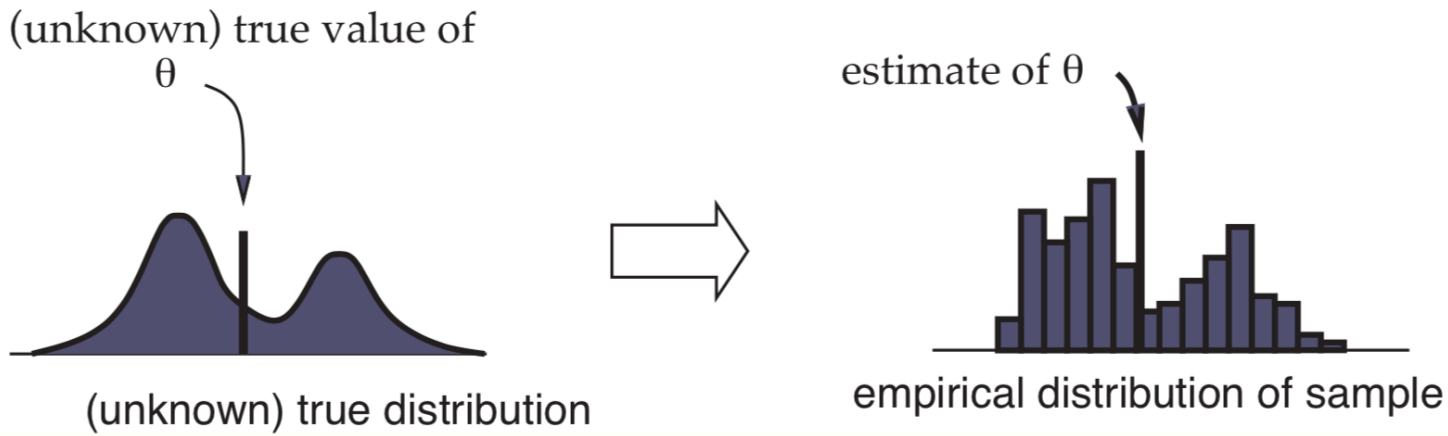
Testing phylogenetic hypotheses

Slides courtesy of Mark Holder and Joe Felsenstein

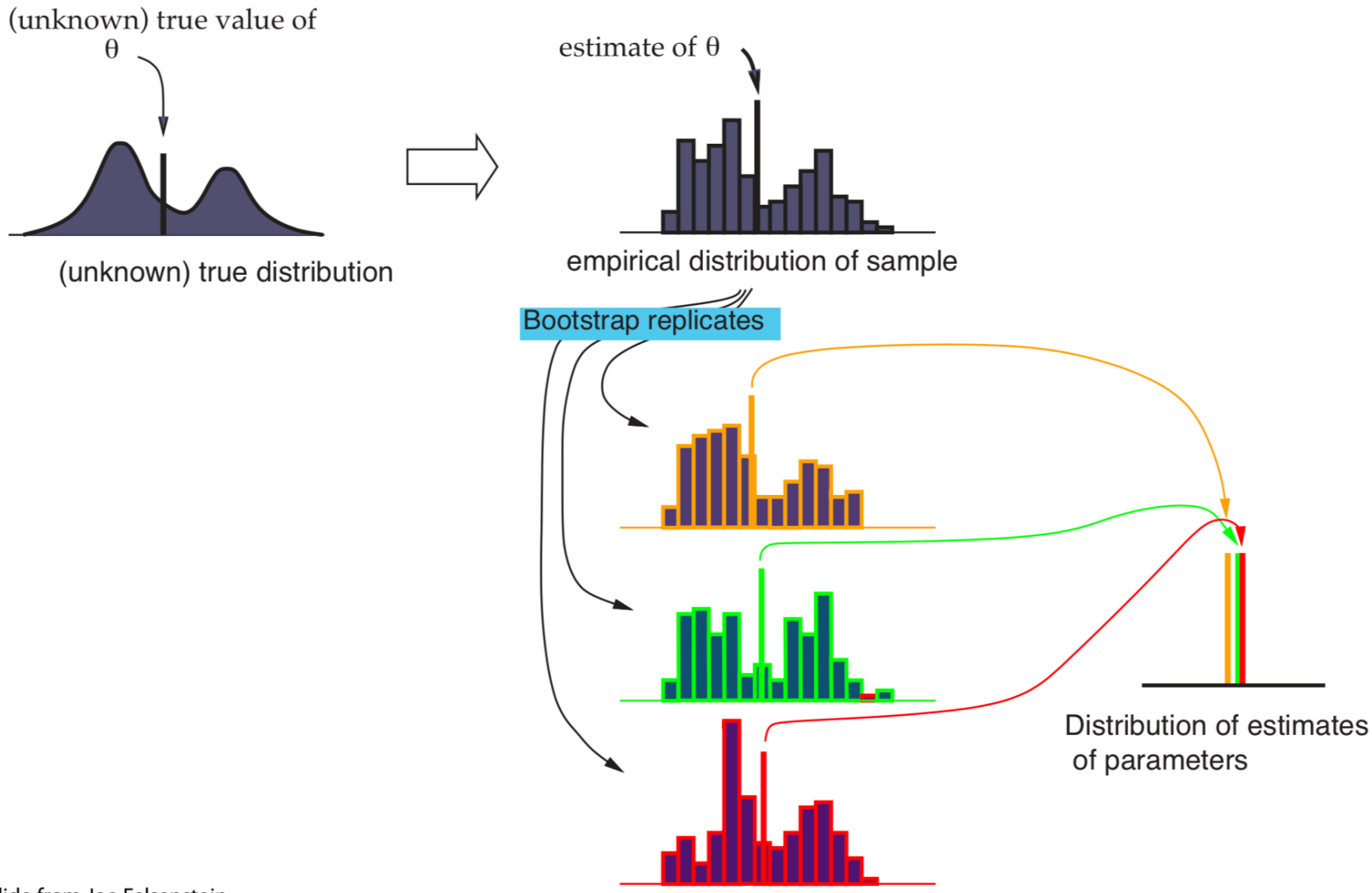
Download the nexus file for this weeks lab and execute it in MrBayes…

```
$ mb RootOfBilateria_Constrained-Bayes.nxs
```

