BIOLOGICAL SCIENCES: GENETICS/CELLULAR AND DEVELOPMENTAL BIOLOGY

SUPPLEMENTAL DATA-TEXT

Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants

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Supplemental text

Determining the rate of healthspan decline

All of the healthspan data was compiled to determine the rate of decline in each of the health parameter. The details of the software and the computational method are described in detail in the methods section. Briefly, for each long-lived mutant, a regression curve was fit to each healthspan analysis data and the rate of decline in health was determined. Different statistical tests were used to determine a model that fit the changes in the parameters with age. This model was designed to take both the age and the genotype of the strain into consideration. Each of the long-lived strains was compared to wild type.

Healthspan Data Analysis: Homeostasis-Oxidative Stress

For the oxidative stress healthspan dataset, the final selected model includes a cubic curve for age, genotype and their interactions. Wild type and *eat-2* mutants, show a similar decline in oxidative stress resistance capacity with age (**Table S1**). Although *daf-2* mutants are more resistant to oxidative stress and *ife-2* mutants are sensitive to oxidative stress than wild type, the rate of decline is similar. *clk-1* mutants have a slower rate of decline compared to wild type.

Considering the fact that worms with different genotypes have different life spans, the absolute age for worms with different genotypes may translate into different stages of life in terms of aging. Therefore, we also normalized the age using the ratio of median life span relative to wild type, and refer to this normalized age as "relative age", which is used to replace age in the above model fitting. The final selected model for the oxidative stress healthspan dataset includes relative age, genotype and their interactions predicted by a quadratic equation. Consistently, for wild type, aging results in a decline in the ability to resist oxidative stress (**Table S2**). *ife-2* and *clk-1* mutants are more susceptible to oxidative stress than wild type, and their probability of

dying at older ages due to oxidative stress is similar to that of age-matched wild type worms. Interestingly, although *daf-2* mutants are more resistant to oxidative stress at Day 1, the rate of decline is significantly faster than wild type in an age matched population. This indicates that when normalized for lifespan, long-lived *daf-2* mutants lose their oxidative stress resistance capacity much faster than wild type. Similarly, *eat-2* mutants become more susceptible to oxidative stress than wild type.

Healthspan Data Analysis: Homeostasis-Heat stress

Similar to oxidative stress, a best fit model was determined by a partial likelihood ratio test. The final selected model for the heat stress healthspan dataset was predicted by a cubic equation taking age, genotype and their interactions into consideration (**Table S3**). Compared to wild type, *clk-1, ife-2* and *eat-2* mutants are sensitive to heat stress at Day 1 while *daf-2* mutants are resistant to heat stress. With increasing age, there is no significant difference in the capacity of *clk-1, ife-2* and *daf-2* strains to maintain homeostasis in response to heat stress. However, *eat-2* mutants are resistant to heat stress in mid life but show a faster rate of decline than wild type when old.

Next, we used the normalized age to replace chronological age in the above model fitting. The final selected model in this case was a quadratic equation taking into consideration relative age, genotype and their interactions **(Table S4).** Compared to wild type, *clk-1* mutants are physiologically more sensitive to heat when young-mid life and slightly better when very old. *ife-2* and *daf-2* mutants respond similar to wild type initially but they lose this resistance at a much accelerated rate in the old age matched population. Compared to wild type, *eat-2* mutants lose capacity to withstand heat stress at a much faster rate with age.

Healthspan Data analysis: Movement in liquid media: Thrashing

The thrashing healthspan dataset could be modeled on the basis of a cubic equation to include age, genotype and their interactions (**Table S5**). In all strains, as the animals aged, there was a significant decline in thrashing capacity. Compared to wild type, *daf-2* mutants thrash significantly less while *clk-1* mutants give a more complex scenario. *clk-1* mutants initially move similar to wild type with a higher ,rate of decline than wild type at younger ages and slower than wild type worms at older ages. *eat-2* and *ife-2* mutants initially thrash similar to wild type. However, the rate of decline is higher than wild type for *eat-2* mutants and slower for *ife-2* mutants when compared chronologically. This suggests that *daf-2*, *ife-2* and *clk-1* mutants improve the health chronologically.

