

**Title:** THE LOCATION OF SUBSTITUTIONS AND BACTERIAL GENOME ARRANGEMENTS

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## Supplementary Material

Further supplemental information and code are available on GitHub at [www.github.com/dlato/Spatial\\_Patterns\\_of\\_Substitutions](https://www.github.com/dlato/Spatial_Patterns_of_Substitutions).

### Software Version Numbers

| Program          | Version Number | Build Date        |
|------------------|----------------|-------------------|
| baseml           | 4.9            | March 2015        |
| codeml           | 4.9            | March 2015        |
| consense         | 3.6b           | NA                |
| dnadist          | 3.6b           | NA                |
| dnaml            | 3.6b           | NA                |
| MAFFT            | v7.045b        | June 5, 2013      |
| neighbor         | 3.6b           | NA                |
| progressiveMauve | Snap Shot      | June 7, 2012      |
| RAxML            | 8.0.25         | June 16, 2014     |
| seqboot          | 3.6b           | NA                |
| trimAl           | v1.4.rev15     | December 17, 2013 |

Table S1: Version numbers and build dates for each of the programs used.

## Sequences

| Bacteria Strain/Species   | Accession Number | Date Accessed      |
|---|------------------|--------------------|
| <i>Escherichia coli</i>   |                  |                    |
| <i>E. coli</i> 0104H4   | CP003289         | September 29, 2016 |
| <i>E. coli</i> 0157H7   | BA000007         | September 29, 2016 |
| <i>E. coli</i> 083H1  | CP001855         | September 29, 2016 |
| <i>E. coli</i> IAI39  | CU928164         | September 26, 2016 |
| <i>E. coli</i> K12 *  | U00096           | September 26, 2016 |
| <i>E. coli</i> UMN026   | CU928163         | September 26, 2016 |
| Outgroup: <i>E. fergusonii</i> ATCC 35469T                                | NC_011740        | August 26, 2020    |
| <i>Bacillus subtilis</i>  |                  |                    |
| <i>B. subtilis</i> 168 *  | NC_000964        | November 10, 2016  |
| <i>B. subtilis</i> BS38   | NZ_CP017314      | November 11, 2016  |
| <i>B. subtilis</i> BSn5   | NC_014976        | November 11, 2016  |
| <i>B. subtilis</i> PY79   | NC_022898        | November 11, 2016  |
| <i>B. subtilis</i> QB928  | NC_018520        | November 11, 2016  |
| <i>B. subtilis</i> RONN1  | NC_017195        | November 11, 2016  |
| <i>B. subtilis</i> W23  | NC_014479        | November 11, 2016  |
| Outgroup: <i>B. cereus</i> FDAARGOS_797                                   | NZ_CP053931      | August 26, 2020    |
| <i>Streptomyces</i>   |                  |                    |
| <i>S. lividans</i> TK24   | NZ_GG657756      | August 26, 2020    |
| <i>S. lividans</i> 1362   | NZ_CM001889      | August 26, 2020    |
| <i>S. coelicolor</i> A3 *   | AL645882         | November 30, 2016  |
| <i>S. coelicolor</i> A32 CFB NCB  | NZ_CP042324      | August 26, 2020    |
| <i>S. coelicolor</i> M1154/pAMX4/pGP1416                                  | NZ_CP050522      | August 26, 2020    |
| Outgroup: <i>S. aureofaciens</i> DM1                                      | NZ_CP020567      | August 26, 2020    |
| <i>S. meliloti</i> Chromosome   |                  |                    |
| <i>S. meliloti</i> 2011   | NC_020528        | April 24, 2017     |
| <i>S. meliloti</i> 1021 *   | NC_003047        | June 3, 2014       |
| <i>S. meliloti</i> AK83   | NC_015590        | June 3, 2014       |
| <i>S. meliloti</i> BL225C   | NC_017322        | June 3, 2014       |
| <i>S. meliloti</i> SM11   | NC_017325        | June 3, 2014       |
| <i>S. meliloti</i> RMO17  | NC_CP009144      | April 24, 2017     |
| Outgroup: <i>Rhizobium leguminosarum</i> trifolii WSM1689 chromosome      | NZ_CP007045      | August 26, 2020    |
| <i>S. meliloti</i> pSymA  |                  |                    |
| <i>S. meliloti</i> 2011   | NC_020527        | April 24, 2017     |
| <i>S. meliloti</i> 1021 *   | NC_003037        | June 3, 2014       |
| <i>S. meliloti</i> AK83   | NC_015591        | June 3, 2014       |
| <i>S. meliloti</i> BL225C   | NC_017324        | June 3, 2014       |
| <i>S. meliloti</i> SM11   | NC_017327        | June 3, 2014       |
| <i>S. meliloti</i> RMO17  | NC_CP009145      | April 24, 2017     |
| Outgroup: <i>Rhizobium leguminosarum</i> trifolii WSM1689 plasmid pRLG202 | NC_0113665       | August 26, 2020    |
| <i>S. meliloti</i> pSymB  |                  |                    |
| <i>S. meliloti</i> 2011   | NC_020560        | April 24, 2017     |
| <i>S. meliloti</i> 1021 *   | NC_003078        | June 3, 2014       |
| <i>S. meliloti</i> AK83   | NC_015596        | June 3, 2014       |
| <i>S. meliloti</i> BL225C   | NC_017323        | June 3, 2014       |
| <i>S. meliloti</i> SM11   | NC_017326        | June 3, 2014       |
| <i>S. meliloti</i> RMO17  | NC_CP009146      | April 24, 2017     |
| Outgroup: <i>Rhizobium leguminosarum</i> trifolii WSM1689 plasmid pRLG201 | NC_011368        | August 26, 2020    |

Table S2: Strains and species used for each replicon analysis. Accession numbers, date accessed, and outgroups for each replicon are provided. An asterisk (\*) indicates the strain that was used as the representative strain.

## Constraints to Number of Sequence Chosen

Computational time constraints and the nature of the data were limiting factors for the number of strains that were chosen for each bacterial species. **progressiveMauve** is a multiple sequence alignment program which is useful for accounting for local and large scale genomic rearrangements. Some of the bacterial strains are very similar and therefore there was no issue finding a sufficient number of locally co-linear blocks (LCBs) without having the genomes broken into an overwhelming number of blocks. *E. coli*, *Bacillus subtilis*, and *S. meliloti* were among the bacteria where this was the case. However, the *Streptomyces* strains were slightly too distantly related so when we tried to use a comparable number of strains to the other bacteria (six genomes), **progressiveMauve** split the genomes into 521 LCBs (Supplementary Figure S1). These blocks were therefore very small in length and resulted in many blocks that were comparing sequences with poor homology. Consequently, we had to reduce the number of genomes used for the *Streptomyces* analysis and after many iterations of genome combinations, we settled on three *Streptomyces* genomes with a total of 6 LCBs (Supplementary Figure S2). This allowed for the correct comparison of homologous sequences, while also accounting for recombination.

The computational time required to run **progressiveMauve** was an additional constraint that needed to be considered. **progressiveMauve** can align multiple whole genomes and identify regions that have been rearranged within the taxa provided. This process happens in relatively quick computational time, however, like most other programs, the addition of more data increased the amount of time required to complete the process. We ran multiple instances of **progressiveMauve** with varying numbers of *E. coli* genomes (Supplementary Figure S3). These data points were connected using a locally estimated scatterplot smoothing method and confidence intervals (Supplementary Figure S3). From this data, we can see that increasing the number of genomes exponentially increases the run time of **progressiveMauve**. It becomes impractical to align more than 27 genomes with **progressiveMauve**, as anything over that would take more than 24h to run. This information combined with **progressiveMauve**'s inability to pair of homologous sequences in LCBs of distantly related taxa, has limited the total number of genomes we can use per taxa to a maximum of 7. This provides the most accurate data and the most reasonable analysis duration.



Figure S1: Visualization of the progressiveMauve alignment of 6 *Streptomyces* genomes (from top to bottom): *S. coelicolor* AL645882, *S. lividans* NZ\_CM001889, *S. lividans* NZ\_GG657756, *S. venezuelae* NC\_018750, *S. venezuelae* NZ\_CP013129, and *S. venezuelae* NC\_CP018074. Each coloured block represents a different locally co-linear block (LCB). Coloured lines connect LCBs that are similar between taxa. The black lines underneath each LCB represent the whole genome sequence of each of the *Streptomyces* taxa. Each LCB can be treated as a rearrangement, there have therefore been 521 rearrangements between these *Streptomyces* genomes.



Figure S2: Visualization of the `progressiveMauve` alignment of the 3 *Streptomyces* genomes chosen for this analysis (from top to bottom): *S. coelicolor* AL645882, *S. lividans* NZ\_CM001889, and *S. lividans* NZ\_GG657756. Each coloured block represents a different locally co-linear block (LCB). Coloured lines connect LCBs that are similar between taxa. The black lines underneath each LCB represent the whole genome sequence of each of the *Streptomyces* taxa. Each LCB can be treated as a rearrangement, there have therefore been 6 rearrangements between these *Streptomyces* genomes.



Figure S3: This graph shows the time to complete a `progressiveMauve` alignment with varying numbers of *E. coli* genomes. The total number of genomes or taxa is along the x-axis and the total time in hours is along the right axis. Each black point represents data from one `progressiveMauve` alignment. All data points are connected by calculating locally estimated scatterplot smoothing (black line) with confidence intervals (grey band).

## progressiveMauve Alignment



Figure S4: Visualization of the **progressiveMauve** alignment of the *B. subtilis* genomes. Each coloured block represents a different locally colinear block (LCB). Coloured lines connect LCBs that are similar between taxa. The black lines underneath each LCB represent the whole genome sequence of each of the *B. subtilis* taxa. From top to bottom the taxa are: *B. subtilis* PY79, *B. subtilis* QB928, *B. subtilis* 168, *B. subtilis* BSn5, *B. subtilis* BS38, *B. subtilis* RONN1, *B. subtilis* W23. Each LCB can be treated as a rearrangement, there have therefore been 12 rearrangements between these *B. subtilis* genomes.

## Poor Sequence Alignment

After a re-alignment of **progressiveMauve** LCBs with **MAFFT** there were still regions of the alignment that were visibly poor. This prompted the additional alignment quality trimming using a custom Python script and **trimAl** (Capella-Gutiérrez et al. 2009). An example of what a “poor” alignment would look like can be found in Figure S5. The FASTA format of this segment of the alignment can be found on GitHub labelled as file “[poor\\_ecoli\\_alignment\\_example.fna](#)”.

This segment of **MAFFT** alignment (Figure S5) appears to have completely misaligned the second sequence (*E. coli* O157H7). When we look at the genes that these regions of DNA are found within (Table S3), we see that the second sequence (*E. coli* O157H7) does not have the same protein sequence as the other bacteria genes. Poor sequence alignments like this, as well as other non-homologous alignment regions were removed from the analysis. Please see the main paper for more detailed methods.

