Subs Paper Things to Do:

- why are the lin reg of dN, dS and ω NS but the subs graphs are...explain!
- 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?

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

✓look into what is up with the chromosome of *S. meliloti*

Edits to the Substitution Paper:

✓change selection and substitution figure captions

✓comment about 0.0001 lines in selection plots

✓add that high values of dS are real

✓edits to methods based on Brian's comments

✓finish personal edits to results section to send to Brian

✓edits to results based on Brian's edits

✓think about and write/edit future directions in discussion

✓think about and edit broad questions in intro and discussion

✓think about and write/edit weakness in discussion

Continuing to look into what is going on with the selection stuff in *S. meliloti* chromosome and basically the only thing I can see is that *S. meliloti* chromosome is really really similar between these taxa. I looked at the progressiveMauve alignment plots of *S. meliloti* replicons, and it basically tells the same story. the two plasmids have more variation in the backbone (which parts are similar between the taxa) than the chromosome.

I also looked at the misc_features in each of the bacterial replicons because these are NOT included as part of my analysis. *S. meliloti* as a whole (all replicons) has about 75-76% of its genes as misc_features, where as all the others are much less (*E. coli* = 0.5%, *B. subtilis* = 1.6% and *Streptomyces* = 46%). The two bacteria that have lower substitutions (*Streptomyces* and *S. meliloti*) are the ones with more misc_features, but since the secondary replicons of *S. meliloti* have about the same percentage of misc_features as the chromosome, I do not think that the misc_features are influencing why the chromosome of *S. meliloti* is so weird.

I looked at dN, dS, and ω values between the chromosome of *S. meliloti* and the secondary replicons (looking at sections of the alignment by hand) and again, it all is real. The massive amounts of zero values for dN and ω in the chromosome are real. So it again looks like the chromosome just has less variation. However, the issue is that because there are so many zero values for ω in the chromosome, anything where ω is $>$ zero is considered an outlier. Which makes all the calculations for linear regressions and such very off. But we can't really do anything about this because we would then have to change the way outliers are calculated for all the bacteria. Additionally, in the genes where ω is > 0 in the chromosome, there are still VERY few substitutions compared to genes where $\omega > 0$ in the other bacteria/replicons. So again, it looks like the chromosome of *S. meliloti* just has less variation.

I created graphs showing the number of sites per 10Kbp for the substitutions analysis to see if maybe the chromosome of *S. meliloti* was under represented (Figures 9 - 13). From these graphs, we can see that all the bacteria have some areas where there are an under representation of sites in different places in the genomes. So I do not think that lack of data is the cause for the weirdness in the chromosome of *S. meliloti*.

Even when looking at the average number of substitutions per genome, we see that the chromosome of *S. meliloti* has the fewest substitutions. *Streptomyces* is on the same order of magnitude, which to a degree makes sense because it is also very similar (based on the progressiveMauve alignment), but is also puzzling because *Streptomyces* appears to not have these issues.

I added *A. tumefaciens* as a taxa to the *S. meliloti* chromosome analysis to test if this changes the values of dN, dS, and ω to be similar in magnitude to the other bacteria (at your request), see Table 1. we see that dS is 3 orders of magnitude bigger than dS, but all the ω values are < 1 (even outliers), which I suppose sort of makes sense because these taxa are more distantly related than the taxa in the other bacterial analysis. **Should I instead use a different *Sinorhizobium* species to add to the analysis? What are your thoughts on all of this extra digging I have done regarding why the chromosome of *S. meliloti* looks so weird?**

I made lots of little edits and wrote more for the substitution paper (see above checklist).

I am still really confused about why the selection graph for *S. meliloti* Chromosome looks so odd and the only explanation I can come up with is that the sequences are just really really similar. **Do you have any thoughts on this or suggestions for other things I could investigate to figure out what is going on?**

This Week

- continue look into whats up with *S. meliloti* chrom bc it does not look right at all
- look into the numerous misc features in *S. meliloti*
- change caption for selection distribution figures so that they match how many bp the averages were calculated over (PA and PB are different)
- fix the results to properly talk about the selection and substitution figs
- make a comment about why there are two lines in the box plot (one at 0 and one at about 0.0001), in caption? or discussion?
- add that high dS values are also real and due to real changes where most of the gene is syn changes with very few non-syn changes and therefore it skews the whole calculation, creating a very high dS value. mention supplemental high subs bar

Next Week

- think about if the selection distribution figs or the summary selection fig should be in the main paper
- re-word captions for all figures so they make sense with current figures
- fix discussion

