Maximum Likelihood Methods in Phylogenetics

ML trees and likelihood-based tree topology testing

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Main Types of Phylogenetic Methods

Data	Method	Evaluation Criterion
Characters (Alignment)	**Maximum Parsimony**	Parsimony
Characters (Alignment)	**Statistical Approaches: Likelihood, Bayesian**	Evolutionary Models
Distances	**Distance Methods**	Evolutionary Models

What is the Maximum Likelihood (ML) Approach?

Having the probabilistic process of evolution and its parameters, we could compute the probability of any outcoming sequence data.

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$$\begin{array}{ccc} \textbf{Probability} & & \textbf{Likelihood} \\ p(\text{Data} \mid \text{Parameter set } \theta) & \equiv & L(\text{Parameter set } \theta \mid \text{Data}) \end{array}$$

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Aim: The ML approach seaches for that parameter set θ for the process (i.e., evolution) which maximizes the probability of our given dataset.

Problem: parameter sets

Problem: In phylogenetic analysis, the parameter set θ comprises:

- evolutionary model
- its parameters
- tree topology
- its branch lengths

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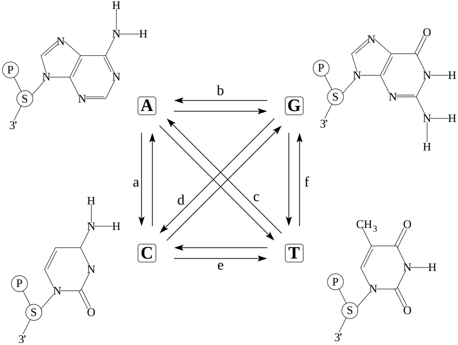
Hence, some parameters like the substitution model and the model parameters are often determined/set separately from the tree.

Evolutionary models are often described using a substitution rate matrix R and character frequencies Π. Here, 4×4 matrix for DNA models:



$$R = \begin{array}{c} \begin{array}{cccc} A & C & G & T \end{array} \\ \begin{pmatrix} - & a & b & c \\ a & - & d & e \\ b & d & - & f \\ c & e & f & - \end{pmatrix} \end{array}$$

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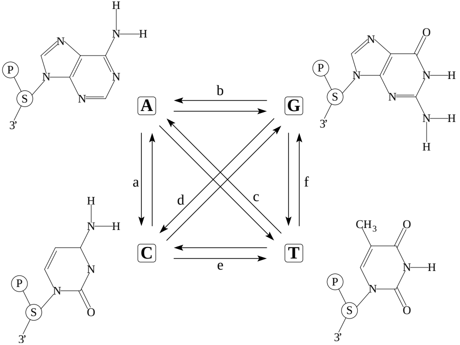

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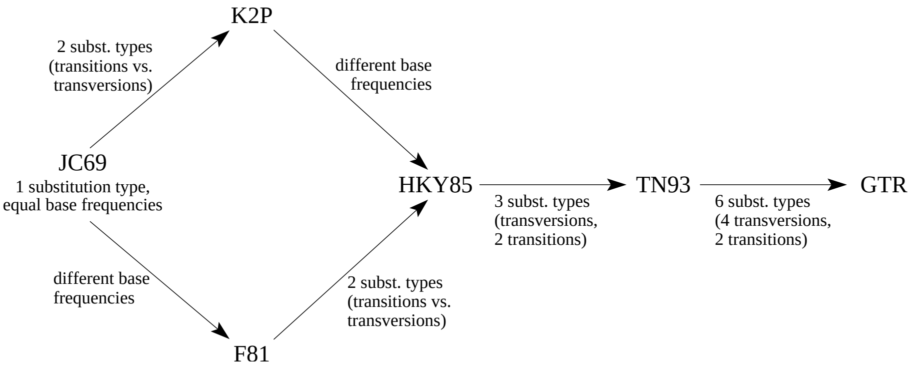
R and Π are then combined to a substitution probability matrix $P(t)$ wich allows us to compute the probability $P_{ij}(t)$ of a change $i \rightarrow j$ over a time t.

DNA substitution models



There are further submodels (see Modeltest) and extensions like models assuming rate heterogeneity and codon models.

Protein Models

Generally this is the same for protein sequences, but with 20×20 matrices. Some protein models are:

- Poisson model ("JC69" for proteins)
- Dayhoff (Dayhoff *et al.*, 1978)
- JTT (Jones *et al.*, 1992)
- mtREV (Adachi & Hasegawa, 1996)
- cpREV (Adachi *et al.*, 2000)
- VT (Müller & Vingron, 2000)
- WAG (Whelan & Goldman, 2000)
- ~~BLOSUM 62 (Henikoff & Henikoff, 1992)~~

Computing ML Distances Using $\mathbf{P_{ij}(t)}$

The Likelihood of sequence s evolving to s' in time t:

$$L(t|s \longrightarrow s') = \prod_{i=1}^{m} \left(\Pi(s_i) \cdot P_{s_i s_i'}(t) \right)$$

