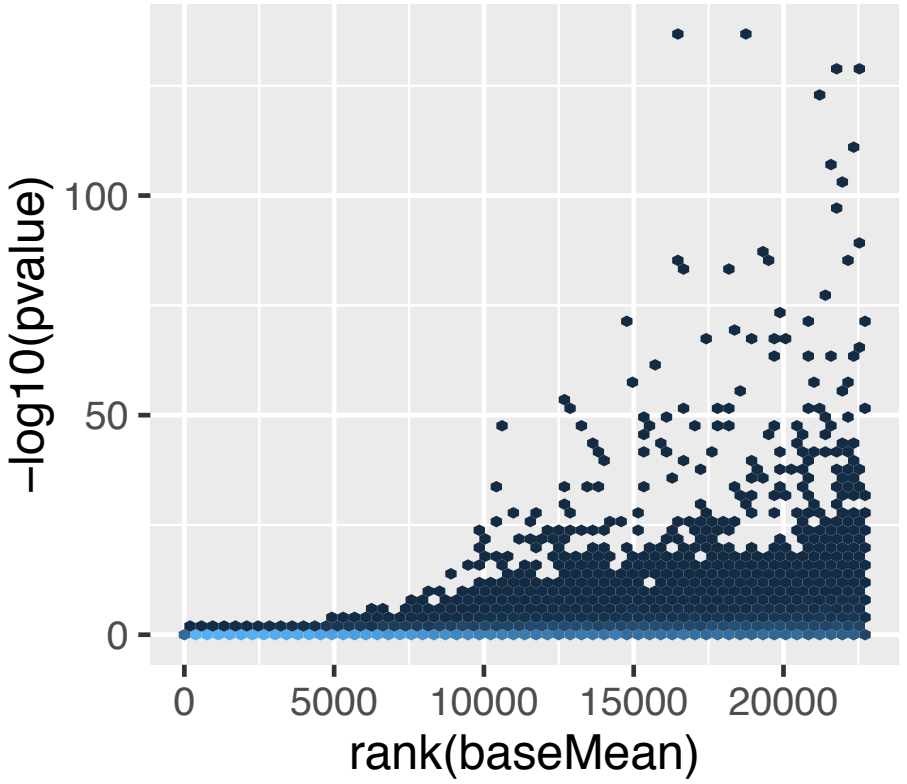


Figure 6.15: Histogram of `baseMean`. We see that it covers a large dynamic range, from close to 0 to around 3.3×10^5.

Covariates - examples

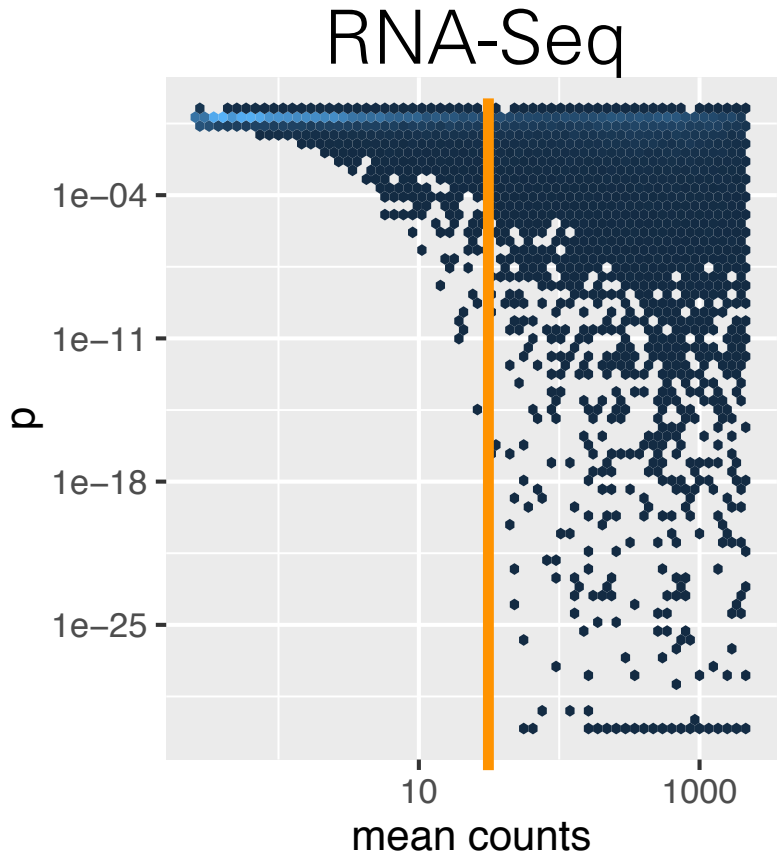
Application	Covariate
Differential RNA-Seq, ChIP-Seq, CLIP-seq, ...	(Normalized) mean of counts for each gene
eQTL analysis	SNP – gene distance
GWAS	Minor allele frequency
t-tests	Overall variance
Two-sided tests	Sign
All applications	Sample size; measures of signal-to-noise ratio