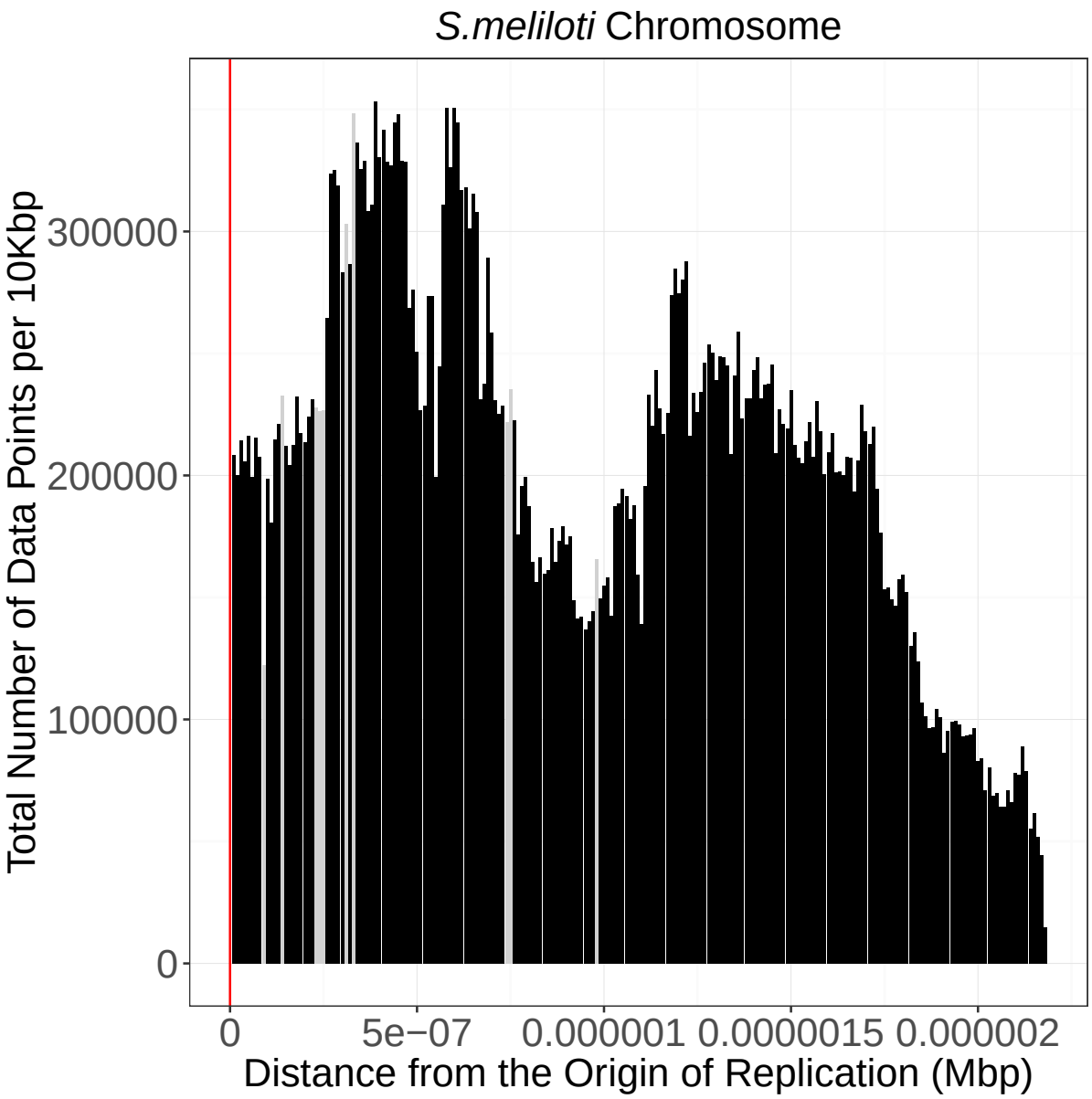


Figure 1: Distribution of total number of substitution data points per 10Kbp in genome.

	Genome Average		
Bacteria and Replicon	dS	dN	ω
S. meliloti* Chrom + *A. tumefaciens	12.5529	0.0553	0.0265
E. coli Chromosome	0.2387	0.0101	0.0441
B. subtilis Chromosome	0.4201	0.0243	0.0714
Streptomyces Chromosome	0.0458	0.0011	0.0335
S. meliloti Chromosome	0.0029	0	0
S. meliloti pSymA	0.0835	0.0099	0.1645
S. meliloti pSymB	0.0940	0.0084	0.1142

Table 1: 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.

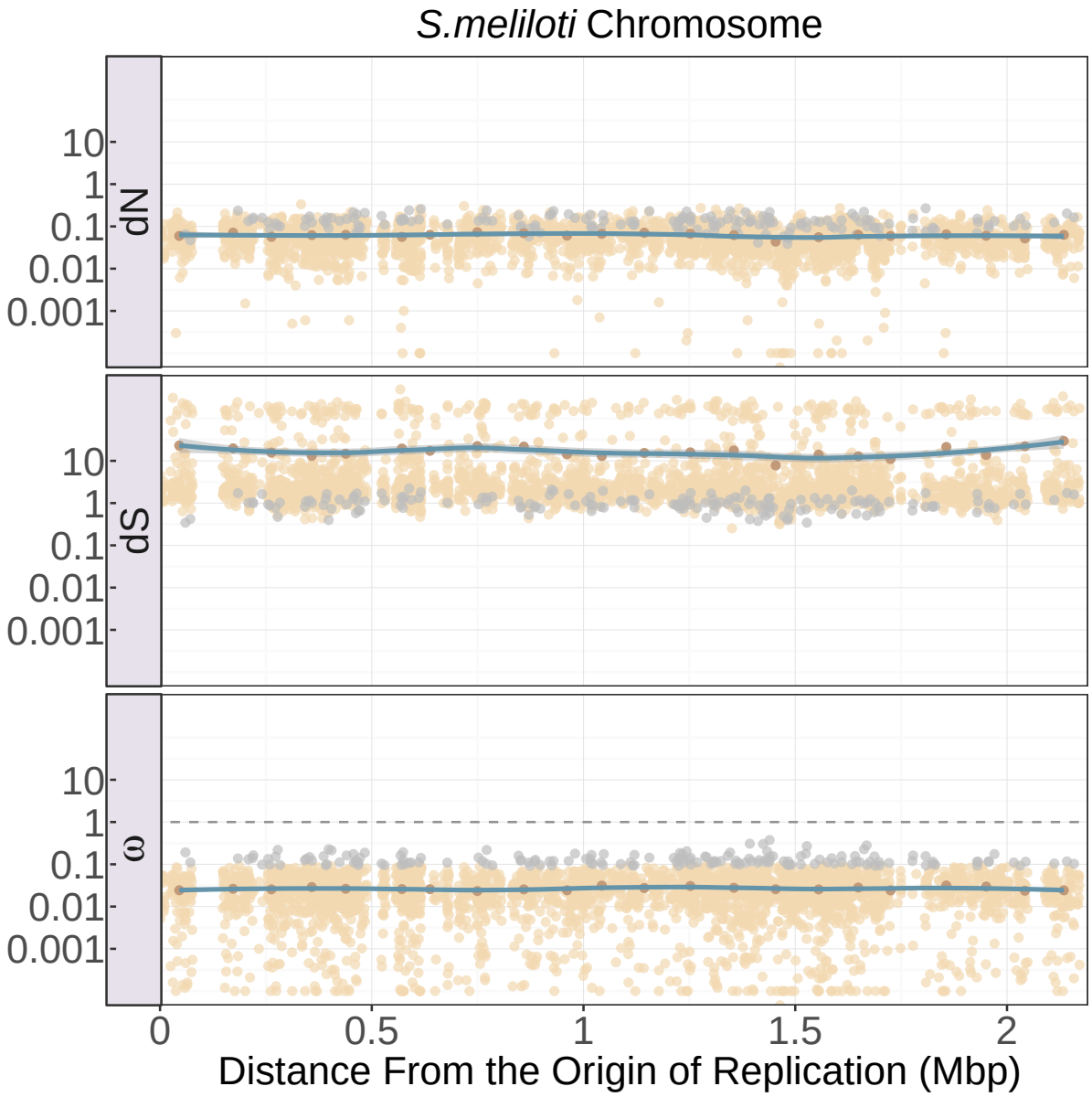
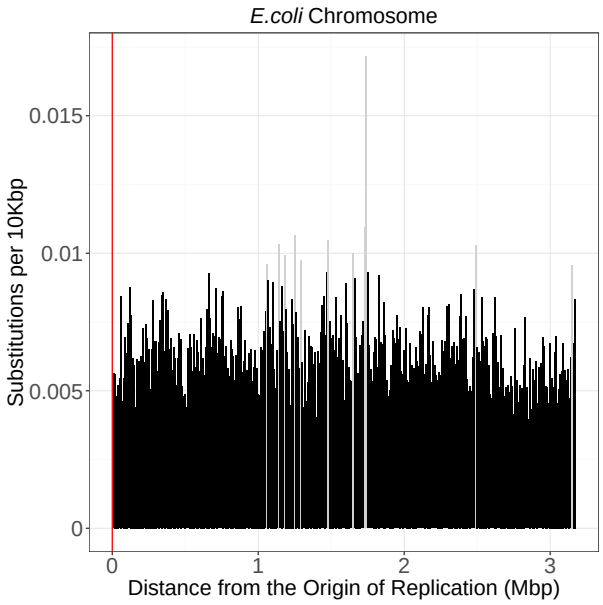
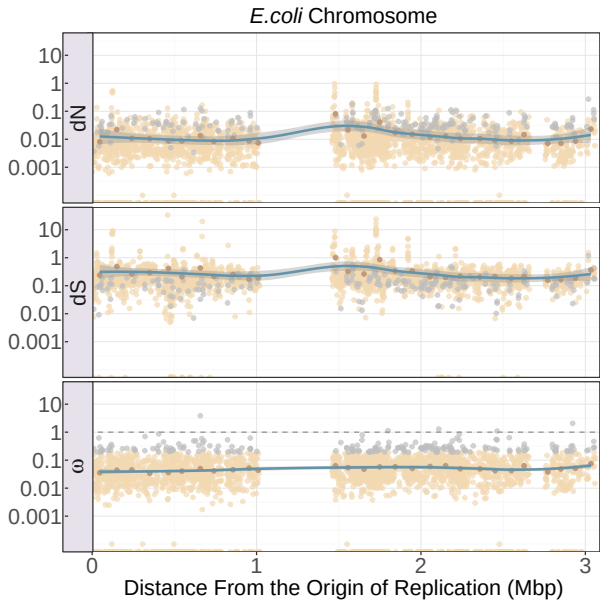


