Supporting Information

Table S1

Summary of the estimates of the number of divisions on plates over 4 and 5 days of growth and fitness in liquid media for each MA line and the ancestral strains. The μ_{max} shown for the ancestors is the average of 9 pseudolines. Missing values correspond to cases where the lines failed to grow from their frozen stocks. In cases where a replicate assay failed, the standard error for G cannot be calculated, and is indicated by (*) in the table.

Line	Total transfers of MA	Number of generations on plates in 4 days (se)	Number of generations on plates in 5 days (se)	Total estimated generations of MA	μ_{max} 1st fitness assay	μ_{max} 2nd fitness assay	Average μ_{max} of both fitness assays
1373 Anc	0	6.47 (*)	10.20 (0.77)	0	0.082	0.070	0.076
CC-1373 1	85	8.08 (*)	10.29(*)	771	0.095	0.082	0.088
CC-1373 2	74	8.06 (*)	10.87 (0.35)	683	0.060	0.073	0.066
CC-1373 3	72	8.59 (*)	11.13 (1.69)	678	0.104	0.094	0.099
CC-1373 4	80	6.79 (*)	9.59 (*)	689	0.045	0.079	0.062
CC-1373 5	77	6.97 (*)	10.68 (1.71)	693	0.080	0.110	0.095
CC-1373 6	84	8.36 (0.40)	9.66 (0.99)	749	0.082	0.081	0.081
CC-1373 7	82	8.90 (0.57)	10.77 (0.40)	766	0.093	0.088	0.090
CC-1373 8	80	8.55 (0.27)	10.02 (1.19)	725	0.086	0.080	0.083
CC-1373 9	79	-	-	-	-	-	-
CC-1373 10	82	5.86 (*)	7.29 (*)	630	0.057	0.061	0.059
CC-1373 11	82	8.79 (0.75)	10.24 (1.18)	751	0.108	0.058	0.083
CC-1373 12	79	-	-	-	-	-	-
CC-1373 13	82	9.39 (0.86)	10.78 (0.45)	773	0.066	0.087	0.077
CC-1373 14	81	6.41 (*)	11.03 (1.11)	732	0.057	0.067	0.062
CC-1373 15	23	8.55(0.58)	11.22 (0.23)	198	0.093	0.058	0.076

Line	Total transfers of MA	Number of generations on plates in 4 days (se)	Number of generations on plates in 5 days (se)	Total estimated generations of MA	μ_{max} 1st fitness assay	μ_{max} 2nd fitness assay	Average μ_{max} of both fitness assays
1952 Anc	0	12.39 (1.92)	14.34 (0.63)	0	0.153	0.130	0.141
CC-1952 1	85	11.86 (1.40)	13.83 (0.73)	1074	-	0.094	0.094
CC-1952 2	85	12.11 (1.32)	14.03 (0.57)	1083	0.125	0.111	0.118
CC-1952 3	85	12.18 (1.43)	13.79 (0.24)	1077	0.124	0.188	0.156
CC-1952 4	85	9.57 (0.44)	10.77 (0.31)	954	0.072	0.093	0.082
CC-1952 5	58	11.09 (*)	13.66 (*)	703	0.060	0.043	0.052
CC-1952 6	85	12.25 (1.35)	13.28 (0.13)	1064	0.112	0.116	0.114
CC-1952 7	85	12.24 (1.42)	12.71 1.02)	1048	0.126	0.091	0.108
CC-1952 8	85	10.64 (*)	11.86 (*)	1000	0.063	0.088	0.075
CC-1952 9	85	-	-	-	-	-	-
CC-1952 10	85	11.15 (0.55)	13.26 (0.49)	1047	0.079	0.107	0.093
CC-1952 11	85	11.01 (0.37)	14.02 (0.53)	1066	0.115	0.091	0.103
CC-1952 12	85	11.06 (0.75)	13.19 (0.28)	1044	0.112	0.141	0.127
CC-1952 13	84	10.35 (1.19)	12.47 (0.17)	1002	0.079	0.083	0.081
CC-1952 14	85	12.78 (1.25)	13.73 (0.05)	1085	0.079	0.089	0.084
CC-1952 15	84	12.55 1.59)	13.03 (0.51)	1049	0.156	0.157	0.156