Likelihood surface for two sequences under JC69:

```
GATCCTGAGAGAAATAAAC
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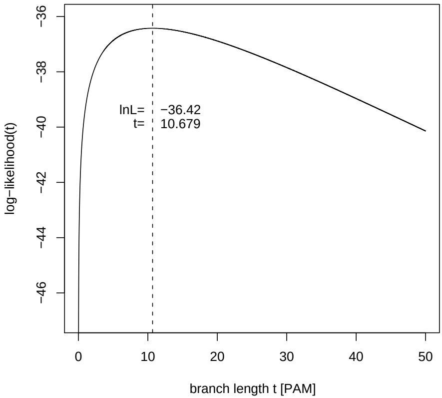
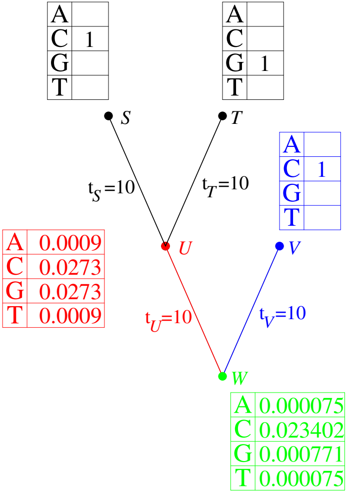
Note: we do not compute the probability of the distance t but that of the data $D = \{s, s'\}$.



Likelihoods of Trees (Single column $\begin{smallmatrix}C\\G\\C\end{smallmatrix}$, given tree)



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$$L_U(i) = [P_{iC}(10) \cdot L(C)] \cdot [P_{iG}(10) \cdot L(G)]$$

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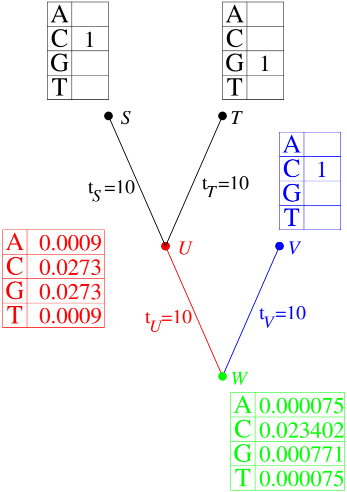

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$$L_W(i) = \quad \left[\sum_{\substack{u= \\ ACGT}} P_{iu}(t_U) \cdot L_U(u)\right] \cdot$$

$$\left[\sum_{\substack{v= \\ ACGT}} P_{iv}(t_V) \cdot L_V(v)\right]$$

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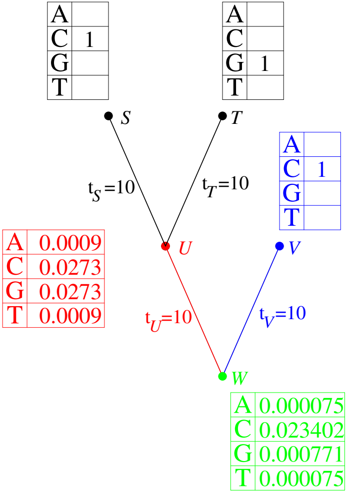

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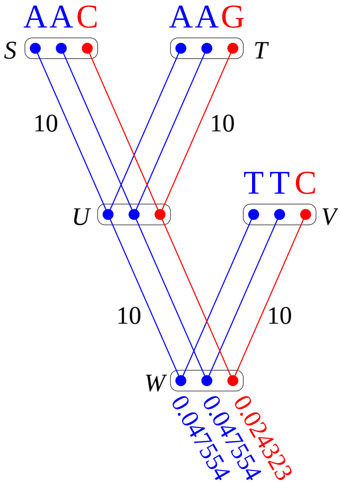
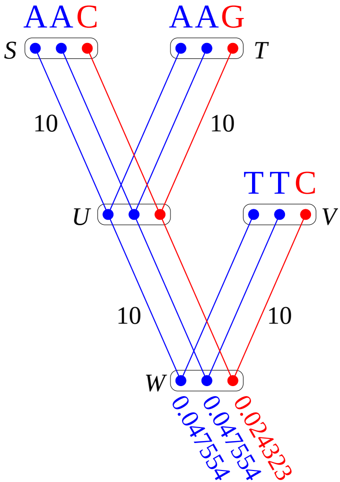
Site-Likelihood of an alignment column k:

$$L^{(k)} = \sum_{\substack{i= \\ ACGT}} \pi_i \cdot L_W(i) = 0.024323$$



Considering this tree with $n = 3$ sequences of length $m = 3$ the tree likelihood of this tree is

$$\mathcal{L}(T) = \prod_{k=1}^{m} L^{(k)}$$



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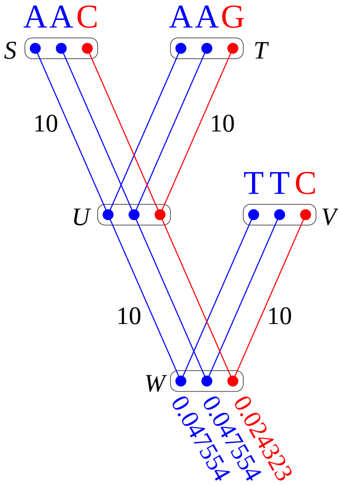
$$= 0.000055$$

Likelihoods of Trees (multiple columns)



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or the log-likelihood

$$\ln \mathcal{L}(T) = \sum_{k=1}^{m} \ln L^{(k)} = -9.80811$$

Adjusting Branch Lengths Step-By-Step

To compute optimal branch lengths do the following. Initialize the branch lengths.
Choose a branch (A.). Move the virtual root to an adjacent node (B.).
Compute all partial likelihoods recursively (C.). Adjust the branch length to maximize the likelihood value (D.).

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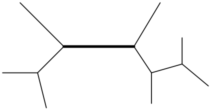

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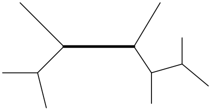

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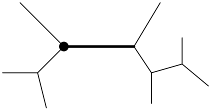

