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

✓supplemental selection analysis including outliers for *S. meliloti* chromosome

Edits to the Substitution Paper:

✓discuss selection linear regression results

✓alter main focus of paper in discussion and conclusion

✓interpret all other results

✓edit discussion and conclusion for flow

✓add in genome clustering figure to supplement to help explain that analysis

✓put new supplementary tables on git

✓bacteria names are italic in references

✓check all references work

✓edit abstract and intro

✓edit methods

✓edit results

✓check black and white look of figures (and alter colours/shapes as necessary)

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

The section below are the results from INCLUDING outliers (non-zero dN and ω values) in the *S. meliloti* chromosome selection analysis. The results are basically the same which is good! Because it means that even with these values, our point still stands that there is no evidence of a correlation between dN, dS and ω and distance from the origin of replication. **I would really appreciate it if you could read it over and let me know of any edits I should make.**

0.1 *S. meliloti* Chromosome Selection Analysis Without Outliers

Due to the extremely high sequence similarity of the *S. meliloti* chromosomes in this analysis, there are a relatively low number of substitutions and therefore many dN and ω values that are equal to zero (see Table **??**). The high number of zero values were included in the original calculation of outliers (see Main Paper for more details) causing all of the non-zero dN and ω values to be classified as outliers (see Figure 6 in the Main Paper). We decided to perform the same calculations on dN, dS, and ω but including the outliers to see what the results would have been. A visualization of the distribution of dN, dS, and ω along the chromosome of *S. meliloti* is seen in Figure 1. The

average values for dN, dS, and ω are found in Table 1 and the linear regression to determine if there is a correlation between distance from the origin of replication and dN, dS, and ω values for the chromosome of *S. meliloti* is found in Table 2. We also looked at the values of dN, dS, and ω in the 20Kbp regions near and far from the origin of replication (including outliers) in the *S. meliloti* chromosome, these results are summarized in Table 3. The methods for these calculations are the same as in the Main Paper and in section **??**, however, outliers were not removed from these calculations.

The results in Tables 1 - 3 closely reflect the results of the *S. meliloti* analysis when outliers were included (see Main Paper). The significant correlation between dS and distance from the origin of replication is small and the lack of significant correlation between dN and ω and distance from the origin of replication suggest that the results are inconclusive. Even when the outliers (non-zero values of dN and ω) are included in the selection analysis, we still can not conclude that there is an overall trend between distance from the origin of replication and dN, dS, and ω values.

	Genome Average		
Bacteria and Replicon	dS	dN	ω
S. meliloti Chromosome	0.0100	0.0007	0.0677

Table 1: Weighted averages of dN, dS, and ω values calculated for *S. meliloti* chromosome using the gene length as the weight. Arithmetic mean was calculated for the per gene averages for *S. meliloti* chromosome. Outliers were included in the calculation.

Bacteria and Replicon	dN	dS	ω
S. meliloti Chromosome	NS	-2.29×10^{-9}*	NS

Table 2: Linear regression to determine the correlations between dN, dS, and ω values and distance from the origin of replication. Outliers were included in the calculation. All results are marked with significance codes as followed: p: < 0.001 = '***', $0.001 < 0.01$ = '**', $0.01 < 0.05$ = '*', > 0.05 = 'NS'.

	Near Origin			Near Terminus		
Bacteria and Replicon	dN	dS	ω	dN	dS	ω
S. meliloti Chromosome	NS	NS	NS	NS	NS	NS

Table 3: Linear regression for dN, dS, and ω calculated for the 20 genes closest and 20 genes farthest from the origin of replication in the *S. meliloti* chromosome. Outliers were included in the calculation. All results are marked with significance codes as followed: p: < 0.001 = '***', $0.001 < 0.01$ = '**', $0.01 < 0.05$ = '*', > 0.05 = 'NS'.

This Week

- incorperate any feedback Brian has about why *S. meliloti* chromosome looks so weird
- discuss selection linear regression results
- alter main focus of paper in discussion
- interpret all other results
- edit discussion for flow
- send Brian discussion to edit

Next Week

- do Brian's edits on discussion
- alter main focus of paper in conclusion
- edit/work on conclusion
- send Brian conclusion to edit
- edit/read through whole paper (in prep to send to Brian)

Bacteria and Replicon	Genome Average 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 4: 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 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 5: 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 6: Average number of protein coding substitutions calculated per base across all bacterial replicons. Outliers and missing data was not included in the calculation.