The bootstrap

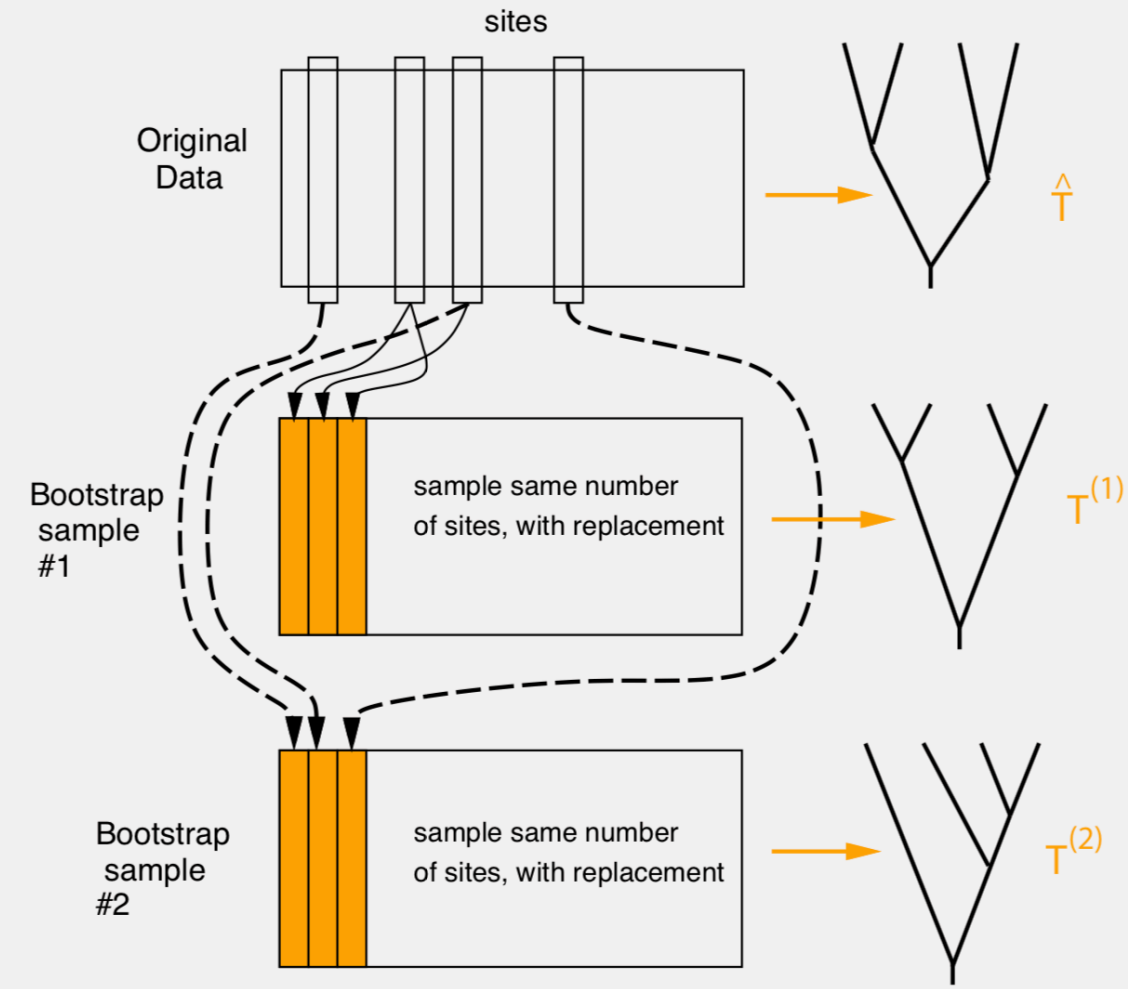


The bootstrap



Slide from Joe Felsenstein

The bootstrap for phylogenies

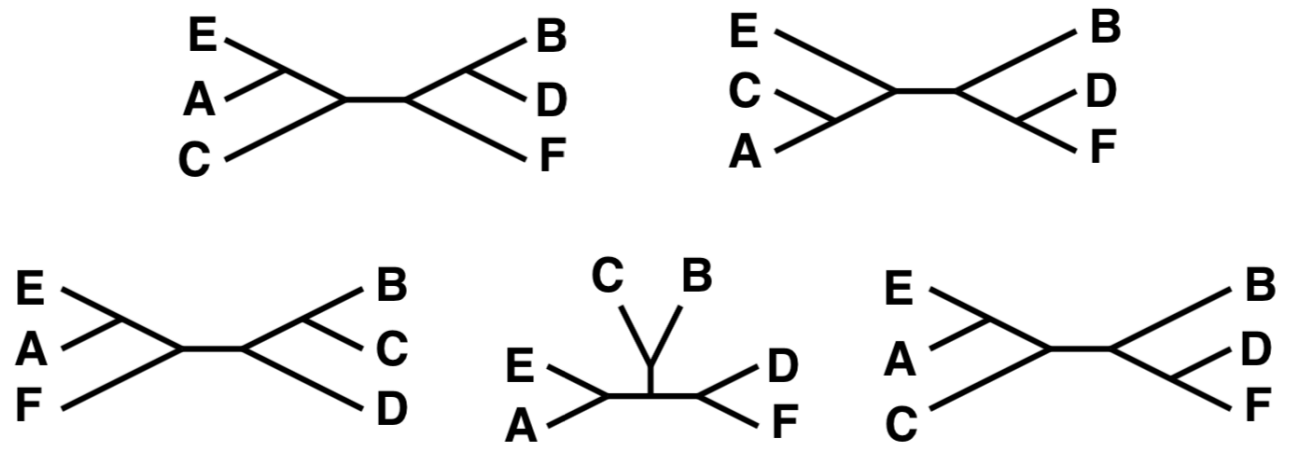


Slide from Joe Felsenstein

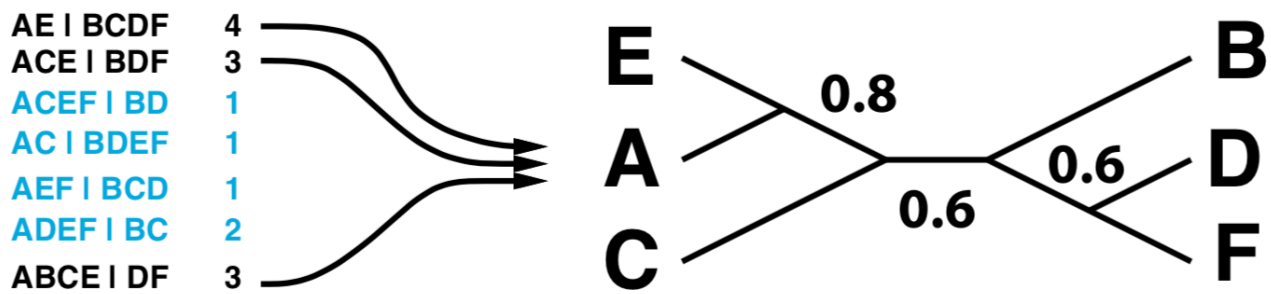
(and so on)

The majority-rule consensus tree

Trees:

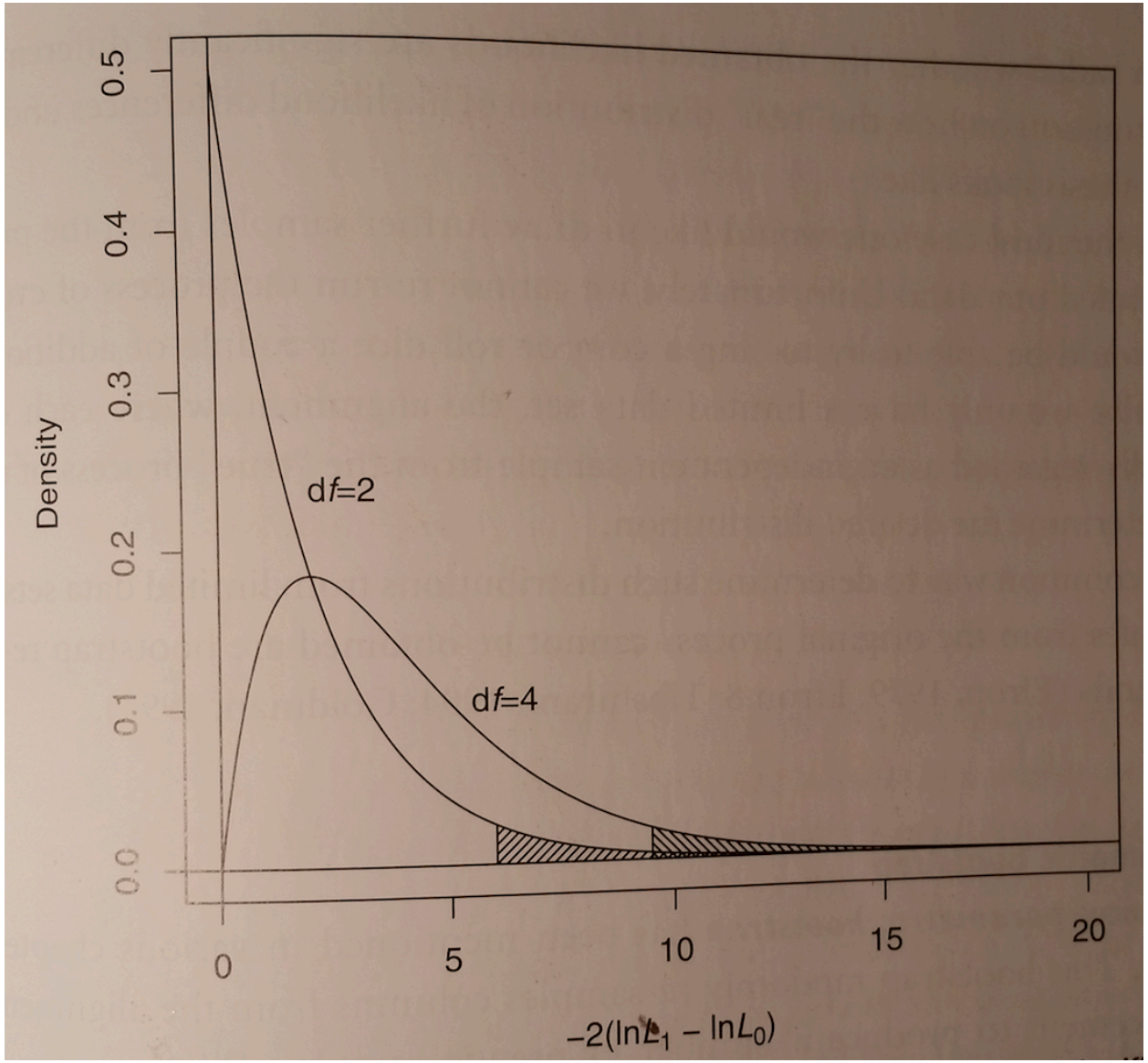


How many times each partition of species is found:



Slide from Joe Felsenstein

Comparing phylogenetic trees using log likelihood ratios



We could use log likelihood ratios to test for fit of substitution model since the models are nested and the test statistic is approx. chi2 distributed.

However, trees are not nested in each other!

The simplest phylogenetic test would compare two trees

Null: If we had no sampling error (infinite data) T_1 and T_2 would explain the data equally well.

Test Statistic:

$$\delta(T_1, T_2 \mid X) = 2\left[\ln L(T_1 \mid X) - \ln L(T_2 \mid X)\right]$$

Expectation under null:

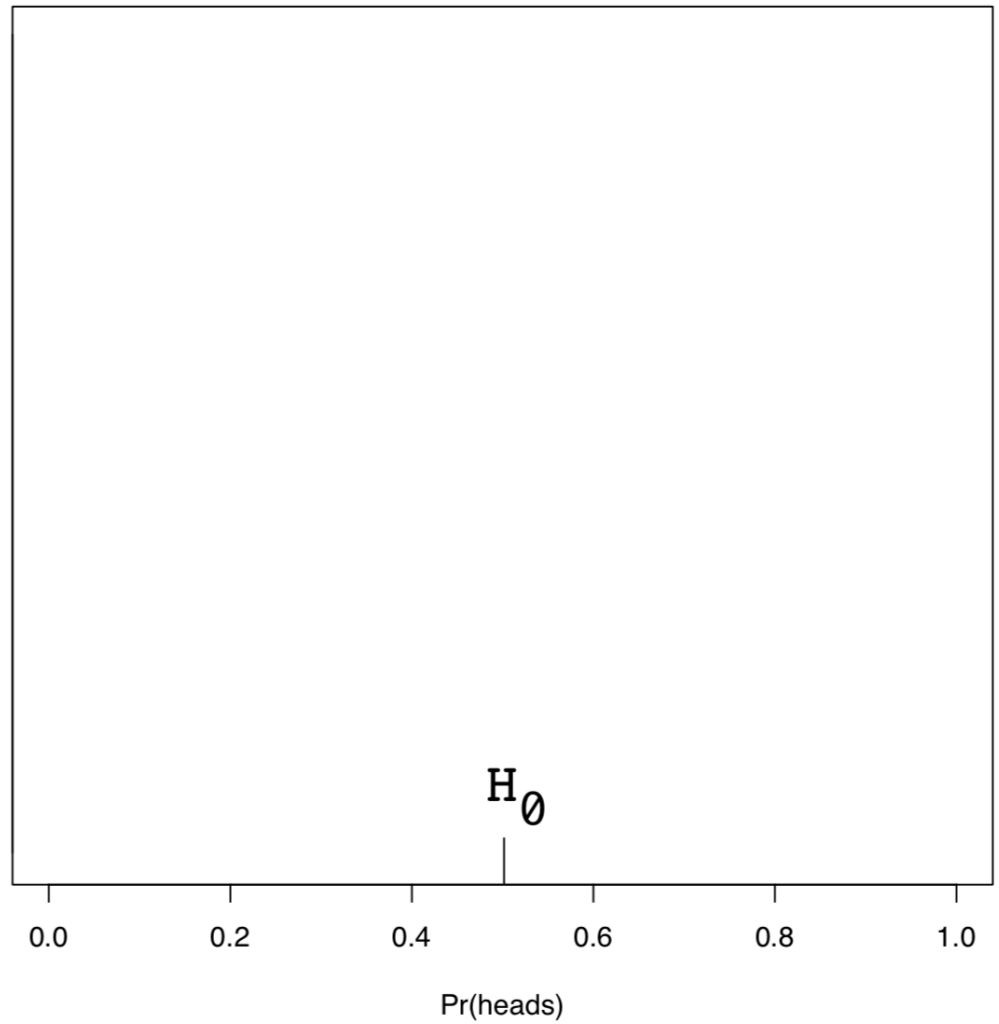
$$\mathbb{E}_{H_0}\left[\delta(T_1, T_2 \mid X)\right] = 0$$