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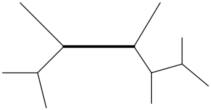

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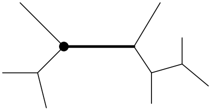

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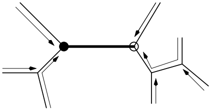

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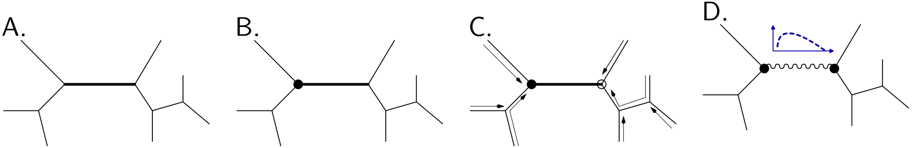

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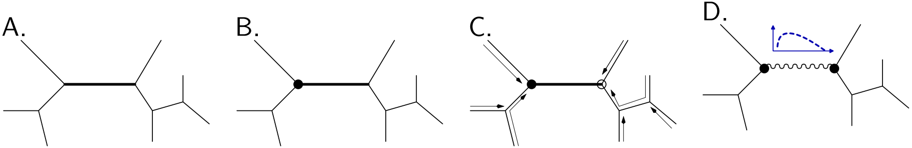
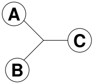


Repeat this for every branch until no better likelihood is gained.

Number of Trees to Examine...



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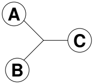

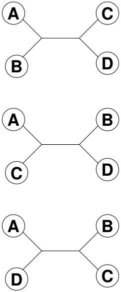

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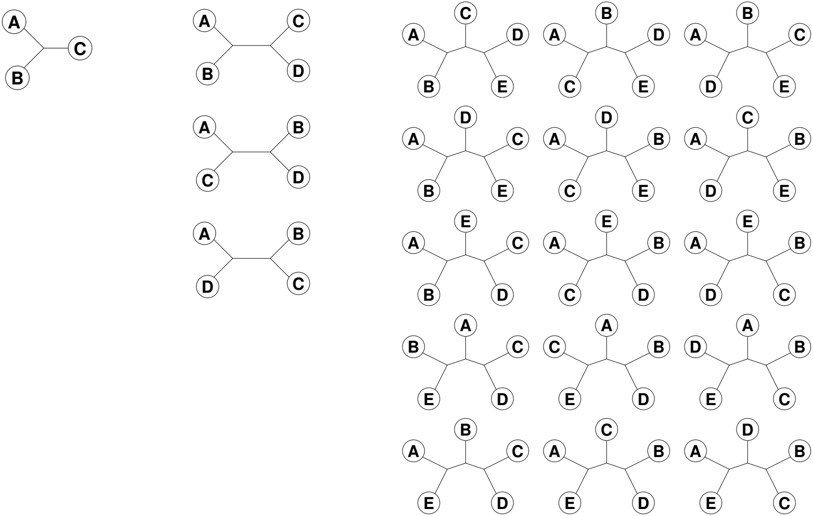

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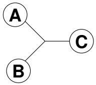

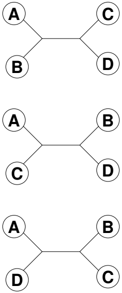

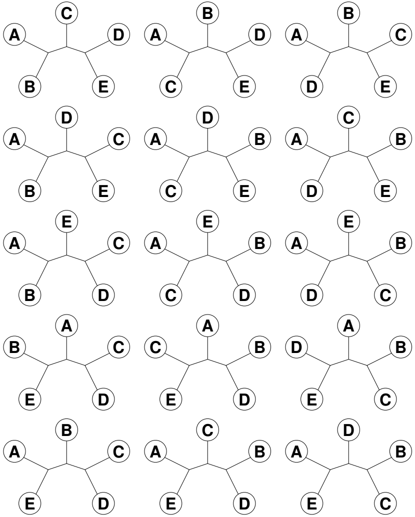

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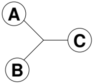
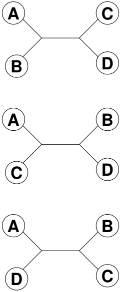
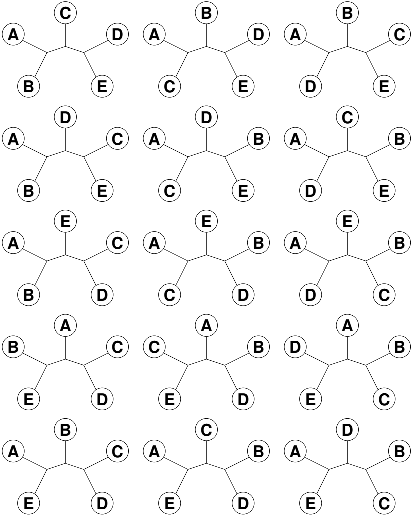




$$
\begin{aligned}
B(n) &= \frac{(2n-5)!}{2^{n-3}(n-3)!} \\
B(10) &= 2027025 \\
B(55) &= 2.98 \cdot 10^{84} \\
B(100) &= 1.70 \cdot 10^{182}
\end{aligned}
$$



Finding the ML Tree

Exhaustive Search: guarantees to find the optimal tree, because all trees are evaluated, but not feasible for more than 10-12 taxa.

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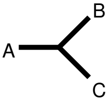
Heuristics: cannot guarantee to find the optimal tree, but are at least able to analyze large datasets.

Build up a tree: Stepwise Insertion



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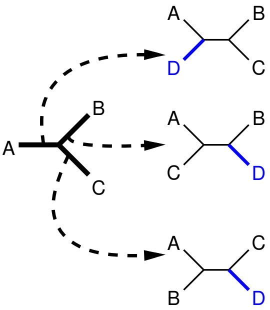

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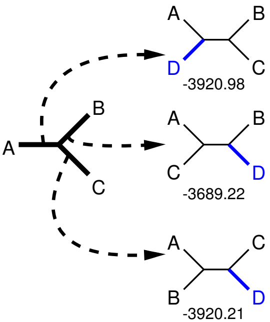

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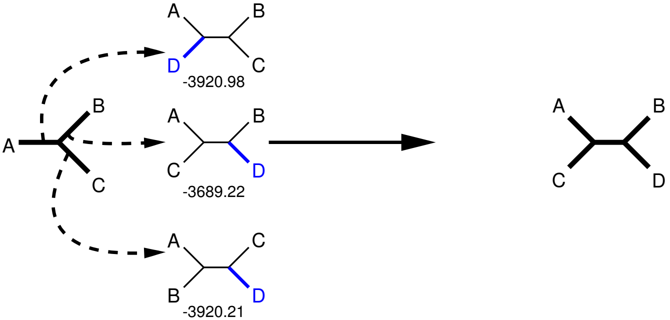

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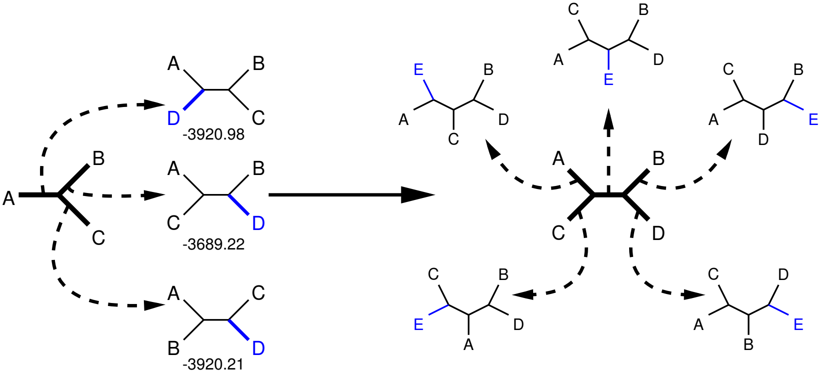

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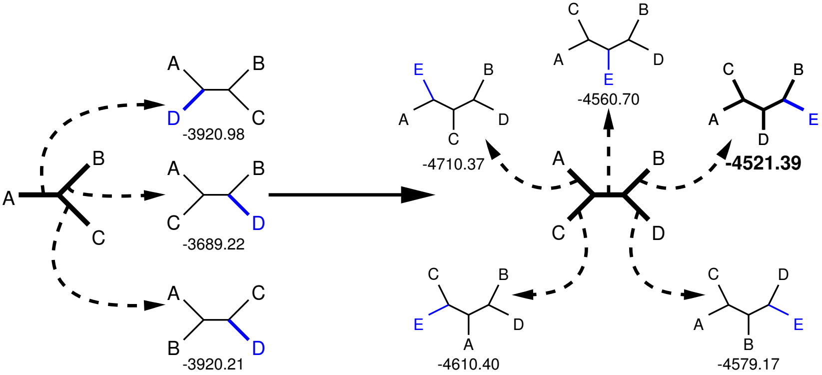
