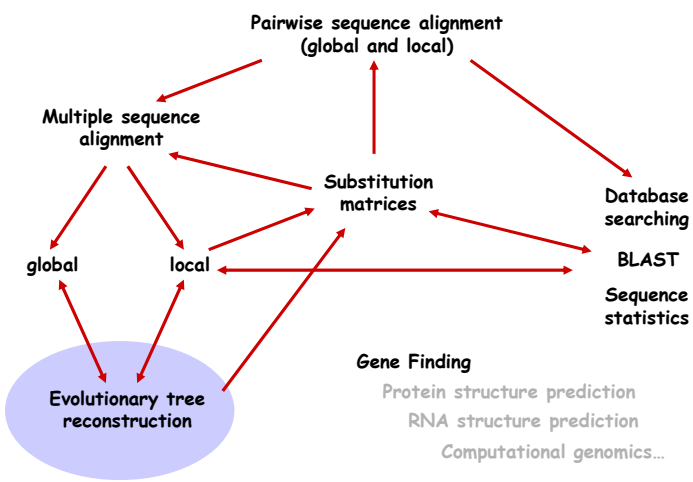


Assumptions:
- Selection dominates
- Mutations are rare
- No multiple substitutions

Parsimony:

Character data

Find the tree that requires the fewest changes to explain the data

Assumptions:
- Neutral mutation dominates
- Multiple substitutions occur

Minimum Evolution:

Distance data

Find the tree that best fits the pairwise distances between taxa

Maximum Likelihood:

Character data

Find the most likely tree

Distance-based methods

- How to obtain a distance matrix
  - Obtain PW distances from a MSA
  - Correct for multiple substitutions
- Find the tree that best fits the distances
  - Conditions for obtaining an exact fit
    - Ultrametric distances
    - Additive distances

How distance matrices are obtained

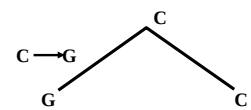
Given sequences from *k* taxa
- Construct a multiple sequence alignment
- Determine pairwise distance from each pair of taxa *using the MSA*
- Correct for multiple substitutions

Multiple Sequence Alignment

```
~~~~ALTEKQEALLKQSWEVLKQNIPAHSLRLFALIIEAA…
~~~MALTEKQEALLKQSWEVLKQNIPAHSLRLFALILEAA…
~~~MALTERQEALLKQSWEVLKQNIPGHSLRLFALIIEAA…
~~~~~~~~~~EALLKQSWEVLKQNIPGHSLCLFALIIEAA…
```

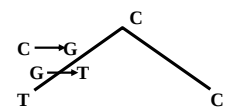
The distance between *taxon i* and *taxon j* is the distance of the pairwise alignment *induced by the MSA.*

Substitution patterns



Single substitution:
- 1 change, 1 difference

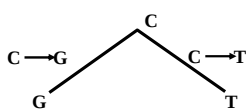
```
...G...
...C...
```



Multiple substitution:
- 2 changes, 1 difference

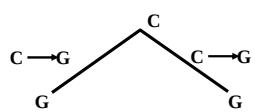
```
...T...
...C...
```

Substitution patterns



Coincidental substitution:
- 2 changes, 1 difference

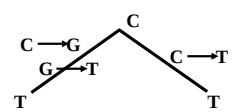
```
...G...
...T...
```



Parallel substitution:
- 2 changes, no difference

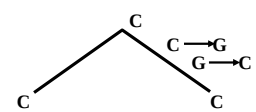
```
...G...
...G...
```

Substitution patterns



Convergent substitution:
- 3 changes, no difference

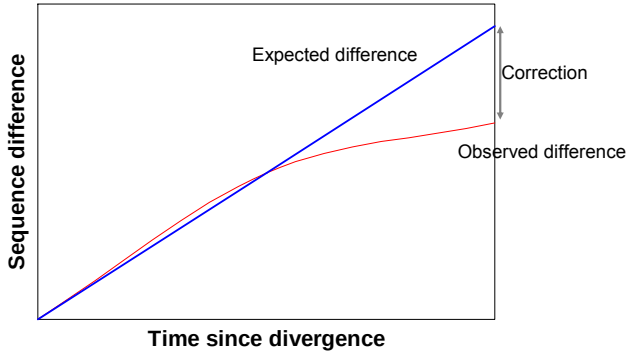
```
...T...
...T...
```



Back substitution:
- 2 changes, no difference

```
...C...
...C...
```

Correcting for multiple substitutions



Correcting for multiple substitutions

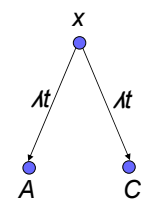
Given *m* mismatches in a PW alignment of length *n*, estimate the actual number of substitutions

