Subs Paper Things to Do:

- why does sinoC have omega lin reg = 0 near and far from the origin?
- create new graphs for selection analysis
- find and example of high substitution bar in *Streptomyces* and put this into supplement as an example of really diverged taxa (and that subs are real!)
- discuss removing omega outliers in methods
- double check that the ter and ori and max genome pos are correct
- ~~make graphs proportional to length of respective cod/non-cod regions~~
- ~~test examples for genes near and far from terminus (robust log reg/results)~~
- ~~linear regression on 10kb regions for weighted and non-weighted substitutions~~
- ~~average number of substitutions in 20kb regions near and far from the origin~~
- ~~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:

- if necessary add a phylogenetic component to the analysis
- codon bias?
- ~~make corrections based on Brian's edits~~
- ~~create a clean copy of the paper (no strikeout) for re-submission~~

Inversions and Gene Expression Letter Things to Do:

- create latex template for paper
- 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)

Last Week

✓lab meeting presentation

✓made edits to gene expression paper you suggested

✓made clean copy of gene expression paper with no red/blue marks

✓checked high dN dS values (they are real!)

✓made new selection graphs

✓still getting Queenie to do some stuff for the inversions and gene expression chapter

✓wrote a bunch for the substitutions paper

✓double check that I am using the correct ori, ter and max genome pos

I made all the appropriate changes for the gene expression paper that you suggested and looked it over again and made sure that we are discussing all the points mentioned by the reviewers. As discussed on friday, I hope that we can re-submit this afternoon.

I looked into some of the very high values fo dN and dS in some of the bacteria and they indeed seem to be real! The genes that I looked at had a lot of either synonymous or non-synonymous changes that is really causing dN or dS to be high! I plan on putting an example of a gene with

high substitutions in the supplement to illusturate this. However, this means that the selection values do truly range wildly, which makes it hard to display without a log scale.

I fixed the distribution graphs for the selection values, and they now include all data points plus averages over 100Kbp. These graphs are found below. Outliers are in grey, actual data points are in beige, average values are in dark brown, and the trendline connecting the average values is in blue. I need to pick better colours for the outliers and actual data points because they are too similar. It looks like for *S. meliloti* there are a lot of gaps in the data across the genome, so I need to look into this more.

I also attempted to re-do the selection summary graphs (boxplots) to make them a bit more accurate with the log scale and wide range of values. The zero and high values are real so we can not classify them as outliers, and removing them from the graph does not change anything (the graph still looks skewed). My two options are below, and I was wondering which one you thought was better? The violin plot does not include the zero values so its still not super representative.

Queenie is still coming around! Although it is very slow going because she is not coming in all that often. Right now she is working on just organizing the gene expression data and normalizing it.

I also did a lot of random writing for the substitutions paper, adding in changes to methods, outliers, updating graphs ... etc. I would like to have a completed draft by the end of the semester at the latest.