Line	Total transfers of MA	Number of generations on plates in 4 days (se)	Number of generations on plates in 5 days (se)	Total estimated generations of MA	μ_{max} 1st fitness assay	μ_{max} 2nd fitness assay	Average μ_{max} of both fitness assays
2342 Anc	0	12.72 (0.06)	13.46 (0.81)	0	0.153	0.130	0.142
CC-2342 1	85	12.39 (0.01)	13.75 (0.34)	1109	0.121	0.108	0.114
CC-2342 2	85	-	-	-	-	-	-
CC-2342 3	83	10.68 (0.30)	13.36 (0.59)	1048	-	0.095	0.095
CC-2342 4	85	12.12 (0.09)	13.34 (0.35)	1093	0.179	0.109	0.144
CC-2342 5	84	12.77 (0.69)	14.42 (0.14)	1121	-	0.120	0.120
CC-2342 6	85	-	-	-	-	-	-
CC-2342 7	85	11.90 (0.26)	14.63 (0.55)	1126	-	0.095	0.095
CC-2342 8	50	7.92 (0.47)	11.10 (0.74)	562	-	0.054	0.054
CC-2342 9	73	12.02 (1.12)	14.35 (0.33)	959	0.126	0.102	0.114
CC-2342 10	85	12.70 (0.47)	14.35 (0.13)	1130	0.052	0.130	0.091
CC-2342 11	85	12.16 (0.52)	13.88 (0.78)	1109	0.126	0.132	0.129
CC-2342 12	75	8.04 (*)	9.96 (0.50)	826	0.034	0.080	0.057
CC-2342 13	85	12.05 (0.01)	14.62 (1.03)	1128	0.090	0.098	0.094
CC-2342 14	84	12.02 (0.29)	13.76 (0.27)	1091	-	0.104	0.104
CC-2342 15	85	11.83 (0.43)	14.50 (1.37)	1122	0.162	0.140	0.151

Line	Total transfers of MA	Number of generations on plates in 4 days (se)	Number of generations on plates in 5 days (se)	Total estimated generations of MA	μ_{max} 1st fitness assay	μ_{max} 2nd fitness assay	Average μ_{max} of both fitness assays
2344 Anc	0	11.39 (0.15)	12.64 (0.09)	0	0.107	0.114	0.110
CC-2344 1	84	10.26 (0.28)	11.40 (0.31)	912	0.079	0.063	0.071
CC-2344 2	84	10.61 (0.31)	11.61 (0.21)	923	0.130	0.093	0.112
CC-2344 3	84	12.12 (0.35)	13.62 (0.49)	1000	0.065	0.124	0.094
CC-2344 4	79	9.30 (0.32)	10.23 (0.32)	809	0.047	0.064	0.055
CC-2344 5	83	11.12 (0.13)	13.04 (0.74)	958	0.040	0.121	0.081
CC-2344 6	85	10.91 (0.36)	12.77 (0.03)	971	0.083	0.110	0.097
CC-2344 7	85	11.65 (0.04)	13.10 (0.28)	991	0.106	0.154	0.130
CC-2344 8	85	11.59 (0.35)	13.81 (0.12)	1010	0.127	0.122	0.125
CC-2344 9	85	7.88 (0.38)	11.91 (2.31)	901	0.047	-	0.047
CC-2344 10	83	10.72 (0.73)	11.79 (*)	917	0.120	0.098	0.109
CC-2344 11	84	9.27 (0.29)	11.08 (0.55)	890	-	0.104	0.104
CC-2344 12	83	11.36 (0.32)	12.65 (0.38)	950	0.094	0.094	0.094
CC-2344 13	85	10.93 (0.04)	12.43 (0.53)	961	0.100	0.117	0.108
CC-2344 14	83	11.31 (0.28)	12.01 (0.30)	931	0.145	0.090	0.118
CC-2344 15	85	11.07 (0.36)	11.83 (0.26)	947	0.118	0.078	0.098

