Supplementary Information

Accumulation of deleterious mutations during bacterial range expansions

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Supplementary Tables

Supplementary Table 1: Next generation sequencing coverage properties

Sample name	Sample size	Sequencing platform	Mean coverage	95% CI	
				Lower bound	Upper bound
HMR exp 1	48	HiSeq2500	315.9	297.61	334.19
HMR exp 2	9	MiSeq	123.51	104.44	142.58
LMR exp 2	10	MiSeq	103.44	85.89	120.99
chemostat	7	HiSeq3000	706.86	590.98	822.73

Supplementary Table 2: Type and number of mutations observed in bacterial strains

	Agar HMR (n=57)	Agar LMR (n=10)	Chemostat HMR (n=7)
Mutation types			
Point substitutions			
Non synonymous substitution	3160	148	57
Non synonymous substitution affecting a start codon	8	-	
Non synonymous substitution affecting a stop codon	69	5	1
Synonymous substitution	1577	69	22
Intergenic substitution	986	58	7
Indels			
Indel in coding region producing a frameshift	737	35	16
Deletion of a codon	3	-	-
Indel in intergenic region	436	25	20
Indel in intragenic region	3	-	-
Complex variants			
Indel in coding region producing a frameshift + loss of a stop codon	3	-	-
Codon change + deletion of a codon	1	-	-
DN/DS[1]	2.003	2.145	2.59
dN/dS[2]	1.014	1.093	1.33
Total no. of mutations	6544	315	123
Total no. of substitutions	5363	253	87
No. of mutations per strain	115.02	31.50	17.57
No. of substitutions per strain	94.28	25.30	12.43

[1] Total number of non-synonymous over synonymous substitutions

[2] Ratio corrected for expected number of synonymous and non-synonymous sites

Supplementary Table 3: Patterns of substitutions

	Transitions		Transversions			
	A:T > G:C	G:C > A:T	A:T > T:A	A:T > C:G	G:C > T:A	G:C > C:G
Agar HMR (n=57)						
Intergenic	377	144	4	17	8	0
Coding	3069	1446	46	121	34	21
Synonymous	1039	527	5	18	6	8
Non-synonymous	2030	945	41	103	28	13
Agar LMR (n=10)						
Intergenic	21	7	2	0	0	1
Coding	134	70	6	5	2	0
Synonymous	47	21	0	0	1	0
Non-synonymous	87	49	6	5	1	0
Chemostat HMR (n=7)						
Intergenic	5	1	0	0	1	0
Coding	55	23	0	1	0	0
Synonymous	15	7	0	0	0	0
Non-synonymous	40	16	0	1	0	0

Supplementary Table 4: Estimation of global mutation rates per genome per generation[a].

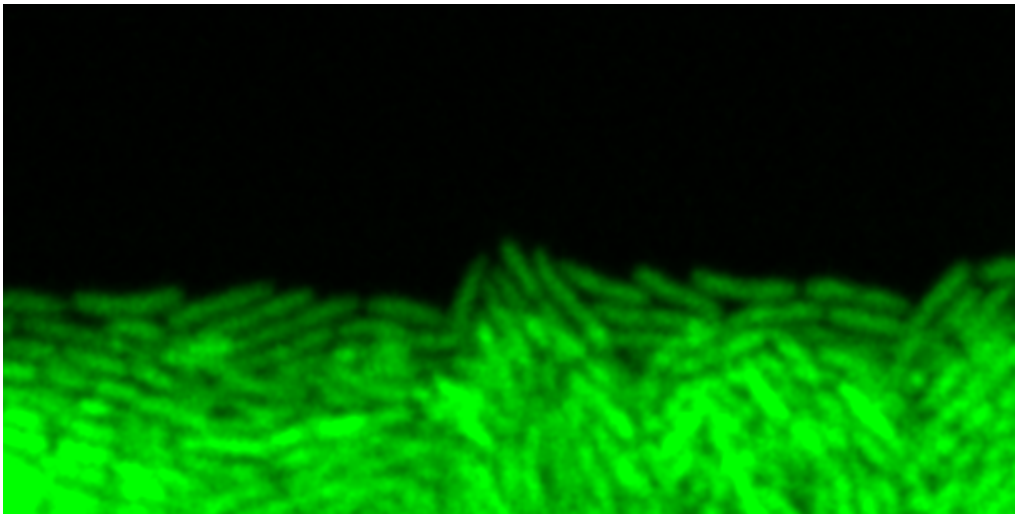
Estimation procedure	Ancestral strain[b]	Agar HMR[c]	Agar LMR[d]	Chemostat HMR[e]
Fluctuation test	0.207	0.216	0.021	0.255
95% CI	[0.151;0.271]	[0.151;0.288]	[0.017;0.043]	[0.109;0.401]
WGS[f]				
All mutations		0.069	0.019	0.0106
		[0.043;0.105]	[0.0094;0.0290]	[0.0082;0.0131]
Substitutions		0.057	0.0153	0.0075
		[0.035;0.089]	[0.007;0.022]	[0.0061;0.0091]