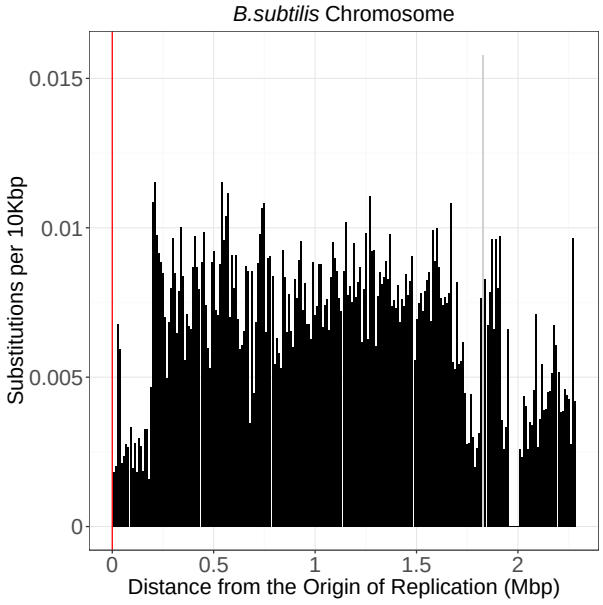
Figure 2: dN, dS, and ω values for *S. meliloti* chromosomes and ***A. tumefaciens***.


E.coli Chromosome
Substitutions per 10Kbp
0.015
0.01
0.005
0
0
1
2
3
Distance from the Origin of Replication (Mbp)

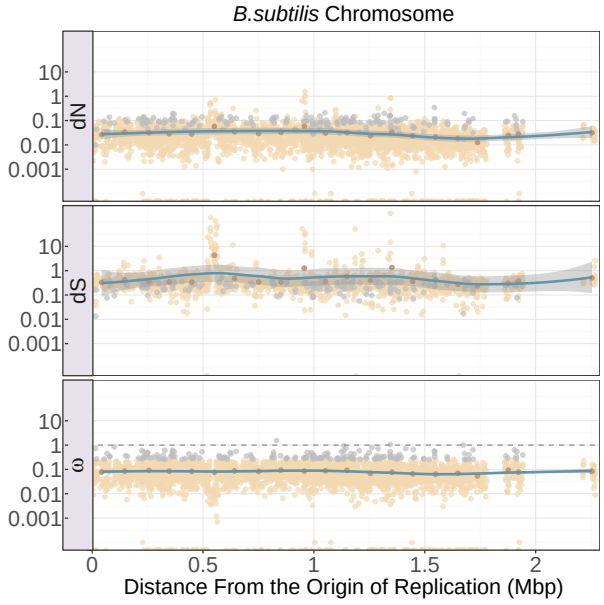
(a)


E.coli Chromosome
dN
dS
ω
10
1
0.1
0.01
0.001
0
1
2
3
Distance From the Origin of Replication (Mbp)

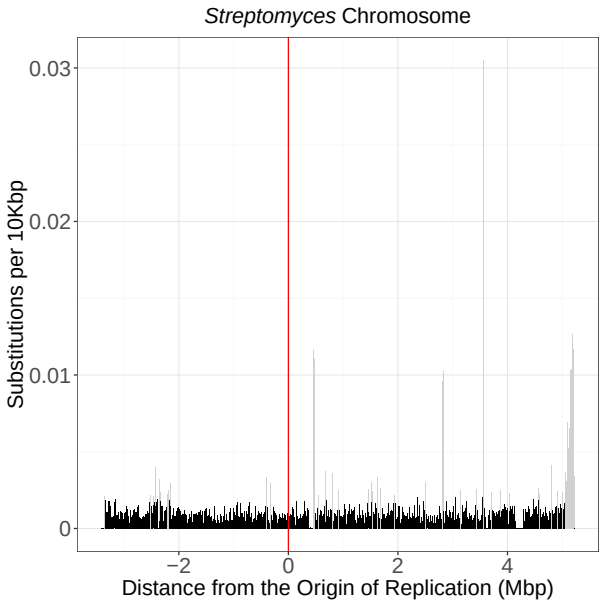
(b)


B.subtilis Chromosome
Substitutions per 10Kbp
0.015
0.01
0.005
0
0
0.5
1
1.5
2
Distance from the Origin of Replication (Mbp)

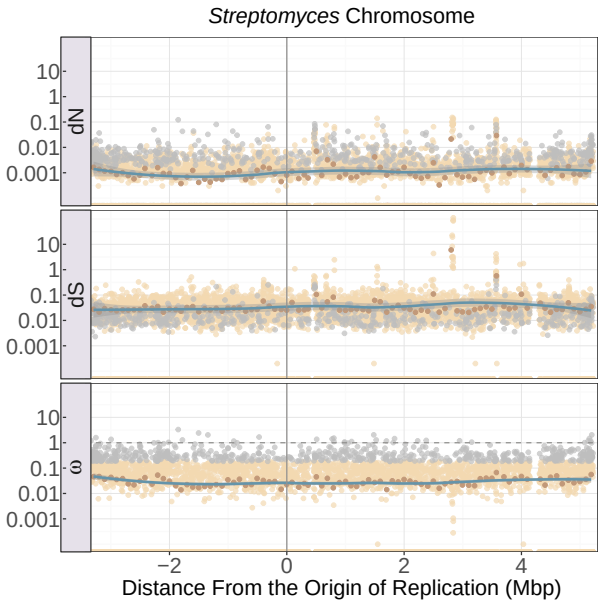
(a)


B.subtilis Chromosome
dN
dS
ω
10
1
0.1
0.01
0.001
0
0.5
1
1.5
2
Distance From the Origin of Replication (Mbp)

