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## The Bioinformatic Analysis of a Cluster S Mycobacterium Smegmatis Bacteriophage Tesla

Janna Fitzgerald  
*Illinois Wesleyan University*

Zoephia Laughlin  
*Illinois Wesleyan University*

Colin Page  
*Illinois Wesleyan University*

Bailey Reichert  
*Illinois Wesleyan University*

Richard Alvey, Faculty Advisor  
*Illinois Wesleyan University*

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**Presenter and Advisor Information**

Janna Fitzgerald; Zoephia Laughlin; Colin Page; Bailey Reichert; Richard Alvey, Faculty Advisor; and David Bollivar, Faculty Advisor





# THE BIOINFORMATIC ANALYSIS OF A CLUSTER S *MYCOBACTERIUM* *SMEGMATIS* BACTERIOPHAGE TESLA

Janna Fitzgerald, Zoephia Laughlin, Colin Page, Bailey Reichert, David Bollivar\*, and Richard Alvey  
Biology Department, Illinois Wesleyan University

## Introduction

The SEA-PHAGES program expands the knowledge of its participants while increasing the diversity of the collection of bacteriophages. The bacteriophage Tesla was collected in Oswego, Illinois by Colin Page, isolated at Illinois Wesleyan University, sequenced at the University of Pittsburgh and annotated at Illinois Wesleyan University. The objective of this project was to interpret the genome of Tesla and then compare the evolutionary relationship existing between the bacteriophage Tesla and the other seven bacteriophages within the S Cluster. A cluster of bacteriophages is a group that shares at least 50 percent of recognizable nucleotide similarities. Using the genomic information of other S cluster phages found on the program Phamerator, it was found that many of Tesla's functions were shared with the other phages. In comparison with Marvin, the original member of the S Cluster, Tesla has 106 genes that are the same and 1 that are different from other annotated phages. In the Tesla genome, 70 are hypothetical proteins and 42 were found to have their functions identified. Based on this information, we have learned that members of the S cluster are highly conserved but have some differences. This supports the mosaic nature of bacteriophage genomes.

## Materials and Methods

- Blast(p) and HHpred were used to compare gene functions with other phages.
- Phamerator was used to compare genomic similarity to other phages in S cluster
- Starterator was used to compare start sites for genes with start sites of homologous genes in other phages
- GeneMark and Glimmer were used to help determine start sites for genes based on coding potential
- DNA Master was used to annotate Tesla's genome.

## Genome Results

- Genes 3-13 are evidence of the mosaic nature of bacteriophage genomes since these genes differed from the other bacteriophages in the S cluster. These genes shared more similarities with genes from bacteriophages in other clusters than those in the S cluster, showing how Tesla is a unique phage.
- There is a translational frameshift that affects genes 45 and 46. Both are tail assembly chaperones.
- Tesla, like other phages in the S cluster, contains genes that function as minor tail proteins that are very close together in the genome.

## Members of S Cluster

Phage	Base Pairs	# of Genes	GC%
Tesla	64,625	112	63.45%
Marvin	65,100	107	63.42%
MosMoris	65,243	111	63.35%