[a] Generation time was measured as mass doubling time on the wave front (see **Supplementary Figure 2**).

[b] n=1; [c] n=9 for the fluctuation test and n=57 for WGS; [d] n=10; [e] n=7.

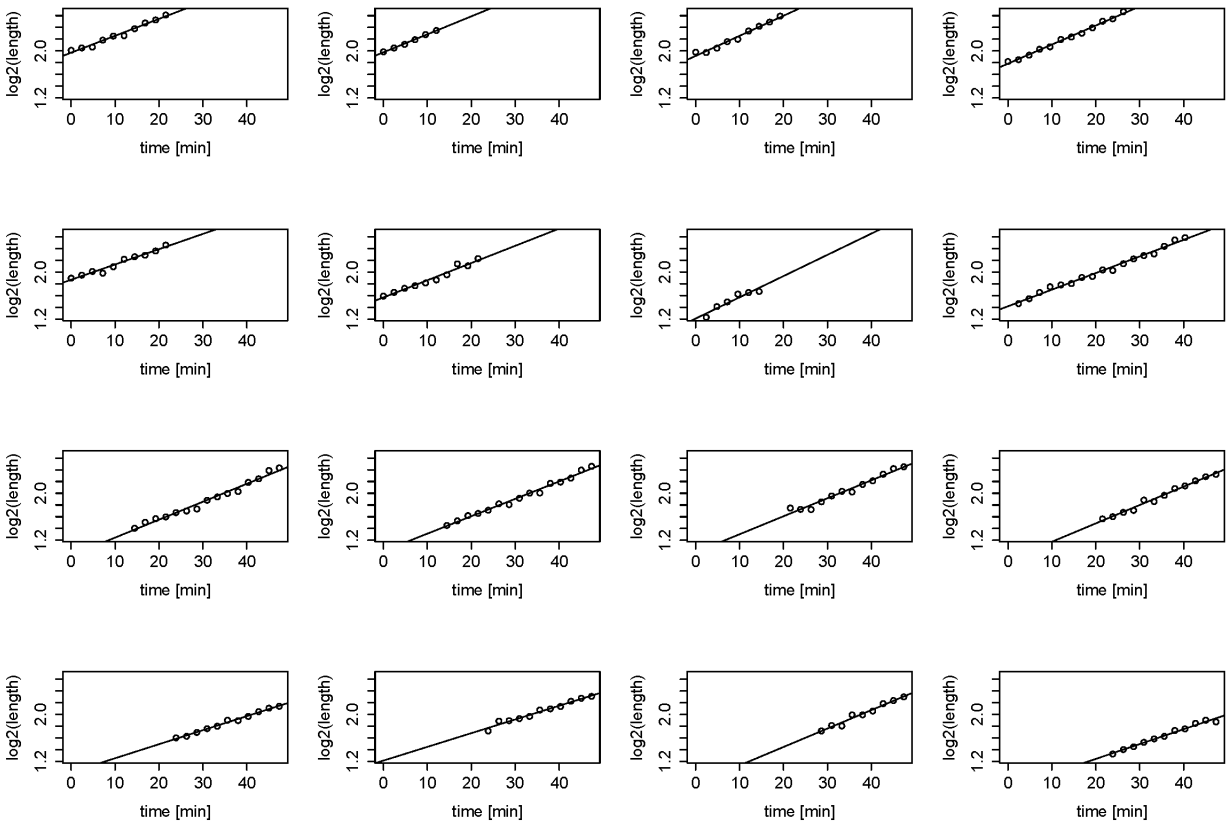
[f] For WGS, we report the 2.5% and 97.5% quantiles of the lineage-specific distributions of mutation rates.