Note that:

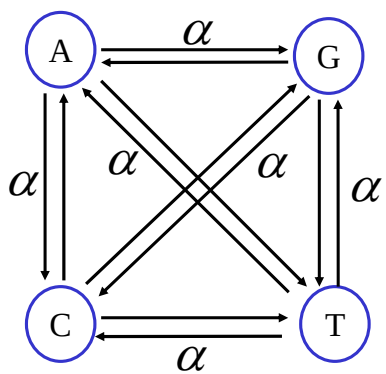
- *p' = m/n* is an estimator for the underlying probability of a mismatch, *p = P(mismatch)*
- The number of substitutions is $2\lambda t$

Strategy

- Propose a Markov model of substitution
- Derive $p=f(\lambda t)$
- $\lambda t = f^{-1}(p)$
- $E[subs/site] = 2 f^{-1}(p)$



Jukes-Cantor model



- α = the rate of substitution (α changes from A to G every t)
- The rate of substitution for each nucleotide is 3α
- In t steps there will be $3\alpha t$ changes

Generalization to any time t

Applies to any time t: $P_{A(t+1)} = (1-3\alpha)\, P_{A(t)} + (1-P_{A(t)})\alpha$

Change as a unit of time: $\Delta P = P_{A(t+1)} - P_{A(t)} = -3\alpha P_{A(t)} + (1-P_{A(t)})\alpha$

$\Delta P = -4\alpha P_{A(t)} + \alpha$

Continuous time model: $dP_{A(t)}/dt = -4\alpha P_{A(t)} + \alpha$

Solution to the 1st order differential expression: $P_{A(t)} = \frac{1}{4} + (P_{A(0)} - \frac{1}{4})e^{-4\alpha t}$

Generalized equations:

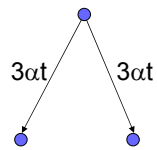
$P_{xx} = \frac{1}{4} + \frac{3}{4}e^{-4\alpha t}$ ← Stay the same

$P_{yx} = \frac{1}{4} - \frac{1}{4}e^{-4\alpha t}$ ← Change

Given the fraction of differences, how many changes occurred?

$p = \frac{3}{4}(1 - e^{-8\alpha t})$

$\alpha t = -1/8 \ln(1 - 4/3p)$



Let K = the number of substitutions since the divergence

$K = 2(3\alpha t)$

$K = -\frac{3}{4} \ln(1 - 4/3p)$

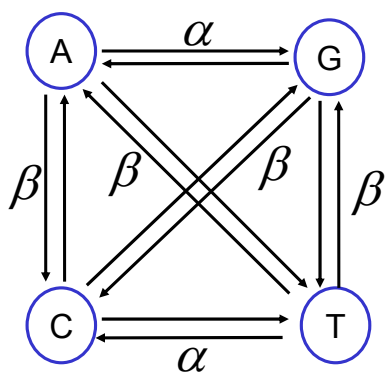
Limitations of the Jukes Cantor Model

- Doesn't take biophysical properties of nucleotides into account

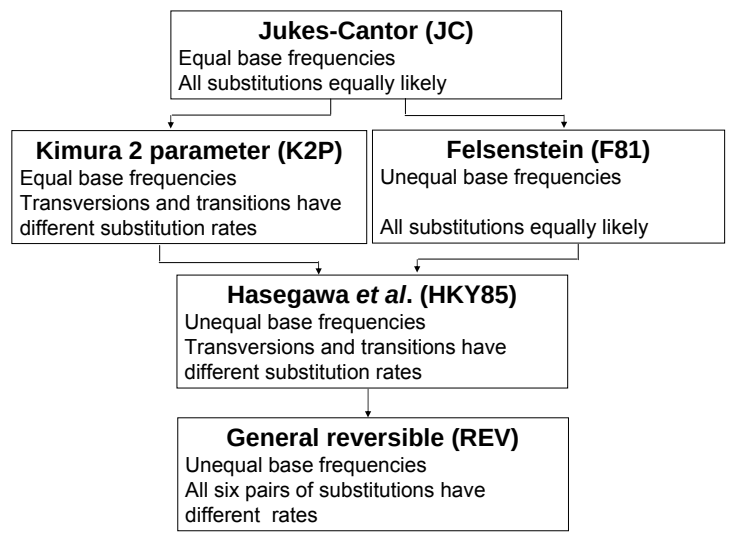
  All substitutions have equal probability
- Doesn't take GC content into account

$$\overline{p} = (0.25, 0.25, 0.25, 0.25)$$

Kimura 2 parameter model:
different probabilities for transitions and transversions