Alignment: poor\_ecoli\_alignment\_example\_TWO.fna  
 Seaview [blocks=10 fontsize=10 A4] on Tue Mar 24 14:14:23 2020



Figure S5: Visualization of a section of MAFFT alignment between the six strains of *E. coli*. This alignment was visualized with the SeaView graphical interface (Gouy et al. 2010).

| <i>E. coli</i> Strain | NCBI Accession Number | Alignment Gene Id |
|-----------------------|-----------------------|-------------------|
| 0104H4                | CP003289              | O3K_04155         |
| O157H7                | BA000007              | ECs3861           |
| 083H1                 | CP001855              | NRG857_18350      |
| IAI39                 | CU928164              | yghE              |
| K12                   | U00096                | yghE              |
| UMN026                | CU928163              | yghE              |

Table S3: *E. coli* strain, NCBI accession number, and Gene Id for the genes in the poor alignment example (Figure S5).



## Phylogenetic Trees

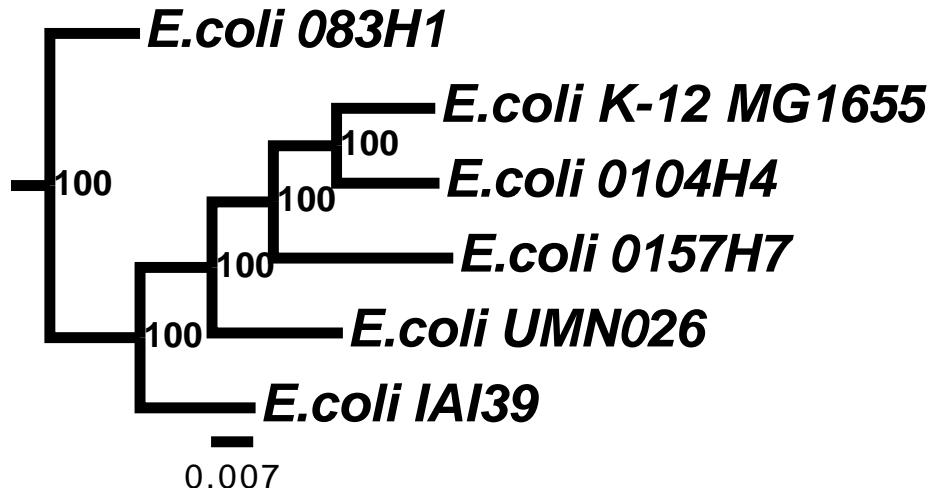


Figure S6: Phylogenetic tree of *E. coli* genomes. *Salmonella enterica* was used as an outgroup to root the tree. Branch lengths are to scale. The numbers at each node indicate the bootstrap value as a percentage. The number of bootstrapped trees was 100.

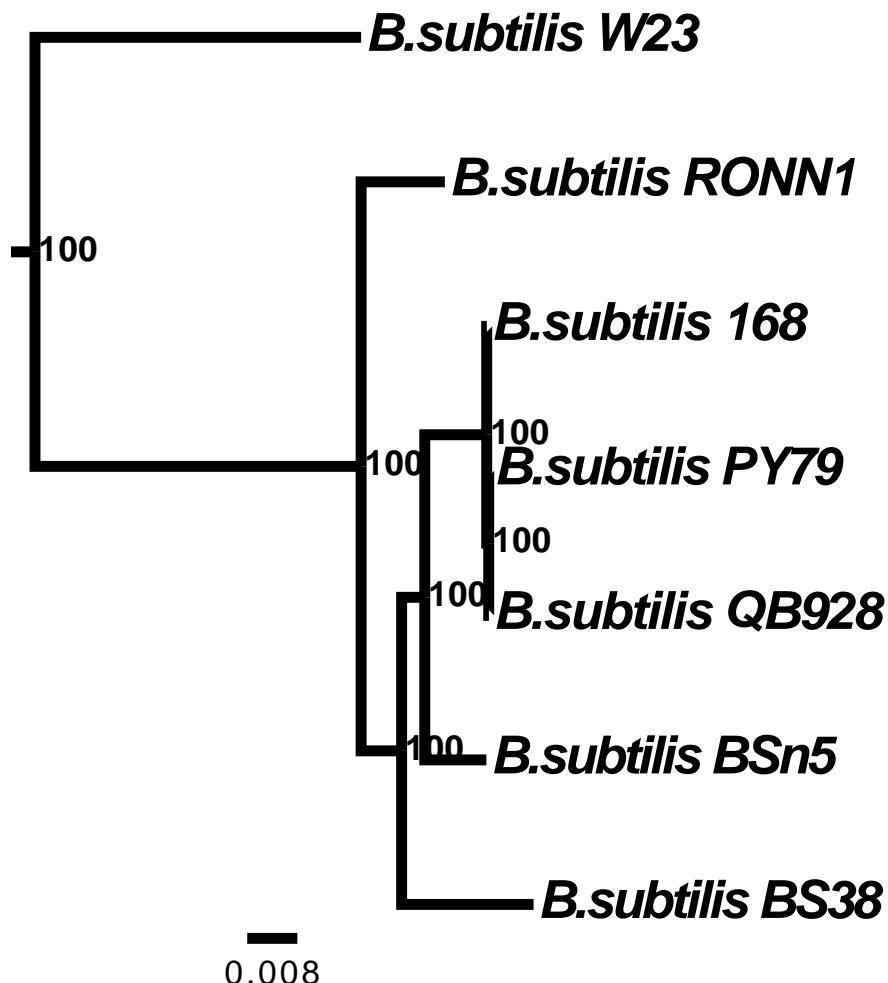


Figure S7: Phylogenetic tree of *B. subtilis* genomes. *Listeria monocytogenes* was used as an outgroup to root the tree. Branch lengths are to scale. The numbers at each node indicate the bootstrap value as a percentage. The number of bootstrapped trees was 100.

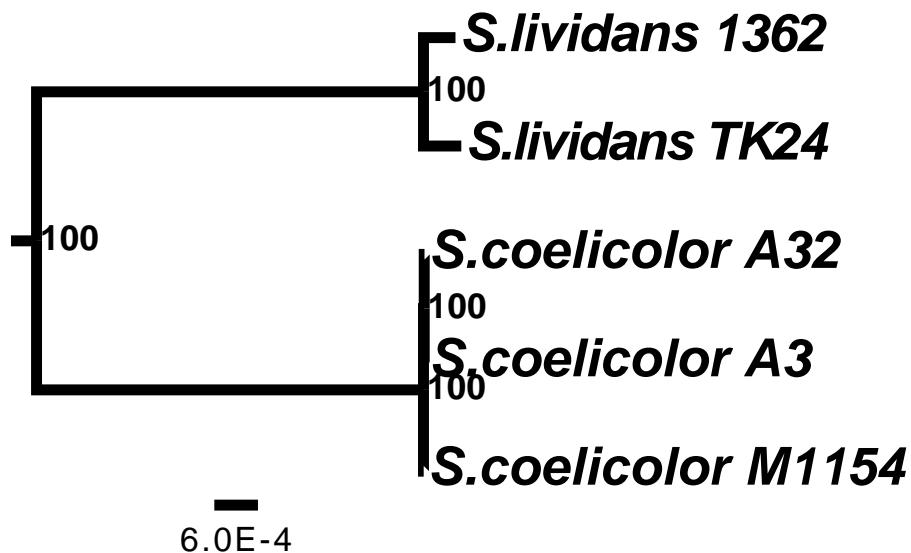


Figure S8: Phylogenetic tree of *Streptomyces* genomes. *Mycobacterium tuberculosis* was used as an outgroup to root the tree. Branch lengths are to scale. The numbers at each node indicate the bootstrap value as a percentage. The number of bootstrapped trees was 100.



Figure S9: Phylogenetic tree using only the chromosomes of *S. meliloti*. *A. tumefaciens* circular chromosome was used as an outgroup to root the tree. Branch lengths are to scale. The numbers at each node indicate the bootstrap value as a percentage. The number of bootstrapped trees was 100.



Figure S10: Phylogenetic tree using only pSymA of *S. meliloti*. *A. tumefaciens* circular plasmid was used as an outgroup to root the tree. Branch lengths are to scale. The numbers at each node indicate the bootstrap value as a percentage. The number of bootstrapped trees was 100.

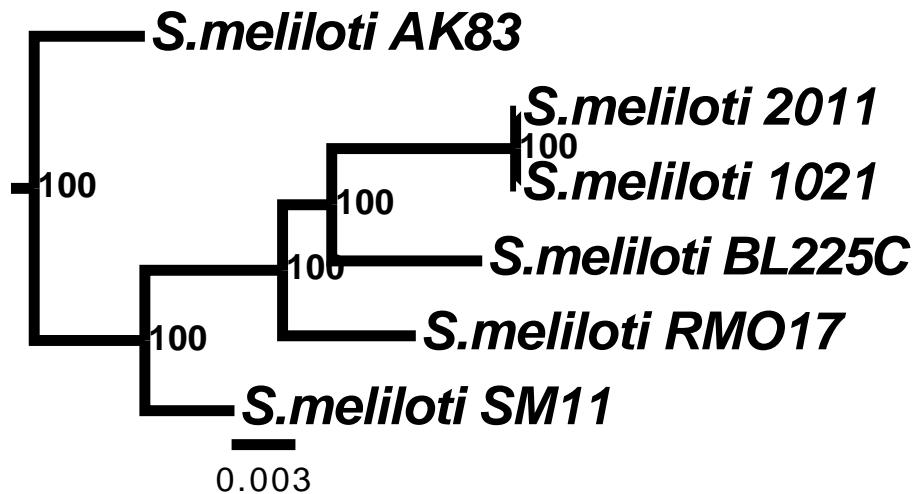


Figure S11: Phylogenetic tree using only pSymB of *S. meliloti*. *A. tumefaciens* circular chromid was used as an outgroup to root the tree. Branch lengths are to scale. The numbers at each node indicate the bootstrap value as a percentage. The number of bootstrapped trees was 100.

| Bacteria Replicon              | # of Total LCBs<br>with Identical<br>Tree | # of Total LCBs<br>with Non-<br>identical Tree | % of Total<br>Alignment<br>Discarded |
|--------------------------------|---|--|--------------------------------------|
| <i>E. coli</i> Chromosome      | 30  | 7  | 25.44%                               |
| <i>B. subtilis</i> Chromosome  | 10  | 2  | 21.62%                               |
| <i>Streptomyces</i> Chromosome | NA  | NA   | NA                                   |
| <i>S. meliloti</i> Chromosome  | 9   | 2  | 25.06%                               |
| <i>S. meliloti</i> pSymA       | 35  | 0  | 0%                                   |
| <i>S. meliloti</i> pSymB       | 8   | 0  | 0%                                   |

Table S4: Number of Locally Colinear Blocks that had identical topologies to the “super sequence” tree, not identical to the “super sequence” tree, and the proportion of the total alignment that was represented by the non-identical tree topologies. Topologies that were not identical were determined to be different at the 5% significant value using an SH-test in RAxML (Stamatakis 2014). The SH-Test could not be preformed on *Streptomyces* as there were only three taxa present and RAxML needs a minimum of 4 taxa for the test (Stamatakis 2014).

