SUPPLEMENTARY MATERIAL

Table S1. Summary of mutation accumulation experiments.

Table S2. Size of the genome used (G*) for mutations calling.

Table S3. Complete list of base-substitution mutations in *O. tauri*.

Table S4. Complete list of base-substitution mutations in *O. mediterraneus*.

Table S5. Complete list of base-substitution mutations in *B. prasinos*.

Table S6. Complete list of base-substitution mutations in *M. pusilla*.

Table S7. Complete list of deletions in the four experiments.

Table S8. Complete list of insertions in the four experiments.

Table S9. RNAseq coverage of exons and non-exonic regions, in mutated and non-mutated sites in *B. prasinos* and *O. tauri*.

Table S10: GC bias between coding and intergenic sequences.

Table S11. Spontaneous base-substitution mutation rates estimated by mutation accumulation experiments or pedigree sequencing in Bacteria and Eukaryotes.

Table S12. Effect of GC departure from equilibrium GC content on the base substitution mutation rate.

Fig. S1. Mutation rate as a function of genome size.

Table S1. *Ne* is the average effective population size during the experiment (estimated as the harmonic mean of cell numbers). Total lines are the number of sequenced lines at the end of the experiment. Total generations are the number of independent generations obtained with all sequenced MA lines. T_0 to Tf is the duration of the experiment since the inoculation of the MA lines.

Species	Total line	Total generations	Mean generations per MA line	N_e	T_0 to T_f (days)
O. tauri RCC4221	40	17 250	431	8.5	378
O. mediterraneus RCC2590	37	8 379	235	7	294
M. pusilla RCC299	37	4 145	112	6	299
B. prasinos RCC1105	36	4994	139	8.5	224

Table S2. Size of the genome used (G*) for mutation calling. G*min is the minimum and G*max the maximum genome size for each species.

Species	G (Mb)	G*average (%)	G*min (%)	G*max (%)
O. tauri RCC4221	13.03	12.60 (97.5)	11.82 (91.54)	12.84 (99.45)
O. mediterraneus RCC2590	13.48	13.10 (97.2)	12.28 (91.10)	13.35 (99.08)
B. prasinos RCC1105	15.07	15.02 (99.6)	14.66 (97.23)	15.03 (99.73)
M. pusilla RCC299	21.11	21.01 (99.5)	20.55 (97.36)	21.07 (99.79)

Table S3. Complete list of base-substitution mutations in *O. tauri*. Ns: non-synonymous mutation, Syn: synonymous mutations, - : intergenic mutations.

Chromosome	Position	Reference	Mutation	Effect
1	281349	T	C	Syn
1	462543	T	A	-
1	462544	A	T	-
1	536352	G	A	-
1	688195	A	G	-
1	762227	A	G	Ns
1	764912	G	A	-
1	934994	C	T	Syn
2	202377	C	A	Ns
2	744253	G	A	Syn
2	759388	C	T	Ns
2	817108	C	T	Ns
2	820022	C	A	Ns
2	868393	G	A	Syn
2	1070559	A	G	Ns
3	41080	C	T	Syn
3	127716	G	A	Ns
3	210680	A	G	-
3	353325	C	T	Ns
3	379647	C	T	Ns
3	779548	T	C	Syn
3	971494	C	T	Ns
4	117	C	A	-
4	366731	C	T	Syn
4	524836	G	A	Ns
4	735752	C	T	Ns
5	5699	C	T	Ns
5	405535	G	A	Syn
5	498490	T	G	Ns
5	600053	T	A	-
5	600056	G	A	-
5	658543	G	A	Ns
5	668815	C	T	Ns
6	12972	C	T	Ns
6	18960	C	T	-
6	46323	T	G	Ns
6	162319	T	C	Ns
6	334481	G	C	-
6	661927	C	T	-
6	716579	C	T	Ns
7	475871	A	G	Ns
7	612454	C	T	Syn
7	702913	A	C	Ns
7	744212	G	A	Ns
8	66027	G	A	-
8	185592	G	C	-
9	31962	G	A	Ns
9	126121	C	T	Syn
9	393218	C	T	Ns