α = transitions β = transversions



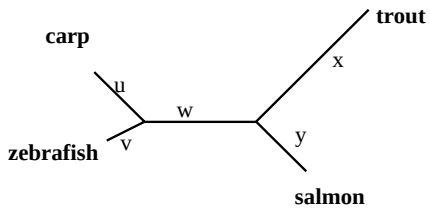
Distance-based methods

- How to obtain a distance matrix
  - Obtain PW distances from a MSA
  - Correct for multiple substitutions
- ➢ Find the tree that best fits the distances
  - Conditions for obtaining an exact fit
    - Ultrametric distances
    - Additive distances

Match distance matrix to branch lengths

	Carp	Zebrafish	Salmon	Trout
Carp	0	3	7	9
Zebrafish		0	6	8
Salmon			0	6
Trout				0

Observed distances



	Carp	Zebrafish	Salmon	Trout
Carp	0	3	7	9
Zebrafish		0	6	8
Salmon			0	6
Trout				0

Observed distances

u + v = 3

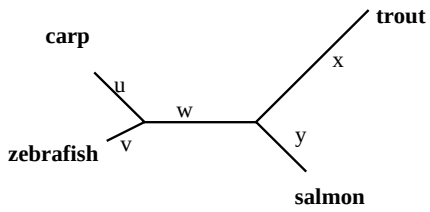
u + w + y = 7

u + w + x = 9

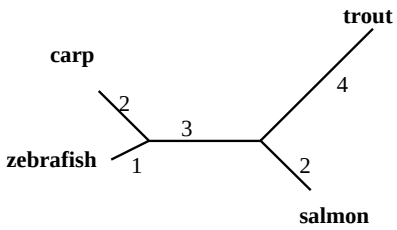
v + w + y = 6

v + w + x = 8

x + y = 6

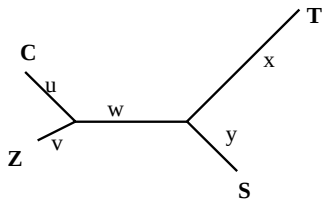


	Carp	Zebrafish	Salmon	Trout
Carp	0	3	7	9
Zebrafish		0	6	8
Salmon			0	6
Trout				0



Can every matrix be fitted to a tree?

	C	Z	S	T
C	0	2	3	3
Z		0	4	3
S			0	2
T				0



Can every matrix be fitted to a tree?

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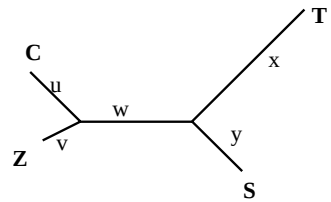


$u + v = 2$

$u + w + y = 3$

$u + w + x = 3$

$v + w + y = 4$

$v + w + x = 3$

$x + y = 2$

$u + v + 2w + x + y = 7$

$u + v + 2w + x + y = 6$

Additive Matrices:

	C	Z	S	T
C	0	2	3	3
Z		0	4	3
S			0	2
T				0

A matrix can be fitted to a tree,
if and only if the equations

```
u + v = 2
u + w + y = 3
u + w + x = 3
v + w + y = 4
v + w + x = 3
x + y = 2
```

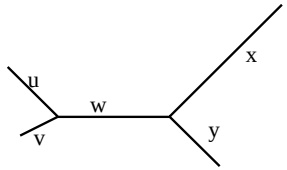
have a solution.

A matrix is *additive* if and only if it satisfies the four point condition.

