Phylogenetic Trees

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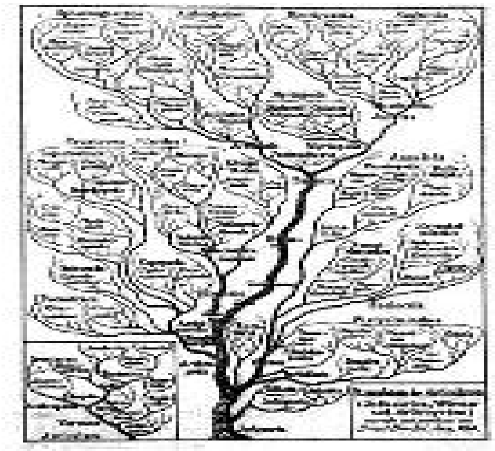
Evolution

- All species of organisms on our planet undergo slow transformation throughout ages. This process has been identified by the biologist as ***evolution***.
- One central problem in biology is to explain the evolutionary history of species and in particular, how species are related to each other and whether or not they shared a common ***ancestor***.

Evolution informs about everything in biology

- Big genome sequencing projects just produce data-- so what?
- Evolutionary history relates all organisms and genes, and helps us understand and predict
  - interactions between genes (genetic networks)
  - drug design
  - predicting functions of genes
  - influenza vaccine development
  - origins and spread of disease
  - origins and migrations of humans

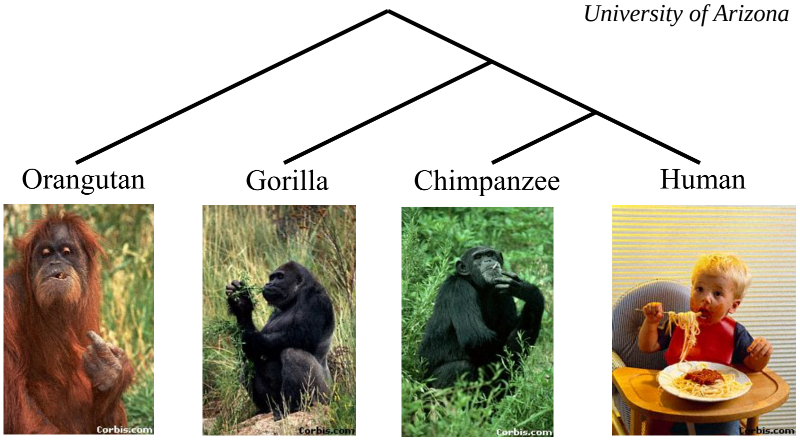
Reconstructing the “Tree” of Life



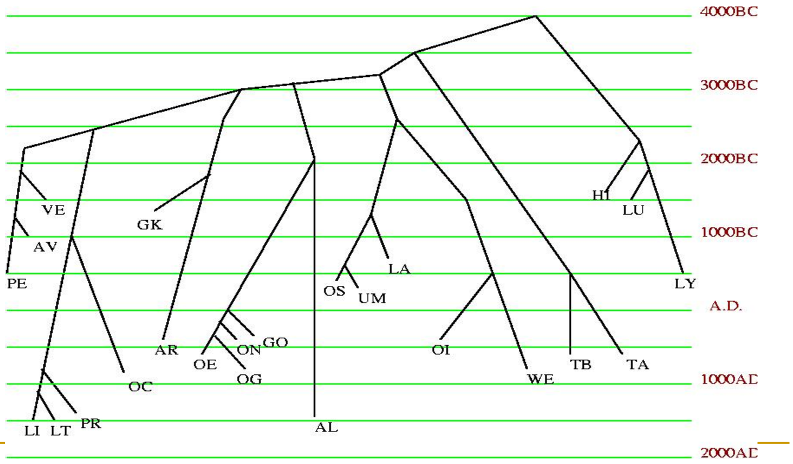
Handling large datasets:
millions of species

Phylogeny

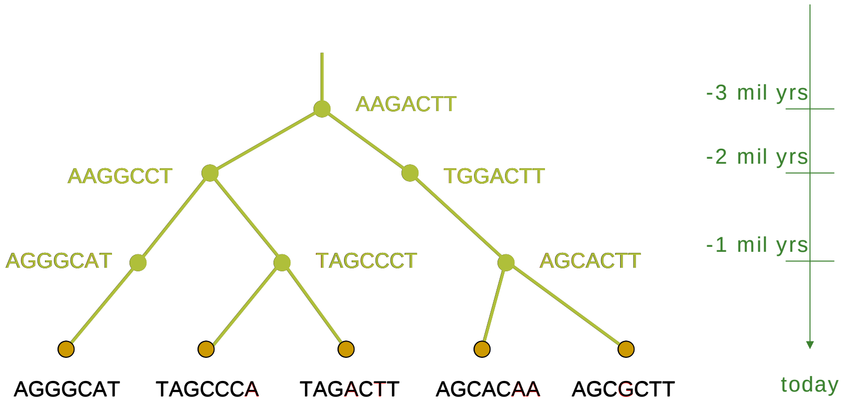
From the Tree of the Life Website,
University of Arizona



Ringe-Warnow Phylogenetic Tree of Indo-European



DNA Sequence Evolution



AAGACTT

AAGGCCT

TGGACTT

AGGGCAT

TAGCCCT

AGCACTT

AGGGCAT

TAGCCCA

TAGACTT

AGCACAA

AGCGCTT

-3 mil yrs

-2 mil yrs

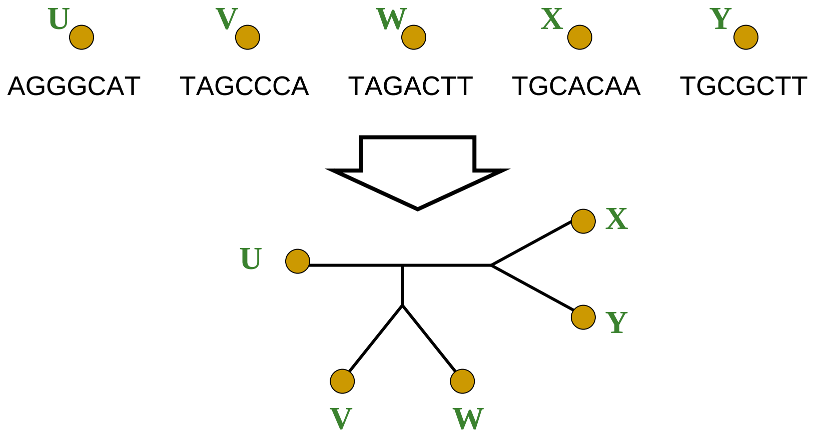
-1 mil yrs

today

Phylogenetic Tree

- The ancestral relationship can be depicted by constructing a tree whose leaves represent the present day species and whose internal nodes represent possible ancestors. Such a tree is called a **phylogenetic tree**.

