SUPPLEMENTARY MATERIAL

Katju and Bergthorsson. ***"Old trade, new tricks: insights into the spontaneous mutation process from the partnering of classical mutation accumulation experiments with high-throughput genomic approaches."***

Table S1. Phenotypic and direct molecular estimates of the genome-wide mutation rate *U* from spontaneous mutation accumulation experiments.

Table S2. Mutation rate from whole-genome sequencing of spontaneous mutation accumulation experiments as a function of genome size and effective population size, N_e.

Table S1. Phenotypic and direct molecular estimates of the genome-wide mutation rate U from spontaneous mutation accumulation experiments. *U* is the number of mutations per genome per generation. BM, MD and ML denote Bateman-Mukai, minimum-distance and maximum likelihood approaches to estimating *U* from phenotypic data, respectively. μ_{bs} is the genome-wide, direct molecular estimate of the base substitution rate from WGS of spontaneous mutation accumulation lines and represented as base substitutions per site per generation. *G* is the genome size in Mb. * indicates fitness assay under stress or competitive conditions.

Group	**Species**	**Phenotypic Trait**	**BM or ML based phenotypic estimate of *U***	**Reference for phenotype-based *U***	**μ_{bs} from MA-WGS**	**Reference for μ_{bs}**	***G***
Prokaryotes							
	E. coli	*r*	0.00017	Kibota & Lynch 1996	2.20×10^{-10}	Lee et al. 2012	4.21
					3.12×10^{-10}	Foster et al. 2015	
	B. cenocepacia	*Competition-TSOY*	0.00007	Dillon & Cooper 2016	1.33×10^{-10}	Dillon et al. 2015	3.48
		Competition-M9MM	0.00006	Dillon & Cooper 2016			
		Competition-M9MM+CA	0.00006	Dillon & Cooper 2016			
Unicellular Eukaryotes							
	B. prasinos	*Cell division rate*	0.00380	Krasovec et al. 2016	3.02×10^{-10}	Krasovec et al. 2017	15.07
	C. reinhardtii	*Maximal growth rate*	0.00202	Morgan et al. 2014	6.76×10^{-11}	Sung et al. 2012a	120.41
					9.63×10^{-10}	Ness et al. 2015	
	D. discoideum	*Plate growth (ML)*	0.00010	Hall et al. 2013	2.90×10^{-11}	Saxer et al. 2012	34.21
		Liquid growth (BM)	0.00390	Hall et al. 2013			
		Liquid growth (ML)	0.00190	Hall et al. 2013			
		Slug distance (BM)	0.00050	Hall et al. 2013			
		Total FBs (BM)	0.00020	Hall et al. 2013			
		Total FBs (ML)	0.00270	Hall et al. 2013			
		Spores per FB (BM)	0.00250	Hall et al. 2013			
		Spores per FB (ML)	0.00260	Hall et al. 2013			
		Spore germination (BM)	0.00330	Hall et al. 2013			
		Competitive ability (ML)	0.00150	Hall et al. 2013			
	M. pusilla	*Cell division rate*	0.00370	Krasovec et al. 2016	8.15×10^{-10}	Krasovec et al. 2017	21.11
	O. mediterraneus	*Cell division rate*	0.00370	Krasovec et al. 2016	4.92×10^{-10}	Krasovec et al. 2017	13.48
	S. cerevisiae	*r*	0.00055	Wloch et al. 2001	3.30×10^{-10}	Lynch et al. 2008	12.16
		w	0.00005	Zeyl & DeVisser 2001	2.90×10^{-10}	Nishant et al. 2010	
		MGR	0.00006	Joseph & Hall 2004	3.60×10^{-10}	Serero et al. 2014	
		MGR	0.00014	Hall et al. 2008	1.67×10^{-10}	Zhu et al. 2014	
		SE	0.00019	Hall & Joseph 2010			
		r	0.00013	Hall & Joseph 2010			
	T. thermophila	*r*	0.03300	Brito et al. 2010	7.61×10^{-12}	Long et al. 2016	103.01
		r	0.00470	Long et al. 2013			

