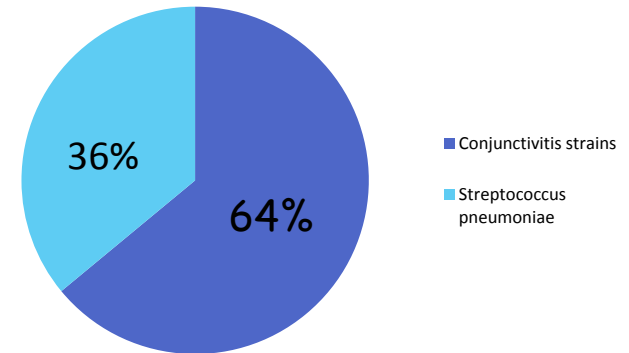


03-727 Project Description

Did horizontal transfer contribute to colonization of the human eye by *Streptococcus pneumoniae*?

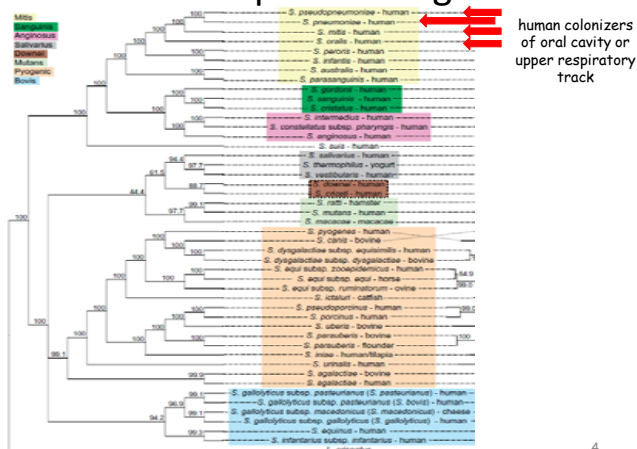
1

Streptococcus pneumoniae is a major cause of conjunctivitis



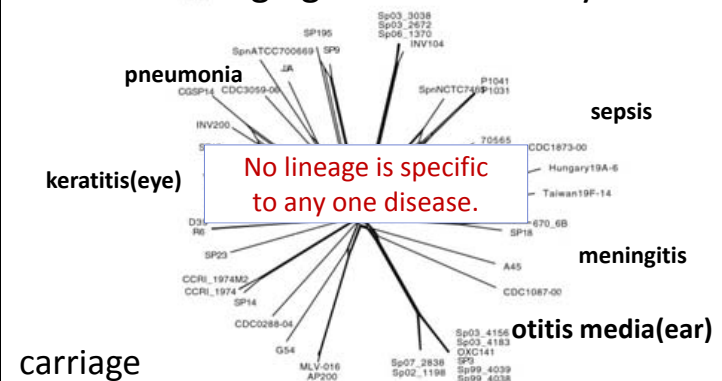
3

Streptococcus pneumoniae in the context of Streptococcal genus



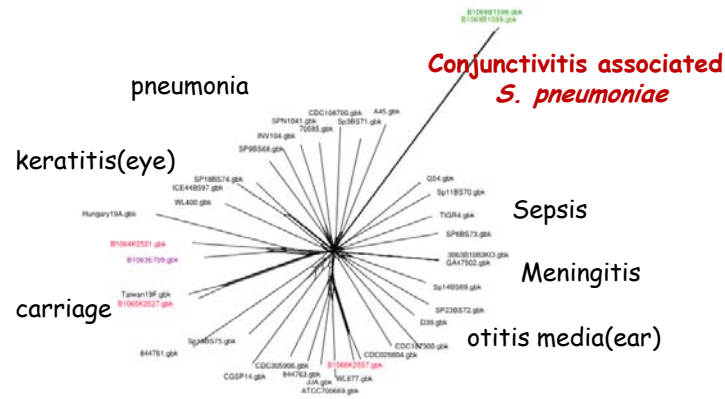
4

S. pneumoniae: wide range of diseases and high genomic diversity



5

First time: link between genomic background with disease phenotype



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Big picture

- What makes conjunctivitis strains unique? Hiller
- Investigating a candidate gene for the conjunctivitis phenotype Hiller, Antic
- What is the origin of this promising candidate?
 - Phylogenetic distribution YOU
 - Inferring the history of transfers