9	398617	C	T	Syn
9	514297	C	T	Ns
9	643568	A	G	Ns
10	108529	G	A	Ns
10	510428	C	T	Ns
10	562914	G	A	Syn
10	587147	G	A	-
11	222249	A	G	-
11	446073	T	C	Syn
11	452825	C	A	Ns
11	472590	G	A	Stop gained
11	474928	A	G	-
12	94	A	G	-
12	419488	C	A	Ns
12	460233	T	G	-
13	145936	G	A	Syn
13	365169	C	T	Ns
13	496008	C	T	Ns
14	75583	G	A	Intron
14	210547	G	A	Ns
14	231150	G	T	-
14	257724	C	A	Ns
14	374547	G	C	Syn
14	495621	G	A	Syn
14	500741	C	T	Ns
15	165137	C	T	Ns
15	177872	G	T	Ns
15	352817	A	G	Syn
16	460708	C	T	Syn
16	325515	G	T	Syn
16	521001	C	T	Ns
17	35411	T	G	Ns
17	410925	C	T	-
17	410926	G	A	-
17	410927	A	G	-
17	410928	C	G	-
18	122316	A	G	-
18	252024	T	G	-
18	345570	T	G	-
18	345641	G	A	-
20	49667	C	T	Ns
20	165267	T	G	-

Table S4. Complete list of base-substitution mutations in *O. mediterraneus*. Ns: non-synonymous mutation, Syn: synonymous mutations, - : intergenic mutations.

Chromosome	Position	Reference	Mutation	Effect
1	240410	T	C	Syn
1	281668	A	T	Ns
1	384332	T	C	-
1	500961	T	G	-
1	504598	G	A	-
1	960787	G	T	Ns
2	783209	C	A	Syn
3	168617	T	G	Ns
3	194253	A	T	Ns
4	4863	A	G	Ns
4	250707	T	C	-
4	271238	A	G	-
4	271242	G	T	-
4	271243	A	G	-
4	271249	A	C	-
4	271258	C	T	-
4	271264	G	T	-
5	204024	G	A	Ns
5	237455	G	A	Ns
5	369175	C	T	Ns
5	561353	C	A	Ns
6	167738	C	T	Ns
6	365873	C	T	Syn
6	638629	C	A	Ns
7	136735	A	C	Ns
7	298488	C	T	Ns
8	59263	G	A	Ns
8	106639	G	A	Ns
8	165454	G	A	Syn
8	246199	T	C	Ns
8	285631	G	A	Syn
8	390757	T	A	Ns
8	425236	T	G	Ns
8	437922	G	C	Ns
8	699362	G	A	Syn
9	552630	G	T	Ns
9	701807	A	C	Ns
9	717500	C	A	-
9	737678	G	T	Ns
10	165842	G	A	Ns
13	107450	T	C	Ns
13	162729	C	T	Syn

13	586916	T	C	Ns
14	60175	T	A	Ns
14	60217	G	A	Ns
14	98081	A	C	Ns
15	112247	C	A	Start gained
16	222414	C	T	Syn
16	297544	C	T	Syn
17	368001	G	T	Ns
17	422490	C	T	-
18	272777	A	G	Ns
18	299852	A	G	Ns
19	81732	C	G	Ns

Table S5. Complete list of base-substitution mutations in *B. prasinos*. Ns: non-synonymous mutation, Syn: synonymous mutations, - : intergenic mutations.

Chromosome	Position	Reference	Mutation	Effect
1	42082	C	A	Ns
1	79489	C	T	Syn
1	418914	T	G	Ns
1	1305044	G	C	Syn
11	525583	C	T	Ns
12	50	T	A	-
12	122406	T	C	-
13	208600	T	A	Ns
17	72	G	C	-
18	999	C	A	Ns
18	235306	T	G	-
19	69819	A	T	-
19	145968	A	T	-
2	556426	G	C	Ns
4	135852	C	T	Syn
4	531741	C	G	Ns
5	87328	C	T	Ns
5	145129	A	T	Intron
5	602981	C	G	Syn
5	797176	A	T	Ns
8	574299	C	A	Ns
9	93038	C	T	Syn

Table S6. Complete list of base-substitution mutations in *M. pusilla*. Ns: non-synonymous mutation, Syn: synonymous mutations, - : intergenic mutations.