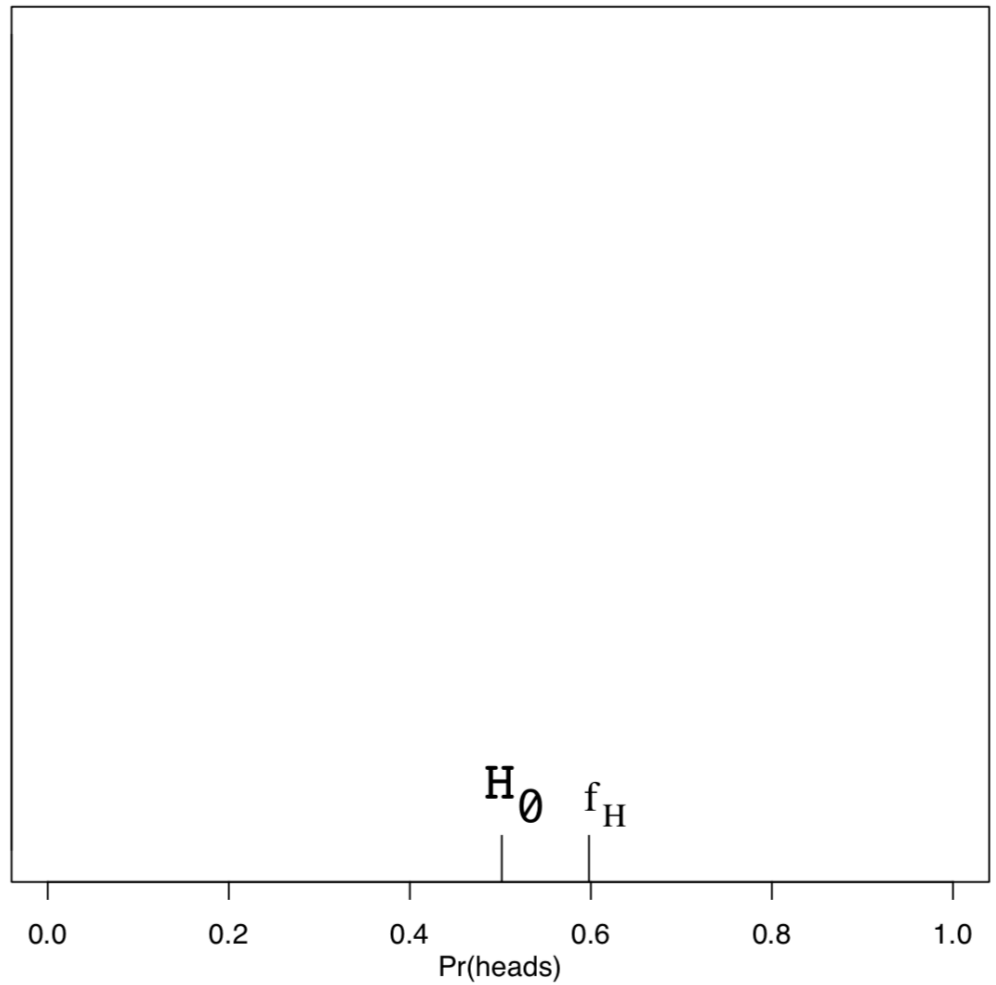
Frequentist hypothesis testing: coin flipping example

$N = 100$ and $h = 60$
Can we reject the fair coin hypothesis? $H_0 : \Pr(\text{heads}) = 0.5$

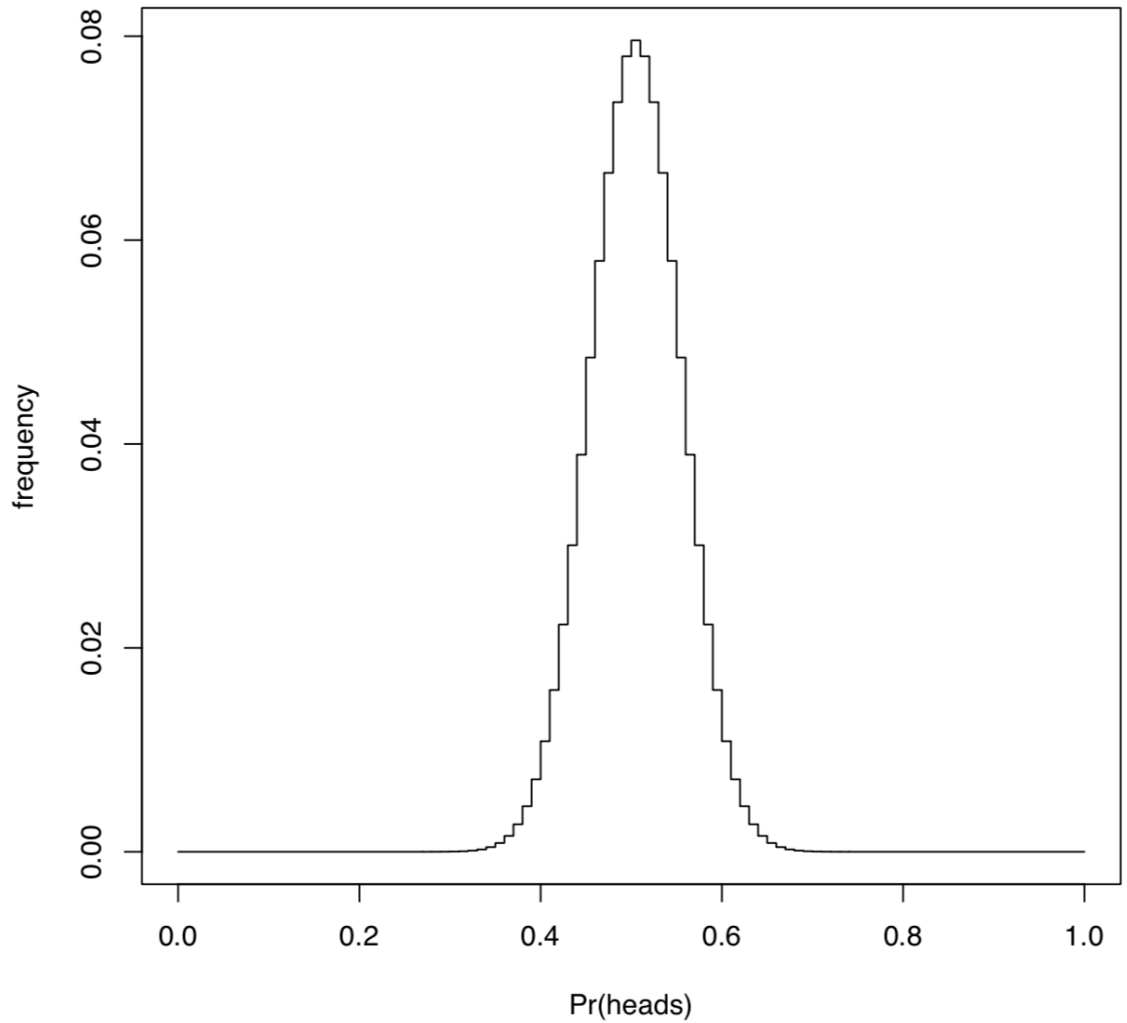
The "recipe" is:

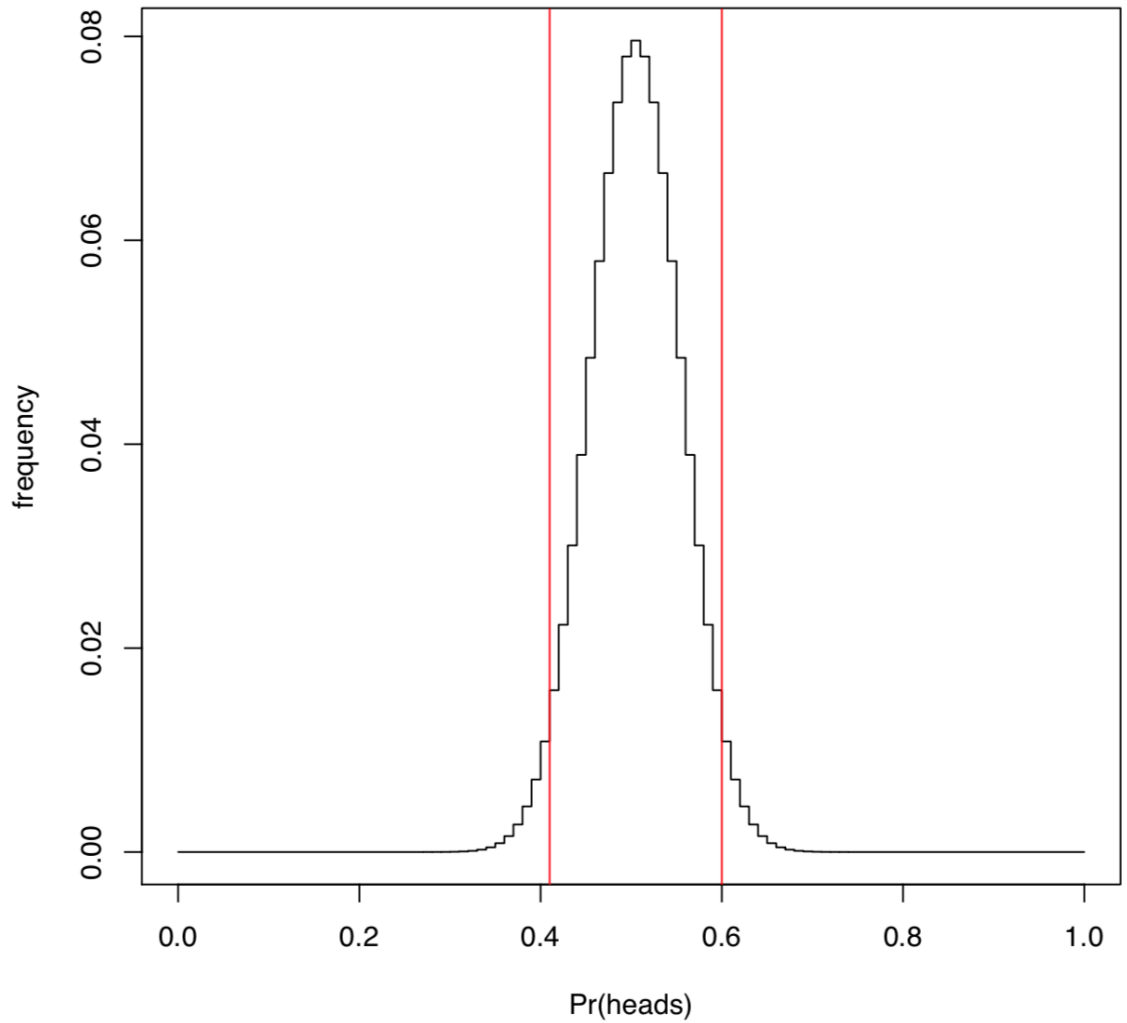
1. Formulate null (H_0) and alternative (H_A) hypotheses.
2. Choose an acceptable Type-I error rate (significance level)
3. Choose a test statistic: $f_H =$ fraction of heads in sample. $f_H = 0.6$
4. Characterize the null distribution of the test statistic
5. Calculate the P-value: The probability of a test statistic value more extreme than f_H arising *even if* H_0 *is true*.
6. Reject H_0 if P-value is $\leq$ your Type I error rate.