### Origin and Terminus Locations

Each of the bacterial strains used in this analysis vary in total genomic length, in some cases this difference is up to 856Kbp like in *E. coli* (Table S5). This will cause the farthest point from the origin of replication to appear larger because of the increased genome size of some strains.

| Bacteria                      | Origin of Replication | Terminus of Replication | Length of Longest Genome (bp) |
|-------------------------------|-----------------------|-------------------------|-------------------------------|
| <i>E. coli</i>                | 3925744               | 1588773                 | 5498450                       |
| <i>B. subtilis</i>            | 1                     | 1942542                 | 4215606                       |
| <i>Streptomyces</i>           | 3419363               | 1 & 8667664             | 8667664                       |
| <i>S. meliloti</i> Chromosome | 1                     | 1735626                 | 3908022                       |
| <i>S. meliloti</i> pSymA      | 1350001               | 672888                  | 1633319                       |
| <i>S. meliloti</i> pSymB      | 55090                 | 896756                  | 1690594                       |

Table S5: Origin of replication and terminus of replication positions in replicons of *E. coli*, *B. subtilis*, *Streptomyces*, and *S. meliloti*. The origin and terminus of replication are values from the representative strain of each bacteria, which can be found in Supplementary Table S2. The linear nature of *Streptomyces* chromosome gives it two termini, one at each end of the chromosome. The lenght of the longest genome is the longest genome length from all strains/species of each bactiera. This is not necessarally the same as the genome length of the representative strain.

| Origin Location   | <i>E. coli</i> Chromosome  | <i>B. subtilis</i> Chromosome | <i>Streptomyces</i> Chromosome | <i>S. meliloti</i> Chromosome | <i>S. meliloti</i> pSymA   | <i>S. meliloti</i> pSymB   |
|-------------------|----------------------------|-------------------------------|--------------------------------|-------------------------------|----------------------------|----------------------------|
| Moved 100kb Left  | -1.445×10 <sup>-7***</sup> | 4.374×10 <sup>-9*</sup>       | 6.909×10 <sup>-9***</sup>      | -1.316×10 <sup>-6***</sup>    | -1.058×10 <sup>-6***</sup> | -2.009×10 <sup>-7***</sup> |
| Moved 90kb Left   | -1.544×10 <sup>-7***</sup> | -1.036×10 <sup>-7***</sup>    | 5.677×10 <sup>-9***</sup>      | -1.32×10 <sup>-6***</sup>     | -1.246×10 <sup>-6***</sup> | -1.357×10 <sup>-7***</sup> |
| Moved 80kb Left   | -1.65×10 <sup>-7***</sup>  | -1.072×10 <sup>-7***</sup>    | 8.11×10 <sup>-9***</sup>       | -1.338×10 <sup>-6***</sup>    | -1.398×10 <sup>-6***</sup> | -6.57×10 <sup>-8***</sup>  |
| Moved 70kb Left   | -1.667×10 <sup>-7***</sup> | -1.102×10 <sup>-7***</sup>    | 6.716×10 <sup>-9***</sup>      | -1.363×10 <sup>-6***</sup>    | -1.405×10 <sup>-6***</sup> | 9.83×10 <sup>-8</sup>      |
| Moved 60kb Left   | -1.64×10 <sup>-7***</sup>  | -1.119×10 <sup>-7***</sup>    | 8.7×10 <sup>-9***</sup>        | -1.324×10 <sup>-6***</sup>    | -1.394×10 <sup>-6***</sup> | 1.129×10 <sup>-7***</sup>  |
| Moved 50kb Left   | -1.446×10 <sup>-7***</sup> | -1.211×10 <sup>-7***</sup>    | 1.045×10 <sup>-8***</sup>      | -1.36×10 <sup>-6***</sup>     | -1.403×10 <sup>-6***</sup> | 1.521×10 <sup>-7***</sup>  |
| Moved 40kb Left   | -1.4×10 <sup>-7***</sup>   | -1.299×10 <sup>-7***</sup>    | 1.214×10 <sup>-8***</sup>      | -1.255×10 <sup>-6***</sup>    | -1.422×10 <sup>-6***</sup> | 1.543×10 <sup>-7***</sup>  |
| Moved 30kb Left   | -1.498×10 <sup>-7***</sup> | -1.292×10 <sup>-7***</sup>    | 1.24×10 <sup>-8***</sup>       | -1.26×10 <sup>-6***</sup>     | -1.392×10 <sup>-6***</sup> | 1.63×10 <sup>-7***</sup>   |
| Moved 20kb Left   | -1.51×10 <sup>-7***</sup>  | -1.1×10 <sup>-7***</sup>      | 1.395×10 <sup>-8***</sup>      | -1.525×10 <sup>-6***</sup>    | -1.412×10 <sup>-6***</sup> | 1.603×10 <sup>-7***</sup>  |
| Moved 10kb Left   | -1.262×10 <sup>-7***</sup> | -2.602×10 <sup>-9</sup>       | 1.563×10 <sup>-8***</sup>      | -1.599×10 <sup>-6***</sup>    | -9.499×10 <sup>-7***</sup> | 2.973×10 <sup>-7***</sup>  |
| Moved 20kb Right  | -1.305×10 <sup>-7***</sup> | -2.045×10 <sup>-8***</sup>    | 1.578×10 <sup>-8***</sup>      | 1.614×10 <sup>-6***</sup>     | -1.026×10 <sup>-6***</sup> | 3.505×10 <sup>-7***</sup>  |
| Moved 20kb Right  | -1.454×10 <sup>-7***</sup> | -1.006×10 <sup>-7***</sup>    | 1.903×10 <sup>-8***</sup>      | -1.634×10 <sup>-6***</sup>    | -1.475×10 <sup>-6***</sup> | 1.649×10 <sup>-7***</sup>  |
| Moved 30kb Right  | -1.548×10 <sup>-7***</sup> | -8.596×10 <sup>-8***</sup>    | 2.046×10 <sup>-8***</sup>      | -1.698×10 <sup>-6***</sup>    | -1.417×10 <sup>-6***</sup> | 1.526×10 <sup>-7***</sup>  |
| Moved 40kb Right  | -1.632×10 <sup>-7***</sup> | -8.378×10 <sup>-8***</sup>    | 2.125×10 <sup>-8***</sup>      | -1.719×10 <sup>-6***</sup>    | -1.367×10 <sup>-6***</sup> | 1.589×10 <sup>-7***</sup>  |
| Moved 50kb Right  | -1.856×10 <sup>-7***</sup> | -7.879×10 <sup>-8***</sup>    | 1.957×10 <sup>-8***</sup>      | -1.735×10 <sup>-6***</sup>    | -1.277×10 <sup>-6***</sup> | 1.654×10 <sup>-7***</sup>  |
| Moved 60kb Right  | -1.91×10 <sup>-7***</sup>  | -6.98×10 <sup>-8***</sup>     | 1.974×10 <sup>-8***</sup>      | -1.788×10 <sup>-6***</sup>    | -1.169×10 <sup>-6***</sup> | 1.645×10 <sup>-7***</sup>  |
| Moved 70kb Right  | -1.892×10 <sup>-7***</sup> | -6.634×10 <sup>-8***</sup>    | 1.934×10 <sup>-8***</sup>      | -1.854×10 <sup>-6***</sup>    | -1.059×10 <sup>-6***</sup> | 1.843×10 <sup>-7***</sup>  |
| Moved 80kb Right  | -1.879×10 <sup>-7***</sup> | -5.814×10 <sup>-8***</sup>    | 2.313×10 <sup>-8***</sup>      | -1.891×10 <sup>-6***</sup>    | -9.07×10 <sup>-7***</sup>  | 1.90×10 <sup>-7***</sup>   |
| Moved 90kb Right  | -1.862×10 <sup>-7***</sup> | -4.314×10 <sup>-8***</sup>    | 2.304×10 <sup>-8***</sup>      | -1.865×10 <sup>-6***</sup>    | -7.171×10 <sup>-7***</sup> | 2.415×10 <sup>-7***</sup>  |
| Moved 100kb Right | -1.799×10 <sup>-7***</sup> | -2.597×10 <sup>-8***</sup>    | 1.945×10 <sup>-8***</sup>      | -1.525×10 <sup>-6***</sup>    | -6.572×10 <sup>-7***</sup> | 3.095×10 <sup>-7***</sup>  |

Table S6: Logistic regression analysis of the number of substitutions along the genome of the respective bacterial replicons after the origin location was moved by the specified increments from the original origin of replication position (listed in Table S5). All results are marked with significance codes as followed: < 0.001 = ‘\*\*\*’, 0.001 < 0.01 = ‘\*\*’, 0.01 < 0.05 = ‘\*’, 0.05 < 0.1 = ‘.’, > 0.1 = ‘ ’. Logistic regression was calculated after the origin of replication was moved to the new location in the genome and all subsequent positions were scaled around the origin accounting for bidirectionality of replication.

| Bacteria Strain                    | Accession Number | Date Accessed      |
|------------------------------------|------------------|--------------------|
| <i>E. coli</i> K12 Chromosome      | U00096           | September 26, 2016 |
| <i>B. subtilis</i> 168 Chromosome  | NC_000964        | November 10, 2016  |
| <i>S. coelicolor</i> A3 Chromosome | AL645882         | November 30, 2016  |
| <i>S. meliloti</i> Chromosome 1021 | NC_003047        | June 3, 2014       |
| <i>S. meliloti</i> pSymA 1021      | NC_003037        | June 3, 2014       |
| <i>S. meliloti</i> pSymB 1021      | NC_003078        | June 3, 2014       |

Table S7: Strains and species used for determining the protein coding regions of each bacterial replicon. GenBank reference annotation was used to determine all protein coding sections of the replicons. NCBI accession numbers and date accessed are provided.

## Genomic Position Clustering

A custom R script was used to cluster genomic positions together based on a user specified genetic distance using single-link clustering. An illustration of the clustering method used in this supplemental test can be found in Figure S12. This clustering was done for genomic distances beginning at 1bp and increasing by one order of magnitude until 1,000,000bp difference exists between the taxa genomic positions. These newly clustered genomic positions were then put into the same substitution analysis as mentioned previously to determine the impact of this position clustering on the spatial substitution trends through a linear regression. A complete table of the statistical results from the clustering assessment are found in Table S8. The results from this analysis indicate that genomic positions up to 1,000,000bp apart can be considered a singular genomic position without altering the overall spatial substitution analysis.