Chromosome	Position	Reference	Mutation	Effect
1	101651	G	T	-
1	319728	T	A	-
1	319729	C	A	-
1	356850	G	C	Ns
1	868786	A	G	-
1	907594	A	G	-
1	989553	G	A	Ns
1	1166944	A	G	-
1	1311961	A	G	Ns
1	1328941	C	T	-
1	1480609	A	C	-
1	1531771	T	C	-
1	1771291	T	C	-
2	296	C	T	-
2	197240	G	C	Ns
2	318653	C	G	Ns
2	318654	A	C	Ns
2	318655	G	C	Ns
2	371001	G	T	Ns
2	629587	T	G	Ns
2	1231590	G	A	Syn
2	1439488	C	T	Ns
2	1898300	G	T	Syn
2	1898302	A	C	Ns
2	1898303	C	A	Ns
2	1898304	T	A	Ns
2	1898305	T	C	Syn
3	1370371	C	G	Ns
3	1375097	G	A	Ns
3	1708043	C	T	Ns
4	3470	C	A	Syn
4	334275	G	A	Syn
4	571514	G	A	Ns
4	1224092	G	A	Syn
5	486431	C	A	Syn
5	508099	G	C	Syn
5	556716	C	G	Ns
5	896616	C	T	-
5	1108981	G	A	Ns
6	1235633	G	A	Ns
7	570664	G	T	Ns

7	570665	A	C	Syn
7	570666	G	T	Ns
7	672236	G	A	-
7	746112	G	C	Syn
8	93285	G	T	Ns
8	273349	C	T	Ns
8	284318	G	T	Ns
8	303063	G	A	Syn
8	310233	T	A	Ns
8	310234	G	C	Ns
8	1077655	G	T	Ns
9	1126769	A	T	Syn
11	173723	G	A	-
11	430804	C	T	Ns
11	899300	A	G	Ns
11	899309	G	A	Ns
12	323466	T	C	Ns
12	797741	C	T	Syn
12	823088	T	G	Ns
13	688257	C	G	Syn
14	346000	G	T	Syn
14	346390	G	C	Syn
14	761603	G	A	Ns
15	146889	A	T	Ns
15	146890	T	G	Ns
15	320895	A	G	Syn
15	408470	A	C	-
15	410306	C	T	Ns
15	504682	G	T	Ns
16	329517	C	T	Ns

Table S7. Complete list of deletions in the four species.

Chromosome	Position	Reference	Mutation	Effect
Bathycoccus prasinos				
1	164711	AGGCGAGCAGTG	A	-
1	164726	AATTCAATTTCAATA	A	-
5	622569	GA	G	Frame shift
5	963834	AAATATCTATTG	A	-
14	329038	TG	T	-
Micromonas pusilla				
1	1787228	TTATTCCTTCGAAGCTTACGTACG	T	-
1	1924354	CA	C	Frame shift
1	319750	GTACCTTCGAAGGTATAA	G	-
3	214	CT	C	-
5	140463	CGCGAGACCTCG	C	Frame shift
6	1235612	AGGAGGAGGAGGGGGAGGAGGG	A	Codons deletion
8	310219	CGATCTGCGTCCGGTG	C	Codons deletion
10	1045182	GCGC	G	-
10	1160336	TCA	T	-
11	899291	ACAGACGGCACAGCTGGCG	A	Codons deletion
14	474	AACCCTTCGT	A	-
16	287228	AACTCGAGTTGACAAGACC	A	-
Ostreococcus tauri				
1	601169	GA	G	-
6	46321	CT	C	Frame shift
8	273361	AAC	A	Frame shift
8	72783	GACACCCGCGTGTACGGGACCGCGACCC	G	-
11	222762	GA	G	Frame shift
12	403601	CGCGAGACCGGCGCACATCGCCGTCGTCGCCACCGTCGGAAACT	C	Frame shift
13	135	CA	C	-
17	87	CT	C	-
Ostreococcus mediterraneus				
6	284415	CG	C	Frame shift
7	156017	GT	G	-
7	1775	TGTTGCC	T	-
8	350046	CGTT	C	Codons deletion
9	185017	CACGGCGACGACGAACGATGGCG	C	Frame shift
12	567140	TCG	T	Frame shift
12	328305	GT	G	-
13	112767	ATCTATCGTCGCGACGGCGGTCGTCTCTATG	A	Codons deletion

Table S8. Complete list of insertions in the four species.