Similar to previous parameters, we used normalized or physiological age, which replaced the "chronological age" in the above model fitting. The final selected model for the thrashing healthspan dataset was a quartic equation, which includes relative age, genotype and their interactions (**Table S6**). Aging resulted in a significant decline in thrashing and the effect is quartic for all genotypes (**Table S6**). Compared to wild type, *daf-2* mutants thrash significantly more initially, and the rate of decline is higher at younger ages and the difference decreases with advanced age, whereas. *clk-1* and *eat-2* mutants decline much faster and *ife-2* mutants decline at the same rate..

Healthspan Data analysis: Movement on solid media: distance travelled

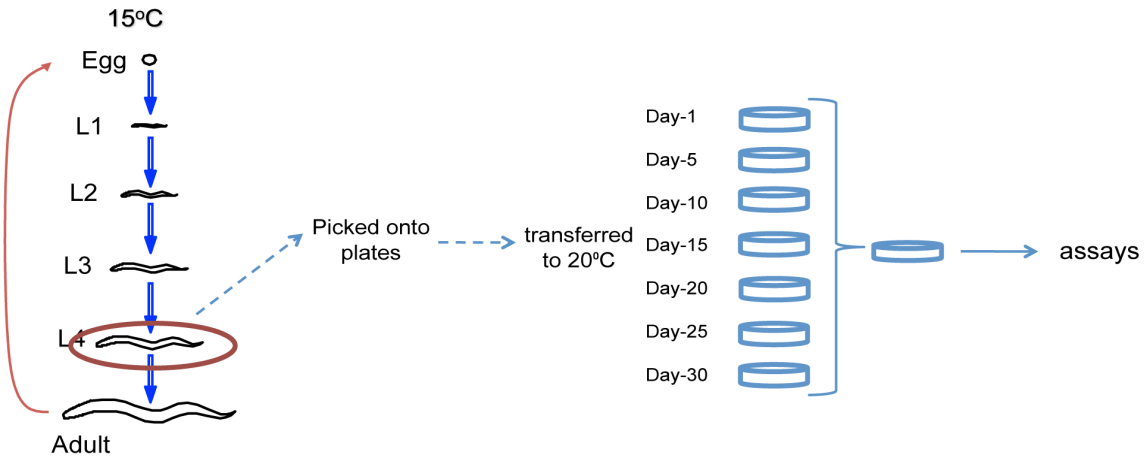
The final selected model for the distance travelled healthspan dataset was a quartic equation which includes age, genotype and their interactions (**Table S7**). Compared to wild type, *eat-2* mutants move significantly less distance initially and then declines faster; *clk-1* and *ife-2* mutants decline slower while *daf-2* mutants show a similar rate of decline (**Table S8**). This

indicates that chronologically *clk-1, ife-2 and daf-2* mutants improve the movement capacity healthspan parameter.

Next we used normalized age that replaces age in the above model fitting. The final selected model for the thrashing dataset includes relative age up to a cubic term, genotype and their interactions (**Table S9).** Compared to wild type, *eat-2* mutants move significantly less cand the rate of decline is much faster in the age-matched population. Both *ife-2 and clk-1* mutants decline slower initially compared to wild type. However, this is followed by a faster rate of decline in *ife-2* mutants than wild type while *clk-1* mutants decline at a similar rate. *daf-2* mutants decline at the same rate as wild type (**Table S10**).

In summary, when comparing the rate of decline of the different healthspan parameters chronologically, the long-lived mutants seem to delay the rate of decline. However, taking the long lifespan of the mutants into account and recalculating the rate of decline gives conflicting results. When compared physiologically, these long-lived animals do not slow down the rate of decline in the healthspan parameters tested. This would result in an expansion of the frailty period indicating a detrimental cost to extending lifespan in the mutants tested in this study.

