Subs Paper Things to Do:

- causes for weird selection and subs results in *Streptomyces*
  - see how often class 4 arises in strep to see what is going on in later portion of the genome (to see if annotation is really a problem)
  - split up the strep data into core and non core and see if results are the same
- ~~make graphs proportional to length of respective cod/non-cod regions~~
- ~~test examples for genes near and far from terminus (robust log reg/results)~~
- ~~figure out why the data is weird for number of cod/non-cod sites~~
- why are the lin reg of dN, dS and ω NS but the subs graphs are...explain!
- grey out outliers in subs graphs?
- mol clock for my analysis?
- GC content? COG? where do these fit?

Gene Expression Paper Things to Do:

- ~~linear regression on 10kb regions~~
- put new 10kb lin reg and # of genes over 10kb lin reg into paper
- write about ↑ in methods and discussion
- put expression lin reg and # coding sites log reg into supplement
- write about ↑ in paper and how results are the same
- update supplementary figures/file
- correlation of gene expression across strains
- if necessary add a phylogenetic component to the analysis
- potentially remove genes that have been recently translocated from the analysis
- model gene exp + position + number of genes
- split up the strep data into core and non core and see if results are the same
- what is going on with *Streptomyces* number of genes changing drastically from core to non-core
- codon bias?
- what is going on with really high gene expression bars
- edit paper

- submit paper

Inversions and Gene Expression Letter Things to Do:

- ~~check if opposite strand in progressiveMauve means an inversions (check visual matches the xmfa)~~
- ~~check if PARSNP and progressiveMauve both identify the same inversions (check xmfa file)~~
- create latex template for paper
- ~~put notes from papers into doc~~
- ~~use large PARSNP alignment to identify inversions~~
- confirm inversions with dot plot
- make dot plot of just gene presence and absence matrix (instead of each site) to see if this will go better
- look up inversions and small RNA's paper Marie was talking about at Committee meeting
- write outline for letter
- write Abstract
- write intro
- write methods
- compile tables (supplementary)
- write results
- write discussion
- write conclusion
- do same ancestral/phylogenetic analysis that I did in the subs paper

General Things to Do:

- summarize references 40 and 56 from Committee meeting report (Brian was asking)
- read and make notes on papers I found for dissertation intro

Last Week

✓linear regression on 10kb regions for gene expression (to match number of genes lin reg)

✓test examples for genes near and far from terminus (robust log reg/results) for number of substitutions

✓make graphs proportional to length of respective cod/non-cod regions

✓figure out why the data is weird for number of cod/non-cod sites

Last week I re-did the gene expression linear regression on 10kb regions instead of using each gene as one data point. This was so that the results are comparable to the number of genes linear regression which was also over 10kb chunks. I showed you this last week and the results are the same and look good! We decided to leave the graphs the same (not to divide the expression by the total number of genes in each 10kb section) because they are already the median expression value so it is already showing some sort of average. These results can be seen in Tables 2 and 4 as well as the per gene linear regression results in Table 3.

I also took 20kb close and far from the origin for each bacteria in both the protein coding and non-protein coding sections and performed a logistic regression on those sub-sections to see how number of substitutions correlates with distance from the origin of replication. The results can be found in Table 1. Most of the results are negative or not significant, but there are a few that are positive... Just wondering what you thoughts are on this? I will come talk to you about this later.

I told you that there was issues with how my data was being read in and processed which was making the proportionate graphs look really wonky. Well, I fixed this! Turns out my indexing was off and the substitutions data was starting from 0 where as the number of protein coding and non-protein coding sites was starting from 10000, and my code was freaking out when there were no substitutions (because of missing data). Additionally, these graphs were stacked bar graphs. So I have separated them out and made two graphs, one for protein coding and one for non-protein coding. This is all fixed and I have some lovely graphs for you that make perfect sense. The non-protein coding regions have more substitutions than the protein coding regions, see Figures below.

I also wanted your opinion about something that was mentioned at the conference. Someone asked me if I was including RNA in my “coding” sections? Because RNA often is under different selective pressures than coding or other “non-coding” sections, they suggested that I do my analysis on just all RNA to see if there is any sort of trend with respect to distance from the origin. I was wondering what you think about this and if you think it is worth it for me to do?

This Week

The new weighted graph for *Streptomyces* is looking a little weird so I need to look into this and make sure that I am not messing something up.