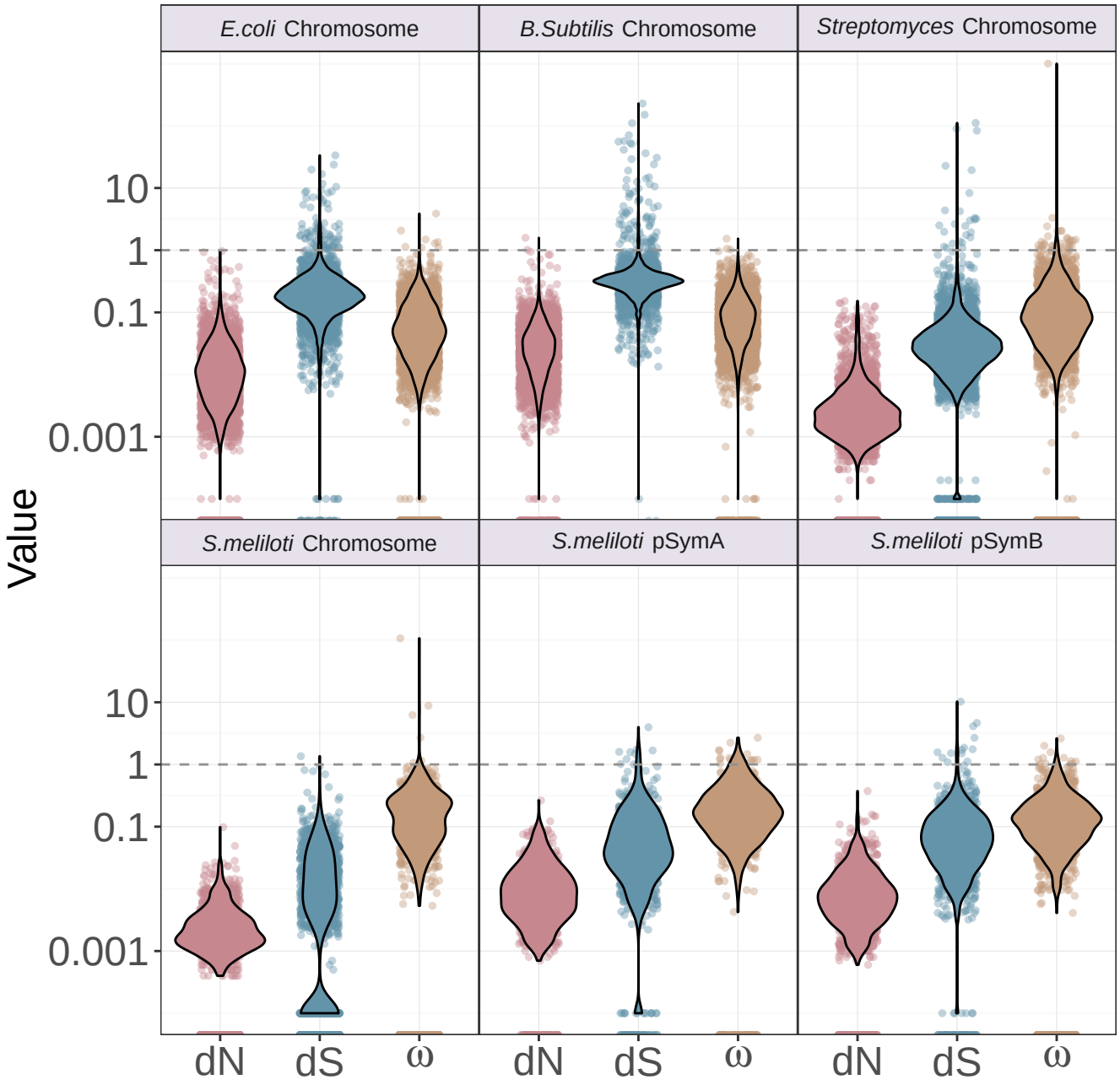
This Week

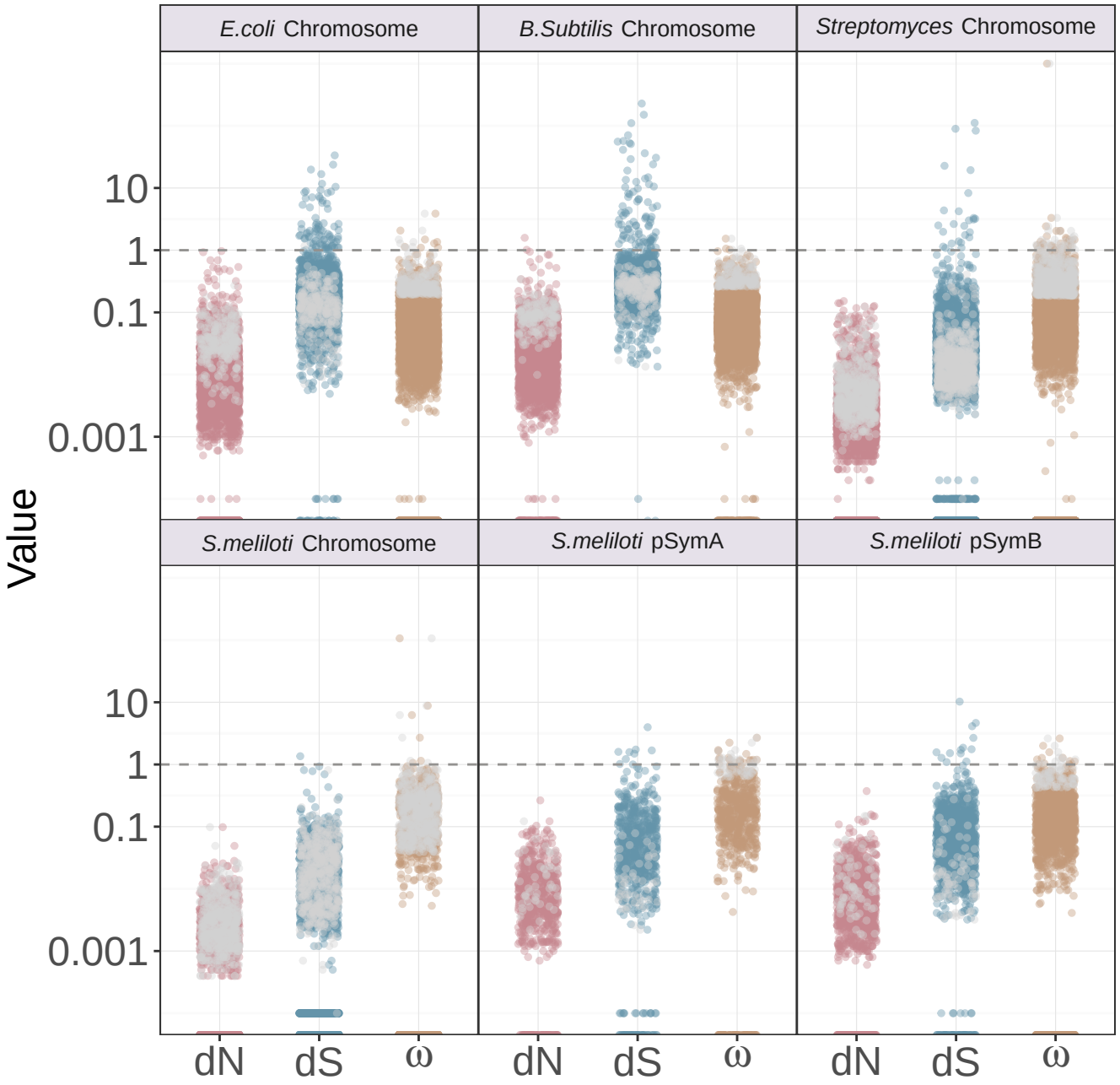
- submit gene expression paper
- change colours for outlier and actual data in selection distribution graphs
- why *S. meliloti* chromosome has an omega linear regression of 0 near and far from the origin
- find high substitution bar in *Streptomyces* as a supplementary example for the paper (to show that high subs that are left are real!)
- make changes to first year presentation