Chromosome	Position	Reference	Mutation	Effect
Bathycoccus prasinos				
2	102	A	AC	-
9	521140	A	AAG	-
13	570953	T	TGCC	Codons insertion
14	272	A	ACC	-
18	523	A	AC	-
Micromonas pusilla				
1	319727	A	AC	-
2	378439	C	CCG	Frame shift
Ostreococcus tauri				
4	76071	C	CG	-
10	455685	T	TCGTCGG	Codons insertion
15	109437	C	CG	-
17	226384	C	CG	-
20	153964	G	GA	-
Ostreococcus mediterraneus				
1	384327	G	GATT	-
8	729406	C	CA	Frame shift
12	432782	C	CTACTG	-

Table S9: RNAseq coverage of coding (cds) and intergenic (non-cds) sequences, in mutated and non-mutated sites in *B. prasinos* and *O. tauri*. The removal of the 10 mutations located in the subtelomeric regions does not change the significance of the transcription bias (Wilcoxon test, P-value=0.016).

Species	Sequence types	*n* mutations	Mutated site coverage	Non-mutated site coverage	P. value Wilcoxon test
Bathycoccus prasinos	non-cds	13	247	646	0.0004
	cds	19	505	667	0.123
Ostreococcus tauri	non-cds	38	26	149	3.45×10^{-7}
	cds	64	127	126	0.382

Table S10: GC bias between coding and intergenic sequences. Data from *B. prasinos* are not presented because of low number of mutations.

Species	Coding sequences			Intergenic sequences		
	n (GC to AT)	n (AT to GC)	μ (GC to AT) / μ (AT to GC)	n (GC to AT)	n (AT to GC)	μ (GC to AT) / μ (AT to GC)
O. tauri	48	13	2.57	12	11	0.76
O. mediterraneus	24	12	1.57	6	6	0.79
M. pusilla	30	11	1.43	7	7	0.79

Table S11. Spontaneous base-substitution mutation rates estimated by mutation accumulation experiments and pedigree. *G* is the genome size in Mb, *µ* is the mutation rate per nucleotide and U is the number of mutations per genome per generation. The data from Ness et al. (2015) is the average of 6 strains. Effective population size come from Lynch supplementary material (Lynch 2010), except for *Mus musculus* (Phifer-Rixey et al. 2012), *Heliconius melpomene* (Keightley, Pinharanda, et al. 2014), *Ficedula albicollis* (Backström et al. 2013), *Arabidopsis thaliana* (Cao et al. 2011), *Caenorhabditis elegans* (Cutter 2006), *Caenorhabditis briggsae* (Cutter et al. 2006), *Drosophila melanogaster* (Shapiro et al. 2007) and O. *tauri* (Romain Blanc-Mathieu, personal communication).

Species	G	µ	U	Ne	References
Homo sapiens	3309.0	1.29E-08	38.5500	2.00E+04	(Besenbacher et al. 2016)
Mus musculus	2671.0	5.40E-09	14.5800	2.00E+05	(Uchimura et al. 2015)
Ficedula albicollis	1118.0	4.60E-09	5.0600	4.50E+05	(Smeds et al. 2016)
Arabidopsis thaliana	134.4	7.00E-09	1.0990	2.50E+05	(Ossowski et al. 2010)
Caenorhabditis elegans	100.3	1.48E-09	0.1479	8.00E+04	(Denver et al. 2012)
Caenorhabditis briggsae	108.4	1.34E-09	0.1447	6.00E+04	(Denver et al. 2012)
Pristionchus pacificus	133.1	2.00E-09	0.2663	-	(Weller et al. 2014)
Drosophila melanogaster	148.0	5.49E-09	0.6698	1.15E+06	(Schrider et al. 2013)
Heliconius melpomene	273.8	2.90E-09	0.7940	2.00E+06	(Keightley, Pinharanda, et al. 2014)
Daphnia pulex	197.2	2.30E-09	0.4536	-	(Flynn et al. 2016)
Ostreococcus tauri	13.0	4.19E-10	0.0054	2.00E+07	This study
Ostreococcus mediterraneus	13.5	4.92E-10	0.0065	-	This study
Bathycoccus prasinos	15.1	3.07E-10	0.0046	-	This study
Micromonas pusilla	21.1	8.15E-10	0.0172	-	This study
Chlamydomonas reinhardtii	112.0	9.63E-10	0.1079	3.10E+07	(Ness et al. 2015)
Saccharomyces cerevisiae	12.3	1.67E-10	0.0021	6.20E+06	(Zhu et al. 2014)
Schizoaccharomyces pombe	12.6	2.00E-10	0.0025	2.60E+06	(Farlow et al. 2015)
Paramecium tetraurelia	72.1	1.94E-11	0.0014	1.24E+08	(Sung, Tucker, et al. 2012)
Tetrahymena thermophila	104.0	7.61E-12	0.0008	1.12E+08	(Long et al. 2016)
Dictyostelium discoideum	34.2	2.90E-11	0.0010	-	(Saxer et al. 2012)
Bacillus subtilis	4.2	3.28E-10	0.0014	6.30E+07	(Sung et al. 2015)
Escherichia coli	4.6	2.20E-10	0.0010	1.80E+08	(Lee et al. 2012)
Mesoplasma florum	0.8	9.78E-09	0.0078	1.10E+06	(Sung, Ackerman, et al. 2012)
Burkholderia cenocepacia	7.7	1.33E-10	0.0010	-	(Dillon et al. 2015)
Pseudomonas aeruginosa	6.6	7.92E-11	0.0005	2.00E+07	(Dettman et al. 2016)
Salmonella typhimurium	4.8	7.00E-10	0.0034	-	(Lind and Andersson 2008)
Mycobacterium tuberculosis	4.4	2.58E-10	0.0011	-	(Ford et al. 2011)
Deinococcus radiodurans	3.2	4.99E-10	0.0016	-	(Long et al. 2015)
Vibrio cholerae	4.02	1.07E-10	0.00043	-	(Dillon et al. 2016)
Vibrio fischeri	4.23	2.07E-10	0.00089	-	(Dillon et al. 2016)