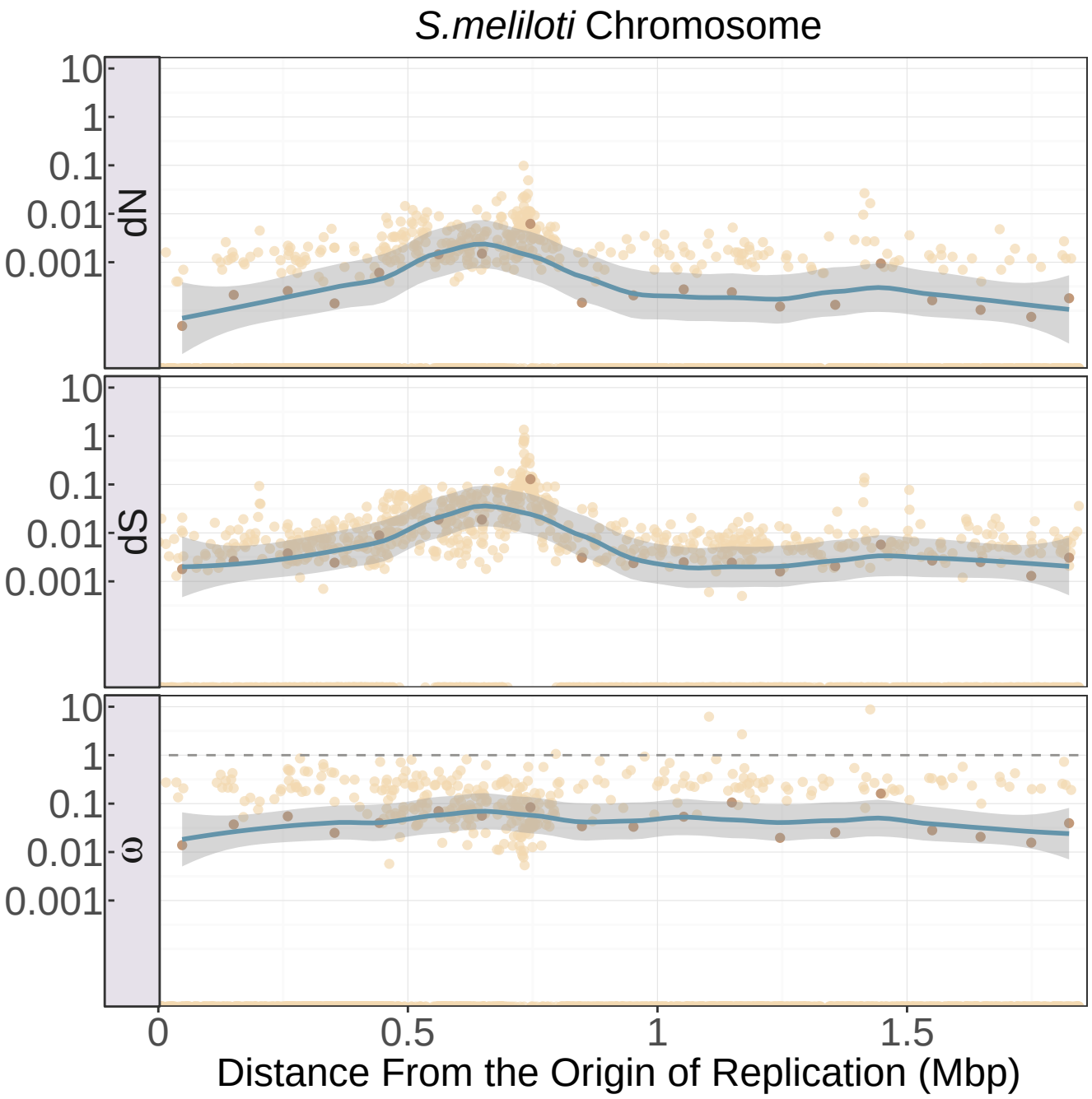


Figure 1: The graph show the values of dN, dS, and ω along the chromosome of *S. meliloti*. Distance from the origin of replication is on the x-axis beginning with the origin of replication denoted by position zero on the left, and the terminus indicated on the far right. The y-axis of the graph indicates the value of dN, dS, and ω found at each gene segment position of the chromosome. Outliers are included in this graph. The average dN, dS, and ω values for each 10,000bp regions of the replicon were calculated and represented by the dark brown points. A trend line represented in blue (using the `loess` method), was fit to these average values and the associated 95% confidence intervals for this line is represented by the grey ribbon around the blue trend line. For a complete list zero value information, please see Supplementary Material.

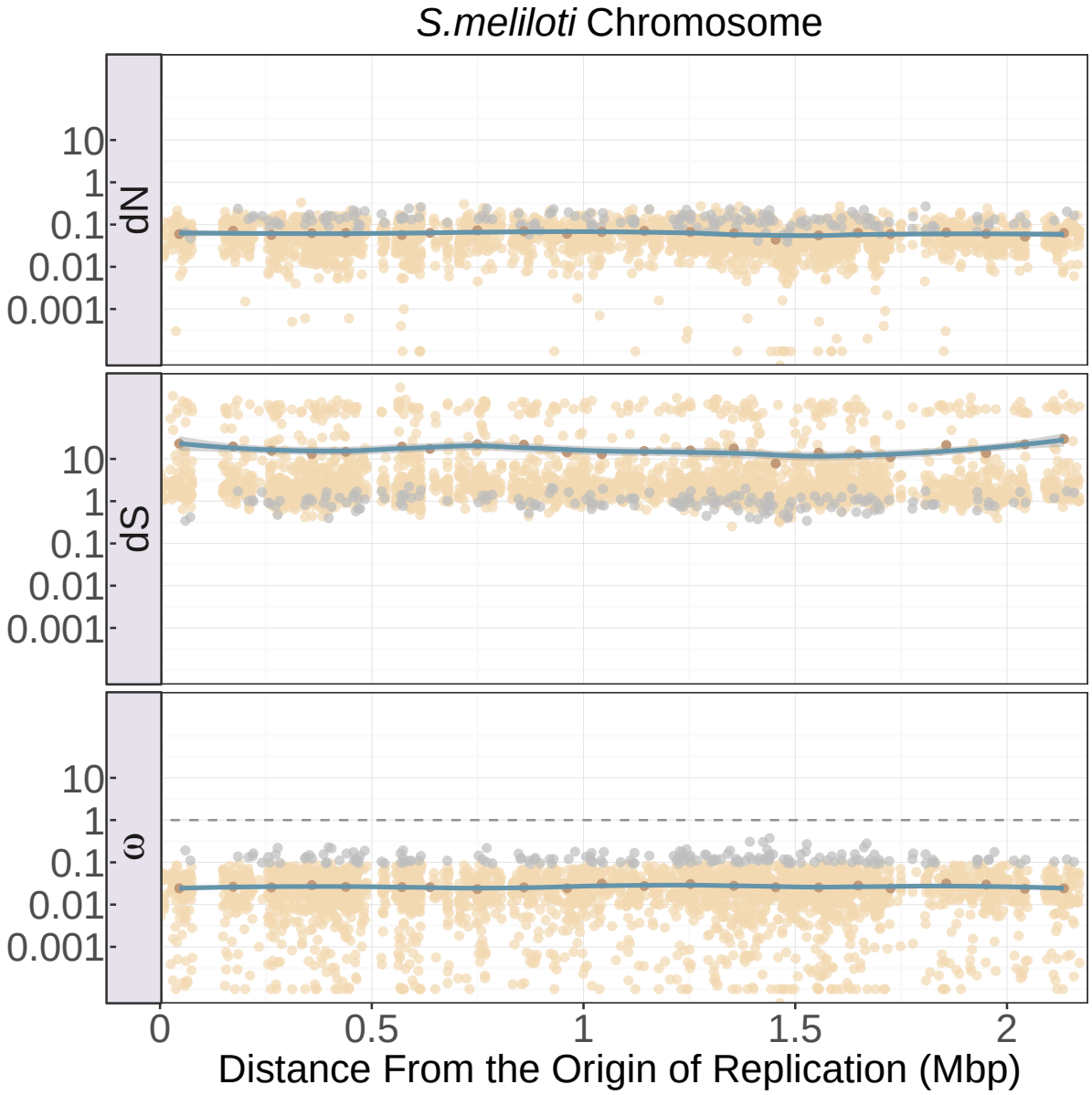
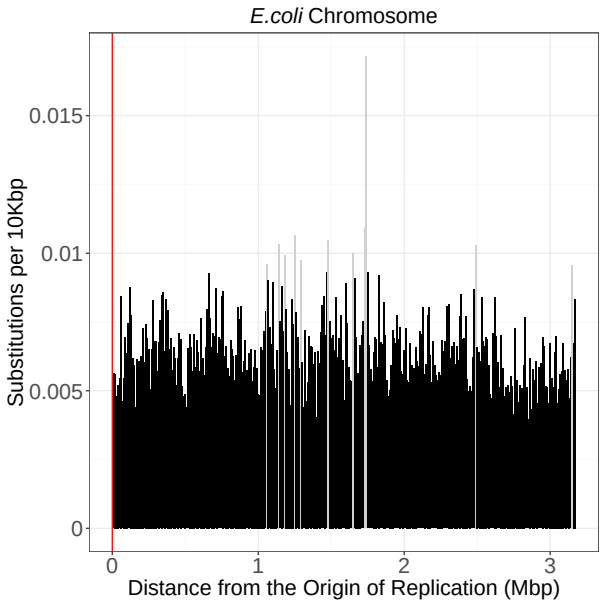
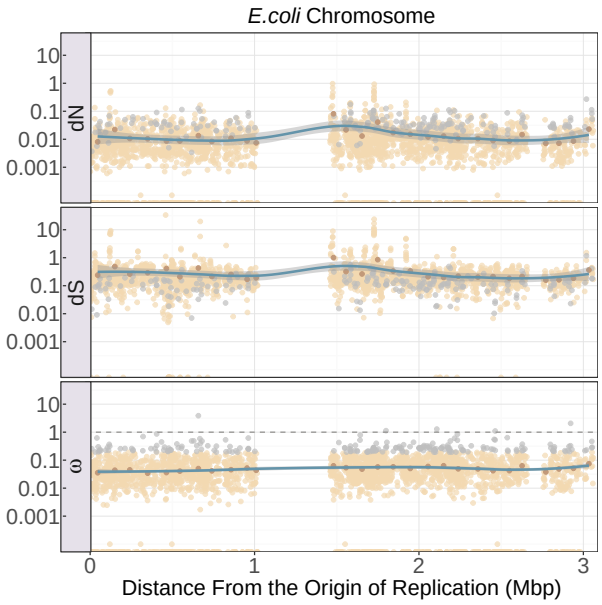