Multicellular Eukaryotes							
	A. thaliana	*LRS*	0.05000	Schultz et al. 1999	7.10×10^{-9}	Ossowski et al. 2010	119.67
		Number of fruits	0.00400	Shaw et al. 2000			
		Seeds/fruit	0.00015	Shaw et al. 2000			
	C. briggsae	*w (BM)*	0.03700	Baer et al. 2005	1.33×10^{-9}	Denver et al. 2012	108.38
		w (BM)	0.01200	Baer et al. 2005			
	C. elegans	*Productivity (BM)*	0.00065	Keightley & Caballero 1997	1.45×10^{-9}	Denver et al. 2012	100.29
		r (BM)	0.00350	Keightley & Caballero 1997			
		Productivity (ML)	0.00350	Keightley & Caballero 1997			
		Productivity (BM)	0.02400	Vassilieva et al. 1999, 2000			
		Productivity (ML)	0.00750	Vassilieva et al. 1999, 2000			
		r (BM)	0.00680	Vassilieva et al. 1999, 2000			
		r (ML)	0.01800	Vassilieva et al. 1999, 2000			
		w (BM)	0.00420	Baer et al. 2005			
		w (BM)	0.00330	Baer et al. 2005			
		Productivity (ML)	0.00700	Katju et al. 2015			
		Survivorship to adult (ML)	0.00280	Katju et al. 2015			
		*Productivity (ML)**	0.00580	Katju et al. 2018			
		*Survivorship to adult (ML)**	0.00440	Katju et al. 2018			
	D. melanogaster	*Viability (BM)*	0.35000	Mukai 1964	3.46×10^{-9}	Keightley et al. 2009	143.73
		Viability (BM)	0.47000	Mukai et al. 1972	5.49×10^{-9}	Schrider et al. 2013	
		Viability (ML)	0.01100	Mukai et al. 1972	6.03×10^{-9}	Sharp & Agrawal 2016	
		Viability (BM)	0.14000	Ohnishi 1977	4.90×10^{-9}	Assaf et al. 2018	
		Viability (ML)	0.01000	Ohnishi 1977			
		Fitness (ML)	0.03000	Houle et al. 1992			
		*w (BM)**	0.00150	García-Dorado et al. 1998			
		Viability (BM)	0.02000	Fernández & López-Fanjul 1996			
		*Viability (BM)**	0.02000	Fry et al. 1999			
		Viability (BM)	0.00310	Caballero et al. 2002			
		Viability (MD)	0.00500	Caballero et al. 2002			
		*w (BM)**	0.03700	Ávila & García-Dorado 2002			
		*Viability (BM)**	0.01200	Charlesworth et al. 2004			
		*Viability (BM)**	0.03000	Charlesworth et al. 2004			
		*Viability (BM)**	0.03900	Charlesworth et al. 2004			
		*Viability (BM)**	0.34000	Gong et al. 2005			
		*Viability (BM)**	0.10000	Gong et al. 2005			
		*Viability (BM)**	0.40000	Gong et al. 2005			

Table S2. Spontaneous base substitution mutation rates generated via whole-genome sequencing (WGS) of mutation accumulation experiments. G is the genome size in Mb. N_e is the estimated effective population size for the species. μ_{bs} is the genome-wide, direct molecular estimate of the base substitution rate from WGS of spontaneous mutation accumulation lines and represented as base substitutions per site per generation. U is the number of mutations per genome per generation.