## S Cluster Map

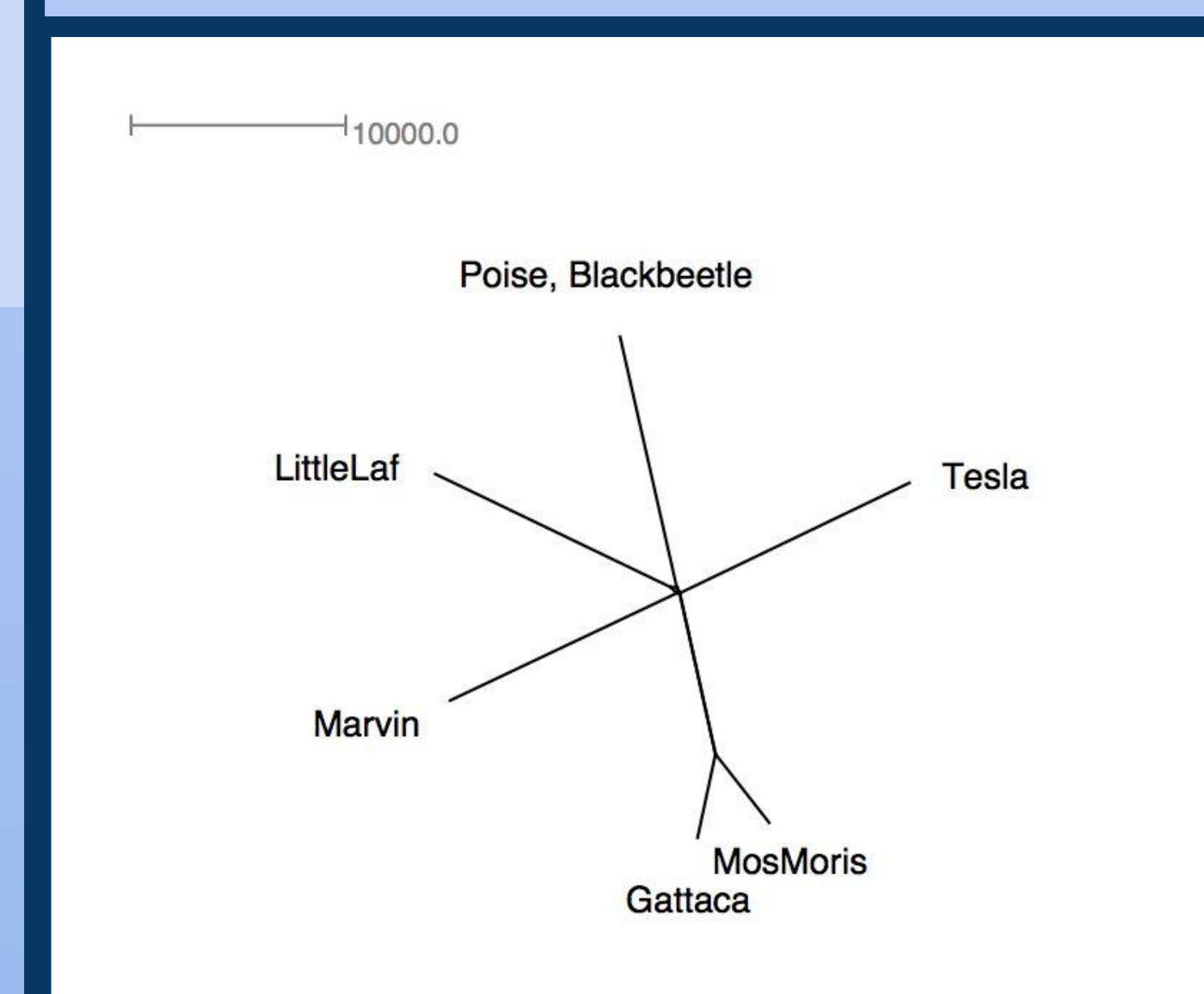


Figure 1: This splitree diagram shows all known phages in the S cluster. This also shows Tesla's close relationship with MosMoris and Marvin, shown additionally in figure 4

## Annotation Results

- Initial results: Tesla is a bacteriophage in the S cluster that contains 64,625 base pairs and 113 genes. Of these genes, 23, 31, 95-107, and 113 were reverse reading genes and the remaining genes were forward reading. This information was provided by the University of Pittsburgh.
- Gene 73 was deleted from the genome because there was not sufficient coding potential and the gap between genes 72 and 74 only included one base pair, lowering the gene total to 112.