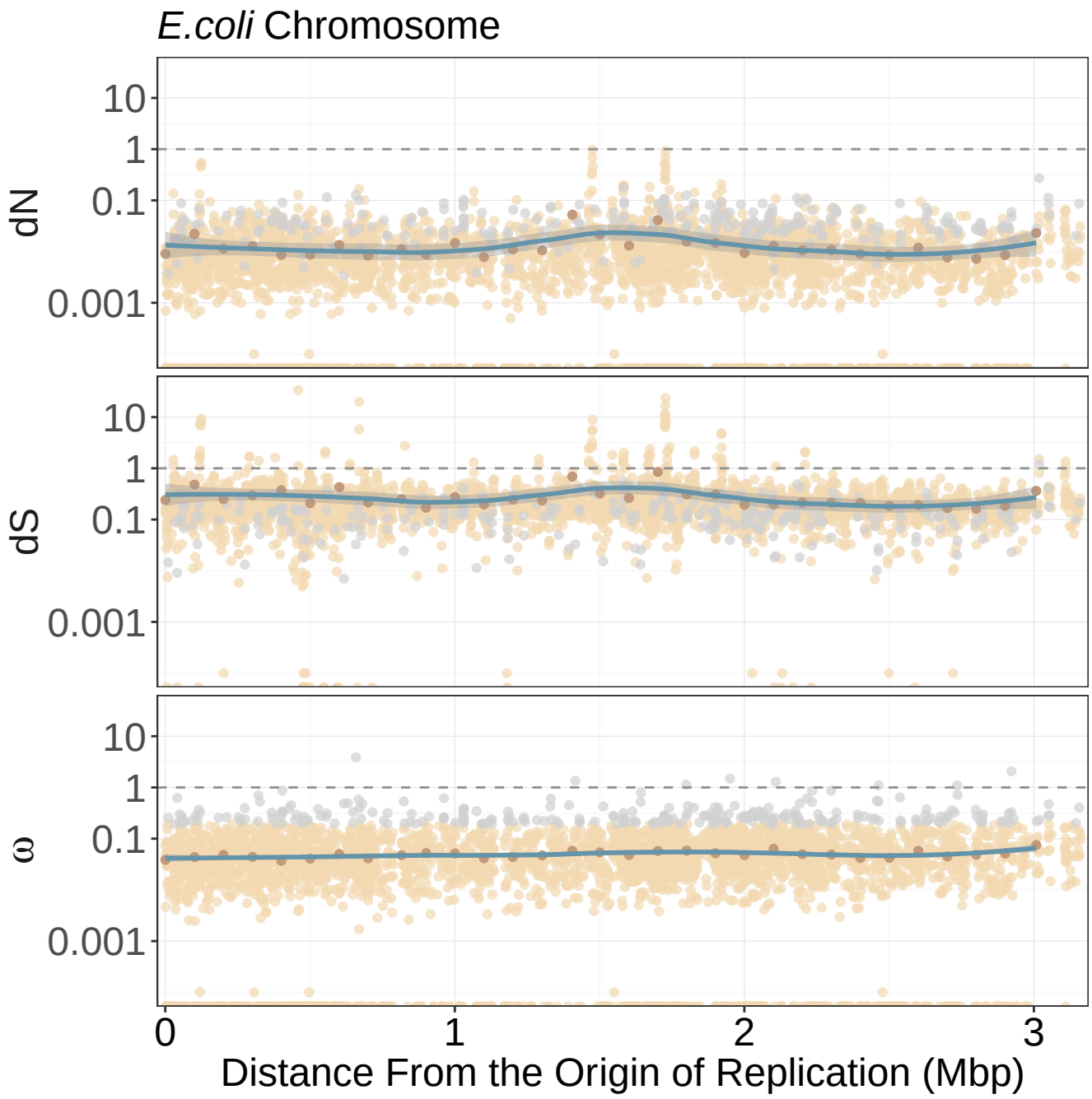
Next Week

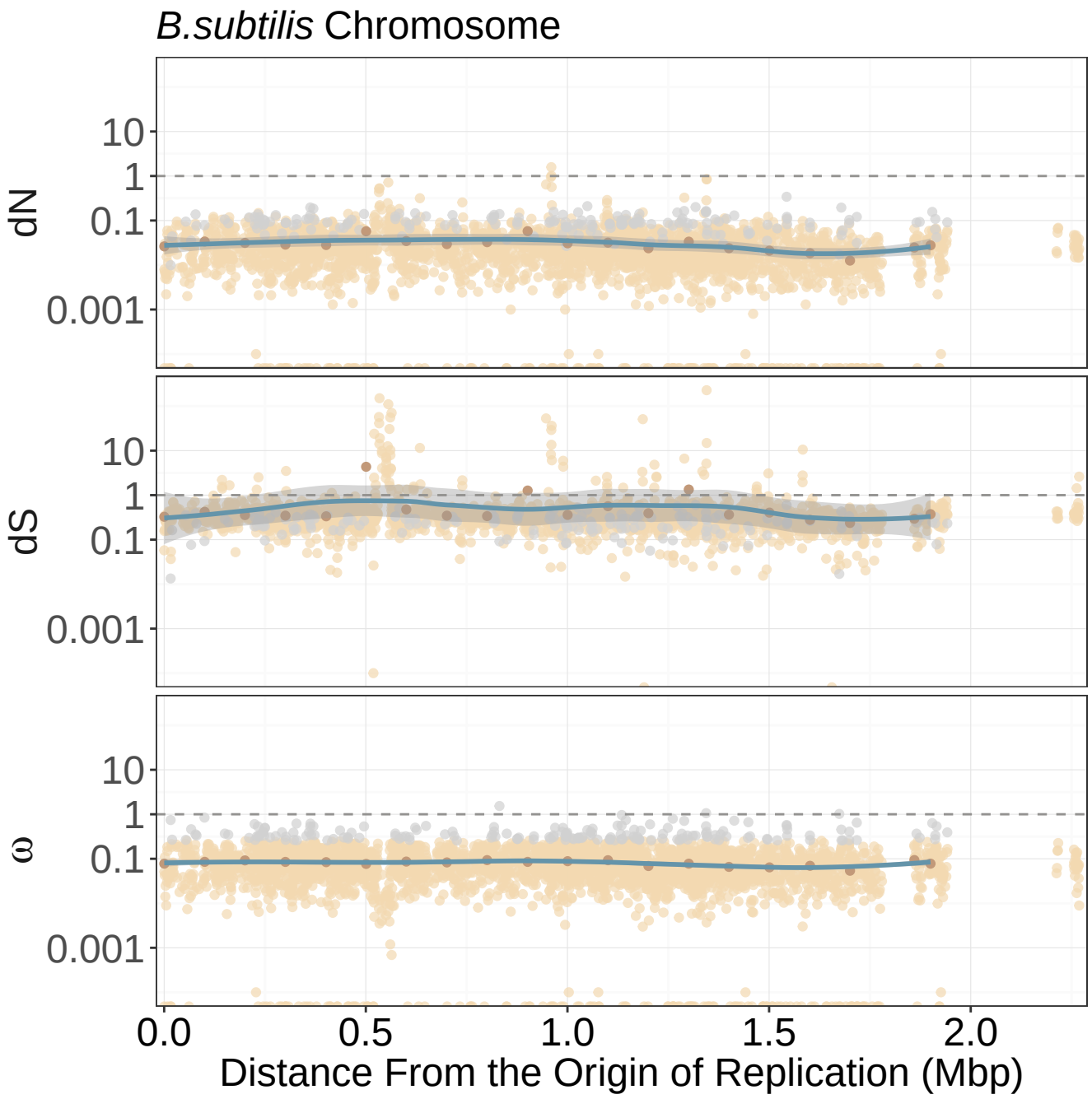
- try re-doing violin plots with log(x +1) to see if zeros will be included in the violin (selection analysis)
- find a block where mauve aligns non-homologous regions and put into supplement
- define a theme for the substitutions graphs (and selection graphs) and re-do these and put in paper