Supplementary Figures



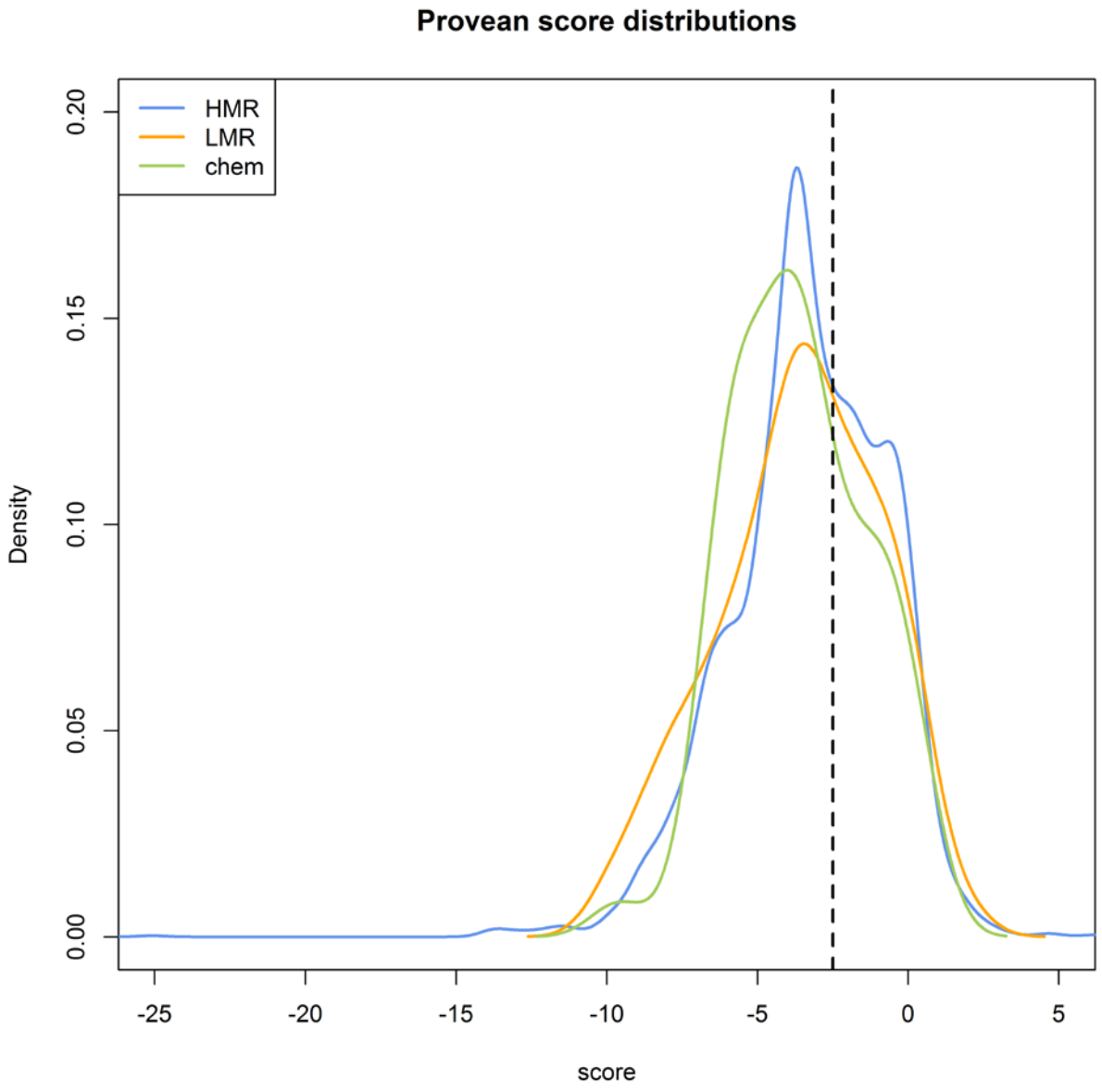
Supplementary Figure 1: Fluorescence image of bacteria on expanding wave front

Image of the wave front of an expanding colony after 24h incubation at 37°C. Images were taken by a confocal microscope (Leica TCS SP5) with a 63x dry objective during incubation direct from the agar plate.