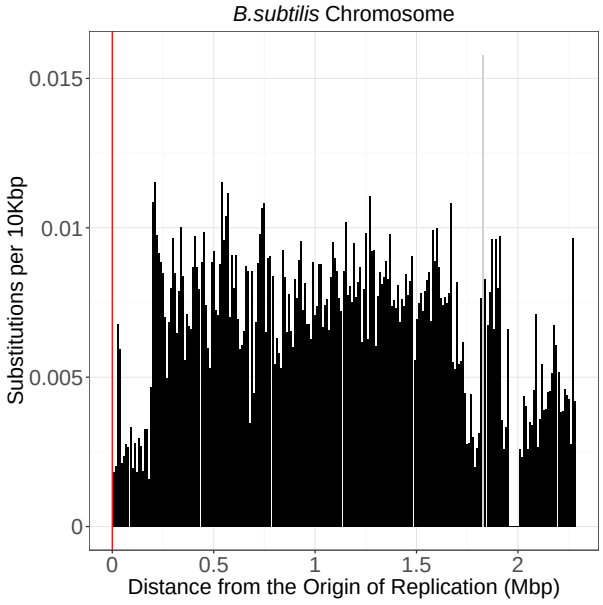
Figure 2: dN, dS, and ω values for *S. meliloti* chromosomes and ***A. tumefaciens***.


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

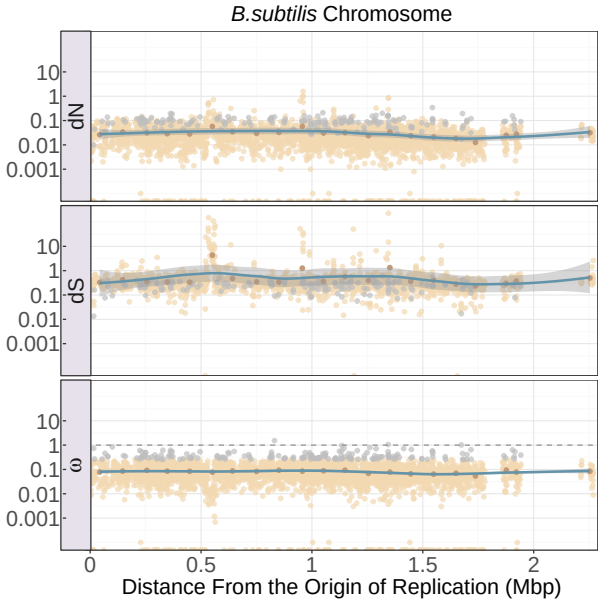
(a)


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)

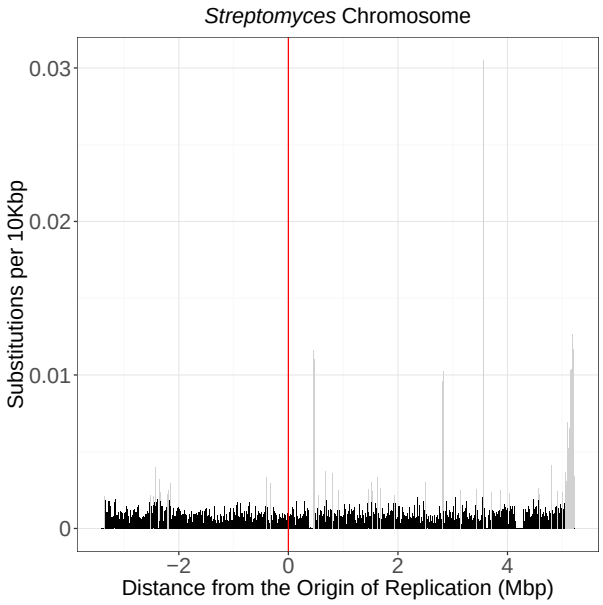
(b)


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)

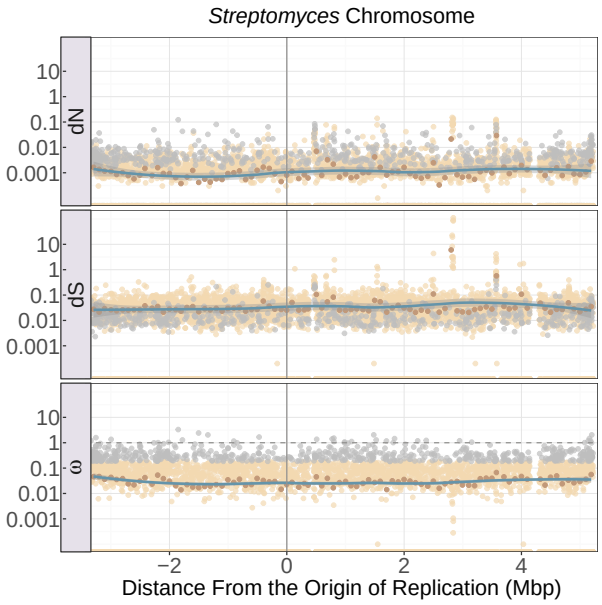
(a)


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)

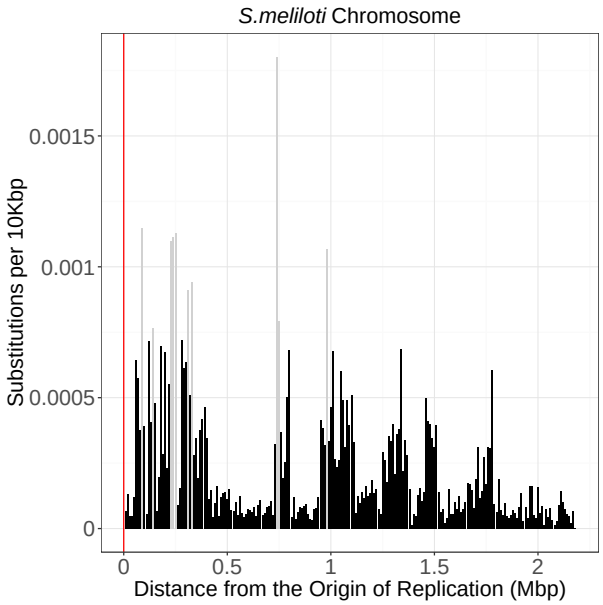
(b)