Line	Total transfers of MA	Number of generations on plates in 4 days (se)	Number of generations on plates in 5 days (se)	Total estimated generations of MA	μ_{max} 1st fitness assay	μ_{max} 2nd fitness assay	Average μ_{max} of both fitness assays
2931 Anc	0	12.06 (0.27)	13.48 (*)	0	0.166	0.130	0.148
CC-2931 1	84	11.42 (0.15)	13.44 (0.23)	1050	0.141	0.120	0.131
CC-2931 2	85	11.49 (0.07)	13.24 (0.45)	1057	0.096	0.113	0.104
CC-2931 3	82	10.89 (0.13)	12.88 (0.21)	1000	0.128	0.123	0.126
CC-2931 4	83	11.54 (0.01)	12.48 (0.07)	1011	0.129	0.125	0.127
CC-2931 5	84	10.60 (1.15)	11.84 (1.18)	994	0.105	0.078	0.091
CC-2931 6	85	11.94 (0.64)	13.94 (0.20)	1083	0.142	0.125	0.134
CC-2931 7	85	11.69 (1.33)	13.51 (0.26)	1067	0.159	0.147	0.153
CC-2931 8	86	-	-	-	-	0.023	0.023
CC-2931 9	85	11.24 (0.22)	14.18 (0.79)	1079	0.152	0.129	0.141
CC-2931 10	85	12.64 (0.65)	14.09 (1.24)	1098	0.104	0.105	0.104
CC-2931 11	85	11.72 (0.11)	11.84 (0.43)	1021	0.151	0.111	0.131
CC-2931 12	81	6.70 (*)	7.59 (*)	787	-	0.033	0.033
CC-2931 13	84	11.13 (0.11)	13.28 (0.07)	1041	0.121	0.122	0.121
CC-2931 14	84	11.32 (0.13)	12.28 (0.67)	1016	0.122	0.100	0.111
CC-2931 15	84	10.76 (1.45)	13.86 (0.15)	1053	0.117	0.118	0.118

Line	Total transfers of MA	Number of generations on plates in 4 days (se)	Number of generations on plates in 5 days (se)	Total estimated generations of MA	μ_{max} 1st fitness assay	μ_{max} 2nd fitness assay	Average μ_{max} of both fitness assays
2937 Anc	0	12.14 (0.27)	13.94 (0.34)	0	0.065	0.110	0.088
CC-2937 1	75	11.29 (0.83)	13.73 (0.38)	925	0.130	0.138	0.134
CC-2937 2	81	11.68 (0.19)	13.06 (0.36)	991	0.114	0.112	0.113
CC-2937 3	81	12.23 (0.78)	12.96 (0.39)	996	0.145	0.096	0.121
CC-2937 4	81	11.92 (0.90)	13.97 (0.56)	1019	0.117	0.101	0.109
CC-2937 5	78	11.79 (0.37)	12.96 (0.17)	951	0.108	0.094	0.101
CC-2937 6	79	11.58 (0.60)	13.62 (1.10)	977	0.144	0.096	0.120
CC-2937 7	79	11.13 (0.58)	12.98 (0.03)	954	0.098	0.071	0.085
CC-2937 8	39	9.10 (0.52)	11.17 (0.09)	404	0.063	0.079	0.071
CC-2937 9	77	12.25 (0.42)	13.85 (0.35)	967	0.122	0.121	0.122
CC-2937 10	78	11.33 (0.82)	13.10 (0.04)	949	0.111	0.099	0.105
CC-2937 11	77	11.52 (0.37)	13.80 (0.40)	956	0.133	0.102	0.117
CC-2937 12	81	10.34 (0.57)	13.36 (*)	981	0.147	0.143	0.145
CC-2937 13	83	10.22 (0.64)	11.97 (0.44)	965	0.113	0.123	0.118
CC-2937 14	79	12.07 (0.77)	13.61 (0.84)	984	-	0.088	0.088
CC-2937 15	79	10.08 (0.68)	12.05 (*)	916	0.102	0.100	0.101

Table S2

Summary of estimates of numbers of generations undergone by each strain between transfers and over the entire MA experiment including CC-1373. Estimates are shown for three, four and five day transfer periods for the ancestors. The three day transfer period was only used in the early part of the study, and so three days measures were not made on the MA lines.

Strain	Number of generations of ancestor in 3 days	Number of generations of ancestor in 4 days	Number of generations of ancestor in 5 days	Average number of generations of MA lines in 4 days (95% CIs)	Average number of generations of MA lines in 5 days (95% CIs)	Total number of generations of MA (95% CIs)
CC-1373	7.11	6.47	10.20	7.95 (7.36, 8.53)	10.28 (9.71, 10.84)	679.89 (597.75, 762.02)
CC-1952	8.68	12.39	14.34	11.49 (11.00, 11.98)	13.12 (12.63, 13.60)	1021.12 (969.37, 1072.88)
CC-2342	10.90	12.72	13.46	11.43 (10.55, 12.31)	13.54 (12.76, 14.31)	1032.65 (942.31, 1122.98)
CC-2344	7.54	11.39	12.64	10.67 (10.11, 11.23)	12.22 (11.73, 12.71)	938.10 (912.50, 963.69)
CC-2931	9.50	12.06	13.48	11.08 (10.36, 11.79)	12.75 (11.87, 13.63)	1025.59 (985.90, 1065.28)
CC-2937	8.17	12.15	13.94	11.24 (10.77, 11.70)	13.08 (12.67, 13.48)	929.07 (854.31, 1003.83)

Table S3

Summary of estimates of mutational variance for fitness assays including CC-1373. Separate columns show estimates from the 1st and 2nd replicate assays (Anc = ancestor, MA= mutation accumulation lines).