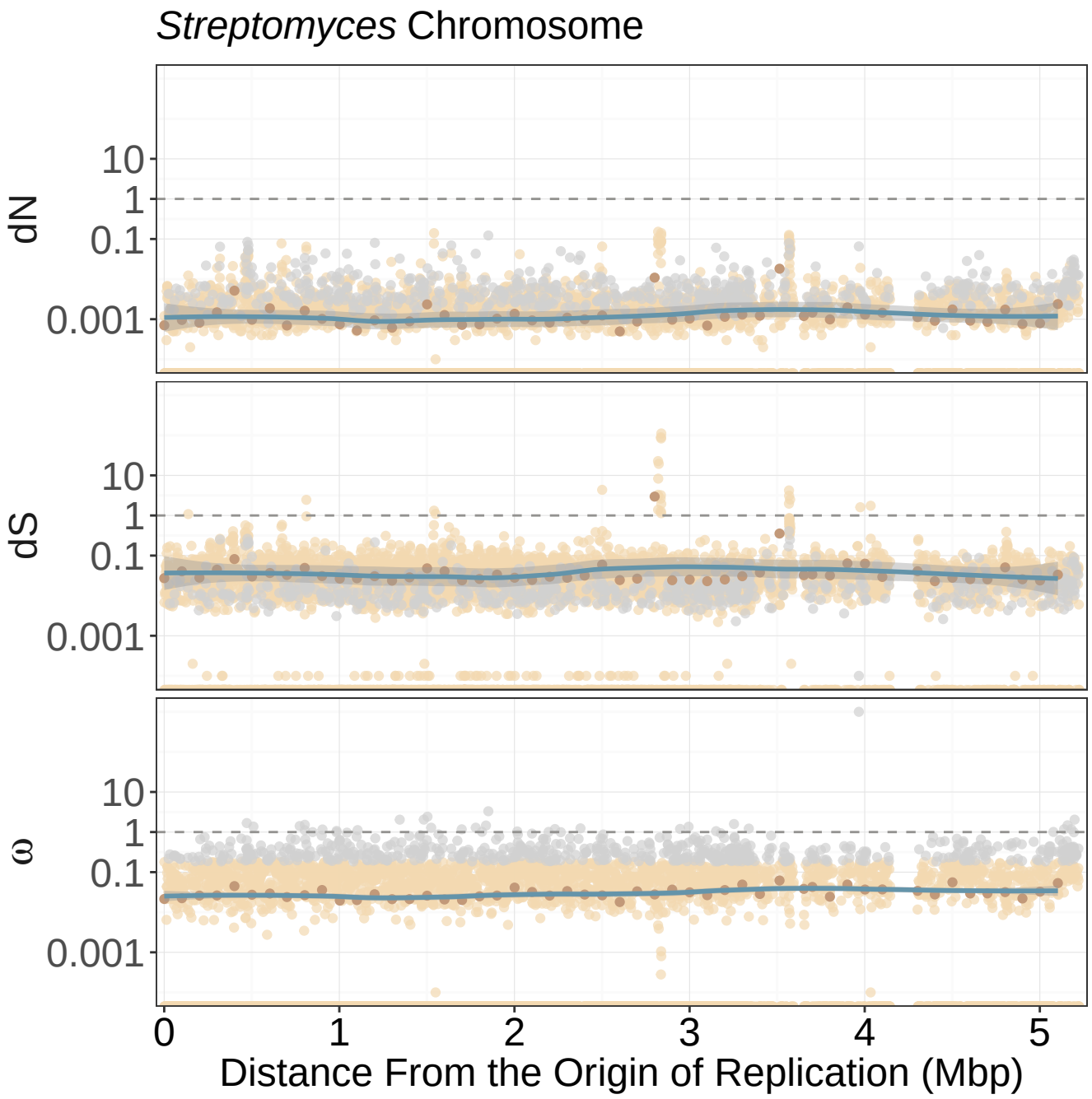
- Why does *S. meliloti* (and *B. subtilis*) have "missing data" in selection distribution graphs