Phylogeny Problem



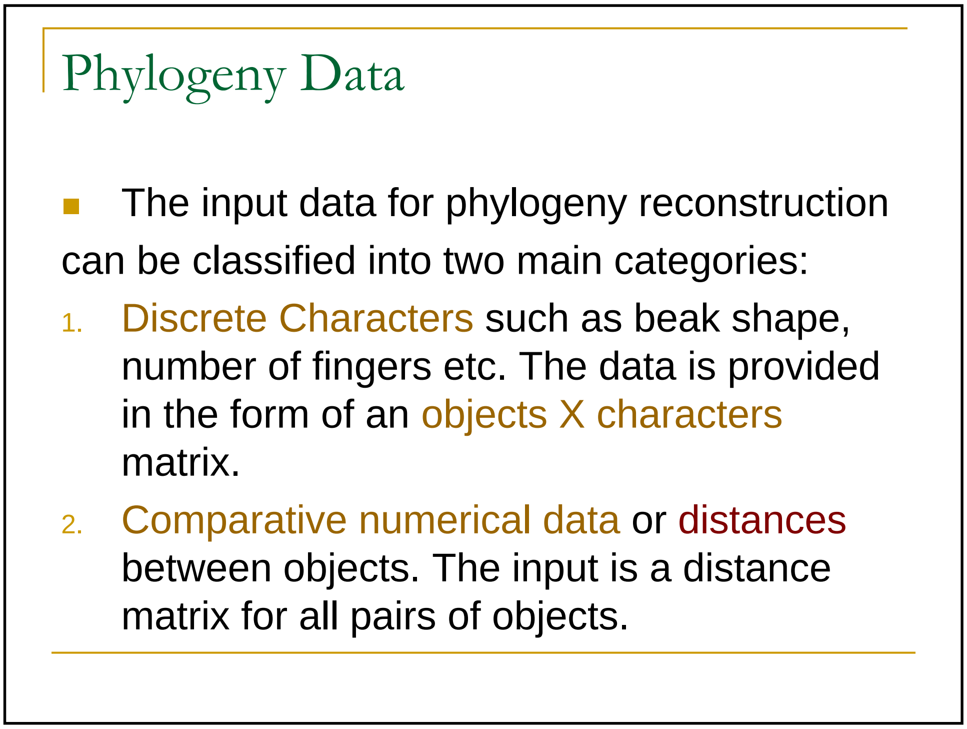
Mutation

- With the advent of molecular biology, the evolutionary processes have been linked to several basic processes at the genome level such as insertion, deletion, substitution, inversion and transposition of its DNA.
- All these operations are grouped under a common name called ***mutation**.*
- In the past, biologists used morphology data (the so-called phenotypes: color of hair, skin, eye, physical characteristics like presence of wings, length of arms, legs etc) or biochemistry data (such as amino acid synthesis pathways) to come up with taxonomy and ancestral relationship.

Phylogenies

- In recent times, use of molecular sequence data has given rise to a more precise science of **phylogenies** which incorporates mathematical and algorithmic approaches.
- Many software tools that have been designed based on these algorithms to re-create phylognetic trees. There is a vast amount of literature and competing theories of evolutionary biology and classification of species.
- Our aim here is very much limited to studies of a few algorithmic approaches for constructing phylogenetic trees.

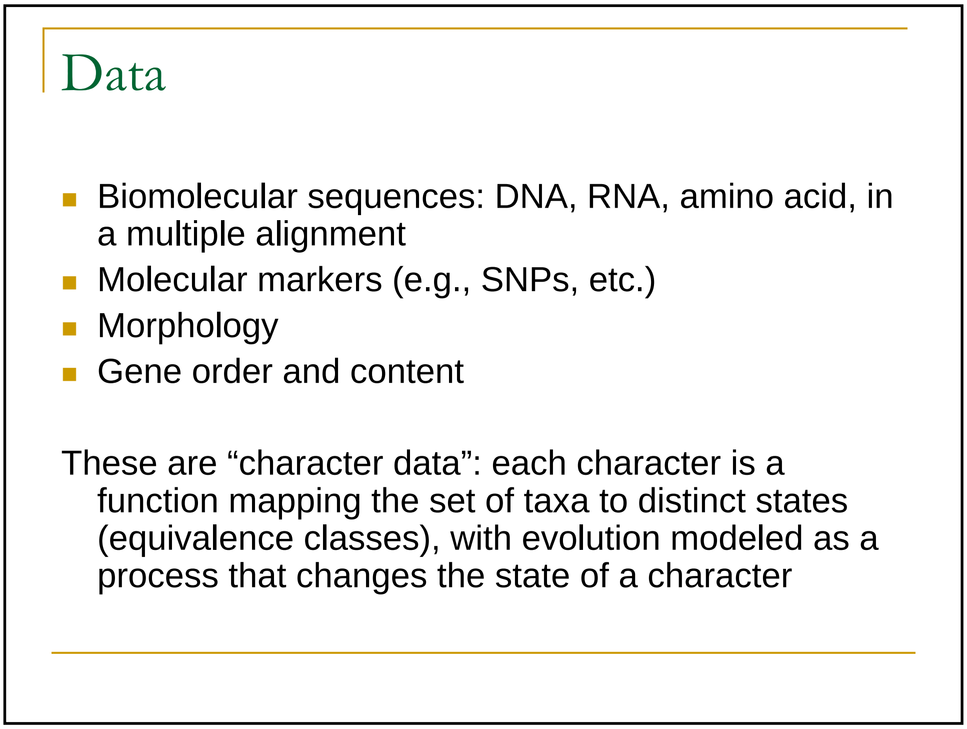
- The tree is an undirected acyclic graph.
- The leaves of the tree denote “objects” under study which could be a group of seemingly related organism, mammals, birds, reptiles or DNA or amino acid sequences which also undergo evolution along with the evolution of the associated organisms.
- An internal node represents a hypothetical common ancestor of all the leaf objects under it.
- The topology or *the* ***branching patterns*** shows the history of evolution of the object via the unique path from it to the *root of the tree* which is supposed to be the common ancestor of all the objects under study.



Phylogeny Data

- The input data for phylogeny reconstruction can be classified into two main categories:

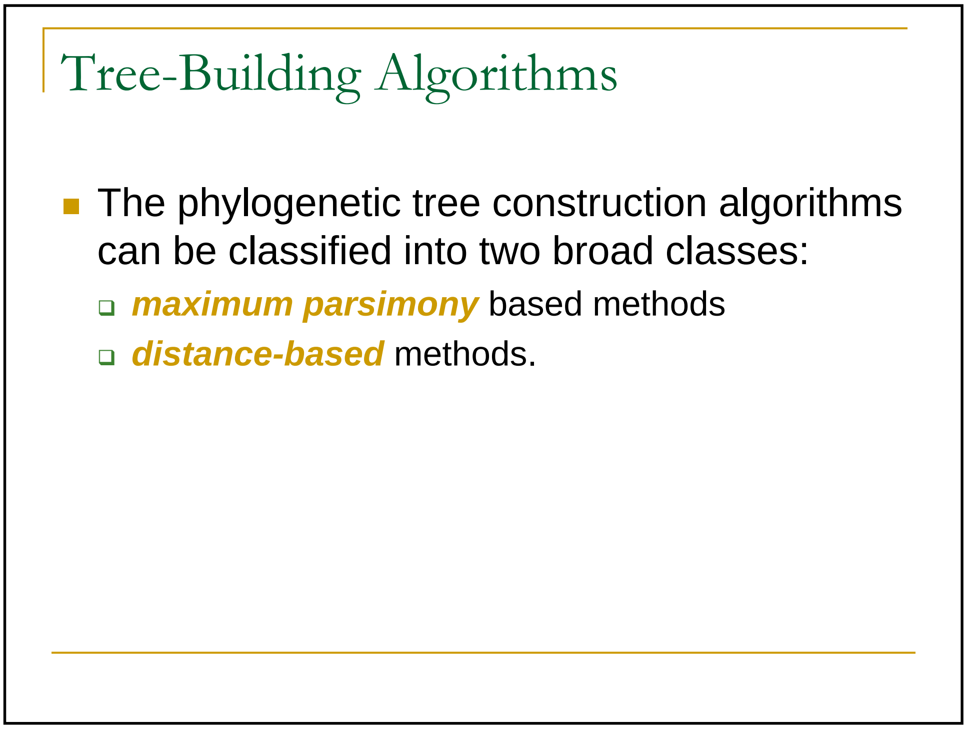
1. Discrete Characters such as beak shape, number of fingers etc. The data is provided in the form of an objects X characters matrix.
2. Comparative numerical data or distances between objects. The input is a distance matrix for all pairs of objects.



