Electronic Supplementary Material

The conflict within: origin, proliferation and persistence of a spontaneously arising selfish mitochondrial genome

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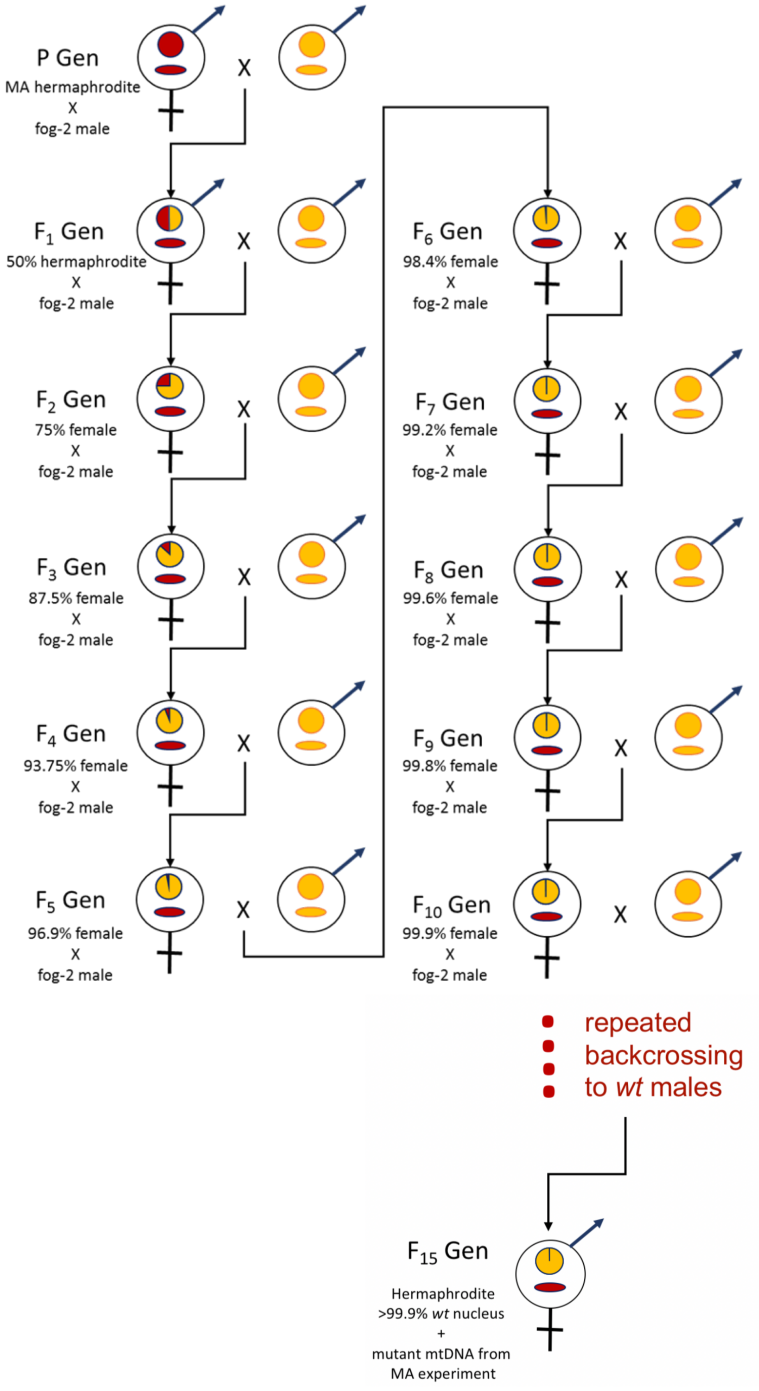

Figure S1. Schematic of the backcrossing experiment to sequester the mutant mitochondrial genome bearing the *ctb-1* deletion within a wild-type nuclear background. After 15 generations of backcrossing mutant mtDNA-bearing *C. elegans* hermaphrodites to wild-type *C. elegans* males (*fog*-2 or *N2*), only 0.003% of the nuclear genome from the original 1G mutation accumulation line is expected to remain, ensuring that the mitochondrial deletion is no longer associated with any other nuclear mutations that arose during the course of the mutation accumulation experiment.

