Lecture 4 – Characters: Molecular

The application of molecular data to estimating phylogenies also has a long history. In fact, as Joe describes in Chapter 10, analyzing molecular data (frequencies of blood-group alleles) was actually the motivation for Cavalli-Sforza & Edwards to begin development of computational methods for phylogeny estimation. (Figure 10.5 is the first phylogeography I know of & it's from 1963).

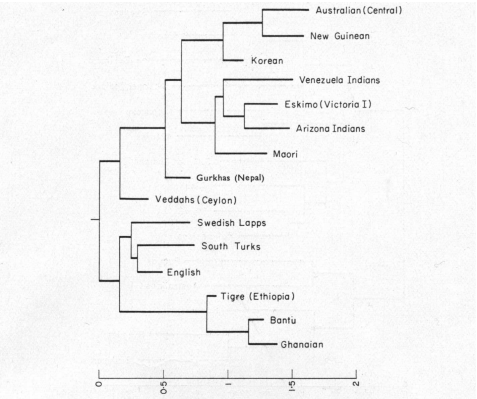


FIG. 5. Tree computed from blood group gene frequencies.

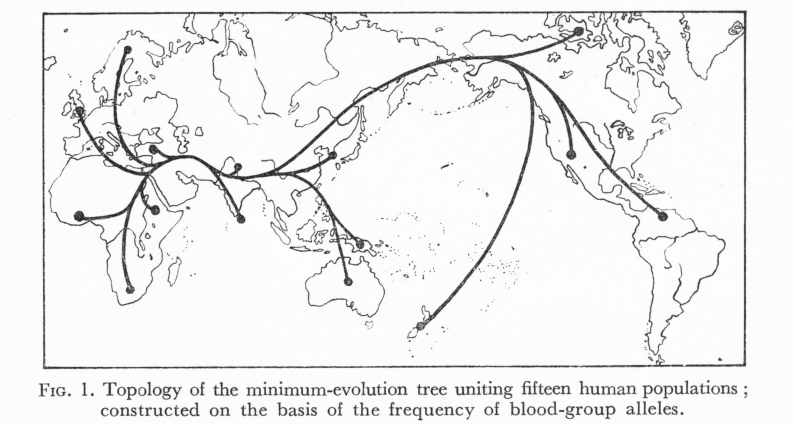


FIG. 1. Topology of the minimum-evolution tree uniting fifteen human populations; constructed on the basis of the frequency of blood-group alleles.

I. Types of Molecular Data: Certainly, the lion's share of molecular data used in phylogeny estimation in is DNA sequence data, but we should examine other types of molecular data, and some of the characteristics of those data that influence phylogeny estimation.

A. Inherently Distance-based Data

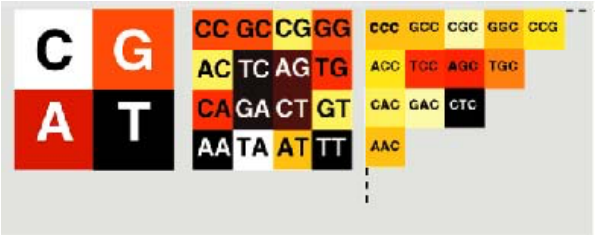
There are molecular phylogenetic approaches in which the nature of the data produced is a matrix of $(n^2-n)/2$ pairwise distances. The units for these distances vary, but the matrix can then be subjected to a number of potential phylogenetic analyses.

	cwk1056	eaa292	cwk1025	eaa448	dsr5032	eaa028	fac1117	cwk1007
cwk1056	-------							
eaa292	0.05840708	-------						
cwk1025	0.01769911	0.05398230	--------					
eaa448	0.08672567	0.08141593	0.08230089	--------				
dsr5032	0.02566372	0.05929204	0.01946903	0.08495575	--------			
eaa028	0.06725664	0.07433628	0.06371681	0.07522124	0.07168142	--------		
fac1117	0.02123894	0.05575221	0.00530973	0.08053097	0.02123894	0.0637168	--------	
cwk1007	0.05221239	0.02920354	0.05132743	0.08230089	0.05486726	0.07610620	0.05132743	--------
eaa667	0.05840708	0.01238938	0.05221239	0.07787611	0.05752213	0.07433628	0.05398230	0.02743363

Again, historically inherently distance based methods have included things like DNA-DNA hybridization and immunological distances. We won't focus on those because they're never used anymore. However, as I mentioned, information on comparative genomics may be presented as inherently distance data.

