

Spatial Patterns of Molecular Trends in Bacteriaal Genomes

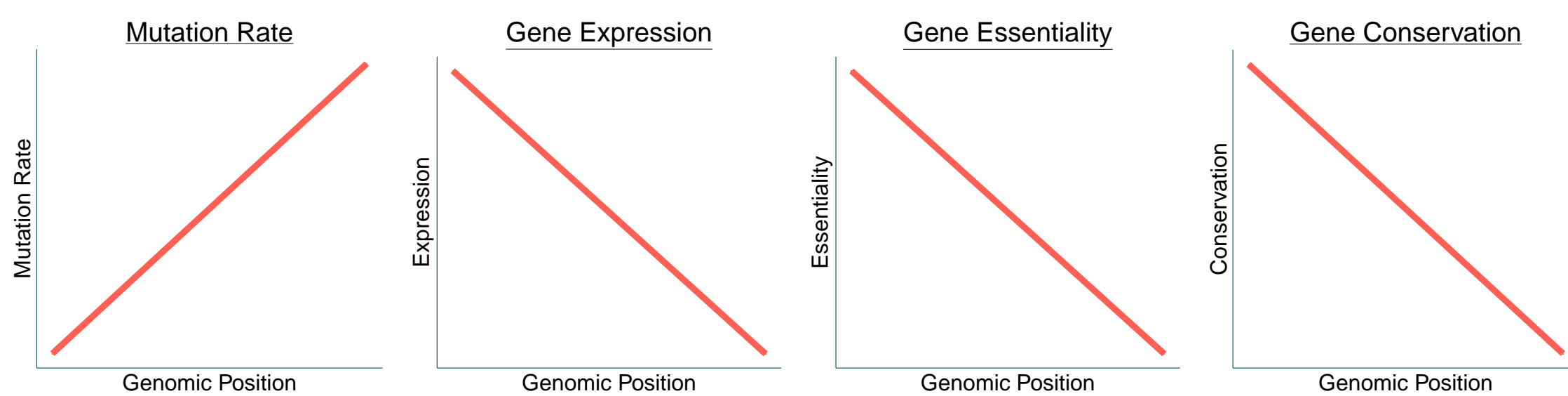
Daniella LATO* G. Brian GOLDING

*latodf@mcmaster.ca

Department of Biology, McMaster University, Hamilton, Ontario, Canada

Molecular Traits Vary With Genomic Position

Molecular traits such as mutation rate and gene expression change with distance from the origin of replication. Genes that are highly conserved, essential, and expressed at higher levels are typically found near the origin of replication^{1;2;3;4;5}. The disparity in molecular features between the origin of replication and the terminus is thought to be due to decreased mutation rate near the origin of replication^{1;3;4} and the requirement to maintain proper gene dosage³ amount and replication timing^{1;5}. These **spatial molecular trends have not been analyzed while accounting for genomic reorganization** such as rearrangements and inversion, which provide bacteria with the opportunity to gain new genetic information.



Objective: In depth analysis of genomic patterns of substitutions and gene expression in bacterial genomes.

Hypothesis: The number of substitutions increases with increasing distance from the origin of replication.