Table S3

Strain	Variance (95% CIs)				F-ratio test		ΔV		ΔV per generation = V_m		CV_m Ancestor (% per generation)		CV_m MA (% per generation)	
	1st Anc	1st MA	2nd Anc	2nd MA	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
CC-1373	0 (0, 0)	3.34×10^{-4} (1.72×10^{-4}, 9.1×10^{-4})	5.28×10^{-5} (2.41×10^{-5}, 1.94×10^{-4})	1.22×10^{-4} (6.28×10^{-5}, 3.33×10^{-4})	NA*	2.31	3.34×10^{-4}	6.92×10^{-5}	4.91×10^{-7}	1.02×10^{-7}	0.86	0.46	0.89	0.41
CC-1952	0 (0,0)	6.93×10^{-4} (3.56×10^{-4}, 1.89×10^{-3})	6.99×10^{-5} (3.19×10^{-5}, 2.57×10^{-4})	5.99×10^{-4} (3.15×10^{-4}, 1.55×10^{-3})	NA*	8.57*	6.93×10^{-4}	5.29×10^{-4}	6.79×10^{-7}	5.18×10^{-7}	0.54	0.55	0.82	0.66
CC-2342	2.86×10^{-17} (1.30×10^{-17}, 1.05×10^{-16})	1.47×10^{-3} (6.42×10^{-4}, 6.08×10^{-3})	2.43×10^{-4} (1.11×10^{-4}, 8.90×10^{-4})	9.82×10^{-4} (5.16×10^{-4}, 2.55×10^{-3})	5.14×10^{13}*	4.04*	1.47×10^{-3}	7.39×10^{-4}	1.42×10^{-6}	7.16×10^{-7}	0.78	0.65	1.06	0.85
CC-2344	0 (0,0)	6.3×10^{-4} (3.31×10^{-4}, 1.64×10^{-3})	0 (0,0)	1.09×10^{-4} (5.75×10^{-5}, 2.84×10^{-4})	NA*	NA*	6.30×10^{-4}	1.09×10^{-4}	6.72×10^{-7}	1.16×10^{-7}	0.77	0.30	0.89	0.32
CC-2931	5.24×10^{-17} (2.39×10^{-17}, 1.93×10^{-16})	1.06×10^{-4} (5.46×10^{-5}, 2.89×10^{-4})	1.99×10^{-4} (9.07×10^{-5}, 7.30×10^{-4})	7.76×10^{-4} (4.16×10^{-4}, 1.93×10^{-3})	2.02×10^{12}*	3.9*	1.06×10^{-4}	5.77×10^{-4}	1.03×10^{-7}	5.63×10^{-7}	0.19	0.58	0.25	0.71
CC-2937	-	-	7.15×10^{-21} (3.26×10^{-21}, 2.62×10^{-20})	2.91×10^{-4} (1.56×10^{-4}, 7.24×10^{-4})	-	4.07×10^{16}*	-	2.91×10^{-4}	-	3.13×10^{-7}	-	0.51	-	0.54

[1] among MA line variance component.
[2] ancestral among pseudoline variance component divided by the between line MA variance component; the critical value = 3.006 with df 15,9 (* = significant difference at the 5% level between MA and ancestral variance).
[3] change in the among line variance component of fitness between the MA lines and their ancestors.
[4] Mutational heritability calculated as ΔV /number of generations.
[5] coefficients of mutational variance calculated as the square root of ΔV per generation divided by the mean fitness of the ancestral lines
[6] coefficients of mutational variance calculated as the square root of ΔV per generation divided by the mean fitness of the MA lines

Table S4

Estimates of fitness and mutational parameters from the fitness assays including CC-1373. Separate columns show estimates from the 1st and 2nd replicate assays.