Table S12. Effect of GC deviation from equilibrium GC content on the base substitution mutation rate. R_1, R_2, R_3 and R_4 (see methods) were used to calculate GC_{eq} and mutation rate at equilibrium μ_{eq}. The ratio μ/μ_{eq} is an estimation of the elevation of the mutation rate due to the distance from equilibrium GC content, *i.e.* in *O. tauri* the mutation rate increases by ~12%. We only included μ_{eq} estimated from more than 8 mutation events per category to avoid spurious variance in GC_{eq} estimates.

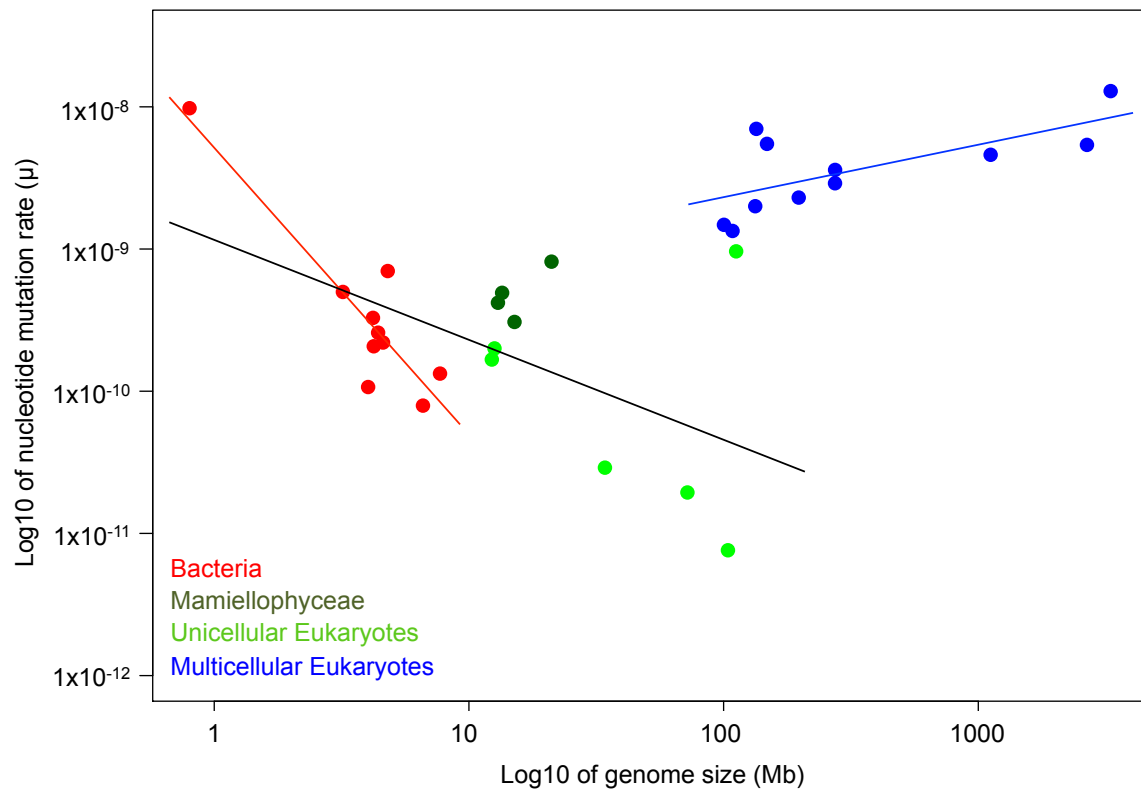| Species	μ_{bs}	GC	GC_{eq}	GC_r	GC>AT relative to AT>GC	μ/μ_{eq}	References
Homo sapiens	1.29E-08	0.415	0.326	1.272	2.064	1.106	(Besenbacher et al. 2016)
Mus musculus	5.40E-09	0.424	0.207	2.051	3.842	1.295	(Uchimura et al. 2015)
Ficedula albicollis	4.60E-09	0.443	0.311	1.426	2.219	1.116	(Smeds et al. 2016)
Arabidopsis thaliana	7.00E-09	0.367	0.138	2.663	6.255	1.640	(Ossowski et al. 2010)
Daphnia pulex	2.30E-09	0.422	0.141	2.997	6.101	1.629	(Flynn et al. 2016)
Caenorhabditis elegans	1.48E-09	0.354	0.193	1.837	4.189	1.225	(Denver et al. 2012)
Caenorhabditis briggsae	1.34E-09	0.377	0.211	1.784	3.732	1.227	(Denver et al. 2012)
Pristionchus pacificus	2.00E-09	0.427	0.157	2.711	5.350	1.381	(Weller et al. 2014)
Drosophila melanogaster	5.49E-09	0.419	0.188	2.228	4.318	1.324	(Schrider et al. 2013)
Ostreococcus tauri	4.19E-10	0.590	0.365	1.615	1.737	1.118	This study