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

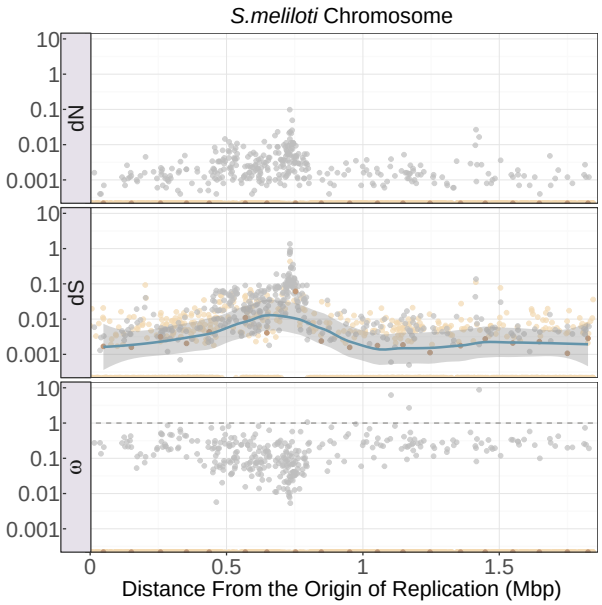
(a)


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

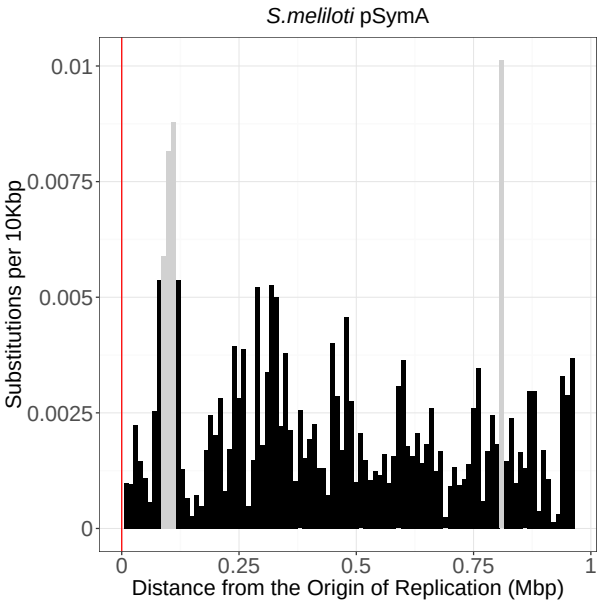
(b)


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)

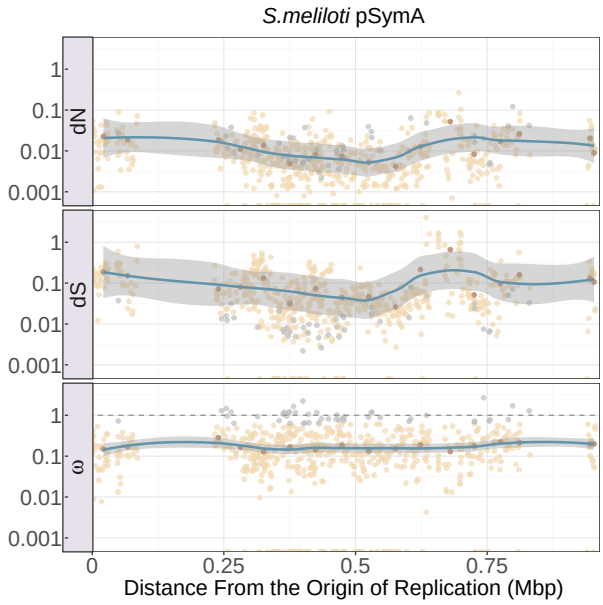
(a)


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)

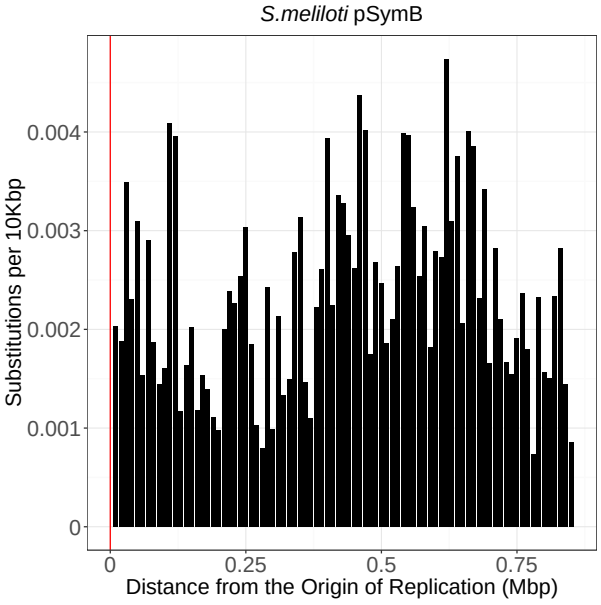
(b)


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)

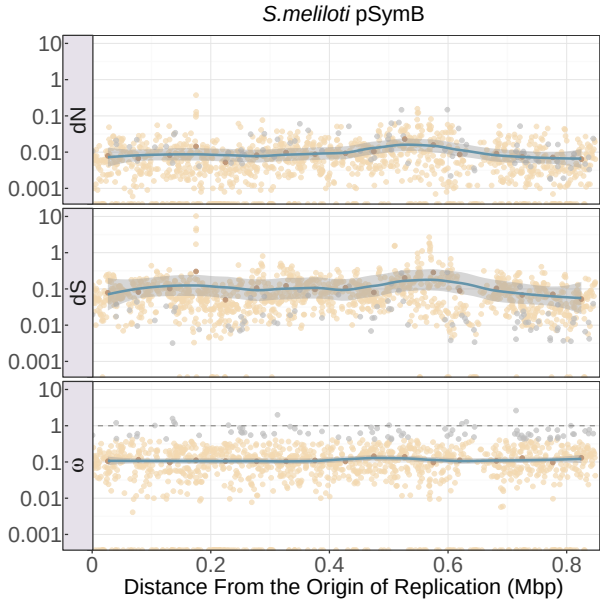
(a)


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)