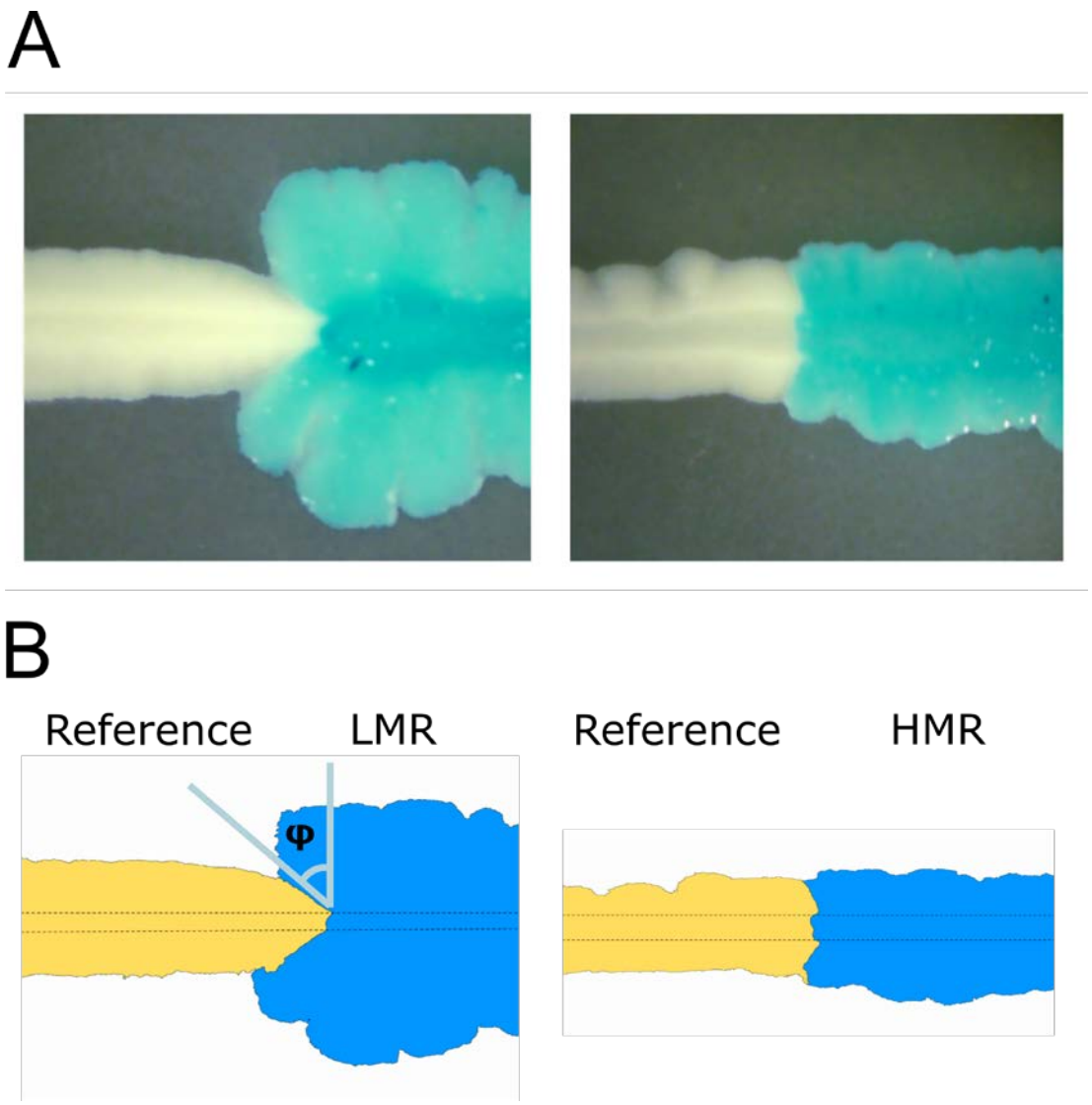


Supplementary Figure 2: Estimation of generation time of bacteria on expanding wave fronts.

