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A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*

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When both genotype and environment are held constant, 'chance' variation in the lifespan of individuals in a population is still quite large. Using isogenic populations of the nematode *Caenorhabditis elegans*, we show that, on the first day of adult life, chance variation in the level of induction of a green fluorescent protein (GFP) reporter coupled to a promoter from the gene *hsp-16.2* predicts as much as a fourfold variation in subsequent survival. The same reporter is also a predictor of ability to withstand a subsequent lethal thermal stress. The level of induction of GFP is not heritable, and GFP expression levels in other reporter constructs are not associated with differences in longevity. HSP-16.2 itself is probably not responsible for the observed differences in survival but instead probably reflects a hidden, heterogeneous, but now quantifiable, physiological state that dictates the ability of an organism to deal with the rigors of living.

Chance has a large and probably ineradicable role in determining variation among individuals in age at death[1,2]. In humans, as well as populations of laboratory animals, 60–90% of the variation in age at death is independent of genotype[3]. In isogenic populations (where genetic variance is essentially zero), in a uniform environment, some individuals die early in life and others live for a long time[1,4]. Differences in individual lifespan in *Caenorhabditis elegans* populations can be as great as 50-fold[4,5] and still have almost as much variation in time of death as do the human population of the US[1,2,6]. Such observations make suspect the popular notion of a "genetic

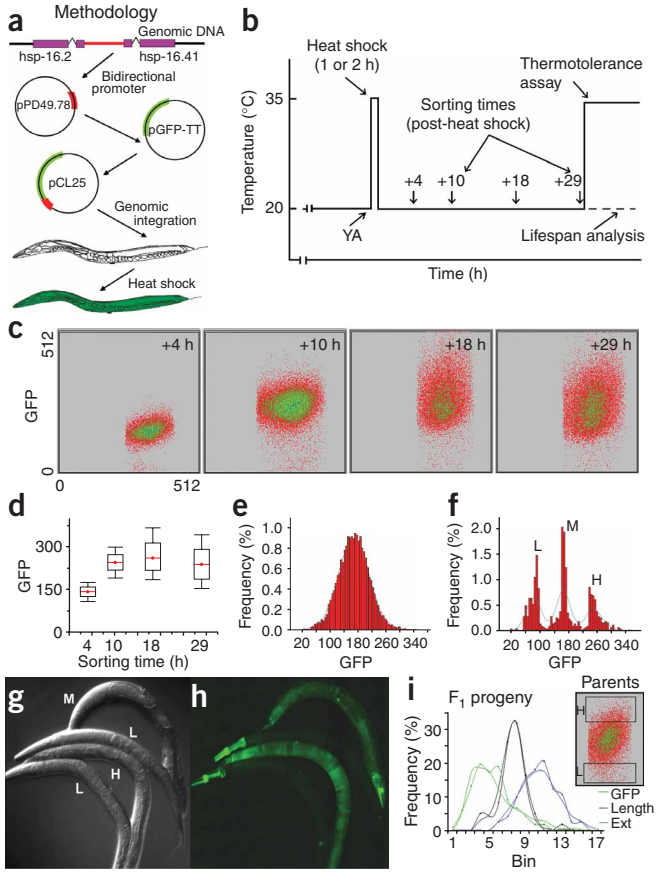

Figure 1 Overview. (**a**) Outline of experimental design and construction of TJ375. (**b**) Schematic of *hsp-16.2*::*GFP* induction, sorting and analysis. (**c**) Individual fluorescence data from a representative experiment. Increasing density of events is color-coded (red to blue). (**d**) Box–and-whisker plots summarizing fluorescence distribution of worm populations at each sorting time after heat shock. (**e**) Distribution of GFP levels in a typical population 19 h after induction by a 2-h pulse at 35 °C (mean ± s.d. = 168.0 ± 44.9 GFP units); the green line shows a normal distribution with the same mean and s.d. (**f**) Distribution of individuals selected in a sort of worms expressing low (L), median (M) and high (H) levels of GFP. (**g**,**h**) Representative worms from each of the three subpopulations in **f**. (**i**) Properties of progeny from parents expressing high or low levels of GFP, showing that level of GFP expression is not heritable. Shown are the population distributions for three parameters: worm length (black lines), extinction (EXT, blue lines) and fluorescence (GFP, green lines). There were no significant differences between progeny derived from the original subpopulations expressing high or low levels of GFP for any of the three parameters ($P > 0.3$, *t*-test).

