Supplementary Materials for:

Reduced lifespan and increased ageing driven by genetic drift in small populations

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Supplementary Methods

Model parameter values

All models used a set of common general parameters: m=0.01, h=0.1, γ=0.05, v=10^{-5}, deme sizes: 16, 40, 100, 190, 251, and 630 individuals. Parameters for the window model were: θ=0.005, number of loci = 2000; and for the lasting-effects model: θ=0.0001, number of loci=100. We assumed that mutations occurred uniformly irrespective of their age of action between age 0 and a maximum age arbitrarily set to 100. The parameters for marker diversity (for both models) were: u=10^{-4}, H=0.67. Deme frequencies were set proportional to 1/n so that all categories contribute equally to the migrant pool. Clearly, the model can only generate qualitative results, as empirical values for several of these parameters are unknown. In addition, for lifespan (but not for the rate of ageing) an additional contribution of mutations with age-independent effects is expected.

Details of life-table assays

To test the qualitative predictions of our models empirically, we assessed the lifespan and age-dependent mortality of water fleas (*Daphnia magna*; Crustacea) from eight European populations with strongly different levels of genetic diversity ("focal populations", Table 1). Genetic diversity was determined using 32 microsatellite markers (following methods described in Haag and Walser 2012). The four least diverse populations originated from physically small ponds (coastal "rock pools") in Finland and Russia (hereafter referred to as "small" populations), while the four more diverse populations are from substantially larger inland ponds in Russia, Germany, and Belgium (hereafter referred to as "large" populations). Pond size is thus bimodally distributed, and the four small populations are also located further

north than the four large populations (Walser and Haag 2012). Hence, genetic diversity is partially confounded with environment (rock pool vs. non-rock pool) and latitude in our data, and therefore we took both these factors into account in the statistical analysis by including them as covariates in our models (for details of the statistical analysis see below).

Lifespan was assessed using standard life-table assays (Ebert and Jacobs 1991; Dudycha 2001), keeping individuals singly in 50 ml tubes under standard laboratory conditions until death. *Daphnia* are ideal for life-table studies, as their cyclical parthenogenetic life cycle permits the assessment of genetically identical individuals (clones). The animals were passed through three generations under experimental conditions in order to remove maternal effects. Specifically, once a mother released her third clutch, one offspring from that clutch was chosen to start the next generation, and this procedure was repeated three times (across three generations). This also synchronises the reproductive cycle of the mothers, allowing the experiment to be started over a small time interval (here 48 hours). Day zero of the experiment was when the offspring of the third-generation females were isolated into new tubes. The data set consists of the life-histories of these fourth-generation offspring, although those that died before the age of maturity were excluded from the analyses (excluding juvenile mortality is common practice in research on ageing, as the age at maturity is considered age zero for the increase in age-specific mortality). Throughout the experiments, medium was changed daily, and the following data were recorded: age at death (the day on which an individual was found dead), age at first reproduction (the day on which the first clutch was released), total number of offspring, clutch sizes (number of offspring released) for all reproduction events, and size of offspring from the third clutch (measured as a straight line from the top of the head to the base of the spine to the nearest μm using a stereomicroscope; offspring conserved in ethanol, done in experiments 1 and 2 only, see below). The

reproductive traits were only recorded for females, as males (experiment 3, see below) did not reproduce in our study. As two summary measures of reproduction, we calculated average daily reproduction (average number of offspring produced per day, calculated across the entire lifespan) and early reproductive effort (number of offspring produced in the first three clutches).

A total of four life-table assays were run, referred to as experiments 1-4. In experiment 1, we used females from five clones per population and cohorts of 20 individuals per clone under moderate food conditions (2,500 cells of the algae *Scenedesmus obliquitus* per day) at 20°C. Experiment 2 was a replication of experiment 1, but with different environmental conditions: a higher food level (5,000 cells per day) and 25°C, with three clones per population and cohorts of 25 individuals per clone. Experiment 3 used males under the same environmental conditions as in experiment 1 with three clones per population and cohorts of 25-50 individuals per clone. The production of males was stimulated by adding (E,E) methyl farnesoate (Echelon Biosciences), a juvenile hormone analogue (Olmstead and LeBlanc 2003) at a final concentration of 400 nM to each tube once the last pre-experimental generation of females reached adulthood. Experiments 1-3 were run to determine the robustness of our results to changing environmental conditions and gender. Details of the environmental conditions in the eight focal populations are unknown, and hence we do not know which experimental conditions best reflect those in the field (and this may indeed vary among the different populations). In addition, running the same experiment three times (under somewhat differing conditions), allowed us to assess the robustness and repeatability of our results.