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Outline

- What makes conjunctivitis strains unique?
- Investigating a candidate gene for the conjunctivitis phenotype
- What is the origin of this promising candidate?
 - Phylogenetic distribution
 - Inferring the history of transfers

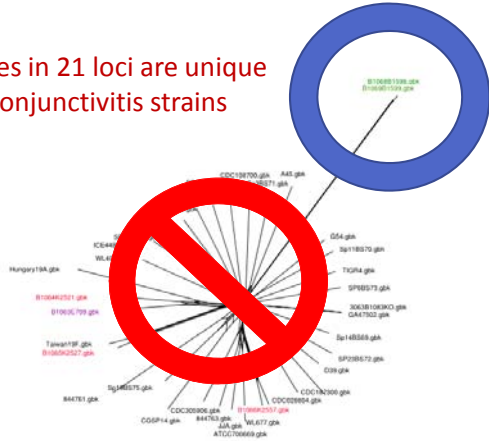
9

- Are there genes unique to conjunctivitis-associated *S. pneumoniae* strains?
- Do conjunctivitis specific genes allow colonization of the human eye?

10

Identified genes present in conjunctivitis strains and absent in other *S. pneumoniae* strains.

93 genes in 21 loci are unique to conjunctivitis strains



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Gene X: A promising candidate that is unique to conjunctivitis strains

- Hypothetical protein
- Surface associated : contains a surface localization motif
- Similarity to host mucus binding proteins: contains an agglutinin receptor domain



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Outline

- What makes conjunctivitis strains unique?
 - 93 genes, including Gene X
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Experimental analysis:

Does deletion of Gene X disrupt adhesion?

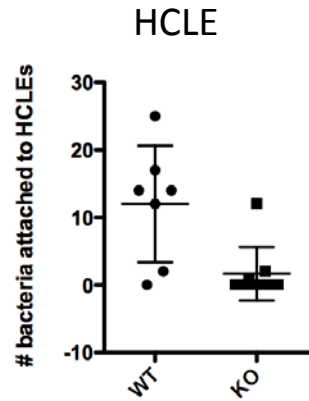
Assayed adhesion to human corneal limbal epithelial (HCLE) cells



- Count number of bacteria attached to HCLES

Collaboration between Hiller and Shank Lab.

Preliminary data: Gene X deletion mutant displays decreased binding to HCLE



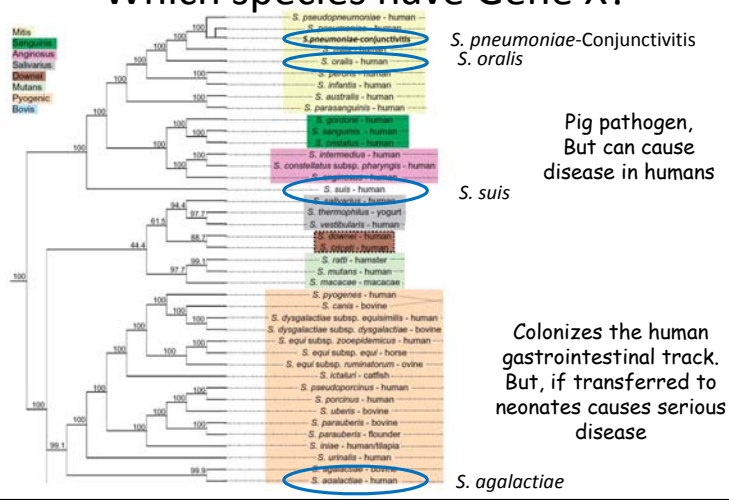
Collaboration between Hiller and Shank Lab