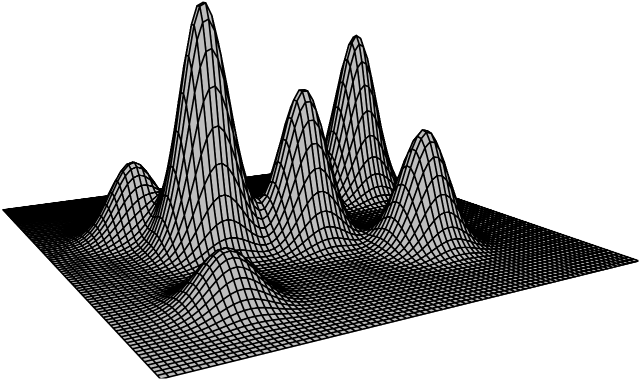


Local Maxima

What if we have multiple maxima in the likelihood surface?



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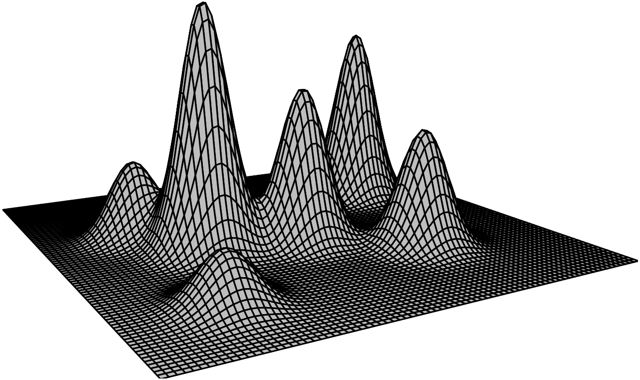
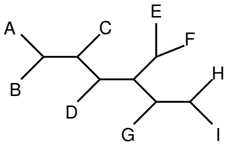
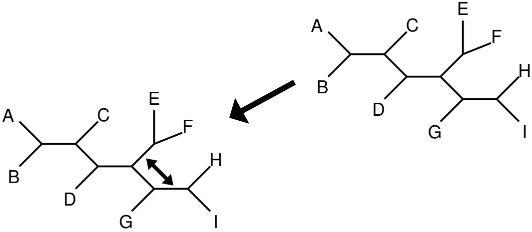


Tree rearrangements to escape local maxima.

Tree Rearrangements: Scanning a Tree's Neighborhood



Tree Rearrangements: Scanning a Tree's Neighborhood



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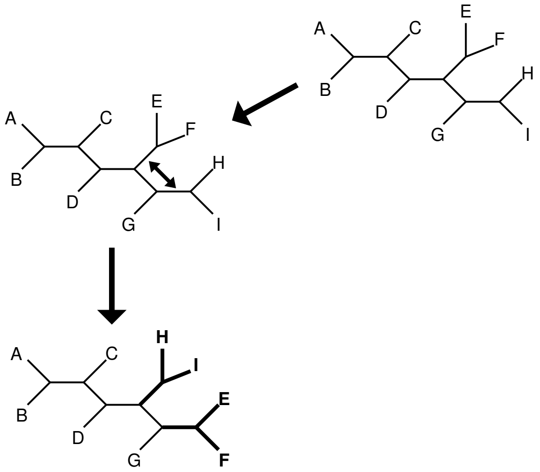

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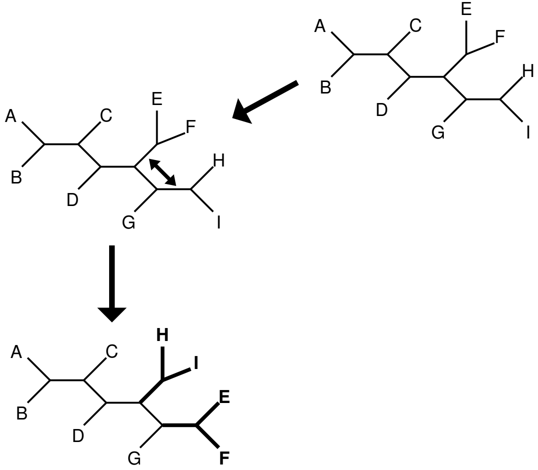

Nearest Neighbor Interchange

Possible NNI trees = O(n)

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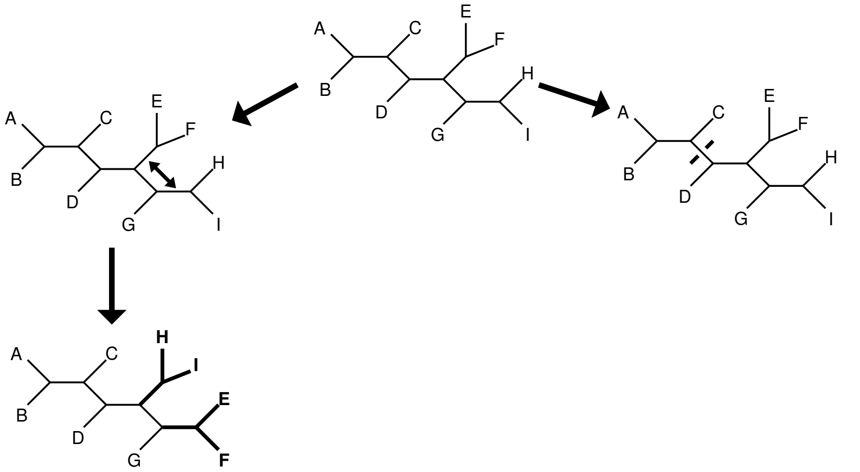

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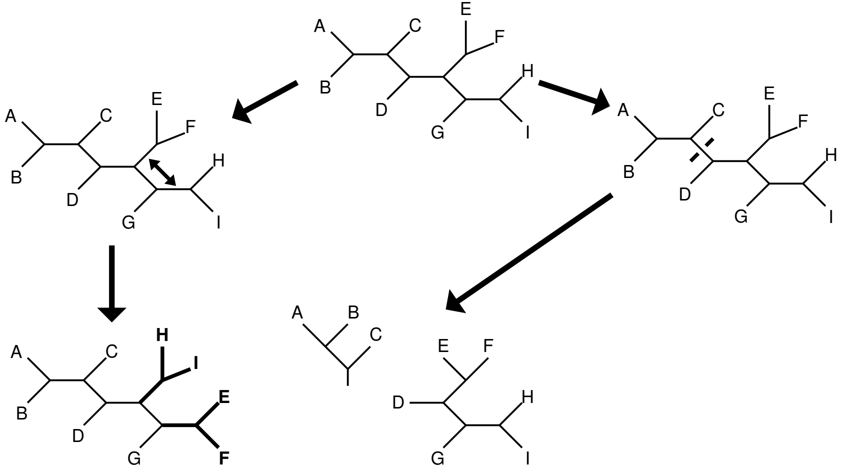

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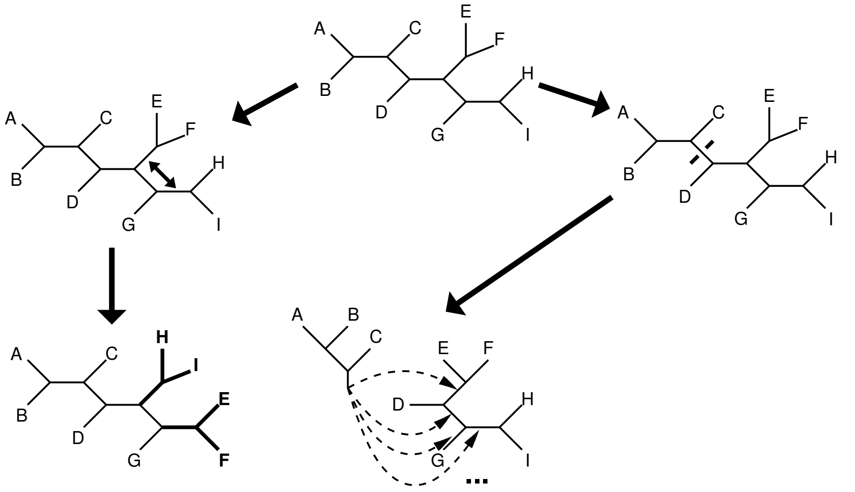

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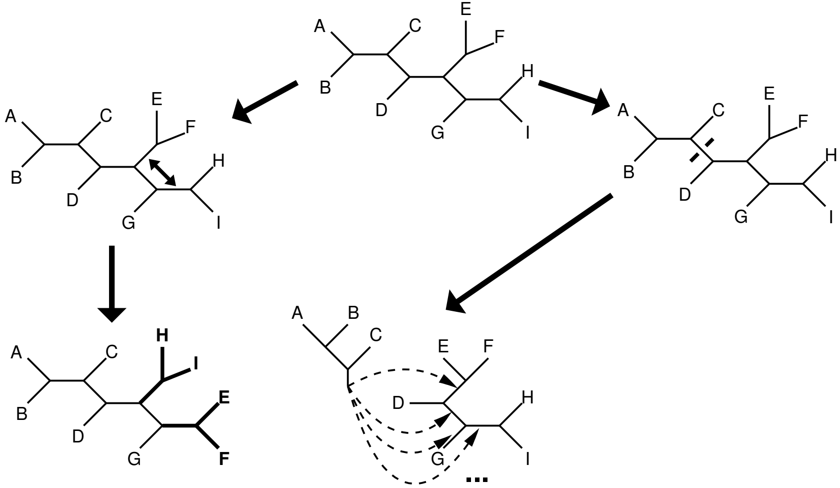

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Possible NNI trees = O(n)

subtree pruning + regrafting

Possible SPR trees = O(n*n)

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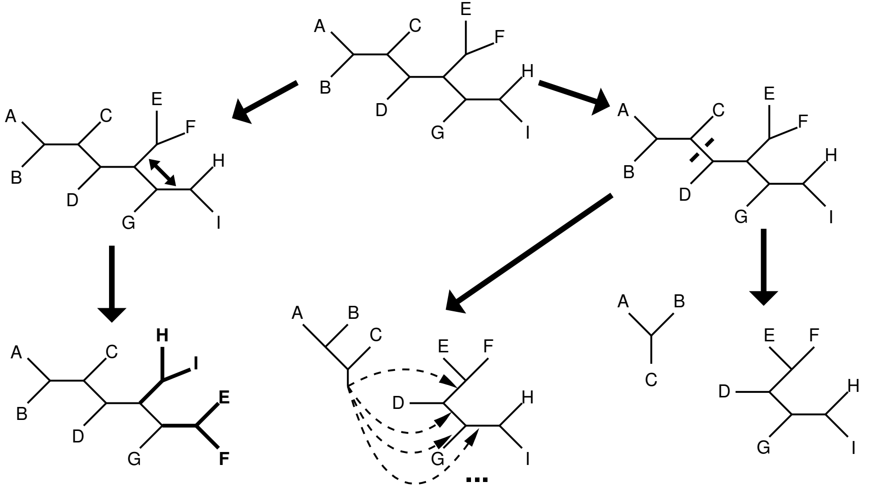

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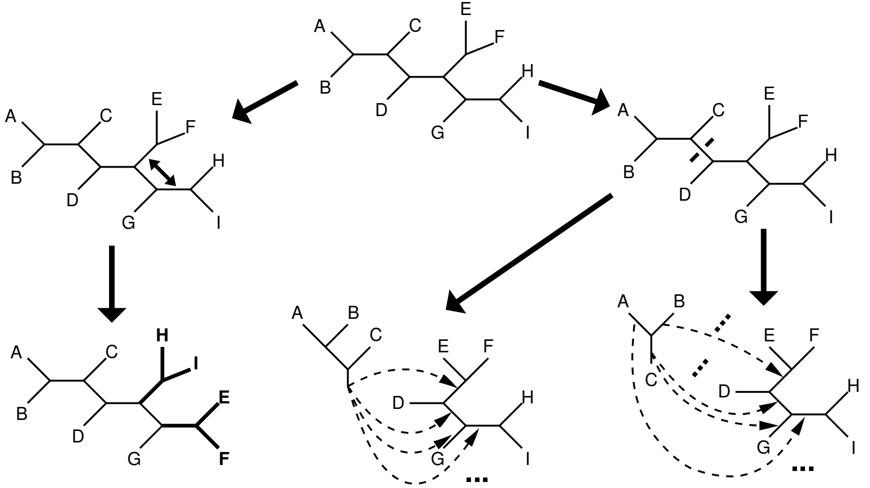

Nearest Neighbor Interchange

Possible NNI trees = O(n)

subtree pruning + regrafting

Possible SPR trees = O(n*n)

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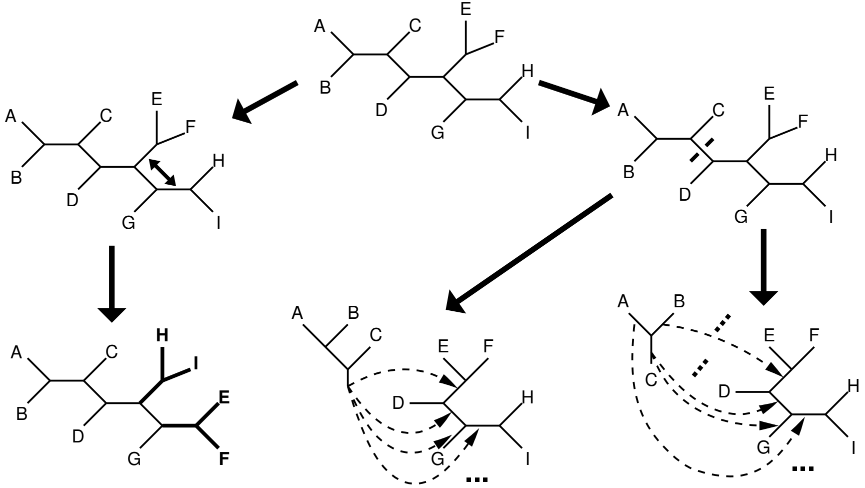

Nearest Neighbor Interchange

Possible NNI trees = O(n)

subtree pruning + regrafting

Possible SPR trees = O(n*n)

tree-bisection + reconnection

Possible TBR trees = O(n*n*n)

ML programs: DNAML (PHYLIP), fastDNAml

Concept: Stepwise insertion + NNI/SPR

1. Build tree with stepwise insertion
   (a) after each insertion optimize using NNI/local rearrangement (default, but user-adjustable gradually up to SPR; only fastDNAml)
   (b) repeat (a) rearrangements until no better tree found.

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Note: To save time, in other methods steps (1) and (2) are usually substituted by swiftly computed trees (e.g., BioNJ).