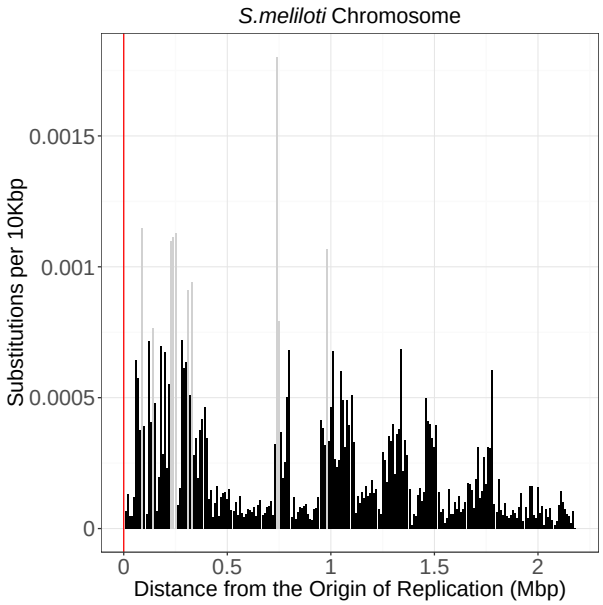
(b)


Streptomyces Chromosome
Substitutions per 10Kbp
0.03
0.02
0.01
0
−2
0
2
4
Distance from the Origin of Replication (Mbp)

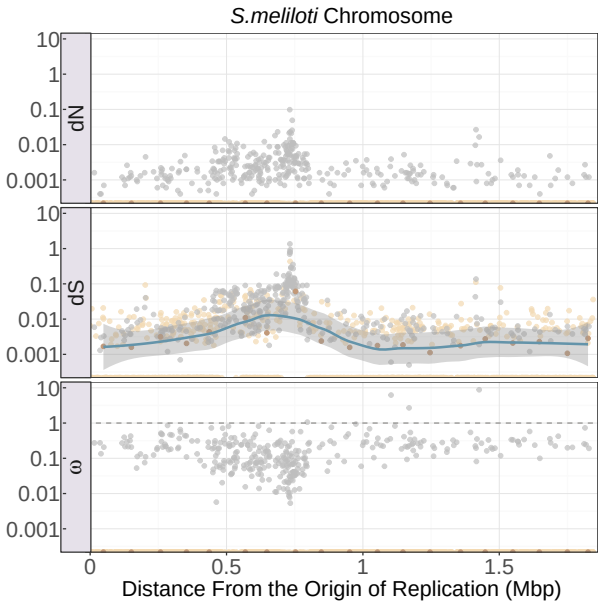
(a)


Streptomyces Chromosome
dN
dS
ω
10
1
0.1
0.01
0.001
−2
0
2
4
Distance From the Origin of Replication (Mbp)

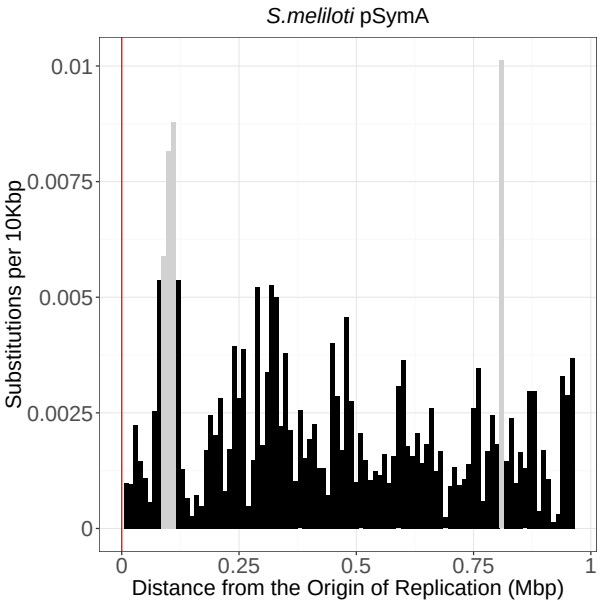
(b)


S.meliloti Chromosome
Substitutions per 10Kbp
0.0015
0.001
0.0005
0
0
0.5
1
1.5
2
Distance from the Origin of Replication (Mbp)

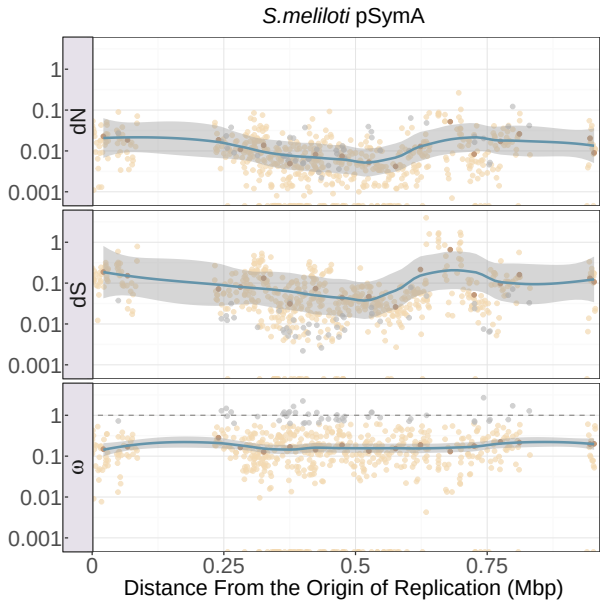
(a)


S.meliloti Chromosome
dN
dS
ω
10
1
0.1
0.01
0.001
0
0.5
1
1.5
Distance From the Origin of Replication (Mbp)

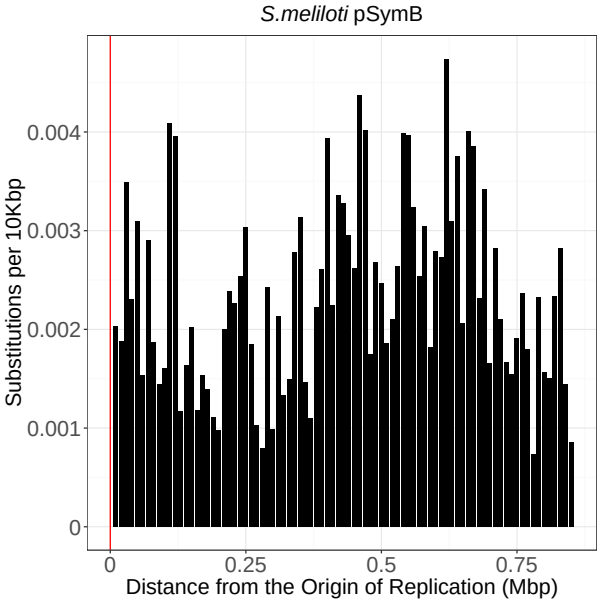
(b)


S.meliloti pSymA
Substitutions per 10Kbp
0.01
0.0075
0.005
0.0025
0
0
0.25
0.5
0.75
1
Distance from the Origin of Replication (Mbp)

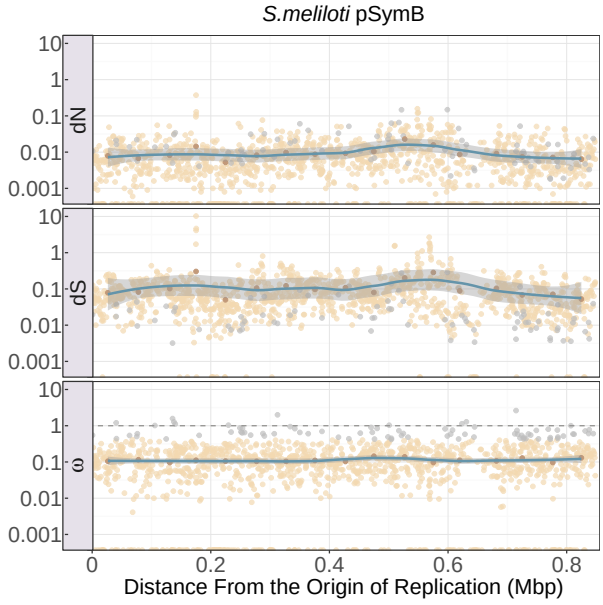
(a)