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program that regulates longevity"[7]. Instead, geriatric, demographic and evolutionary evidence suggests an alternative paradigm of aging that encompasses a variety of often highly plastic processes, influenced by genetic, environmental and stochastic phenomena[1,2,6]. Here we show that the ability of individual isogenic worms to respond to stress on the first day of adult life has a large stochastic component and is a good predictor of their subsequent longevity.

The optical transparency of *C. elegans* allows for noninvasive visual assessment of living worms without compromising subsequent measurement of longevity. We used a chromosomally integrated transgenic strain (TJ375) containing the 400-bp *hsp-16.2* promoter coupled to the gene encoding green fluorescent protein (GFP) but encoding no HSP-16.2 product (**Fig. 1a**). This reporter provides an accurate assessment of the total amount of native HSP-16.2 protein[8]. We observed no detectable GFP in uninduced worms (**Supplementary Fig. 1** online), but GFP became readily apparent after exposure to a 1- to 2-h heat shock at 35 °C (**Fig. 1b**) and peaked 15–18 h later (**Fig. 1c**,**d**).

Heat-shocked populations had a wide and essentially normally distributed variation in individual GFP fluorescence (**Fig. 1c–g**), even though individuals were isogenic and grown in an environment designed to minimize environmental heterogeneity. This variation was observed as soon as GFP expression was detectable and continued until GFP had completely dissipated (several days, data not shown). The degree of heterogeneity increased markedly with time (**Fig. 1c**,**d**) and was replicable, quantifiable and heritable (**Fig. 1g–i**).

We considered whether *hsp-16.2::GFP* expression might predict longevity. Because our initial findings (on individual, isogenic worms measured manually; **Supplementary Fig. 2** online) suggested that there was a significant correlation between GFP expression and subsequent longevity ($r = 0.48$; $P = 0.002$), we extended our studies to large populations. We sorted worms into classes with high, median or low GFP expression at various times after heat induction (**Fig. 1b**,**f**) and then tested their resistance to a lethal thermal stress or kept them for longevity analysis. We routinely observed significant differences in longevity and thermotolerance among worms that expressed GFP at high, median or low levels (**Figs. 2** and **3**). When sorted after a 2-h induction at 35 °C, worms expressing different levels of GFP showed large differences in mean remaining lifespan and thermotolerance. In a typical experiment, we found mean remaining lifespans of 16.4 d in worms expressing the highest levels of GFP after heat shock but only ~3.2 d in worms expressing the lowest levels of GFP after heat shock (**Fig. 2a** and **Supplementary Table 1** online). Worms expressing the highest GFP levels also had greater thermotolerance (9.5 h) than the average (6.7 h) or than worms expressing the lowest levels of GFP (4.0 h; $P < 0.001$; **Fig. 2d**).

We next determined whether sorting at different time intervals after the heat shock affected differences in survival. We sorted worms at various times after induction and found difference in mean remaining lifespan to be as much as 10–15 d (**Fig. 2b** and **Supplementary Table 2** online), averaging 8.0 d over all 19 experiments (**Fig. 2c**). After 2 h of induction, lifespan averaged 15.1 d in worms expressing the highest levels of GFP and only 7.1 d in worms expressing the lowest levels of GFP, more than a twofold difference ($P = 8.0 \times 10^{-29}$; **Fig. 2c** and **Supplementary Table 1** online). Sorting earlier than 9 h after induction gave nonsignificant results. Worms expressing different levels of GFP also had significantly different thermotolerance 9–36 h after induction (**Fig. 2d**,**e** and **Supplementary Tables 1** and **3** online) in 14 of 16 replicates. This difference in thermotolerance was very robust, averaging ~3.4 h ($P = 1.7 \times 10^{-28}$; **Fig. 2f** and **Supplementary Table 1** online). Differences in survival and thermotolerance were highest ~18 h after induction, when variance was maximal.

In previous studies, incubation of the TJ375 reporter strain for 2 h at 35 °C resulted in subsequent thermotolerance and increased longevity in a process called hormesis[8]. In the experiments reported here, the 2-h heat induction also resulted in hormesis for both longevity and thermotolerance, but only in a subpopulation of worms. Using a discrete two-population frailty model, we found that 27% of the worms were damaged by this treatment (D.W., S.L.R., T.E.J. & J.W.V., unpublished data). These results are different because we used new induction conditions to maintain a more uniform environment (abrupt versus slow temperature shift). When we decreased induction time to 1 h, we observed significant differences in survival between worms expressing the highest and lowest levels of GFP in seven of nine experiments (**Fig. 3a–e** and **Supplementary Tables 1**, **2** and **4** online). Average lifespan differed by as much as 14 d in one study (**Fig. 3b**). Furthermore, after 1 h of heat shock, we observed a robust hormesis effect, consistent with previous observations[9,10].

