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REDUCED LIFESPAN AND INCREASED AGEING DRIVEN BY GENETIC DRIFT IN SMALL POPULATIONS

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Explaining the strong variation in lifespan among organisms remains a major challenge in evolutionary biology. Whereas previous work has concentrated mainly on differences in selection regimes and selection pressures, we hypothesize that differences in genetic drift may explain some of this variation. We develop a model to formalize this idea and show that the strong positive relationship between lifespan and genetic diversity predicted by this model indeed exists among populations of *Daphnia magna*, and that ageing is accelerated in small populations. Additional results suggest that this is due to increased drift in small populations rather than adaptation to environments favoring faster life histories. First, the correlation between genetic diversity and lifespan remains significant after statistical correction for potential environmental covariates. Second, no trade-offs are observed; rather, all investigated traits show clear signs of increased genetic load in the small populations. Third, hybrid vigor with respect to lifespan is observed in crosses between small but not between large populations. Together, these results suggest that the evolution of lifespan and ageing can be strongly affected by genetic drift, especially in small populations, and that variation in lifespan and ageing may often be nonadaptive, due to a strong contribution from mutation accumulation.

KEY WORDS: ***Daphnia*, genetic drift, mutation accumulation, population size, senescence.**

Ageing can be defined as the progressive deterioration in function and reproduction, accompanied by increasing mortality with age. At the cellular level, ageing is believed to be caused by the build-up of macromolecular damage and to be modulated by a large number of genes (Kennedy 2008). By definition, ageing limits the reproductive potential of individuals, thus raising the question of how and why it has evolved (Partridge and Barton 1993; Zwaan 1999). Closely connected with this is the observation that the rate of ageing and lifespan (usually assessed in the absence of principal extrinsic mortality factors such as starvation, predation, and parasites) vary greatly within and between species (Finch 1990; Stearns 1992). Yet, despite recent progress in determining the genetic basis of ageing, the ultimate evolutionary reasons for the strong variation in lifespan and ageing remain unresolved (Jones et al. 2013).

Central to all evolutionary explanations of ageing is the notion that the strength of natural selection decreases with age, once reproductive maturity is reached (Fisher 1930; Haldane 1941; Medawar 1952; Rose 1991). Even if reproduction and mortality remain constant throughout life (i.e., there is no ageing), old age classes will contain fewer individuals (due to nonzero mortality) and thus contribute fewer offspring than younger age classes. Hence, old individuals are less relevant for natural selection than young individuals (Fisher 1930). As a result, alleles with harmful effects restricted to late life are only weakly counter selected and may increase in frequency either because the same alleles have beneficial effects early in life (the Antagonistic Pleiotropy or AP hypothesis Williams 1957), or because their overall effect on fitness is so small that they can accumulate by mutation and genetic drift, even when they carry no beneficial effect early in life

(the Mutation Accumulation or MA hypothesis; Medawar 1952). Similar to the AP hypothesis, the disposable soma hypothesis (Kirkwood 1977) predicts trade-offs between investments into somatic maintenance and investments into early-life functions (e.g., reproduction), and therefore, we treat this hypothesis as being part of AP (Fabian and Flatt 2011).

There is a continuing debate over which of the two processes—MA or AP—is more important in the evolution of ageing and lifespan, in particular because this debate touches on a central question of biology. Do finite lifespan and ageing represent adaptations that increase fitness through enhanced survival and/or reproductive performance early in life or are they non-adaptive (neutral or maladaptive) by-products of evolution (Rose and Charlesworth 1980; Partridge and Barton 1993; Moorad and Promislow 2008)? Most attempts to explain the strong variation in lifespan and ageing among organisms concentrate on ecological variables that are supposed to either select for a shift between "slow" and "fast" life histories via AP or to allow for the accumulation of early or late-expressed deleterious alleles via MA (e.g., Gustafsson and Part 1990; Abrams 1993; Reid et al. 2003; Nussey et al. 2006; Flatt and Promislow 2007; Nussey et al. 2008). One of the first ideas stated that high-risk environments (i.e., environments with high external mortality risks) may lead to short lifespans, due to either AP or MA (Abrams 1993; Keller and Genoud 1997; Reznick et al. 2004). However, this relationship depends on the details of demographic processes, including density dependence (Abrams 1993; Charlesworth 1994; Williams et al. 2006; Ronce and Promislow 2010). In addition, there is empirical support for a close connection between ageing and generation time (Jones et al. 2008), and it is hypothesized that this relationship is maintained by trade-offs, as stated by the AP hypothesis (Braendle et al. 2011). The common feature of these studies is that they concentrate on how ecology may affect the evolution of lifespan and ageing via a change in selection pressure on early versus late-life performance.