An example of a simple genomic distance is provided by Edwards et al. (2002; Systematic Biology, 51:599).

This method is based on taking large amounts of sequence data that is **assumed to be a random sample from each respective genome** and calculating the frequency of *n* bp words (sometimes called *k*-mers) in each taxon. If $n = 1$ there are 4 "words", G, A, T, C (so the data are the base frequencies). If $n = 2$ there are 16 possible dinucleotide words (4^n).



Edwards et al. (2002) use 5 bp words, so there are $4^5 = 1024$ possible words, and the frequency of each word is calculated from the genome sample for each OTU.

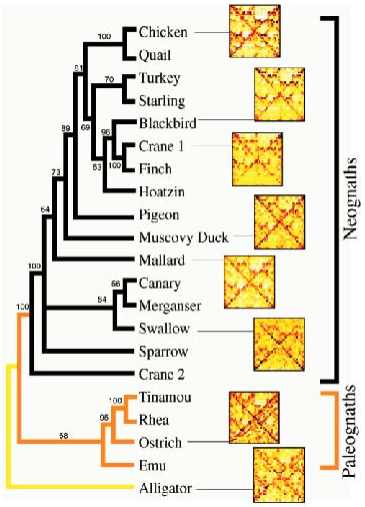
The frequency of each of the 1024 penta-nucleotides is calculated.

The Euclidian distance between each pair of genomes in calculated to generate a distance matrix.

$$d_{ij} = \left\{ \sum_{x=1}^{1024} (f_{xi} - f_{xj})^2 \right\}^{1/2}$$

where f_{xi} is the frequency of word x in taxon i and f_{xj} is the frequency of word x in taxon j.

This matrix is then subjected to one of a number of tree estimation methods (that we'll cover later).



The well-established deep split in bird phylogeny (Paleognathous birds) is reflected in the genomic signature.

It remains to be seen to what extent such approaches will find application in phylogenetics. There are a number of potential problems (calculating distances based on non-homologous characters), and these are discussed in the paper. Even though it's over 18 years old, it's still a good read as an introduction to alignment-free genomic approaches.

B. Potential Character Data – Many molecular approaches are amenable to erecting a character-by-taxon matrix.

1. Allozymes (isozymes) represent one of the first types of molecular character data. Allozymes are simply allelic forms of enzymes that differ in electronic charge (because of an amino-acid substitution) and have different electrophoretic mobility. {For the math/c.s. folks, electrophoresis is a standard lab procedure in which molecules (e.g., proteins or DNA fragments) in a solution are subjected to an electromagnetic field, usually through some type of gel matrix. These molecules migrate through the matrix at a rate that is dependent on size and ionic charge.}

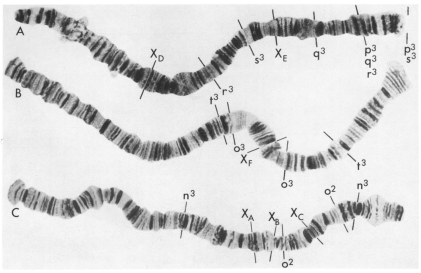
Throughout the 70's and 80's, allozymes were the most widely used molecular data type, and a ton was written on how best to use the data this approach provided. It's not used much for phylogeny anymore but is still used some in population genetics.