Group	Species	G	N_e	Reference for N_e	μ_{bs} from MA-WGS	Reference for μ_{bs}	U
Prokaryotes							
	B. subtilis NCIB 3610	4.21	6.30×10^{7}	Sung et al. 2015	3.28×10^{-10}	Sung et al. 2015	0.00138
	B. cenocepacia	8.01	2.47×10^{8}	Dillon et al. 2015	1.33×10^{-10}	Dillon et al. 2015	0.00102
	D. radiodurans	3.26	–	–	4.99×10^{-10}	Long et al. 2015	–
	E. coli K12	4.64	2.50×10^{7}	Charlesworth & Eyre-Walker 2006	2.20×10^{-10}	Lee et al. 2012	0.00112
					3.12×10^{-10}	Foster et al. 2015	
	M. florum L1	0.79	1.10×10^{6}	Sung et al. 2012a	9.78×10^{-9}	Sung et al. 2012a, 2015	0.00782
	P. aeruginosa	6.26	2.00×10^{7}	Dettman et al. 2016	7.90×10^{-11}	Dettman et al. 2016	0.00052
	S. typhimurium LT2	4.86	–	–	7.00×10^{-10}	Lind & Andersson 2008	–
	V. cholerae 2740-80	4.09	4.78×10^{8}	Dillon et al. 2017	1.07×10^{-10}	Dillon et al. 2017	0.00043
	V. fischeri ES114	4.27	1.62×10^{8}	Dillon et al. 2017	2.07×10^{-10}	Dillon et al. 2017	0.00088
Unicellular Eukaryotes							
	B. prasinos RC1105	15.07	–	–	3.02×10^{-10}	Krasovec et al. 2017	–
	C. reinhardtii	120.41	3.10×10^{7}	Ness et al. 2015	6.76×10^{-11}	Sung et al. 2012a	0.06201
					9.63×10^{-10}	Ness et al. 2015	
	D. discoideum	34.21	6.90×10^{6}	Flowers et al. 2010	2.90×10^{-11}	Saxer et al. 2012	0.00099
	M. pusilla RCC299	21.11	–	–	8.15×10^{-10}	Krasovec et al. 2017	–
	O. mediterraneus RCC2590	13.48	–	–	4.92×10^{-10}	Krasovec et al. 2017	–
	O. tauri RCC4221	13.03	2.00×10^{7}	Krasovec et al. 2017	4.19×10^{-10}	Krasovec et al. 2017	0.00545
	P. tetraurelia	72.09	1.24×10^{8}	Sung et al. 2012b	1.94×10^{-11}	Sung et al. 2012b	0.00140
	S. cerevisiae	12.16	6.20×10^{6}	Zhu et al. 2014	3.30×10^{-10}	Lynch et al. 2008	0.00349
					2.90×10^{-10}	Nishant et al. 2010	
					1.67×10^{-10}	Zhu et al. 2014	
	S. pombe	12.59	2.60×10^{6}	Farlow et al. 2015	2.13×10^{-10}	Farlow et al. 2015	0.00241
					1.70×10^{-10}	Behringer & Hall 2016	
	T. thermophila	103.01	1.12×10^{8}	Long et al. 2016	7.61×10^{-12}	Long et al. 2016	0.00078
Multicellular Eukaryotes							

A. thaliana	119.67	2.50×10^{5}	Ossowski et al. 2010	7.10×10^{-9}	Ossowski et al. 2010	0.84966
C. briggsae	108.38	2.67×10^{5}	Lynch et al. 2016	1.33×10^{-9}	Denver et al. 2012	0.14415
C. elegans	100.29	5.41×10^{5}	Lynch et al. 2016	2.10×10^{-9}	Denver et al. 2009	0.17801
				1.45×10^{-9}	Denver et al. 2012	
D. pulex	197.21	7.82×10^{5}	Lynch et al. 2017	3.80×10^{-9}	Keith et al. 2016	0.74940
D. melanogaster	143.73	8.63×10^{5}	Lynch et al. 2016	3.46×10^{-9}	Keightley et al. 2009	0.72152
				5.49×10^{-9}	Schrider et al. 2013	
				6.03×10^{-9}	Sharp & Agrawal 2016	
				4.90×10^{-9}	Assaf et al. 2018	
M. musculus	2818.97	1.77×10^{5}	Lynch et al. 2016	5.40×10^{-9}	Uchimura et al. 2015	15.22244
P. pacificus	154.96	1.75×10^{6}	Lynch et al. 2016	2.00×10^{-9}	Weller et al. 2014	0.30992

Literature Cited

Assaf ZJ, Tilk S, Park J, Siegal ML, Petrov DA. 2018. Deep sequencing of natural and experimental populations of *Drosophila melanogaster* reveals biases in the spectrum of new mutations. Genome Res. 27:1988–2000.