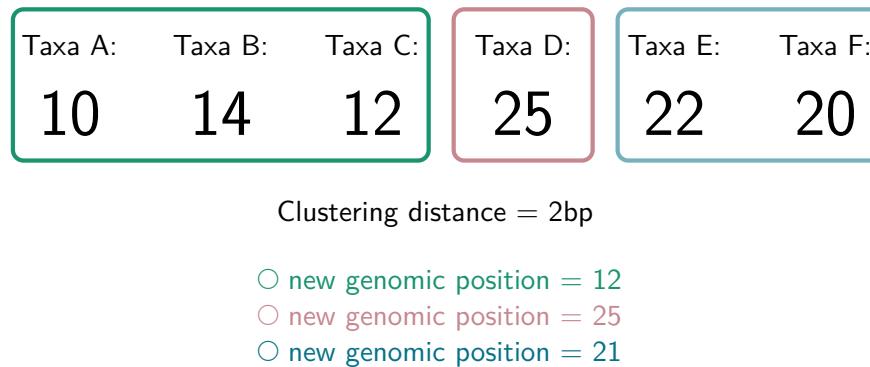


Figure S12: Visualization of the genomic position clustering method. In this example, the user specified the genetic distance to be 2, all genomic positions within 2 base pairs would be clustered together. In this example we are looking at 6 taxa with genomic positions 10, 14, 12, 25, 22, and 20. Based on the clustering algorithm, positions 10, 14 and 12 would be grouped into a cluster (outlined in green), position 25 would be its own cluster (outlined in pink), and positions 22 and 20 would be grouped into another cluster (outlined in blue). Once the clusters are determined, a new genomic position for each of the clusters is calculated using the average of all positions within that cluster. In this example, the green cluster would have a new genomic position of 12 (the average between those three positions), the pink cluster would have the same genomic position of 25, and the blue cluster would have a new genomic position of 21. The new list of genomic positions for the 4 taxa would be: 12, 12, 12, 25, 21 and 21.

| Position Difference | <i>E. coli</i> Chromosome  | <i>B. subtilis</i> Chromosome | <i>Streptomyces</i> Chromosome | <i>S. meliloti</i> Chromosome | <i>S. meliloti</i> pSymA   | <i>S. meliloti</i> pSymB   |
|---------------------|----------------------------|-------------------------------|--------------------------------|-------------------------------|----------------------------|----------------------------|
| 1bp                 | $-1.394 \times 10^{-7}***$ | $-2.538 \times 10^{-8}**$     | $1.736 \times 10^{-8}**$       | $-1.541 \times 10^{-6}**$     | $-9.130 \times 10^{-7}**$  | $2.488 \times 10^{-7}***$  |
| 10bp                | $-1.394 \times 10^{-7}***$ | $-2.518 \times 10^{-8}***$    | $-4.484 \times 10^{-9}***$     | $-1.627 \times 10^{-6}***$    | $-9.13 \times 10^{-7}***$  | $3.487 \times 10^{-7}***$  |
| 100bp               | $-1.764 \times 10^{-7}***$ | $-1.417 \times 10^{-8}***$    | $1.448 \times 10^{-8}***$      | $-1.605 \times 10^{-6}***$    | $-1.166 \times 10^{-6}***$ | $4.021 \times 10^{-7}***$  |
| 1000bp              | $-1.784 \times 10^{-7}***$ | $-1.417 \times 10^{-8}***$    | $1.505 \times 10^{-8}***$      | $-1.605 \times 10^{-6}***$    | $-1.153 \times 10^{-6}***$ | $4.021 \times 10^{-7}***$  |
| 10000bp             | $-1.712 \times 10^{-7}***$ | $-3.496 \times 10^{-8}***$    | $4.790 \times 10^{-8}***$      | $-1.605 \times 10^{-6}***$    | $-3.570 \times 10^{-8}$    | $3.784 \times 10^{-7}***$  |
| 100000bp            | $-2.061 \times 10^{-7}***$ | $-3.561 \times 10^{-8}***$    | $4.167 \times 10^{-9}***$      | $-1.605 \times 10^{-6}***$    | $-4.676 \times 10^{-7}***$ | $3.784 \times 10^{-7}***$  |
| 1000000bp           | $4.229 \times 10^{-8}***$  | $-7.710 \times 10^{-9}***$    | $6.083 \times 10^{-8}***$      | $-1.605 \times 10^{-6}***$    | $4.285 \times 10^{-6}***$  | $-8.888 \times 10^{-7}***$ |

Table S8: Results from the position clustering analysis. Logistic regression analysis of the number of substitutions along the genome of the respective bacteria replicons to test position differences. The “Position Difference” column denotes different base pair distances that the positions in the genome were clustered together as. All results are marked with significance codes as followed:  $< 0.001 = ***$ ,  $0.001 < 0.01 = **$ ,  $0.01 < 0.05 = *$ ,  $0.05 < 0.1 = .$ ,  $> 0.1 = '$ . Logistic regression was calculated after the positions in the genome were determined to be the same at each position difference listed in the first column.

| Bacteria and Replicon          | Average Replicon Length | Number of Sites | Number of Substitutions |
|--------------------------------|-------------------------|-----------------|-------------------------|
| <i>E. coli</i> Chromosome      | 5082529                 | 2318259         | 353740                  |
| <i>B. subtilis</i> Chromosome  | 4077077                 | 2032176         | 185060                  |
| <i>Streptomyces</i> Chromosome | 8494093                 | 6057063         | 24046                   |
| <i>S. meliloti</i> Chromosome  | 3426881                 | 1892874         | 11210                   |
| <i>S. meliloti</i> pSymA       | 1455940                 | 571278          | 13132                   |
| <i>S. meliloti</i> pSymB       | 1664597                 | 1248879         | 28941                   |

Table S9: Total number of protein coding sites in each replicon for this analysis and the number of those sites that have a substitution (multiple substitutions at one site are counted as two substitutions).

## High Substitutions Gene Example

Throughout this analysis there are a few genes/gene segments in all the bacterial replicons that have relatively high numbers of substitutions when compared to other genes or gene segments. These high numbers of substitutions are indeed real changes seen in homologous genes. To illustrate this, we have chosen a segment of alignment from *Streptomyces*. Information about the genes involved in this segment can be found in Table S10. A protein alignment for these genes can be found on GitHub ([https://github.com/dlato/Spatial\\_Patterns\\_of\\_Substitutions](https://github.com/dlato/Spatial_Patterns_of_Substitutions)) under the file name “*Streptomyces\_high\_substitutions\_gene\_example.txt*”.

Both *S. lividans* strains have 100% sequence identity at the DNA level, while the *S. coelicolor* species has 87.2% sequence identity with the *S. lividans* strains for this particular alignment. Despite this high sequence identity and almost identical protein alignment (Figures S13 and S14), there are a total of 31 substitutions (across all nodes of the phylogenetic tree, Figure S8) within this short stretch of sequence. It is segments like these that are resulting in the appearance of extremely high numbers of substitutions in sections of all the bacterial repliconic genomes.

| Species                 | NCBI Acession Number | Gene Id      |
|-------------------------|----------------------|--------------|
| <i>S. coelicolor</i> A3 | AL645882             | SCO6334      |
| <i>S. lividans</i> 1362 | NZ_CM001889          | SLI_RS32020  |
| <i>S. lividans</i> TK24 | NZ_GG657756          | SSPG_RS06405 |

Table S10: Information about the example gene segment with high number of substitutions.

Alignment: *Streptomyces\_high\_substitutions\_gene\_example.txt*  
Seaview [blocks=10 fontsize=10 A4] on Thu Apr 30 13:24:40 2020



Figure S13: Visualization of the nucleotide alignment of *Streptomyces* genes with high numbers of substitutions. Alignment visualization was performed with SeaView (Gouy et al. 2010)

Alignment: PROTEIN\_ALN.txt  
 Seaview [blocks=10 fontsize=10 A4] on Mon Mar 9 15:02:33 2020



Figure S14: Visualization of the protein alignment of *Streptomyces* genes with high numbers of substitutions. Alignment visualization was performed with SeaView (Gouy et al. 2010)

## High Substitution Distribution

| Bacteria and Replicon          | Bidirectional Genomic Position (bp) | Protein/Gene Examples                       |
|--------------------------------|-------------------------------------|---|
| <i>E. coli</i> Chromosome      | 1130000 - 1140000                   | Uncharacterized protein                     |
|                                |                                     | Hypothetical protein                        |
|                                |                                     | Lipoprotein                                 |
|                                | 1720000 - 1740000                   | Transcriptional activator                   |
|                                |                                     | Hypothetical protein                        |
|                                |                                     | Predicted protein                           |
| <i>B. subtilis</i> Chromosome  | 560000 - 570000                     | Small toxic polypeptide                     |
|                                |                                     | Hypothetical protein                        |
|                                |                                     | Derived by automated computational analysis |
|                                | 1820000 - 1380000                   | Membrane protein                            |
| <i>Streptomyces</i> Chromosome | 3550000 - 3570000                   | Derived by automated computational analysis |
|                                |                                     | Hypothetical protein                        |
|                                |                                     | Putative integral membrane protein          |
|                                |                                     | Reductase                                   |
| <i>S. meliloti</i> Chromosome  | 80000 - 90000                       | Hypothetical proteins                       |
|                                | 730000 - 740000                     | Hypothetical proteins                       |
|                                |                                     | Putative proteins                           |
| <i>S. meliloti</i> pSymA       | 100000 - 110000                     | Hypothetical proteins                       |
|                                | 800000 - 810000                     | Hypothetical protein                        |
|                                |                                     | Transporter protein                         |
| <i>S. meliloti</i> pSymB       | 450000 - 460000                     | Hypothetical protein                        |
|                                |                                     | Putative oxidoreductase                     |
|                                |                                     | Hypothetical proteins                       |
|                                | 610000 - 620000                     | Hypothetical protein                        |
|                                |                                     | Putative transport regulator                |
|                                |                                     | Predicted membrane protein                  |

Table S11: Table of high number of substitutions per 10Kbp genomic regions for each bacterial replicon and examples of the associated proteins/gene functions found in that region. The genomic position begins at the origin of replication and continues in both directions until the terminus of replication (bidirectional replication).

## Weighted, Non-weighted, and 20Kbp Near and Far From the Origin Substitution Linear Regression Analysis

| Bacteria and Replicon          | Protein Coding Window Size            |                                       |                                      |                                       |               |               |
|--------------------------------|---------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|---------------|---------------|
|                                | 10Kbp                                 | 25Kbp                                 | 50Kbp                                | 100Kbp                                | 200Kbp        | 400Kbp        |
| <i>E. coli</i> Chromosome      | $-2.27 \times 10^{-9}***$<br>(0.039)  | $-2.54 \times 10^{-10}**$<br>(0.078)  | $-2.32 \times 10^{-10}*$<br>(0.112)  | $-2.36 \times 10^{-10}***$<br>(0.133) | NS<br>(0.200) | NS<br>(0.361) |
| <i>B. subtilis</i> Chromosome  | NS<br>(0.002)                         | NS<br>(0.004)                         | NS<br>(0.0002)                       | NS<br>(0.002)                         | NS<br>(0.019) | NS<br>(0.484) |
| <i>Streptomyces</i> Chromosome | $2.38 \times 10^{-11}*$<br>(0.006)    | NS<br>(0.008)                         | $4.41 \times 10^{-11}**$<br>(0.175)  | NS<br>(0.033)                         | NS<br>(0.050) | NS<br>(0.010) |
| <i>S. meliloti</i> Chromosome  | $-1.21 \times 10^{-10}***$<br>(0.076) | $-1.71 \times 10^{-10}***$<br>(0.137) | $-1.86 \times 10^{-10}**$<br>(0.253) | $-2.78 \times 10^{-10}**$<br>(0.350)  | NS<br>(0.150) | NS<br>(0.397) |
| <i>S. meliloti</i> pSymA       | NS<br>(0.013)                         | NS<br>(0.001)                         | NS<br>(0.024)                        | NS<br>(0.279)                         | NS<br>(0.355) | NS<br>(0.240) |
| <i>S. meliloti</i> pSymB       | NS<br>(0.010)                         | NS<br>(0.020)                         | NS<br>(0.043)                        | NS<br>(0.025)                         | NS<br>(0.002) | NS<br>(0.039) |

Table S12: Linear regression on various sections of the genome (10Kbp, 25Kbp, 50Kbp, 100Kbp, 200Kbp, and 400Kbp) with increasing distance from the origin of replication after accounting for bidirectional replication. The total number of substitutions in each section of the genome was divided by the total number of protein coding sites in that genomic region. All results are marked with significance codes as followed:  $< 0.001 = '***'$ ,  $0.001 < 0.01 = '**'$ ,  $0.01 < 0.05 = '*'$ ,  $> 0.05 = 'NS'$ . The  $R^2$  value for each coefficient estimate is found below the value in brackets () .