A good review was provided by Murphy et al. (1996; pp. 51-120 in *Molecular Systematics*, 2nd Edition [Hillis et al., eds.] Sinauer).

2. Another data type, that's seeing a bit of a resurgence, is **chromosomal data**.

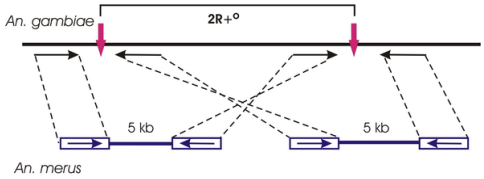
Historically, these data ranged from simple karyotypes (which included things like counts and gross morphology of chromosomes), to FISH (fluorescent in-situ hybridization), which can be used to identify the locality of genes of interest on the complement of chromosomes.

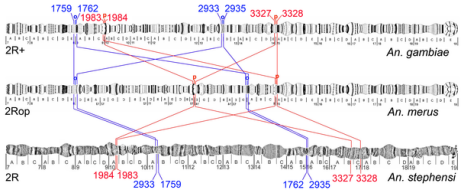
Usually some inference is made with respect to inversion events, and the events are used as characters. A great early example is Carson (1983. Genetics 103: 465), who applied inferred inversions of g-banded polytene chromosomes to the phylogeny of Hawaiian *Drosophila*.



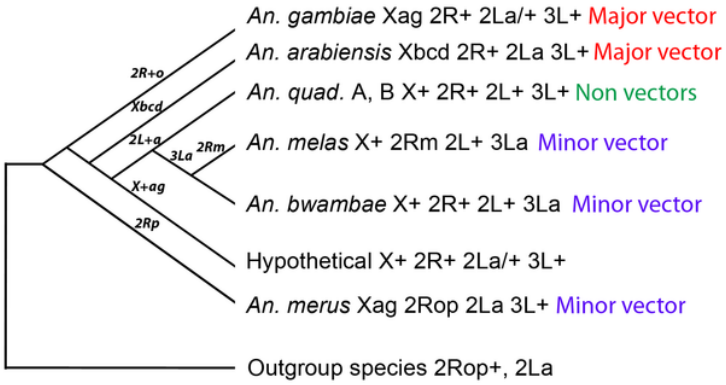
A good overview of these techniques was provided by Sessions (1996. pp121-167 in *Molecular Systematics*, 2nd Edition [Hillis et al., eds.] Sinauer).

A more recent example is from Kamail et al. (2012. PLoS Pathogens), in which the authors use sequences flanking break points, to produce a very well supported reconstruction of inversions among several species of *Anopheles*, which are the vectors of human malaria.





They then use these in a classical Hennigian analysis that indicates the major vectors are not sister species.



3. The most common type of molecular data is **sequence data**. These can obviously be from DNA or from proteins.

a. Gene sequences (DNA sequences) are the most common.

This is quite straightforward; nucleotide positions are the characters, and the four nucleotides are the character states (there are only four possible states).

We'll spend the majority of the semester with this type of data.

b. Protein sequences (amino-acid sequences) are also used, particularly to estimate old phylogenetic relationships.

So, amino-acid positions are the characters and the 20 amino acids are the character states. There's a much bigger character state space, and this is seen by some as an advantage. However, a disadvantage is that there may be more than one path of nucleotide substitutions from one amino acid to another, and this has led some to view this as an inferior character type.

Typically, nucleotide sequence data are collected and amino-acid sequences are inferred. This is due to the fact that the DNA sequencing technology is far superior.

There have been controversies over which approach is better. Even if nucleotide data are generated, it's easy enough to translate them into protein sequences and analyze these data.

4. Higher order molecular characters – Rare Genomic Changes.

There are several different types of higher order molecular characters. A good (but aging) review is provided by Rokas and Holland (2000. TREE, 15:454).