H_0
0.0
0.2
0.4
0.6
0.8
1.0
Pr(heads)


H0
fH
0.0
0.2
0.4
0.6
0.8
1.0
Pr(heads)

Null distribution





P-value ≈ 0.058

Making similar plots for tree inference is hard.

- Our parameter space is trees and branch lengths.
- Our data is a matrix of characters.
- It is hard to put these objects on the same plot.

The simplest phylogenetic test would compare two trees

Null: If we had no sampling error (infinite data) T_1 and T_2 would explain the data equally well.

Test Statistic:

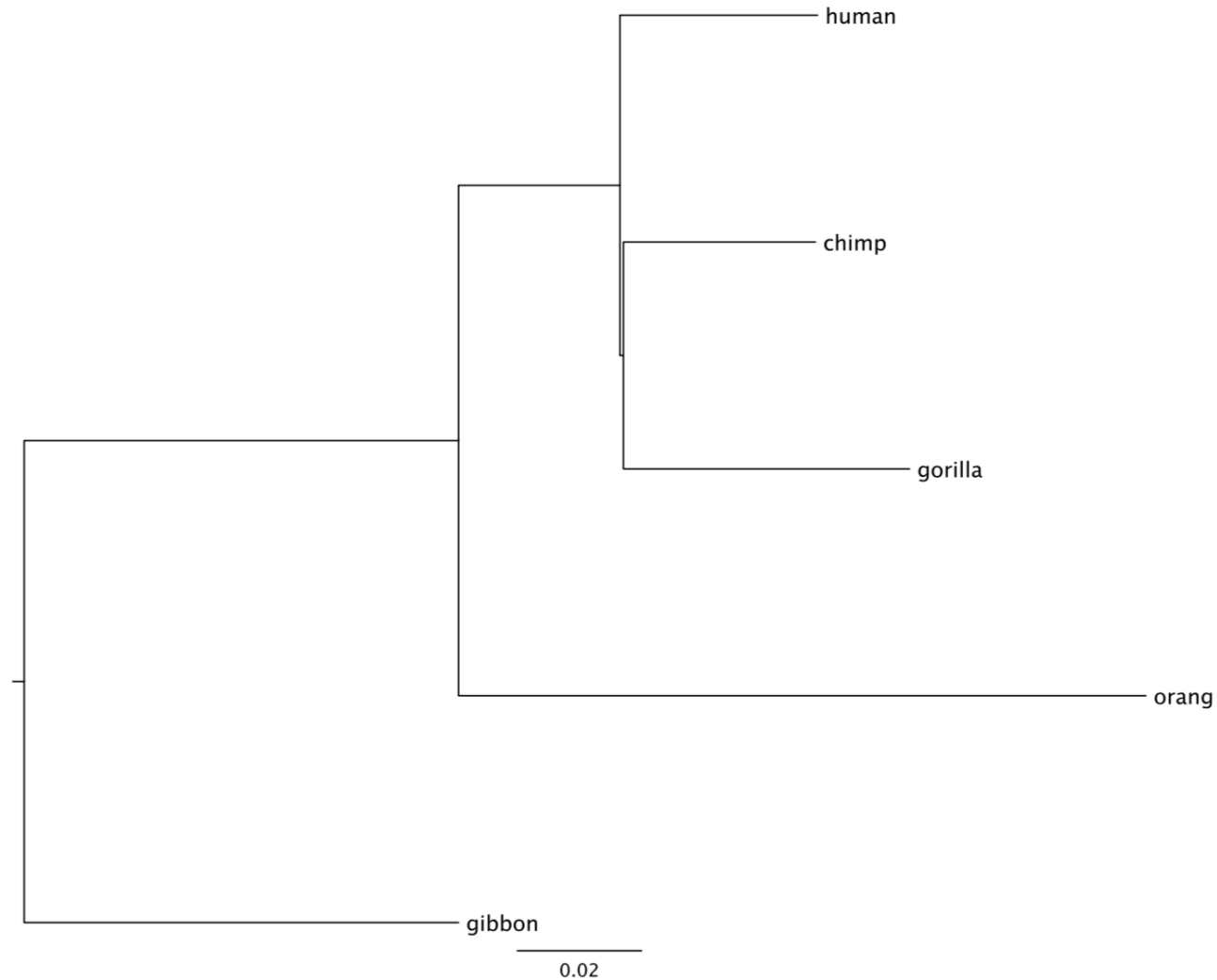
$$\delta(T_1, T_2 \mid X) = 2\left[\ln L(T_1 \mid X) - \ln L(T_2 \mid X)\right]$$

Expectation under null:

$$\mathbb{E}_{H_0}\left[\delta(T_1, T_2 \mid X)\right] = 0$$

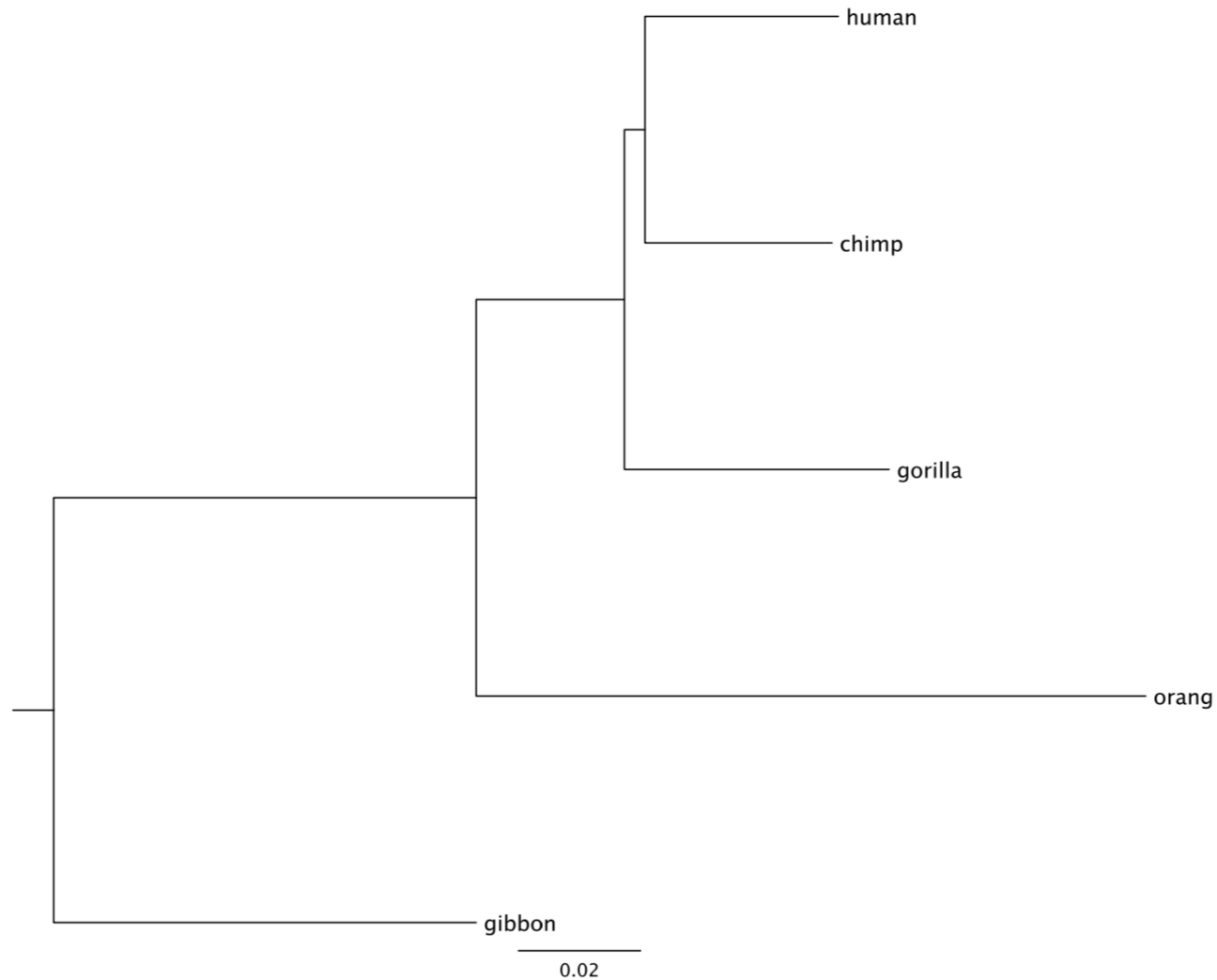
Using 3000 sites of mtDNA sequence for 5 primates

T_1 is ((chimp, gorilla), human)



Using 3000 sites of mtDNA sequence for 5 primates

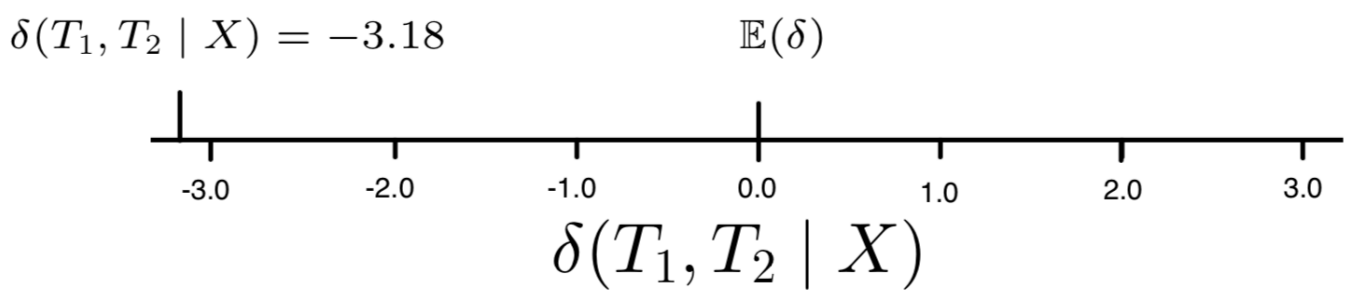
T_2 is ((chimp, human), gorilla)