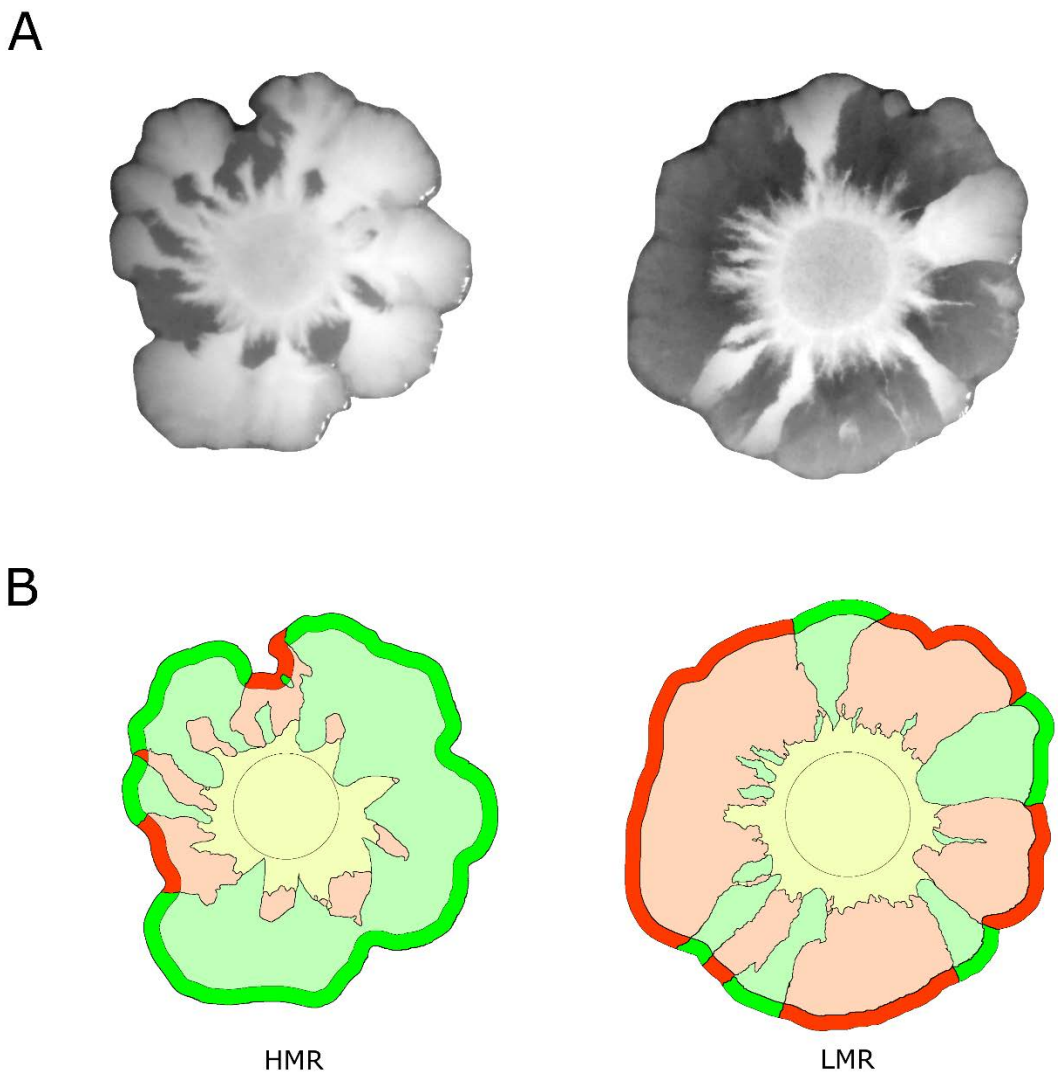
Generation time was estimated from bacterial mass doubling time, which was computed from video analysis of single bacteria growing on agar wave fronts. The growth rate was determined by calculating the elongation rate under a mixed-effect linear regression model. Estimated generation time: 34.2', 95%CI [33.0, 35.6]



Supplementary Figure 3: Distribution of PROVEAN scores at non-synonymous sites in HMR, LMR, and chemostat lines. The distributions between HMR, LMR and chemostat lines are not significantly different from each other according to pairwise Kolmogorov-Smirnov tests. Mutations with a score lower than -2.5 (on the left of the vertical dashed line) are usually considered as having a strong impact on protein function.