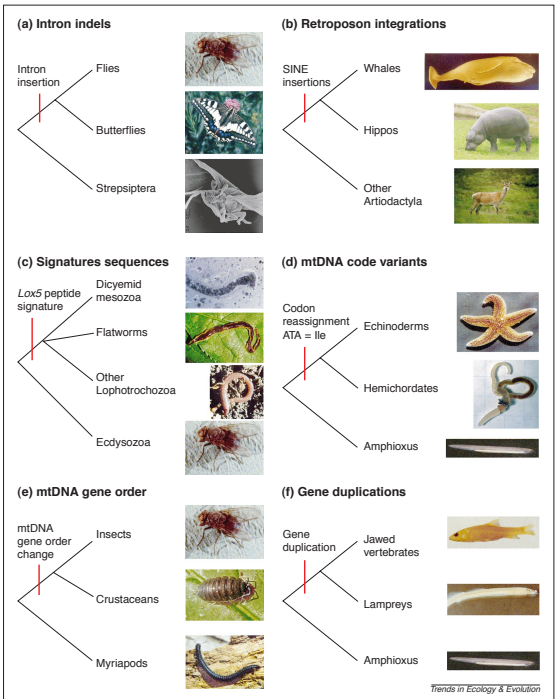
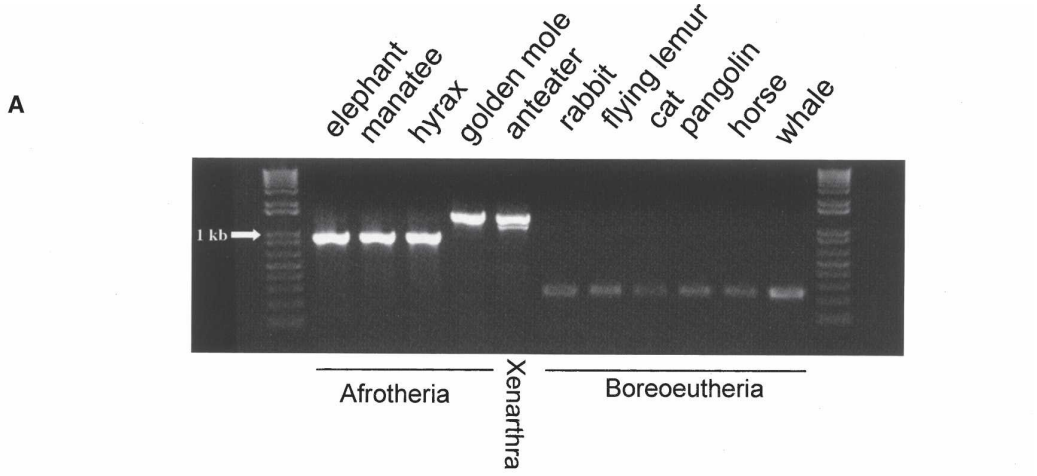


Fig. 1. Examples of rare genomic changes (RGCs) as phylogenetic markers. (a) An intron insertion in the gene *engrailed* suggests that Strepsiptera are not a sister group to Diptera (flies)[14]. (b) Retroposon integrations using short interspersed elements (SINEs) have established the sister group relationship of whales and hippos, to the exclusion of other Artiodactyla[20]. (c) Hox gene signature sequences have robustly supported the division of protostome invertebrates into the lophotrochozoan and ecdysozoan clades[25], and have identified dicyemid mesozoa as lophotrochozoans[26]. (d) A codon reassignment in the mitochondrial genetic code suggests that echinoderms and hemichordates are sister groups[34], a result supported by sequence data[35]. (e) mtDNA gene order in mitochondrial genomes supports the common grouping of insects and crustaceans, with myriapods as an outgroup[28]. (f) Repeated events of gene duplication have occurred in the lineage leading to vertebrates[46]. *Photographs reproduced, with permission, from G. Brown (fly)/whale/flatworm/earthworm/crustacean/myriapod/starfish/fish/lamprey), S. Ferguson (hippo), P. Holland (butterfly), A. Rokas (Strepsiptera), M. Kobayashi (mesozoa), D. Remsen and the Marine Biological Laboratory (hemichordate) and J. Pemberton (deer).*

a. Insertions/Deletions in/of introns.