S.meliloti pSymA
dN
dS
ω
1
0.1
0.01
0.001
0
0.25
0.5
0.75
Distance From the Origin of Replication (Mbp)

(b)


S.meliloti pSymB
Substitutions per 10Kbp
0.004
0.003
0.002
0.001
0
0
0.25
0.5
0.75
Distance from the Origin of Replication (Mbp)

(a)


S.meliloti pSymB
dN
dS
ω
10
1
0.1
0.01
0.001
0
0.2
0.4
0.6
0.8
Distance From the Origin of Replication (Mbp)

(b)

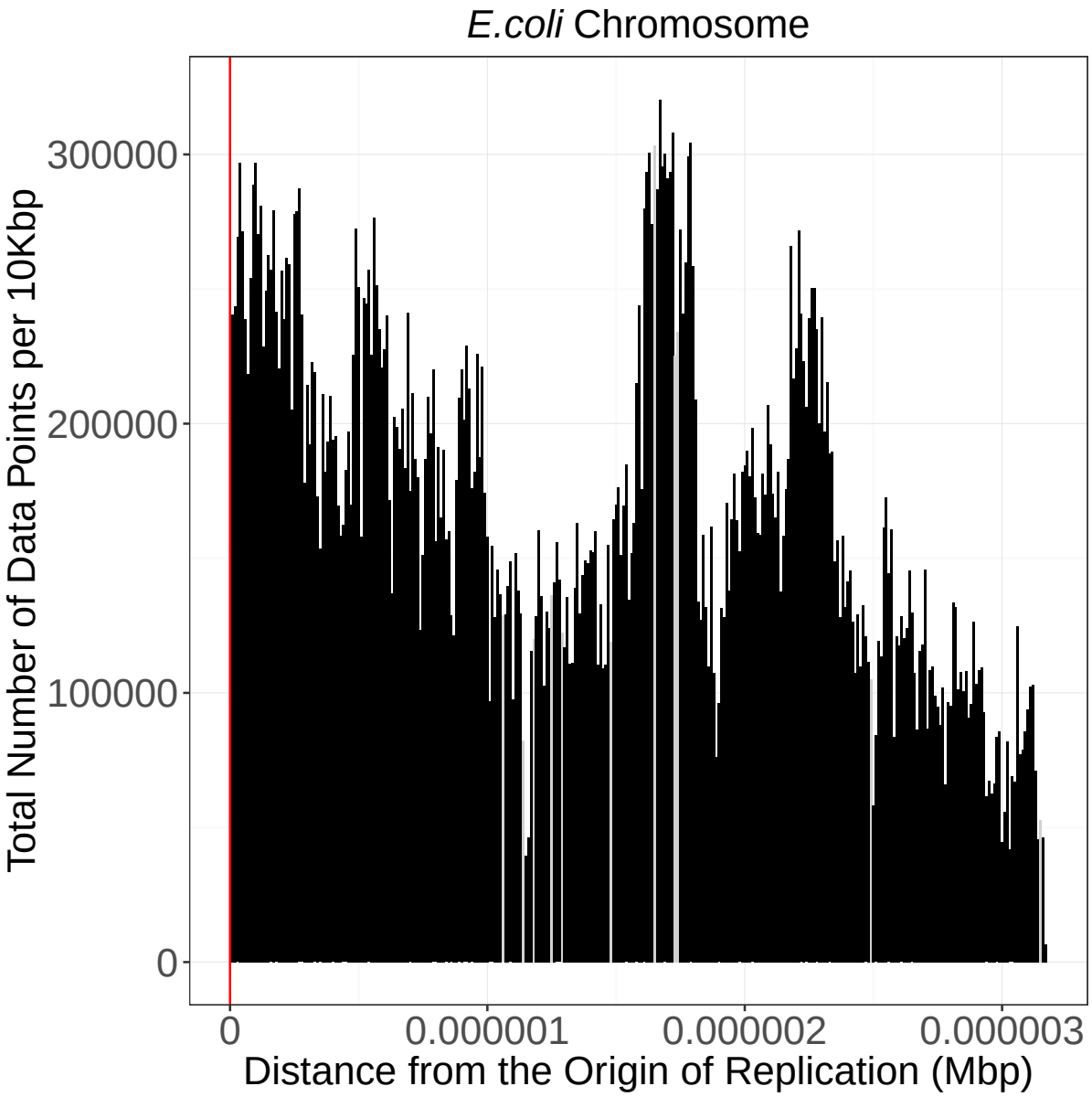


Figure 9: Distribution of total number of substitution data points per 10Kbp in genome.