Strain	Fitness (95% CIs)				ΔM (x 10^{-3}) per generation[1] (95% CIs (x 10^{-3}))		U^2 (se)		$E(a)^3$ (se)	
	1st Anc	1st MA	2nd Anc	2nd MA	1st	2nd	1st	2nd	1st	2nd
CC-1373	0.082 (0.068, 0.096)	0.079 (0.068, 0.090)	0.069 (0.058, 0.081)	0.078 (0.066, 0.090)	-0.007 (-0.031, +0.017)	+0.018 (-0.006, +0.042)	1.01×10^{-4} (1.53×10^{-4})	*	-6.98×10^{-2} (6.77×10^{-2})	$+5.71 \times 10^{-3}$ (5.54×10^{-3})
CC-1952	0.153 (0.127, 0.178)	0.100 (0.079, 0.121)	0.130 (0.102, 0.158)	0.108 (0.071, 0.145)	-0.051 (-0.071, -0.031)	-0.019 (-0.036, -0.002)	3.86×10^{-3} (4.55×10^{-3})	7.08×10^{-4} (1.07×10^{-3})	-1.33×10^{-2} (1.29×10^{-2})	-2.70×10^{-2} (2.62×10^{-2})
CC-2342	0.153 (0.138, 0.168)	0.112 (0.061, 0.163)	0.130 (0.112, 0.148)	0.099 (0.078, 0.120)	-0.042 (-0.066, -0.018)	-0.017 (-0.034, 0.001)	1.26×10^{-3} (1.91×10^{-3})	4.27×10^{-4} (6.46×10^{-4})	-3.36×10^{-2} (3.26×10^{-2})	-4.09×10^{-2} (3.97×10^{-2})
CC-2344	0.107 (0.089, 0.125)	0.092 (0.062, 0.121)	0.114 (0.100, 0.128)	0.105 (0.086, 0.124)	-0.016 (-0.036, +0.004)	-0.011 (-0.030, +0.007)	3.71×10^{-4} (5.61×10^{-4})	1.13×10^{-3} (1.71×10^{-3})	-4.26×10^{-2} (4.13×10^{-2})	-1.01×10^{-2} (9.80×10^{-2})
CC-2931	0.166 (0.145, 0.187)	0.129 (0.110, 0.148)	0.130 (0.111, 0.149)	0.106 (0.088, 0.124)	-0.036 (-0.055, -0.016)	-0.017 (-0.034, 0.000)	1.22×10^{-2} (1.84×10^{-2})	$5.02x\ 10^{-4}$ (7.59×10^{-4})	-2.91×10^{-3} (2.72×10^{-3})	-3.35×10^{-2} (3.25×10^{-2})
CC-2937	-	-	0.109 (0.098, 0.121)	0.104 (0.090, 0.118)	-	-0.003 (-0.021, +0.014)	-	3.13×10^{-5} (4.73×10^{-5})	-	-9.16×10^{-2} (8.89×10^{-2})

[1] change in mean fitness per generation between the ancestral pseudolines (Anc) and the MA lines (MA) for each strain.
[2] Bateman-Mukai estimates of the genomic deleterious mutation rate per generation, se calculated using the delta method
[3] Bateman-Mukai estimates of the mean mutational effect per generation, se calculated using the delta method.

Table S5. Coefficient of mutation variance (CV_m) and genomic properties of model organisms used for MA experiments. The organisms listed are those for which genomic and phenotypic outcomes of MA are available. The figures reported for CV_m are the mean value for each species as summarised in Halligan and Keightley (2009 and references therein). Using available estimates of the mutation rate, genome size and, proteome size we calculate the variance in fitness contributed per mutation.

Organism	μ (/bp*gen.)	genome size (bp)	Coding positions [1]	Ploidy	cell div. / gen.[2]	Mut. / div. * genome	Mut./div.* proteome	CV_m	CV_m / div.	CV_m / genome *mut.	CV_m / proteome *mut.
Arabidopsis thaliana	6.50×10^{-9}	157,000,000	20,098,557.72	2	35	0.0292	0.0037	4.15	0.119	4.1	31.8
Caenorhabditis elegans	5.75×10^{-9}	97,000,000	15,058,140.93	2	9	0.062	0.0096	1.49	0.165	2.7	17.2
Drosophila melanogaster [3]	4.65×10^{-9}	139,500,000	10,649,322.34	2	36	0.018	0.0014	1.54	0.043	2.4	31.0
Chlamydomonas reinhardtii	2.08×10^{-10}	112,000,000	19,641,831.08	1	1	0.0233	0.0041	0.54	0.540	23.2	132.2
Saccharomyces cerevisiae	3.30×10^{-10}	12,495,682	4,357,469.27	1	1	0.0041	0.0014	0.09	0.087	21.1	60.5
E. coli	2.60×10^{-10}	4,639,675	1,972,335.83	1	1	0.0012	0.0005	0.02	0.020	16.6	39.0

[1] Proteome sizes were taken from Massey 2008 and references therein. The number of coding positions was estimated as 2/3 of CDS to account for synonymous changes

[2] Cell divisions per generation were taken from Lynch (2010) and references therein.

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