A.



B.

EXTERNAL STRESS RESPONSE	INTERNAL STRESS RESPONSE	SARCOPENIA	FEEDING RATE
Heat stress resistance	Autofluorescence or accumulation of AGE products	Movement capacity	Pharyngeal pumping
Oxidative stress resistance		Muscle integrity	

Figure S1. Experimental design for measuring healthspan

A: Protocol for cross-sectional study.

B. Different categories and assays used to quantify health.

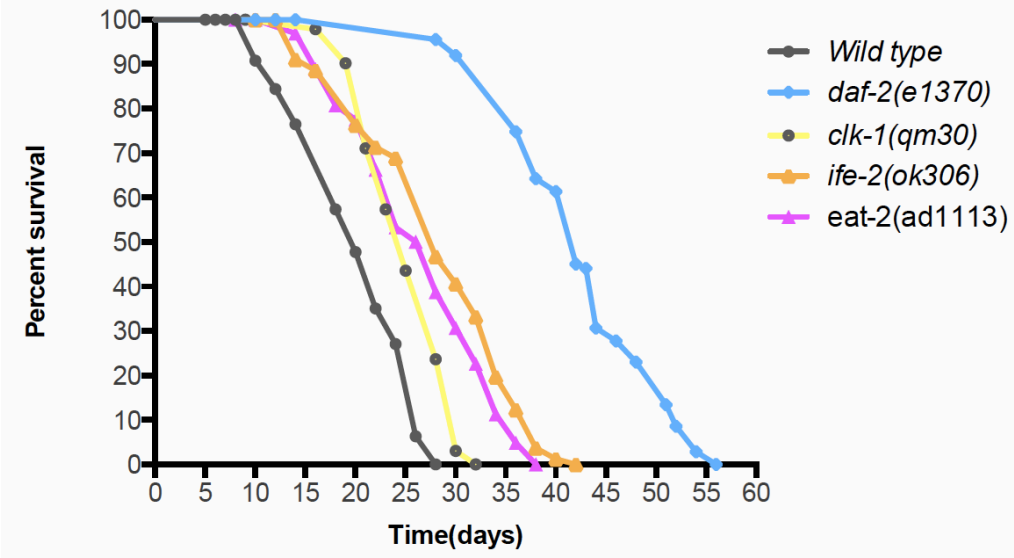


Figure S2: Survival analyses of strains used in the study without FudR.

This analysis was done without FudR at 20°C. The animals were transferred to fresh plates everyday initially and every 2-4 days after they stopped laying eggs. The worms were examined every two days for touch-provoked movement until death. The experiment was repeated twice and graph is the representative of two biological repeats