Independent Filtering

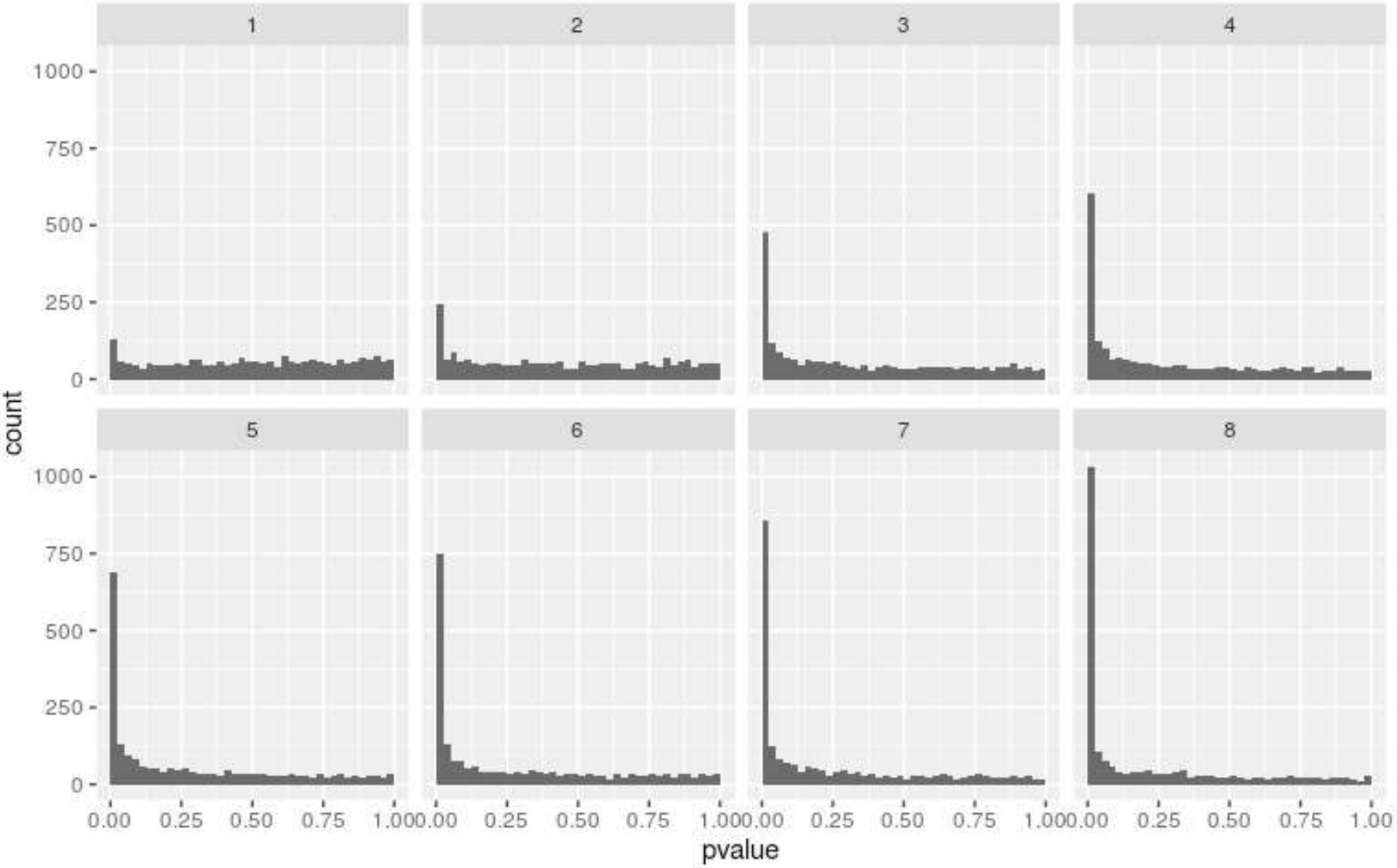
Two steps:

- All hypotheses H_i with $X_i < x$ get filtered.
- Apply BH to remaining hypotheses.