An alternative explanation for the variation in lifespan and ageing can be derived from the MA hypothesis (Hughes 2010). Harmful alleles accumulate by genetic drift when drift overwhelms selection (i.e., when selection is inefficient). This is the case if the selection coefficient (s) against a harmful allele (i.e., the percentage fitness decrease relative to the wild type) is considerably less than $1/(2N_e)$ (Hartl and Clark 1997). In this case, expected allele frequency changes become like those for neutral alleles, leading to a potentially large increase in the frequency of deleterious alleles compared to when selection is efficient (i.e., when $s > 1/2N_e$) and also to increased homozygosity of these alleles. In this way genetic drift decreases the efficacy of selection across all life stages. However, because the strength of natural selection decreases with age, alleles with deleterious effects restricted to late life should, on average, have lower selection coefficients and thus experience greater amounts of mutation accumulation due to genetic drift. As the strength of drift varies strongly among species and populations, differences in lifespan and ageing may result. In particular, mutations that accumulate by drift in small populations can have stronger deleterious effects than those that can accumulate in large populations. This can be understood by considering that the range $s << 1/2\text{Ne}$, in which deleterious mutations are effectively neutral (and thus their fate mainly determined by drift), includes mutations with larger selection coefficients in small populations than in large populations. Hence, the age after which the expression of e deleterious mutation is effectively neutral occurs at an earlier age, with the result that individuals from small populations are expected to age at a faster rate.

Here, we develop a model to formalize this idea and to show that, as genetic drift reduces genetic diversity within populations, a positive correlation between genetic diversity and lifespan is expected. We then show empirically that there indeed exists a strong positive relationship between lifespan and genetic diversity among populations of *Daphnia magna* and that ageing is accelerated in populations with relatively low genetic diversity (small populations). The *Daphnia* used in the experiments came from populations of various physical sizes, ranging form small rock pools up to large ponds and showing a well-established positive relationships between pond size, effective population size, and genetic diversity (Vanoverbeke et al. 2007; Walser and Haag 2012).

To assess whether this correlative relationship is based on a causative relationship between drift and longevity (as predicted by our model), we investigated the alternative hypothesis that the decreased lifespan (and increased rate of ageing) in small populations may not be caused by increased drift but rather by adaptation to environments favoring faster life histories (which may happen to coincide with small population size). To do so, we first used a statistical approach to correct for variables that were partially confounded with genetic diversity in our data (latitude and pond size class) and which might (for unknown reasons) also be correlated with faster life histories. Second, we investigated other life-history traits (average daily reproduction, early reproduction, age at first reproduction, and offspring size) in order to test for potential trade-offs. Finally, we used another prediction derived from the MA hypothesis, namely that hybrid vigor for lifespan and ageing would be expected in crosses between populations (Escobar et al. 2008). This prediction is based on the expectation that, under MA, alleles contributing to ageing are neutral or slightly deleterious with respect to total fitness and thus accumulate only by chance. Hence different alleles are expected to accumulate in different populations. Because deleterious alleles tend to be at least partially recessive (Wright 1977; Charlesworth and Charlesworth 1999), hybrids between populations, which should

be primarily heterozygous at these loci, are expected to have an increased fitness and longer lifespan compared to offspring of within-population crosses (Escobar et al. 2008). As our model predicts decreased lifespan in small populations compared to large populations as a consequence of the increased accumulation of deleterious alleles, it follows that hybrid vigor should be stronger in crosses between small populations compared to crosses between large populations.