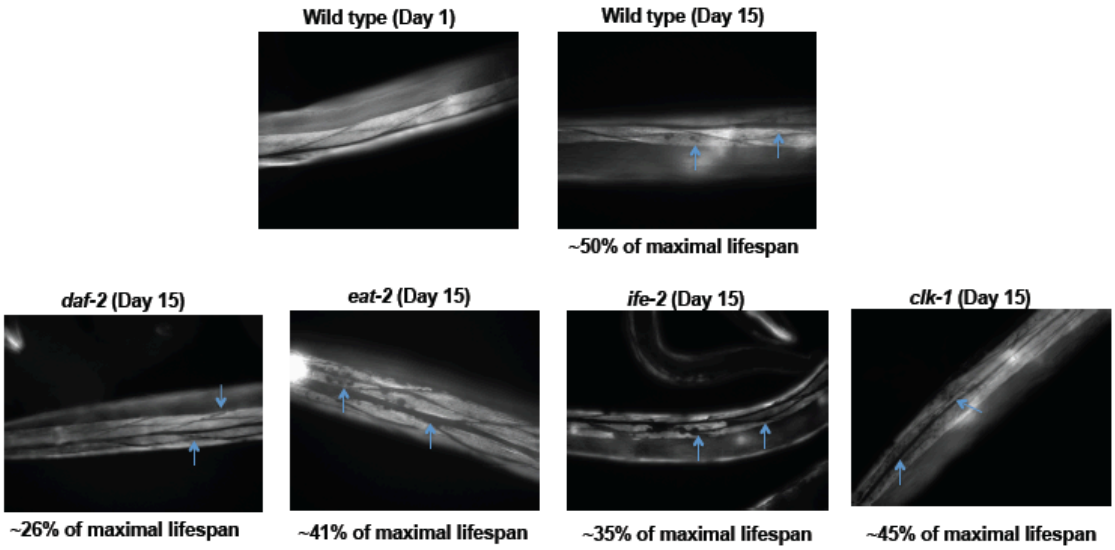


Figure S3: Movement capacity of aging worms: Muscle architecture of animals at Day 15

Phalloidin staining comparing Day 15 for wild type and the long-lived mutants. Wild type worm shows visible degradation at Day 15 (50%) of maximal lifespan (as indicated by the arrows). However, other long-lived mutants show degradation much earlier when normalized for their lifespan.

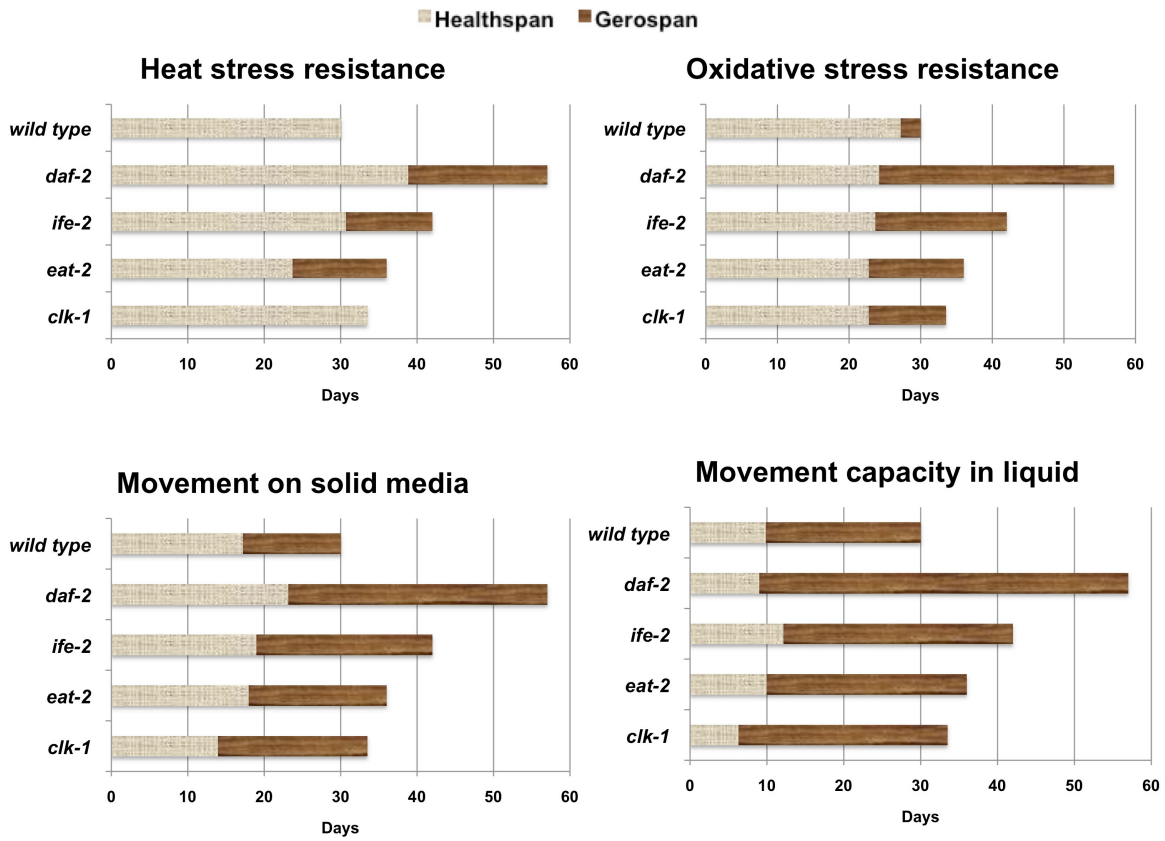


Figure S4. Comparing healthspan vs gerospan in long-lived mutants chronologically (gerospan cut off is set at 30% of wild type functional capacity).