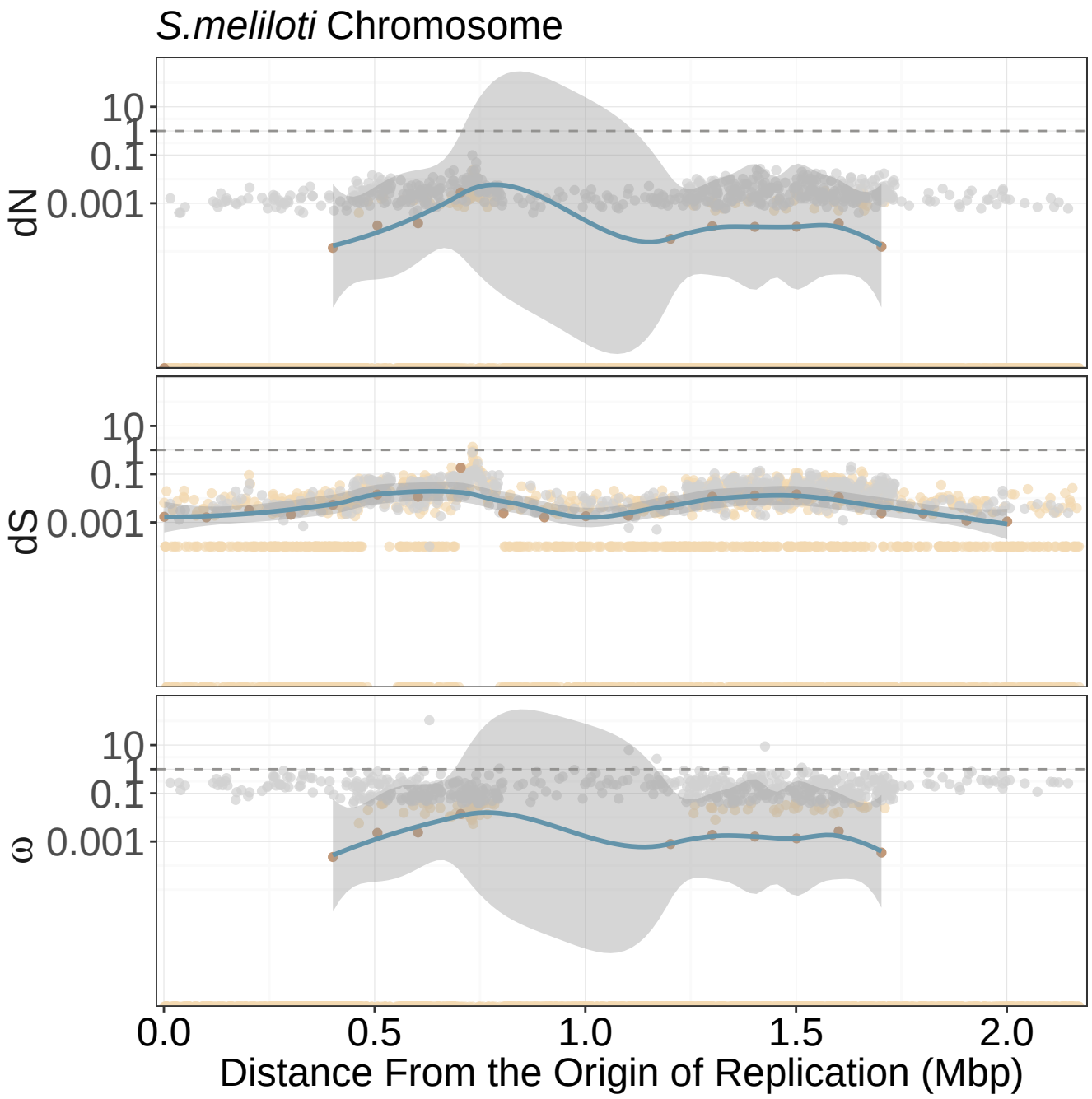

E.coli Chromosome
B.Subtilis Chromosome
Streptomyces Chromosome
S.meliloti Chromosome
S.meliloti pSymA
S.meliloti pSymB
Value
10
1
0.1
0.001
dN
dS
ω