	A	B	C	D
A	0	2	3	3
B		0	4	3
C			0	2
D				0

Four point condition:

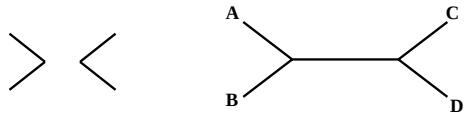
```
AB+CD <= max(AC+BD,AD+BC)
AC+BD <= max(AB+CD,AD+BC)
AD+BC <= max(AC+BD,AB+CD)
```



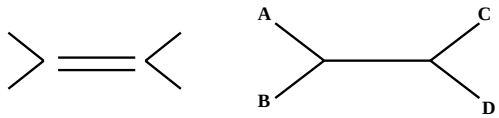
The four-point condition: a test for *additivity*…

```
AB+CD <= max(AC+BD,AD+BC)
AC+BD <= max(AB+CD,AD+BC)
AD+BC <= max(AC+BD,AB+CD)
```

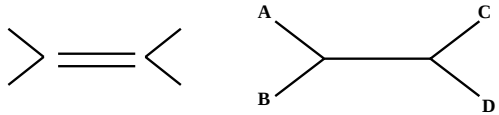
AB + CD



AC + BD



AD + BC




```
AB+CD <= max(AC+BD,AD+BC)

AC+BD <= max(AB+CD,AD+BC)

AD+BC <= max(AC+BD,AB+CD)
```

	A	B	C	D
A	0	2	3	3
B		0	4	3
C			0	2
D				0

Does this matrix satisfy the four point condition?

```
AB+CD <= max(AC+BD,AD+BC)

AC+BD <= max(AB+CD,AD+BC)

AD+BC <= max(AC+BD,AB+CD)
```

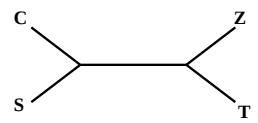
	A	B	C	D
A	0	3	9	7
B		0	8	6
C			0	6
D				0

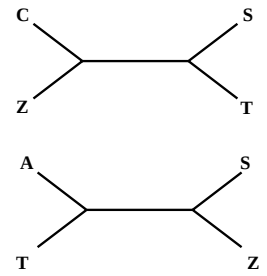
Does this matrix satisfy the four point condition?

We know the matrix

	Carp	Salmon	Zebrafish	Trout
Carp	0	6	3	7
Salmon		0	7	7
Zebrafish			0	8
Trout				0

We don't know the tree topology



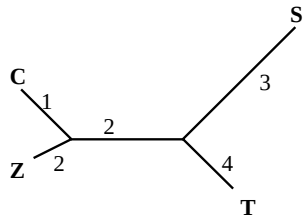


	Carp	Salmon	Zebrafish	Trout
Carp	0	6	3	7
Salmon		0	7	7
Zebrafish			0	8
Trout				0

```
AB+CD <= max(AC+BD,AD+BC)

AC+BD <= max(AB+CD,AD+BC)

AD+BC <= max(AC+BD,AB+CD)
```

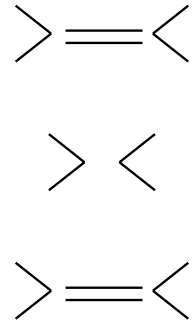


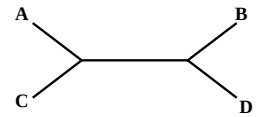
The four-point condition also gives the topology:

AB + CD

AC + BD

AD + BC





The matrix is additive

The four point condition holds for all quartets in *t* :

```
AB+CD <= max(AC+BD,AD+BC)
AC+BD <= max(AB+CD,AD+BC)
AD+BC <= max(AC+BD,AB+CD)
```

The equations

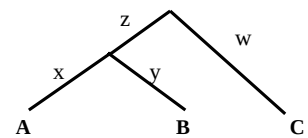
```
u + v = AB        v + w + y = BC
u + w + y = AC    v + w + x = BD
u + w + x = AD    x + y = CD
```

Equivalent statements

have a solution.

The topology and branch lengths are uniquely determined.

Ultrametric distances



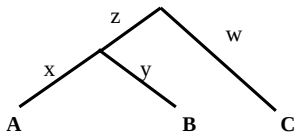
Consider

- a rooted tree with
- constant mutation rate on all branches (molecular clock)

Note:

1. Same distance from the root to every leaf
2. $D[A,B] < D[A,C] = D[B,C]$
3. $x+y < x+z+w = y+z+w$

Three point condition



$x+y < x+z+w = y+z+w$

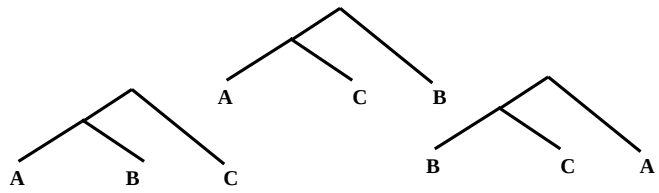
For every triple, {A,B,C} in *T*

- $AB \leq max(AC,BC)$
- $AC \leq max(AB,BC)$
- $BC \leq max(AC,BC)$

We know the matrix

	A	B	C
A	0	2	3
B		0	4
C			0

We don't know the tree topology



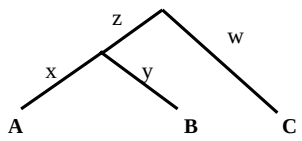
Is the matrix ultrametric?