Healthspan is defined as the period of time where the animal has greater than 30% of the maximal functional capacity of wild type (when the mutants have lost 70% of the functional capacity as compared to wild type). Gerospan is defined as the period of time where the animal has less than 30% of the maximal functional capacity of wild type. The mean values of number of days of healthspan and gerospan are plotted in the bar graphs.

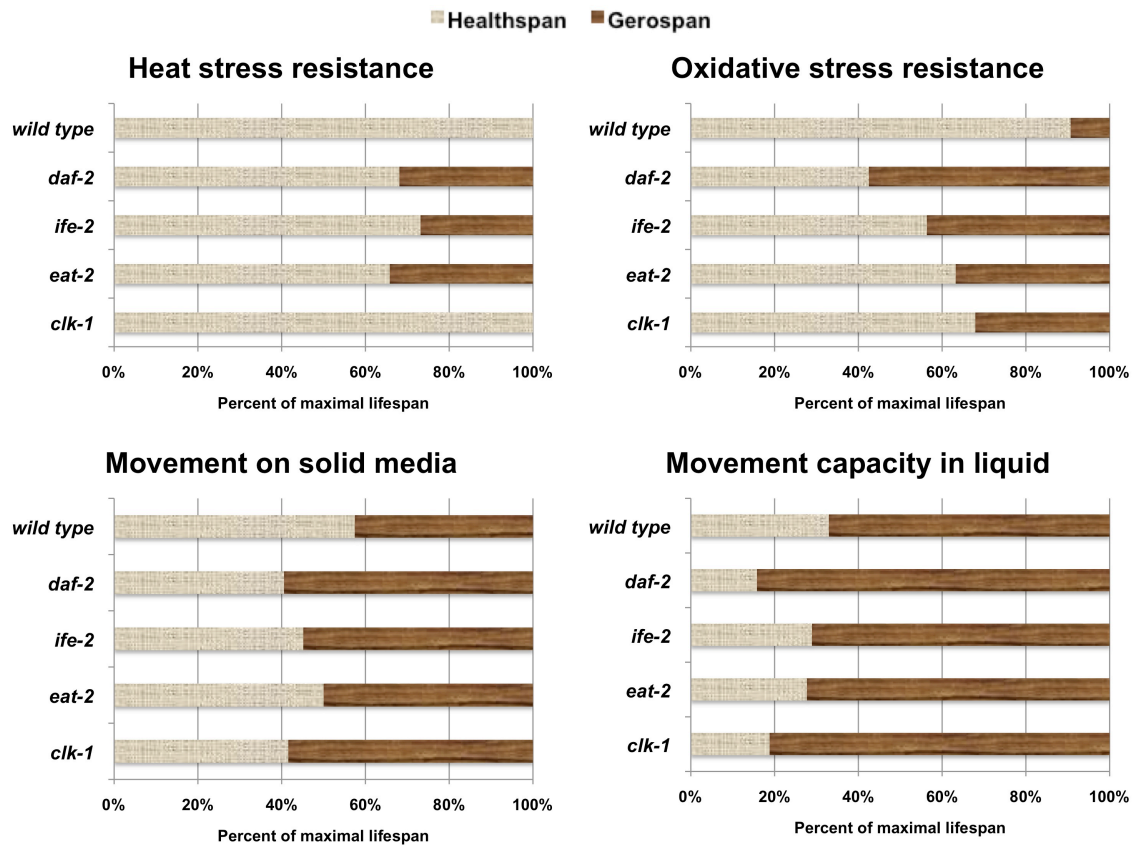


Figure S5. Comparing healthspan vs gerospan in long-lived mutants physiologically (gerospan cut off is set at 30% of wild type functional capacity30%).