(Bourgon, Gentleman, Huber *PNAS* 2010)



RNA-Seq p-value histogram stratified by average read count



Weighted Benjamini-Hochberg method

- Let $w_i \geq 0$ and $\frac{1}{m}\sum_{i=1}^{m} w_i = 1$ ("weight budget").
- Define $Q_i = P_i / w_i$.
- Apply BH to Q_i instead of P_i.
- Proven Type-I error (FDR) control (Genovese, Roeder, Wasserman *Biometrika* 2006).
- If $w_i > 1$, then H_i is easier to reject.
- $Q_i \leq t \Leftrightarrow P_i \leq w_i t =: t_i$

Weighted Benjamini-Hochberg method

- Let $w_i \geq 0$ and $\frac{1}{m}\sum_{i=1}^{m} w_i = 1$ ("weight budget").
- Define $Q_i = P_i/w_i$.
- Apply BH to Q instead of P
- Proven Ty der, Wasserma
- If $w_i > 1$,
- $Q_i \leq t \Leftrightarrow$

Weighted Benjamini-Hochb g method

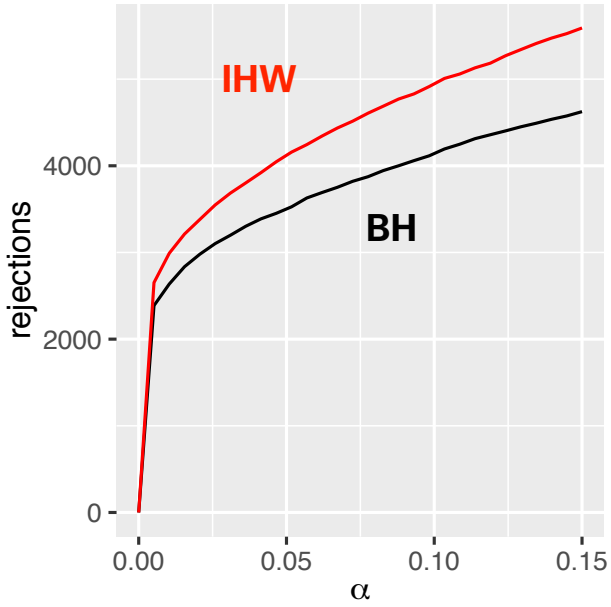
- Let $w_i \geq 0$ and $\frac{1}{m} \sum^{n}$ udget").
- Define $Q_i =$
- Apply
- Proven der, Wasserma
- If $w_i > 1$,
- $Q_i \leq t \Leftrightarrow$

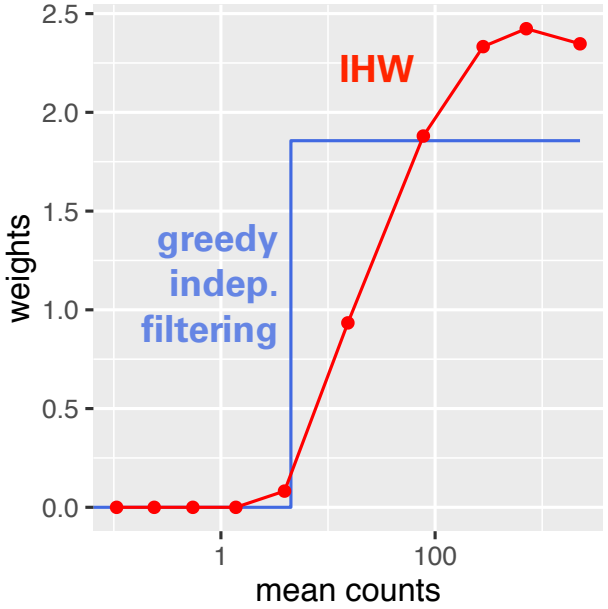
Problem: how to know the weights?