Using 3000 sites of mtDNA sequence for 5 primates

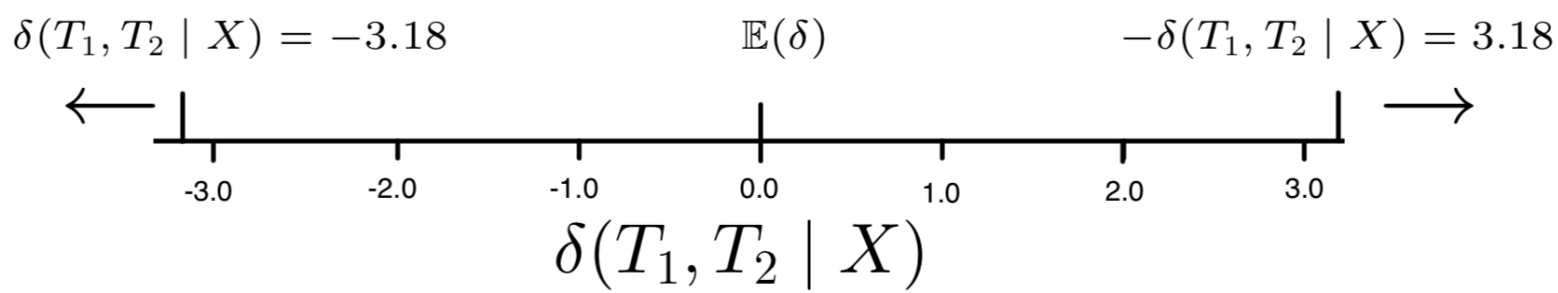
T_1 is ((chimp, gorilla), human) $\quad \ln L(T_1 \mid X) = -7363.296$

T_2 is ((chimp, human), gorilla) $\quad \ln L(T_2 \mid X) = -7361.707$



To get the P-value, we need to know the probability:

$$\Pr\left(\left|\delta(T_1, T_2 \mid X)\right| \geq 3.18 \middle| H_0 \text{ is true}\right)$$



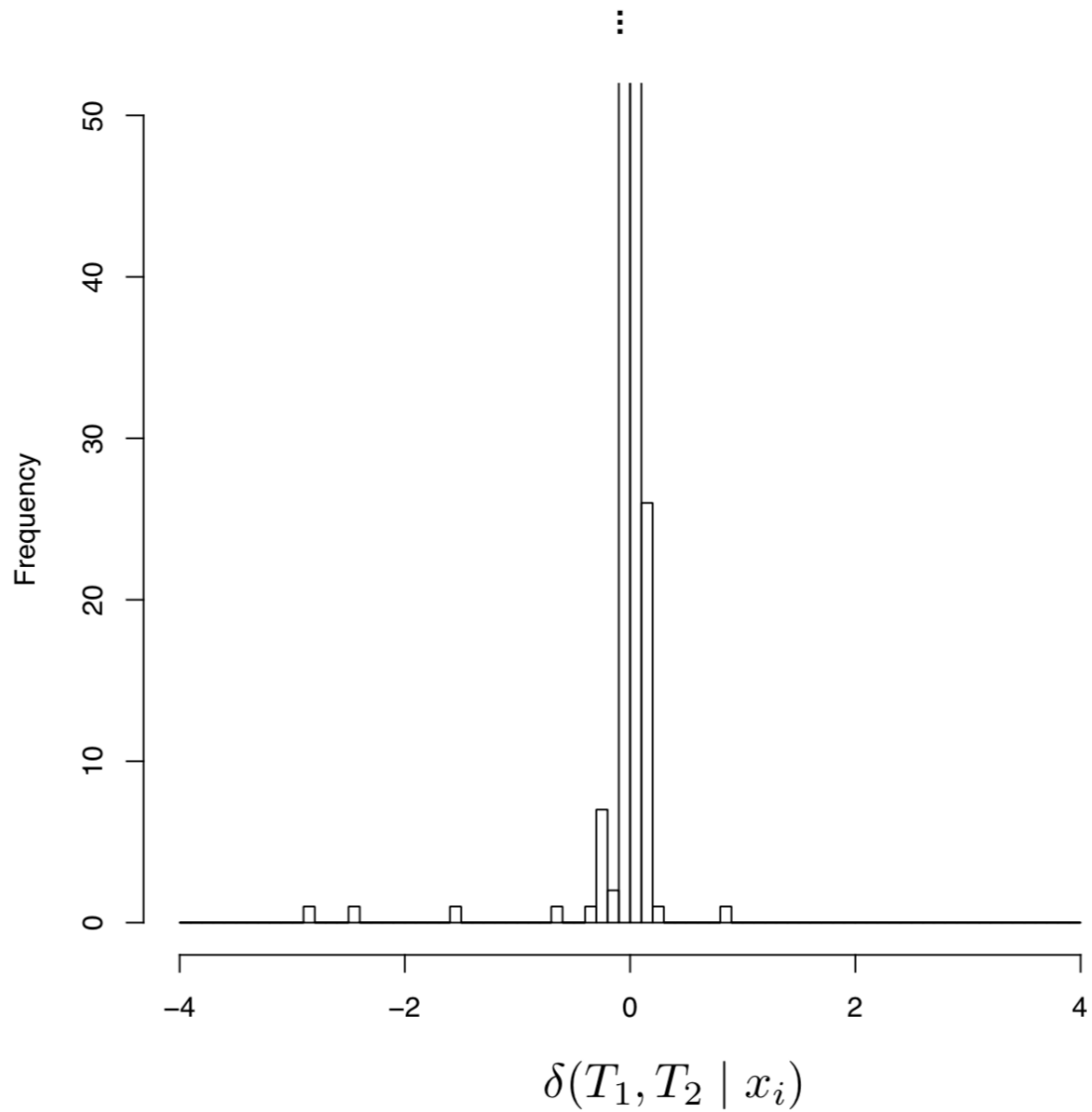
KH Test

1. Examine the difference in $\ln L$ for each site: $\delta(T_1, T_2 \mid X_i)$ for site i.
2. Note that the total difference is simply a sum:

$$\delta(T_1, T_2 \mid X) = \sum_{i=1}^{M} \delta(T_1, T_2 \mid X_i)$$

3. The variance of $\delta(T_1, T_2 \mid X)$ will be a function of the variance in "site" $\delta(T_1, T_2 \mid X_i)$ values.

$\delta(T_1, T_2 \mid X_i)$ for each site, i.

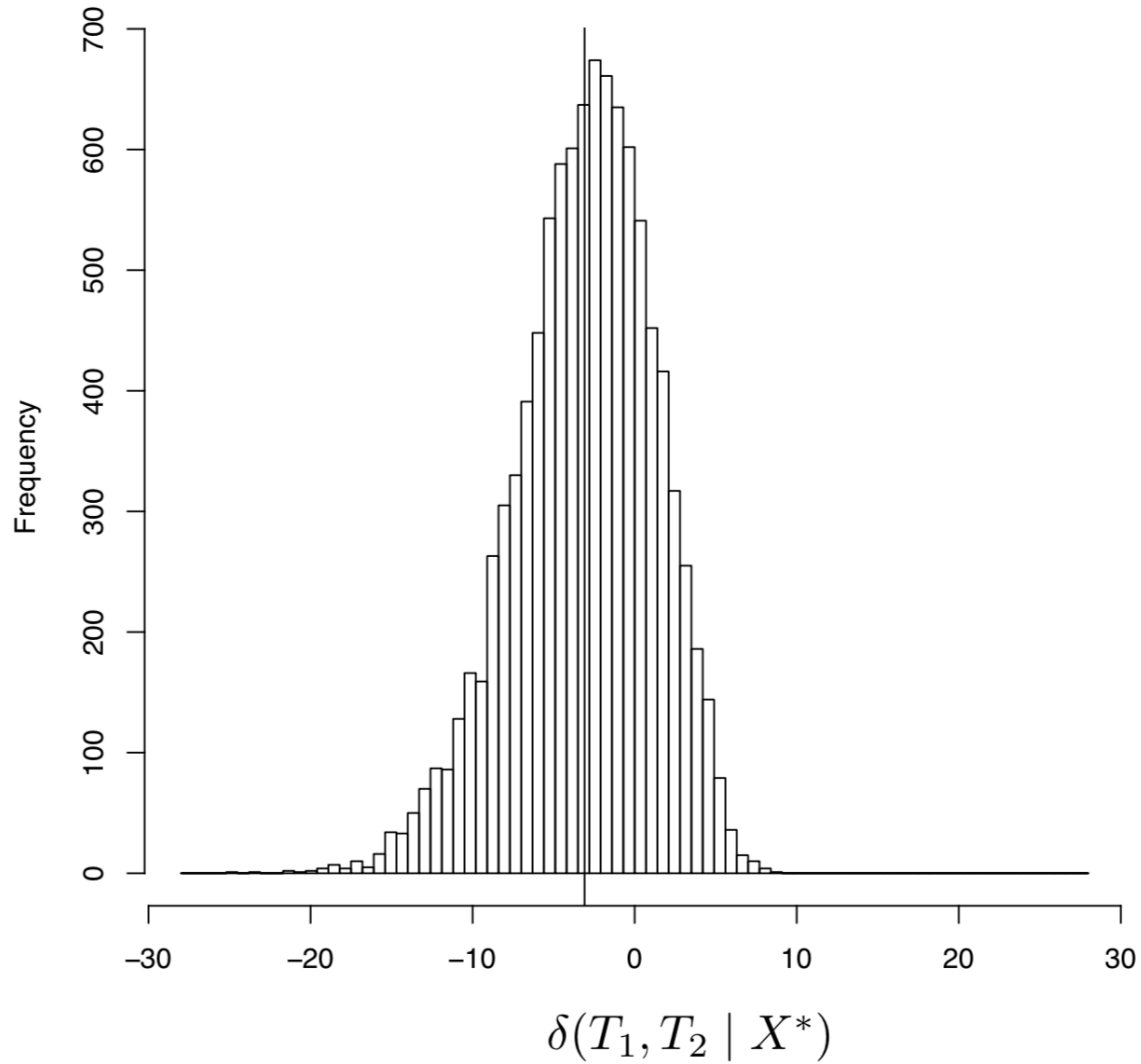


KH Test - the variance of $\delta(T_1, T_2 \mid X)$

To approximate variance of $\delta(T_1, T_2 \mid X)$ under the null, we could:

1. use assumptions of Normality (by appealing to the Central Limit Theorem). Or
2. use bootstrapping to generate a cloud of pseudo-replicate $\delta(T_1, T_2 \mid X^*)$ values, and look at their variance.

δ for many (RELL) bootstrapped replicates of the data



RELL bootstrap

Often, the MLE of numerical parameters (including branch lengths) do not change much when we bootstrap.