Outline

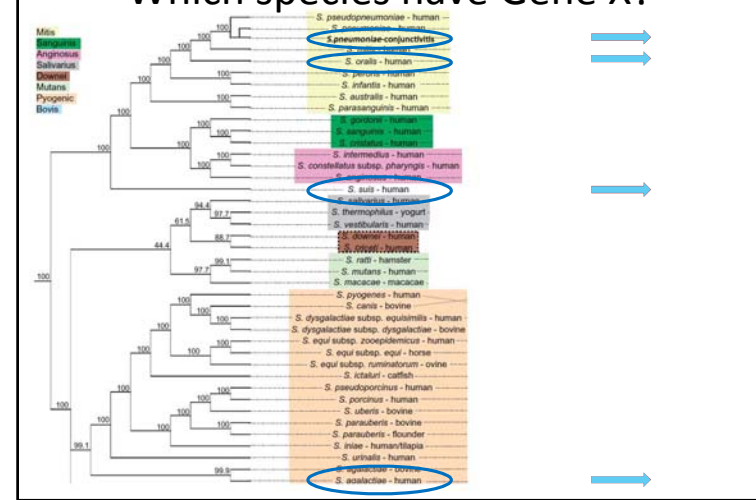
- What makes conjunctivitis strains unique?
 - 93 genes, including Gene X
- Investigating Gene X for its role in the conjunctivitis phenotype
 - Preliminary Experimental data: decreases in adhesion to HCLE for Gene X mutants
- What is the origin of this promising candidate?
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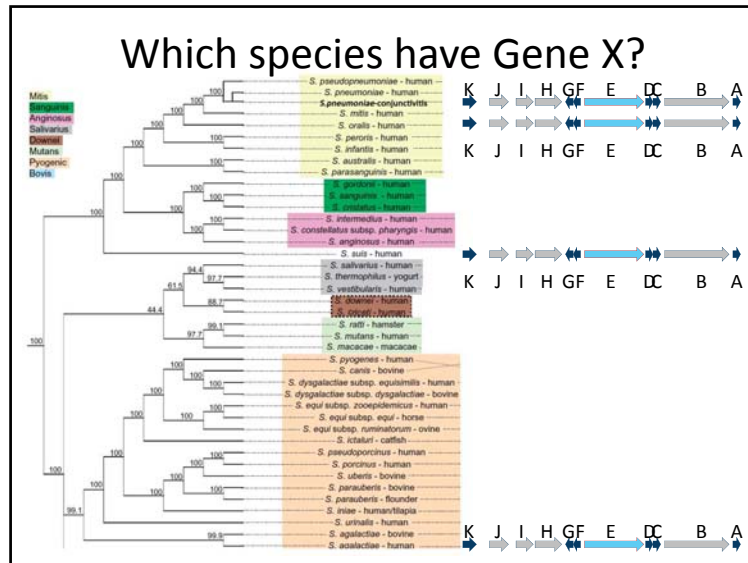
18

Which species have Gene X?



Which species have Gene X?



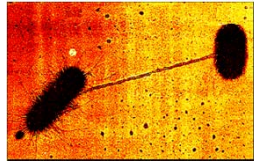


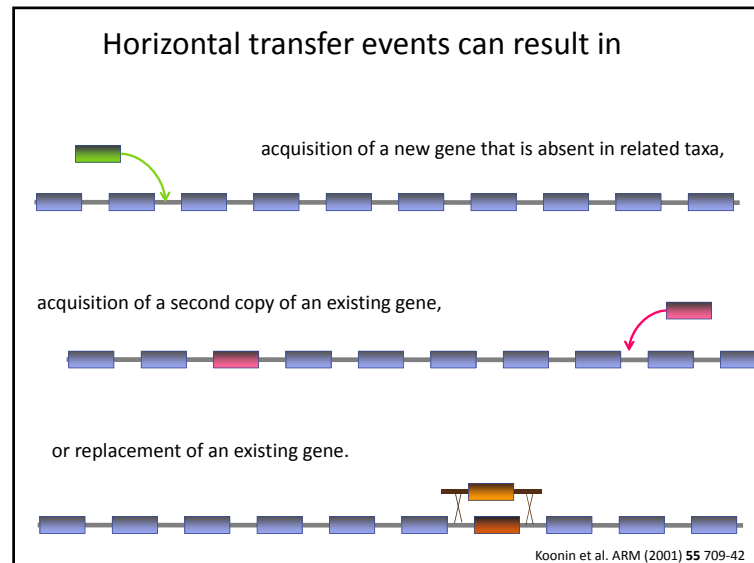
Hypothesis: Gene X was acquired by Horizontal Gene Transfer (HGT)

Quick review

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Mechanisms of horizontal transfer

- 1) Transformation** – prokaryotes can take up free DNA from their surroundings
- 2) Conjugation** – plasmids are transferred through a tube-like structure, called the pilus.
 
