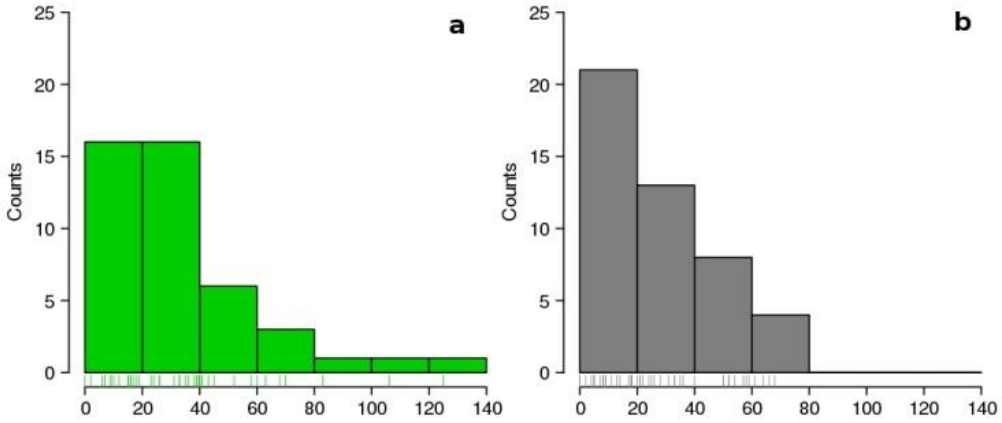
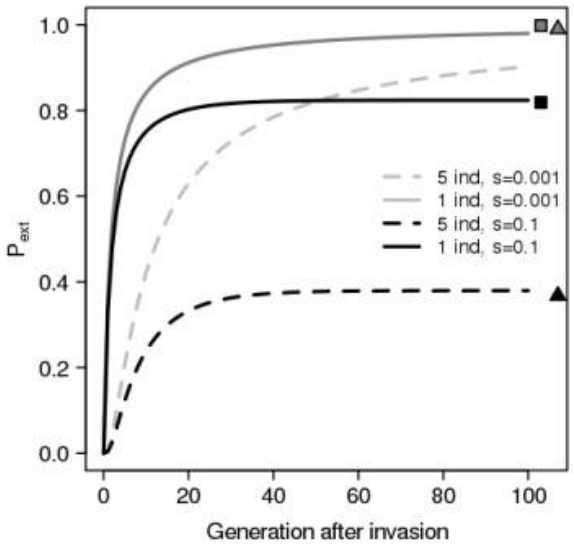
Supplementary Information for:

An experimental test on the probability of extinction of new genetic variants

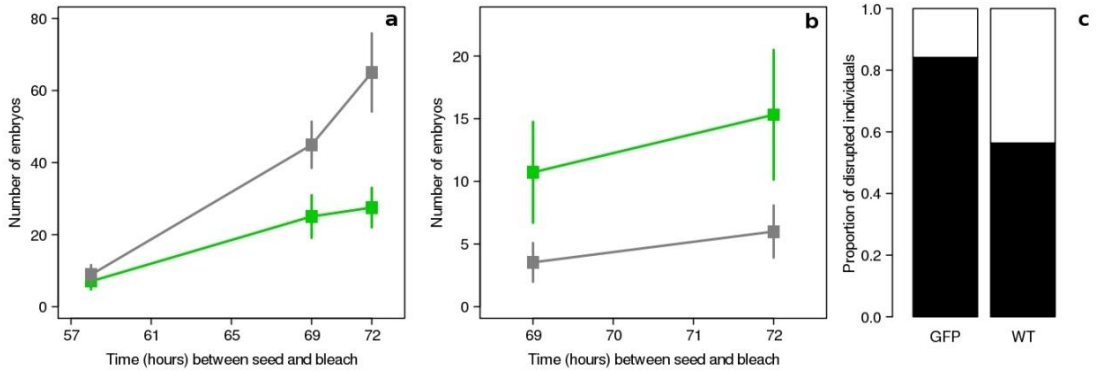
Ivo M. Chelo, Judit Nédli, Isabel Gordo and Henrique Teotónio