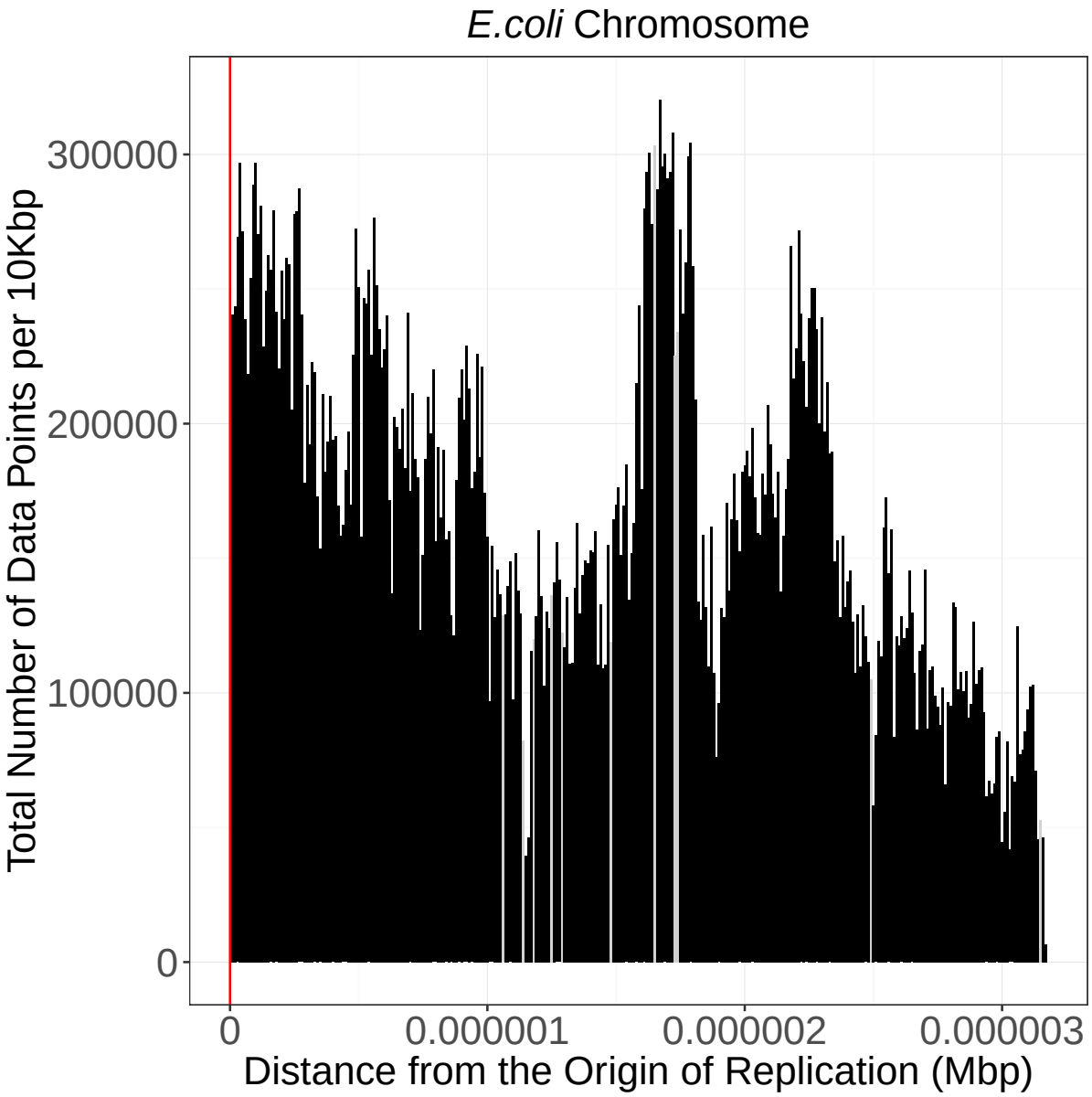


Figure 9: Distribution of total number of substitution data points per 10Kbp in genome.

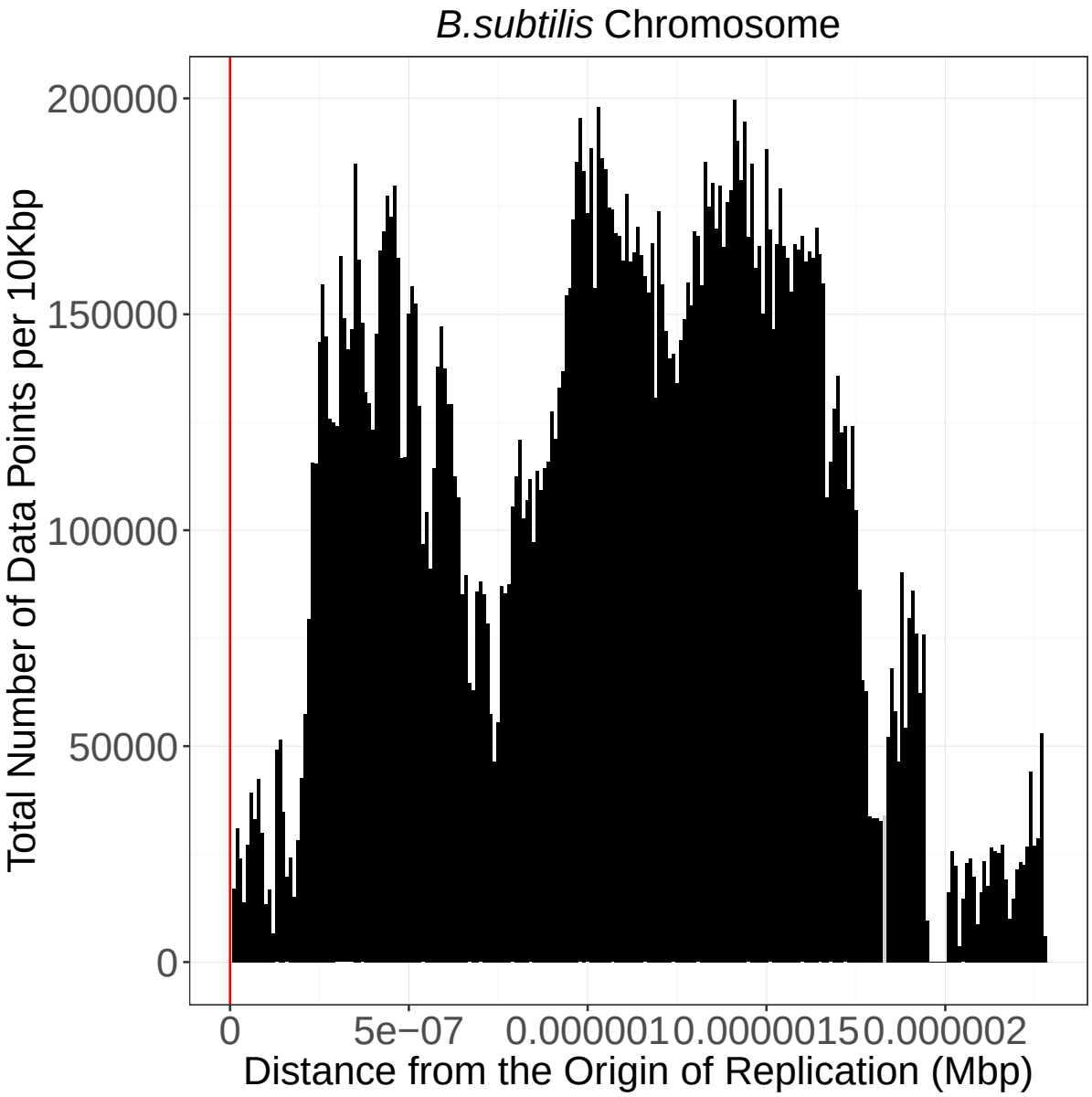


Figure 10: Distribution of total number of substitution data points per 10Kbp in genome.