Ávila V, García-Dorado A. 2002. The effects of spontaneous mutation on competitive fitness in *Drosophila melanogaster*. J Evol Biol. 15:561–566.

Baer CF, et al. 2005. Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. Proc Natl Acad Sci U S A. 102:5785–5790.

Behringer MG, Hall DW. 2016. Genome-wide estimates of mutation rates and spectrum in *Schizosaccharomyces pombe* indicate CpG sites are highly mutagenic despite the absence of DNA methylation. G3. 12:149–160.

Brito PH, Guilherme E, Soares H, Gordo I. 2010. Mutation accumulation in *Tetrahymena*. BMC Evolutionary Biol. 10:354.

Caballero A, Cusi E, García C, García-Dorado A. 2002. Accumulation of deleterious mutations: additional *Drosophila melanogaster* estimates and a simulation of the effects of selection. Evolution. 56:1150–1159.

Charlesworth B, Borthwick H, Bartolomé C, Pignatelli P. 2004. Estimates of the genomic mutation rate for detrimental alleles in *Drosophila melanogaster*. Genetics. 167: 815–826.

Charlesworth J, Eyre-Walker A. 2006. The rate of adaptive evolution in enteric bacteria. Mol Biol Evol. 23:1348–1356.

Denver DR, et al. 2009. A genome-wide view of *Caenorhabditis elegans* base-substitution mutation processes. Proc Natl Acad Sci U S A. 106:16310–16314.

Denver DR, et al. 2012. Variation in base-substitution mutation in experimental and natural lineages of *Caenorhabditis* nematodes. Genome Biol Evol. 4:513–522.

Dettman JR, Sztepanacz JL, Kassen R. 2016. The properties of spontaneous mutations in the opportunistic pathogen *Pseudomonas aeruginosa*. BMC Genomics. 17:27.

Dillon MM, Cooper VS. 2016. The fitness effects of spontaneous mutations nearly unseen by selection in a bacterium with multiple chromosomes. Genetics. 204:1225–1238.

Dillon MM, Sung W, Lynch M, Cooper VS. 2015. The rate and molecular spectrum of spontaneous mutations in the GC-Rich multichromosome genome of *Burkholderia cenocepacia*. Genetics. 200:935–946.

Dillon MM, Sung W, Sebra R, Lynch M, Cooper VS. 2017. Genome-wide biases in the rate and molecular spectrum of spontaneous mutations in *Vibrio cholerae* and *Vibrio fischeri*. Mol Biol Evol. 34:93–109.

Farlow A, et al. 2015. The spontaneous mutation rate in the fission yeast *Schizosaccharomyces pombe*. Genetics. 201:737–744.

Fernández J, López-Fanjul C. 1996. Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. Genetics. 143:829–837.

Flowers JM, et al. 2010. Variation, sex, and social cooperation: molecular population genetics of the social amoeba *Dictyostelium discoideum*. PLoS Genet. 6:e1001013.

Foster PL, Lee H, Popodi EM, Townes JP, Tang H. 2015. Determinants of spontaneous mutation in the bacterium *Escherichia coli* as revealed by whole-genome sequencing. Proc Natl Acad Sci U S A. 112:E5990–E5999.

Fry JD, Keightley PD, Heinsohn SL, Nuzhdin SV. 1999. New estimates of the rates and effects of mildly deleterious mutation *in Drosophila melanogaster*. Proc Natl Acad Sci U S A. 96:574–579.

García-Dorado A, Monedero JL, López-Fanjul C. 1998. The mutation rate and the distribution of mutational effects of viability and fitness in *Drosophila melanogaster*. Genetica. 103:255–265.