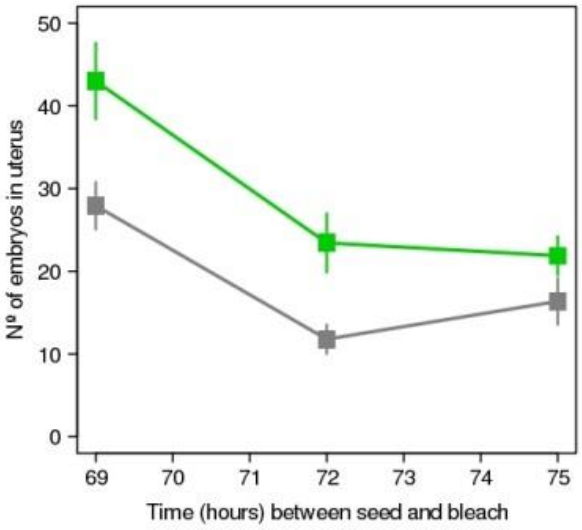
Supplementary Figure S1. Fertility distributions of the two inbred lines used in the present study. Inbred lines were revived from -80ºC stocks in separate assays and expanded for two generations. In the third generation after revival, single individuals were handpicked, 48 hours after seeding of the L1s (see **Methods** in main text), and placed into single wells of 96 well plates, containing NGM-lite agar and seeded with *E. coli*. The 96 well plates were then sealed with Parafilm, kept for 24 hours, after which worms were subjected to a 1M KOH: 5% NaOCl solution for 5 minutes. 150µl of M9 buffer was then added to each well, rinsed three to six times and transferred to new 96 well plates with M9. 24 hours after, plates were spun down, photographs were taken for each well and the number of live L1s scored. The number of L1s per hermaphrodite is a fertility estimate since it includes fecundity and egg to L1 viability. The assays were done in conditions that attempt to mimic those of the invasion and intermediate frequency experiments reported in the main text. Fertility estimates are plotted for GFP (**a**) and wild type (**b**) individuals. Counts (GFP n=44, wild type n=46) were clustered into different classes and represented as histograms. Individual counts are shown as vertical lines below the histograms. These were used to calculate likelihoods under the assumption that the underlying distributions were either Poisson or negative binomial. Likelihood ratio tests indicate that the negative binomial is a better fit to both the GFP data (D= 629, χ_1^2 p<0.001) and the wild-type data (D=555, χ_1^2 p<0.001). For negative binomial distributions: GFP mean=35.1±4.1SD and wild-type mean=27.7±3.5SD; GFP dispersion=1.76 ±0.38 SD and wild-type dispersion=1.42 ±0.31SD. In the context of the constant populations sizes maintained during the experiments the probability that each L1 is represented in the following generation is extremely low, which suggests that the number of offspring that each adult effectively contributes to the following generation may follow a Poisson distribution.