Equivalent statements

A matrix

- is ultrametric
- satisfies the three point condition
- fits a rooted tree with equal distances from the root to all leaves
- mutation rates are the same in all lineages.

Three point condition an example



	A	B	C
A	0	7	4
B		0	7

For every triple, {A,B,C} in *T*

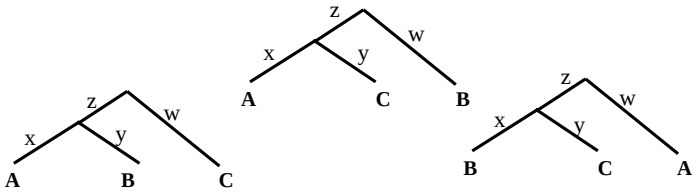
- $AB \leq max(AC,BC)$
- $AC \leq max(AB,BC)$
- $BC \leq max(AC,BC)$

Three point condition an example

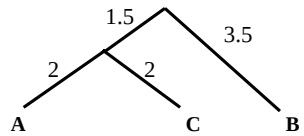
For every triple, {A,B,C} in *T*

- *AB ≤ max(AC,BC)*
- *AC ≤ max(AB,BC)*
- *BC ≤ max(AC,BC)*

	A	B	C
A	0	7	4
B		0	7



Three point condition



	A	B	C
A	0	7	4
B		0	7

For every triple, {A,B,C} in *T*

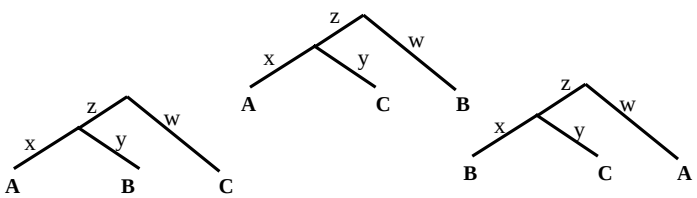
- *AB ≤ max(AC,BC)*
- *AC ≤ max(AB,BC)*
- *BC ≤ max(AC,BC)*

Three point condition an example

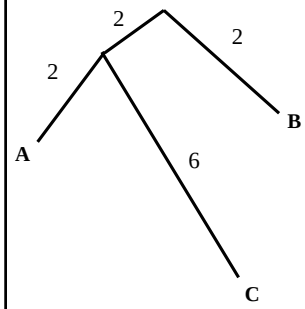
For every triple, {A,B,C} in *T*

- *AB ≤ max(AC,BC)*
- *AC ≤ max(AB,BC)*
- *BC ≤ max(AC,BC)*

	A	B	C
A	0	6	8
B		0	10



Three point condition another example



	A	B	C
A	0	6	8
B		0	10

All ultrametric matrices fit rooted trees
*but n*ot all rooted trees are ultrametric.

If the matrix is not ultrametric,
the closest pair may not be neighbors

Summary

- A matrix is *additive* if it satisfies the four point condition.
- A tree defines a *tree metric, T[i,j]; i.e.,* the pairwise distances between all pairs of leaves.
- All tree metrics are additive.
- If a matrix, *O[i,j],* is additive
  - there exists a unique tree topology with branch lengths such that *T[i,j] = O[i,j].*
  - This tree can be obtained in polynomial time.
- In real life, observed distance matrix, *O[i,j]* is never additive.

Summary, cont'd

- A matrix is *ultrametric* if it satisfies the three point condition.
- All ultrametric matrices fit rooted trees.
- Not all rooted tree metrics are ultrametric.
- An ultrametric tree
  - satisfies the molecular clock hypothesis.
  - All distances from the root to a leaf are the same.
  - Its branch lengths are proportional to time.
- For *k > 3,*
  - All ultrametric matrices are additive
  - But, an additive matrix is *not necessarily* ultrametric.