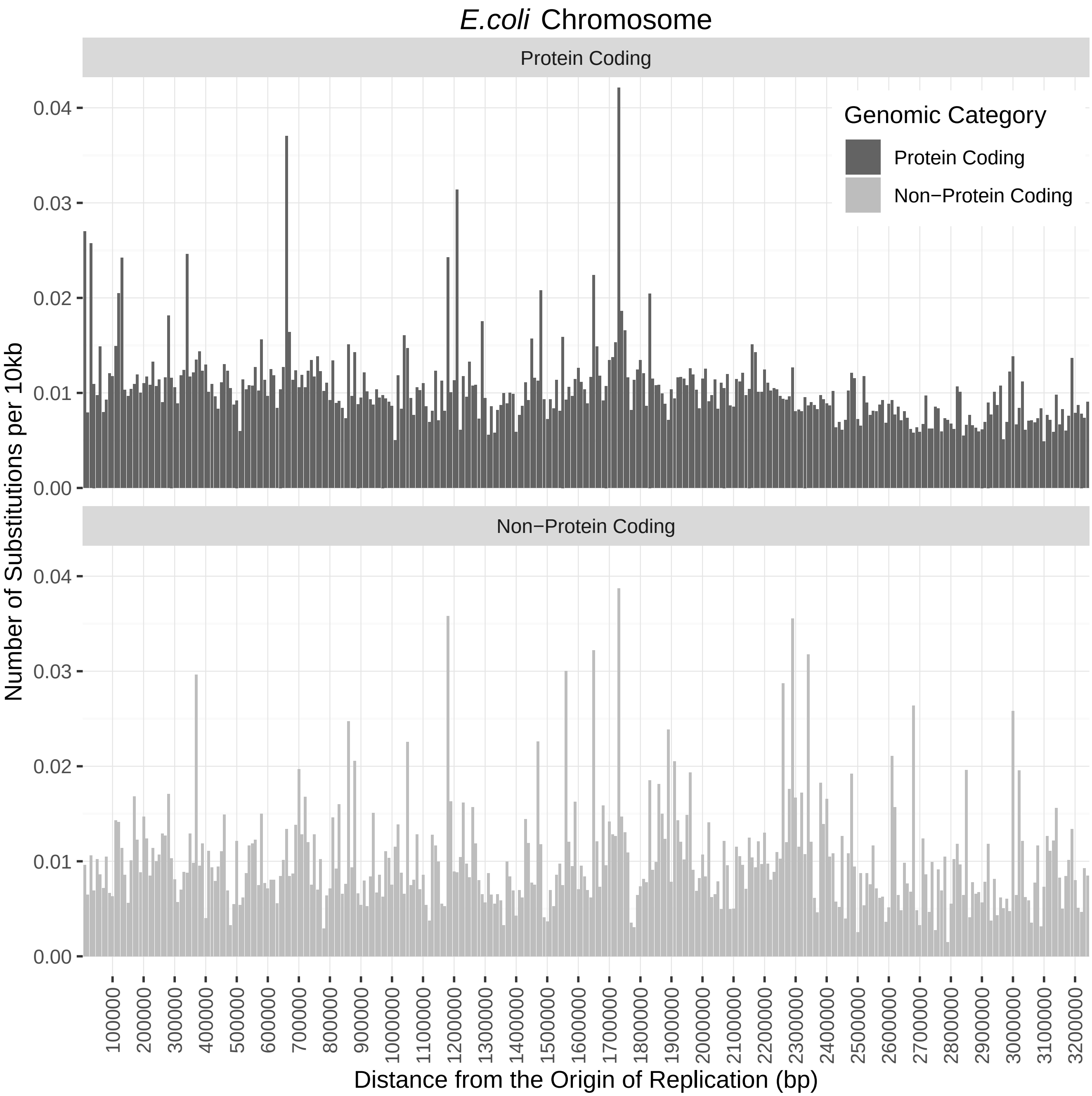
I would like to check if all strains have roughly the same gene expression values per gene to ensure that they are comparable. I am not sure if I need to do this statistically or if I can just do it "by eye". Thoughts?