We tested this prediction in an additional experiment, assessing the lifespan of individuals derived from within- and between-population crosses. Together, our results provide compelling evidence that the correlation between genetic diversity and lifespan is due to increased drift in small populations rather than due to adaptation to environments that favor faster life histories, and that the short lifespan and increased rate of ageing in *D. magna* from small populations reflect mutation load rather than adaptation. We discuss our results in relation to the AP/MA and the adaptive/nonadaptive debates and conclude that the results of our experiments lend strong support for a prominent role of mutation accumulation in the evolution of lifespan and ageing.

Methods

MODEL BACKGROUND

To predict the effects of genetic drift on lifespan and ageing, we used a model of mutation accumulation (MA) in a metapopulation with local populations (demes) of unequal sizes and thus with locally variable amounts of genetic drift. Building on classical models of the evolution of ageing (Charlesworth 2001), we derived deme-size dependent equilibrium frequencies of mutations that increase mortality at older ages. We assume all local populations ("demes") pertain to the same metapopulation and are connected to each other by a constant migration rate via a migrant pool to which a fixed proportion of each population contributes. In this metapopulation model only variation in deme size can generate differences in genetic drift among demes, with stronger drift in smaller demes. The rationale of the model is that deleterious, partially recessive mutations affecting mortality are less efficiently counter-selected when their age of expression increases (Hamilton's principle; Hamilton 1966). For this reason late-expressed mutations may occasionally reach high frequencies by genetic drift in small demes, resulting in a faster increase in mortality with age. To model this situation we proceed in three steps: (i) we compute the selection coefficient of a mutation affecting mortality at age t, (i.e., how the strength of selection declines with age), (ii) we model the distribution of allele-frequencies as a result of the interplay of migration, selection and drift, and the resulting age-dependent mortalities, for different deme sizes, and (iii) we derive the relationship between deme size and genetic diversity at neutral markers; this last step is to mimic empirical data in which deme size is estimated indirectly through neutral genetic diversity.

DERIVATION OF MODEL

(i) The selection coefficient of a mutation that increases mortality by a small constant $d\mu(x)$ at age x has been described by Charlesworth (2001). His model assumes stable population sizes, and hence that lifetime reproductive success is a good measure of fitness. It also assumes that, in natural populations, extrinsic mortality (γ) is much higher than increases in mortality due to age-dependent mutations (this increase will nevertheless not be negligible in a laboratory context, in which extrinsic mortality due to predation, stress, etc. is much reduced). The sensitivity of fitness to mortality at age x, $dw/d\mu(x)$ is then

$$\partial w/\partial \mu(x) = Min(e^{-\gamma(x-b)}, 1) \quad (1)$$

where b is the age at first reproduction (from Charlesworth 2001, equation (6)). For simplicity, we will focus on adult mortality and rescale so that age 0 corresponds to the onset of reproduction. Thus, $b = 0$ and the sensitivity of fitness is simply $e^{-\gamma x}$. This sensitivity simply represents the fact that at age x only a fraction $e^{-\gamma x}$ of the population is alive to be affected by the mutation.

Real mutations are not expected to affect mortality at a single, precise age, but may act for a certain duration or from a given age; thus, the selection coefficient that applies to these mutations can be found by integrating their effect on fitness (computed above) over the age interval at which they are expressed. Following Charlesworth (2001), we consider two possible models for age-dependent mutations: mutations that increase the mortality by a small constant during a short-time window around age x (window model), and mutations that increase the mortality by a small constant at all ages $\geq x$ (lasting effects model, slightly simplified from the "cumulated effects" model of Charlesworth 2001). In both cases the change in fitness due to one homozygous mutation will be of the form

$$s(x) = \theta e^{-\gamma x} \quad (2)$$

although the constant θ will represent different things, depending on the model. In the window model θ is approximately equal to the total effect of the mutation $d\mu$, that is, the increase in mortality rate due to the mutation multiplied by the duration of its time window of action. In the lasting-effects model, θ represents the relative increase in mortality due to the mutation, that is, the increase in mortality divided by the baseline mortality γ. Both models however are computationally similar, in that the strength of selection decreases exponentially with the age of action of the mutation.