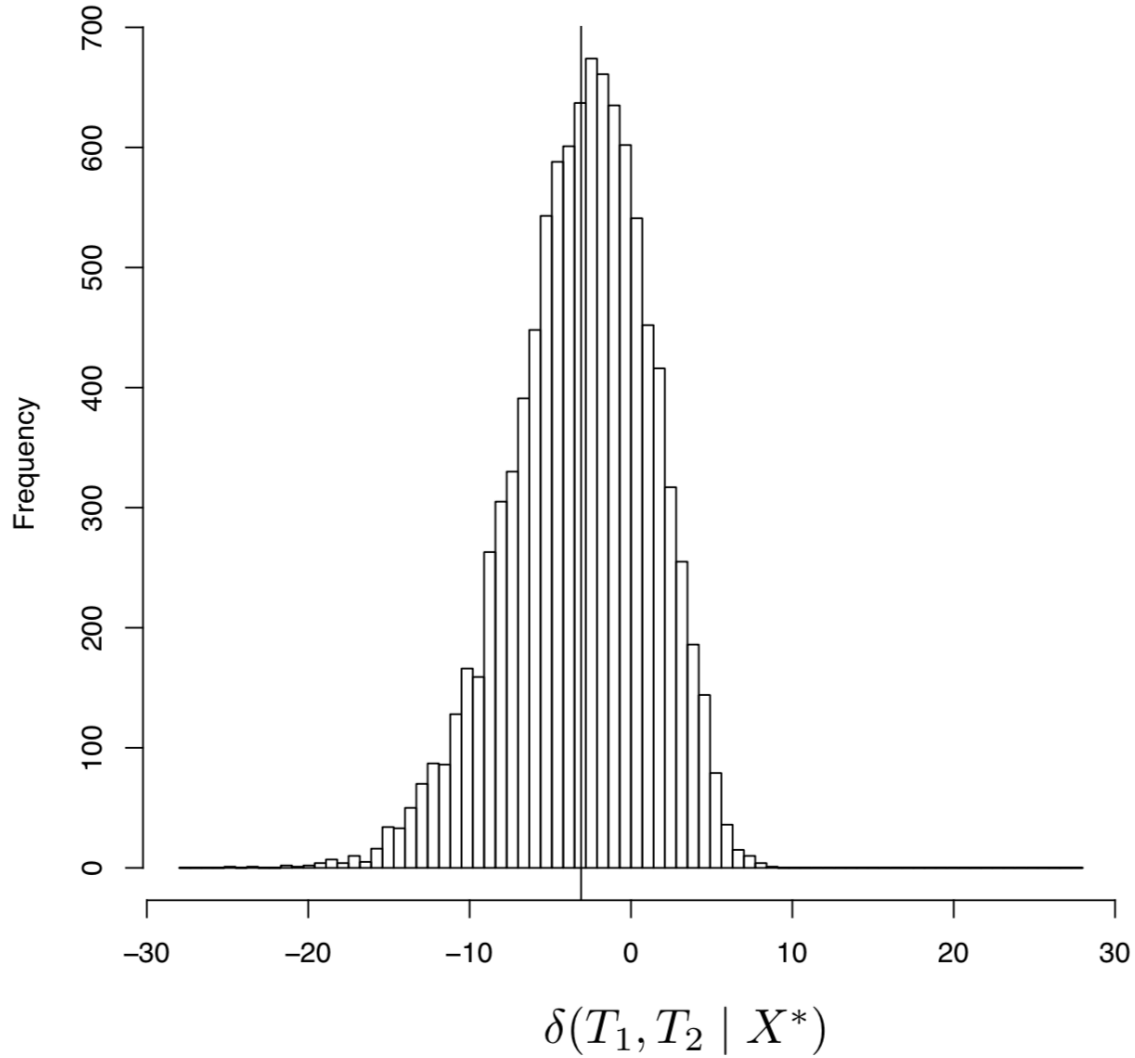
So, we can simply resample the site $\ln L$ values and sum them (rather than reoptimizing parameters).

This is called the RELL bootstrap (Kishino et al., 1990, and Felsenstein). It is not a "safe" replacement for normal bootstrapping (especially on large trees; Stamatakis et al., 2008) when you want to estimate clade support.

But it should be good enough for helping us learn about the standard error of the $\ln L$.

And it is really fast.

The (RELL) bootstrapped sample of statistics.
Is this the null distribution for our δ test statistic?



KH Test - 'centering'

H_0 gives us the expected value:

$$\mathbb{E}_{H_0}\left[\delta(T_1, T_2 \mid X)\right] = 0$$

Bootstrapping gives us a reasonable guess of the variance under H_0

By subtracting the mean of the bootstrapped $\delta(T_1, T_2 \mid X^*)$ values, we can create a null distribution.

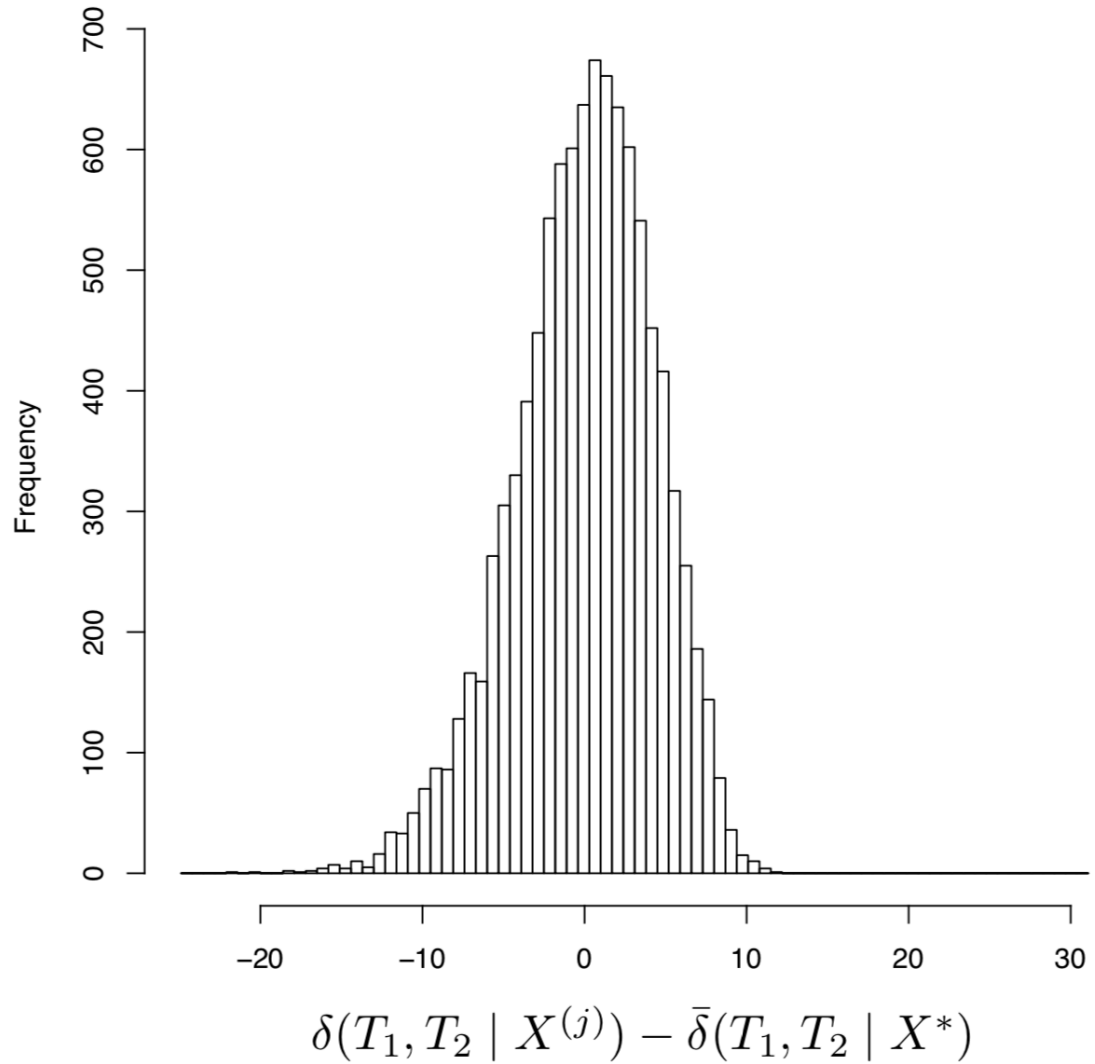
For each of the j bootstrap replicates, we treat

$$\delta(T_1, T_2 \mid X^{*j}) - \bar{\delta}(T_1, T_2 \mid X^*)$$

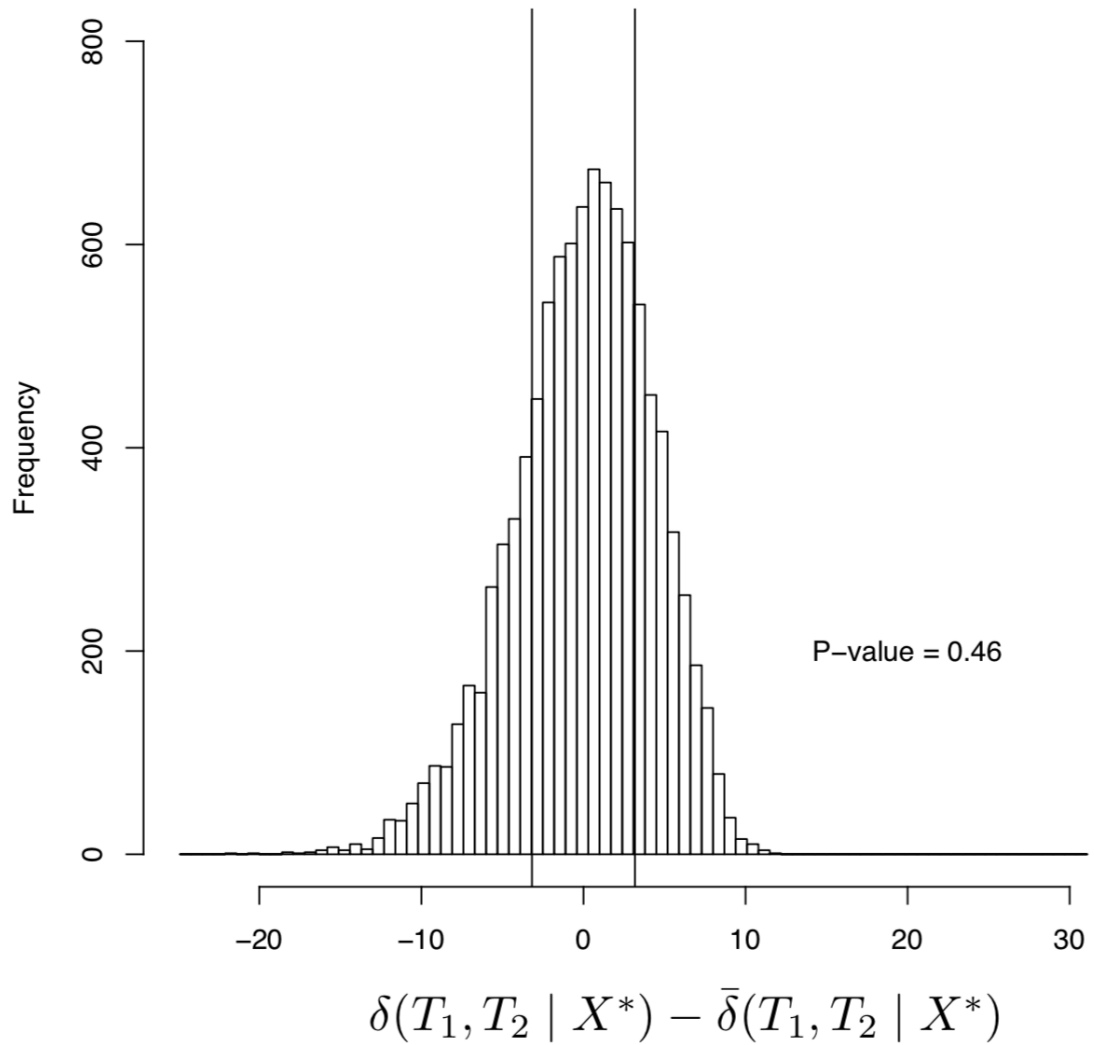
as draws from the null distribution.