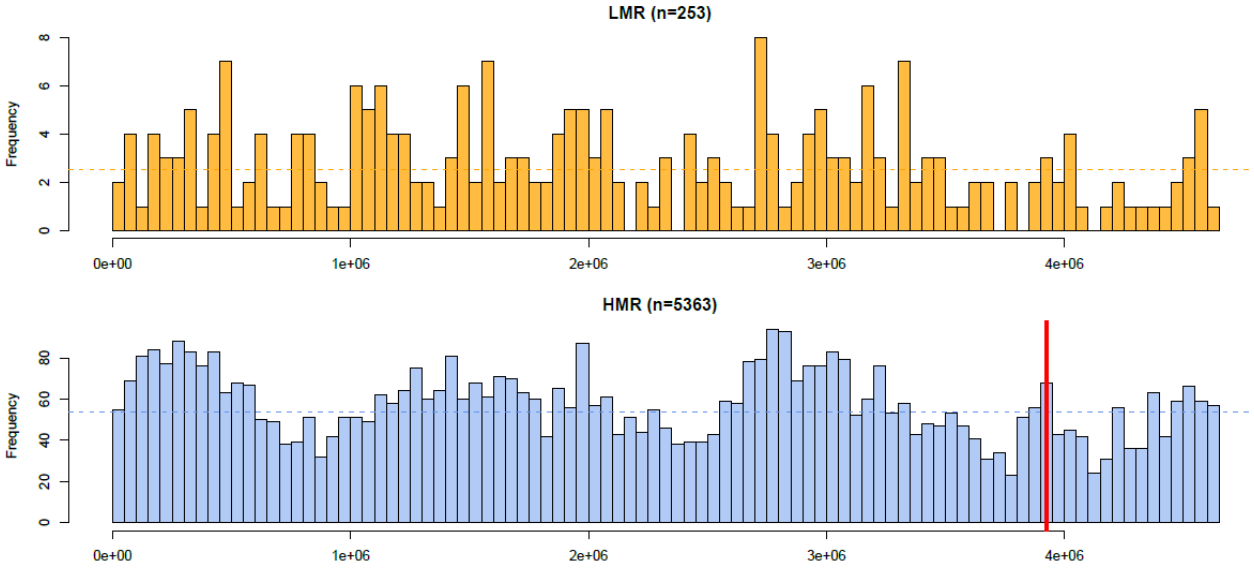


Supplementary Figure 4: Linear expansion competition experiment on agar. **A**: Competitive growth of a reference strain (white) and evolved strains (blue) inoculated linearly side by side on agar. **B**: Image processing of the images in B, allowing one to determine the angle (φ) forming between the reference and evolved strain, allowing one to compute the selection coefficient s associated to the LMR line according to $\tan(\varphi) = \sqrt{s(2+s)}$ (KOROLEV *et al.* 2012). In this case, the Low Mutation Rate strain (LMR) on the left pane is clearly growing faster than the reference strain, indicative of a much higher fitness, whereas the High Mutation Rate (HMR) strain on the right pane is growing in a way similar to the reference strain suggesting similar fitness. Note that the reference strain is not the ancestral strain of the HMR and LMR lines.