(ii) Now, we derive equilibrium distributions of the frequencies of mutant alleles as a function of their homozygous effect (s) on fitness (computed in the previous step), the migration rate m, the mutation rate v (wild type to mutant, reverse mutation is neglected), and deme size. We assume that the metapopulation is made of different types of demes $i = 1 \ldots k$, each type with a different size n_i, and representing a fraction p_i of all demes. At mutation-migration-selection-drift equilibrium (MMSDE), the allele frequency q (of the mutant allele) differs among demes as a result of genetic drift; allele frequencies are expected to be more variable in small demes. Different types (i) of demes have different distributions of allele frequencies, which can be described by probability densities $\phi_i(q)$. At MMSDE, the latter are given by the diffusion approximation (Wright 1931) as

$$\varphi_i(q) = \frac{c_i}{q(1-q)} Exp\left[4n_i \int \frac{\Delta q}{q(1-q)} dq\right] \qquad (3)$$

where Δq is the per generation change in allele frequency due to mutation, migration, and selection, and c_i is an integration constant such that $\int \phi_i(q) \mathrm{d}q = 1$. In our case, assuming that h represents the dominance coefficient of the mutation, and $\bar{q}$ is the frequency of the mutant allele in the pool of immigrants

$$\begin{aligned} \frac{\Delta q}{q(1-q)} &= \frac{-sq^2 - hsq(1-q) + v(1-q) + m(\bar{q} - q)}{q(1-q)} \\ &= s(1-h) + \frac{v + m\bar{q}}{q} - \frac{m(1-\bar{q}) + s}{1-q} \end{aligned} \qquad (4)$$

(Escobar et al. 2008).

This equation simply represents the addition of three processes: the removal of mutant alleles by selection (on homozygotes and heterozygotes, respectively, the two terms in s in the numerator of the first line), the input of new mutant alleles by mutation (term in v) and the change towards the metapopulation mean due to migration (term in m). The average allele frequency in type i demes is then given by taking the expectation of ϕ_i. In a stationary metapopulation, the pool of immigrants is stable and is constituted at each generation by contributions from all kinds of populations, proportional to $p_i n_i$. Thus, $\bar{q}$, the frequency of the mutant allele in the pool of immigrants, is given by a weighted average:

$$\bar{q} = \frac{1}{\sum_{i=1}^{k} n_i p_i} \left(\sum_{i=1}^{k} n_i p_i \int_0^1 q \varphi_i(q) dq \right). \qquad (5)$$

To obtain the distribution of allele frequencies, we need to determine $c_1, c_2 \ldots c_k$, and $\bar{q}$. There is no simple analytical solution, but numerical solutions are easily obtained: For a given value of $\bar{q}$ the c_i's can be obtained by numerical integration of equation (3) and then used to obtain an updated value of $\bar{q}$ (eq. 5) which may be above or below the initial value; at equilibrium the two values are equal. We programmed a simple dichotomy procedure in Mathematica to obtain rapid numerical convergence. This procedure was used to obtain equilibrium distributions for mutations affecting mortality at various ages, by substituting the selection coefficient s using equation (2).

Finally, we derived the age-dependent mortality load induced by mutation at one locus, which can be obtained as

$$\Delta\bar{\mu}_i(x) = \int_0^1 \left(2q(1-q)hd\mu(x) + q^2 d\mu(x)\right) \varphi_i(q) dq, \quad (6)$$

where $\mathrm{d}\mu(x)$ is the homozygous effect of the mutation on mortality at age x. This equation represents the total increase in mortality at age x in the population due to the mutation, both in the heterozygous form (left term) and homozygous form (right term), averaged over all demes of a given type i. The effects of all mutations are then summed over the number of loci to obtain total mutational increases in mortality as a function of age and deme size.