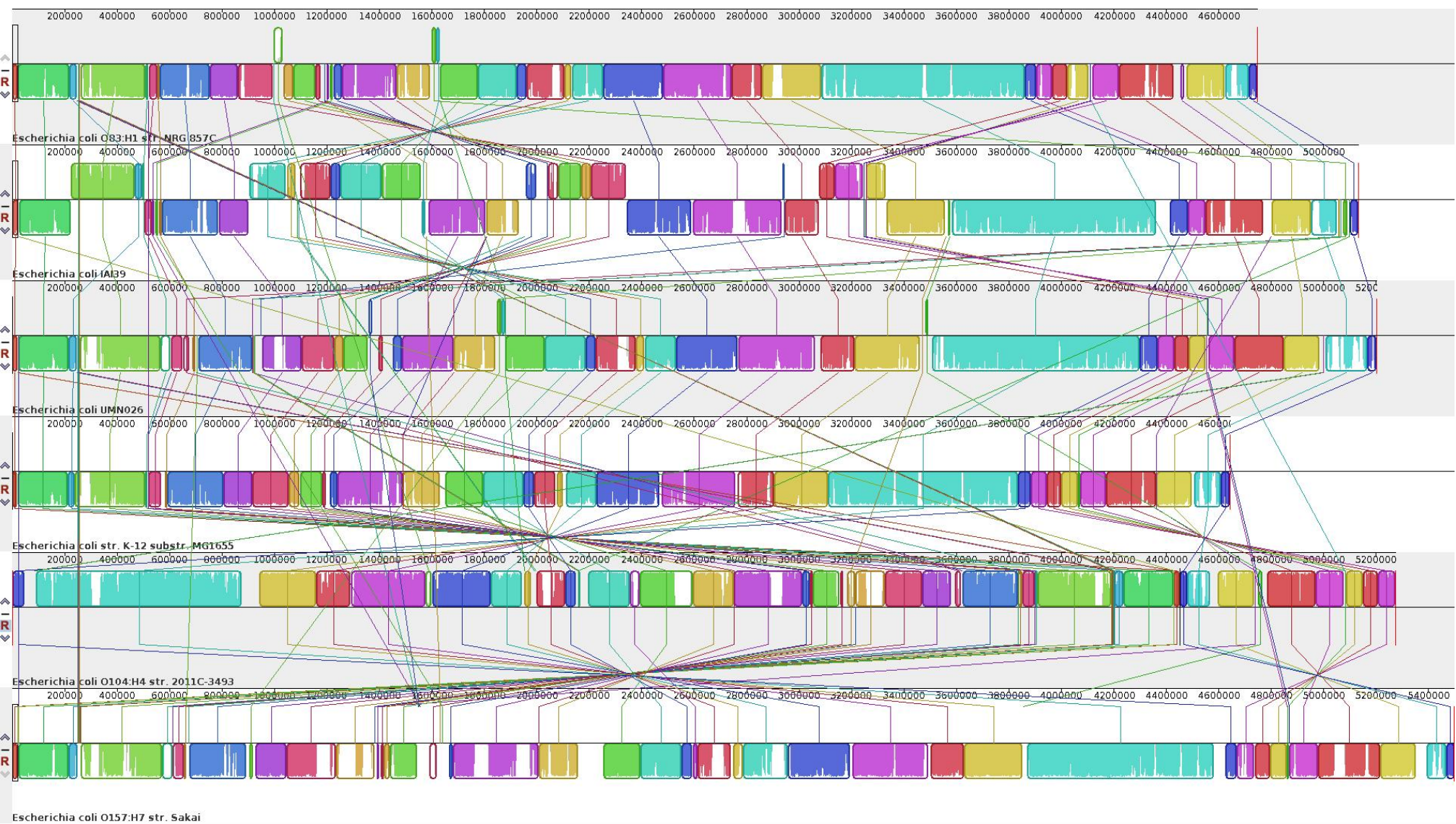
1. Methods

25 bacterial genomes and gene expression data from 9 bacterial species.

Bacteria and Replicon	Number of Strains	Genome Structure
<i>Escherichia coli</i> Chromosome	6	○
<i>Bacillus subtilis</i> Chromosome	7	○
<i>Streptomyces</i> Chromosome	6	—
<i>Sinorhizobium meliloti</i> Chromosome	6	○ ○
<i>Sinorhizobium meliloti</i> pSymA	6	○ ○
<i>Sinorhizobium meliloti</i> pSymB	6	○ ○

Alignment:

- Each whole genome was **globally aligned** to obtain **Locally Co-linear Blocks (LCB)** which **allows for genome reorganization** such as inversions and rearrangements (progressiveMauve⁶).
- Each **LCB** was **locally re-aligned** with MAFFT⁷ for a more accurate alignment.