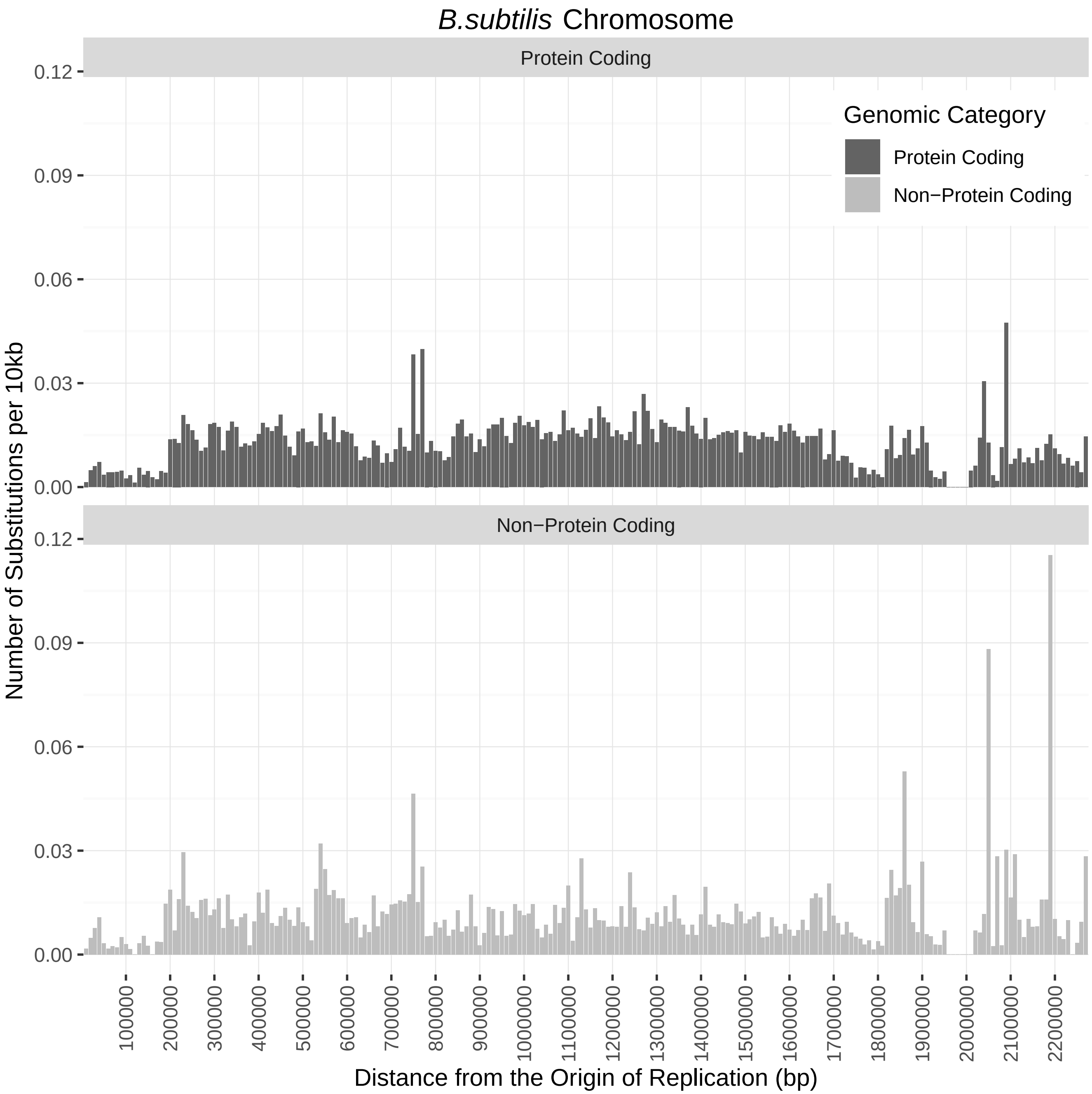
You might have noticed that the logistic regression for number of substitutions near and far from the origin is missing values for the average substitutions. I still need to calculate this to ensure that the number of substitutions is higher near the origin than at the terminus. I plan on doing that this week.