ML programs: RAxML

Concept: MP tree + LSR
Descendant on fastDNAml, but ...

1. Starting with MP tree.
2. Uses *lazy subtree rearrangements* (only the 3 insertion branches are optimized), collecting candidates.
3. Candidates are evaluated.
4. Iterating (2)-(4).

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Pro: Fast,
smart algorithmic and numerical optimized ML computation.

Con: Only few trees fully evaluated trees.

ML programs: PHYML

Concept: BioNJ tree + fastNNI

1. Start with BioNJ tree.
2. Do fastNNIs to optimize trees, i.e., evaluate all NNIs simultaneously and then merge all best ones which are non-conflicting.
3. Repeat (1) until no better tree found anymore.

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Pro: Fast

Con: Prone to get stuck on local optima due to NNI-only.
(SPR-based version PhyML-SPR has not been released yet.)

ML programs: IQPNNI

Concept: BioNJ tree + randomization + fastNNI

1. Start with BioNJ tree.
2. Do fastNNIs to optimize trees, i.e., evaluate all NNIs simultaneously and then accept all best ones which are non-conflicting. (after first round, identical to PHYML).
3. Remove randomly a certain amount of taxa and re-insert them by a fast and rough quartet-based method. (some randomization)
4. Repeat (2)-(3) until stop criterion is met.

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Pro: Can evade local optima,
offers automatic stopping criterion,
hints when search didn't run enough,
numerically optimized ML computation,
offers codon models

Con: slower than PhyML/RAxML

ML programs: Other strategies

- Genetic Algorithms (GARLI, GAML, MetaPIGA)
- Simulated Annealing (SSA, RAxML-SA)
- Quartet-based trees (TREE-PUZZLE, Qstar)
- . . .

Note: The first two are also based on NNI/SPR/TBR.

How reliable is the reconstructed tree:

- Usually programs deliver a single tree, but without confidence values for the subtrees.
- How can we assess reliability for the subtree?

- We can now reconstruct ML trees, but how comparable are the likelihoods, how reliable the groupings?
- Branch reliability can be checked, support values computed using:
  - Randomizing input orders in stepwise insertions (e.g., TREE-PUZZLE).