- 3) Transduction** – genes can be moved from one prokaryote species to another via viruses.



Genomic view of microbiology

- Gene content varies dramatically between individuals within the same species/population.
- Bacterial infections are diverse, polyclonal populations.

Pan-genome:

The complete set of all genes in a species.

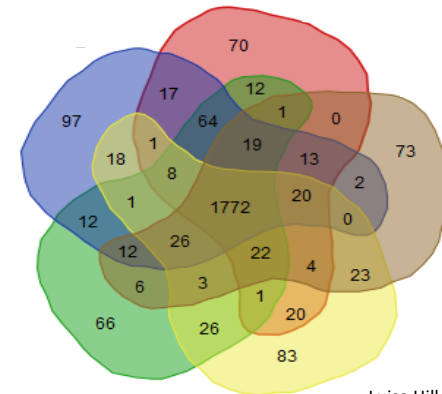
Core genome:

Genes shared by ALL strains in a species.

Distributed genome:

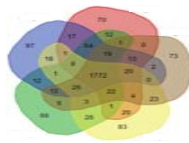
Genes found only in a subset of strains in a species.

Result: Extensive Genic Differences among Strains



Extensive Variability in the *Streptococcus pneumoniae* Pangenome

44 *S. pneumoniae* strains

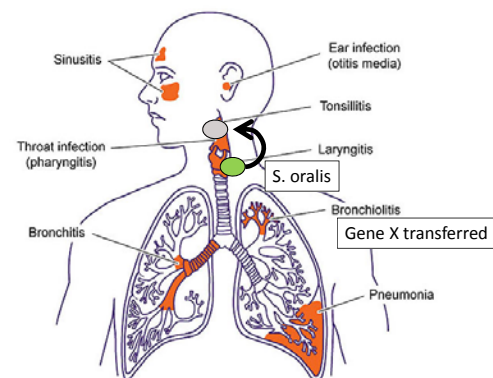


Genes in pangenome	3221
Core genes	1666 (52%)
Distributed genes	1555 (48%)
Average genes per strain	2107

Luisa Hiller

Donati et al., 2011

Hypothesis: horizontal transfer of Gene X leads to niche expansion



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Hypothesis: Gene X was acquired by Horizontal Gene Transfer (HGT)

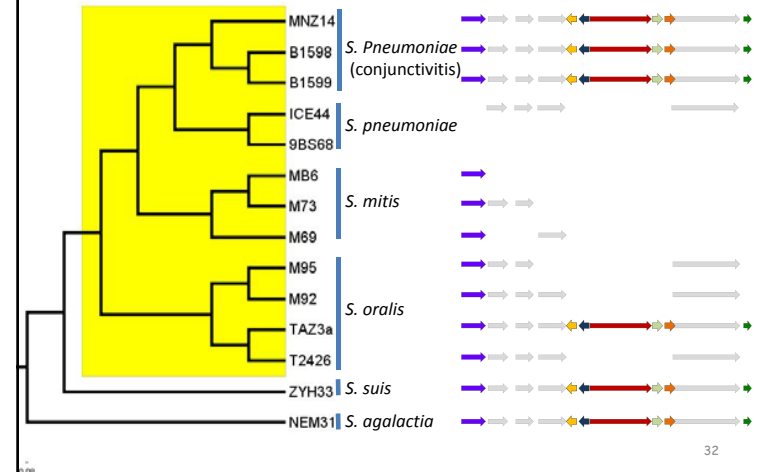
- What is the region that was transferred?
- What is the phylogenetic evidence to support the hypothesis?



The light grey genes are not unique to conjunctivitis strains. Were they part of the transfer?

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Phylogenetic distribution of the genes



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Hypothesis: Gene X was acquired by Horizontal Gene Transfer (HGT)

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What is the phylogenetic evidence to support the transfer hypothesis?