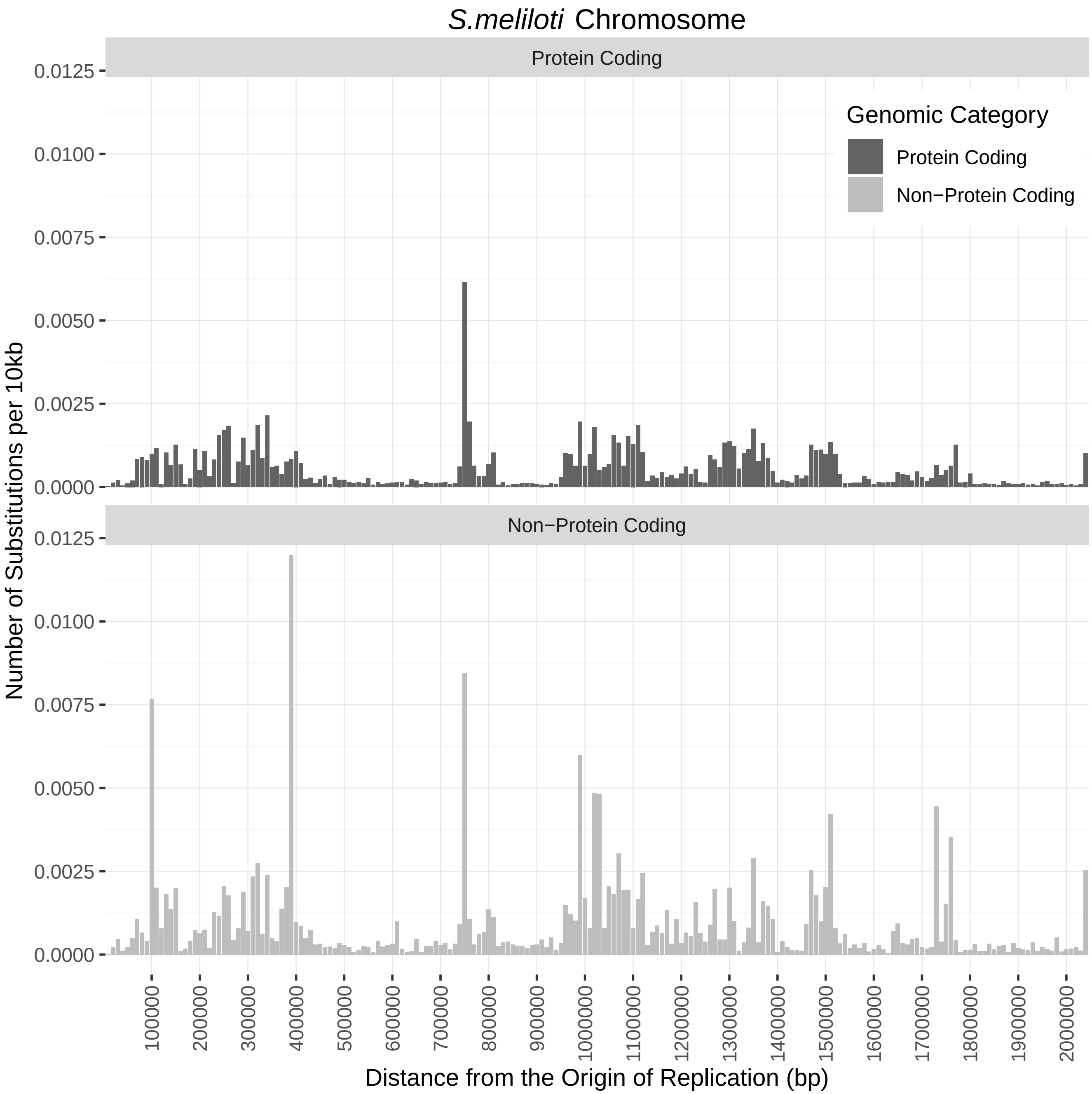
I would also like to perform a linear regression on the average (or total?) number of substitutions per 10kb section of the genome. This would be another check to ensure that the substitution results are robust.