Multiple linear regressions were performed to determine if there was any correlation between number of substitutions and distance from the origin of replication. A linear regression to determine how the weighted and non-weighted total number of substitutions in various sections of the genome (10Kbp, 25Kbp, 50Kbp, 100Kbp, 200Kbp, and 400Kbp) changes with genomic position was performed (Tables S12 and S13). All additional linear regression results (Tables S12 and S13) mirror the results from the logistic regression on presence or absence of substitutions and changes in genomic position (see the Main Paper results section for more information). The results from these supplemental tests are consistent with the results from the linear regression found in the Main Paper, most bacterial replicons have a decreasing number of substitutions when moving away from the origin of replication.

To calculate the non-weighted values of the total number of substitutions per 10Kbp region of the genome, the total number of substitutions was summed up over each region of the genome (10Kbp, 25Kbp, 50Kbp, 100Kbp, 200Kbp, and 400Kbp), while accounting for bidirectional replication (see Main Paper for details). A linear regression on these total number of substitutions in each section of the genome (10Kbp, 25Kbp, 50Kbp, 100Kbp, 200Kbp, and 400Kbp) was performed to see how the number of substitutions changes with distance from the origin of replication (Table S13). The weighted values of the total number of substitutions per various region of the genome, the total number of substitutions was summed up over each region of the genome (10Kbp, 25Kbp, 50Kbp, 100Kbp, 200Kbp, and 400Kbp) while accounting for bidirectional replication (see Main Paper for details). These summed values were then divided by the total number of protein coding sites in each region to obtain the weighted value. A linear regression on these weighted total number of substitutions in each section of the genome was performed to see how the number of substitutions changes with distance from the origin of replication (Table S12).

We took a closer look at 20Kbp regions of the replicons close and far from the origin of replication. We performed a logistic regression on the presence or absence of a substitution with distance from the origin of replication. Data points from the 20Kbp regions closest to the origin of replication and data points from the 20Kbp regions closest to the terminus of replication were used for this portion of the analysis. Outliers were removed from this analysis. The number of substitutions per site was also calculated in each of these 20Kbp regions for each bacterial replicon. We were unable to determine a consistent spatial substitution trend when considering only the 20Kbp near and far from the origin of replication in all bacterial replicons. Some bacterial replicons had a positive correlation coefficient,

| Bacteria and Replicon          | Protein Coding Window Size           |                                      |                                      |                                      |                                     |                                    |
|--------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|------------------------------------|
|                                | 10Kbp                                | 25Kbp                                | 50Kbp                                | 100Kbp                               | 200Kbp                              | 400Kbp                             |
| <i>E. coli</i> Chromosome      | $-1.66 \times 10^{-4}***$<br>(0.398) | $-4.18 \times 10^{-4}***$<br>(0.476) | $-8.64 \times 10^{-4}***$<br>(0.563) | $-1.71 \times 10^{-3}***$<br>(0.509) | $-3.42 \times 10^{-3}**$<br>(0.534) | $-6.71 \times 10^{-3}*$<br>(0.592) |
| <i>B. subtilis</i> Chromosome  | NS<br>(0.004)                        | NS<br>(0.005)                        | NS<br>(0.0008)                       | NS<br>(0.001)                        | NS<br>(0.014)                       | NS<br>(0.027)                      |
| <i>Streptomyces</i> Chromosome | NS<br>(0.001)                        | $2.75 \times 10^{-6}**$<br>(0.028)   | NS<br>(0.022)                        | NS<br>(0.006)                        | NS<br>(0.044)                       | NS<br>(0.033)                      |
| <i>S. meliloti</i> Chromosome  | $-8.97 \times 10^{-6}**$<br>(0.040)  | $-3.72 \times 10^{-5}**$<br>(0.098)  | $-7.76 \times 10^{-5}*$<br>(0.126)   | $-1.64 \times 10^{-4}*$<br>(0.188)   | NS<br>(0.082)                       | NS<br>(0.427)                      |
| <i>S. meliloti</i> pSymA       | NS<br>(0.035)                        | NS<br>(0.062)                        | $-4.98 \times 10^{-4}*$<br>(0.217)   | NS<br>(0.295)                        | NS<br>(0.408)                       | NS<br>(0.957)                      |
| <i>S. meliloti</i> pSymB       | NS<br>(0.011)                        | NS<br>(0.018)                        | NS<br>(0.032)                        | NS<br>(0.196)                        | NS<br>(0.389)                       | NS<br>(0.527)                      |

Table S13: Linear regression on various sections of the genome (10Kbp, 25Kbp, 50Kbp, 100Kbp, 200Kbp, and 400Kbp) with increasing distance from the origin of replication after accounting for bidirectional replication. The linear regression was performed on the total number of substitutions in each section of the genome without accounting for the number of sites in each genomic region. All results are marked with significance codes as followed:  $< 0.001 = '***'$ ,  $0.001 < 0.01 = '**'$ ,  $0.01 < 0.05 = '*'$ ,  $> 0.05 = 'NS'$ . The  $R^2$  value for each coefficient estimate is found below the value in brackets ().

indicating that the number of substitutions increases with increasing distance from the origin of replication (Table S14). Other replicons had a negative correlation coefficient, suggesting that the number of substitutions decreases with increasing distance from the origin of replication (Table S14). Additionally, it was unclear if the number of substitutions per site locally were higher near the origin of replication or near the terminus. Some bacteria had higher number of substitutions per site near the origin (*E. coli*, *S. meliloti* chromosome and pSymB), while other replicons has the opposite trend (*B. subtilis*, *Streptomyces* and *S. meliloti* pSymA) (Table S14). These results suggest that on a small local scale, there are varying patterns of substitutions with respect to distance from the origin of replication. This varies between bacteria, and in some cases even within the same bacteria (*E. coli*). This variation locally does not allow us to make any overarching statements about the local distribution of substitutions in bacterial genomes. It is therefore more useful to consider the global (genome wide) pattern of substitutions when making overarching statements about genomic substitution arrangements.

## Non-linear Analysis of Number of Substitutions and Distance From the Origin of Replication

Using a simple smoothed conditional means method (`geom_smooth()` function in R), a non-linear trend analysis was performed on all bacterial replicons. The previous mentioned weighted data (see the previous subsection), was used in this analysis. The weighted data represents the total number of substitutions divided by the total number of protein-coding sites in 10Kbp segments of the genomes. Outliers were removed. The results from this non-linear analysis can be seen in Figures S15 - S19. The visual results from this analysis mirror the findings from the main paper, the total number of substitutions varies with distance from the origin of replication, but the direction of this trend is unclear and inconsistent between bacterial replicons.

## Total Number of Sites Linear Regression

We performed a linear regression on the total number of protein coding sites and distance from the origin of replication. We found that the total number protein coding sites decreases with distance from the origin of replication in majority of the bacterial replicons in this analysis. We were unable to detect a significant relationship between the number of

| Bacteria and Replicon          | Protein Coding                       |                           |  |                       |
|--------------------------------|--------------------------------------|---------------------------|--|-----------------------|
|                                | Correlation Coefficient<br>20kb Near |                           | Number of Substitutions<br>per 20kb Near |                       |
|                                | Origin                               | Terminus                  | Origin                                   | Terminus              |
| <i>E. coli</i> Chromosome      | $6.51 \times 10^{-6}**$              | $6.16 \times 10^{-6}**$   | $5.85 \times 10^{-3}$                    | $6.47 \times 10^{-3}$ |
| <i>B. subtilis</i> Chromosome  | NS                                   | $1.57 \times 10^{-5}***$  | $4.23 \times 10^{-3}$                    | $5.01 \times 10^{-3}$ |
| <i>Streptomyces</i> Chromosome | NS                                   | $7.48 \times 10^{-4}***$  | $6.43 \times 10^{-4}$                    | $1.95 \times 10^{-3}$ |
| <i>S. meliloti</i> Chromosome  | $7.11 \times 10^{-6}***$             | NS                        | $1.51 \times 10^{-3}$                    | $3.86 \times 10^{-5}$ |
| <i>S. meliloti</i> pSymA       | $-6.94 \times 10^{-5}***$            | NS                        | $2.03 \times 10^{-3}$                    | $3.28 \times 10^{-3}$ |
| <i>S. meliloti</i> pSymB       | $1.58 \times 10^{-5}***$             | $-7.10 \times 10^{-5}***$ | $3.06 \times 10^{-3}$                    | $1.25 \times 10^{-3}$ |

Table S14: Logistic regression on 20kb closest and farthest from the origin of replication after accounting for bidirectional replication and outliers. Number of substitutions was calculated by taking the total number of substitutions in each of the 20Kbp regions and dividing by the total number of sites in those regions. All results are marked with significance codes as followed:  $< 0.001 = '***'$ ,  $0.001 < 0.01 = '**'$ ,  $0.01 < 0.05 = '*'$ ,  $> 0.05 = 'NS'$ . The  $R^2$  values for each estimate are in brackets.

protein coding sites and distance from the origin of replication in pSymB of *S. meliloti*.

| Bacteria and Replicon          | Coefficient Estimate      | $R^2$                 |
|--------------------------------|---------------------------|-----------------------|
| <i>E. coli</i> Chromosome      | $-2.33 \times 10^{-2}***$ | 0.423                 |
| <i>B. subtilis</i> Chromosome  | NS                        | 0.001                 |
| <i>Streptomyces</i> Chromosome | $-1.24 \times 10^{-3}***$ | 0.069                 |
| <i>S. meliloti</i> Chromosome  | NS                        | 0.013                 |
| <i>S. meliloti</i> pSymA       | NS                        | 0.002                 |
| <i>S. meliloti</i> pSymB       | NS                        | $8.08 \times 10^{-7}$ |

Table S15: 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:  $< 0.001 = '***'$ ,  $0.001 < 0.01 = '**'$ ,  $0.01 < 0.05 = '*'$ ,  $> 0.05 = 'NS'$ .