Healthspan is defined as the period of time where the animal has greater than 30% of the maximal functional capacity of wild type (when the mutants have lost 70% of the functional capacity as compared to wild type). Gerospan is defined as the period of time where the animal has less than 30% of the maximal functional capacity of wild type. The fraction of the maximal lifespan spent in healthspan and gerospan was calculated by setting the maximal lifespan equal to 100%.

Variable	Log(Hazard Ratio)	Hazard Ratio	P-value	95% confidence interval
daf-2	-3.0110238	0.04924	0.0000000	(0.02739,0.08854)
ife-2	0.5060030	1.65865	0.0317485	(1.04519,2.63216)
Age.Linear	0.2138678	1.23846	0.0007097	(1.09424,1.40168)
clk-1:Age.Quadratic	-0.0190775	0.98110	0.0189986	(0.96559,0.99687)
clk-1:Age.Cubic	0.0004956	1.00050	0.0168632	(1.00009,1.00090)

Table S1. The final variables selected and their hazard ratio estimated from fitting Cox Proportional Hazards Model using absolute age for oxidative stress resistance.

Variable	Log(Hazard Ratio)	Hazard Ratio	P-value	95% confidence interval
clk-1	0.602487	1.82666	1.899e-03	(1.24889,2.67171)
daf-2	-3.322938	0.03605	0.000e+00	(0.02192,0.05927)
ife-2	0.775743	2.17221	1.548e-05	(1.52795,3.08810)
Relative.Age.Linear	0.209459	1.23301	9.226e-14	(1.16692,1.30285)
daf-2:Relative.Age.Linear	0.357051	1.42911	3.775e-15	(1.30741,1.56213)
eat-2:Relative.Age.Linear	0.166664	1.18136	2.529e-03	(1.06024,1.31631)
daf-2:Relative.Age.Quadratic	-0.006970	0.99305	7.857e-05	(0.98962,0.99650)
eat-2:Relative.Age.Quadratic	-0.006308	0.99371	3.161e-02	(0.98801,0.99944)

Table S2. The final variables selected and their hazard ratio estimated from fitting Cox Proportional Hazards Model using relative age for oxidative stress resistance.

Variable	Log (Hazard Ratio)	Hazard Ratio	P-value	95% confidence interval
clk-1	0.515415	1.67433	4.898e-020	(1.00230,2.7970)
daf-2	-2.433932	0.08769	0e+00	(0.05272,0.1459)
eat-2	1.554193	4.73127	2.082e-10	(2.92962,7.6409)
eat-2:Age	-0.600639	0.4846	5.661e-11	(0.45827,0.6564)
eat-2:Age,quadratic	0.060399	1.06226	1.031e-12	(1.04476,1.08010)
eat-2:Age,cubic	-0.001389	0.99861	7.624e-11	(0.99819,0.9990)

Table S3. The final variables selected and their hazard ratio estimated from fitting Cox Proportional Hazards Model using absolute age for heat stress resistance.

Variable	Log (Hazard Ratio)	Hazard Ratio	P-value	95% confidence interval
clk-1	0.494660	1.63994	1.18e-02	(1.11557,2.4108)
daf-2	-2.526621	0.07993	0e+00	(0.05287,0.1208)
eat-2	0.593881	1.81100	3.195e-03	(1.22029,2.6877)
ife-2	0.953624	2.59510	6.664e-08	(1.83584,3.6684)
Relative age;quadratic	0.004841	1.00485	3.647e-06	(1.00280,1.0069)
clk-1:relative age;linear	0.128444	1.13706	3.164e-03	(1.04409,1.2383)
ife-2:relative age;linear	-0.263570	0.76830	7.503e-13	(0.71490,0.8257)
clk-1:relative age;quadratic	-0.05463	0.99455	5.440e-03	(0.99073,0.9984)
daf-2;relative age;quadrative	0.016049	1.01618	0e+00	(1.101302,1.0193)
eat-2;relative age;quadrative	0.017300	1.01745	3.886e-15	(1.01307,1.0218)
ife-2;relative age;quadrative	0.14461	1.01457	0e+00	(1.101163,1.0175)

Table S4. The final variables selected and their hazard ratio estimated from fitting Cox Proportional Hazards Model using relative age for heat stress resistance.