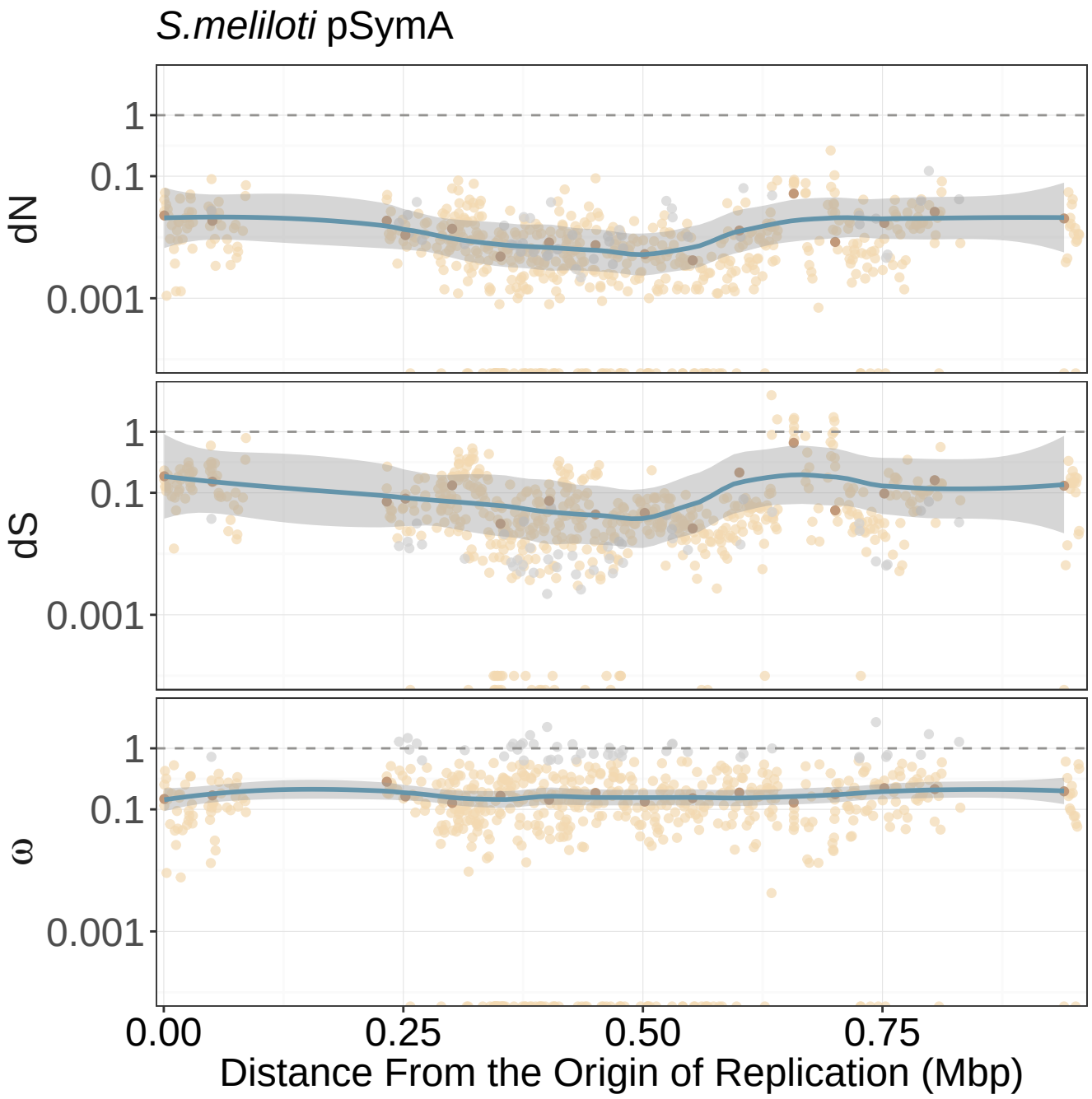

E.coli Chromosome
B.Subtilis Chromosome
Streptomyces Chromosome
S.meliloti Chromosome
S.meliloti pSymA
S.meliloti pSymB
Value
10
1
0.1
0.001
dN
dS
ω