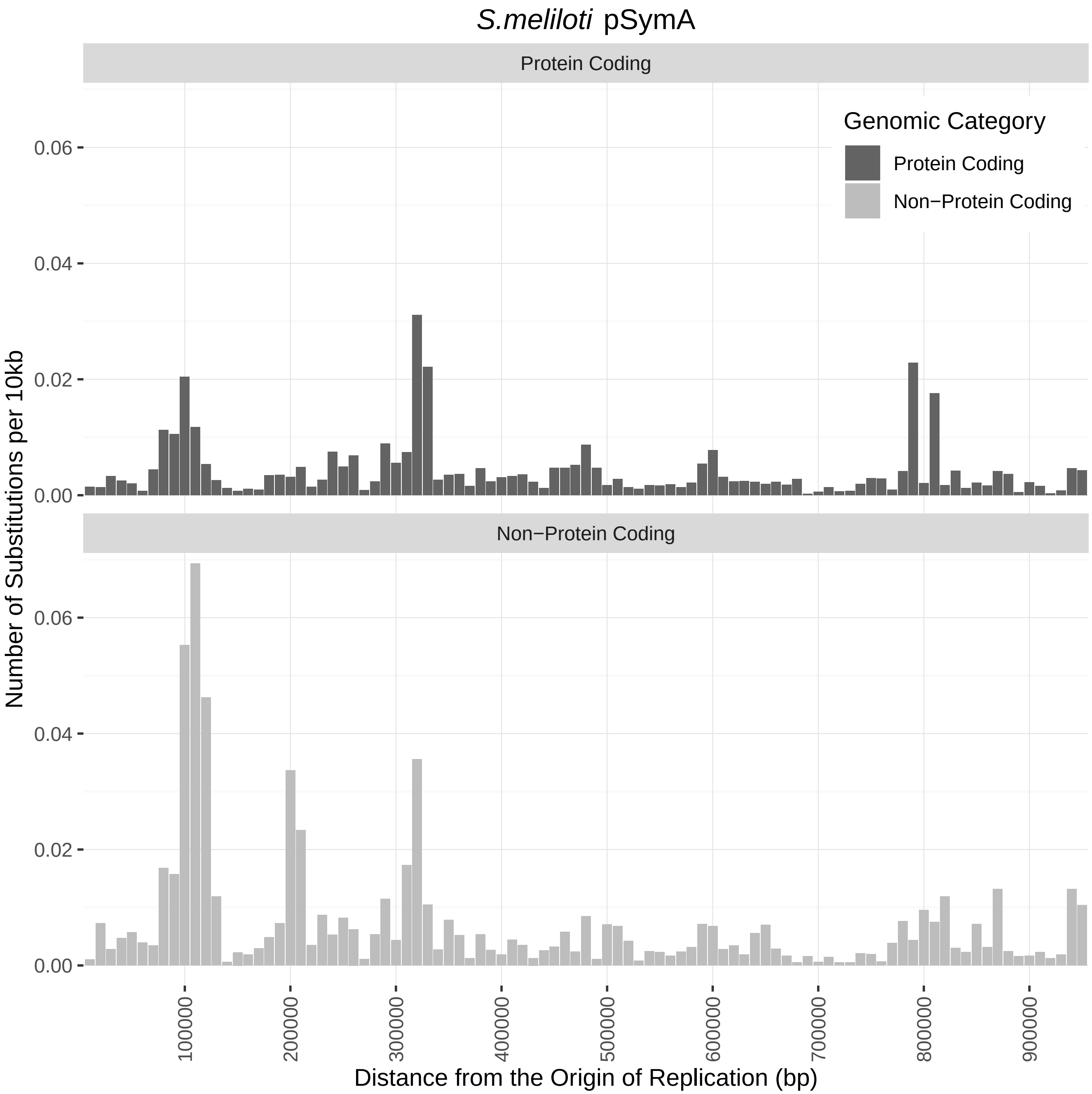
Next Week

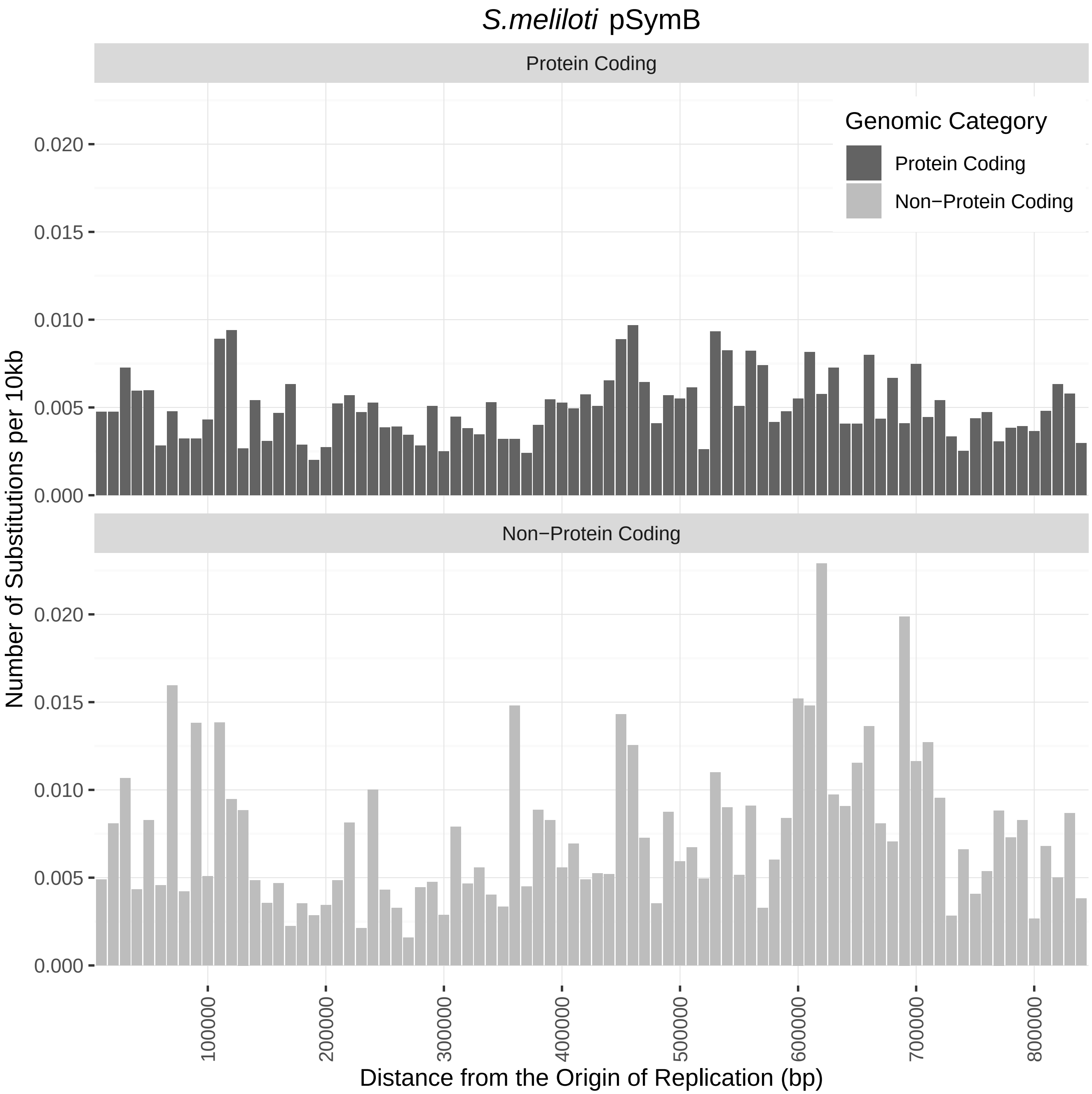
I would like to really investigate why *Streptomyces* has such weird values for dN, dS, and ω. I plan on first checking that for all the bacteria ω is not > 1 for any gene. Then calculating dN, dS, and ω by hand for a few *Streptomyces* genes to see what happens.


E.coli Chromosome
Protein Coding
Non−Protein Coding
Genomic Category
Protein Coding
Non−Protein Coding
Number of Substitutions per 10kb
0.00
0.01
0.02
0.03
0.04
100000
200000
300000
400000
500000
600000
700000
800000
900000
1000000
1100000
1200000
1300000
1400000
1500000
1600000
1700000
1800000
1900000
2000000
2100000
2200000
2300000
2400000
2500000
2600000
2700000
2800000
2900000
3000000
3100000
3200000
Distance from the Origin of Replication (bp)


B.subtilis Chromosome
Protein Coding
Non−Protein Coding
Genomic Category
Protein Coding
Non−Protein Coding
Number of Substitutions per 10kb
0.12
0.09
0.06
0.03
0.00
100000
200000
300000
400000
500000
600000
700000
800000
900000
1000000
1100000
1200000
1300000
1400000
1500000
1600000
1700000
1800000
1900000
2000000
2100000
2200000
Distance from the Origin of Replication (bp)


S.meliloti Chromosome
Protein Coding
Non−Protein Coding
Genomic Category
Protein Coding
Non−Protein Coding
Number of Substitutions per 10kb
0.0000
0.0025
0.0050
0.0075
0.0100
0.0125
Distance from the Origin of Replication (bp)
100000
200000
300000
400000
500000
600000
700000
800000
900000
1000000
1100000
1200000
1300000
1400000
1500000
1600000
1700000
1800000
1900000
2000000


S.meliloti pSymA
Protein Coding
Non−Protein Coding
Genomic Category
Protein Coding
Non−Protein Coding
Number of Substitutions per 10kb
0.00
0.02
0.04
0.06
100000
200000
300000
400000
500000
600000
700000
800000
900000
Distance from the Origin of Replication (bp)