$\delta(T_1, T_2 \mid X^{(j)}) - \bar{\delta}(T_1, T_2 \mid X^*)$

for many (RELL) bootstrapped replicates of the data

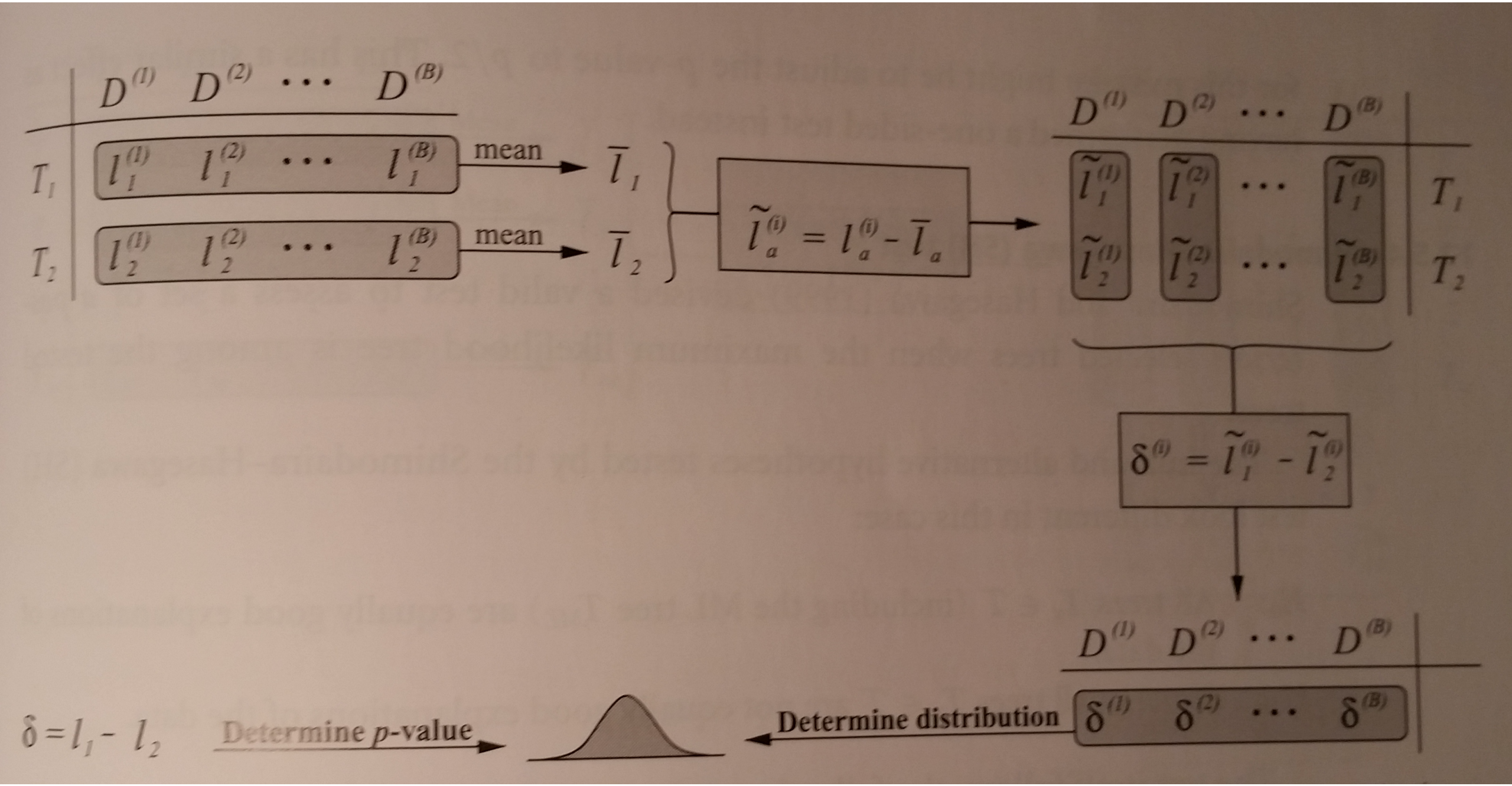


Approximate null distribution with tails (absolute value ≥ 3.18) shown



Summary - Part 1

- $\delta(T_1, T_2 \mid X) = 2\left[\ln L(T_1 \mid X) - \ln L(T_2 \mid X)\right]$ is a powerful statistic for discrimination between trees.
- We can assess confidence by considering the variance in signal between different characters.
- Bootstrapping helps us assess the variance in $\ln L$ that we would expect to result from sampling error.


$D^{(1)}$ $D^{(2)}$ $\cdots$ $D^{(B)}$
T_1
T_2
$l_1^{(1)}$ $l_1^{(2)}$ $\cdots$ $l_1^{(B)}$
$l_2^{(1)}$ $l_2^{(2)}$ $\cdots$ $l_2^{(B)}$
mean
mean
$\bar{l}_1$
$\bar{l}_2$
$\tilde{l}_a^{(i)} = l_a^{(i)} - \bar{l}_a$
$\tilde{l}_1^{(1)}$ $\tilde{l}_1^{(2)}$ $\cdots$ $\tilde{l}_1^{(B)}$
$\tilde{l}_2^{(1)}$ $\tilde{l}_2^{(2)}$ $\cdots$ $\tilde{l}_2^{(B)}$
$\delta^{(i)} = \tilde{l}_1^{(i)} - \tilde{l}_2^{(i)}$
$\delta^{(1)}$ $\delta^{(2)}$ $\cdots$ $\delta^{(B)}$
Determine distribution
Determine p-value
$\delta = l_1 - l_2$

Scenario

1. A (presumably evil) competing lab scoops you by publishing a tree, T_1, for your favorite group of organisms.

2. You have just collected a new dataset for the group, and your ML estimate of the best tree, T_2, differ's from T_1.

3. A KH Test shows that your data **significantly** prefer T_2 over T_1.

4. You write a (presumably scathing) response article.

Should a *Systematic Biology* publish your response?

What if start out with only one hypothesized tree, and we want to compare it to the ML tree?

The KH Test is **NOT** appropriate in this context (see Goldman et al., 2000, for discussion of this point)

Multiple Comparisons: lots of trees increases the variance of $\delta(\hat{T}, T_1 \mid X)$

Selection bias: Picking the ML tree to serve as one of the hypotheses invalidates the centering procedure of the KH test.

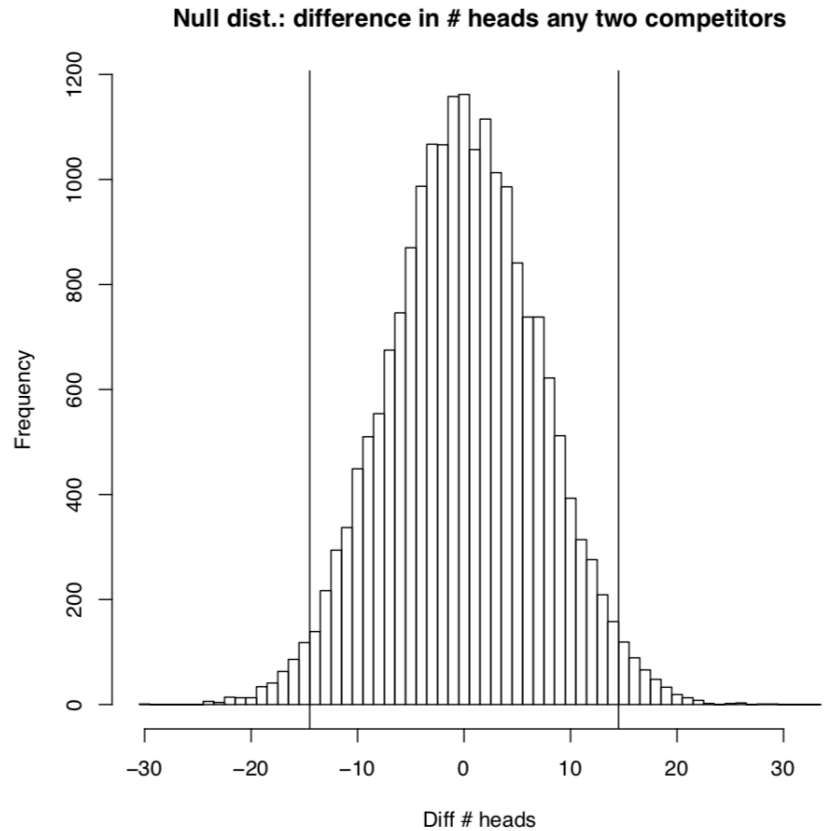
Using the ML tree in your test introduces selection bias

Even when the H_0 is true, we do not expect $2\left[\ln L(\hat{T}) - \ln L(T_1)\right] = 0$

Imagine a competition in which a large number of equally skilled people compete, and you compare the score of one competitor against the highest scorer.