Most of the examples I've seen apply these characters to already existing phylogenetic hypotheses.

For example, Murphy et al. (2007. Genome Res., 17: 413) collected comparative genomic data on several mammals to try to test alternative placements at the root of placental mammals.

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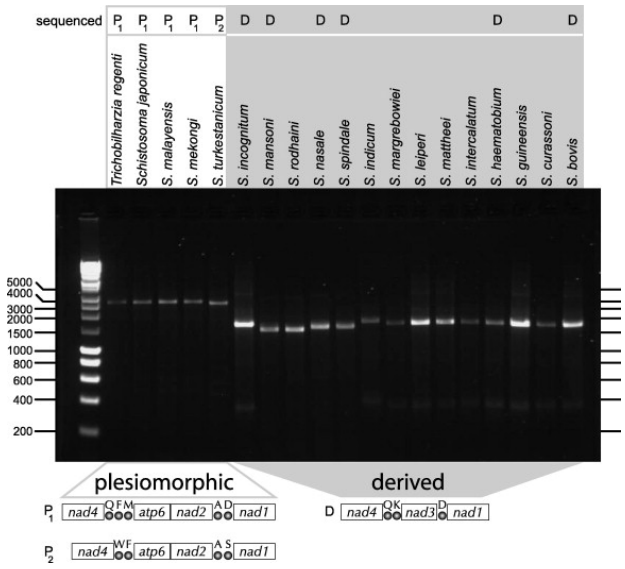
human          CAAAGGTAGGGAAACTTTTTGCCTTGAAA----------------------------//--------------------------CTA-ACCCCTCTCTTAAATCATCCTCAGATG
macaque        CAAAGGTAGGGAAACTTTTTGCCTTGAAA----------------------------//--------------------------CTA-ACCCCTCTCTTAAATCATCCTCAGATG
flying lemur   CAAAGGTAAGGAAACTTTCTGCCTTGAAA----------------------------//--------------------------CTA-ACTCC-CTCTTAAGTCATCCGTAGTTG
mouse          CAAAGGTAGGAACACTGTCTTGCCTGAAA----------------------------//--------------------------AAA-TAT-CTCTGTTGA-------TCAGAGG
chipmunk       CAAAGGTAGGGAAATTTGCTGCCTTGATA----------------------------//--------------------------TGA-AC--CCCATTTAAA------TCACATG
squirrel       CAAAGGTAGGGAAATTTTCTACCTTGAAA----------------------------//--------------------------TGA-AC-TCCCACTTAAA------TCATATG
guinea pig     CAAAGGTATGCAAACTTTCTGCCTTAAAG----------------------------//--------------------------ATACACTTCCCACTTAAG------TCAGATG
rabbit         CAAAGGTAGGAAAGCGTCCTGCCTCGACA----------------------------//--------------------------CTA-AC-CCCCTCTTAAATCATACCCCAATG
whale          CAAAGGTAGGGAAACTTTCTGCCTTGAAA----------------------------//--------------------------CTA-ACCCCC-TTTGAAATCATTCCCAGATG
cow            CAAAGGTAGGGAAACTTTCTGCCTTGAAA----------------------------//--------------------------CTA-ACCCCCCTTTGAAAGCATTCCCAGATA
horse          CAAAGGTAGGGAAACTTTCTGCCTTGAAA----------------------------//--------------------------CTA-ACTC-TCTCTTAAATCATCCCCACATG
cat            CAAAGGTAGGGAAAATCTCTTTGTTGAGA----------------------------//--------------------------CTA-ACTC-TCTCTTAAATCAT-CCCAGATG
dog            CAAAGGTAGGGGAACTTCCTGCCCTGAAA----------------------------//--------------------------CTA-AGCCCC-TCTTAAATCAT-CCCAGTTG
pangolin       CAAAGGTAGGGAAACTTTCTGCCTTGAAA----------------------------//--------------------------CTA-ACCCCC-TCTCAAATCATCCCTAGATG
bat            CAAAGGTAGGGAAACTTTCTGCCTGGAAA----------------------------//--------------------------CTA-CCCCCCACTTTAAGTCATCCCCGGATG