Variable	Df	Sum e	Mean e	F-value	P-value
Experiment	1	1519	1519	13.43	0.00026
Age.Linear	1	592375	59237	5239.95	<2e-16
Age.Quadratic	1	134346	13434	1188.38	<2e-16
Age.Cubic	1	10190	10190	90.14	<2e-16
Genotype	4	9676	2419	21.4	<2e-16
Genotype:Age.Linear	4	7084	1771	15.67	1.9e-12
Genotype:Age.Quadratic	4	2464	616	5.45	0.00024
Genotype:Age.Cubic	4	6597	1649	14.59	1.4e-11
Residuals	1021	115424	113		

Table S5. Analysis of Variance Table using absolute age for movement in liquid.

Variable	**Estimate**	**P-value**
Intercept	87.726773	1.573e-172
Experiment2	-2.485196	1.714e-04
Age.Linear	-5.074239	1.046e-07
Age.Cubic	0.006149	5.405e-03
daf-2	-7.446643	2.256e-02
clk-1:Age.Linear	-8.025711	2.814e-09
daf-2:Age.Linear	-2.343741	2.775e-02
clk-1:Age.Quadratic	0.842777	1.502e-11
daf-2:Age.Quadratic	0.337242	2.603e-04
*ife-2:*Age.Quadratic	0.209932	2.848e-02
clk-1:Age.Cubic	-0.021271	1.451e-11
daf-2:Age.Cubic	-0.009188	4.892e-05
eat-2:Age.Cubic	-0.007618	1.486e-02
ife-2:Age.Cubic	-0.006584	4.565e-03

Table S6. The final variables selected and their coefficients estimate using absolute age for movement in liquid.

Variable	Df	Sum Square	Mean Square	F-value	P-value
Experiment	1	1519	1519	14.65	0.00014
Relative.Age.Linear	1	580805	580805	5602.53	<2e-16
Relative.Age.Quadratic	1	115776	115776	1116.79	<2e-16
Relative.Age.Cubic	1	4251	4251	41.01	2.3e-10
Relative.Age.Quartic	1	0	0	0.00	0.95918
Genotype	4	40054	10013	96.59	<2e-16
Genotype:Age.Linear	4	8292	2073	20.00	7.3e-16
Genotype:Age.Quadratic	4	3467	867	8.36	1.2e-06
Genotype:Relative.Age.Cubic	4	13429	3357	32.39	<2e-16
Genotype:Relative.Age.Quartic	4	6755	1689	16.29	6.1e-13
Residuals	1016	105327	104		

Table S7. Analysis of Variance Table using Relative Age for movement in liquid

Variable	Estimate	P-value
Intercept	78.086479	2.065e-109
Experiment2	-2.506825	7.601e-05
Relative.Age.Quadratic	-1.700208	2.961e-07
Relative.Age.Cubic	0.104801	9.838e-08
Relative.Age.Quartic	-0.001904	4.413e-07
clk-1	18.359689	2.994e-05
daf-2	7.996943	3.999e-02
clk-1:Relative.Age.Linear	-29.495306	8.263e-19
daf-2:Relative.Age.Linear	-27.134624	3.004e-21
eat-2:Relative.Age.Linear	-10.588568	2.380e-03
clk-1:Relative.Age.Quadratic	5.033247	6.292e-14
daf-2:Relative.Age.Quadratic	4.698633	4.648e-17
clk-1:Relative.Age.Cubic	-0.300790	1.221e-09
daf-2:Relative.Age.Cubic	-0.283921	1.929e-12
clk-1:Relative.Age.Quartic	0.006016	6.717e-07
daf-2:Relative.Age.Quartic	0.005786	3.082e-09

Table S8. The final variables selected and their coefficients estimate from fitting linear model using relative age for movement in liquid.