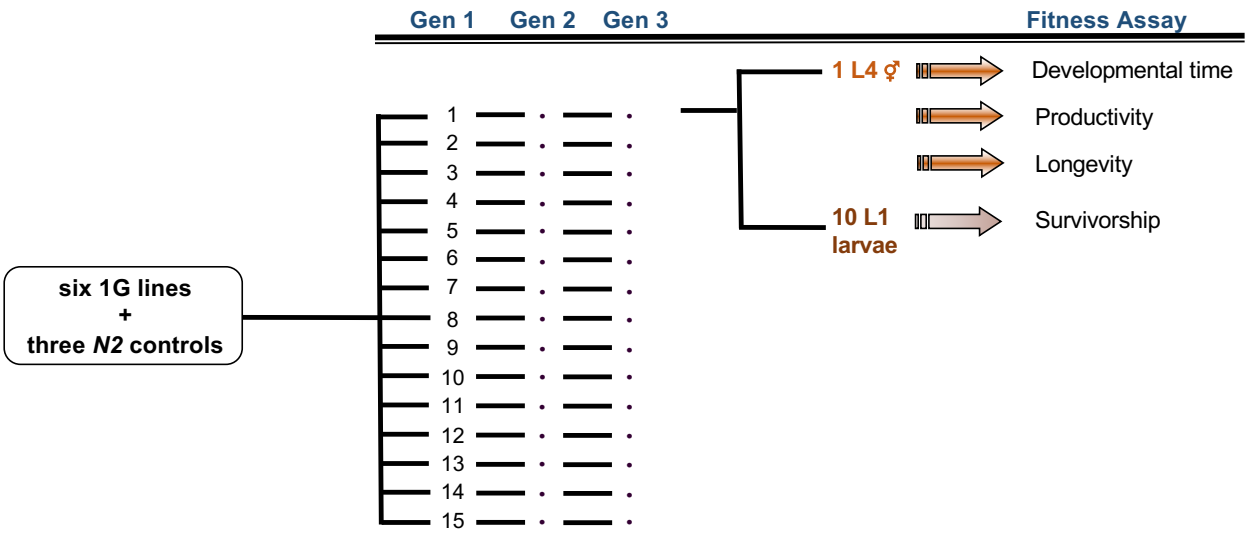

Figure S2. Design of assays for four fitness-related traits. Three *N2* control and six replicate lines of 1G with the *ctb-1* deletion bearing mitochondrial genome sequestered in a wild-type nuclear background were assayed. For experimental or control lines, 15 within line replicates were established and propagated for two additional generations by single-progeny descent to remove environmental effects. The F3 generation worms were utilized for the fitness assays. 10 L1 larvae per line were isolated soon upon hatching and utilized for measuring survivorship to adulthood. One L4 larva, a sibling of the ten larvae utilized for the survivorship assay was used for measuring three additional fitness-related traits, namely developmental time, productivity and longevity.