shrew          CAAAGGT-GGGAAACTTTCTTCCTTGAAA----------------------------//--------------------------CTA-ACCCCTTTATAAGGCCAT-CCCAGATT
hedgehog       CAAAGGT-AGGAAACTTTCTGCCTCGAAA----------------------------//--------------------------CTC-AC--------TTAAATGAT---CAGATT
armadillo      CAAAGGTAAGGAAACGTTCTGCCATGAAATTATTCCCCTATTTTTTTTT-TGAGGTG//----------------------------------------TCTTAAATCATTCCCAGATG
anteater       CAAAGGTAGTGAAACTTTCTGCAATGAAACTATTTCTGTCTTCTTTTTTTTTTAGGTG//GAAGATTCCAATTTCTACACACCCAGCTA-ACCCCC-TCTTTAATCACTCCCAGATC
sloth          CAAAGGTAGGGAAAATTTCTGCTATGAAGCTATCACCCTCTTTTTTATA-TG---TG//----------------------------A-ACCCCC-TCTTAAATCACTCCCAGATG
Afr.elephant   CAAAGGTAGCGAAACCTTCTGCCATGAAACAATCTCCCACTTATTTTTACTGAGGTG//AAAGGTTCCAATTTCTCCACATT-ATCTA-ATCCCC-TCTTAAATCACCCCCAGATG
Asi.elephant   CAAAGGTAGCGAAACCTTCTGCCATGAAACAGTCTCCCACTTATTTTTACTGAGGTG//AAAGGTTCCAATTTCTCCACGTT-ATCTA-ATCCCC-TCTTAAATCACCCCCAGATG
manatee        CAAAGGTAGAGAAAGCTTCTGCCATGAAACAATCCCCCACTTATTTTTATTGAGGTG//AAAGGTTCCAATTTCTCCACATT-ATCTA-ATCCCC-TCTTAAATCACTCCCAGATG
hyrax          CAAAGGTAGGGAAACCTTCTGCTGTGAAAAAATACCCCATTTATTTTTTATAGAGGTG//AAAGCCTCCAATTTCTCCACATT-GTCTA-ATCCCC-TCTTAGATCACTCTCAGATG
tenrec         CAAAGGTAGGGAAACATTCTACCATGACACAATCCCCCA-TTAATTTTATTGCGTTG//AAAGTTATCAATTTCTGAATATC-ATCTA-ATCCCT-TCTTAAATTACCCCCAGGTG
golden mole    -------------------------------------GACTTATTTTTATTGCGGTG//AAAGGTTCCAATTTCTCCACATC-GCCTA-ACTCCC-TCTTGAATTACCCGCAGATA
```

They identified lots of these sites to support a particular resolution in the deepest part of the tree, that is, that Afrotheria & Xenarthra form a clade that is the sister-taxon to the rest of the placental mammals.

b. Gene order – lots of work has been done on inferring phylogenies from gene order.

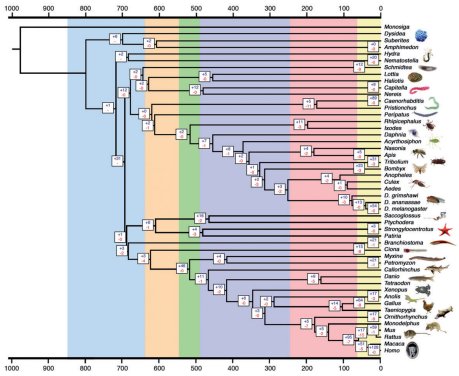
These have often focused on organellar genomes (e.g., rearrangement of genes in mtDNA genome), and here again, shared rearrangements can be treated as Hennigian

synapomorphies. (Example – Webster & Littlewood. 2012. Int. J. Parasit. 42:313-321.) Similar to the chromosomal inversion approaches we just addressed.



c. microRNA Profiles – miRNAs are small (~70 bp) non-coding RNA that form hairpins. These will bind to mRNA and inhibit translation; this is an important regulatory mechanism during development in metazoans (Reviewed by Tarver et al., 2013. MB&E, 30:2369).