Data

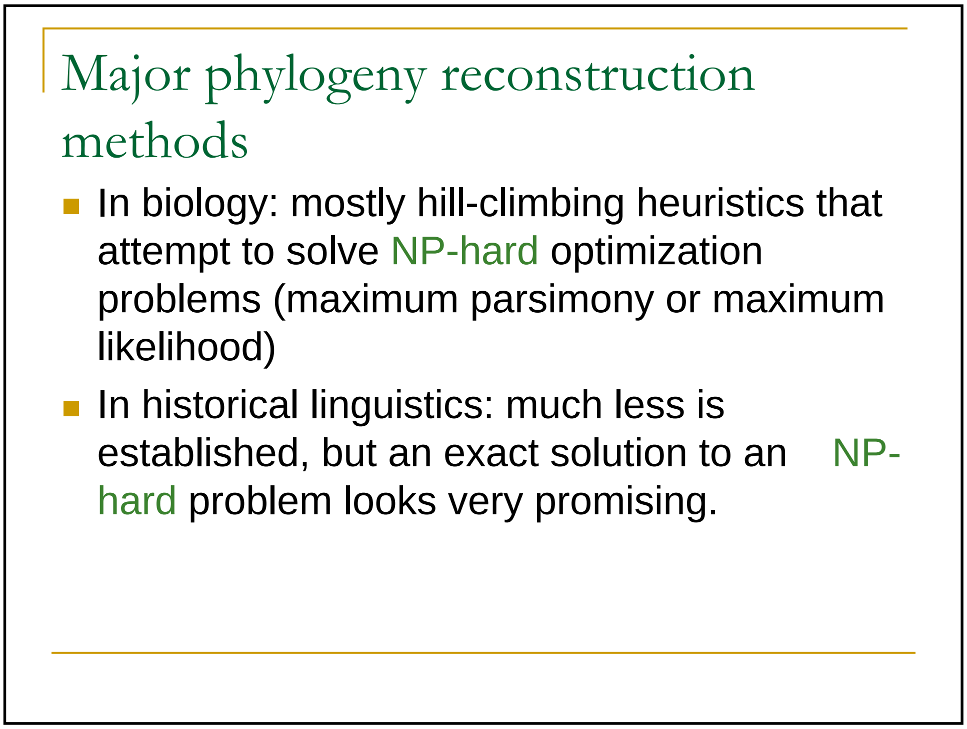
- Biomolecular sequences: DNA, RNA, amino acid, in a multiple alignment
- Molecular markers (e.g., SNPs, etc.)
- Morphology
- Gene order and content

These are “character data”: each character is a function mapping the set of taxa to distinct states (equivalence classes), with evolution modeled as a process that changes the state of a character



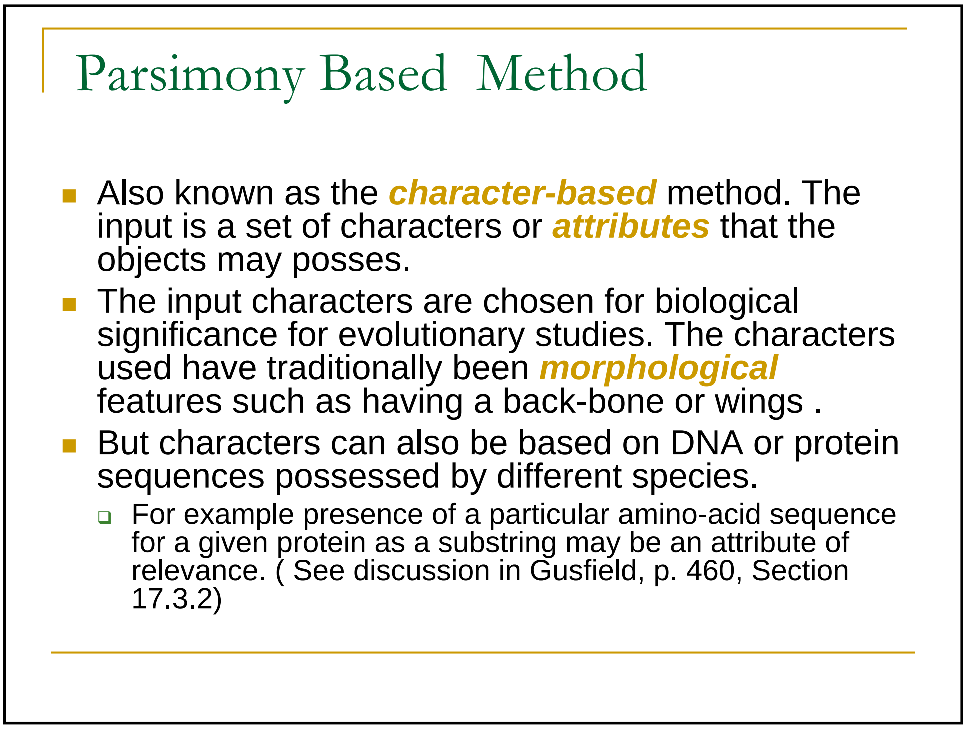
Tree-Building Algorithms

- The phylogenetic tree construction algorithms can be classified into two broad classes:
  - ***maximum parsimony*** based methods
  - ***distance-based*** methods.