### Average $dN$ , $dS$ , and $\omega$ per Gene Values

The average  $dN$ ,  $dS$ , and  $\omega$  values per gene were calculated. For genes that were split into multiple parts (due to the presence of gaps or poor homology in the alignment), the  $dN$ ,  $dS$ , and  $\omega$  values for each gene part were averaged to obtain a single average value per gene. A complete list of these values can be found on GitHub ([www.github.com/dlato/Spatial\\_Patterns\\_of\\_Substitutions](https://www.github.com/dlato/Spatial_Patterns_of_Substitutions)) under the file name “Supplementary\_table\_per\_gene\_dN\_dS\_omega.pdf”.

### Distribution of $dN$ , $dS$ , and $\omega$

| Bacteria and Replicon          | Outliers (%) | Zero Value (%) |       |          |
|--------------------------------|--------------|----------------|-------|----------|
|                                |              | $dN$           | $dS$  | $\omega$ |
| <i>E. coli</i> Chromosome      | 7.13         | 14.33          | 1.19  | 14.33    |
| <i>B. subtilis</i> Chromosome  | 5.33         | 4.09           | 0.09  | 4.09     |
| <i>Streptomyces</i> Chromosome | 8.71         | 49.66          | 28.52 | 49.65    |
| <i>S. meliloti</i> Chromosome  | 16.20        | 65.58          | 66.72 | 65.58    |
| <i>S. meliloti</i> pSymA       | 6.90         | 11.39          | 9.45  | 11.39    |
| <i>S. meliloti</i> pSymB       | 6.06         | 13.01          | 5.16  | 13.01    |

Table S16: Percent of data that was calculated to be an outlier or had a selection variable ( $dN$ ,  $dS$ , and  $\omega$ ) value of zero.

### 20Kbp Near and Far From Origin Selection Linear Regression Analysis

We additionally took a closer look at 20 genes close and far from the origin of replication. We performed a linear regression on the change in selection values ( $dN$ ,  $dS$ , and  $\omega$ ) with distance from the origin of replication in these genes (Table S17). For majority of the bacterial replicons we failed to find a trend, which is not surprising since there was no evidence of an overall genomic trend when looking at these values (see Main Paper for results). Again, we are unable to conclude that there is a consistent overall trend for any of the selection values,  $dN$ ,  $dS$ , and  $\omega$ .

| Bacteria and Replicon          | Near Origin                      |      |          | Near Terminus                   |                                |          |
|--------------------------------|----------------------------------|------|----------|---------------------------------|--------------------------------|----------|
|                                | $dN$                             | $dS$ | $\omega$ | $dN$                            | $dS$                           | $\omega$ |
| <i>E. coli</i> Chromosome      | NS                               | NS   | NS       | $3.13 \times 10^{-7}*$ (0.254)  | NS                             | NS       |
| <i>B. subtilis</i> Chromosome  | NS                               | NS   | NS       | NS                              | NS                             | NS       |
| <i>Streptomyces</i> Chromosome | $-1.35 \times 10^{-7}**$ (0.449) | NS   | NS       | NS                              | NS                             | NS       |
| <i>S. meliloti</i> Chromosome  | NS                               | NS   | NS       | NS                              | NS                             | NS       |
| <i>S. meliloti</i> pSymA       | NS                               | NS   | NS       | $-2.53 \times 10^{-7}*$ (0.238) | NS                             | NS       |
| <i>S. meliloti</i> pSymB       | NS                               | NS   | NS       | NS                              | $4.92 \times 10^{-6}*$ (0.232) | NS       |

Table S17: Linear regression for  $dN$ ,  $dS$ , and  $\omega$  calculated for each bacterial replicon for the 20 genes closest and 20 genes farthest from the origin of replication. All results are marked with significance codes as followed:  $p < 0.001 = '***'$ ,  $0.001 < p < 0.01 = '**'$ ,  $0.01 < p < 0.05 = '*'$ ,  $p > 0.05 = 'NS'$ . The  $R^2$  values for each estimate are in brackets.

### *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  $\omega$  values that are equal to zero (see Table S16). 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  $\omega$  values to be classified as outliers (see Figure 6 in the Main Paper). We decided to perform the same calculations on  $dN$ ,  $dS$ , and  $\omega$  but including the outliers to see what the results would have been. A visualization of the distribution of  $dN$ ,  $dS$ , and  $\omega$  along the chromosome of *S. meliloti* is seen in Figure S21. The average values for  $dN$ ,  $dS$ , and  $\omega$  are found in Table S18 and the linear regression to determine if there is a correlation between distance from the origin of replication and  $dN$ ,  $dS$ , and  $\omega$  values for the chromosome of *S. meliloti* is found in Table S19. We also looked at the values of  $dN$ ,  $dS$ , and  $\omega$  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 S20. The methods for these calculations are the same as in the Main Paper and in section 20Kbp Near and Far From Origin Selection Linear Regression Analysis, however, outliers were not removed from these calculations.

The results in Tables S18 - S20 closely reflect the results of the *S. meliloti* analysis when outliers were included (see Main Paper). We were unable to determine a significant correlation between  $dN$  and  $\omega$  values and distance from the origin of replication. The significant correlation between  $dS$  and distance from the origin of replication is small. Even when the outliers (non-zero values of  $dN$  and  $\omega$ ) are included in the selection analysis, we still can find no evidence for an overall trend between distance from the origin of replication and  $dN$ ,  $dS$ , and  $\omega$  values.

| Genome Average                |        |        |          |
|-------------------------------|--------|--------|----------|
| Bacteria and Replicon         | $dS$   | $dN$   | $\omega$ |
| <i>S. meliloti</i> Chromosome | 0.0100 | 0.0007 | 0.0677   |

Table S18: Weighted averages of  $dN$ ,  $dS$ , and  $\omega$  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         | $dN$                           | $dS$                                      | $\omega$                     |
|-------------------------------|--------------------------------|---|------------------------------|
| <i>S. meliloti</i> Chromosome | NS ( $-1.96 \times 10^{-10}$ ) | <b>-2.29 <math>\times 10^{-9}*</math></b> | NS ( $5.96 \times 10^{-9}$ ) |

Table S19: Linear regression to determine the correlations between  $dN$ ,  $dS$ , and  $\omega$  values and distance from the origin of replication. Outliers were included in the calculation. All results are marked with significance codes as followed: p:  $< 0.001 = '***'$ ,  $0.001 < 0.01 = '**'$ ,  $0.01 < 0.05 = '*'$ ,  $> 0.05 = 'NS'$ .

| Bacteria and Replicon         | Near Origin                  |                               |                              | Near Terminus                 |                              |                               |
|-------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|
|                               | $dN$                         | $dS$                          | $\omega$                     | $dN$                          | $dS$                         | $\omega$                      |
| <i>S. meliloti</i> Chromosome | NS ( $2.16 \times 10^{-8}$ ) | NS ( $-7.05 \times 10^{-8}$ ) | NS ( $3.07 \times 10^{-6}$ ) | NS ( $-1.50 \times 10^{-8}$ ) | NS ( $2.22 \times 10^{-7}$ ) | NS ( $-2.06 \times 10^{-6}$ ) |

Table S20: Linear regression for  $dN$ ,  $dS$ , and  $\omega$  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:  $< 0.001 = '***'$ ,  $0.001 < 0.01 = '**'$ ,  $0.01 < 0.05 = '*'$ ,  $> 0.05 = 'NS'$ .

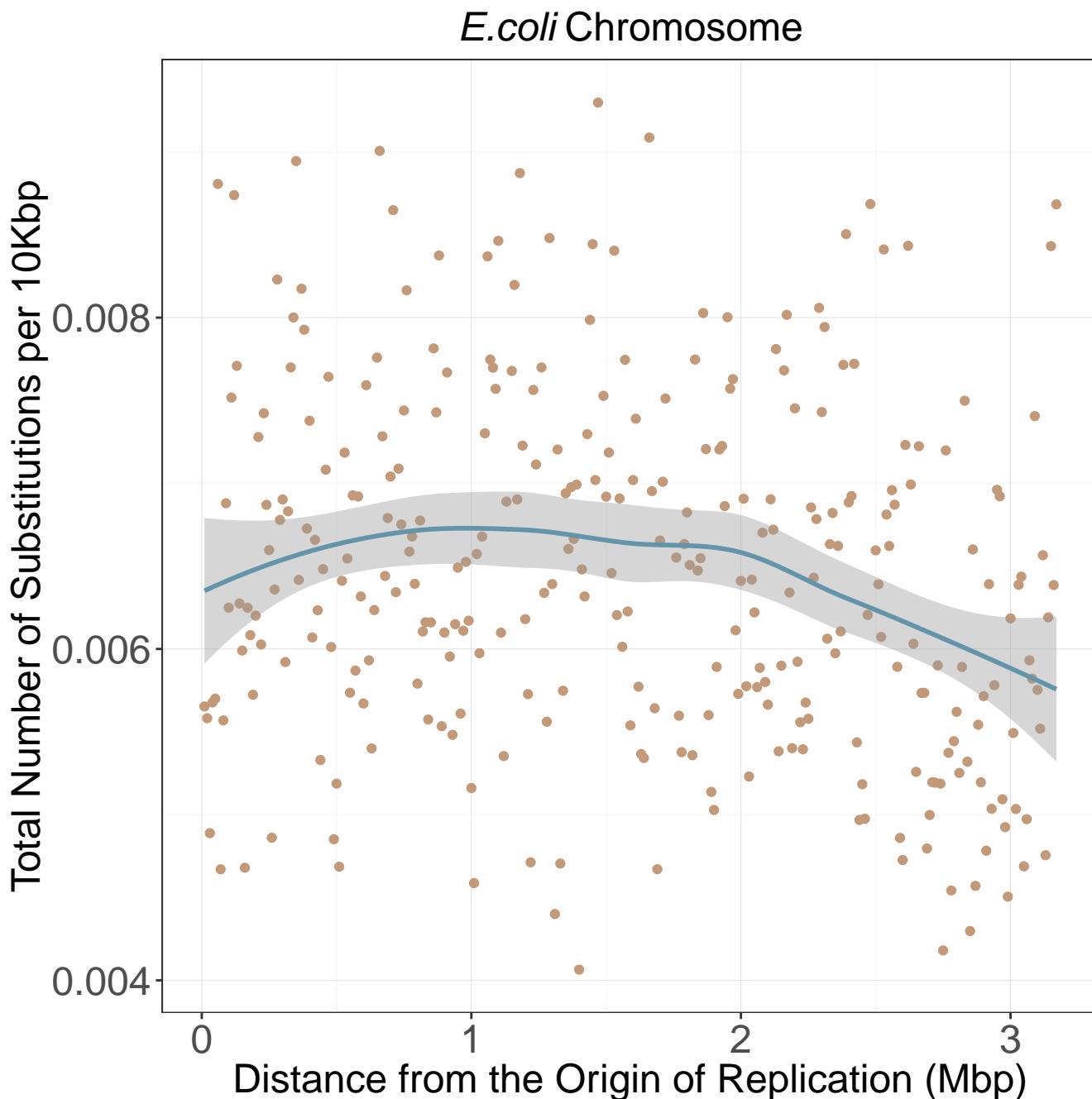


Figure S15: The graph shows the total number of substitutions weighted by the total number of protein-coding sites per 10Kbp segments of the genome. Each of these individual values are represented by beige coloured circles. A non-linear trend line (using the `geom_smooth()` function in R), was fit to these average values and the associated 95% confidence intervals for this line is represented by the grey ribbon around the blue trend line. Outliers were removed from this graph.