For each of the 11 genes in locus

- Search for other gene family members in *S.oralis*, *S.pseudopneumoniae*, *S.pneumoniae*, *S.mitis*,
- Construct a gene family tree
- Compare gene tree with species tree to infer horizontal transfer events



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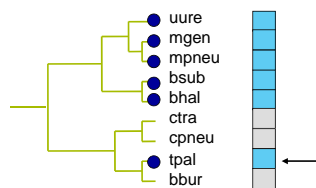


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Detecting Horizontal Transfers

- Unusual phyletic pattern
- Conservation of gene order
- Anomalous DNA composition
- Unexpected phylogenetic tree topology

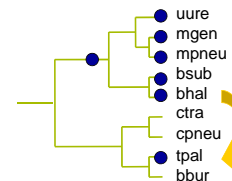
Unusual phyletic pattern



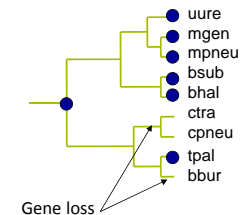
- Independent origin? – (for SNPs, fusion events)
- Gene loss?
- Horizontal transfer?

Problems for detection by incongruent tree topology Ruling out other sources of tree disagreement

Hypothesis 1:
Horizontal transfer



Hypothesis 2:
Lineage-specific gene loss

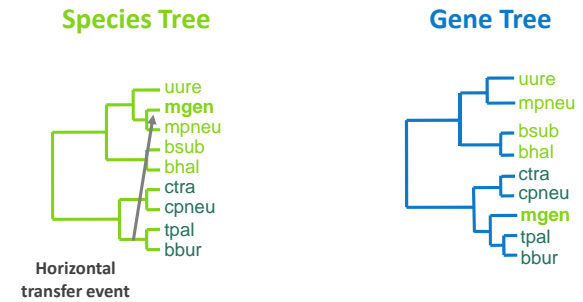


Transfer is the most *parsimonious* explanation because only one transfer event needs to be hypothesized while two gene loss events need to be assumed in the gene loss model.

Detecting Horizontal Transfers

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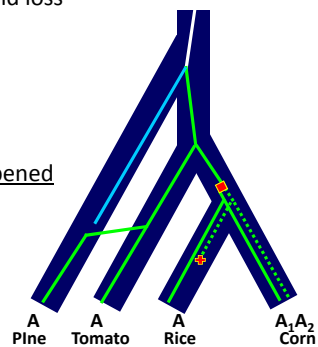
The signature of horizontal transfer is incongruence between the species tree and the gene tree



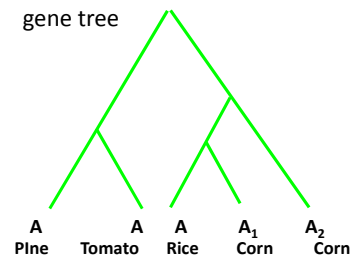
Gene family evolution in the context of a species tree

- Sequence evolution
- Gene duplication and loss
- Horizontal transfer

What actually happened



The result of phylogenetic inference

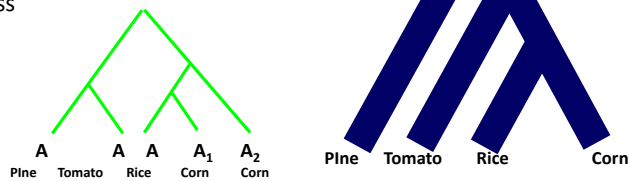


Reconciliation: Find the events that best explain the incongruence between gene tree and species tree.