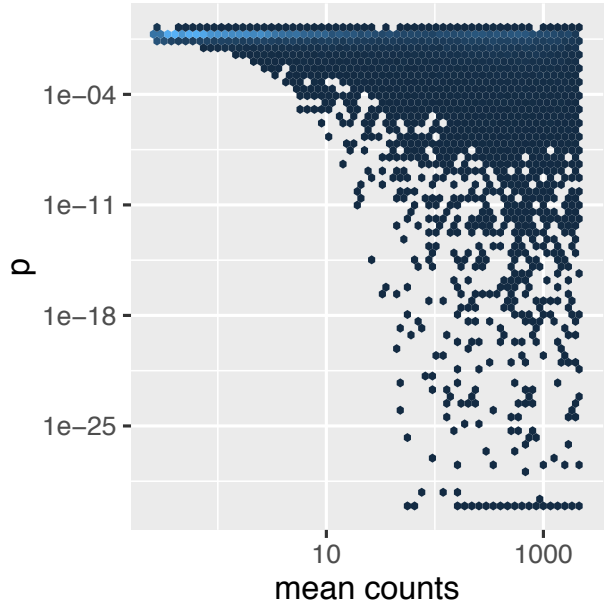
Independent hypothesis weighting (IHW): basic idea

- Stratify the tests into G bins, by covariate X
- Choose α
- For each possible weight vector $\mathbf{w} = (w_1, \ldots, w_G)$ apply weighted BH procedure. Choose $\mathbf{w}$ that maximizes the number of rejections at level α.
- Report the result with the optimal weight vector $\mathbf{w}^*$.

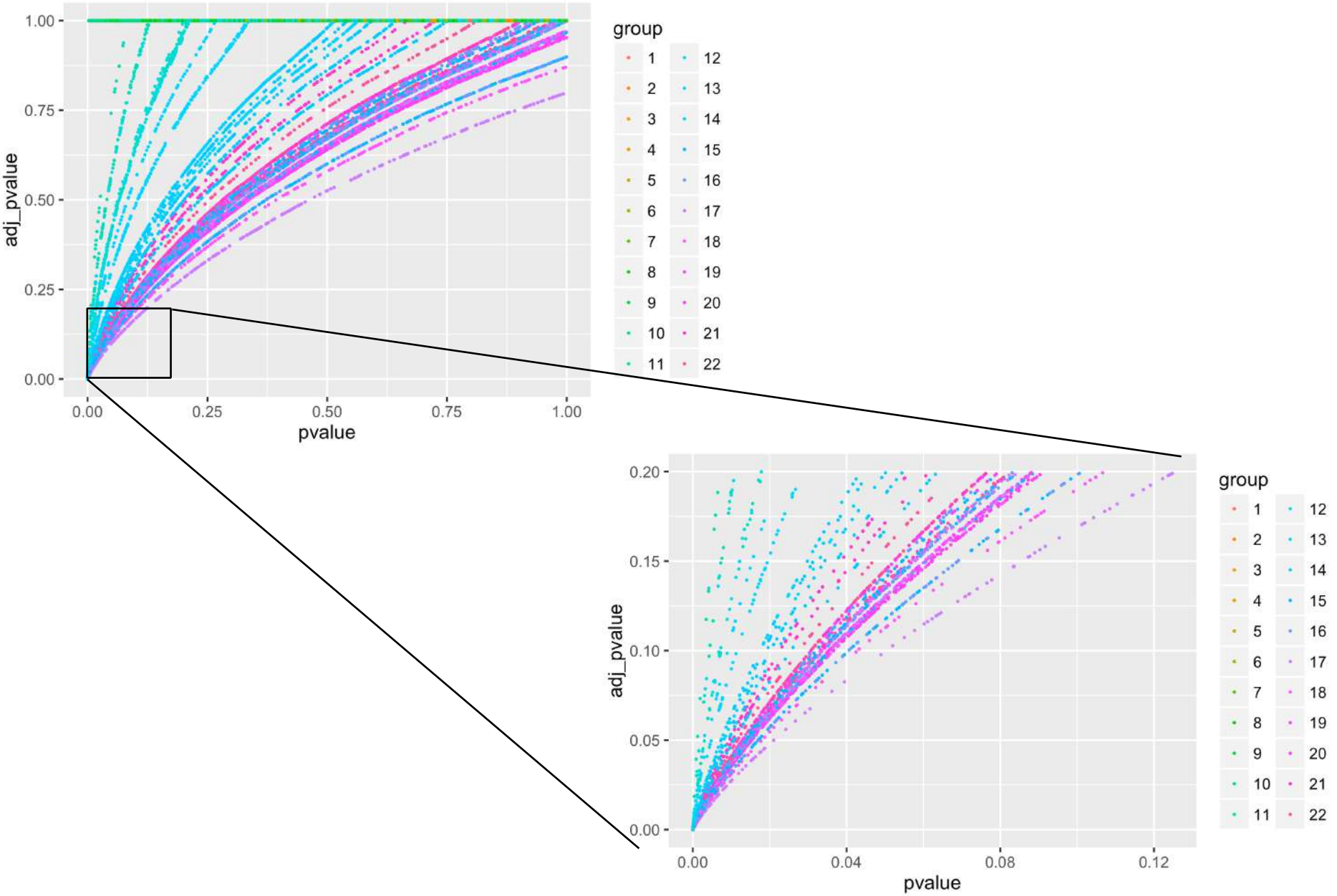
RNA-Seq example (DESeq2)