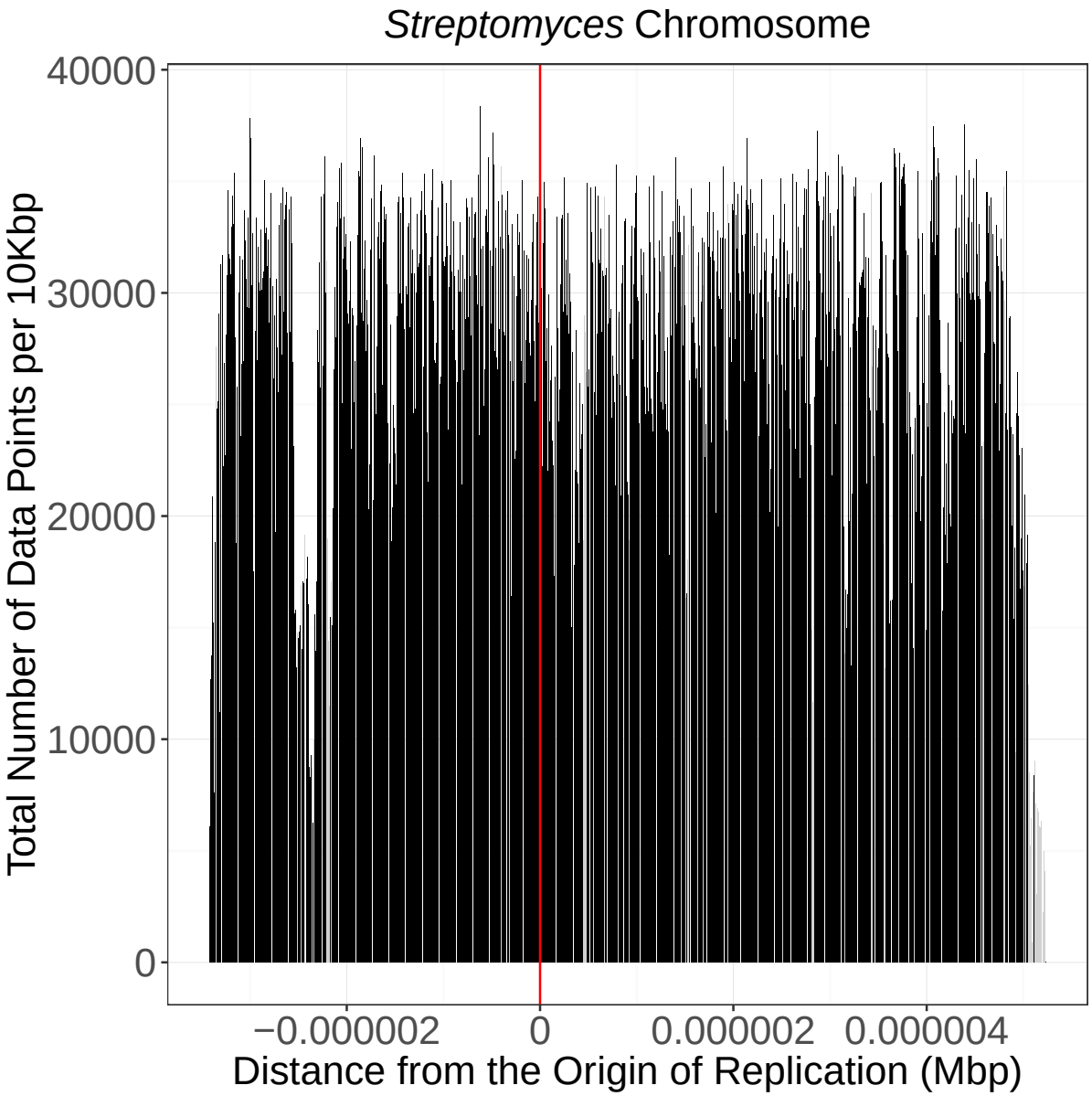


Figure 11: Distribution of total number of substitution data points per 10Kbp in genome.

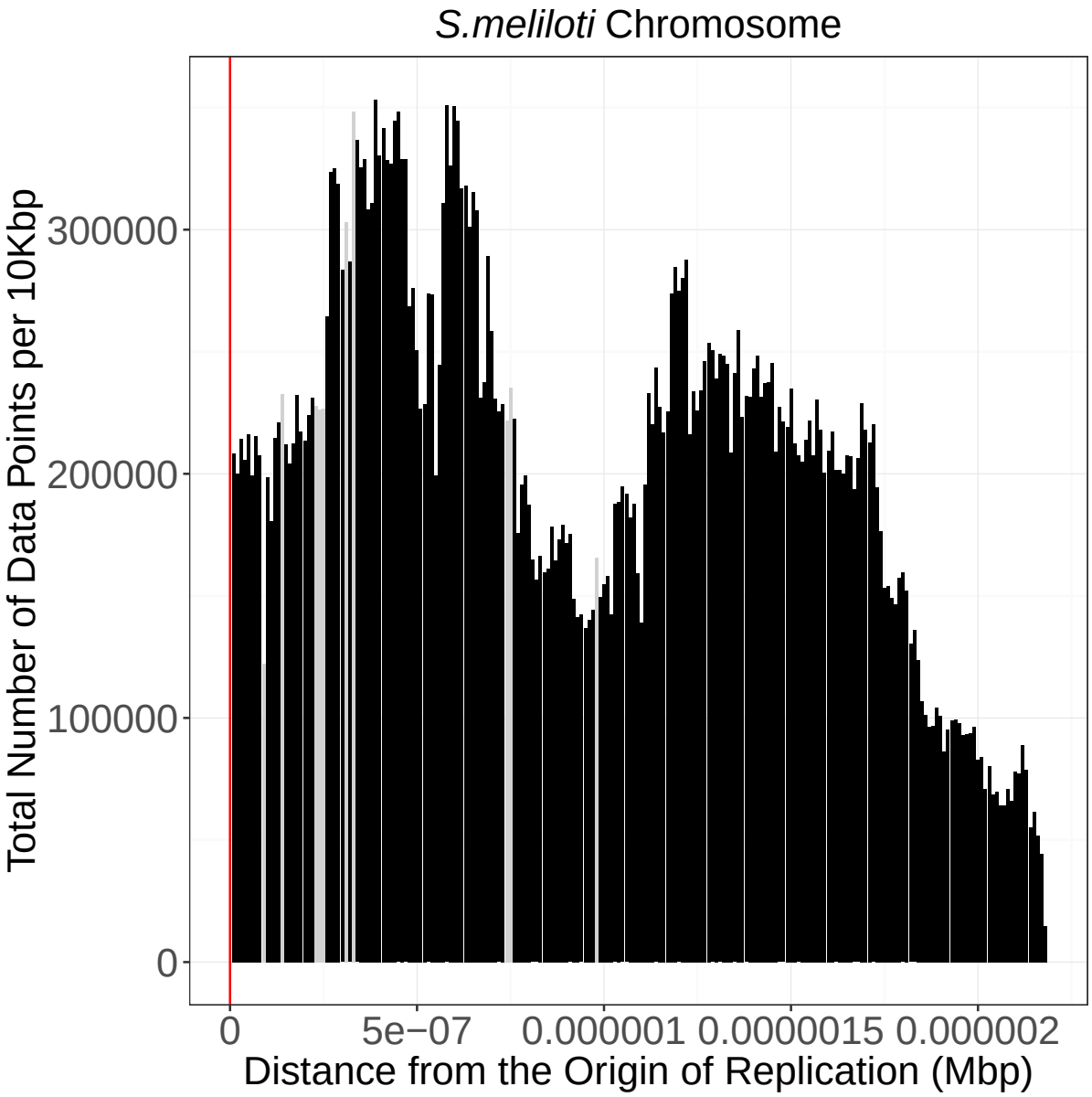


Figure 12: Distribution of total number of substitution data points per 10Kbp in genome.