They're said to be lost only rarely, and not susceptible to convergence, so these are the most recent "next big thing."



In fact, a science writer at *Nature* has gone all in with these (Dolgin 2012. Nature, 486:460)

However, critical examination of the utility of miRNA leads to a more measured conclusion (Thompson et al. 2014. doi 10.1073/pnas.1407207111; Penny. 2013. GB&E, 5:819).

Losses are more frequent than reported, there is large heterogeneity in rates of gains and losses, there's ascertainment bias, and silly mistakes have been made in interpretation (i.e., with respect to rooting).

d. Gene Content - Increasingly**,** gene-content comparisons have been applied to the growing database of prokaryotic genomes.

Here, the methods are distance based (and the term "genomic distance," as opposed to "genetic distance," is used).

Some distances are simple comparisons of the number of shared genes, such as this:

$$D_{i,j} = 1 - \left(\frac{Genes_i \cap Genes_j}{Genes_i \cup Genes_j}\right).$$

The numerator is the number of genes shared by a pair of genomes (the number of genes in the *intersection* of the two sets of genes) and the denominator is the sum of the number of shared genes and the numbers of genes unique to each genome (the number of genes in the *union* of the two sets of genes).

Other distances are more complex and try to measure the number of transformations (gene loss, duplications, gene gain via HGT, etc.) required for two genomes to be identical in terms of content (e.g., Bohnenkamper et al. 2021. J. Comp. Biol. 28. doi:10.1089/cmb.2020.0434).

So, among many molecular systematists, there's a great deal of enthusiasm for these RGC approaches. The idea is that many of them are caused by changes in complex molecular genetic machinery (e.g., DNA repair mechanisms) and are unlikely to be subject to homoplasy

(convergence and/or reversal). Therefore, they should provide great classical Hennigian synapomorphies.

My opinion is less enthusiastic for two reasons. First, by their nature RGC's are rare and we shouldn't expect to find enough of them to provide resolution of very large phylogenies (and phylogenies are growing all the time). That is, for *n* taxa, there are *n*-3 internal branches in an unrooted tree; for a modern analysis of moderate size (say 80 taxa), we would need 77 synapomorphies and have them distributed perfectly across the tree (i.e., one on each internal branch).