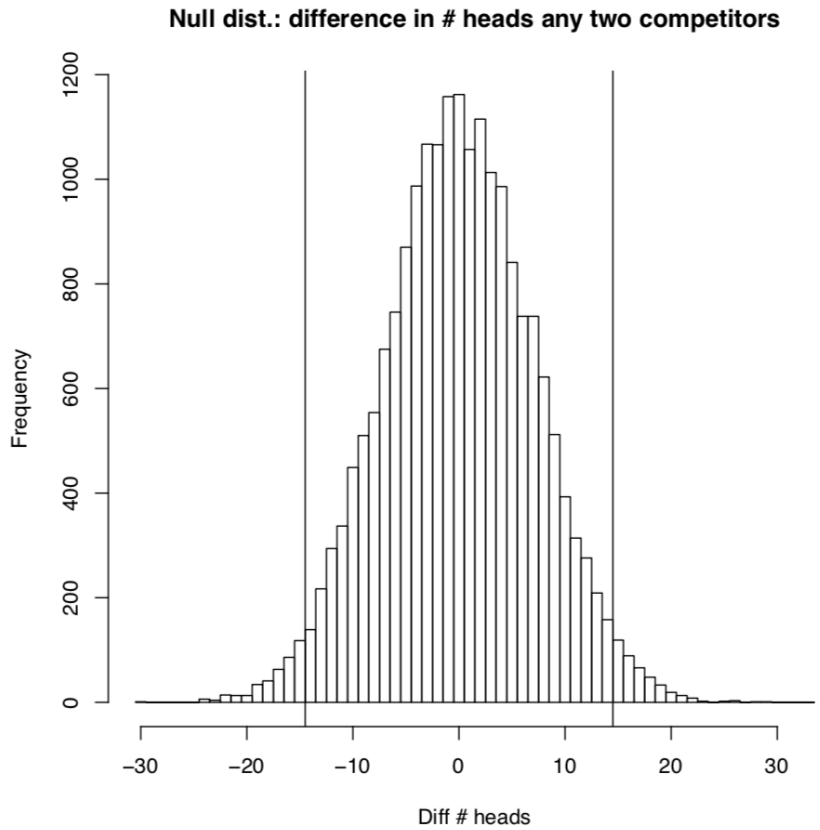
Experiment: 70 people each flip a fair coin 100 times and count # heads.

$$h_1 - h_2$$

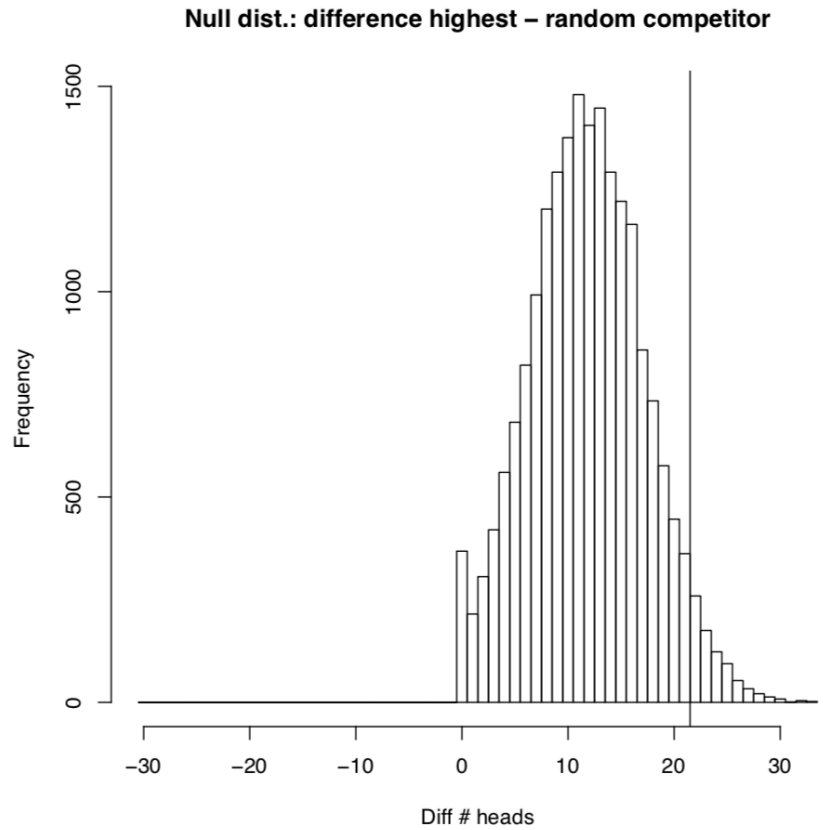


Experiment: 70 people each flip a fair coin 100 times and count # heads.

$h_1 - h_2$



$\max(h) - h_1$



Shimodaira and Hasegawa proposed the SH test which deals the "selection bias" introduced by using the ML tree in your test

You have to specify of a **set of candidate trees** - inclusion in this set **must not** depend on the dataset to be analyzed.

The null hypothesis is that all members of the candidate set have the same expected score.

The test makes worst-case assumptions, so the SH test **is conservative**.

SH test candidate set selection

- Should be all trees that you would have seriously entertained before seeing the data (considering a subset of trees for computational convenience can invalidate the test).
- Using all trees is safe.
- If a tree has low $\ln L$ and low variance of site-log-likelihoods then it can probably be safely removed without affecting the P-values of other trees[1]

[1] Because such a tree would be unlikely to ever be the tree that is the determines the maximum diplacement from the centered value, $m^{(j)}$.

SH Test details

- For each tree T_i in the candidate set calculate $\delta(\hat{T}, T_i \mid X)$
- Bootstrap to generate $\ln L(T_i \mid X^{(j)})$ for each bootstrap replicate j.
- For each tree T_i, use the mean, $\bar{\ln} L(T_i \mid X^*)$, over all bootstrap replicates to center the bootstrapped collection of log-likelihoods:

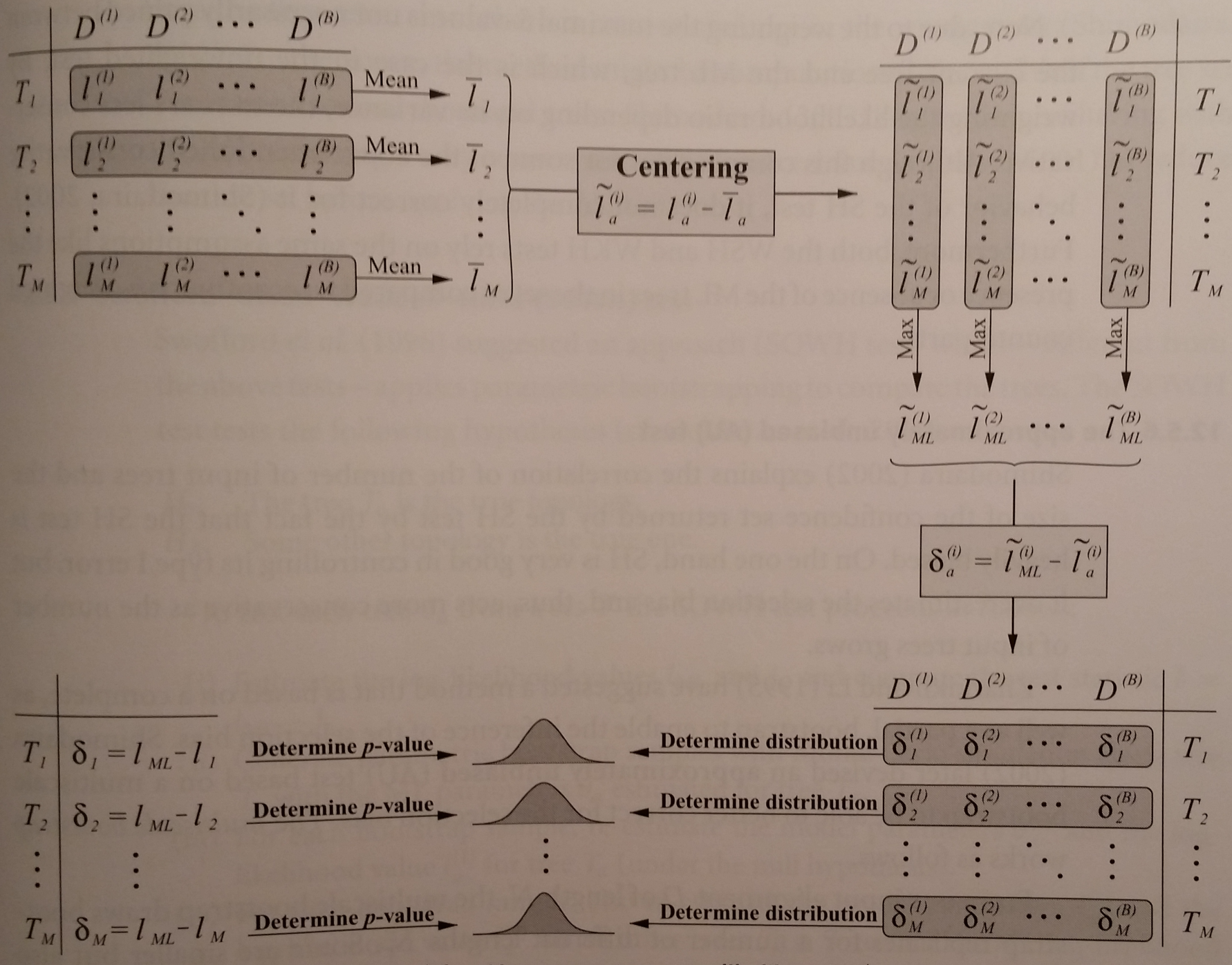
$$c_i^{(j)} = \ln L(T_i \mid X^{(j)}) - \bar{\ln} L(T_i \mid X^*)$$

- For each bootstrap replicate, j, pick the highest value from the centered distributions (this mimics the selection bias):

$$m^{(j)} = \max \left[c_i^{(j)} \right] \text{ over all } i$$

- Then for each tree and replicate, you get a sample from the null $\delta_i^{(j)} = m^{(j)} - c_i^{(j)}$
- P-value for tree T_i is approximated by the proportions of bootstrap reps for which:

$$\delta_i^{(j)} \leq \delta(\hat{T}, T_i \mid X)$$


Mean
Mean
Mean
Centering
Max
Max
Max
Determine p-value
Determine p-value
Determine p-value
Determine distribution
Determine distribution
Determine distribution

KH Test

H0: The two trees are equally supported

HA: The two trees are not equally supported

SH Test

H0: All trees (including the ML tree) are equally good explanations of the data

HA: Some or all trees are not equally good explanations of the data

Let’s run tree-puzzle to perform the KH and SH tests…

```
$ tree-puzzle PHYLIP.Alignment PHYLIP.tre
```

To get the PHYLIP.tre file open all MrBayes consensus tree files in figtree, save the tree as a Newick tree, then concatenate the Newick trees.