Variable	Df	Sum Square	Mean Square	F-value	P-value
Experiment	1	6.36e+07	6.36e+07	53.26	5.8e-13
Age; linear	1	5.47e+09	5.47e+09	4575.62	<2e-16
Age.Quadratic	1	2.52e+08	2.52e+08	210.93	<2e-16
Age.Cubic	1	6.32e+07	6.32e+07	52.87	7.0e-13
Age.quartic	1	4.49e+07	4.49e+07	37.58	1.3e-09
Genotype	4	2.30e+08	5.75e+07	48.08	<2e-16
Genotype:Age.Linear	4	3.78e+07	9.46e+06	7.91	2.8e-06
Genotype:Age.Quadratic	4	7.23e+07	1.81e+07	15.12	5.1e-12
Genotype:Relative.Age.Cubic	4	2.75e+07	6.88e+06	5.76	0.00014
Residuals	1030	1.23e+09	1.20e+06		

Table S9. Analysis of Variance Table using absolute age for distance travelled experiment

Variable	Estimate	P-value
Intercept	7.226e+03	8.548e-125
Experiment2	-5.031e+02	1.794e=13
Age.Quadratic	-4.767e+01	9.709e-07
Age.Cubic	1.515e+00	1.56e-06
Age.Quartic	-1.025e-01	1.066e-02
eat-2	-1.243e+03	7.596e-04
clk-1:Age.Linear	-4.320e+02	1.637e-03
eat-2:Age.Linear	3.169e+02	2.167e-02
ife-2:Age.Linear	-2.926e+02	1.403e-02
clk-1:Age.Quadratic	3.651e+01	4.068e-02
ife-2:Age.Quadratic	-2.926e+02	1.403e-02
clk-1:Age.Cubic	-7.390e-01	2.149e-02
ife-2:Age.Cubic	-6.215e-01	1.459e-02

Table S10. The final variables selected and their coefficients estimate using absolute age for distance travelled

Variable	Df	Sum Square	Mean Square	F-value	P-value
Experiment	1	6.36e+07	6.36e+07	52.97	6.7e-13
Relative Age.Linear	1	5.47e+09	5.47e+09	4550.26	<2e-16
Relative Age.Quadratic	1	1.67e+08	1.67e+08	138.69	<2e-16
Relative Age.Cubic	1	8.73e+07	8.73e+07	72.65	<2e-16
Genotype	4	3.12e+08	7.80e+07	64.95	<2e-16
Genotype:Relative Age.Linear	4	2.05e+07	5.12e+06	4.26	0.002
Genotype:Relative Age.Quadratic	4	9.65e+07	2.41e+07	20.08	0.00024
Genotype:Relative Age.Cubic	4	3.79e+07	9.47e+06	7.88	6.2e-16
Residuals	1031	1.24e+09	1.20e+06		3e-06

Table S11: Analysis of Variance table using relative age for distance travelled.

Variable	Estimate	P-value
Intercept	7278.1400	3.169e-126
Experiment2	-502.9616	2.107e-13
Relative Age.quadratic	-38.9708	1.944e-05
Relative Age.Cubic	0.9826	2.135e-05
eat-2	-1242.9895	7.878e-04
clk-1;relative age;linear	-540.2387	7.958e-04
eat-2;relative age;linear	503.9392	3.451e-03
ife-2;relative age;linear	-497.2107	1.561e-04
eat-2;relative age;quadratic	-85.2408	5.194e-05
ife-2;relative age;quadratic	31.3118	1.131e-02
eat-2;relative age;cubic	3.1968	1.265e-05

Table S12. The final variables selected and their coefficients estimate using relative age for distance travelled.