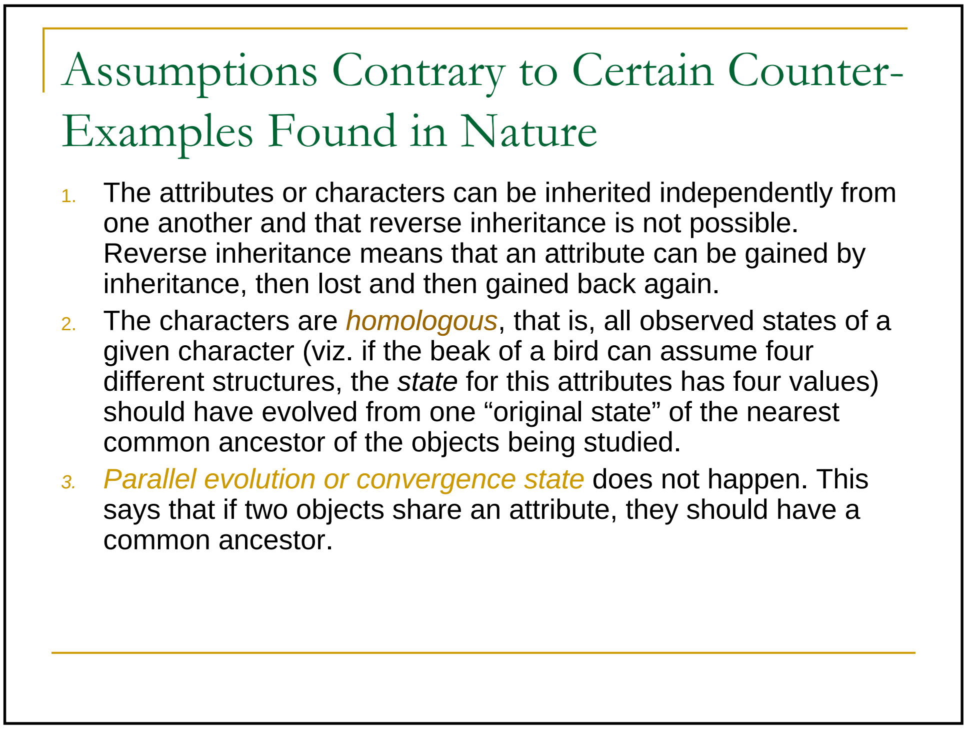
Major phylogeny reconstruction methods

- In biology: mostly hill-climbing heuristics that attempt to solve NP-hard optimization problems (maximum parsimony or maximum likelihood)
- In historical linguistics: much less is established, but an exact solution to an NP-hard problem looks very promising.



Parsimony Based Method

- Also known as the ***character-based*** method. The input is a set of characters or ***attributes*** that the objects may posses.
- The input characters are chosen for biological significance for evolutionary studies. The characters used have traditionally been ***morphological*** features such as having a back-bone or wings .
- But characters can also be based on DNA or protein sequences possessed by different species.
  - For example presence of a particular amino-acid sequence for a given protein as a substring may be an attribute of relevance. (See discussion in Gusfield, p. 460, Section 17.3.2)



Assumptions Contrary to Certain Counter-Examples Found in Nature

1. The attributes or characters can be inherited independently from one another and that reverse inheritance is not possible. Reverse inheritance means that an attribute can be gained by inheritance, then lost and then gained back again.
2. The characters are *homologous*, that is, all observed states of a given character (viz. if the beak of a bird can assume four different structures, the *state* for this attributes has four values) should have evolved from one “original state” of the nearest common ancestor of the objects being studied.
3. *Parallel evolution or convergence state* does not happen. This says that if two objects share an attribute, they should have a common ancestor.

Properties of Characters

- The characters could be *ordered* or *unordered*. In general, if a character has *r* values, the character can assume any one of the *r* values.
  - For an unordered characters, any state can change to any state (although the same state may not repeat – reverse inheritance).
  - For an ordered character, the state changes may follow a particular specified total or partial order. For example, a linear order 3↔4↔1↔2 means that a transition from state 3 to 1 or from 1 to 3 has to go through an intermediate state 4.

Properties of Characters

- Even with ordered characters, note the state transitions are not directed**,** simply ordered. Characters for which the direction of change is known are called **directed.** The unordered, ordered and directed characters are also known as qualitative, cladistic and polar characters , respectively.

Character State Matrix

Definition:

- Let *M* be an *n* by *m* -*r*- ary matrix, representing *n* objects each having *m* attributes or characters. A given row of the matrix is called a state vector for an object. The *i* -th row of *M* represents the *i* -th object. The *j* -th column represents the *j* -th character. Since we will assign states to interior (inferred) nodes in the tree, they are also associated with state vectors.

Perfect Phylogeny

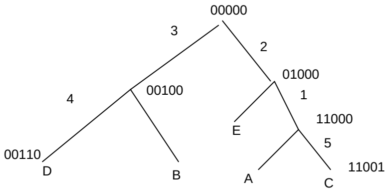
- A ***perfect phylogenetic*** tree for *M* is a rooted tree *T* with exactly *n* leaves that obeys the following properties:
  - Each of the *n* objects labels exactly one leaf of *T.*
  - For each state *s* (*s*=0,1,..,*r)* of each character *c*(*c*=1,2,…, *m),* the set of all nodes (leaves and interior nodes) for which the state is *s* with respect to *c* must form a subtree of *T* (that is, a connected subgraph of *T*). This means that the edge leading to this subtree is uniquely associated with a transition from some state *w* to state *s*.

Example

	1	2	3	4	5
A	1	1	0	0	0
B	0	0	1	0	1
C	1	1	0	0	1
D	0	0	1	1	0
E	0	1	0	0	1

	1	2	3	4	5
A	1	1	0	0	0
B	0	0	1	0	0
C	1	1	0	0	1
D	0	0	1	1	0
E	0	1	0	0	1

The first matrix has no perfect phylogenetic tree. The second matrix *M* has a solution tree.(Note the leaf nodes B and D and the interior node 00100 all have 3rd character value 1 and is a subtree etc.)



Perfect Phylogeny Problem

- For *n* objects, it can be shown that there are

$$\prod_{i=3}^{n}(2i-5)$$

possible labeled trees which grows faster than *n*! , so building all trees and then deciding which one of them is a perfect phylogeny is out of question.

- For unordered characters, the problem is NP-complete.
- For ordered characters, there exist algorithms with complexity polynomial in *n, m* and *r.*

Perfect Phylogeny Problem for Binary Matrix

- For the binary matrix case, the problem is: Given a *n* by *m* 0-1 matrix *M*, determine whether there is a phylogenetic tree for *M*, and if so, build one. This problem has a *O*(*nm*) solution.
  - For convenience of proof, it will be simpler to transform *M* to a matrix whose columns are sorted in a in decreasing order from left to right.

Example

Cols. of M Cols. of $\overline{M}$	2 1	1 2	3 3	5 4	4 5
A	1	1	0	0	0
B	0	0	1	0	0
C	1	1	0	1	0
D	0	0	1	0	1
E	1	0	0	0	0

It is obvious if M has a perfect tree so does $\overline{M}$

which is simply a reordering of the columns. The edges of the tree has to be renamed according to the permutation given on top of the table.

- **Definition**: For any column *k* of *M* , let O_k be the set of objects (rows) that have ‘1’ in column *k.*
- ***Theorem**:* Matrix M has a phylogenetic tree if and only if for every pair of columns *i,j* either O_i and O_j are disjoint or one contains the other.
- Proof: (See class presentation)
  - The proof can be understood easily if we note that each character or attribute appears only once in the phylogenetic tree. Thus, the characters that appear in the left side of a branching point must be disjoint from those appearing on the right side of the same branching point and this is true for every interior node.
  - Second, once a character c appears in an edge, this introduces a ‘1’ in all object vectors that are in the leaves of the subtree below this edge. The succeeding nodes in any path of this subtree must obey the containment requirement.

- A straightforward algorithm to test the validity of the above theorem would take $\Omega(nm^2)$ since there are $O(m^2)$ pairs of columns and testing for the validity of the conditions will take $O(n)$ time for each pair. A more efficient algorithm exists which is described below.