Ostreococcus mediterraneus	3.73E-10	0.560	0.433	1.293	1.310	1.017	This study
Micromonas pusilla	8.15E-10	0.638	0.462	1.382	1.166	1.030	This study
Chlamydomonas reinhardtii	9.63E-10	0.619	0.259	2.392	2.864	1.428	(Ness et al. 2015)
Saccharomyces cerevisiae	1.67E-10	0.384	0.311	1.235	2.216	1.067	(Zhu et al. 2014)
Schizoaccharomyces pombe	2.00E-10	0.360	0.264	1.364	2.790	1.122	(Farlow et al. 2015)
Bacillus subtilis	3.28E-10	0.437	0.443	0.986	1.256	0.999	(Sung et al. 2015)
Escherichia coli	2.20E-10	0.506	0.450	1.124	1.222	1.010	(Lee et al. 2012)
Mesoplasma florum	9.78E-09	0.270	0.059	4.582	15.970	2.628	(Sung et al. 2012a)
Burkholderia cenocepacia	1.33E-10	0.669	0.551	1.213	0.814	0.978	(Dillon et al. 2015)
Pseudomonas aeruginosa	7.92E-11	0.662	0.396	1.671	1.524	1.083	(Dettman et al. 2016)
Deinococcus radiodurans	4.99E-10	0.668	0.643	1.039	0.555	0.983	(Long et al. 2015)
Vibrio cholerae	1.07E-10	0.476	0.281	1.694	-	-	(Dillon et al. 2016)
Vibrio fischeri	2.07E-10	0.384	0.202	1.901	-	-	(Dillon et al. 2016)

Fig. S1. Mutation rate as a function of genome size G (data available Table S11). *Blue*: positive correlation between μ and G in multicellular eukaryotes (n=10, Pearson correlation, ρ=0.67, P-value<0.03. *Black*: negative correlation between μ and G in microorganisms (n=19, Pearson correlation, ρ=-0.57, P-value=0.01). *Red*: negative correlation between μ and G in bacteria (n=10, Pearson correlation, ρ=-0.95, P-value=0.001).

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