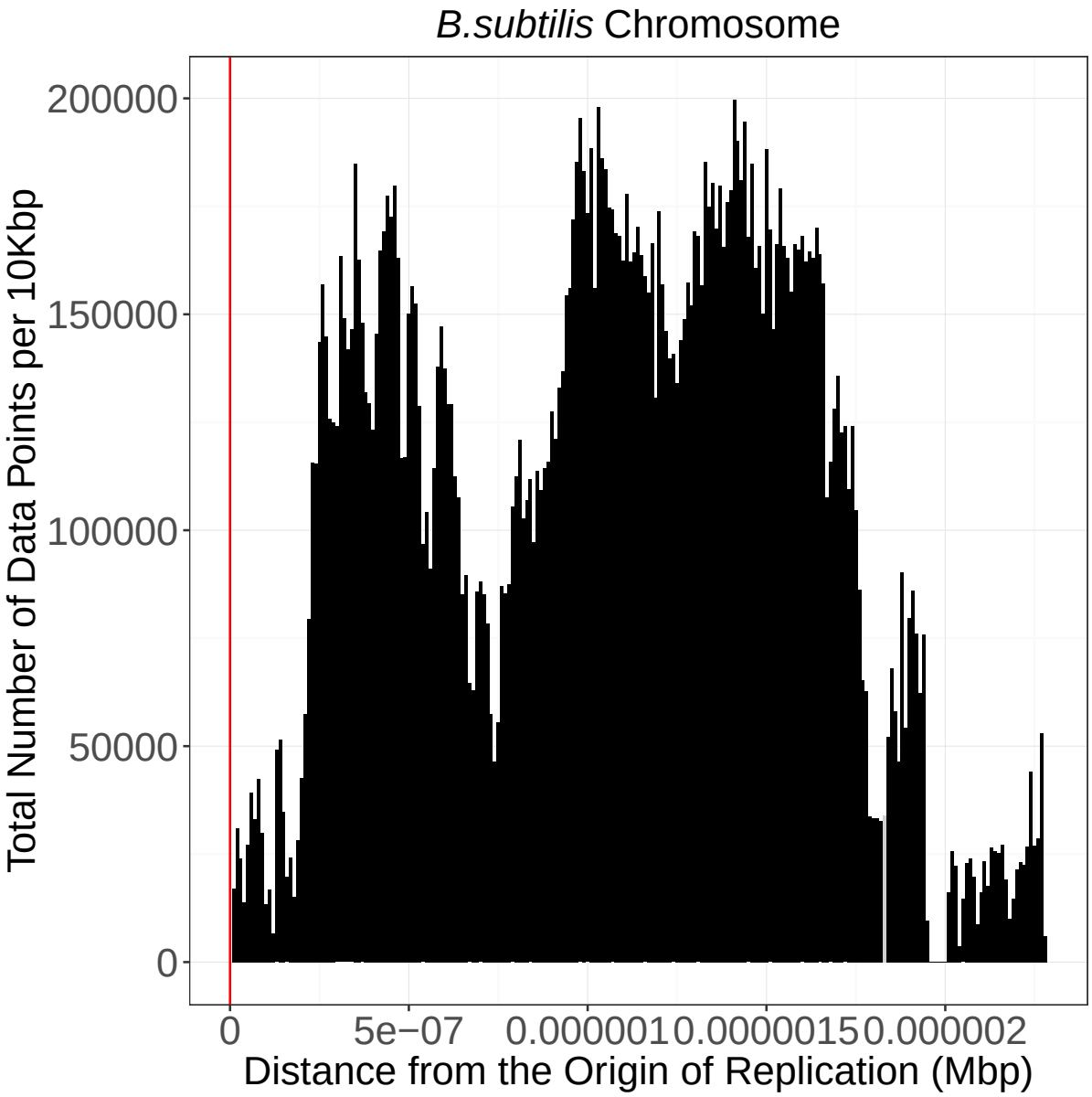


Figure 10: Distribution of total number of substitution data points per 10Kbp in genome.

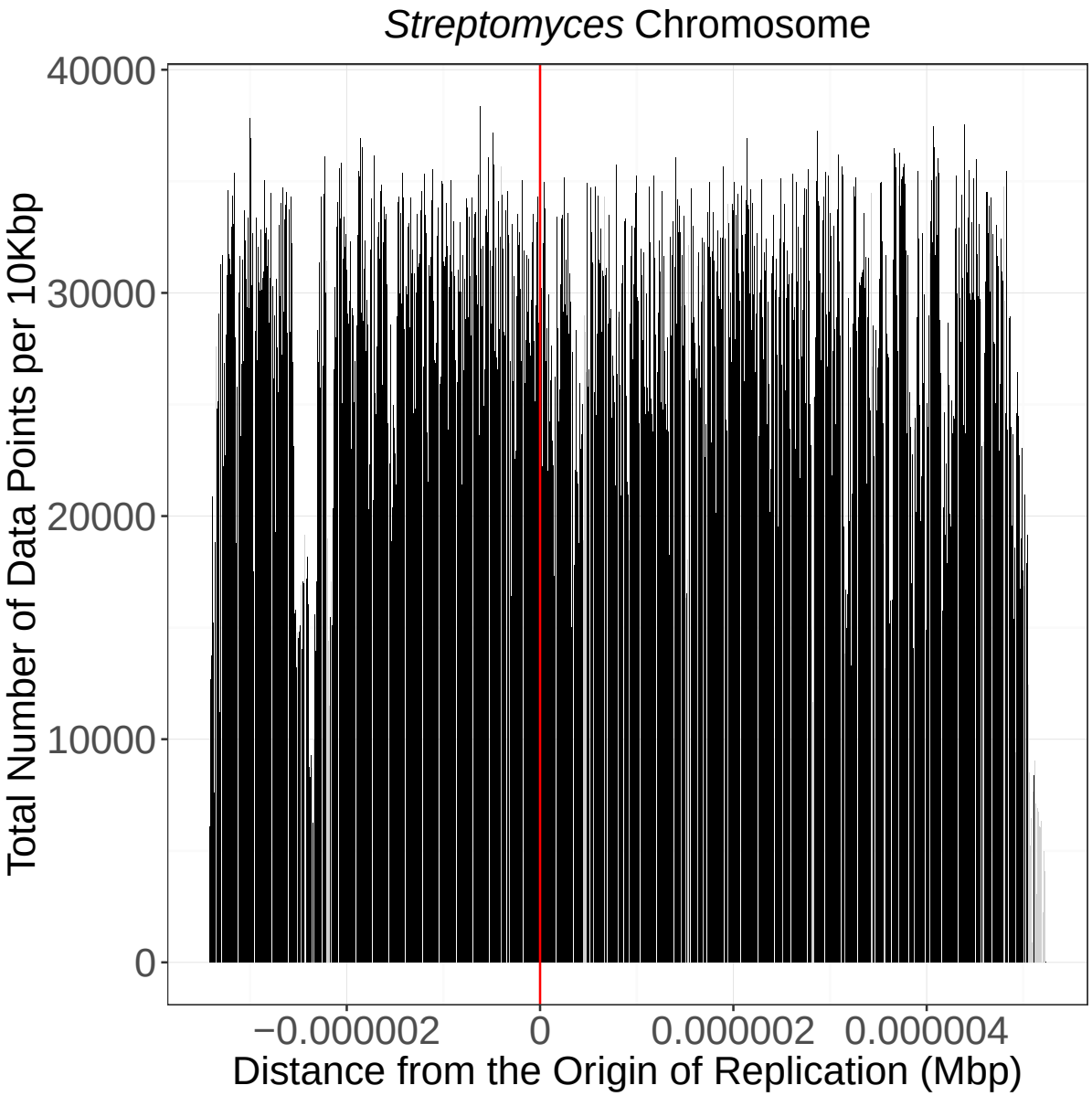


Figure 11: Distribution of total number of substitution data points per 10Kbp in genome.