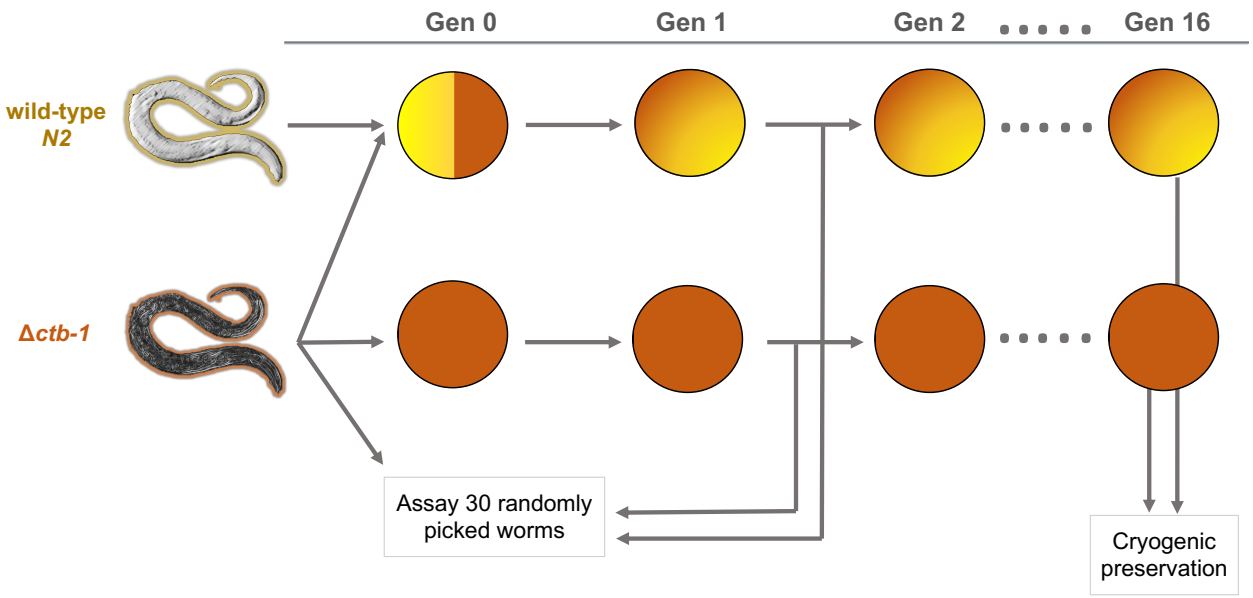

Figure S3. Design of the competition experiment. 50 WT *N2* hermaphrodites and 50 Δ*ctb-1* mtDNA-bearing 1G hermaphrodites from the backcrossing experiment were used to establish each of the five replicate populations comprising the competition assays. Non-competed control populations of 1G replicate lines were established with 50 L4 hermaphrodites from each of five Δ*ctb-1* mtDNA bearing replicate lines of 1G. Three days after establishing each new generation, 30 randomly picked worms were each analyzed for the presence of the Δ*ctb-1* mitotype. On day 4, the entire worm population on the NGM plate was bleached and the eggs transferred to a new plate to establish the next generation. The bleaching procedure was repeated every four days for 16 generations and the frequency analysis was performed in each of the 16 generations.

Table S1. Probes for Bio-Rad ddPCR assay for quantification of mtDNA copy-number.

Gene	Assay ID	Quencher	Probe Fluorophore	Location	MIQE Context
actin-2	dCNS59442090	Iowa Black	FAM	Chr. V: 11077658.. 11077779	CACCCAGTACTTCTCACTGAAGCCCCACTCAATCCAAAGGCTAACCGTG AAAAGATGACCCAAATCATGTTCGAGACCTTCAATACCCCAGCCATGTA TGTCGCCATCCAAGCTGTCCTCTCC
ctb-1	dCNS821148280	Iowa Black	FAM	mtDNA: 5056..5177	AGCTATTCTAGTTATTGTATTGGGGCATTTAATTTTTTTACACAGAACTGG TAGAACATCTAGGTTATATTGCCACGGTGATTATGATAAAGTTTGTTTTA GACCTGAGTACTTAGGTAAAGA
cox-1	dCNS484374227	Iowa Black	HEX	mtDNA: 8189..8310	CATCTATATTATTAATTTTAGATGCTTGTTTTGTAGATATAGGTTGTGGGA CTAGGTGAACAGTCTACCCACCTTTAAGAACAATGGGGCACCCTGGAAG TAGAGTAGATTTAGCTATTTTTA

Table S2. Fitness of Δ*ctb-1* bearing worms relative to WT *N2* controls. Fitness was tested under benign laboratory conditions. Four fitness-related traits namely productivity, survivorship to adulthood, developmental time and longevity were assayed in three *N2* controls (C1-C3) and six experimental lines of 1G with the *ctb-1* deletion bearing mitochondrial genome sequestered in a wild-type nuclear background. Where possible, data was collected for 15 replicates per experimental and control line. Grey cells signify missing data.

Line	Replicate	Survivorship	Developmental Time (hrs)	Longevity (days)	Productivity
C1	1	0.9	51	19	279
C1	2	1	53	12	274
C1	3	1	53	21	286
C1	4	0.9	53	13	312
C1	5	0.9	51	9	282
C1	6	1	49	21	326
C1	7	1	51	18	381
C1	8	1	49	11	301
C1	9	1	53	17	285
C1	10	1	53	11	300
C1	11	1	55	11	286
C1	12	1	51	22	276
C1	13	1	49	13	244
C1	14	1	51	11	281
C1	15	1	53	18	311
C2	1	1	42	26	323
C2	2	1	40	25	308
C2	3	1	42	18	387
C2	4	1	42	21	334
C2	5	1	40	10	340
C2	6	1	46	17	339
C2	7	1	42	18	320
C2	8	1	42	10	349
C2	9	1	44	17	317
C2	10	1	44	13	326
C2	11	1	38	23	303
C2	12	1	40	18	262
C2	13	1	44	21	348
C2	14	1	46	12	277
C2	15	1	42	20	362
C3	1	1	40	14	363
C3	2	1	42	15	314