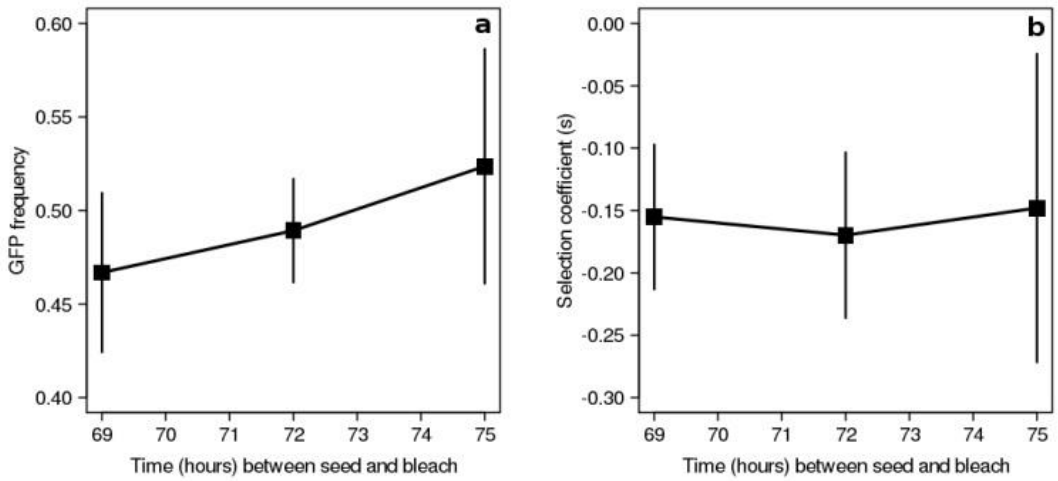
Supplementary Figure S2. Probability of extinction of beneficial alleles at different generations. Following Fisher (Proc Royal Soc Edinburgh 1922, 42: 321), the probability of a single beneficial allele to be present in *x* individuals in the following generation is $f(x) = e^{m(x-1)}$, where *m* equals 1+s .The probability of extinction after two generations is f(f(x)) and three generations f(f(f(x))) and so on. If the allele is initially present in *r* copies the function becomes $[f(x)]^r$. We iterated f(x) for different numbers of generations (from 1 to 50), two different selection coefficients (s= 0.001 or 0.1) and two different initial numbers (r = 1 or 5). The probability of extinction at different generations is shown with the invasion of 1 (solid lines) or 5 (dashed lines) individuals into a resident population. The magnitude of selection and the initial number of invaders influences the probability of extinction, most clearly seen at later generations (s=0.001 for gray lines and s=0.1 for black lines). Note that the experiments reported in the main text followed extinction during 5 generations to assure that the input of novel mutations was negligible and thus keep the assumption only one type of invader allele and one type of resident allele are present during the competitions. The ultimate probabilities of extinction, obtained with the approximation of Barrett et al. (Genetics 2006, 174: 2071): $P_{ext} = e^{-2sn}$ **,** where n=5 invaders (triangles) or n=1 invader (square) and s=0.1 (filled) or s=0.001 (gray). See also **Figure 4c** in the main text.



