



OPERON MEMBERSHIP AND TRANSCRIPTION TIMING INCREASE LOCAL PURIFYING SELECTION AND CODON-BIAS IN BACTERIA

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CENTRAL QUESTION

How do the structural and transcriptional changes imposed by operons influence the way genes evolve?

OBJECTIVES

1. Understand if genes in operons experience different codon biases and rates of evolution than individual genes.
2. Examine variation in codon bias and evolutionary rate within operons and elucidate the reasons why genes in operons evolve differently.

ACKNOWLEDGEMENTS

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INTRODUCTION

- Operons increase gene expression and the propensity of mRNA to form secondary structure, which may impose increased evolutionary constraints on genes (Figure 1) (1).
- Within operons, genes that are transcribed earlier are expressed more because translation begins first and occurs in the ribosome rich regions near DNA (2).
- Understanding intra-genome variation in the strength of purifying selection can provide insights into how structural aspects of the genome evolve, the evolutionary constraints that they impose, and will improve the overall legibility of genomes (1,3).

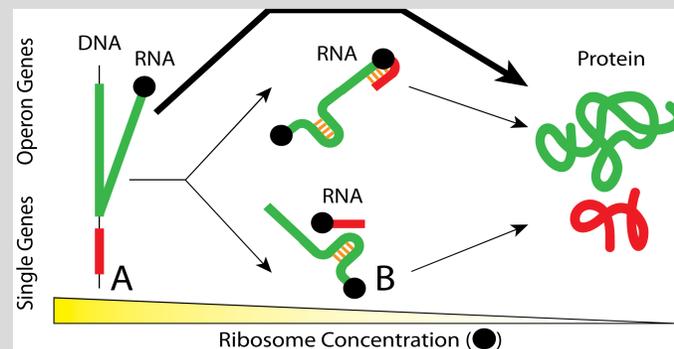


Figure 1: Because of their increased transcript length, operons are expressed more from increased transcriptional translation (A), and are subject to increased mRNA secondary structure during post-transcriptional translation (B), which can fundamentally change local purifying selection.

DESIGN & METHODS

- Sequences and Gene Information were obtained from the Integrated Microbial Genome Database (JGI) (Figure 2).
- *Burkholderia cenocepacia* HI2424
- *Burkholderia multivorans* 17616
- *Burkholderia ambifaria* AMMD
- Orthologs Identification:
 - Reciprocal BLAST best hits, Synteny.
- Operon Identification:
 - Database for Prokaryotic Operons (DOOR).
- Codon Adaptation Index:
 - Codon Usage Bias Database (CUB-DB).
- Evolutionary Rate:
 - Aligned with ClustalW.
 - PAML code-ml module.
 - Excluded if dN or dS > 2.

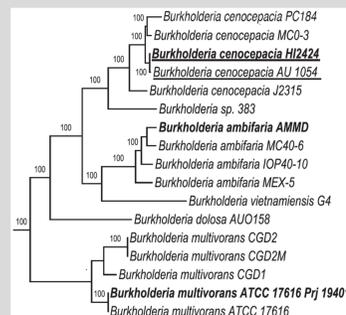


Figure 2: Phylogeny of a select portion of the *Burkholderia cepacia* complex.

RESULTS

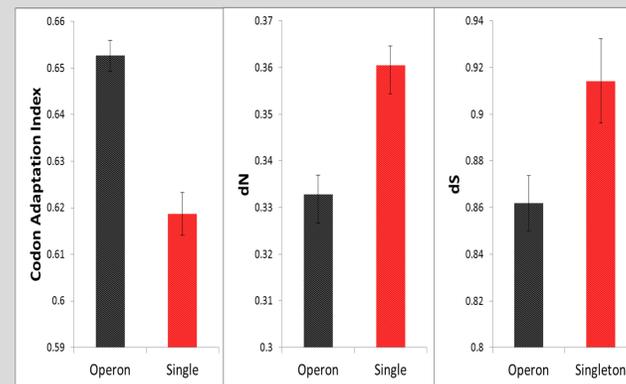


Figure 3: Codon adaptation and evolutionary rate (dN/dS) in genes located in operons (black) relative to single genes (red) ($p < 0.0001^*$).