Example progressiveMauve alignment of *E. coli* genomes.

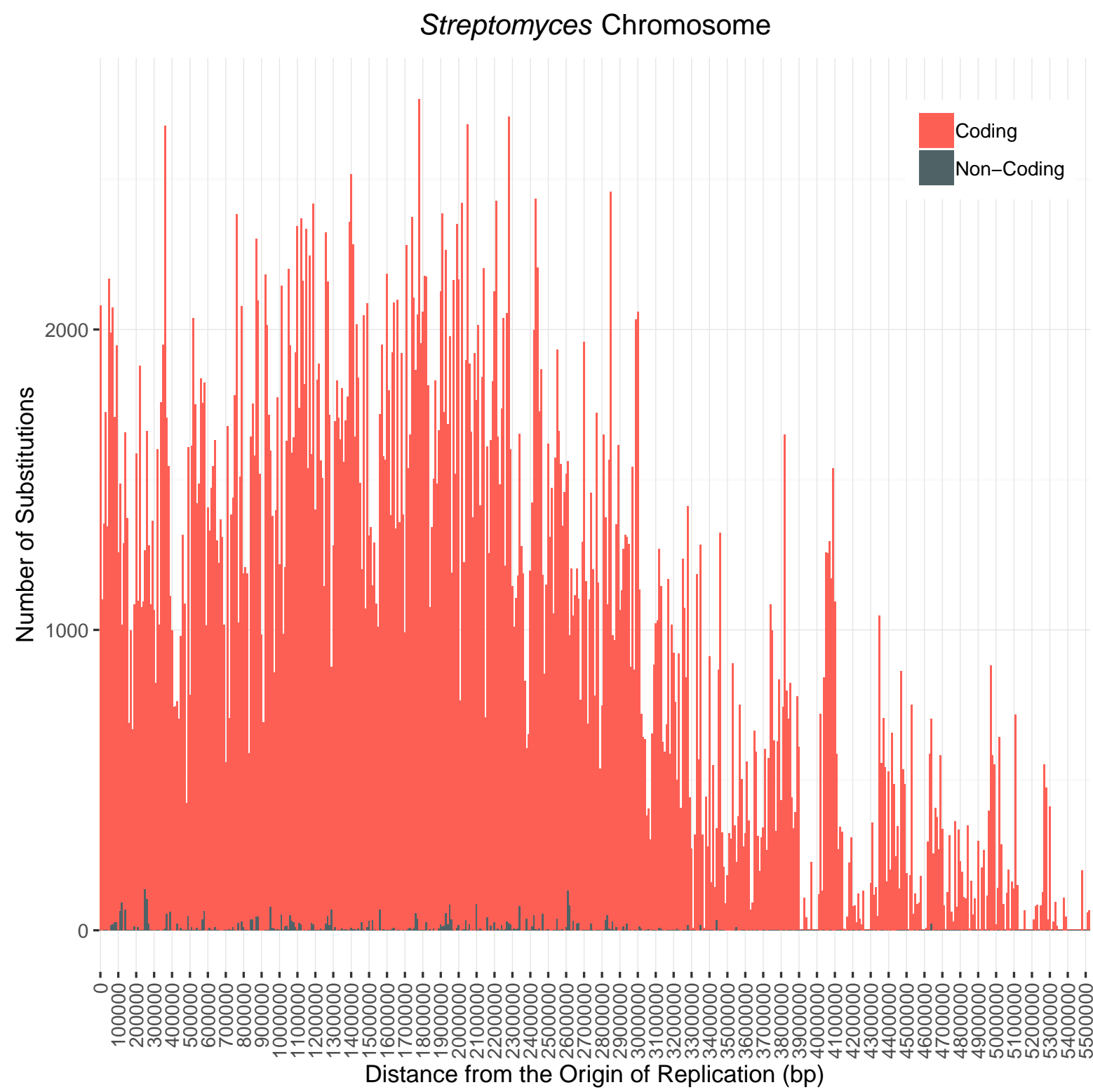
Phylogeny:

- Bootstrap phylogenetic trees** were constructed for each bacterial replicon (PHYLP⁸).