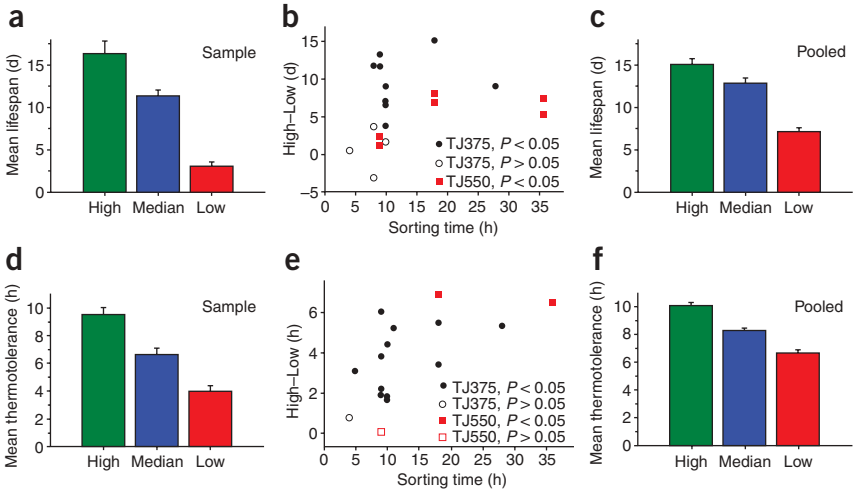

Figure 2 Survival and thermotolerance of worms previously sorted on differential *hsp-16.2*::GFP expression after a 2-h heat shock. (**a**) A representative longevity assessment showing mean remaining adult lifespan after heat shock (mean ± s.e.m.; high = 16.4 ± 1.5 d, median = 11.3 ± 0.7 d, low = 3.2 ± 0.4 d; $N = 30$; $P < 0.001$). (**b**) The difference in average longevity of subpopulations of worms expressing high and low levels of GFP for every study is shown as a point. Significant differences in survival are shown as filled symbols; nonsignificant differences, as open symbols. TJ375 symbols are black and TJ550 are red. Details are given in **Supplementary Table 2** online. (**c**) Combined data for all 13 longevity experiments using TJ375 (high = 15.13 ± 0.61 d, $N = 530$; median = 12.92 ± 0.56 d, $N = 545$; low = 7.13 ± 0.49 d, $N = 550$). Data for TJ550 are given in **Supplementary Table 2** online. (**d**) Thermotolerance (survival at 35 °C) of worms derived from the same populations that were sampled to generate the longevity data in **a** (mean ± s.e.m.; high = 9.5 ± 0.5 h, median = 6.7 ± 0.4 h, low = 4.0 ± 0.4 h; $N = 31$–33; $P < 0.00001$). (**e**) The difference in thermotolerance of subpopulations of worms expressing high and low levels of GFP for each experiment is shown as a point (symbols as in **b**). (**f**) Combined data for all thermotolerance experiments (high = 10.07 ± 0.20 h, $N = 394$; median = 8.27 ± 0.15 h, $N = 401$; low = 6.68 ± 0.18 h, $N = 404$; $P < 10^{-10}$).

To correct for possible inter-relationships between the effect of heat on survival and its effect on fertility[11], we crossed two temperature-sensitive fertility mutations, *fer-15(b26)* and *spe-9(hc88)*, into the TJ375 reporter strain to form a new strain, TJ550. At the nonpermissive temperature, the combination of both mutations completely blocked reproduction but not germ-cell formation or proliferation[5]. We still observed significant differences in survival between worms on this background expressing the highest and lowest levels of GFP in all six replicates (**Fig. 2b** and **Supplementary Table 2** online).