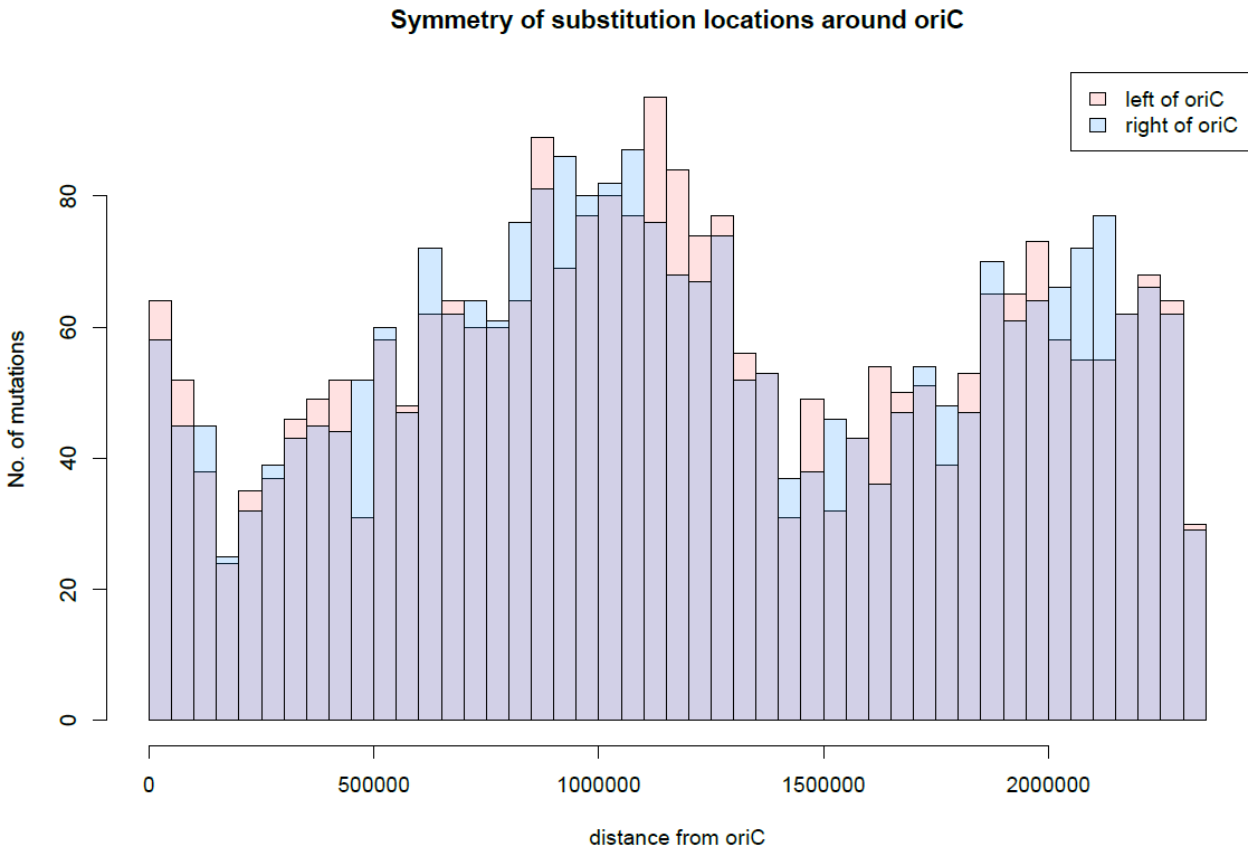


Supplementary Figure 5: Radial expansion competition experiment on agar. **A**: Competitive growth of the ancestor strain (white) and evolved strain (black) marked with different fluorescent plasmids. The left panel shows a High Mutation Rate (HMR) strain and the right panel a Low Mutation Rate (LMR) strain. The ancestral and evolved strains were mixed in a 1:1 ratio and inoculated at the center of an agar plate and then let grown for 3 days, like in the long-term experiment. **B:** Processed images. Green: ancestor strain; red: evolved strain, yellow: both strains mixed. The darker colors on the edge of the