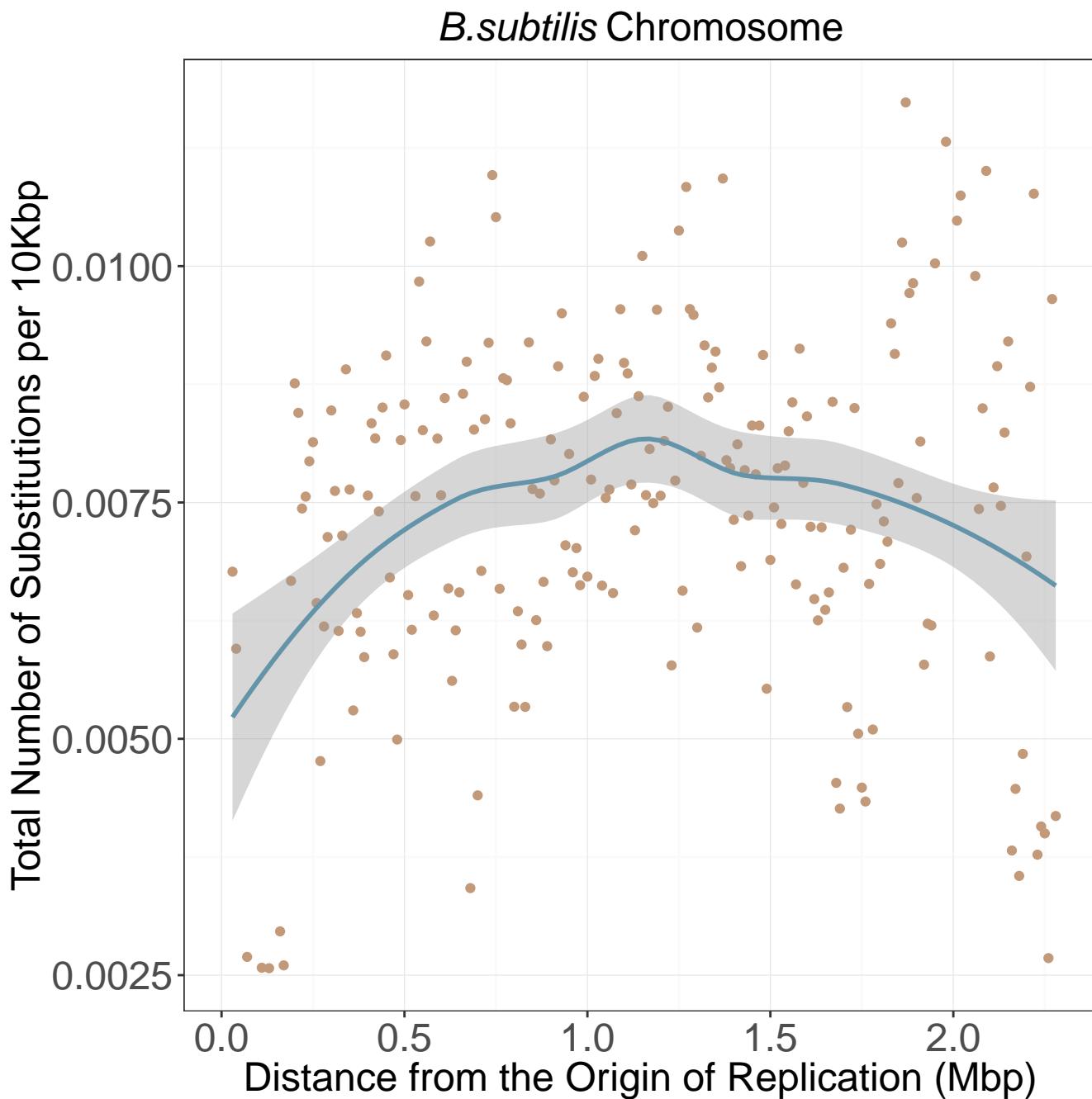


Figure S16: The graph shows the total number of substitutions weighted by the total number of protein-coding sites per 10Kbp segments of the genome. Each of these individual values are represented by beige coloured circles. A non-linear trend line (using the `geom_smooth()` function in R), was fit to these average values and the associated 95% confidence intervals for this line is represented by the grey ribbon around the blue trend line. Outliers were removed from this graph.



Figure S17: The graph shows the total number of substitutions weighted by the total number of protein-coding sites per 10Kbp segments of the genome. Each of these individual values are represented by beige coloured circles. A non-linear trend line (using the `geom_smooth()` function in R), 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. Outliers were removed from this graph.

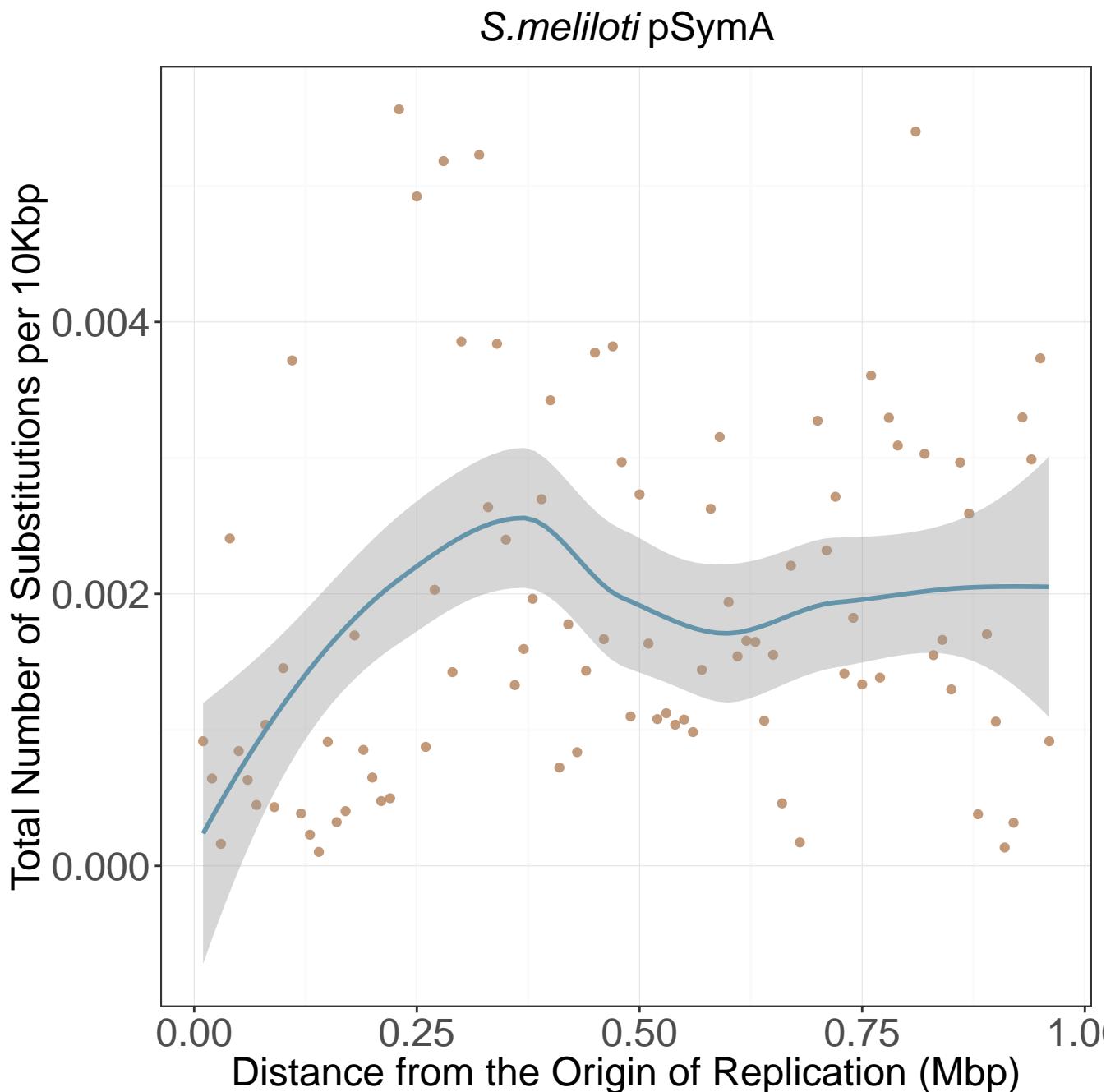


Figure S18: The graph shows the total number of substitutions weighted by the total number of protein-coding sites per 10Kbp segments of the genome. Each of these individual values are represented by beige coloured circles. A non-linear trend line (using the `geom_smooth()` function in R), was fit to these average values and the associated 95% confidence intervals for this line is represented by the grey ribbon around the blue trend line. Outliers were removed from this graph.