Individual differences in *hsp-16.2::GFP* reporter expression may result from genetic variation: epigenetic changes may occur in isogenic individuals during propagation that lead to differential inactivation or expression of one or more of the large number of repeats in the transgenic array present in the reporter strains[12]. To address this possibility, we determined whether differences in levels of GFP expression were heritable. We sorted a population 11 h after heat shock into subpopulations containing a few hundred of the initial population of 60,000 worms (**Fig. 1i**). We collected progeny, allowed them to grow to maturity, induced them by heat shock and assessed their levels of GFP expression. Progeny of worms expressing the highest and lowest levels of GFP had almost identical average levels (298.0 versus 288.6 GFP units) and variance in GFP expression ($P = 0.5$, χ^2 test for distributional difference; **Fig. 1i**), essentially recapitulating observations from the parental population. Therefore, precise level of GFP expression is not heritable. Although further experimentation may identify discrete causal factors that determine variance in GFP expression, our results were obtained from an isogenic population, maintained in a uniform environment during their propagation. Nonheritability of GFP expression level suggests that there is a large underlying stochastic component specifying level of GFP expression in individual worms, similar to that observed in bacteria[13,14].

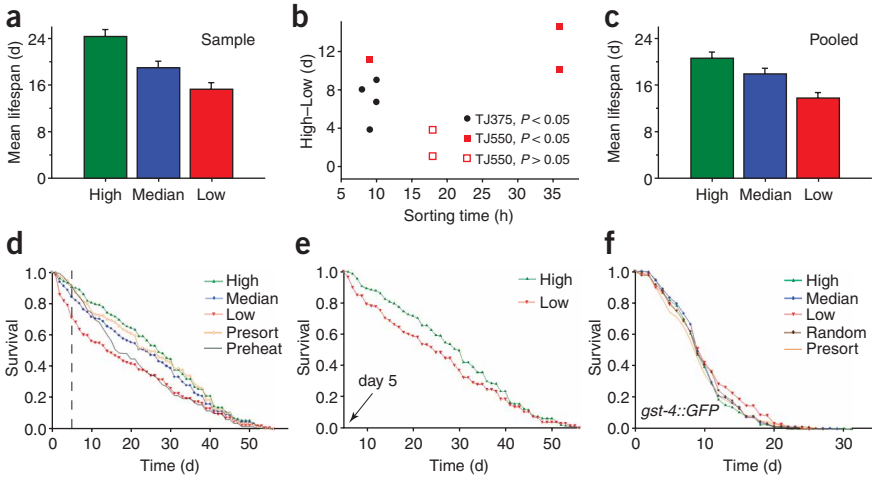

Figure 3 Survival of worms previously sorted on differential *hsp-16.2*::GFP expression after 1 h of induction at 35 °C. Sorting and other conditions were as described for **Figure 2**. (**a**) Data from a typical longevity analysis shown (mean ± s.e.m.; high = 24.4 ± 1.1 d, median = 18.7 ± 1.1 d, low = 15.35 ± 1.0 d; $N = 40$; $P < 0.025$). (**b**) The difference in average longevity of the subpopulations of worms expressing high and low levels of GFP for each of nine experiments is shown by a point. Details are given in **Supplementary Table 2** online. (**c**) Combined data for all longevity experiments (mean ± s.e.m.; high: 20.86 ± 0.93 d, median = 18.20 ± 0.80 d, low = 14.03 ± 0.85 d; $N = 149$–150; $P = 0.03$ for high versus median and $P < 0.001$ for other comparisons). (**d**) Survival trajectories of worms used to generate the data in **a** (see also **Supplementary Tables 1 and 4** online). (**e**) Survival trajectories of subpopulations of worms expressing high and low levels of GFP (as in **d**) plotted from 5 d onward. The curves are significantly different ($P < 0.05$). (**f**) Not all GFP reporter constructs are biomarkers for longevity. Worms expressing the oxidative stress reporter *gst-4::GFP* were sorted into populations constitutively expressing high, median or low levels of GFP, and their survival was then assessed. Shown is the combined data ($N = 291$ for each subpopulation) from five independent experiments. Controls include an unsorted population (Presort) and a sorted but unselected population (Random). The curves are not significantly different ($P > 0.1$; see also **Supplementary Table 1** and **Supplementary Fig. 3** online).

Finally, we also asked whether level of GFP fluorescence was a predictor of longevity when GFP was tagged to promoters of non-stress-inducible genes (*myo-2* and *mtl-2*). It was not. Because GFP fluorescence depends on redox activation, we also tagged the promoter of a gene normally activated in response to oxidative stress (*gst-4*) and again found no relationship between GFP levels and subsequent longevity (**Fig. 3f** and **Supplementary Fig. 3** and **Supplementary Table 1** online). We conclude that HSP-16.2 expression level in young adults is a robust predictor of remaining lifespan and that variation in this reporter is not heritable.