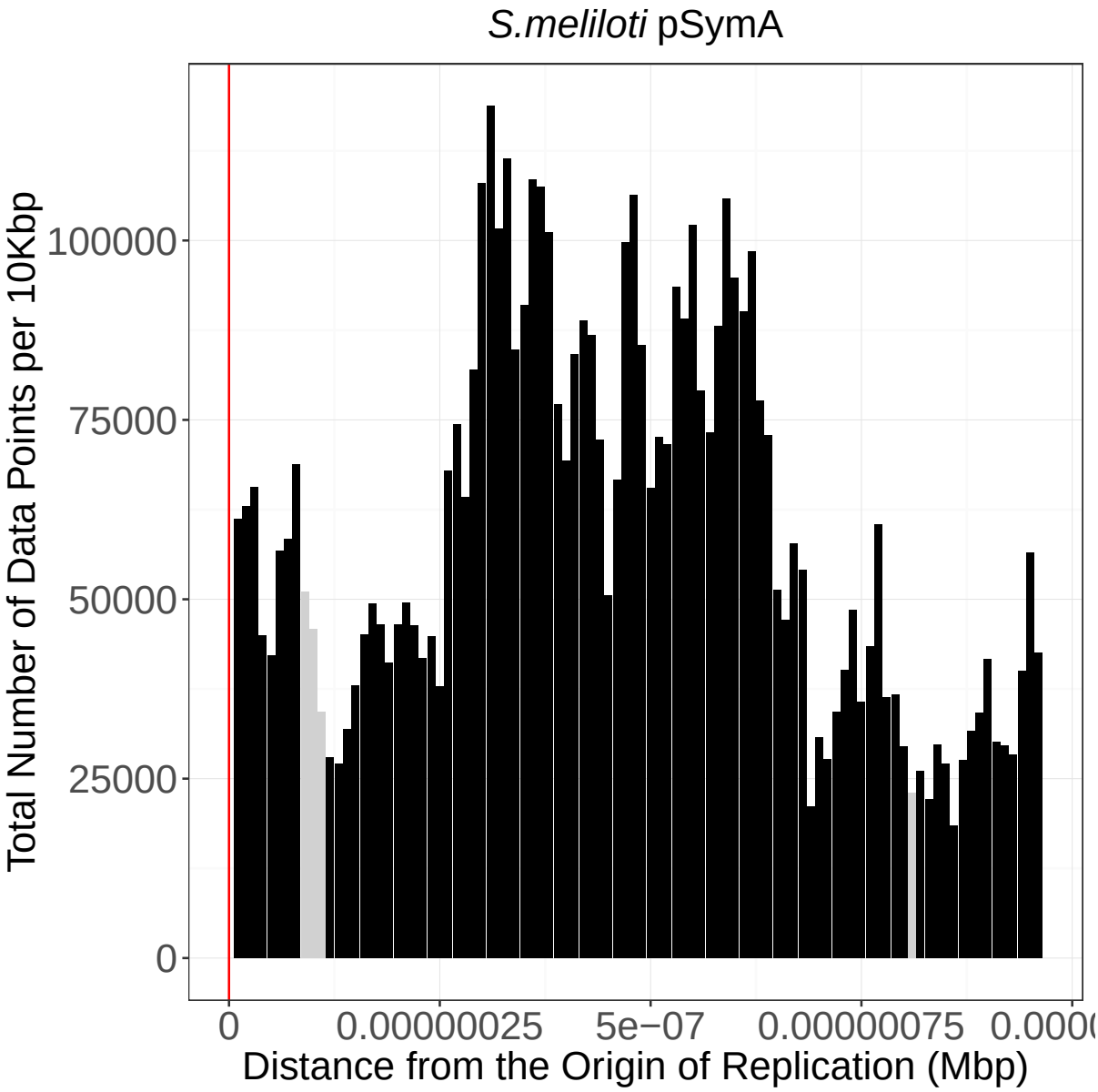


Figure 13: Distribution of total number of substitution data points per 10Kbp in genome.

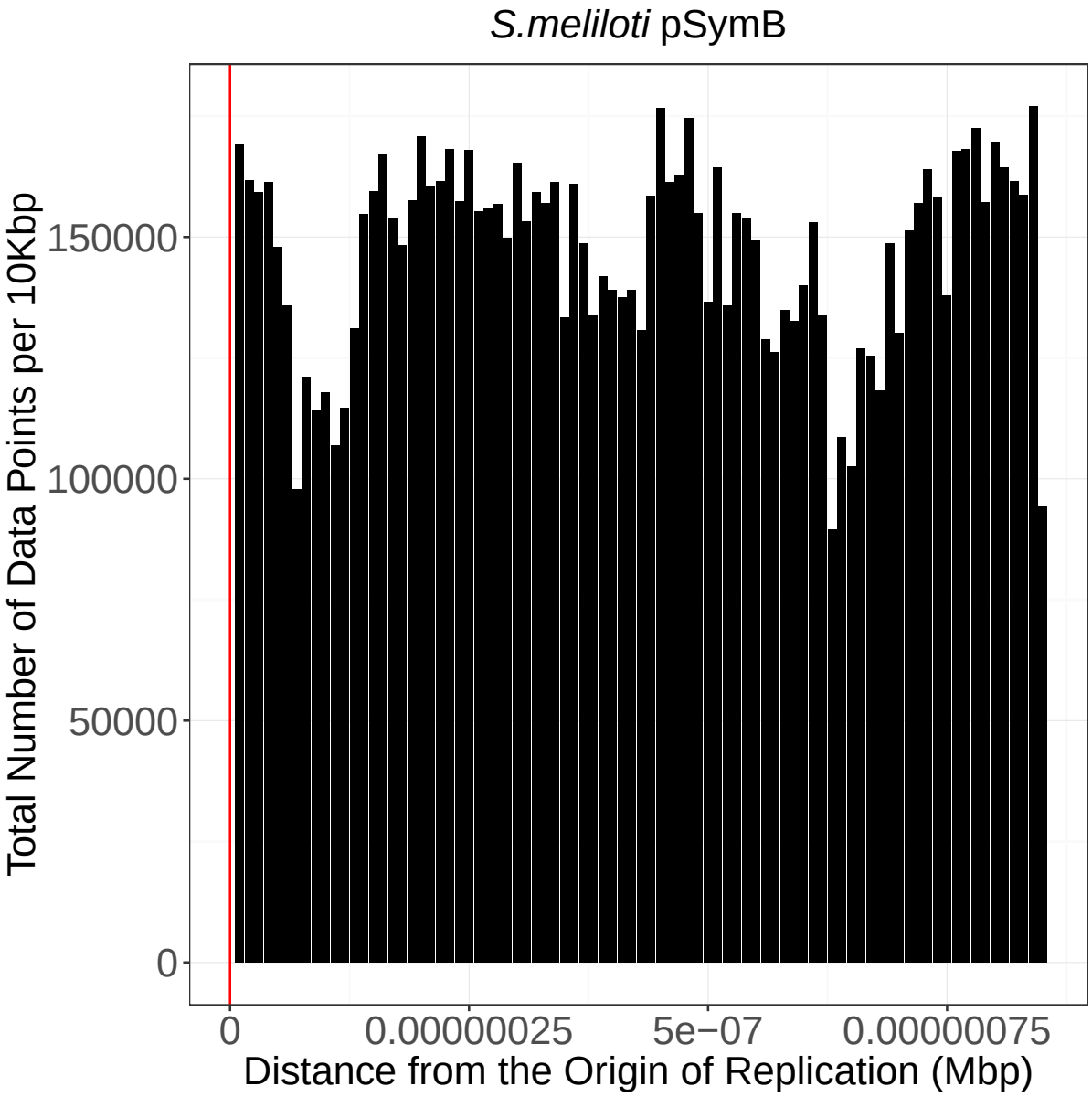


Figure 14: Distribution of total number of substitution data points per 10Kbp in genome.