Perfect Phylogeny Algorithm: *O*(*nm*) Time

1. Sort matrix *M* to obtain matrix $\overline{M}$ using radix sort taking *O* (*nm*) time.
2. For each row of $\overline{M}$, construct the character strings corresponding to '1' in the row from left to right. Use the characters of original *M* to write these strings. There will be *n* such strings each of maximum length *m.* (For our example, these strings are '21', '3','215', '34', '2').
3. Build a 'Keyword Tree *T* for these 'patterns' constructed in step 2. This step takes *O*(*nm*) time.
4. Test whether *T* is a perfect phylogeny (each character appears once and only once in an edge in the tree, the tree is rooted and connected).

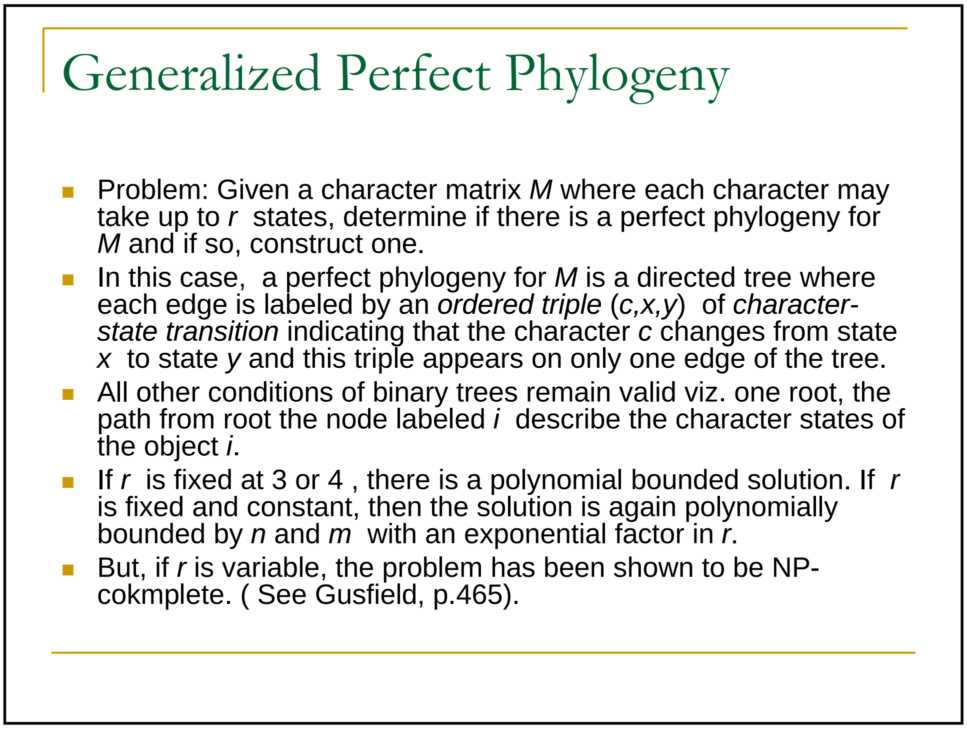
- If the characters are unordered, one can also develop a *O*(*nm*) algorithm to obtain a perfect phylogeny. (Prove)

Tree Compatibility Problem

- Given two or more phylogenetic trees, do they represent a consistent evolution history and if so how to connect these trees into one phylogenetic tree incorporating all the evolutionary history of the constituent trees?
- Problems like this arises in handling real data because people use different tree building methods .

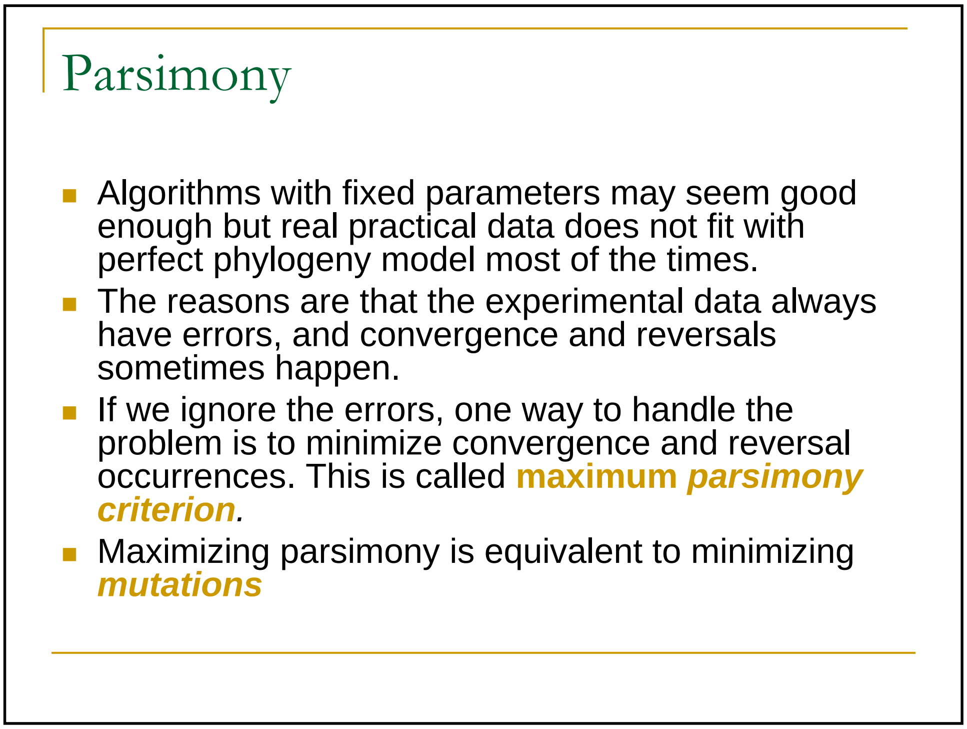
- Definition: A phylogenetic tree *T’* is a refinement of *T* if *T* can be obtained by a series of contraction of edges of *T’.*
  - If *T’* refines *T* then *T’* contains all the revolutionary history contained in *T* and it will have additional history not displayed in *T.*
  - Let *T1* and *T2* be two phylogenetic trees on *n* objects in “reduced” form (both binary trees, no node except the root node can have exactly one child).
- Definition:Trees *T1* and *T2* are **compatible** if there exists a phylogenetic tree *T3* refining both *T1* and *T 2*.

- Let M_1 and M_2 be matrices corresponding to the given trees T_1 and T_2. Let M_3 be a matrix formed as the union of matrices M_1 and M_2. Then, prove
- Theorem: Trees T_1 and T_2 are compatible if and only if there is a phylogenetic tree for M_3. Further, a phylogenetic tree T_3 is a refinement of both T_1 and T_2.
- See Gusfield for example and further discussion.