Experiment 4 was a test of hybrid vigour, where we used the same eight focal populations as

93 above along with eight additional populations used for outcrossing. The populations chosen
94 for outbreeding were of similar genetic diversity and size as the focal population and came
95 from nearby, yet distinct ponds ("outcross partners", Table 1). Due to the lack of a sufficient
96 number of appropriate outcross partners, one of the focal populations was also used as an
97 outcross partner for another focal population (Table 1). Outcrossing was performed in mass
98 cultures in the laboratory. For each pair of populations (i.e., focal and outcross partner), four
99 independent crosses (each using a different pair of clones, one from the focal population and
100 one from the outcross partner) were performed by introducing ~100 females of each of the
101 two clones together into a bucket. This resulted in a total of 32 bucket populations. Sexually
102 produced diapause stages were collected from these bucket populations, dried for two weeks,
103 and then hatched by placing them in fresh medium under high light conditions (16L:8D).
104 Hatchlings were raised individually until they produced their first clonal offspring, upon
105 which their hybrid status was verified using microsatellite markers. Four hybrid clones per
106 population (one per each bucket population) were used in the life table assay (25 individuals
107 per clone). In addition, the life-table assay also included individuals from the eight focal
108 populations and their eight outcross partners, using four clones per population and 25
109 individuals per clone (these 16x4 clones were called "parental clones" in this experiment to
110 distinguish them from the hybrid clones). The experiment was run under the same conditions
111 as experiment 1. For all experiments, age zero refers to the first day of the experiment. At age
112 zero the *Daphnia* are 0-24 hours old.

113

114 **Statistical analysis of hybrid vigour**

115 To test whether hybrid vigour correlated with genetic diversity, we run linear mixed effects
116 models as described in the main text. However, these models had to be modified to account

for the specific data structure of this experiment, which included "triplets of clones" (one parental clone from the focal population, one parental clone from the outcross partner, and one hybrid clone resulting from crossing these two specific parental clones) within "pairs of populations" (one focal, one outcross partner). In total, there were 8 pairs of populations and four independent triplets of clones per pair with clones within each triplet being non-independent. We thus specified for each clone its breed (parental, hybrid), population, pair of populations, triplet of clones, clone, and genetic diversity of its population. The population factor for hybrids was specific to the pair of populations (like a third population within the given pair), and the genetic diversity of the hybrid was the average of their two parent populations. This allows testing the specific hypothesis that hybrid vigor (i.e., the relative performance of parentals and hybrids) depends on the average genetic diversity of the parents by assessing the significance of the interaction between breed and genetic diversity. First a full model was run, for instance for age at death (ad): ad~genetic_diversity*breed, random= ~ (1+breed|pair_of_pops) + (1+breed|triplet_of_clones)+ (1|pop)+(1|clone). We then removed all non-significant interaction terms with random factors, but kept the main factors, even in non-significant, to account for data structure.

Tables

Table S1. Results of the linear mixed effect models testing for a relationship between the Gompertz parameters *a* and *b* and genetic diversity in experiments 1-4. Shown are the *t*-ratios and associated *P*-values in the simple as well as full models (see methods) for each trait. A star indicates a significant *P*-value. Note that the *df* refers to those used in the *t*-test, i.e., the residual *df* of the model and *N* refers to the number of clones, not the number of individuals tested, as Gompertz parameters cannot be estimated per individual.

	Experiment 1 *N = 39*			**Experiment 2** *N* = 24			**Experiment 3** *N* = 23			**Experiment 4** *N* = 95		
	t	*df*	*P*	*t*	*df*	*P*	*t*	*df*	*P*	*t*	*df*	*P*
Gompertz *b*												
Simple model												
Genetic diversity	-2.9	6	0.038*	-2.8	6	0.023*	-0.4	6	0.685	-3.6	6	0.003*
Full model												
Genetic diversity	-0.3	4	0.778	-3.4	4	0.027*	1.2	4	0.281	-2.4	4	0.033*
Latitude	-2.0	4	0.110	-2.5	4	0.067	-0.8	4	0.468	-0.9	4	0.389
Pond size	1.9	4	0.133	-0.8	4	0.472	1.7	4	0.159	-0.5	4	0.655
Gompertz *a*												
Simple model												
Genetic diversity	-0.6	6	0.544	-0.4	6	0.715	-0.9	6	0.407	-2.2	6	0.047*
Full model												
Genetic diversity	-1.7	4	0.174	-0.7	4	0.550	-1.5	4	0.217	-0.3	4	0.762
Latitude	1.1	4	0.332	0.6	4	0.554	0.6	4	0.556	0.1	4	0.910
Pond size	-1.9	4	0.130	-0.8	4	0444	-1.4	4	0.247	0.3	4	0.752

Table S2. Results of the linear mixed effect models testing for a relationship between the Gompertz parameters *a* and *b*, breeding type ("breed": parental, outbred) and genetic diversity in experiments 4. Shown are the *t*-ratios and associated *P*-values for the two fixed factors as well as their interaction. Note that the *df* refers to those used in the *t*-test, i.e., the residual *df* of the model. The interaction tests whether the relative performance of the parents and hybrids changes with genetic diversity. A star indicates a significant *P*-value.

	t	*df*	*P*
Gompertz b			
Genetic diversity	0.4	13	0.161
Breed	5.8	77	<0.001*
Breed*Genetic diversity	-2.9	77	0.005*
Gompertz a			
Genetic diversity	0.2	13	0.872
Breed	0.4	77	0.663
Breed*Genetic diversity	-2.1	77	0.042*

Literature cited

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Figure legends

Figure S1 | Life-history trait comparison between the two parental lines and their hybrid offspring in experiment 4. Starting in top left corner: Age at death, in days; Average daily reproduction, in number of offspring per mother per day; Early reproduction, in number of offspring in the first 20 days following onset of reproduction; Age at first reproduction, in days. Error bars show the standard error of the clonal means per population.