Statistical Analysis:

- Each **genomic position** was **scaled** to the origin of replication and accounted for bidirectional replication (R^9).
- To track genome rearrangements, **nucleotide substitutions and genomic positions were reconstructed** in extinct taxa and **ancestors** on the given phylogenies (custom Perl and Python scripts, PAML¹⁰).
- Logistic regression and linear regression** were performed to compute changes in substitutions and gene expression across all coding and non-coding segments of the genome (R^9).

2. Substitutions ↘ with Genomic Position



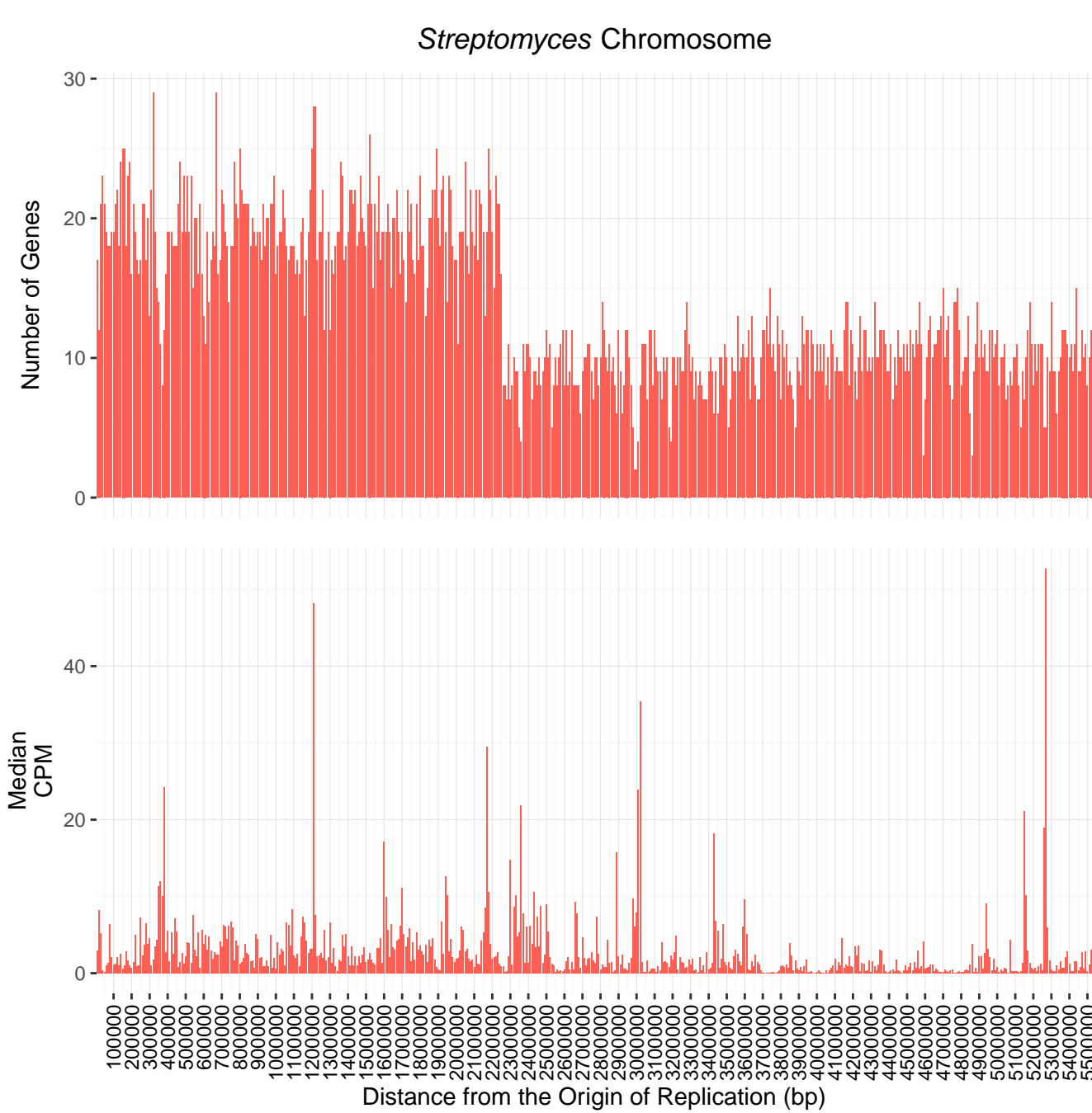
Bacteria and Replicon	Coefficient Estimate		Genome Structure
	Coding Sites	Non-Coding Sites	
<i>E. coli</i> Chromosome	$-9.119 \times 10^{-8}***$	$7.022 \times 10^{-8}***$	○
<i>B. subtilis</i> Chromosome	$-1.273 \times 10^{-7}***$	$-9.861 \times 10^{-8}***$	○
<i>Streptomyces</i> Chromosome	$-7.945 \times 10^{-9}***$	$3.637 \times 10^{-7}***$	—
<i>S. meliloti</i> Chromosome	$-1.550 \times 10^{-7}***$	$-1.510 \times 10^{-7}*$	○ ○
<i>S. meliloti</i> pSymA	$-1.156 \times 10^{-7}*$	NS	○ ○
<i>S. meliloti</i> pSymB	$2.587 \times 10^{-7}***$	$8.591 \times 10^{-7}***$	○ ○