Ranking is not monotonous in raw p-values



Avoiding overfitting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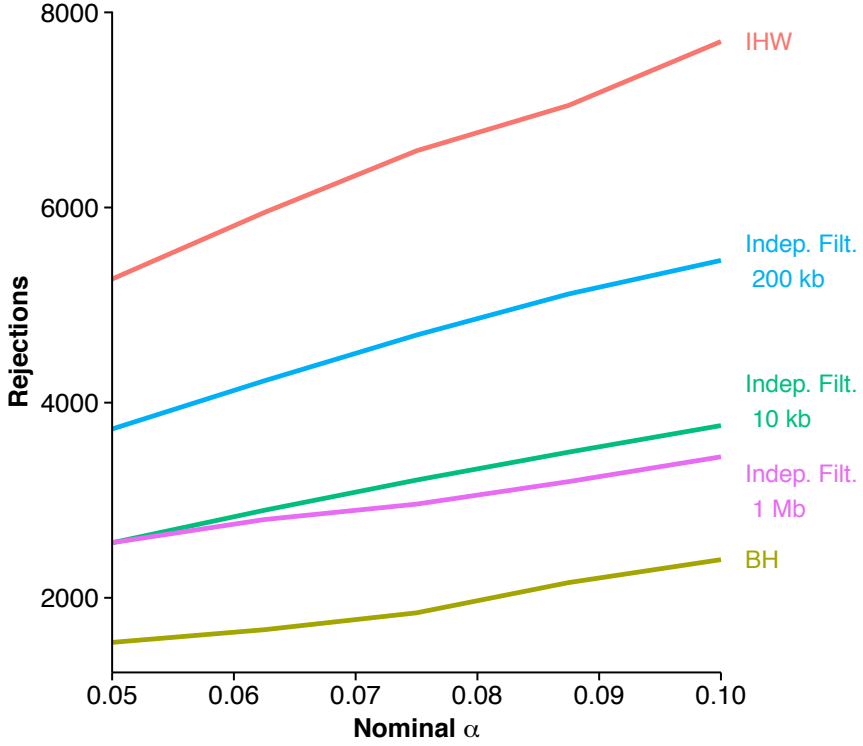
Hypothesis splitting: randomly split hypotheses into k folds. Learn weights for the hypotheses in a fold from the other k–1 folds

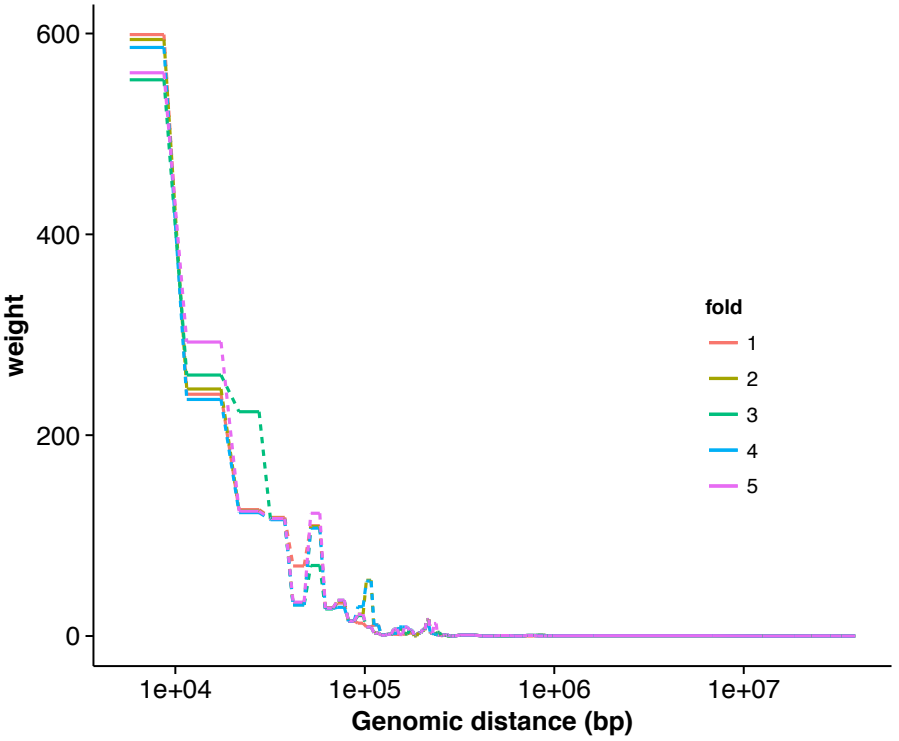
Regularisation:

- for ordered covariate: $\Sigma_g |w_g - w_{g-1}| \leq \lambda$
- for categorical covariate: $\Sigma_g |w_g - 1| \leq \lambda$

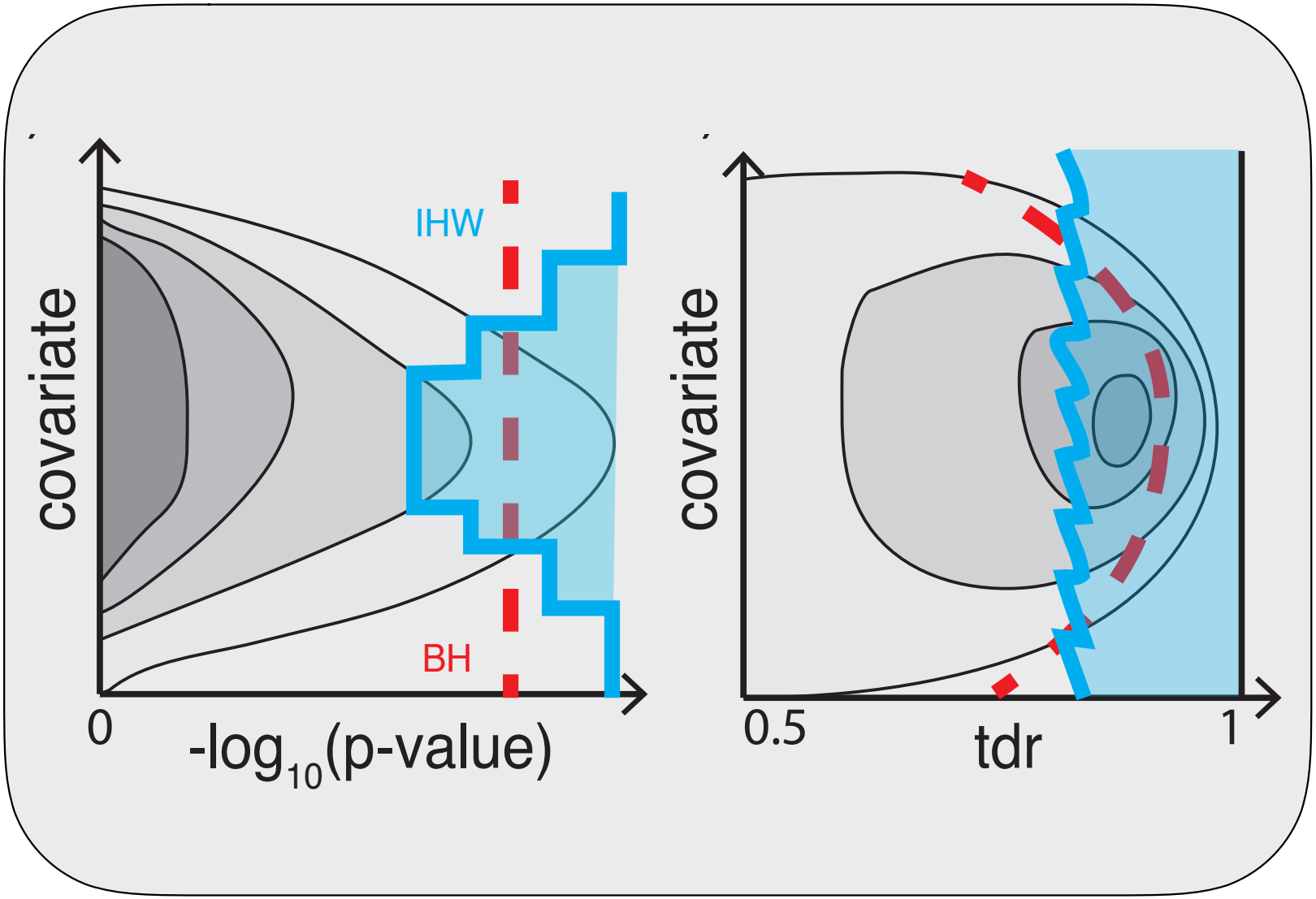
Convex relaxation: for weight optimisation (only), replace ECDFs of the p-values with Grenander estimators (least concave majorant of the ECDF)