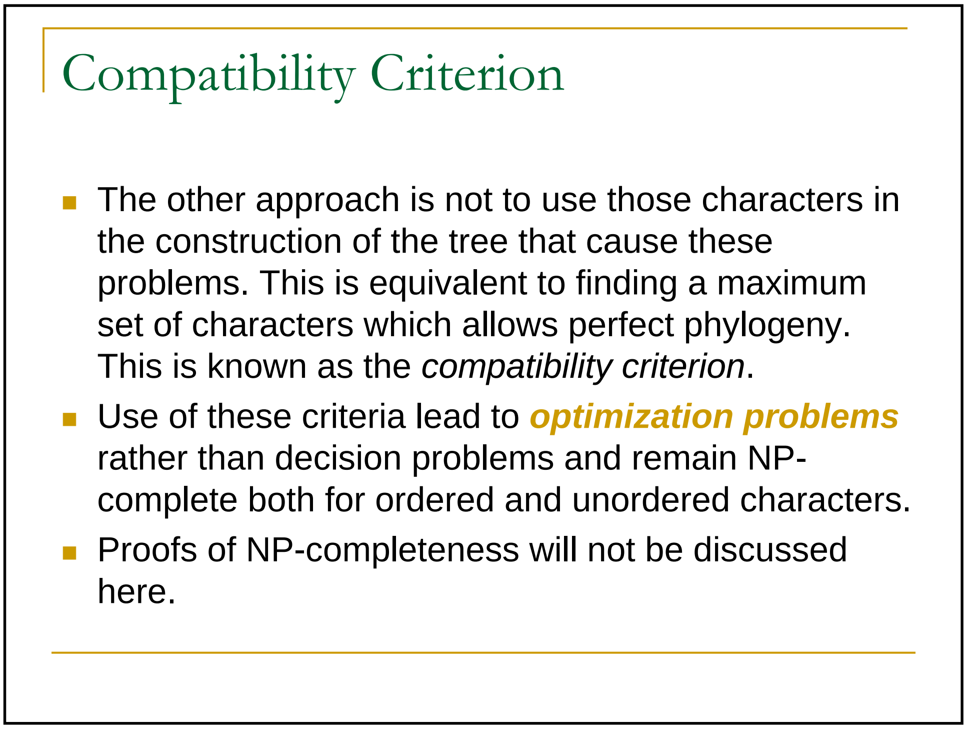
Generalized Perfect Phylogeny

- Problem: Given a character matrix *M* where each character may take up to *r* states, determine if there is a perfect phylogeny for *M* and if so, construct one.
- In this case, a perfect phylogeny for *M* is a directed tree where each edge is labeled by an *ordered triple* (*c,x,y*) of *character-state transition* indicating that the character *c* changes from state *x* to state *y* and this triple appears on only one edge of the tree.
- All other conditions of binary trees remain valid viz. one root, the path from root the node labeled *i* describe the character states of the object *i*.
- If *r* is fixed at 3 or 4 , there is a polynomial bounded solution. If *r* is fixed and constant, then the solution is again polynomially bounded by *n* and *m* with an exponential factor in *r*.
- But, if *r* is variable, the problem has been shown to be NP-cokmplete. (See Gusfield, p.465).



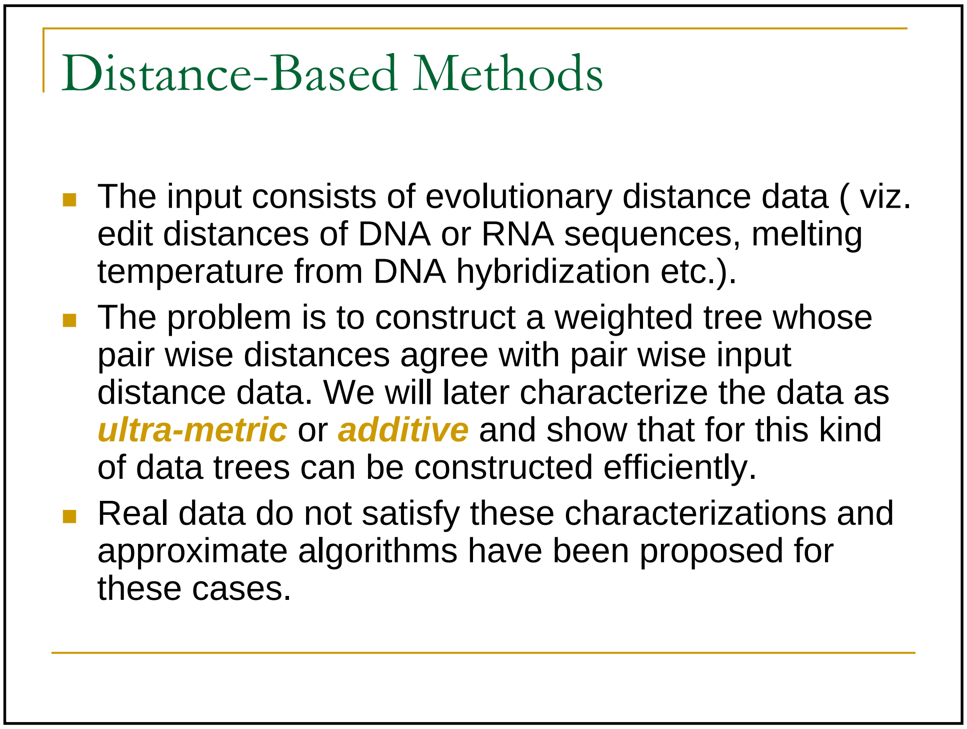
Parsimony

- Algorithms with fixed parameters may seem good enough but real practical data does not fit with perfect phylogeny model most of the times.
- The reasons are that the experimental data always have errors, and convergence and reversals sometimes happen.
- If we ignore the errors, one way to handle the problem is to minimize convergence and reversal occurrences. This is called **maximum *parsimony criterion.***
- Maximizing parsimony is equivalent to minimizing ***mutations***



Compatibility Criterion

- The other approach is not to use those characters in the construction of the tree that cause these problems. This is equivalent to finding a maximum set of characters which allows perfect phylogeny. This is known as the *compatibility criterion*.
- Use of these criteria lead to ***optimization problems*** rather than decision problems and remain NP-complete both for ordered and unordered characters.
- Proofs of NP-completeness will not be discussed here.



Distance-Based Methods

- The input consists of evolutionary distance data (viz. edit distances of DNA or RNA sequences, melting temperature from DNA hybridization etc.).
- The problem is to construct a weighted tree whose pair wise distances agree with pair wise input distance data. We will later characterize the data as ***ultra-metric*** or ***additive*** and show that for this kind of data trees can be constructed efficiently.
- Real data do not satisfy these characterizations and approximate algorithms have been proposed for these cases.

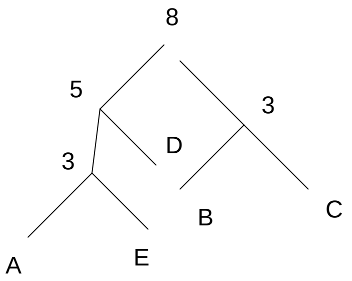
Ultrametric Tree

- Ultrametric trees have applications in many numerically-based tree construction methods, and can be used to find the branching patterns of evolutionary history and measures of elapsed time among nodes in the tree.
- Although the input data is a set of numbers, these numbers are usually the output of some string algorithm such as sequence comparisons or pair wise distance data of multiple alignment of a set of sequences.

- Definition: Let *D* be a *n* by *n* symmetric matrix of real numbers. An ultrametric tree for *D* is a rooted tree *T* with the following properties:
  - *T* contains *n* leaves, each labeled by a unique row of *D.*
  - Each internal node of the tree is labeled by one entry in *D* and has two children.
  - The numbers labeling internal nodes in the tree along any path from the root to leaf are *strictly decreasing*.
  - For any two leaves *i* and *j* of *T* , *D*(*i*,*j*) is the label of the least common ancestor of *i* and *j* in *T*.