  - Jackknifing alignment columns + consensus.
  - Bootstrapping alignment columns + consensus.
  - Trees from Bayesian MCMC sampling + consensus.

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These questions can be assesed by hypothesis testing.

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  - Say should I use the JC model or the GTR model?
  - Or perhaps, Is tree T_a statistically different from tree T_b?
- It is important to note that you should know the null hypothesis/hypotheses **before** you “collect” the data.

Testing Tree Topologies with LRT?

- **Only nested models** can be tested by ordinary LRT:
  One model (H_0, Null-model, constraint model) is nested in another model (H_A, alternative, unconstraint model) if the model H_0 can be produced by restricting parameters in model H_A.

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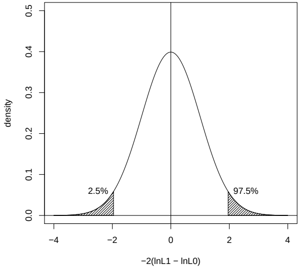
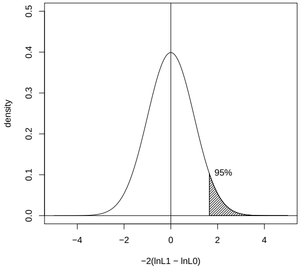
- two differrent topologies are not nested.
- Thus, LRT cannot be used on different topologies, because the assumption of the χ^2 distribution does not fit.
- Hence, other (bootstrap-bases) methods have been devised to determine the distribution of log-likelihood differences for testing (e.g., KH or SH test).

Usual Null-Hypotheses:





First the Null hypothesis has to be stated, for example:

- **top:** The two likelihood are not significantly different – i.e. their expected difference $E(\ln L_1 - \ln L_0) = 0$.
- **bottom:** The 2nd likelihood is not significantly worse – i.e. their expected difference $E(\ln L_1 - \ln L_0) \leq 0$.

If the observed value falls into the white area, the Null hypothesis cannot be rejected. If it falls into the grey area, this is interpreted as support for the alternative by rejecting the Null hypothesis.

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- Use the distribution of $\delta^{(i)}$ to test your trees.

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- During the bootstrap the already estimated site-log-likelihoods are sampled and added to produce $L_x^{(i)}$.
- The resampling of estimated log-likelihoods (RELL) has been shown to be often sufficient to produce the distribution of log-likelihood differences.