For *C. elegans*, mutational analysis has long been the preferred approach for understanding gene action and biological function[15], no less so for aging and lifespan. Despite the success of the genetic approach in explaining lifespan extension between distinct genotypes in *C. elegans*, most lifespan variation is not under genetic control. Even under rigidly controlled laboratory conditions, 60% of the variation in longevity in F_2 intercrosses in nematodes is not genetic[16]. Similar observations have been made in all species that have been studied[1,3]; in humans, only ~25% of the variation in lifespan (even after excluding early deaths due to childhood disease and accident) is due to measurable genetic effects[1,2,17], leaving most variation in lifespan unexplained or 'environmental', some of which results from chance or stochastic events in individuals[1,2].

Stochastic variation arises from fundamental thermodynamic and statistical mechanical considerations. A large fraction of individual variation in lifespan must stem from the fact that life results from an integrated series of metabolic reactions that themselves are under physical constraints of the specificity and rigidity with which they, too, can be regulated[18]. At the molecular level, two points are germane to this study. First, when the number of molecules regulating a biological process becomes countably small, chance distributions come into play such that some regulatory molecules can vary severalfold between individual cells[19]. Second, the Maxwell-Boltzmann (M-W) equation specifies the distribution of kinetic energies among molecules and requires kinetic energy to be a distributed function. This equation was used to develop a general theory explaining mortality kinetics[20]. Several sources of variation at the molecular level could conceivably alter GFP (HSP-16.2) expression level and simultaneously affect more global processes. These include intracellular differences and fluctuations in the rates of molecular processes such as transcription, ribosome loading and translation (as previously postulated[21]). Chance variation in the number of HSF effecter molecules present in each cell at the time of heat shock also could have marked phenotypic consequences. Variation in the frequency of mitochondrial genomic rearrangements, as previously observed in isogenic populations of

C. elegans[22–24], could have an effect. There is a growing body of research describing variation among isogenic individuals at the molecular level, typically in microbial or yeast cultures where such effects can be visualized[13,14,25]. Substantial variation among genetically identical individuals is a fact of nature, and inherent molecular variability implies that biochemical and molecular genetic processes must have inherent variability.

From the earliest studies of aging *C. elegans* populations[26], it has been apparent that individual age at death varies greatly in isogenic populations, with several weeks separating those dying on the first day of adult life (excluding larval and embryonic death) from the last to die. In the case of long-lived Age mutants, this span can be several months. Stochastic variation provides a means by which we can start to understand this huge variation in lifespan. Genetic regulatory systems can be viewed in terms of robustness or sensitivity toward chance environmental fluctuations, either maintaining expression of a single phenotype or leading to the expression of a distributed phenotype. When multiple phenotypes are useful, such as for sampling changing environments, genetic systems that have a built-in capacity to uncover variance, or indeed amplify it, could be selected[27]. Such systems might be of particular use to self-fertilizing organisms such as *C. elegans*.

A biomarker of aging is defined as "a biological parameter of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some later age than will chronological age"[28]. This study suggests that level of HSP-16.2 production may be such a biomarker of aging and that it is a robust predictor of individual longevity. The *hsp-16.2::GFP* reporter construct used in this study provided no functional HSP-16.2 protein. It is unlikely that GFP conferred the longevity effect, because other GFP reporters we tested resulted in no differences in longevity. It is also unlikely that endogenous HSP-16.2 proteins alone were responsible for the differences in longevity, because overexpression of HSP-16.2 increases longevity by only a few days[29]. Instead, the *hsp-16.2::GFP* reporter is probably conveying information about the general physiological state of the cell or organism with respect to its ability to withstand stress and its likelihood of subsequent survival. Future studies will probably identify additional biomarkers for longevity in *C. elegans* that also indicate something about the physiologic state of the organism.