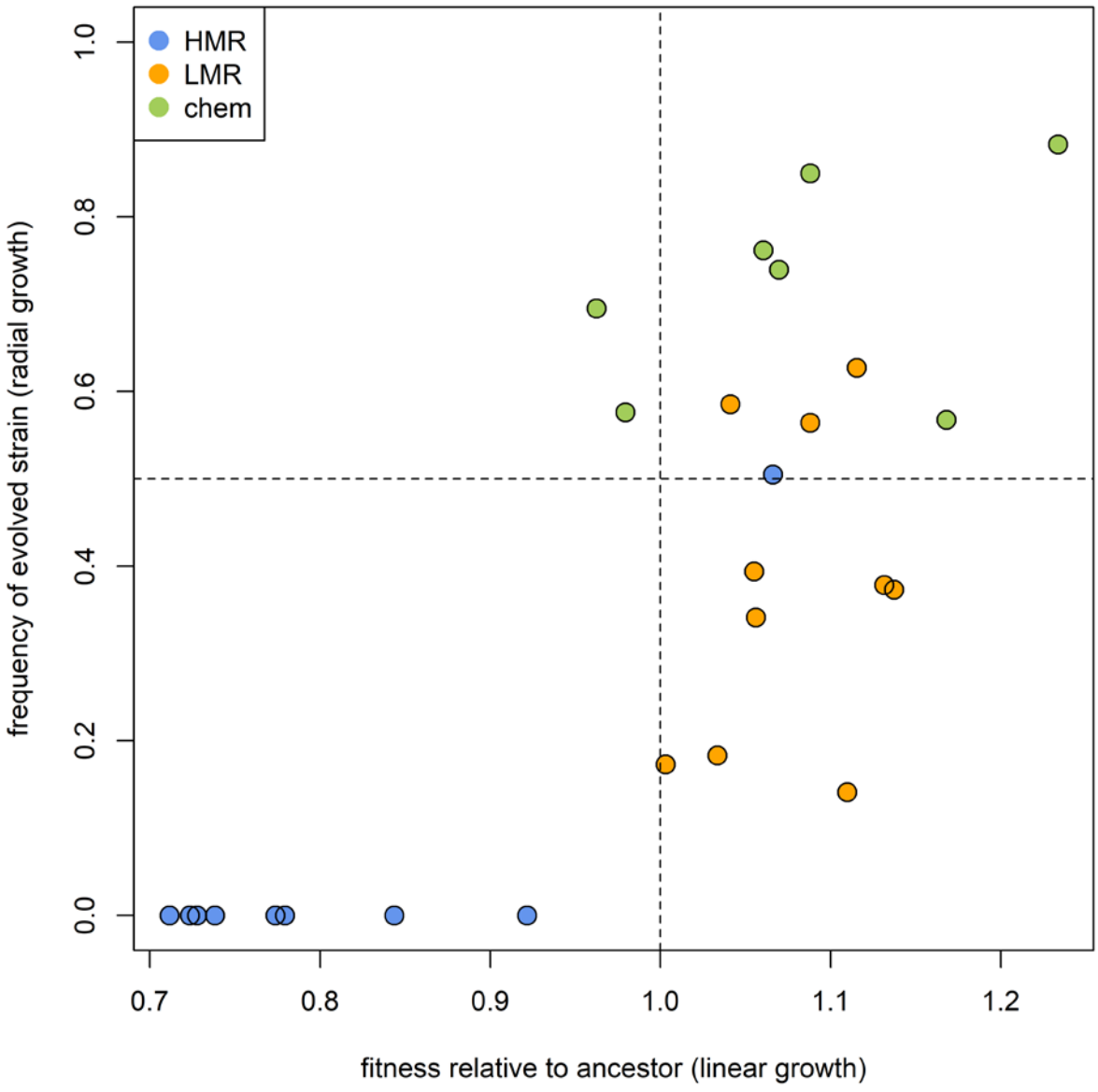
colony delimit the wave front where we estimated the relative proportions of the two strains, as reported in **Fig. 4B**.



Supplementary Figure 6: **Genomic distribution of the number of mutations occurring in LMR and HMR lines**. Dashed lines represent the genomic average in each condition. The position of the origin of genome replication (oriC) is marked with a vertical red line.



Supplementary Figure 7: Symmetry in the distribution of substitutions around *oriC*. The total number of substitution is symmetric (R^2=0.7, *p-val*=2.05×10^{-13}) around the origin of replication *oriC* (map position 3,880,349-3,881,752, as shown in **Supplementary Figure 2**), as previously shown by FORSTER *et al*. (2013).



Supplementary Figure 8. Comparison of frequency of evolved strain in a radial competition assay and linear spatial growth rate. Each point shows the relative frequency of the evolved strain after a radial competition experiment with the ancestral strain (as shown in **Fig. 4A**) and the linear spatial growth rate relative to the ancestral strain (as shown in **Fig. 4B**) for a single evolved HMR, LMR or chemostat line.

References

FOSTER, P. L., A. J. HANSON, H. LEE, E. M. POPODI AND H. TANG, 2013 On the mutational topology of the bacterial genome. G3 (Bethesda) **3:** 399-407.

KOROLEV, K. S., M. J. MULLER, N. KARAHAN, A. W. MURRAY, O. HALLATSCHEK *et al.*, 2012 Selective sweeps in growing microbial colonies. Phys Biol **9:** 026008.