Second, because they're rare, we often don't have enough information to model them and it's therefore difficult to apply statistical phylogenetics.

C. More and more genomicists are trying to get away from alignment-based phylogenies (e.g., Bromberg et al. 2016. PLoSCompBiol, 12(6): e1004985.) In many ways Scott Edwards was ahead of the curve, although the genomicists don't cite the phylogenetic literature (and the reverse is probably true).

II. Homology of Molecular Characters – We need to focus on issues of homology, just as we did for morphology. For molecular characters, there are two levels of homology that we have to deal with.

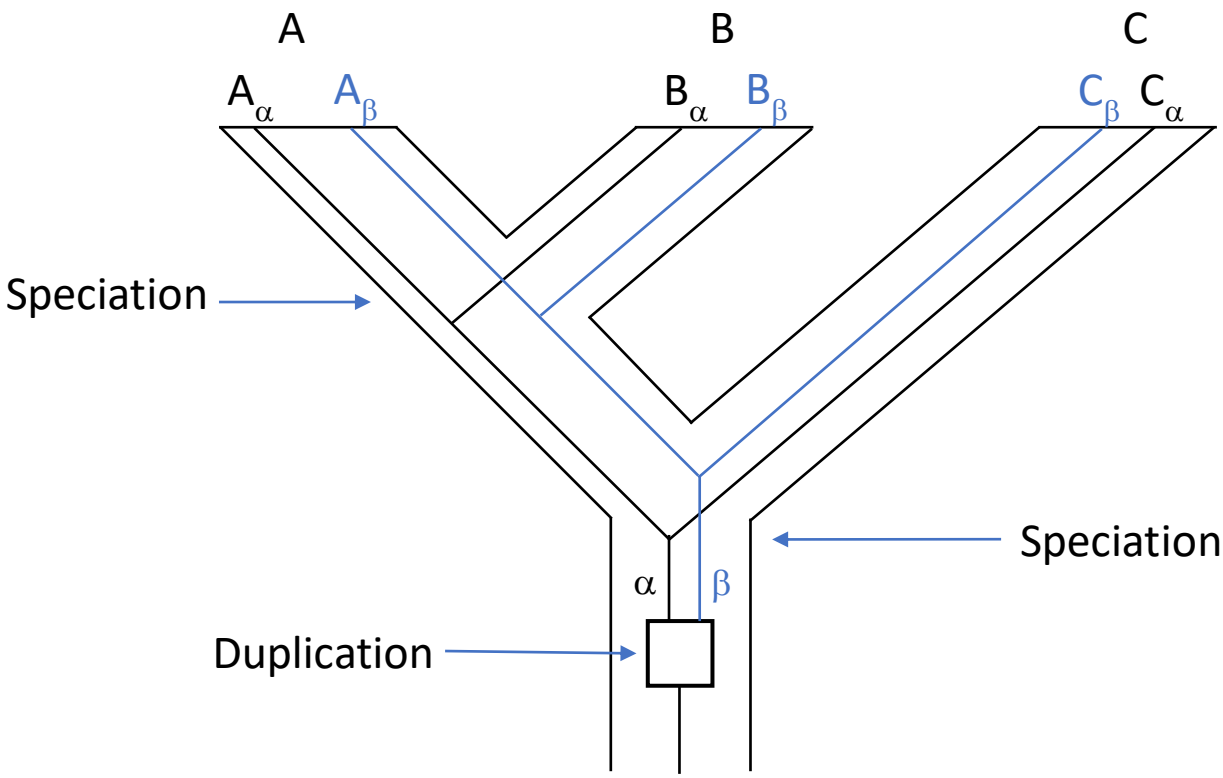
A. Alignment and homology of characters (e.g., nucleotide sites).

Because of its centrality to molecular phylogenetics, alignment will be addressed separately in Lecture 5. Alignment of sequences determines positional homology (or, more precisely, provides hypotheses of positional homologies).

B. At a higher level, we have to worry about homology of the source of the sequence data (i.e, homology of the genes).

Remember that homology is similarity due to descent from a common ancestor.

Paralogy is homology due to gene duplication.
Orthology is homology due to speciation,
Gene duplication has been an incredibly important and widespread evolutionary process.



It's easy to demonstrate that if a mixture of orthologous and paralogous genes are compared, the true history of the genes (a.k.a. the gene tree) won't correspond to the species phylogeny (a.k.a. the species tree) that we're trying to estimate.

This isn't too much of a problem if all copies are present in all species and orthology can be assigned.

This can be relatively straight-forward if the time between the duplication(s) and the first speciation is long (e.g., hemoglobin genes).

It can be very difficult if this time is short.

However, if copy loss has occurred in a different manner in different members of a phylogenetic study, it would create big problems.

Because gene duplication is such a widespread phenomenon, there have been several methods devised to try to take advantage of them a character data. The community is just starting to model the process now, so statistical approaches should be forthcoming.