Significance Codes: $p < 0.001 = '***'$, $0.001 < 0.01 = '**'$, $0.01 < 0.05 = '*'$, NS = Not Significant

Discussion: Increased transposon insertion events¹¹ and potential genomic and pathogenicity islands^{12;13} near the origin of replication can cause asymmetry in nucleotide composition¹⁴, GC content¹⁵, and mutation rate^{1;3;4}. This could be why we see an increase in the number of substitutions near the origin of replication.

Conclusion: The number of substitutions decreases with increasing distance from the origin of replication in most bacterial replicons at most coding sites.

3. Gene Expression ↗ with Genomic Position



Bacteria and Replicon	Coefficient Estimate	Genome Structure
<i>E. coli</i> Chromosome	$-6.03 \times 10^{-5}***$	○
<i>B. subtilis</i> Chromosome	$-9.7 \times 10^{-5}***$	○
<i>Streptomyces</i> Chromosome	$-1.17 \times 10^{-6}***$	—
<i>S. meliloti</i> Chromosome	NS	○ ○
<i>S. meliloti</i> pSymA	$1.39 \times 10^{-3}***$	○ ○
<i>S. meliloti</i> pSymB	NS	○ ○

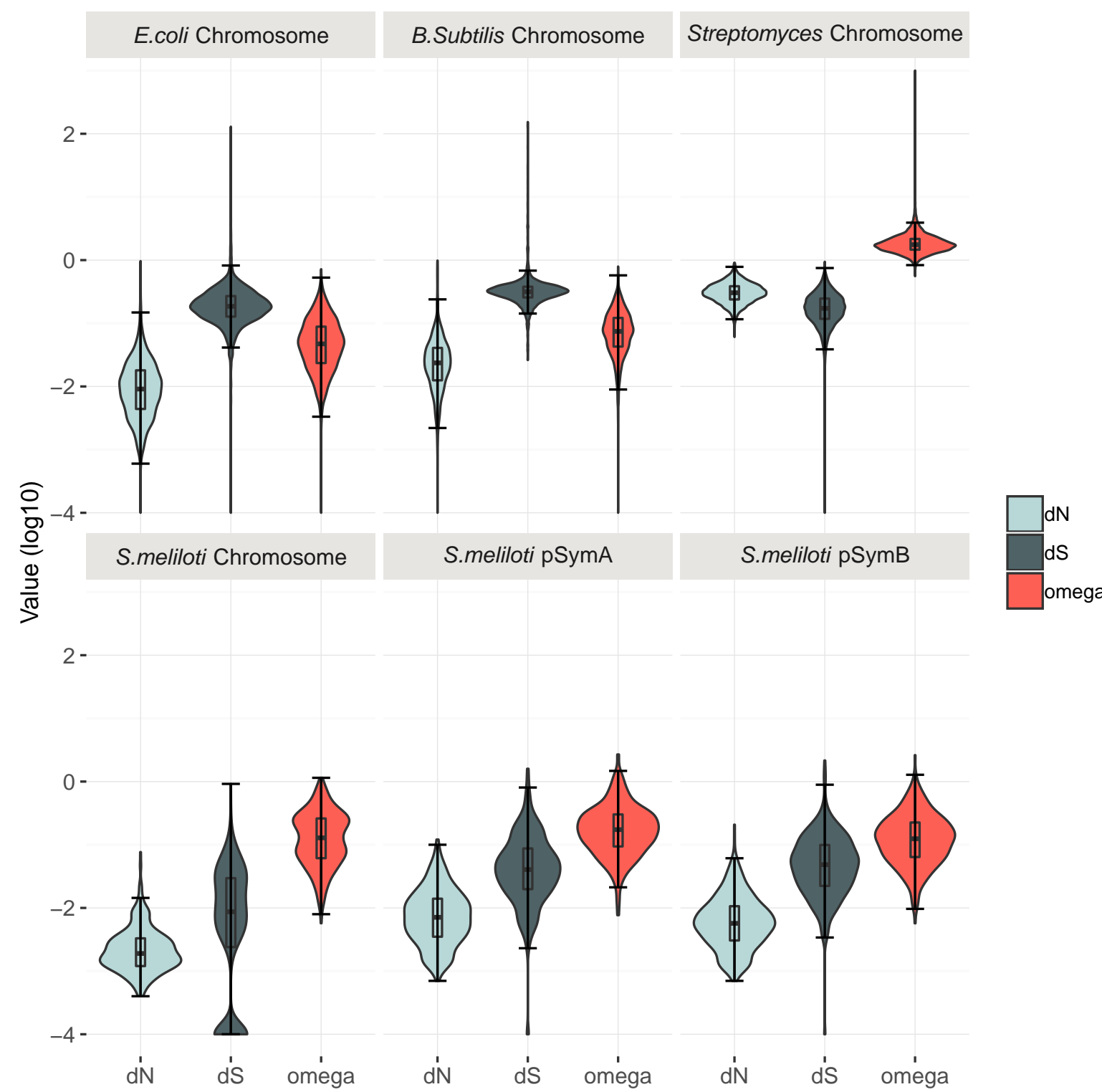
Significance Codes: $p < 0.001 = '***'$, $0.001 < 0.01 = '**'$, $0.01 < 0.05 = '*'$, NS = Not Significant

Discussion: Replication error increases when moving away from the origin of replication, therefore, genes that are highly expressed are often located near the origin.^{16;17;18}

Conclusion: Gene expression decreases with increasing distance from the origin of replication in most bacterial replicons.

4. Preliminary Selection Results

Distribution of dN , dS , and ω for the coding regions (codeml PAML¹⁰).



dN = # of non-synonymous substitutions per site, dS = # of synonymous substitutions per site, ω = ratio dN/dS

Most of the bacterial replicons are under **purifying selection** ($\omega < 1$).

Conclusions and ongoing research

Determining how the number of substitutions are distributed spatially throughout bacterial genomes broadens our understanding of their evolution. Most replicons considered in this study have the number of substitutions and gene expression decrease with increasing distance from the origin of replication. The exceptions to this were pSymB of *S. meliloti* and *Streptomyces* which had the number of substitutions increase when moving away from the origin of replication. These spatial substitution and gene expression results can be used to determine if all bacteria possess the same evolutionary patterns.