(iii) The previous computations allow us to derive mortality patterns in demes of different sizes in a metapopulation. To express mortality as a function of genetic diversity at neutral loci (which is the usual surrogate for deme size; Nei and Takahata 1993), we need to obtain genetic diversity as a function of deme size. From the standard infinite-allele model of mutation this function can be written as

$$H_{e,i} \approx 1 - \frac{1}{1 + 4n_i(m+u)} - \left(1 - \frac{1}{1 + 4n_i m}\right)(1 - H), \quad (7)$$

where u is the mutation rate at the marker locus and H is the genetic diversity across the whole metapopulation (or, equivalently, in the pool of propagules), which depends on the total number of demes, and can be estimated directly from empirical data.

The model deliberately excludes viability mutations with age-independent effects, which are expected to contribute to limited lifespan by increasing mortality hazard by a constant, but not to ageing. Several models already exist on the effects of such mutations in demes of varying size (Kimura et al. 1963; Glémin 2003). Generally, their average frequency may be somewhat lower in small compared to large demes (due to "purging by drift" sensu Glémin 2003), but their negative impact on lifespan is generally higher because the higher variance in allele frequencies in small populations leads to increased expression of the homozygous effects of these mutations. At intermediate deme sizes also the impact on lifespan might be slightly reduced, but the definition of "intermediate" depends on values of selection and dominance coefficients, so that in real populations in which a range of mutations with different values of these parameters occur, a reduction in deme size almost always causes a decreased lifespan in these models (Glémin 2003). For details on the specific parameters used in the model see the Supplementary Materials.

Table 1. Population IDs, origins, and outcross partners for the eight focal populations used in the life-history experiments. Given are also the genetic diversities (H_E: expected heterozygosity, H_O, observed heterozygosity) for all populations. The full names as well as the countries of origin and geographical coordinates of the populations are listed in the footnote.

Type	Focal	H_E[1]	H_O[2]	Outcross	H_E[1]	H_O[2]
Large	**AST**	0.642	0.500	**VOL**	0.449	0.501
	WTE	0.583	0.622	**BEOM**	0.530	0.595
	MOS	0.449	0.360	**AST**	0.642	0.500
	ISM-6	0.437	0.361	**ISM-12**	0.428	0.350
Small	**KOR**	0.242	0.171	**BOL**	0.162	0.0552
	KMG	0.161	0.0688	**N-45**	0.140	0.0729
	N-49	0.137	0.0625	**SK-1**	0.141	0.0721
	VR1	0.0722	0.0591	**K-10**	0.154	0.150

AST: Astrakhan, Russia (N45.9036,E47.6564); BEOM: Belgium (N50.8667, E4.6834); BOL: Bolshoi Asafiy, Russia (N66.4250,E33.8334); ISM-6: Ismaning, Germany (pond 6; N48.2034,E11.6835); ISM-12: Ismaning, Germany (pond 12; N48.2078,E11.7110); K-10: Tvärminne, Finland (island K, pond 10; N59.8238,E23.2521); KOR: Korablik, Russia (N66.4308,E33.7834); KMG: Tvärminne, Finland (island Kummelgrundet; N59.8218,23.E2050); MOS: Moscow, Russia (N55.7636,E37.5816); N-45: Tvärminne, Finland (island Storgrundet, pond 45; N59.8221,E23.2601); N-49: Tvärminne, Finland (island Storgrundet, pond 49; N59.8220,E23.2599); SK-1: Tvärminne, Finland (island Skallotholmen, pond 1; N59.8326 E23.2581); VR1: Vääränmaanruskea, Finland (N60.2716,E21.8963); VOL: Volgograd, Russia (N48.5300,E44.4871); WTE: Witte Hoeve, Belgium (N50.8285,E4.6393).