Event parsimony: The history with the fewest events is the best explanation

Event models

- DL: Duplication and loss
- DTL: Duplication, transfer, loss

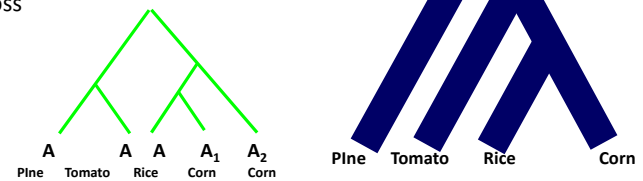


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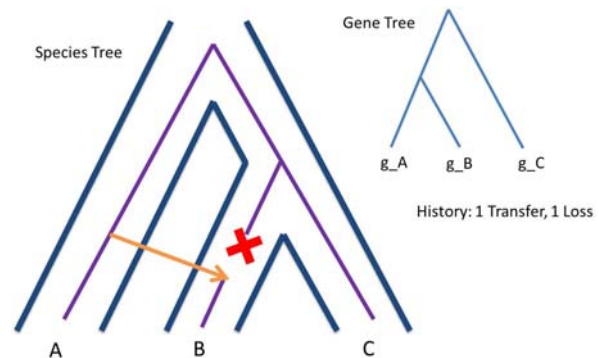
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Compare gene tree with species tree to infer horizontal transfer events



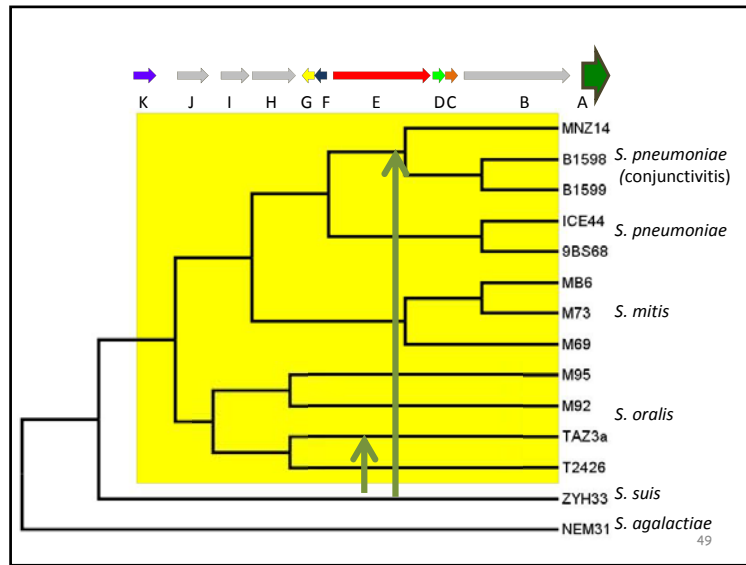
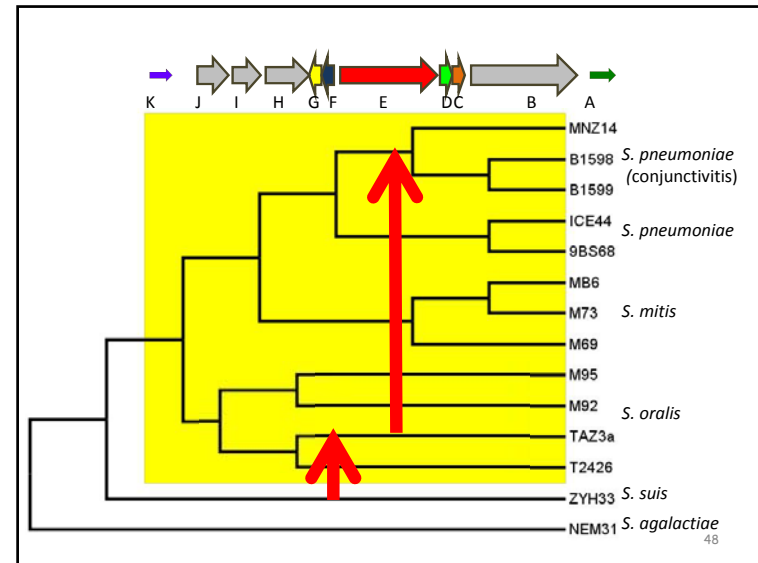
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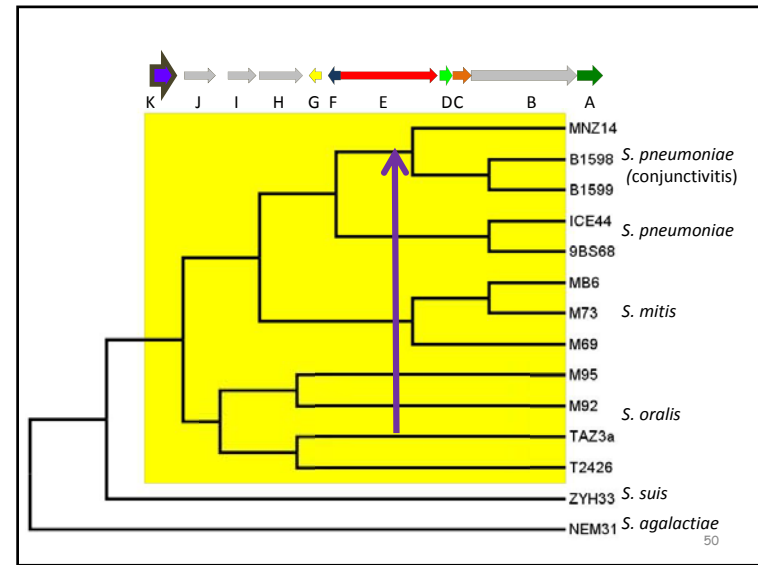
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Expected outcome: If the entire region was transferred together, then the inferred transfers in each gene family should be consistent.