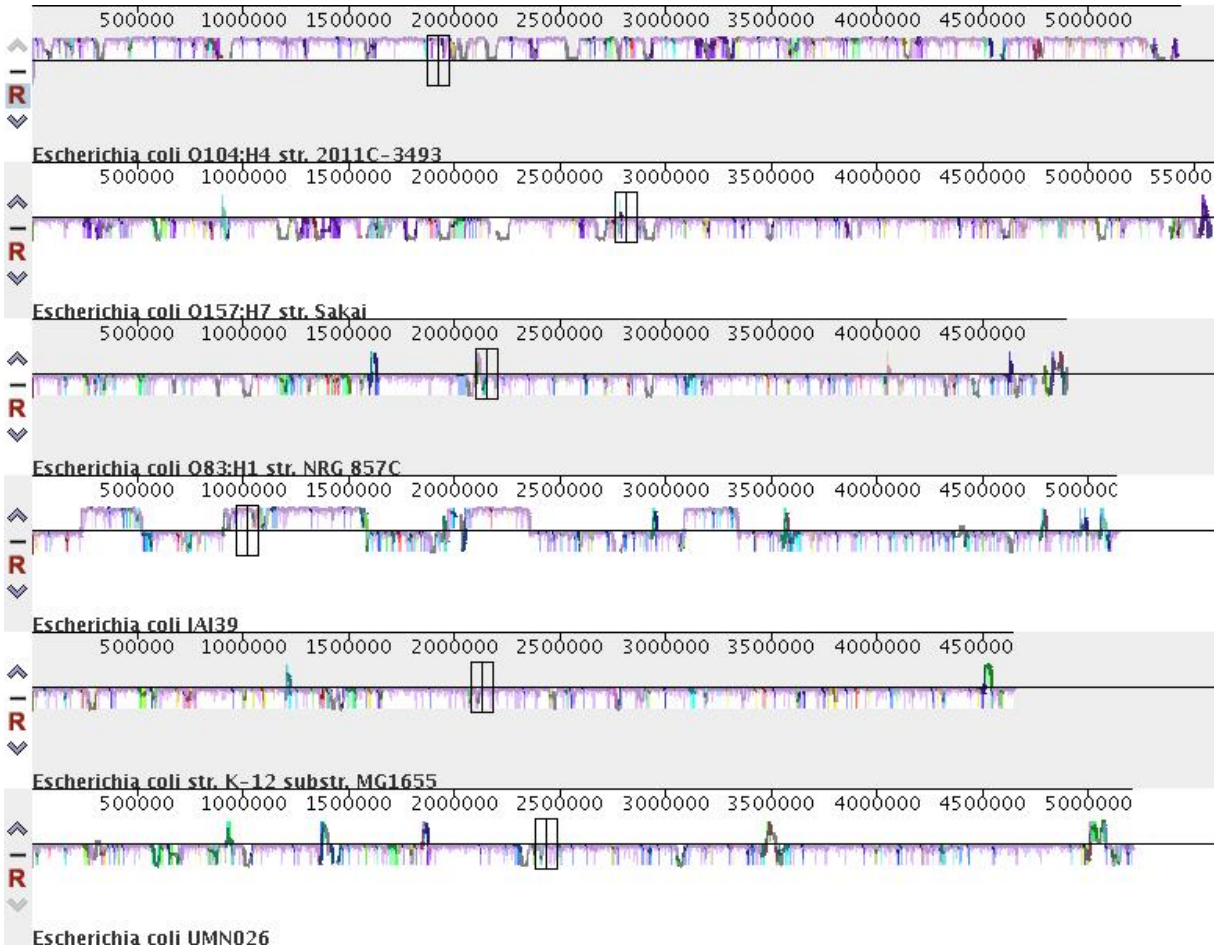


Figure 15: progressiveMauve alignment of *Escherichia coli* genomes highlighting the "backbone" of the alignment (matching regions).

Figure 16: progressiveMauve alignment of *S. meliloti* Chromosomes highlighting the "backbone" of the alignment (matching regions).

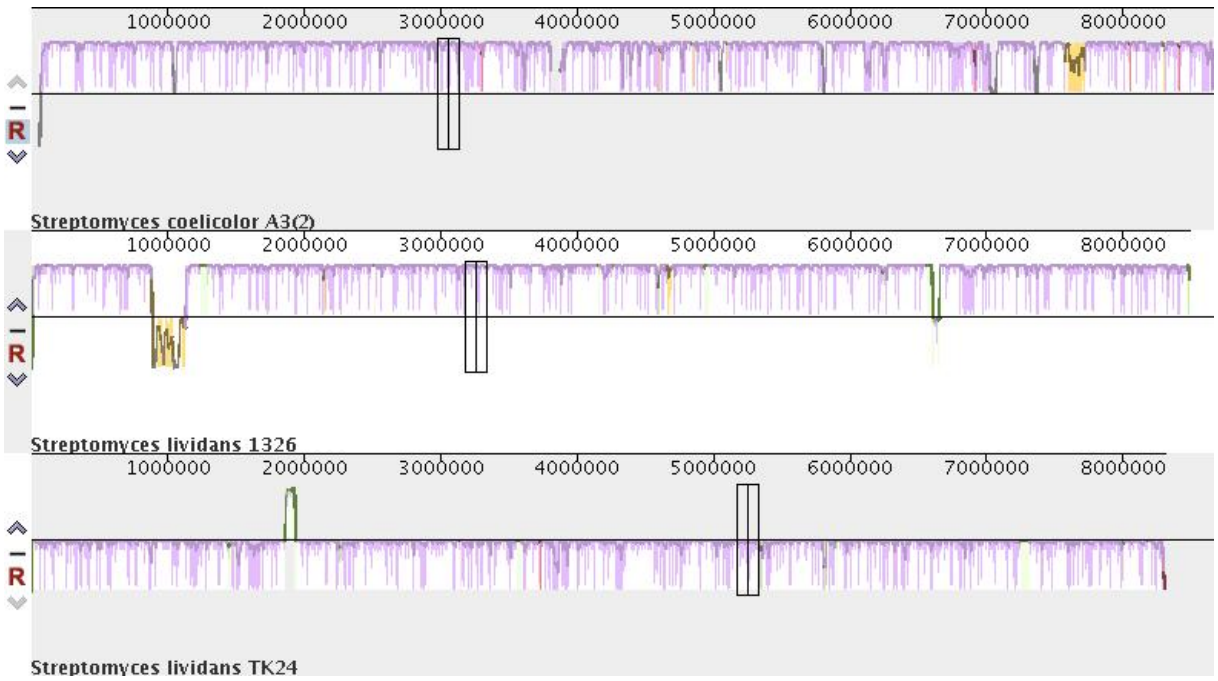


Figure 17: progressiveMauve alignment of *Streptomyces* genomes highlighting the "backbone" of the alignment (matching regions).