## Gene Function Chart

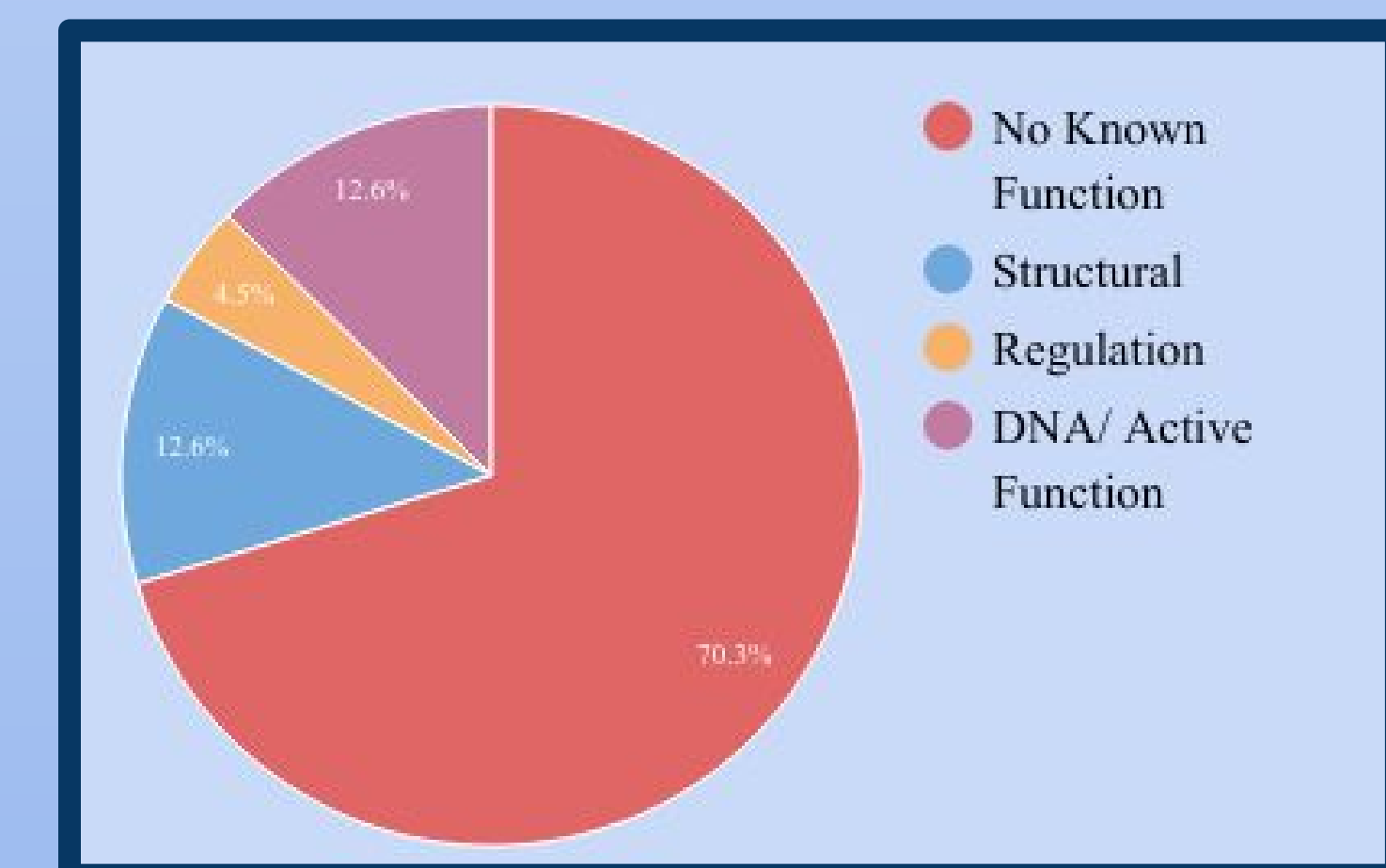


Figure 3: This chart shows the percentages of each function within the genome. The majority of the genes have no known function.