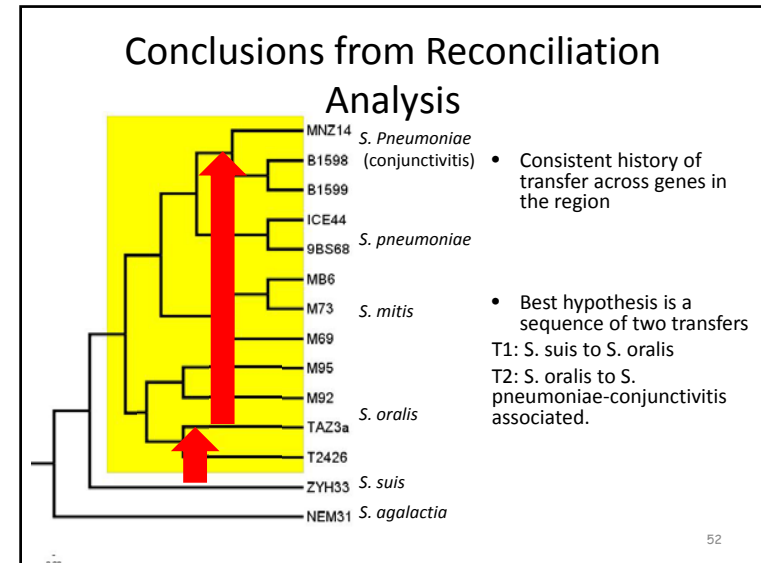
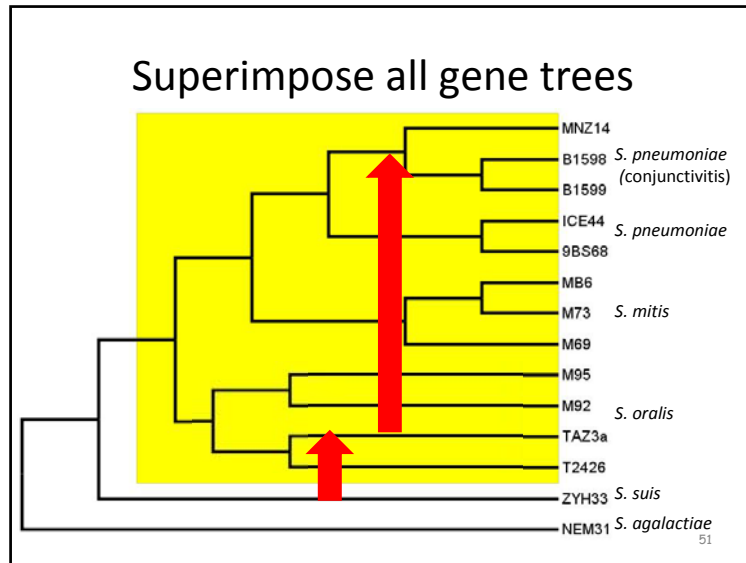
47



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- ### Big picture
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 - Investigating a candidate gene for the conjunctivitis phenotype Hiller, Antic
 - What is the origin of this promising candidate? YOU
 - Phylogenetic distribution
 - Inferring the history of transfers
- 20 loci remain to be analyzed!
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