METHODS

Strains and construction: TJ375 [*hsp-16.2::GFP(gpIs1)*]. The 431-bp *Hin*DIII-*Bam*H1 fragment from pPD49.78 (a gift from A. Fire, Carnegie Institution of Washington), containing the 393-bp bidirectional promoter from *hsp-16.2/hsp-16.41*, was cloned into pGFP-TT (S65T, I176T) to form pCL25. GFP in pCL25 is in the direction of *hsp-16.2*. pCL25 was integrated into strain N2 (wild-type) using standard injection and γ-irradiation techniques. One integrant was back-crossed into strain N2 ten times (subsequently referred to as TJ375), and it behaved indistinguishably from N2 except that it uniformly fluoresced green (excluding gonad region) when heat-shocked. Expression lasted several days, depending on the length of the heat pulse (data not shown). No GFP expression was observed in the absence of a heat pulse in worms cultured at 16–25 °C. The strains were constructed by S. Henderson and C. Link (University of Colorado).

TJ550 [*spe-9(hc88ts)I; fer-15(b26ts)II; [hsp-16.2::GFP(gpIs1)]*]. TJ375 males were mated to TJ1060 *spe-9(hc88ts)I; fer-15(b26ts)II* worms, and F_2 progeny doubly homozygous with respect to *spe-9(hc88ts)* and *fer-15(b26ts)* were identified on the basis of reduced fertility at 23 °C. Double mutants homozygous with respect to *hsp-16.2::GFP* also were identified by a sublethal heat shock (30 min at 35 °C). The TJ550 line was selected. Fertility assessment of F_1 progeny from TJ550 crossed with BA671 *spe-9(hc88ts)I* and BA713 *fer-15(b26ts)II* independently confirmed homozygosity with respect to the *spe-9* and *fer-15* loci.

Other strains constitutively expressing GFP. CL2122 *dvIs15* [*mtl-2::GFP, pPD30.38 (unc-54* (expression vector))] expresses GFP under the control of the intestinal-specific metallothionine-2 (*mtl-2*) promoter. PD4788 *mIs13* [*myo-2::GFP, pes-10::GFP, gut::GFP*] expresses GFP under the control of the pharynx-specific myosin-2 (*myo-2*) promoter, the germline-specific pes-10 promoter as well as a gut specific enhancer. In the adult, expression is restricted to the pharynx only. CL2166 *dvIs19* [*K08F4.7::GFP*] expresses GFP under the direction of the glutathione-S-transferase 4 (*gst-4*) promoter. All three strains are transgenic derivatives of Bristol N2 and were gifts from C. Link (University of Colorado).

Nematode maintenance and mass cultures. We used standard techniques to maintain nematode strains[15,16]. For large-scale production of synchronized populations (>120,000 worms), we seeded 16 10-cm NGM/*E. coli* (OP50) plates with 1,200 arrested first-stage larvae (L1) each. After 76 h at 20 °C, we collected gravid adults in (8 ml of S-Basal medium per plate), transferred them into a single tube and allowed them to pellet under gravity (~5 min at room temperature). After washing the pellet to remove eggs and hatched L1 progeny (five times in 50 ml of S-Basal medium), we resuspended the gravid adults in ~1 ml of S-Basal medium and then spread them onto four × 10-cm NGM/OP50 plates. We provided sufficient food for cultures to starve and stop laying eggs after 4 h. After ~24 h, we washed hatched L1 larvae and gravid adults from the plates (in 8 ml of S-Basal medium) and separated and discarded the gravid adults by allowing them to differentially pellet under gravity (~5 min). After determining the concentration of L1 larvae, we seeded them onto 16 new starter NGM/OP50 plates (1,200 larvae per plate) or 12 10-cm NGM/RW2 (wild-type *E. coli*) plates supplemented with 2% peptone (RNGM, 12,000 larvae per plate). We incubated the plates at 20 °C (TJ375) or 25 °C (TJ550), allowed the cultures to reach adulthood (65 h or 50 h, respectively), collected the adults (in 8 ml of S-Basal medium per plate), pooled them, washed them (twice with 50 ml of S-Basal medium) and finally resuspended them in 3 ml of S-Basal medium. We supplemented the populations with 1×10^{11} OP50 per RNGM plate at +52 h to prevent starvation.

Heat shock. We heat-shocked the pooled gravid adults (35 °C) for 1 or 2 h (with rotation, 100 r.p.m.) after transferring them into preheated S-Basal medium (35 °C) containing 1×10^9 OP50 ml^{-1} and 10 μg of cholesterol ml^{-1} (liquid food, 300 worms per ml). After heat shock, we pelleted the worms by gravity (~ 5 min) and then quickly transferred them to prechilled (20 °C) liquid food. We maintained cultures at 20 °C with rotation (120 r.p.m.) until ready for sorting. We refed the cultures every 12–16 h with fresh liquid food. For each experiment, we retained a subsample of worms not exposed to the heat treatment in 4 ml of liquid food (20 °C) in a 6-cm dish for later use.