## Cluster Tree

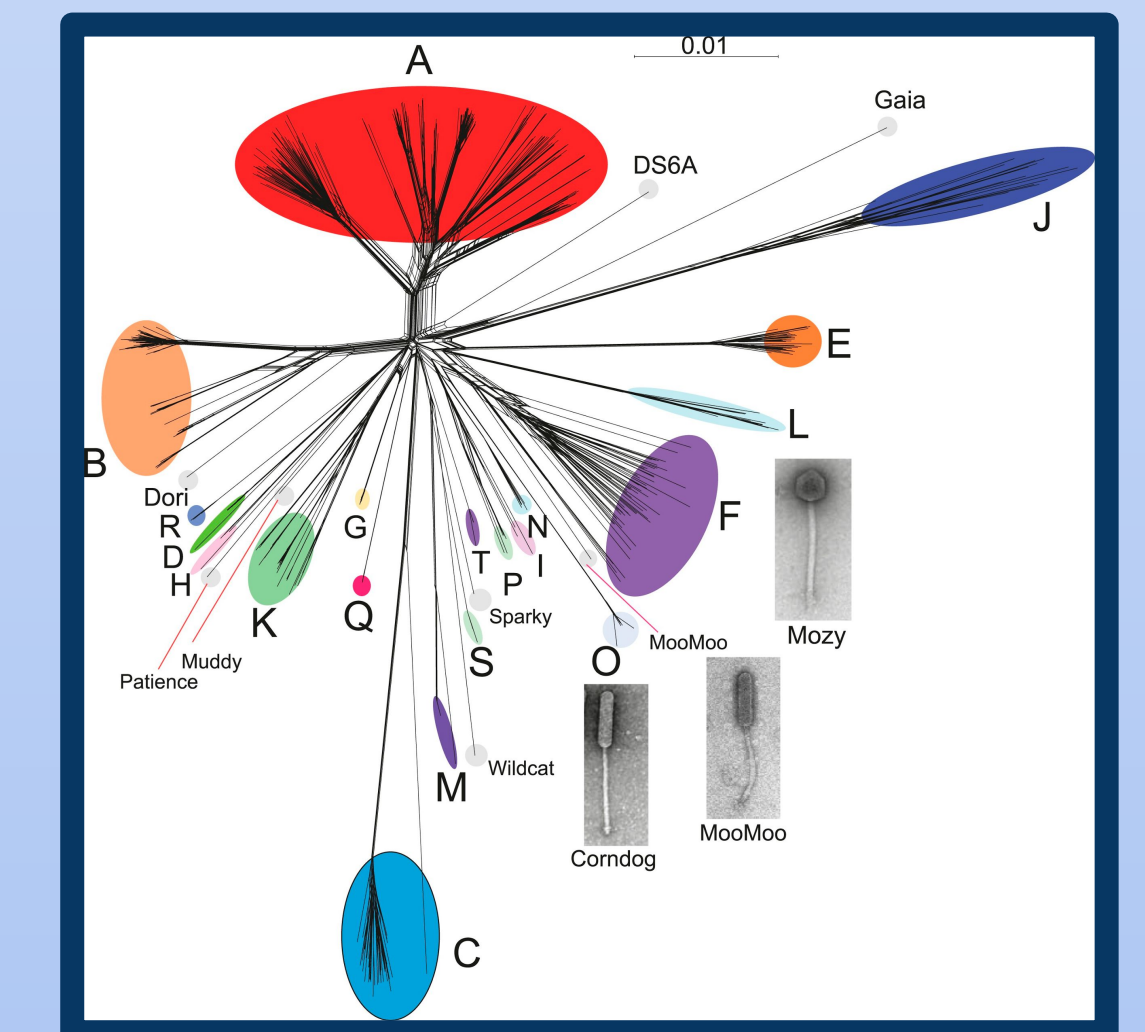


Figure 2: This figure shows how large the S cluster is compared to other bacteriophages.

## Conclusion

- Tesla has thus been shown to demonstrate many hallmarks of an S cluster bacteriophage
- These similarities (see above "Members of S Cluster" table), which include gene location, genome length, types of genes present, frameshifts, etc. support the model of highly conserved genetic mosaicism that characterizes S cluster mycobacteriophages.

## Gene Function Table

Gene function	Placement	Length	Clusters with Gene
Helix-Turn-Helix Binding Domain	Beginning	234 bp	S Cluster
Lysin A	Middle	1380 bp	B4, B7, K2, S, & X Cluster
HNH Homing Endonuclease	End	327 bp	S Cluster
Minor Tail Protein	End	2142 bp	S & V Clusters

## Acknowledgements

- TA's were Ellen Stumph, Brooke Koebele, and Niyant Vora
- Lab manuals used were the DNA Master guide and the Phages Discovery Guide (provided by the SEA phages program)
- Splitree diagram was provided by Dr. Bollivar
- Cluster tree provided by elifesciences.org in their article "Whole genome comparison of a large collection of mycobacteriophages reveals a continuum of phage genetic diversity"

Figure 4: This image is the Phamerator comparison between the genomes of Tesla, MosMoris, and Marvin. Tesla is very similar to the annotated bacteriophages Marvin and MosMoris. It is clear that almost all of the genes align in these three genomes. Genes 3-13 are evidence of the mosaic nature of bacteriophage genomes since they differ from the others in the cluster. The frameshift that affects genes 45 and 46 can be seen below in all three of the genomes. Both of these genes are tail assembly chaperones.