Example

	A	B	C	D	E
A	0	8	8	5	3
B		0	3	8	8
C			0	8	8
D				0	5
E					0



•If the property 3) above is changed to : labels in the internal nodes must strictly increase, then the tree is called a ***min-ultrametric tree*** (in absence of any better name).
•Obviously, not all matrices are untra- or min-ultrametric. Since with *n* leaf nodes the tree must have only *n*-1 internal nodes, if the matrix has more than *n*-1 distinct values in *D,* the matrix cannot be untra- or min-ultrametric.

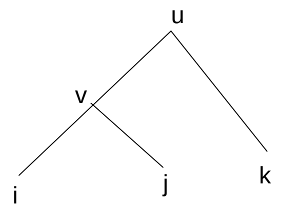
Evolutionary Trees as Ultrametric Trees

- If the evolutionary history of *n* taxa form a rooted directed tree, with extant taxa represented at the leaf nodes, then each internal node can be looked upon as a ***divergence event****.*
- A divergent point is a point *v* in time when two taxa p and q diverge. It simply means that before the point *v* the two taxa *p* and *q* shared a common ancestor.
- If we know the absolute or relative times when the divergences took place and if we append these times at the internal nodes (the branching nodes), these times must be strictly increasing for the nodes in a path from root to a leaf .

- The label at common ancestor *v* of leaf nodes *p* and *q*, is the time when *p* and *q* diverged. Thus, *T* is a min-ultrametric tree for the *n* by *n* matrix *D* and *D*(*p,q*) is the time that *p* and *q* diverged.
- On the other hand, if we want to interpret the label at each node to represent *elapsed time* since the divergent *v* happened, then the times must be strictly decreasing in the path from the root to the leaf node and the corresponding matrix becomes ultrametric.

Test for an Ultrametric Tree

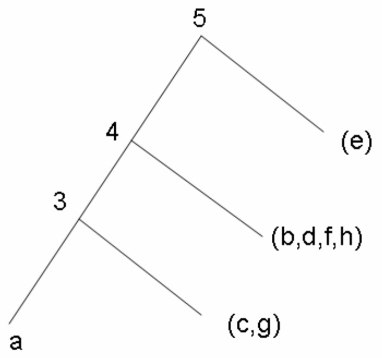
- Definition: A symmetric matrix *D* of real numbers defines an ***ultrametric distance*** if and only if for every three indices *i, j* and *k* , there is a tie for the maximum of *D*(*i,j*), *D*(*i, k*) and *D*(*j,k*). Similarly, *D* defines a *min-ultrametric* distance if and only if for every three indices *i, j* and *k* , there is a tie for the miniumum of *D*(*i,j*), *D*(*i, k*) and *D*(*j,k*).
- **Theorem:** A symmetric matrix *D* has an ultrametric tree (or a min-ultrametric tree) if and only if *D* is an ultrametric (or min-ultrametric) matrix.
  - Proof: First, show that if *D* has an ultrametric tree, then *D* is an ultrametric matrix.



Here *v* and *u* are common ancestors of *i* and *j*, and *i* and *k*, respectively. Since it is an ultrametric tree, *u*>*v*, strictly. By definition of *D*, *D*(*i,k*)= *D*(*j,k*)=maximum. Hence *D* is an ultrametric matrix.

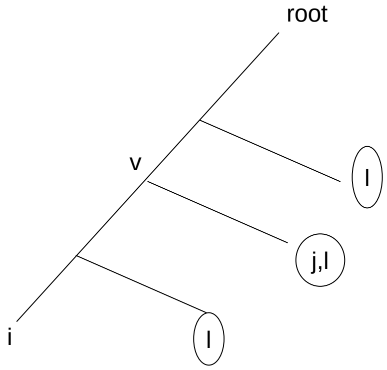
- Now, assume *D* is an ultrametric matrix. We have to show that there exists an ultrametric tree.
- By definition, if in a row *a* of *D*, there are *d* distinct entries, then any ultrametric tree for *D* must have these numbers from the leaf representing the row *a* to the root in strictly increasing order. This also induces a partition of the remaining (besides row a) rows in *d*-1 disjoint partitions, as shown below

	a	b	c	d	e	f	g	h
a	0	4	3	4	5	4	3	4
b		0	4	2	5	1	4	4
c								
d								
e								
f								
g								
h								



Now, if we can find ultrametric tree for each of these partitions, we can simply attach these trees and get an ultrametric tree for the entire matrix. We would like to apply the procedure recursively. We show now that the approach works correctly.

- Consider the class defined by the internal node *v* and assume the leaf node *j* is a member of this class . Let *l* be some other leaf node. We have three cases:
  1. *l* is in the same class as that of *j*: This means that *D*(*i, j*)= *D*(*i,l*). There fore,since *D* is ultrametric. If we can now attach an ultrametric subtree containing *j* and *l*, we are done. If , then node *v* will have degree greater than 2
  2. *l* is in a class between the leaf node *i* and node *v*: In this case, *D*(*i,l*)<*D*(*i,j*) and and *v* must be the least common ancestor of *j* and *l.* Therefore, if the ultrametric tree containing *j* is connected at *v* , then *D*(*j,l*) will be correctly written at the least common ancestor of leaves *j* and *l*.
  3. *l* is in a class between *v* and the root node : In this case, *D*(*i,l*)>*D*(*i,j*) and so . So, if an ultrametric tree for the class containing *j* is connected at node *v* , *D*(*j,l*) will be correctly the least common ancestor of *j* and *l*.



Additional Notes: http://www.cs.ucdavis.edu/~gusfield/ultraerrat/ultraerrat.html

- On page 451, the proof of Theorem 17.1.1 presents an algorithm for building an ultrametric tree. The algorithm is correct, however Theorem 17.1.3 states that the algorithm can be implemented in $O(n^2)$ time.
- In fact, I don't see how to do that, although $O(n^2 log\ n)$ is an easy bound for the algorithm.
- Rather than continuing to try to find a clever implementation of that algorithm, here is another combinatorial algorithm that I claim is correct and that does run in $O(n^2)$ time. The algorithm is described in terms of a graph G, based on matrix D, but it can be implemented without an explicit graph.

- Let each row *i* of matrix *D* be represented by a node *i* in *G*, and each edge (*i*,*j*) be given the value *D*(*i*,*j*). In *O*(n^2) time, the algorithm will find a very particular path in graph *G*:
  - set *N* equal to all the indices 1 through *n*; set *L* to the empty path; set *i* to any node.
  - repeat *n*-1 times: begin remove *i* from *N*; find an index *j* in *N* such that $D(i,j) \leq D(i,k)$ for any *k* in *N*. place edge (*i*,*j*) in the path *L*; set *i* to *j*; end;
- What this produces is a path *L* of exactly *n* edges, and the algorithm can be implemented in *O*(n^2) time. It turns out that *L* is a minimum spanning tree of *G*, but that fact is not needed.

Create Ultrametric Tree Recursively Using L

- Concentrate on an edge (*p*,*q*) in the path *L* with the largest edge weight of all edges in *L*, and let *P* be the set of nodes at or to the left of *p* in *L*, and let *Q* be the set of nodes at or to the right of *q* in *L*.
- The fact that *D* is an ultrametric matrix implies that for any pair of nodes (*i*,*j*) where *i* is in *P* and *j* is in *Q*, *D*(*i*,*j*) = *D*(*p*,*q*).
  - One way to prove this is by induction on the number of edges between *i* and *j* in *L* (applying the ultrametric condition that the in any triangle, the max of the three edge weights is not unique).
- What this means is that in the ultrametric tree we are building (and in any ultrametric tree for *D*), any pair of leaves (*i*,*j*) where *i* is in *P* and *j* is in *Q* must have their least common ancestor at the root of the ultrametric tree, and that root must be labelled *D*(*p*,*q*).