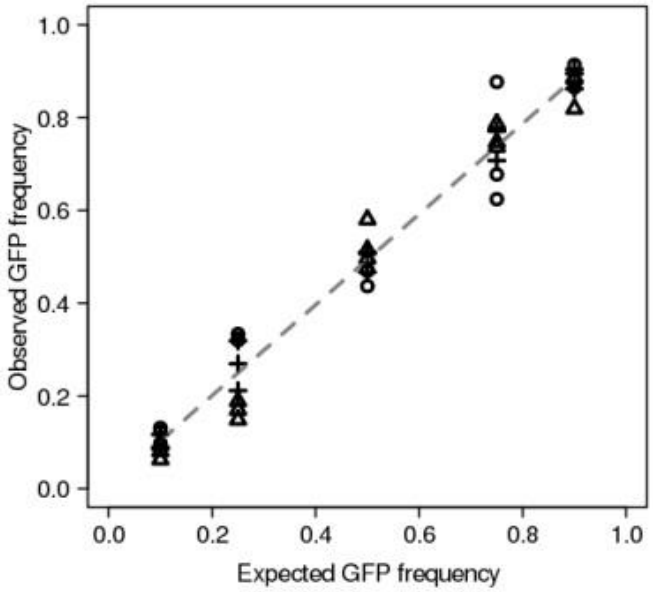
Supplementary Figure S3. Embryo laying rates and successful embryo passaging after bleach. Inbred lines were revived from -80ºC stocks and expanded for two generations under a common environment. In the third generation, 48h after seeding of L1s (see **Methods** in main text), worms were handpicked to 6cm NGM-lite plates spotted with 10uL of *E. coli*. These were left to lay embryos for 17h, 21h, or 24h until scoring of number of embryos and L1s took place for the estimation of embryo laying rates. In parallel, from the same growth plates, single individuals were handpicked at 58 hours, 68 hours and 72 hours after seeding of L1s, and placed into single wells of 96 well plates, containing 10 µl of the M9 buffer. Worms were subjected to a 1M KOH: 5% NaOCl solution for 5 minutes and 300µl of M9 buffer was then added to each well. Plates were spun down, photographs were taken for each well and the number of embryos was scored for number to estimate the success of the "bleaching" protocol. Embryo laying rates as the number of embryos left on the assay plate and released from the adults upon bleaching. GFP data is shown in green lines and wild-type data in gray. Error bars are 2SEM based on 40 (**a**) or 48 (**b**) replicates. Rates of embryo laying until the time of bleach is higher in the wild-type line (panel **a**; ANCOVA: line $F_{1,262}$=62.9, p<0.001; time to bleach $F_{1,262}$=155.0, p<0.001, line*time to bleach $F_{1,262}$=29.1, p<0.001). However, the number of embryos retained and released upon bleaching is higher in the GFP lines at both time points (panel **b**; ANOVA, line $F_{1,169}$=27.19, p<0.001; time to bleach $F_{1,169}$=4.7, p=0.03). GFP hermaphrodites are more sensitive to the bleach solution (panel **c**, proportion of adults disrupted by the bleach solution shown in black bars, those that remained intact after bleach in white bars; χ_1^2 =14.59, p=0.0001). This assay shows that the wild-type inbred line had higher embryo laying rates than the GFP line. By the time of the bleach at 72h, the usual time of bleach in all experiments reported in the main text, the wild type hermaphrodites have laid more embryos than the GFP hermaphrodites. However, after bleaching the number of embryos that are passaged is higher for the GFP line. It thus appears that the GFP hermaphrodites retain a larger proportion of embryos than wild type hermaphrodites (see next **Supplementary Figure S4**). Though wild type hermaphrodites have higher fecundity they are more resistant to the bleach solution. Variation in the numbers of embryos that are successfully passaged can thus explain positive selection on the GFP line.



Supplementary Figure S4. Embryo retention rates in inbred lines. Inbred lines were revived from -80ºC stocks and expanded for two generations under a common environment. On the third generation, GFP individuals were mixed with wild type individuals at equal proportions as L1 larvae. After 69 hours, 72 hours or 75 hours after seeding of the L1s, individuals were handpicked and placed on a slide, scored for GFP expression, and scored for the number of embryos present in the uterus. The number of embryos is shown for GFP (green) and wild-type (gray) individual uterus. Error bars are 2SEM based on 46 to 56 replicates. GFP individuals retain a higher number of embryos than wild-type individuals. In both lines this number reduces with time and an apparently higher rate in GFP individuals than wild-type individuals (ANCOVA: line $F_{1,310}$=58.7, $p<0.001$; time $F_{1,310}$=88, $p<0.001$ and line*time $F_{1,310}$=8.1, $p<0.01$). The GFP line retains more embryos after fertilization than the wild type line. This variation is apparent both before and after the usual time of the bleach protocol (until 72h), but not after (at 75h). Together with the results of Figure S3, at 72h it is likely that the GFP hermaphrodites leave more offspring than the wild type hermaphrodites. Though this trait is only a measure of embryo retention rates it might be positively correlated with ovulation rates in *C. elegans*, and is sometimes used as its proxy (see for example, J. McCarter et al. Develop. Biology 1999, 205:111). Variation among lines in embryo retention rates could explain positive selection on the GFP line.