Gong Y, Woodruff RC, Thompson JN. 2005. Deleterious genomic mutation rate for viability in *Drosophila melanogaster* using concomitant sibling controls. Biol Lett. 1: 492–495.

Hall DW, Fox S, Kuzdzal-Fick JJ, Strassmann JE, Quellar DC. 2013. The rate and effects of spontaneous mutation on fitness traits in the social amoeba, *Dictyostelium discoideum*. G3 (Bethesda). 3:1115–1127.

Hall DW, Joseph SB. 2010. A high frequency of beneficial mutations across multiple fitness components in *Saccharomyces cerevisiae*. Genetics. 185:1397–1409.

Houle D, Hoffmaster DK, Assimacopoulos S, Charlesworth B. 1992. The genomic mutation rate for fitness in *Drosophila*. Nature. 359:58–60.

Joseph SB, Hall DW. 2004. Spontaneous mutations in diploid *Saccharomyces cerevisiae*: more beneficial than expected. Genetics. 168:1817–1825.

Joseph S, Hall DW. 2008. Spontaneous mutations in diploid *Saccharomyces cerevisiae*: another thousand cell generations. Genet Res. 90:229–241.

Katju V, Packard LB, Bu L, Keightley PD, Bergthorsson U. 2015. Fitness decline in spontaneous mutation accumulation lines of *Caenorhabditis elegans* with varying effective population sizes. Evolution. 69:104–116.

Katju V, Packard LB, Keightley PD. 2018. Fitness decline under osmotic stress in

Caenorhabditis elegans populations subjected to spontaneous mutation accumulation at varying population sizes. Evolution. 72:1000–1008.

Keightley PD, Caballero A. 1997. Genomic mutation rates for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 94:3823–3827.

Keightley PD, et al. 2009. Analysis of the genome sequences of three *Drosophila melanogaster* spontaneous mutation accumulation lines. Genome Res. 19:1195–1201.

Keith N, et al. 2016. High mutational rates of large-scale duplication and deletion in *Daphnia pulex*. Genome Res. 26:60–69.

Kibota TT, Lynch M. 1996. Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. Nature. 381:694–696.

Krasovec M, et al. 2016. Fitness effects of spontaneous mutations in picoeukaryotic marine green algae. G3 (Bethesda). 6:2063–2071.

Krasovec M, Eyre-Walker A, Sanchez-Ferandin S, Piganeau G. 2017. Spontaneous mutation rate in the smallest photosynthetic eukaryotes. Mol Biol Evol. 34:1770–1779.

Lee H, Popodi E, Tang H, Foster PL. 2012. Rate and molecular spectrum of of spontaneous mutations in the bacterium *Escherichia coli* as determined by whole-genome sequencing. Proc Natl Acad Sci U S A. 109:E2774–E2783.

Lind PA, Andersson DI. 2008. Whole-genome mutational biases in bacteria. Proc Natl Acad Sci U S A. 105:17878-17883.

Long H-A, Paixao T, Azevedo RBR, Zufall RA. 2013. Accumulation of spontaneous mutations in the ciliate *Tetrahymena thermophila*. Genetics. 195:527–540.

Long H, et al. 2015. Background mutational features of the radiation-resistant bacterium *Deinococcus radiodurans*. Mol Biol Evol. 32:2383–2392.

Long et al. 2016. Low base-substitution mutation rate in the germline genome of the ciliate *Tetrahymena thermophila*. Genome Biol Evol. 8:3629–3639.

Lynch M, et al. 2008. A genome-wide view of the spectrum of spontaneous mutations in yeast. Proc Natl Acad Sci U S A. 105:9272–9277.

Lynch M, et al. 2016. Genetic drift, selection and the evolution of the mutation rate. Nat Rev Genet. 17:704–714.

Lynch M, et al. 2017. Population Genomics of *Daphnia pulex*. Genetics. 206:315–332.