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 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. nonrock 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. 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). For details on how maternal effects were removed as well as further details on the life-table assays see the Supplementary Materials.

A total of four life-table assays were run, referred to as experiments 1–4. Experiment 1, 2, and 3 used the same clones, but were run under different conditions. Experiment 3, in addition, used male *daphnia*. Running the same experiment three times (under different 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 above along with eight additional populations used for outcrossing. The populations chosen for outbreeding were of similar genetic diversity and size as the focal population and came from nearby, yet distinct ponds ("outcross partners," Table 1). Further details on the life-table assays and experimental procedures for outcrossing can be found in the Supplementary Materials.

STATISTICAL TESTS OF LIFE-HISTORY TRAITS

The relationships between genetic diversity and the life-history traits age at death (AD), average daily reproduction (ADR), early reproduction (ER), age at first reproduction (AFR), and offspring size (OS) were evaluated using linear mixed-effects models in the program R, with the package nlme (R Development Core Team 2013). In each case, we started with a simple model, which included genetic diversity as a fixed factor, and population and clone as random factors, with clone nested within population. We then also included latitude and pond size class (large, small) as fixed factors, as well as their interaction terms (changing the order of these factors in the models had no effect on the results). However, the interactions were in no case significant and were not retained in the model selection. Thus, what we report in the result tables as "full model" includes all the main effects, but no interaction terms. The model selection was done via likelihood-ratio testing using the maximum likelihood function of the nlme package. For each retained model, plots of the residuals were inspected to ensure no skew or pattern, plots of the response

variables were inspected to ensure the relationship was linear, and finally, quantile–quantile plots per population were inspected to ensure each was reasonably normally distributed.

The primary analysis of the life-history traits from experiment 4 included only the parental clones. To further compare life-history traits between hybrid and parental clones and to test whether the degree of hybrid vigor changed with genetic diversity, we repeated the same linear-mixed models as outlined above on the entire dataset from the experiment and included the factor breeding type (parental, hybrids) and the interaction between breeding type and genetic diversity in the models. In addition, in this experimental design we had, within each pair of populations (focal and outcross population), four independent triplicates of clones (two parental clone and the corresponding outcrossed F1 offspring between those two parental clones). This data structure was incorporated into the linear-mixed effects model by explicitly specifying each triplicate of clones and each pair of populations (all random effects; see Supplementary Materials). The interaction between breeding type and genetic diversity tests whether the relative performance of the parents and hybrids changes with genetic diversity. In order to keep the models sufficiently simple, latitude, and pond size class were not included in these tests for hybrid vigor.

STATISTICAL TESTS OF AGEING

In order to analyse patterns of ageing, age-specific mortality rates were parameterized using the Gompertz mortality model: $u(x) = ae^{bx}$ (Pletcher and Curtsinger 1998; Pletcher 1999), where a is the frailty or baseline mortality and b is the rate at which age-specific mortality increases with age and thus represents ageing. When log-transformed, the Gompertz mortality model becomes linear, with the y-axis intercept representing the frailty and the slope representing ageing. The Gompertz mortality model was fit using the statistical package WinModest (Pletcher 1999). Model selection in WinModest chose the Gompertz model as the best fitting model (among several other available mortality models) for all experiments. We used this method to obtain estimates of the Gompertz parameters at the level of each clone, which were then used in further analyses (see below). We also used the likelihood ratio tests in WinModest to examine overall differences in Gompertz parameters between the small and large populations (this fits a single a and a single b for to all data, and then tests whether an alternative model that fits separate a and b for small and large populations performs better; Pletcher 1999). Using the same likelihood ratio test implemented in WinModest, we also tested for overall differences in Gompertz parameters between breeding types (parentals vs. hybrids) in experiment 4.