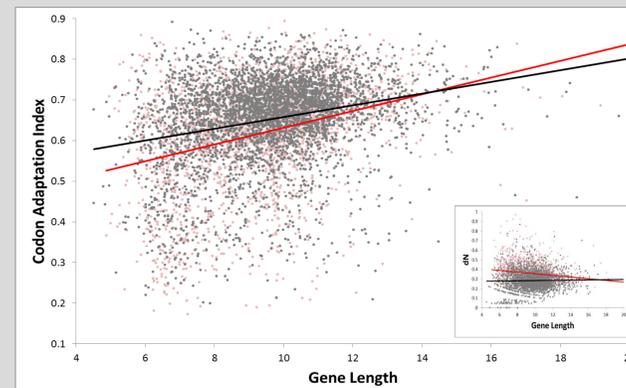


Figure 4: Regression analysis of the effect of gene length on the extent of codon adaptation of genes in operons (black) and single genes (red) (Operons: $r^2 = 0.07$, $p < 0.0001^*$; Single Genes: $r^2 = 0.11$, $p < 0.0001^*$).

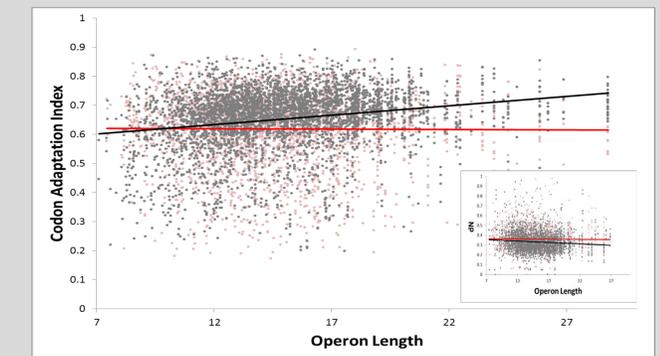


Figure 5: Regression analysis of the effect of the length of the operon on the codon adaptation of the genes within it (black) ($r^2 = 0.05$, $p < 0.0001^*$). The non-significant regression of the effect of identically sized sets of local single genes is also shown as a local control ($r^2 = 1.8 * 10^{-5}$, $p = 0.82$).

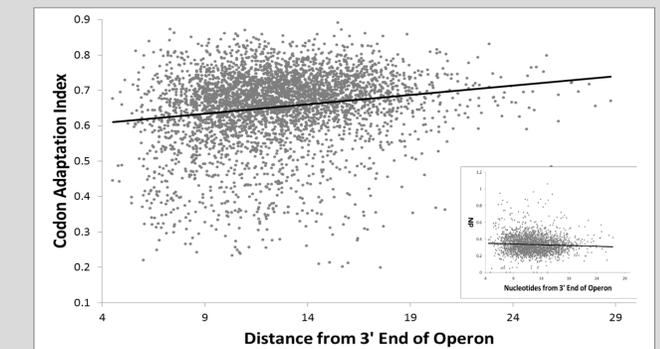


Figure 6: Regression analysis of the effect of the distance of a gene from the 3' end of its operon on the extent of codon adaptation ($r^2 = 0.03$, $p < 0.0001^*$).

CONCLUSIONS

Operons vs. Individual Genes

1. Genes in operons are more codon adapted and evolve more slowly than individual genes (Figure 3).
2. Longer genes are more codon adapted in both operons and individual genes, but the effect is stronger in individual genes (Figure 4).
3. Genes in longer operons are more codon adapted than genes in shorter operons, but surrounding gene length has no effect on the codon adaptation of individual genes (Figure 5).

Intra-Operon Variation

1. A general increase in codon adaptation as you move from the 3' transcription terminus to the 5' transcription initiation site of operons was observed (Figure 6).

In summary...

We suggest that genes in operons experience different rates of evolution and patterns of codon usage bias because of their increased expression and increased probability of forming mRNA secondary structure. Genes in operons, particularly those farthest from the 3' end, are expressed more because they undergo more transcriptional translation in ribosome rich regions (2). Furthermore, we provide evidence that because of their longer mRNA transcripts, genes in operons are subject to increased purifying selection to prevent extensive mRNA secondary structure from causing translational errors (3). Future research will seek to determine the relative role of increased expression and potential secondary structure in dictating the different evolutionary patterns observed in operons and individual genes.