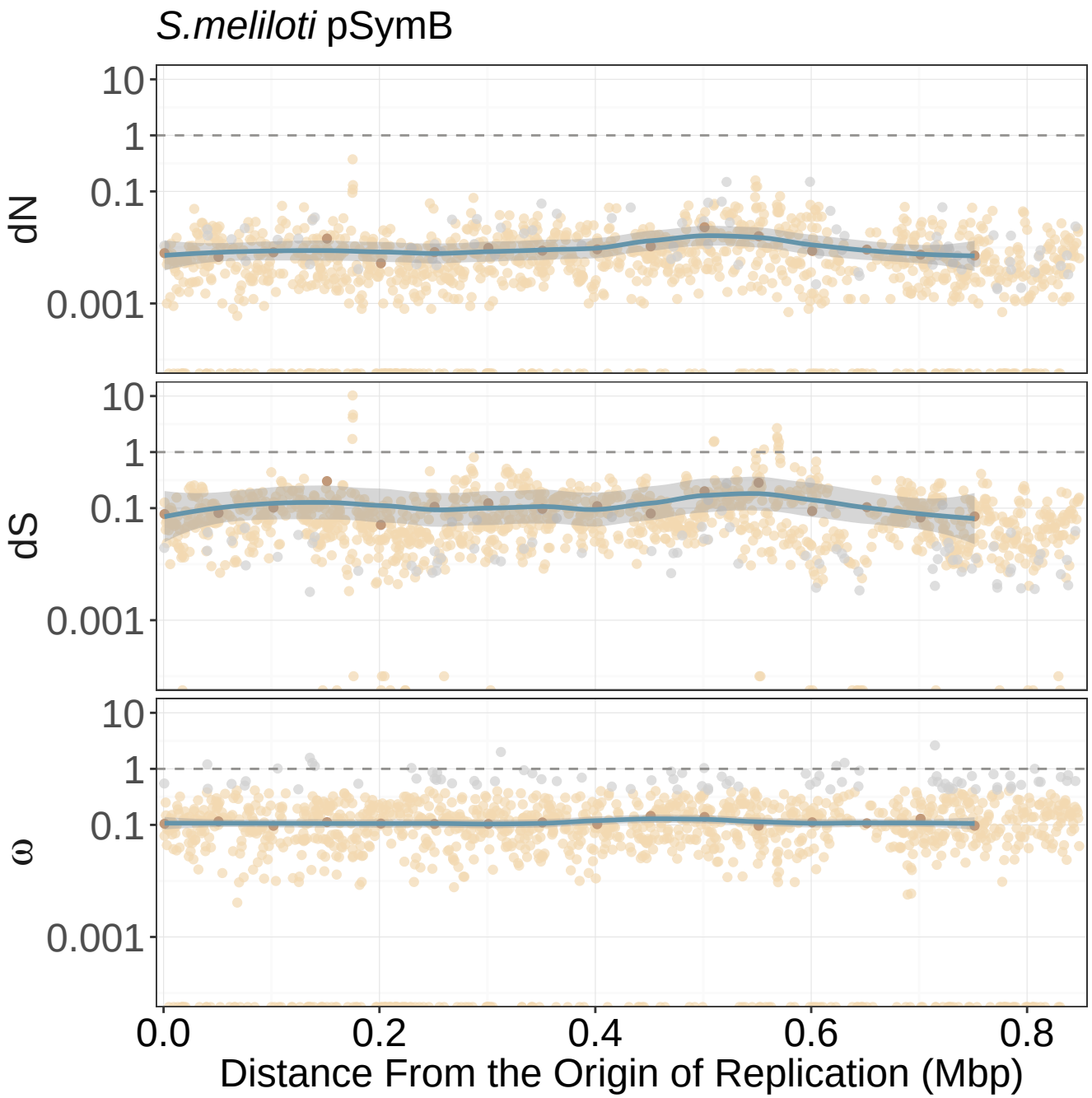

E.coli Chromosome
dN
dS
ω
10
1
0.1
0.001
0
1
2
3
Distance From the Origin of Replication (Mbp)


B.subtilis Chromosome
dN
dS
ω
10
1
0.1
0.001
0.0
0.5
1.0
1.5
2.0
Distance From the Origin of Replication (Mbp)


Streptomyces Chromosome
dN
dS
ω
10
1
0.1
0.001
0
1
2
3
4
5
Distance From the Origin of Replication (Mbp)


S.meliloti Chromosome
dN
dS
ω
10
1
0.1
0.001
0.0
0.5
1.0
1.5
2.0
Distance From the Origin of Replication (Mbp)


S.meliloti pSymA
dN
dS
ω
1
0.1
0.001
0.00
0.25
0.50
0.75
Distance From the Origin of Replication (Mbp)


S.meliloti pSymB
dN
dS
ω
10
1
0.1
0.001
0.0
0.2
0.4
0.6
0.8
Distance From the Origin of Replication (Mbp)

Bacteria and Replicon	Protein Coding Sequences
E. coli Chromosome	-1.98×10^{-8}***
B. subtilis Chromosome	-5.55×10^{-8}***
Streptomyces Chromosome	7.49×10^{-8}***
S. meliloti Chromosome	-4.19×10^{-7}***
S. meliloti pSymA	-5.18×10^{-7}***
S. meliloti pSymB	1.67×10^{-7}***

Table 1: 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'.

	Protein Coding			
	Correlation Coefficient 20kb Near		Number of Substitutions per 20kb Near	
Bacteria and Replicon	Origin	Terminus	Origin	Terminus
E. coli Chromosome	NS	NS	5.62×10^{-3}	6.66×10^{-3}
B. subtilis Chromosome	NS	-8.37×10^{-5}***	1.95×10^{-3}	9.10×10^{-3}
Streptomyces Chromosome	7.91×10^{-5}***	-1.32×10^{-4}***	6.74×10^{-4}	6.73×10^{-3}
S. meliloti Chromosome	8.26×10^{-5}*	NS	9.79×10^{-5}	5.07×10^{-5}
S. meliloti pSymA	NS	NS	9.75×10^{-4}	3.23×10^{-3}
S. meliloti pSymB	-1.44×10^{-5}*	-6.32×10^{-5}***	1.96×10^{-3}	1.24×10^{-3}

Table 2: 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'.

	Protein Coding	
Bacteria and Replicon	Weighted	Non-Weighted
E. coli Chromosome	-2.28×10^{-10}***	-1.65×10^{-4}***
B. subtilis Chromosome	-7.96×10^{-10}**	-1.73×10^{-4}**
Streptomyces Chromosome	2.38×10^{-11}*	NS
S. meliloti Chromosome	-1.05×10^{-10}***	-1.24×10^{-5}***
S. meliloti pSymA	NS	NS
S. meliloti pSymB	NS	NS

Table 3: Linear regression on 10kb sections of the genome with increasing distance from the origin of replication after accounting for bidirectional replication. Weighted columns have the total number of substitutions in each 10kb section of the genome divided by the total number of protein coding and non-protein coding sites in the genome. Non-weighted columns are performing a linear regression on the total number of substitutions in each 10kb section of the genome. 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
E. coli Chromosome	-2.32×10^{-2}***
B. subtilis Chromosome	-1.93×10^{-2}**
Streptomyces Chromosome	-1.24×10^{-3}***
S. meliloti Chromosome	-1.88×10^{-2}***
S. meliloti pSymA	-2.50×10^{-2}*
S. meliloti pSymB	NS

Table 4: Linear regression analysis of the total number of protein coding sites 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'.

	Gene/Genome Average		
Bacteria and Replicon	dS	dN	ω
E. coli Chromosome	0.2351	0.0101	0.0444
B. subtilis Chromosome	0.4201	0.0243	0.0714
Streptomyces Chromosome	0.0458	0.0011	0.0335
S. meliloti Chromosome	9.0094	0.0001	0.0015
S. meliloti pSymA	0.0872	0.0099	0.1642
S. meliloti pSymB	0.0940	0.0084	0.1142

Table 5: 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.