To specifically test for a relationship between genetic diversity and ageing (as well as for differences in the rate of ageing between hybrids and parental clones in experiment 4), we used the estimates of the Gompertz parameters obtained for each clone separately. The analysis was then carried out as described above for the life-history data, except that the number of observations was lower (one observation per clone instead of per individual).

Results

As expected (Charlesworth 2001), our model predicts that mutations acting late in life reach higher frequencies and mortality rates increase with age in all cases. However, our model also predicts large differences in lifespan and ageing among demes of different size (Fig. 1). In the window model, small demes have a higher initial mortality rate than large demes, and this difference increases with age, indicating faster senescence in small demes. This is not due to a higher frequency of mutations in small demes; on the contrary small demes tend to have slightly lower average mutation frequencies than large ones (due to "purging by drift" sensu Glémin 2003). However, the impact of the mutations is higher because extreme allele frequencies, including near-fixation of mutations, are more frequent in small demes, so that homozygous effects of mutations are more often expressed. In the lasting-effects model, similar results were obtained, although initial mortality rates do not differ; it is indeed a property of this model that mortality at age zero cannot be affected by mutation, simply because mortality at age x is affected only by the accumulation of mutations whose action starts before age x and we assumed no mutation acting before our conventional age zero.

Irrespective of the mutation model, the same patterns are found when neutral genetic diversity is used as a proxy of deme size. Both lifespan and genetic diversity respond nonlinearly to deme size, in such a way that a quasi-linear positive relationship between them arises (Fig. 1). Note that all these properties are likely to be conserved in laboratory settings, because laboratory conditions are likely to modify the baseline mortality (shifting log-mortalities by a constant) but not the effects of mutations that are responsible for the patterns described here.

In all life-table experiments, individuals from the small, genetically less diverse populations had substantially (up to 50%) reduced average lifespans compared to those from the large, more diverse populations. Within each of the experiments, there was a positive relationship between lifespan and genetic diversity (Fig. 2; Table 2). This relationship remained significant, after accounting for latitude and pond size class (Table 2). The best-supported model included all three fixed factors (genetic diversity, latitude, and pond size class), but no interaction terms.

For age at first reproduction we found a negative linear relationship with genetic diversity and for offspring size a positive one (Fig. 2; Table 2), though these relationships were less pronounced than for age at death. The relationships between