- If there are *k* > 1 ties for the global max edge weight in *L*, then removing those *k* edges creates *k*+1 subpaths of nodes, and applying the above argument, any two nodes *i* and *j* which are in different subpaths must have their least common ancestor at the root of the tree, which again must be labeled *D*(*p*,*q*).
- Hence, any ultrametric tree *T* for *D* must have exactly *k*+1 edges out of *D*, and the leaf set below any such edge must be exactly the (distinct) set of nodes in one of the *k*+1 subpaths.
- No matter what *k* is, removing the *k* max weight edges in *L*, and partitioning *N*, takes only *O*(*n*) time.

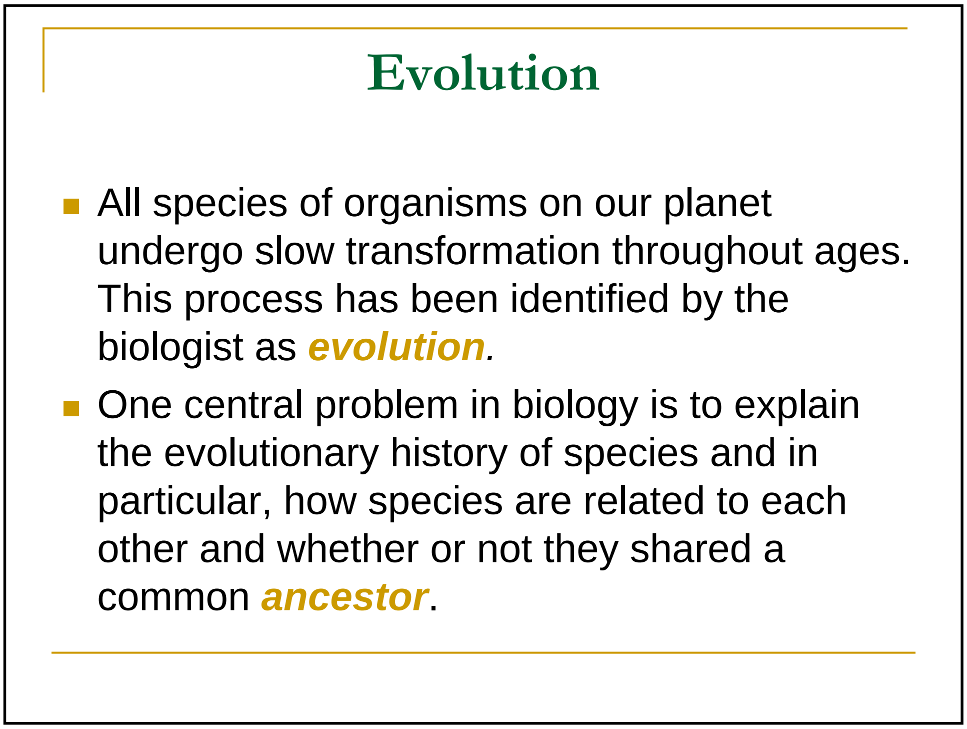
- Assume for convenience that *k* = 1.
- Let LP and LQ denote the two subpaths created by removing the max weight edge in *L*. we want to find an ultrametric tree for set *P* and one for set *Q*; these two ultrametric trees will then be attached to the root to creat the full ultrametric tree for *D*.
- But note that we already have the needed paths LP and LQ that would be created if we were to recursively apply the above method (clearly LP could result if we applied the path building algorithm to *P* alone, and similarly for LQ and *Q*).
- So we only need to find the max weight edge(s) in LP and the max weight edge(s) in LQ. Those two edges can be found in *O*(*n*) total time.
  - because the nodes were partitioned in the first step, this time bound holds even for *k* > 1.

Build Ultrametric Tree in $O(n^2)$

- Note that at each step of the algorithm, the node partitions that are created, and the associated edges that are put into *T*, are forced. Hence if *D* is an ultrametric matrix, the ultrametric tree *T* for *D* is unique.

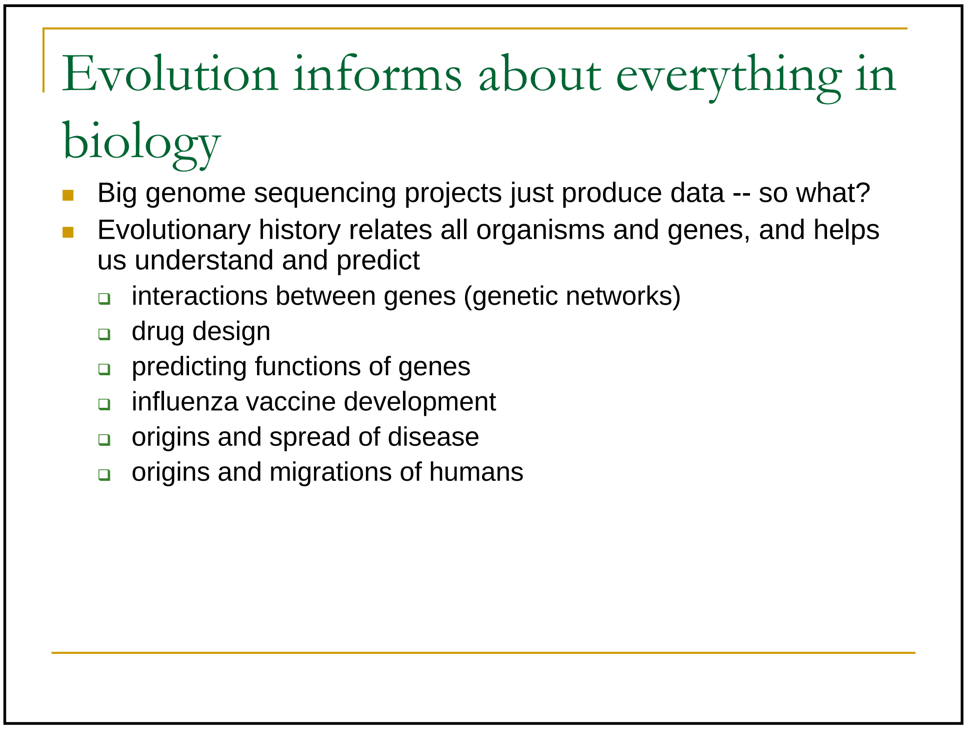
Additive Distance Tree

- If the data giving time-since-divergence is correct, the ultrametric tree gives the true evolutionary history.
- But, in practice, data is rarely ultrametric. This is handled by imposing a weaker requirement on the evolutionary data, that is, data is ***additive***.



Evolution

- All species of organisms on our planet undergo slow transformation throughout ages. This process has been identified by the biologist as ***evolution***.
- One central problem in biology is to explain the evolutionary history of species and in particular, how species are related to each other and whether or not they shared a common ***ancestor***.



Evolution informs about everything in biology

- Big genome sequencing projects just produce data -- so what?
- Evolutionary history relates all organisms and genes, and helps us understand and predict
  - interactions between genes (genetic networks)
  - drug design
  - predicting functions of genes
  - influenza vaccine development
  - origins and spread of disease
  - origins and migrations of humans