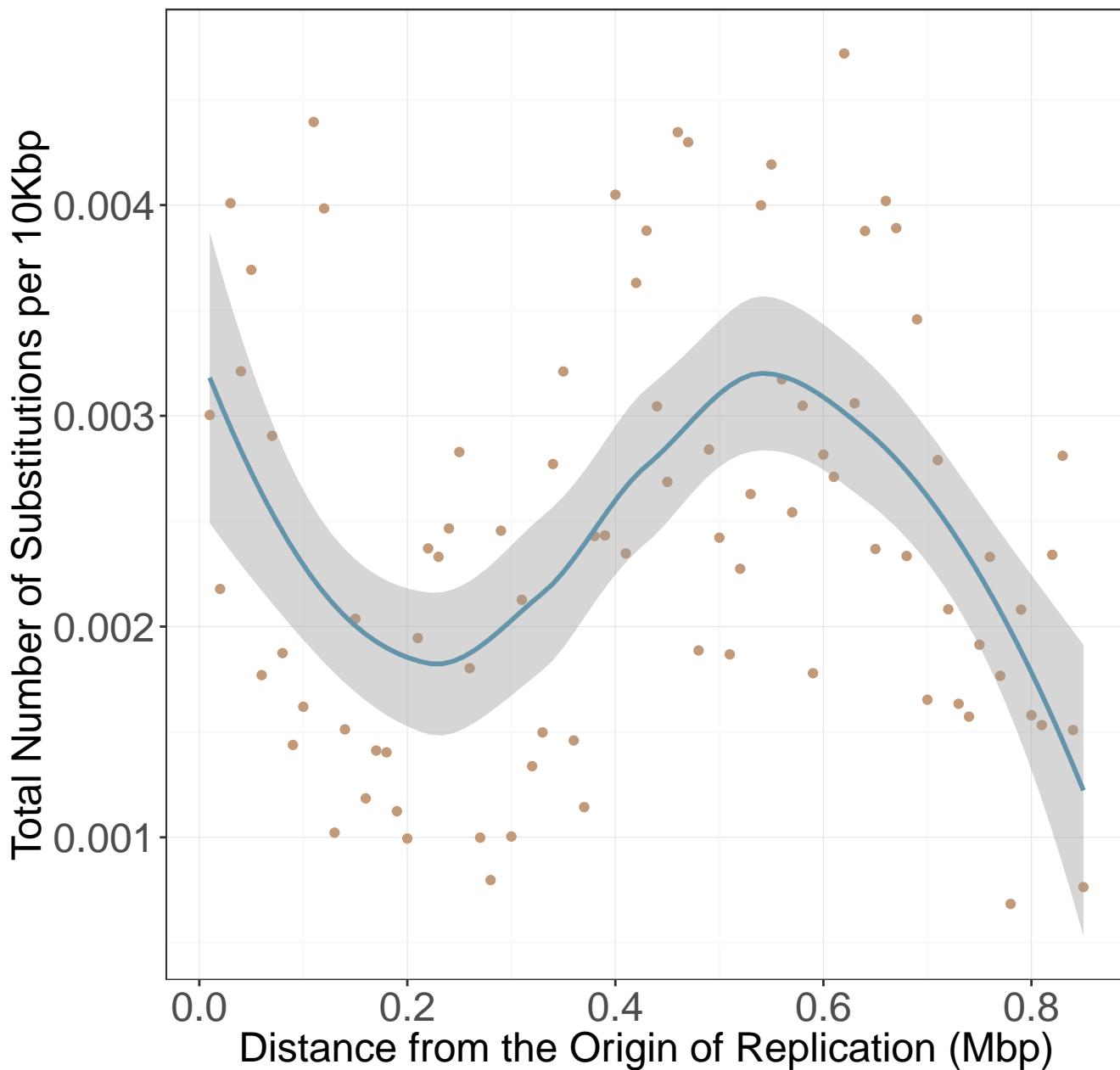
*S.meliloti pSymB*

Figure S19: The graph shows the total number of substitutions weighted by the total number of protein-coding sites per 10Kbp segments of the genome. Each of these individual values are represented by beige coloured circles. A non-linear trend line (using the `geom_smooth()` function in R), 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. Outliers were removed from this graph.

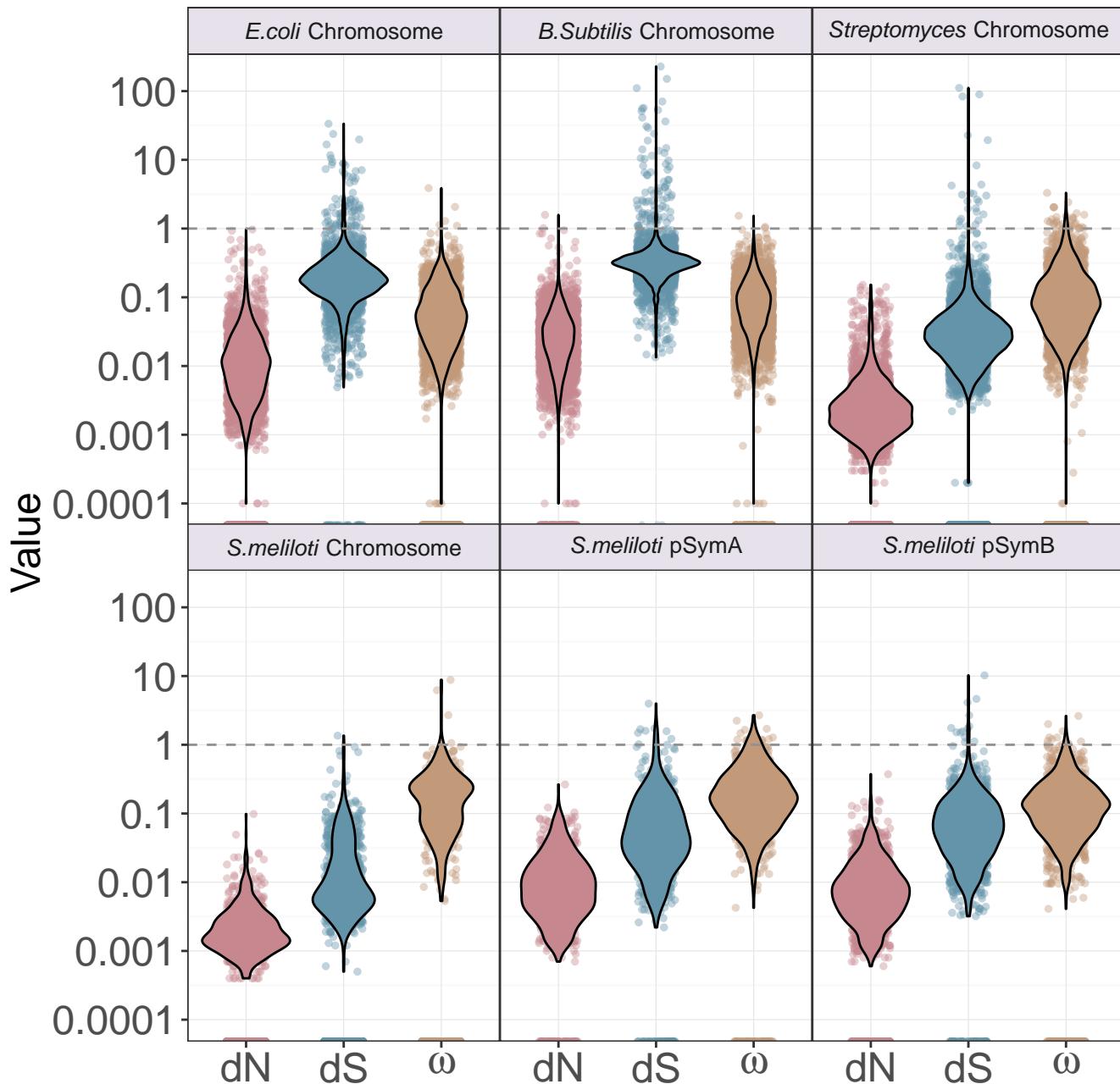


Figure S20: Distribution of all  $dN$ ,  $dS$ , and  $\omega$  values on a log base 10 scale for each replicon. Individual points are shown as a strip chart (which has been jittered in the x-direction in R (Wickham et al. 2019)), and the density of these selection values is shown in the overlaid violin plot. All points are included in this graphic including outliers. For more information on how outliers were calculated, please see the main paper. Any  $dN$ ,  $dS$ , or  $\omega$  values that had a value of zero is pushed to the bottom of the x-axis. Since these values will not appear on a log base 10 scale, they are not included in the violin portions of this graphic. For a complete list of zero values in each of the selection categories please refer to Table S16. In these graphs there is a horizontal line of values at 0.0001 for most of the selection coefficients in most of the bacterial replicons. This is due to rounding practices when `codeml` (Yang 1997) calculates  $dN$ ,  $dS$ , and  $\omega$  values.

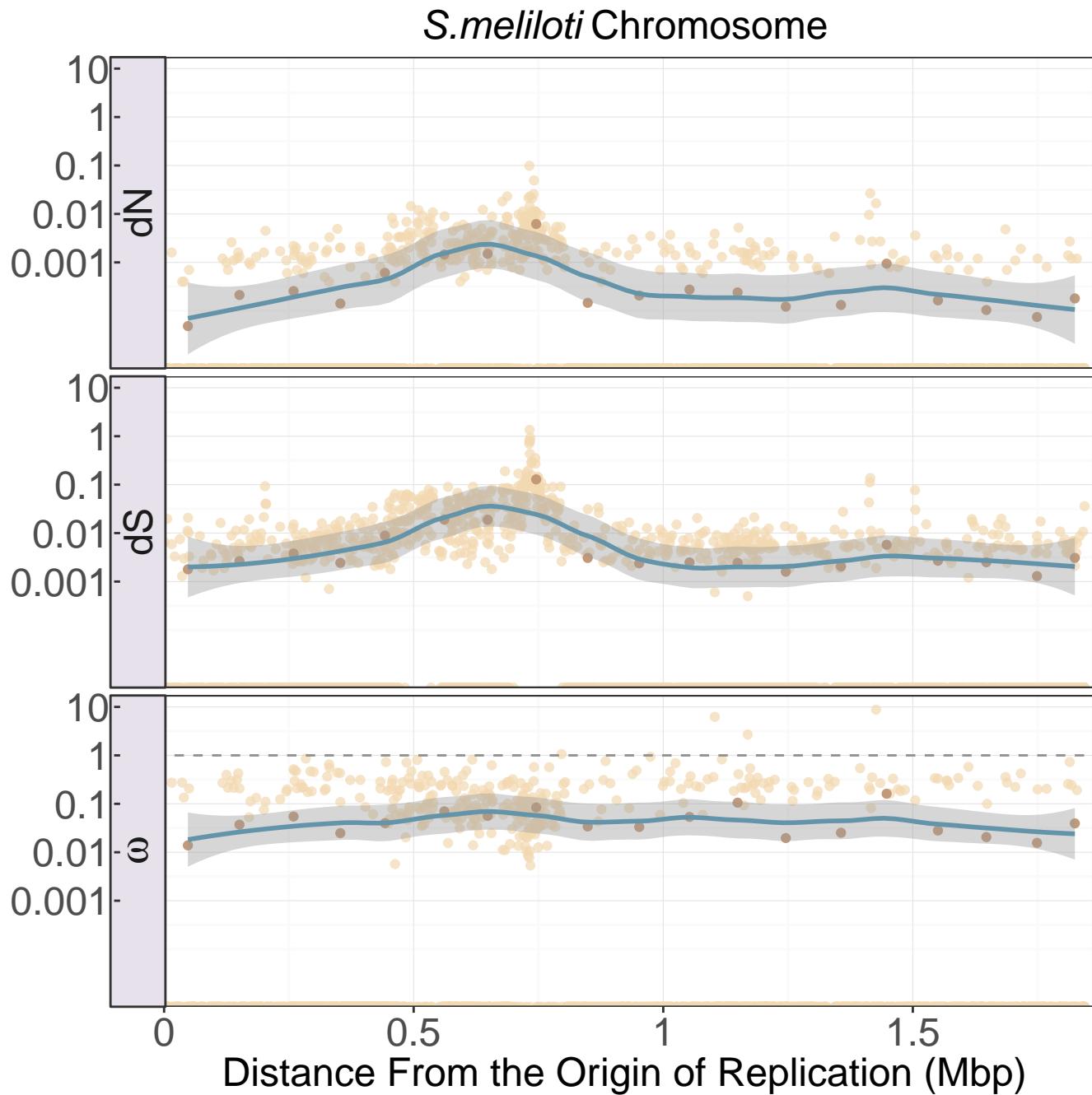


Figure S21: The graph show the values of  $dN$ ,  $dS$ , and  $\omega$  along the chromosome of *S. meliloti*. Distance from the origin of replication is on the x-axis beginning with the origin of replication denoted by position zero on the left, and the terminus indicated on the far right. The y-axis of the graph indicates the value of  $dN$ ,  $dS$ , and  $\omega$  found at each gene segment position of the chromosome. Outliers are included in this graph. The average  $dN$ ,  $dS$ , and  $\omega$  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 Table S16.

## References

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