Morgan AD, Ness RW, Keightley PD, Colegrave N. 2014. Spontaneous mutation accumulation in multiple strains of the green alga, *Chlamydomonas reinhardtii*. Evolution. 68:2589–2602.

Mukai T. 1964. The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics. 50:1–19.

Mukai T, Chigusa SI, Mettler LE, Crow JF. 1972. Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. Genetics. 72:335–355.

Ness RW, Morgan AD, Vasanthakrishnan RB, Colegrave N, Keightley PD. 2015. Extensive de novo mutation rate variation between individuals and across the genome of *Chlamydomonas reinhardtii*. Genome Res. 25:1739–1749.

Nishant KT, et al. 2010. The baker's yeast diploid genome is remarkably stable in vegetative growth and meiosis PLoS Genet. 6:e1001109.

Ohnishi O. 1977. Spontaneous and ethyl methanesulfonate-induced mutations controlling viability in *Drosophila melanogaster*. II. Homozygous effect of polygenic mutations. Genetics. 87:529–545.

Ossowski S, et al. 2010. The rate and molecular spectrum of spontaneous mutations in

Arabidopsis thaliana. Science 327: 92–94.

Saxer G, et al. 2012. Whole genome sequencing of mutation accumulation lines reveals a low mutation rate in the social amoeba *Dictyostelium discoideum*. PLoS One 7:e46759.

Schrider DR, Houle D, Lynch M, Hahn MW. 2013. Rates and genomic consequences of spontaneous mutational events in *Drosophila melanogaster*. Genetics. 194:937–954.

Schultz ST, Lynch M, Willis JH. 1999. Spontaneous deleterious mutation in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A. 96:11393–11398.

Serero A, Jubin C, Loeillet S, Legoix-Né P, Nicolas AG. 2014. Mutational landscape of yeast mutator strains. Proc Natl Acad Sci U S A. 111:1897–1902.

Sharp NP, Agrawal AF. 2016. Low genetic quality alters key dimension of the mutational spectrum. PLoS Biol. 14:e1002419.

Shaw RG, Byers DL, Darmo E. 2000. Spontaneous mutational effects on reproductive traits of *Arabidopsis thaliana*. Genetics. 155:369–378.

Sung W, et al. 2012a. Drift-barrier hypothesis and mutation-rate evolution. Proc Natl Acad Sci U S A. 109:18488–18492.

Sung W, et al. 2012b. Extraordinary genome stability in the ciliate *Paramecium tetraurelia*. Proc Natl Acad Sci U S A. 109:19339–19344.

Sung W, et al. 2015. Asymmetric context-dependent mutation patterns revealed through mutation-accumulation experiments. Mol Biol Evol. 32:1672–1683.

Uchimura A, et al. 2015. Germline mutation rates and the long-term phenotypic effects of mutation accumulation in wild-type laboratory mice and mutator mice. Genome Res. 25:1125–1134.

Vassilieva LL, Hook AM, Lynch M. The fitness effects of spontaneous mutations in

Caenorhabditis elegans. Evolution. 2000 54:1234–1246.

Vassilieva LL, Lynch M. 1999. The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. Genetics. 151:119–129.

Weller AM, Röderlsperger C, Eberhardt G, Molnar RI, Sommer RJ. 2014. Opposing forces of A/T-biased mutations and G/C-biased gene conversions shape the genome of the nematode *Pristionchus pacificus*. Genetics 196:1145–1452.

Wloch DM, Szafraniec K, Borts R, Korona R. 2001. Direct estimate of the mutation rate and distribution of fitness effects in the yeast *Saccharomyces cerevisiae*. Genetics. 159:441–452.

Zeyl C, DeVisser JAGM. 2001. Estimates of the rate and distribution of fitness effects of spontaneous mutation in *Saccharomyces cerevisiae*. Genetics. 157:53–61.

Zhu YO, Siegal ML, Hall DW, Petrov DA. 2014. Precise estimates of mutation rate and spectrum in yeast. Proc Natl Acad Sci U S A. 111:E2310–E2318.