	Protein Coding				Non-Protein Coding			
	Correlation Coefficient 20kb Near		Number of Substitutions per 20kb Near		Correlation Coefficient 20kb Near		Number of Substitutions per 20kb Near	
Bacteria and Replicon	Origin	Terminus	Origin	Terminus	Origin	Terminus	Origin	Terminus
E. coli Chromosome	-3.018×10^{-5}*	NS			-2.884×10^{-5}**	-5.276×10^{-5}*		
B. subtilis Chromosome					NS	5.960×10^{-5}**		
Streptomyces Chromosome	-1.988×10^{-5}***	-5.986×10^{-5}***			3.154×10^{-5}***	NS		
S. meliloti Chromosome	5.109×10^{-6}**	NS			NS	NS		
S. meliloti pSymA	NS	NS			1.425×10^{-4}***	-1.867×10^{-4}*		
S. meliloti pSymB	NS	-4.411×10^{-5}***			NS	-4.669×10^{-5}**		

Table 1: Logistic regression on 20kb closest and farthest from the origin of replication after accounting for bidirectional replication and outliers. All results are marked with significance codes as followed: < 0.001 = '***', $0.001 < 0.01$ = '**', $0.01 < 0.05$ = '*', > 0.05 = 'NS'.

Bacteria and Replicon	Gene Expression 10kb
E. coli Chromosome	-2.742×10^{-5}**
B. subtilis Chromosome	-2.198×10^{-5}*
Streptomyces Chromosome	-5.230×10^{-7}***
S. meliloti Chromosome	NS
S. meliloti pSymA	NS
S. meliloti pSymB	NS

Table 2: Linear regression analysis of the median counts per million expression data for 10kb segments of the genome of the respective bacteria replicons. Linear regression was calculated after the origin of replication was moved to the beginning of the genome and all subsequent positions were scaled around the origin accounting for bidirectionality of replication. All results are marked with significance codes as followed: < 0.001 = ‘***’, $0.001 < 0.01$ = ‘**’, $0.01 < 0.05$ = ‘*’, > 0.05 = ‘NS’.

Bacteria and Replicon	Coefficient Estimate	Standard Error	P-value
E. coli Chromosome	-6.03×10^{-5}	1.28×10^{-5}	2.8×10^{-6}
B. subtilis Chromosome	-9.7×10^{-5}	2.0×10^{-5}	1.2×10^{-6}
Streptomyces Chromosome	-1.17×10^{-6}	1.04×10^{-7}	$<2\times10^{-16}$
S. meliloti Chromosome	3.97×10^{-5}	4.25×10^{-5}	NS (3.5×10^{-1})
S. meliloti pSymA	1.39×10^{-3}	2.53×10^{-4}	4.9×10^{-8}
S. meliloti pSymB	1.46×10^{-4}	2.03×10^{-4}	NS ($5.34.7\times10^{-1}$)

Table 3: Linear regression analysis of the median counts per million expression data along the genome of the respective bacteria replicons. Linear regression was calculated after the origin of replication was moved to the beginning of the genome and all subsequent positions were scaled around the origin accounting for bidirectionality of replication.

Bacteria and Replicon	Coefficient Estimate
E. coli Chromosome	NS
B. subtilis Chromosome	-2.682×10^{-6}***
Streptomyces Chromosome	-2.360×10^{-6}***
S. meliloti Chromosome	-2.074×10^{-6}***
S. meliloti pSymA	NS
S. meliloti pSymB	-4.19×10^{-6}*

Table 4: Linear regression analysis of the total number of protein coding genes per 10kb along the genome of the respective bacteria replicons. Linear regression was calculated after the origin of replication was moved to the beginning of the genome and all subsequent positions were scaled around the origin accounting for bidirectionality of replication. All results are marked with significance codes as followed: < 0.001 = ‘***’, 0.001 < 0.01 = ‘**’, 0.01 < 0.05 = ‘*’, > 0.05 = ‘NS’.