Supplementary Figure S5. L1s left on the competition plates and selection. In the same assay as that shown in Supplementary Figure S4, worms were subjected to 1M KOH: 5% NaOCl solution for 5 minutes, washed with M9 buffer and kept for 24 hours as in the invasion and competition experiments. GFP proportions were measured both in the individuals that were left on the plates, after washing and those that resulted from the egg hatching as in the competitions done at intermediate frequencies reported in the main text (see **Methods** in main text). (**a**) GFP proportions of L1s left on the competition plates is shown as a function of time between seed of L1s and the bleach protocol. GFP proportions tend to increase with time between seeding and bleaching, a result however that is not statistically significant (ANCOVA: time $F_{1,28}$=3.08, p=0.09, using the logit transformed frequencies) (**b**) Selection coefficients estimated by using **equation 1** (see **Methods** in main text) on the GFP proportions observed as L1s in the following generation. No effects of time of bleach on selection are detected (ANCOVA: time $F_{1,28}$=0.01, p=0.9). GFP hermaphrodites lay fewer embryos (**Supplementary Figure S3**) but retain more embryos (**Supplementary Figure S4**) than the wild type hermaphrodites. There seems however to be a tendency for those GFP embryos that are laid to stick more to the culture plates than the wild type embryos when the washing to the bleaching protocol is performed. These "unwashed" embryos are not passaged to the following generation. Variation in this trait among lines could explain negative selection on the GFP line.



Supplementary Figure S6. Titration of the GFP counts on L1s. 5 different mixtures of GFP and wild type L1s were done from separate cultures of the GFP and wild type inbred lines. After mixing, GFP proportions were scored under a microscope, using a similar protocol as that used for the experiments (see Methods). For each mixture three replicates were done by re-estimating densities (different symbols), and for each replicate three photographs were obtained for scoring. A mean of 105 individuals (±14SD) was used per estimate. Regression analysis reveals that the expected GFP proportions explain most of the variation (R^2=0.97), with the slope being equal to 0.97. Our method to estimate GFP proportions at the L1 stage is adequate and should introduce little error when estimating selection. The GFP genetic construct appears to be fully penetrant.

Supplementary Table S1. Counts of GFP individuals with 2 GFP invaders at generation 0 (G0).

Replicate	G3	G4	G5
1	0	0	0
2	3	5	5
3	7	5	3
4	1	7	16
5	0	0	0
6	4	5	4
7	0	0	0
8	11	8	5
9	7	4	6
10	9	2	3
11	0	0	0
12	1	1	2
13	5	3	5
14	4	1	1
15	22	11	19
16	10	1	5
17	13	1	0
18	0	0	0
19	11	3	6
20	8	4	2
21	20	4	3
22	9	14	12
23	0	0	0
24	5	8	7
25	1	0	0
26	9	1	1
27	14	5	9
28	7	6	10
29	7	2	9
30	0	0	0
31	8	1	1
32	1	0	0
33	0	0	0
34	0	0	0
35	0	0	0

Supplementary Table S2. Counts of GFP individuals with 5 GFP invaders at generation 0 (G0).

Plate	G3	G4	G5
1	2	2	3
2	9	9	6
3	10	5	9
4	11	3	7
5	7	12	4
6	2	4	4
7	11	5	1
8	0	0	0
9	3	2	1
10	0	0	0
11	0	0	0
12	4	6	7
13	31	23	64
14	1	6	15
15	0	0	0
16	2	8	56
17	4	10	17
18	6	13	20
19	5	10	56
20	16	8	31
21	0	0	0
22	1	2	4
23	8	14	33
24	3	6	6
25	0	0	0
26	9	16	90
27	8	24	11
28	1	12	22
29	3	11	25
30	2	13	18
31	9	9	7
32	5	5	6
33	2	1	3
34	6	7	16
35	18	22	35
36	0	0	0
37	2	14	14
38	10	4	10
39	5	24	34
40	7	8	22
41	3	3	4
42	4	7	12
43	6	5	20
44	19	16	118
45	2	4	8
46	11	19	44
47	5	16	61
48	4	3	2
49	9	34	24

50	10	19	55
51	25	28	83
52	15	13	33
53	2	1	1
54	20	24	132
55	4	6	5
56	5	8	15