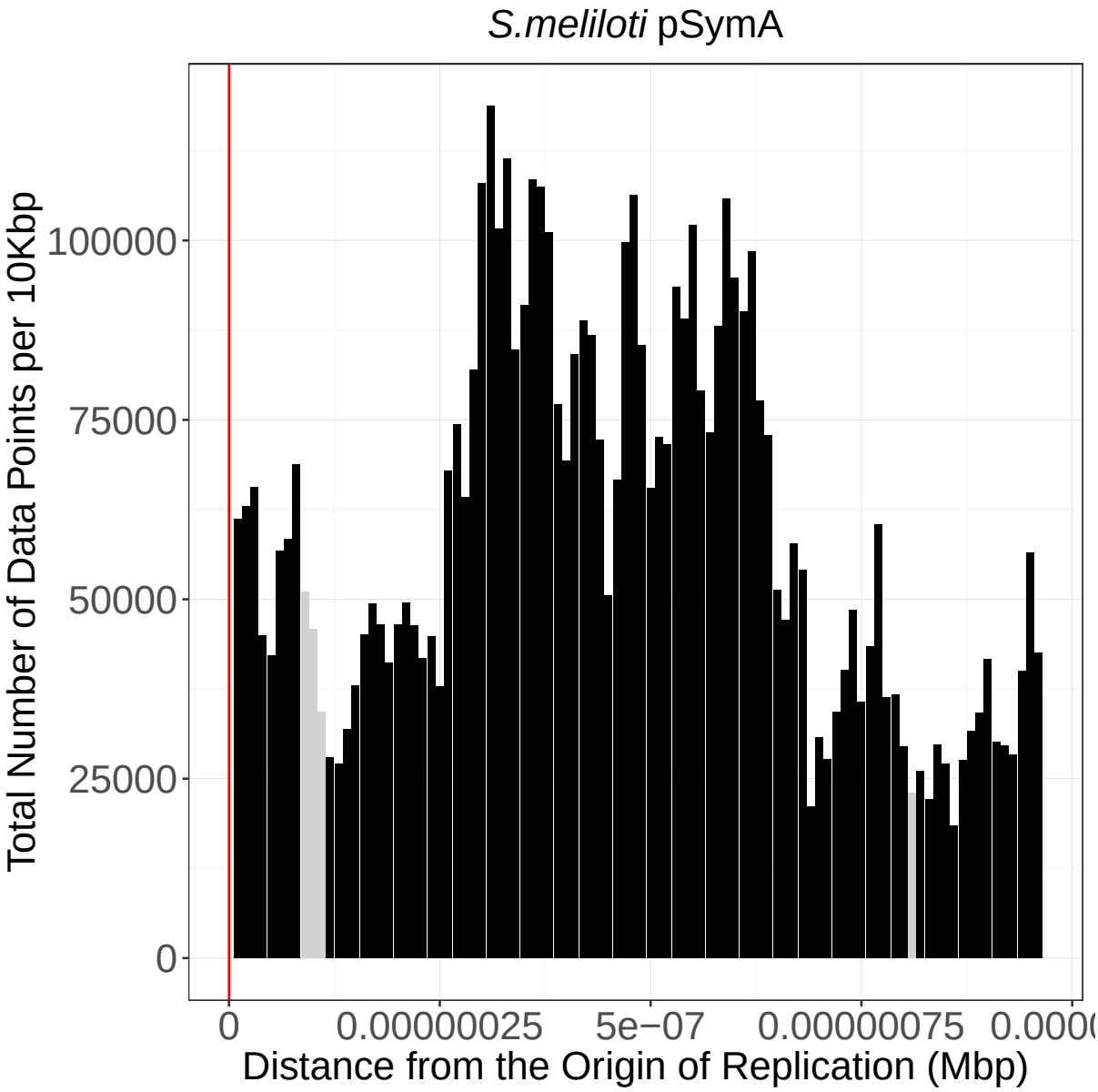


Figure 12: Distribution of total number of substitution data points per 10Kbp in genome.

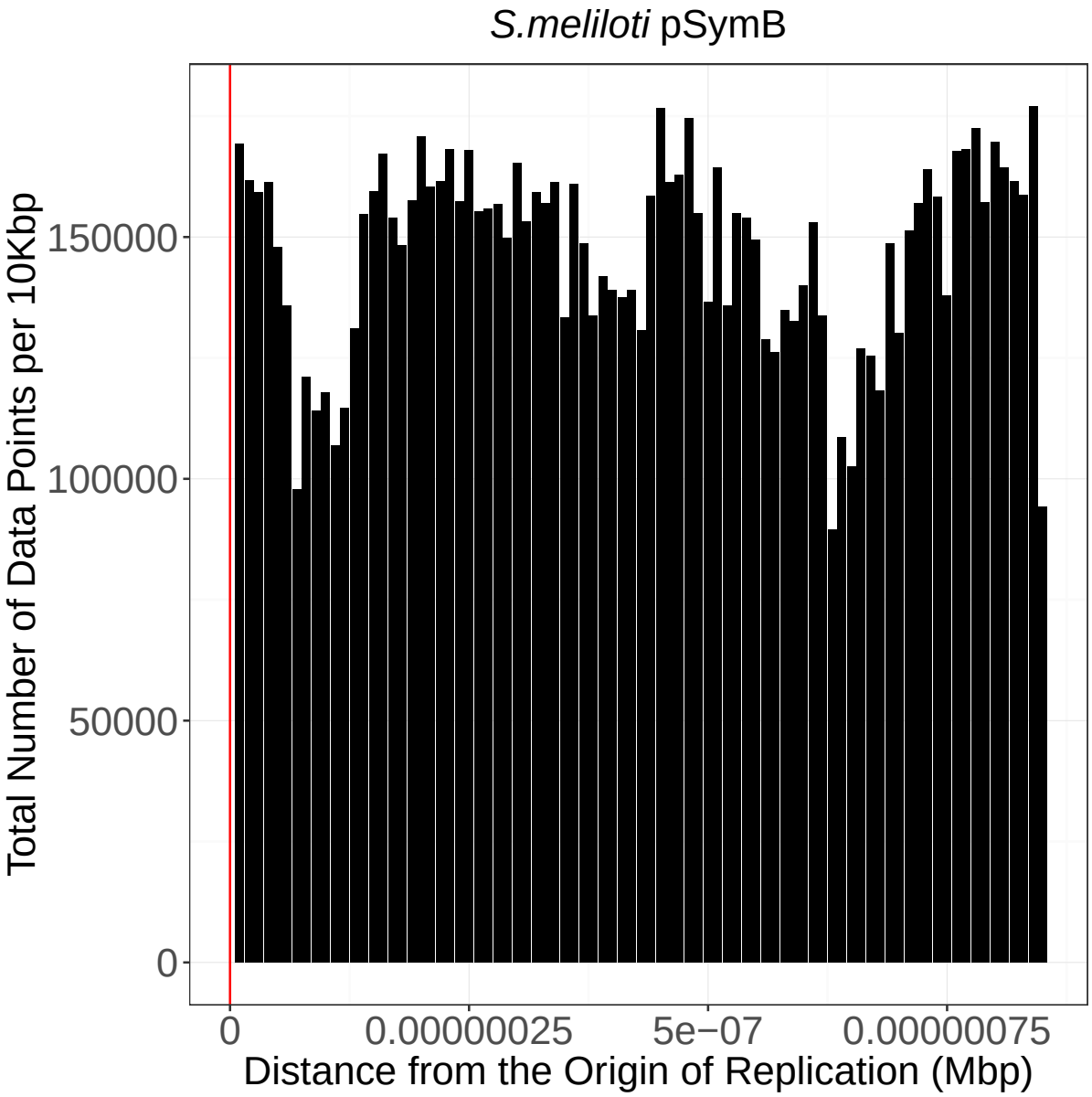


Figure 13: Distribution of total number of substitution data points per 10Kbp in genome.

Bacteria and Replicon	Protein Coding Sequences
E. coli Chromosome	-1.43×10^{-8}***
B. subtilis Chromosome	-5.55×10^{-8}***
Streptomyces Chromosome	7.49×10^{-8}***
S. meliloti Chromosome	-5.99×10^{-7}***
S. meliloti pSymA	-5.18×10^{-7}***
S. meliloti pSymB	1.67×10^{-7}***

Table 2: Logistic regression analysis of the number of substitutions along all protein coding positions of the genome of the respective bacteria replicons. 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 bidirectional 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	Average Number of Substitutions per bp
E. coli Chromosome	1.97×10^{-4}
B. subtilis Chromosome	1.93×10^{-4}
Streptomyces Chromosome	2.74×10^{-6}
S. meliloti Chromosome	9.72×10^{-5}
S. meliloti pSymA	6.54×10^{-5}
S. meliloti pSymB	1.99×10^{-4}

Table 3: Average number of protein coding substitutions calculated per base across all bacterial replicons. Outliers and missing data was not included in the calculation.