C3	3	1	48	11	346
C3	4	1	48	12	153
C3	5	1	40	10	346
C3	6	1	42	15	325
C3	7	1	42	10	330
C3	8	1	42	18	331
C3	9	1	40	7	250
C3	10	1	44	21	307
C3	11	1	50	20	358
C3	12	1	46	12	340
C3	13	1	44	10	308
C3	14	1	42	18	308
C3	15	1	46	9	311
1G.C	1	0.9	58	14	0
1G.C	2	0.6	58	14	3
1G.C	3	1	46	13	141
1G.C	4	1	52	5	26
1G.C	5	0.9	52	13	149
1G.C	6	1	48	14	0
1G.C	7	1	44	14	247
1G.C	8	1	58	27	0
1G.C	9	0.7	58	20	5
1G.C	10	1	50	7	151
1G.C	11	0.8	56	5	18
1G.C	12	0.8	54	14	320
1G.C	13	1	42	7	116
1G.C	14	0.9	42	7	199
1G.C	15	0.5	58	7	1
1G.L	1	1	53	6	138
1G.L	2	0.9	55	9	295
1G.L	3	0.9	61	13	0
1G.L	4	0.7		0	0
1G.L	5	0.9	67	23	0
1G.L	6	1	63	22	0
1G.L	7	0.9	63	6	5
1G.L	8	1	55	4	58
1G.L	9	1	53	4	76
1G.L	10	1	51	13	136
1G.L	11	1	61	25	96

1G.L	12	1	59	19	13
1G.L	13	1	55	18	255
1G.L	14	1	49	10	217
1G.L	15	0.9	57	25	164
1G.M	1	1	59	4	43
1G.M	2	1	55	5	57
1G.M	3	0.8	61	9	0
1G.M	4	1	61	4	14
1G.M	5	1	63	16	0
1G.M	6	0.9	55	11	37
1G.M	7	1	55	11	185
1G.M	8	1	55	25	230
1G.M	9	1	53	10	178
1G.M	10	1	63	25	0
1G.M	11	0.9	65	11	155
1G.M	12	1		2	0
1G.M	13	0.9	53	18	182
1G.M	14	0.9	55	26	216
1G.M	15	0.9	61	19	8
1G.N	1	1	48	18	0
1G.N	2	0.9	44	4	49
1G.N	3	0.2	50	5	79
1G.N	4	0.8	48	17	183
1G.N	5	0.4	50	16	47
1G.N	6	0.4	56	4	56
1G.N	7	0.5	54	16	56
1G.N	8	0.6	42	15	229
1G.N	9	1	46	4	26
1G.N	10	1	48	24	0
1G.N	11	0.9	56	7	114
1G.N	12	1	40	11	205
1G.N	13	0.8	40	11	186
1G.N	14	1		0	0
1G.N	15	0.9	58	13	57
1G.T	1	1	48	13	214
1G.T	2	1	40	7	230
1G.T	3	1	44	15	167
1G.T	4	0.8	46	5	130

1G.T	5	1	52	14	4
1G.T	6	1	40	18	230
1G.T	7	1	54	19	172
1G.T	8	0.9	44	16	332
1G.T	9	1	48	5	133
1G.T	10	1	48	16	276
1G.T	11	0.8	44	21	249
1G.T	12	0.8	44	4	124
1G.T	13	1	56	13	166
1G.T	14	1	48	4	36
1G.T	15	1	46	16	86
1G.U	1	1	54	29	0
1G.U	2	1	58	7	3
1G.U	3	1	44	5	171
1G.U	4	1	42	7	143
1G.U	5	1	44	14	193
1G.U	6	1	46	17	304
1G.U	7	1	52	21	103
1G.U	8	0.6	58	18	0
1G.U	9	1	52	18	87
1G.U	10	1	58	15	119
1G.U	11	1	50	16	230
1G.U	12	1	48	14	182
1G.U	13	1	58	5	23
1G.U	14	0.7	52	12	175
1G.U	15	1	52	7	83