Original Kishino and Hasegawa test (KH test)

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  H_A: the expected $\delta = L_1 - L_2 \neq 0$.
  KH assumes that the ML tree is not among the trees.

Kishino-Hasegawa test:

	$D^{(1)}$	$D^{(2)}$	$\cdots$	$D^{(B)}$
T_1	$l_1^{(1)}$	$l_1^{(2)}$	$\cdots$	$l_1^{(B)}$
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centering

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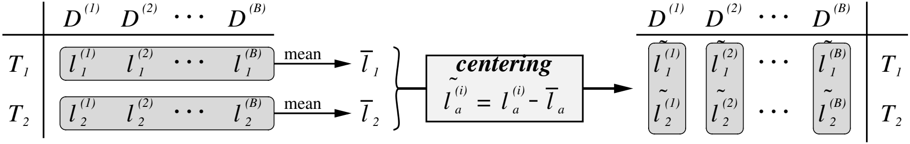

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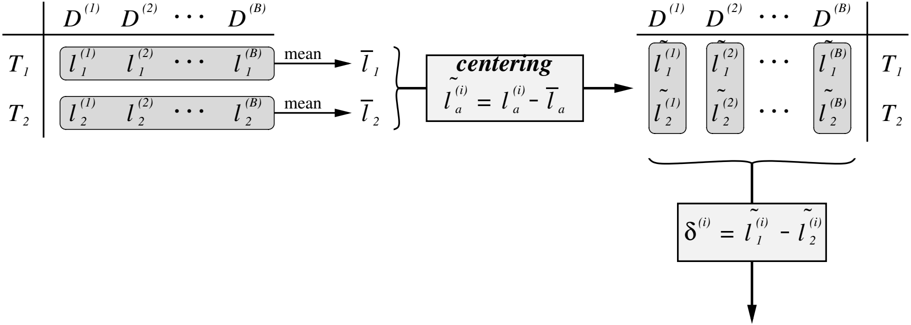

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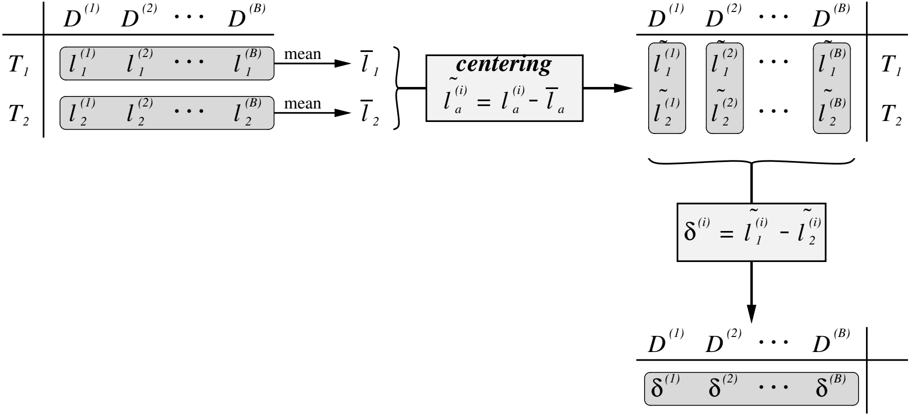

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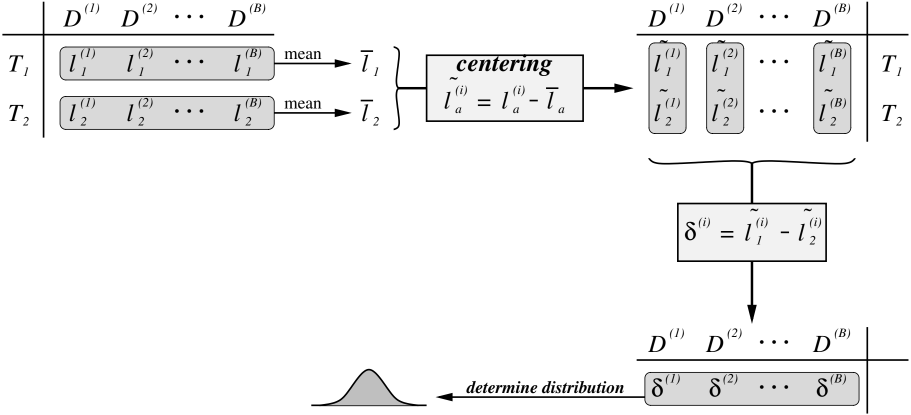

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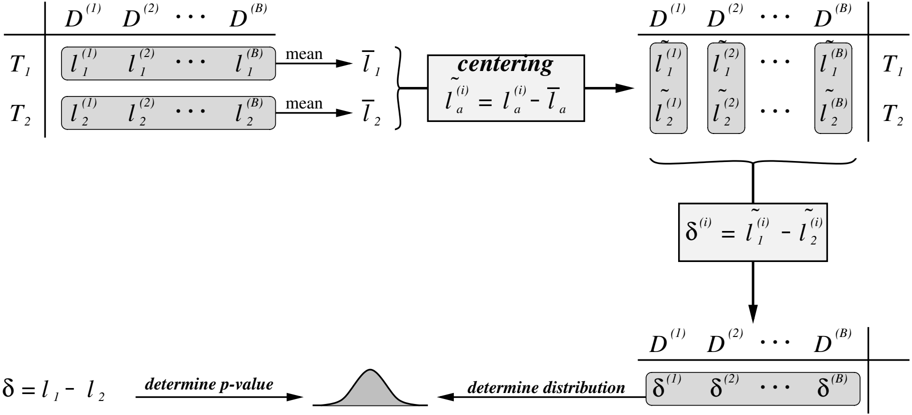

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- H_0: the expected $\delta = L_1 - L_2 = 0$. H_A: the expected $\delta = L_1 - L_2 > 0$.

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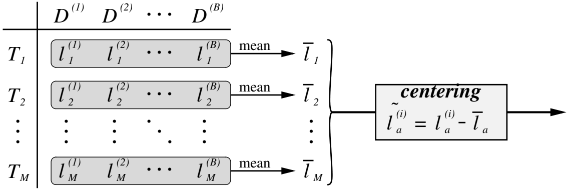

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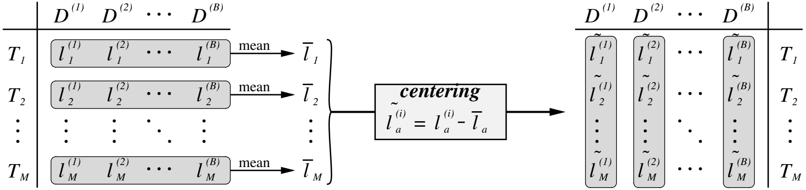

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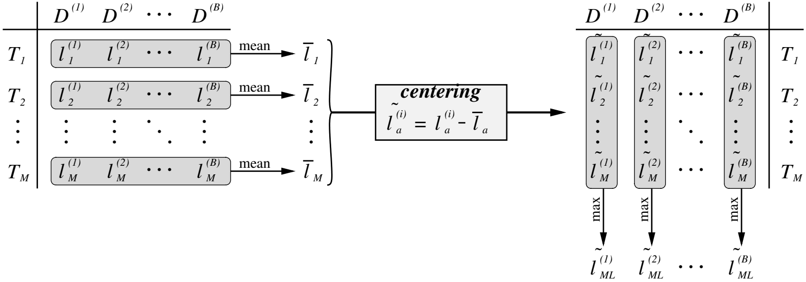

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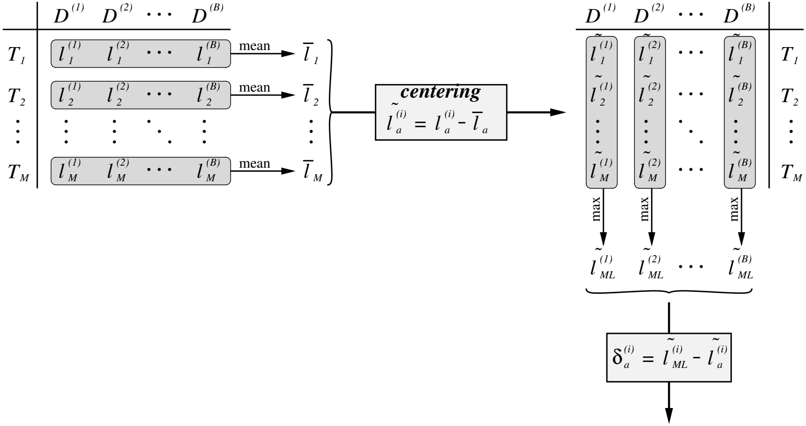

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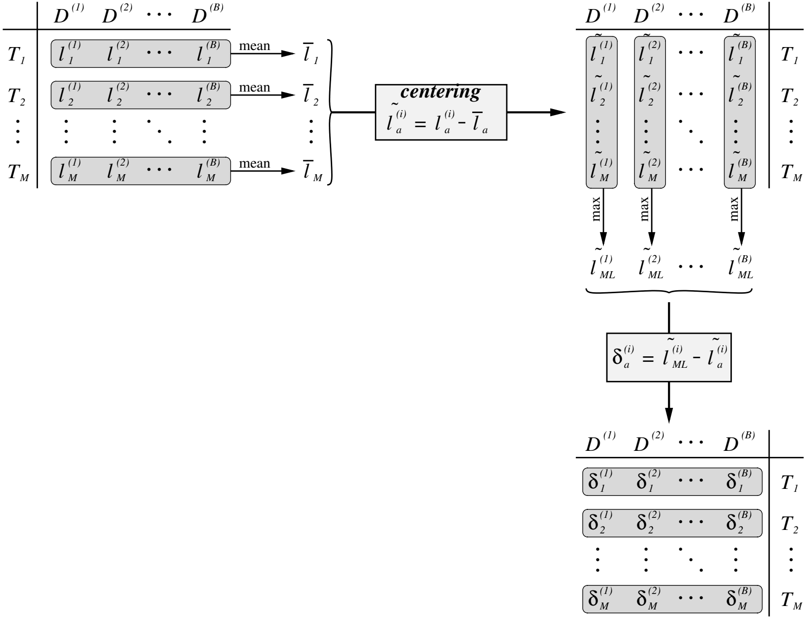

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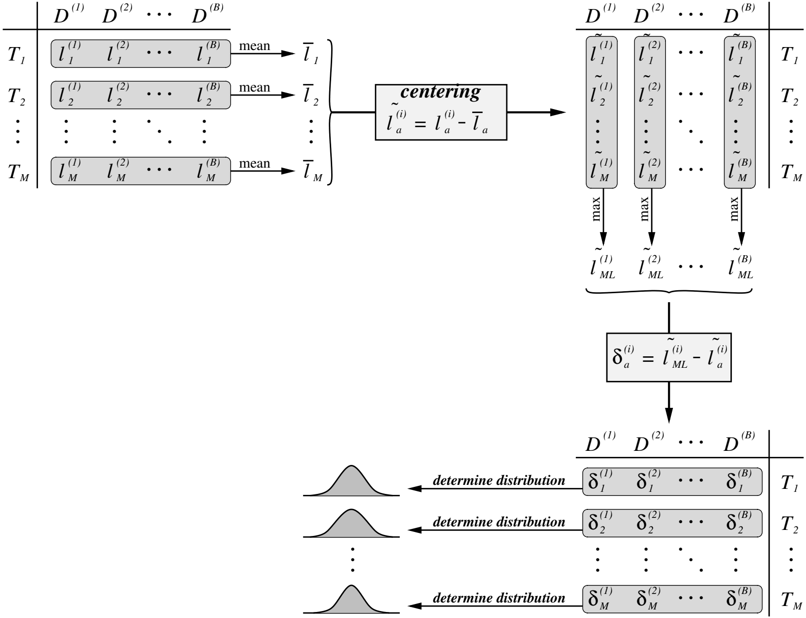

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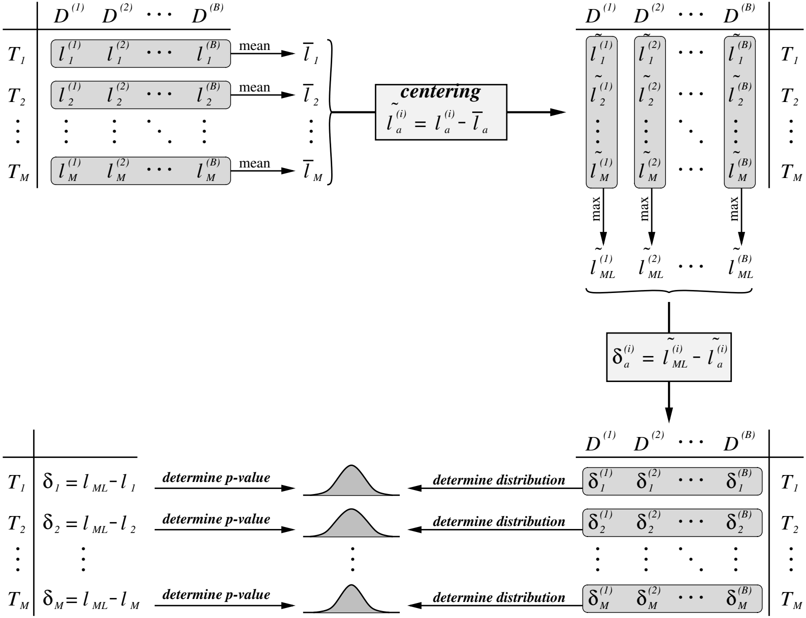

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- Adjust the log-likelihoods with the mean over the samples i by setting $\tilde{L}_x^{(i)} = L_x^{(i)} - \bar{L}_x^{(i)}$ (Centering)
- For each sample i, find $\tilde{L}_{ML}^{(i)}$ over all topologies T_x.
- and compute $\delta_x^{(i)} = \tilde{L}_{ML}^{(i)} - \tilde{L}_x^{(i)}$.
- For each tree T_x, test whether δ_x is a plausible sample from the distribution of $\delta_x^{(i)}$ (over all replicates i).

The SH procedure:

- Compute log-likelihood values and the differences $\delta_x = L_{ML} - L_x$ for your trees.
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- For each sample i, find $\tilde{L}_{ML}^{(i)}$ over all topologies T_x.
- and compute $\delta_x^{(i)} = \tilde{L}_{ML}^{(i)} - \tilde{L}_x^{(i)}$.
- For each tree T_x, test whether δ_x is a plausible sample from the distribution of $\delta_x^{(i)}$ (over all replicates i).
- We use a single sided test, since $\tilde{L}_{ML}^{(i)} \geq \tilde{L}_x^{(i)}$.

Expected Likelihood weights:

	$D^{(1)}$	$D^{(2)}$	$\cdots$	$D^{(B)}$
T_1	$L_1^{(1)}$	$L_1^{(2)}$	$\cdots$	$L_1^{(B)}$
T_2	$L_2^{(1)}$	$L_2^{(2)}$	$\cdots$	$L_2^{(B)}$
$\vdots$	$\vdots$	$\vdots$	$\ddots$	$\vdots$
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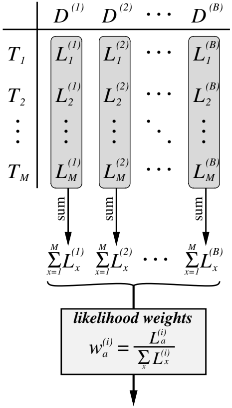
$\vdots$	$\vdots$	$\vdots$	$\ddots$	$\vdots$
T_M	$L_M^{(1)}$	$L_M^{(2)}$	$\cdots$	$L_M^{(B)}$
	sum $\downarrow$	sum $\downarrow$		sum $\downarrow$
	$\sum_{x=1}^{M} L_x^{(1)}$	$\sum_{x=1}^{M} L_x^{(2)}$	$\cdots$	$\sum_{x=1}^{M} L_x^{(B)}$

Expected Likelihood weights:



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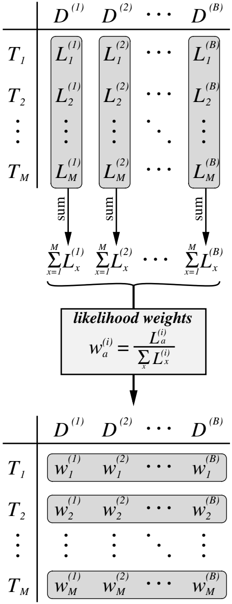

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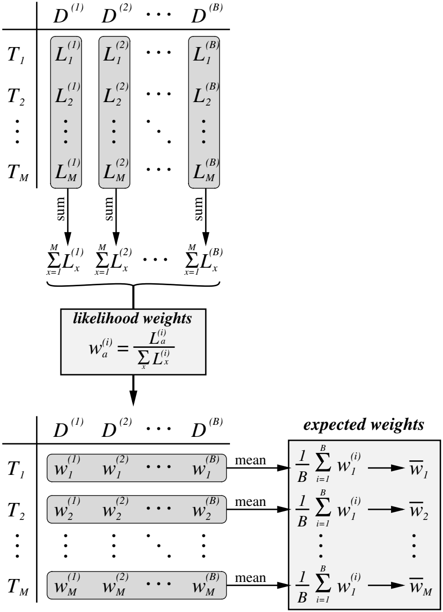
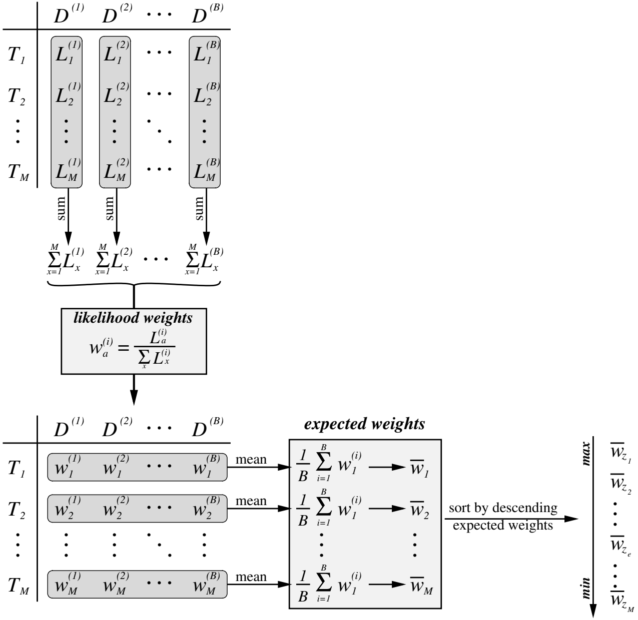


Expected Likelihood weights:



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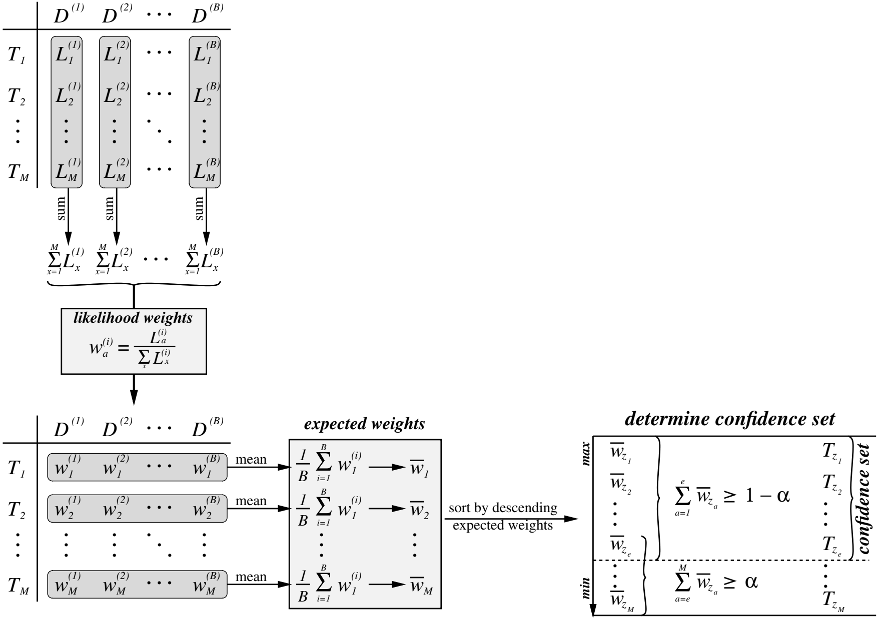

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- **Expected likelihood weights (ELW)** – less conservative than SH, but the impact of model mis-specification unclear.
- **Approximately unbiased test (AU)** – fixes the conservativeness issue of SH, but many similarly good trees can lead to artificial over-confidence.

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- Proper experimental design and proper use of tests is required.
- One should alway be aware of the hypothesis a test assesses and should make sure that this answers the question asked.
- Testing tree topologies can be used to assess whether two competing hypotheses are really substantially different. If they are not, one cannot be prefered over the other.

Exercises:

the exercises can be found at

`http://www.cibiv.at/~hschmidt/VEME/ML-test`