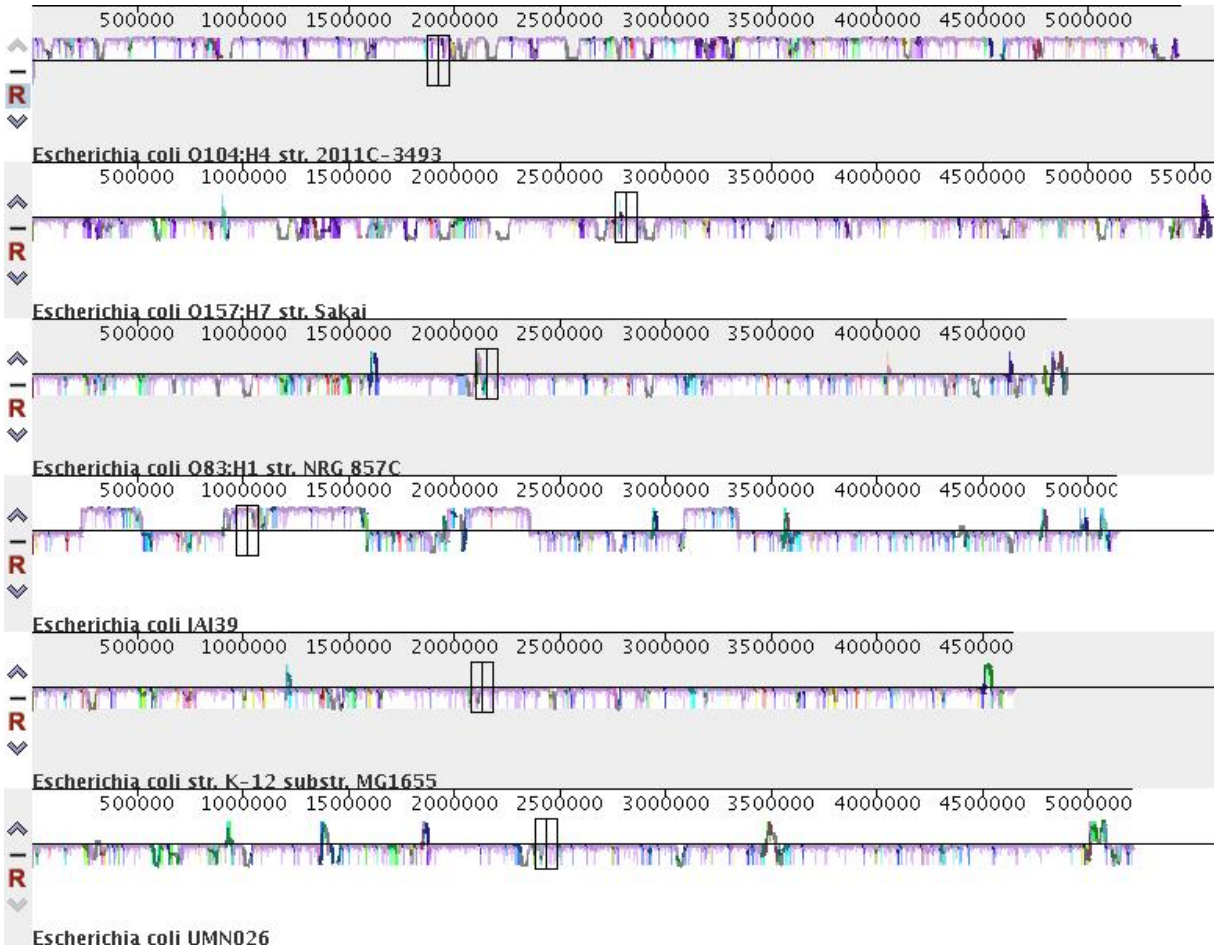


Figure 14: progressiveMauve alignment of *Escherichia coli* genomes highlighting the "backbone" of the alignment (matching regions).

Figure 15: progressiveMauve alignment of *S. meliloti* Chromosomes highlighting the "backbone" of the alignment (matching regions).

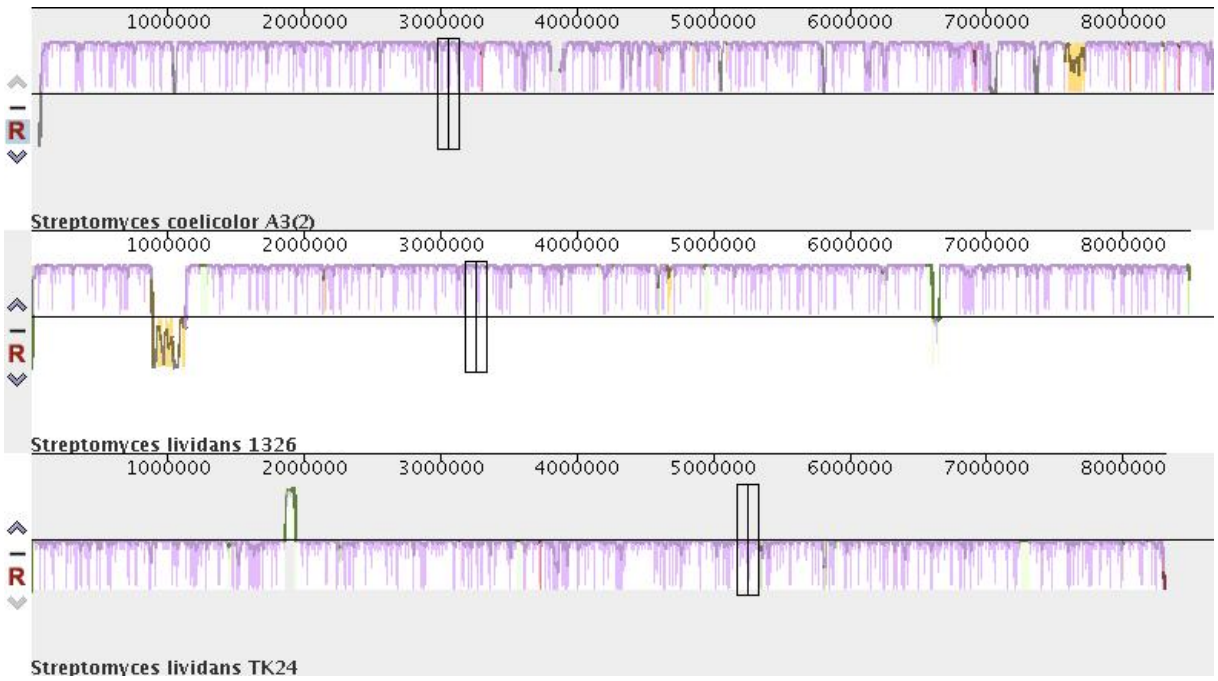


Figure 16: progressiveMauve alignment of *Streptomyces* genomes highlighting the "backbone" of the alignment (matching regions).