Bacteria and Replicon	Protein Coding Sequences	Non-Protein Coding Sequences
E. coli Chromosome	-1.354×10^{-7}***	NS
B. subtilis Chromosome	-6.735×10^{-8}***	NS
Streptomyces Chromosome	4.105×10^{-7}***	1.635×10^{-7}***
S. meliloti Chromosome	-9.185×10^{-8}***	-1.749×10^{-7}***
S. meliloti pSymA	-8.121×10^{-7}***	-1.247×10^{-6}***
S. meliloti pSymB	1.655×10^{-7}***	4.105×10^{-7}***

Table 5: Logistic regression analysis of the number of substitutions along all positions of the genome of the respective bacteria replicons. These genomic positions were split up into the coding and non-coding regions of the genome. Grey coloured boxes indicate a negative logistic regression coefficient estimate. All results are statistically significant. Logistic regression was calculated after the origin of replication was moved to the beginning of the genome and all subsequent positions were scaled around the origin accounting for bidirectionality of replication. All results are marked with significance codes as followed: < 0.001 = ‘***’, 0.001 < 0.01 = ‘**’, 0.01 < 0.05 = ‘*’, > 0.05 = ‘NS’.

Bacteria and Replicon	dN	dS	ω
E. coli Chromosome	NS	NS	NS
B. subtilis Chromosome	NS	NS	$-9.08\times10^{-6}*$
Streptomyces Chromosome	NS	NS	NS
S. meliloti Chromoeom	NS	NS	NS
S. meliloti pSymA	NS	NS	NS
S. meliloti pSymB	NS	NS	$1.163\times10^{-5}*$

Table 6: Linear regression for dN, dS, and ω calculated for each bacterial replicon on a per genome basis. All results are marked with significance codes as followed: p: < 0.001 = '***', 0.001 $<$ 0.01 = '**', 0.01 $<$ 0.05 = '*', $>$ 0.05 = 'NS'.

Bacteria and Replicon	Average Expression Value (CPM)
E. coli Chromosome	160.500
B. subtilis Chromosome	176.400
Streptomyces Chromosome	6.084
S. meliloti Chromosome	271.400
S. meliloti pSymA	690.100
S. meliloti pSymB	595.700

Table 7: Arithmetic gene expression calculated across all genes in each replicon. Expression values are represented in Counts Per Million.

	Gene Average			Genome Average		
Bacteria and Replicon	dS	dN	ω	dS	dN	ω
E. coli Chromosome	1.0468	0.1330	1.3183	0.6491	0.0364	0.2432
B. subtilis Chromosome	4.652	0.2333	2.4200	1.0879	0.0703	0.3852
Streptomyces Chromosome	13.4950	2.0973	21.0423	5.1256	0.8911	8.9146
S. meliloti Chromosome	0.0184	0.0012	0.1069	0.0187	0.0013	0.0962
S. meliloti pSymA	1.0602	0.7451	5.1290	0.4100	0.0863	0.8311
S. meliloti pSymB	3.2602	0.0256	0.3878	0.1436	0.0100	0.1943

Table 8: Weighted averages calculated for each bacterial replicon on a per genome basis using the gene length as the weight. Arithmetic mean calculated for the per gene averages for each bacterial replicon.

Bacteria Strain/Species	GEO Accession Number	Date Accessed
E. coli K12 MG1655	GSE60522	December 20, 2017
E. coli K12 MG1655	GSE73673	December 19, 2017
E. coli K12 MG1655	GSE85914	December 19, 2017
E. coli K12 MG1655	GSE40313	November 21, 2018
E. coli K12 MG1655	GSE114917	November 22, 2018
E. coli K12 MG1655	GSE54199	November 26, 2018
E. coli K12 DH10B	GSE98890	December 19, 2017
E. coli BW25113	GSE73673	December 19, 2017
E. coli BW25113	GSE85914	December 19, 2017
E. coli O157:H7	GSE46120	August 28, 2018
E. coli ATCC 25922	GSE94978	November 23, 2018
B. subtilis 168	GSE104816	December 14, 2017
B. subtilis 168	GSE67058	December 16, 2017
B. subtilis 168	GSE93894	December 15, 2017
B. subtilis 168	GSE80786	November 16, 2018
S. coelicolor A3	GSE57268	March 16, 2018
S. natalensis HW-2	GSE112559	November 15, 2018
S. meliloti 1021 Chromosome	GSE69880	December 12, 2017
S. meliloti 2011 pSymA	NC_020527 (Dr. Finan)	April 4, 2018
S. meliloti 1021 pSymA	GSE69880	November 15, 18
S. meliloti 2011 pSymB	NC_020560 (Dr. Finan)	April 4, 2018
S. meliloti 1021 pSymB	GSE69880	November 15, 18

Table 9: Summary of strains and species found for each gene expression analysis. Gene Expression Omnibus accession numbers and date accessed are provided.