Current work involves:

- Determining the dN/dS ratio for each coding section of the bacterial genomes and if this changes with genomic position.
- Identifying inversions within bacterial genomes and how they might alter gene expression
- Performing ancestral reconstruction of gene expression in several *Escherichia coli* species

See GitHub for more information.

[www.github.com/dlato/SMBE_Mutation_Rate_Evolution_Poster](https://github.com/dlato/SMBE_Mutation_Rate_Evolution_Poster)

figs/doi_url-eps-convert

References

- [1] J D Morrow and V S Cooper. Evolutionary effects of translocations in bacterial genomes. *Genome Biology and Evolution*, 4(12):1256–1262, 2012.
- [2] P M Sharp, E Bailes, R J Grocock, J F Peden, and R E Sockett. Variation in the strength of selected codon usage bias among bacteria. *Nucleic Acids Research*, 33(4):1141–1153, 2005.
- [3] E Couturier and E P Rocha. Replication-associated gene dosage effects shape the genomes of fast-growing bacteria but only for transcription and translation genes. *Molecular Microbiology*, 59(5):1506–1518, 2006.
- [4] V S Cooper, S H Vhor, S C Wrocklage, and P J Hatcher. Why genes evolve faster on secondary chromosomes in bacteria. *PLoS Computational Biology*, 6(4):e1000732, 2010.
- [5] K M Flynn, S H Vohr, P J Hatcher, and V S Cooper. Evolutionary rates and gene dispensability associate with replication timing in the archaeon *Sulfolobus islandicus*. *Genome Biology and Evolution*, 2:859–869, 2010.
- [6] A E Darling, B Mau, and N T Perna. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS one*, 5(6):e11147, 2010.
- [7] K Katoh, K Misawa, K Kuma, and T Miyata. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30(14):3059–3066, 2014.
- [8] J Feisenstein. PHYLIP - Phylogeny Inference Package (Version 3.2). *Cladistics*, 5:164–166, 1989.
- [9] R Development Core Team. R: a language and environment for statistical computing. 2014.
- [10] Z Yang. PAML: a program package for phylogenetic analysis by maximum likelihood. *Bioinformatics*, 13(5):555–556, 1997.
- [11] S Y Gerdes, M D Scholle, J W Campbell, G Balazsi, E Ravasz, M D Dougherty, A L Somera, N C Kyrpides, I Anderson, M S Gelfand, A Bhattacharya, V Kapatral, M D'Souza, M V Baev, Y Grechkin, F Mseeh, M Y Fonstein, R Overbeek, A L Barabasi, Z N Oltvai, and A L Osterman. Experimental determination and system level analysis of essential genes in *Escherichia coli* MG1655. *Journal of Bacteriology*, 185(19):5673–5684, 2003.
- [12] S Karlin. Detecting anomalous gene clusters and pathogenicity islands in diverse bacterial genomes. *Trends in Microbiology*, 9(7):335–343, 2001.
- [13] A Mira, A B Martin-Cuadrado, G D'Auria, and F Rodriguez-Valera. The bacterial pan-genome: a new paradigm in microbiology. *International Microbiology*, 13(2):45–57, 2010.
- [14] P Mackiewicz, A Gierlik, M Kowalczyk, M R Dudek, and S Cebart. How does replication-associated mutational pressure influence amino acid composition of proteins? *Genome Research*, 9(5):409–416, 1999.
- [15] H Ikeda, J Ishikawa, A Hanamoto, M Shinose, H Kikuchi, T Shiba, Y Sakaki, M Hattori, and S Omura. Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nature Biotechnology*, 21(5):526–531, 2003.
- [16] E P C Rocha. The replication-related organization of bacterial genomes. *Microbiology*, 150(6):1609–1627, 2004.
- [17] R S Washburn and M E Gottesman. Transcription termination maintains chromosome integrity. *Proceedings of the National Academy of Sciences*, 108(2):792–797, 2011.
- [18] D H S Block, R Hussein, L W Liang, and H N Lim. Regulatory consequences of gene translocation in bacteria. *Nucleic acids research*, 40(18):8979–8992, 2012.