Sorting procedure. We collected recovering worms by gravity, washed them (twice in 50 ml of S-Basal medium) and then resuspended them (1,000 worms per ml) in S-Basal medium plus 1×10^9 OP50 (this concentration of bacteria did not interfere with subsequent sorting). We sorted worms using a COPAS Biosort 250 Worm Sorter (Union Biometrica Inc., Harvard Biosciences) using three criteria: length, optical absorbance (extinction) and integrated fluorescence intensity at 488 nm (GFP). We defined values for length and extinction (gating) before beginning each sort experiment; typically 60–75% of the entire population was included. We then sorted four subpopulations, each expressing differing amounts of GFP: highest 2%, lowest 2%, a median sample and a gated-only sample. Percentages represent fraction of the total starting population. We also kept worms that were not subjected to the COPAS sorting as controls in each experiment; we found negligible changes in this population compared with the gated-only sample (data not shown). Worms were not recycled through the sorter, and so a population of 30,000 individuals yielded ~150 worms per group. Worms were sorted directly into 6-cm dishes containing 4 ml of liquid food and incubated at 20 °C until further use. All sorting was done at room temperature.

Thermotolerance assay. After sorting, and at defined times after the original 1-h or 2-h heat shock, we transferred groups of 30 worms from each sample from liquid food onto 6-cm NGM/OP50 plates and then immediately placed them into an incubator at 35 °C. We scored survivorship until 100% death at periodic intervals (typically over a span of 16 h).

Longevity assay. All lifespan analyses were done in liquid food as described previously[16]. We cultured 60–80 worms per subpopulation in 4 ml of liquid food, transferred them every day to fresh food while egg-laying and then transferred them every third day until none remained alive.

Microscopy. We collected fluorescent images using a Zeiss Axioskop. We immobilized nematodes with 1 mM sodium azide, mounted them on 2% agarose cushions and then captured images using a PCO SensiCam charge-coupled diode camera.

Heritability of fluorescence intensity. We grew 60,000 arrested L1 larvae to adulthood (72 h, 20 °C), heat-shocked them for 1 h at 35 °C, allowed them to recover for 11 h at 20 °C and then sorted them according to GFP expression (highest 2% and lowest 2%; ~350 worms each) exactly as described above. We transferred each subpopulation sequentially onto two NGM/OP50 6-cm plates and allowed them to lay eggs for 20 h at a time (this strategy was adopted to increase the number of F_1 progeny for resorting). We pooled each set of F_1 progeny, heat-shocked them for 1 h at 35 °C, allowed them to recover for 16 h at 20 °C and then sequentially sorted them. We collected and analyzed statistics for individual F_1 worms.

Statistical analyses. We derived longevity and thermotolerance information for 5,836 and 2,389 individuals, respectively, from a combination of 30 TJ375 data sets (nine were completely independent and sourced from populations of 120,000 worms each). We collected 14 longevity and thermotolerance data sets for TJ550 from a single population of 120,000 individuals. We used a total of almost 2,000,000 worms for this study.

We estimated mean lifespan using the formula

$$MLS = \frac{1}{N}\sum_j \frac{x_j + x_{j+1}}{2} * d_j,$$

where d_j is the number of worms that died in the age interval (x_j, x_{j+1}) and N is the total number of worms. We calculated the standard error of the mean lifespan estimate using the equation

$$SE = \sqrt{\frac{1}{N(N-1)}\sum_j \left(\frac{x_j + x_{j+1}}{2} - MLS\right)^2 d_j}.$$

We compared mean lifespan estimates for two populations by calculating the normalized statistic

$$z = (MLS_1 - MLS_0)/\sqrt{SE_1^2 + SE_0^2},$$

where MLS_0 and MLS_1 are mean lifespan estimates and SE_0 and SE_1 are standard error estimates for populations 1 and 2, respectively.

We used a log-rank test to assess differences in survival curves and a χ^2 test to assess the difference in GFP fluorescence distributions for the progeny experiments. Use of the discrete two-population frailty model mentioned in this paper will be described elsewhere (T.E.J., unpublished data). We carried out all other significance testing using Statistica (1999 edition) software (StatSoft, Inc.) and Gauss (Aptech Systems, Inc).

GenBank accession number. *hsp-16.2*, AC006774.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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