Histone-QTL example (H3K27ac)





2D decision boundaries



Summary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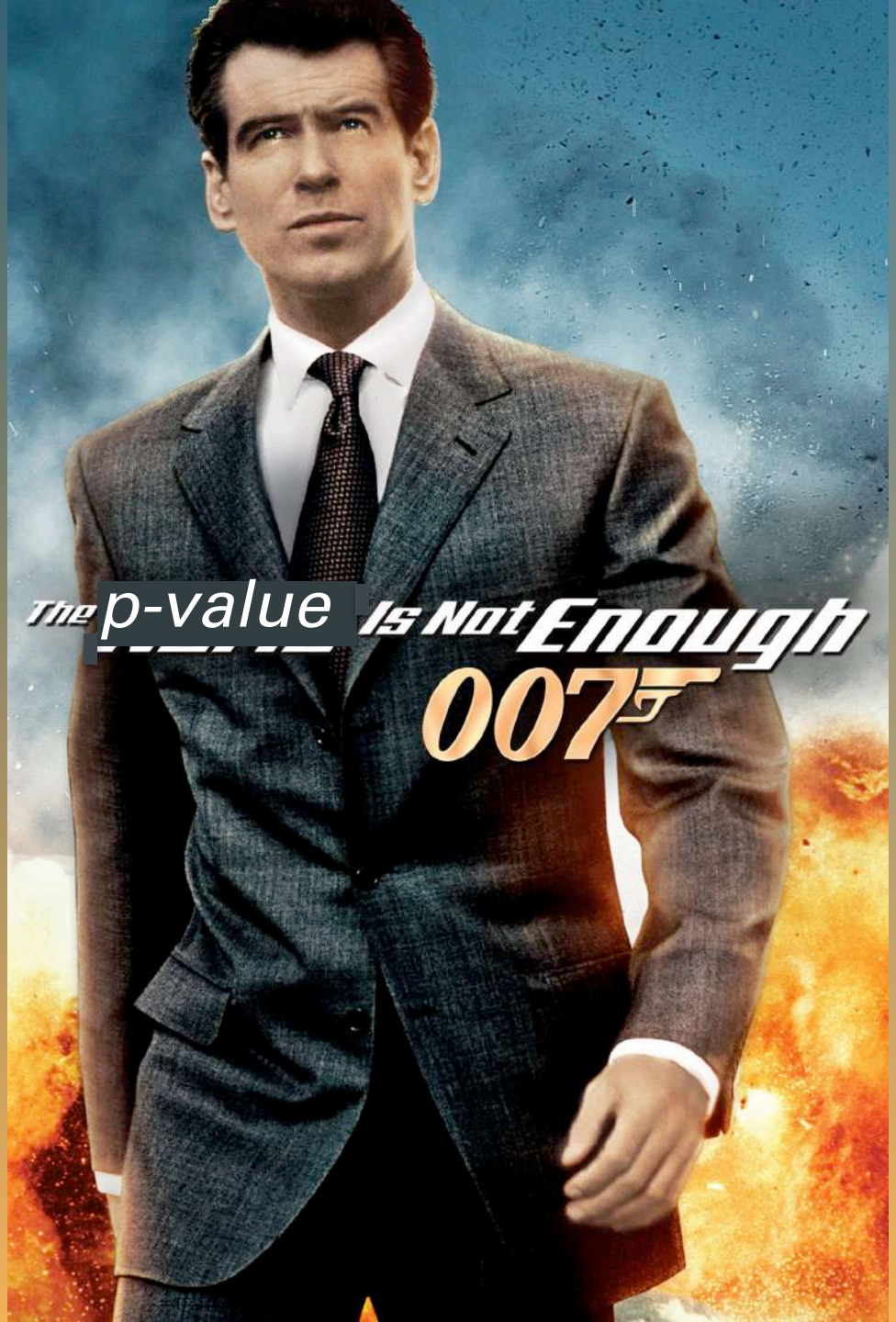
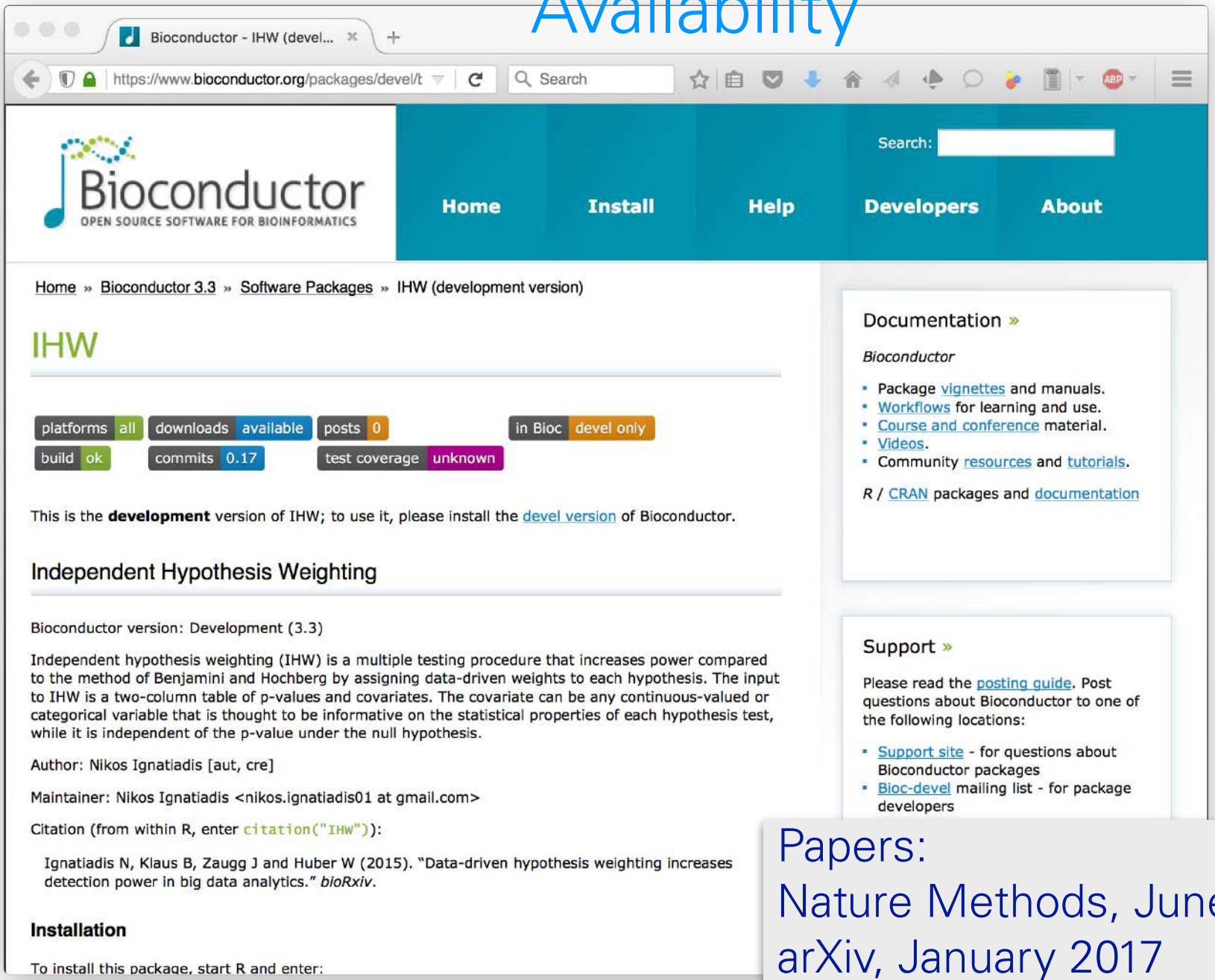
- Multiple testing is not a problem but an opportunity
- Heterogeneity across tests
- Informative covariates are often apparent to domain scientists
  - independent of test statistic under the null
  - informative on π_1, F_{alt}
- Data-driven weighting
- Scales well to millions of hypotheses
- Controlling 'overoptimism'


The p-value Is Not Enough
007

Availability



Papers:
Nature Methods, June 2016
arXiv, January 2017