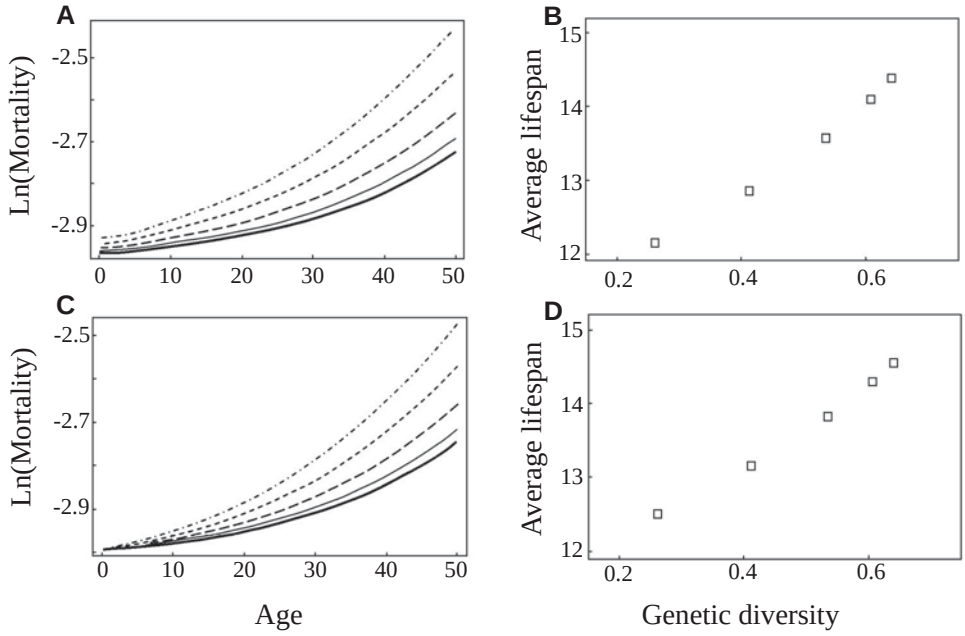

Figure 1. **Mortality curves expected for different deme sizes in a metapopulation (A, B) and the expected relationship between genetic diversity at neutral markers and average lifespan (C, D). (A) and (C) represent the "window model," in which each mutation increases the mortality rate during a restricted time window between two ages. (B) and (D) represent the "lasting-effects" model, in which each mutation increases mortality after a given age. In both cases, the age of effect of a mutation is uniformly distributed between age 0 and 100. Deme sizes: *n* = 16, 40, 100, 190, 251, and 630 individuals from top (small dashes) to bottom (continuous thick lines) in (A–B) and from left to right in (C–D).**

average daily reproduction and genetic diversity as well as early reproduction and genetic diversity were, on the other hand, rather weak, though there was a tendency toward positive relationships between these traits and genetic diversity (Fig. 2; Table 2). The best-supported linear mixed-effect models generally included latitude and in some cases pond size, however, in most cases the relationship with genetic diversity remained significant upon their inclusion.

In addition to the striking lifespan differences, individuals from the genetically less diverse populations also aged at a faster rate (Fig. 3). Overall the Gompertz slope, *b*, was steeper for the small populations, indicating a faster increase in mortality with age than in the large populations (likelihood ratio tests: experiment 1: $\chi^2 = 213.4$, $df = 1$, $N = 751$, $P < 0.001$; experiment 2: $\chi^2 = 139.3$, $df = 1$, $N = 563$, $P < 0.001$; experiment 3: $\chi^2 = 83.3$, $df = 1$, $N = 694$, $P < 0.001$; parental clones of experiment 4: $\chi^2 = 321.4$, $df = 1$, $N = 1267$, $P < 0.001$; Figs. 3 and 4). Furthermore, there was a negative relationship between genetic diversity and the Gompertz *b* in experiments 1, 2, and 4, though this was not significant for the males phenotyped in experiment 3 (Fig. 5, Supplementary Table S1). In addition, although there were significant differences between large and small pond size classes in Gompertz *a* (likelihood ratio tests: experiment 1: $\chi^2 = 217.1$, df = 1, $N = 751$, $P < 0.001$; experiment 1: $\chi^2 = 99.7$, $df = 1$, $N = 563$, $P < 0.001$; experiment 1: $\chi^2 = 78.3$, $df = 1$, $N = 694$, $P < 0.001$; experiment 1: $\chi^2 = 200.1$, $df = 1$, $N = 1270$, $P < 0.001$), there was not a strong relationship between Gompertz *a* and genetic diversity in experiments 1–4 (Supplementary Table S1).

When comparing life-history traits between the hybrid and parental individuals of experiment 4, there is a clear pattern of hybrid vigor in crosses between the small populations, but not in crosses between the large populations (Supplementary Fig. S1; Table 3). Strikingly, hybrids from the small populations showed an almost complete rescue phenotype (i.e., similar phenotype to parental individuals from large populations) and this was true not just for lifespan, but for the other life-history traits as well (Supplementary Fig. S1; Table 3).

Comparisons of the Gompertz *b* from the hybrid and parental individuals shows the same pattern of hybrid vigor in the small populations (likelihood ratio test: hybrids vs. parental in small populations: $\chi^2 = 16.7$, $df = 1$, $P < 0.001$; Fig. 4). Accordingly, the linear mixed-effects model found a significant interaction between breed and genetic diversity (indicating that hybrid vigor correlates with genetic diversity; Supplementary Table S2). The Gompertz *a*, on the other hand, differed little between breeding types (likelihood ratio tests: hybrid vs. parental in small populations: $\chi^2 = 0.05$, $df = 1$, $P = 0.820$, hybrids vs. parental

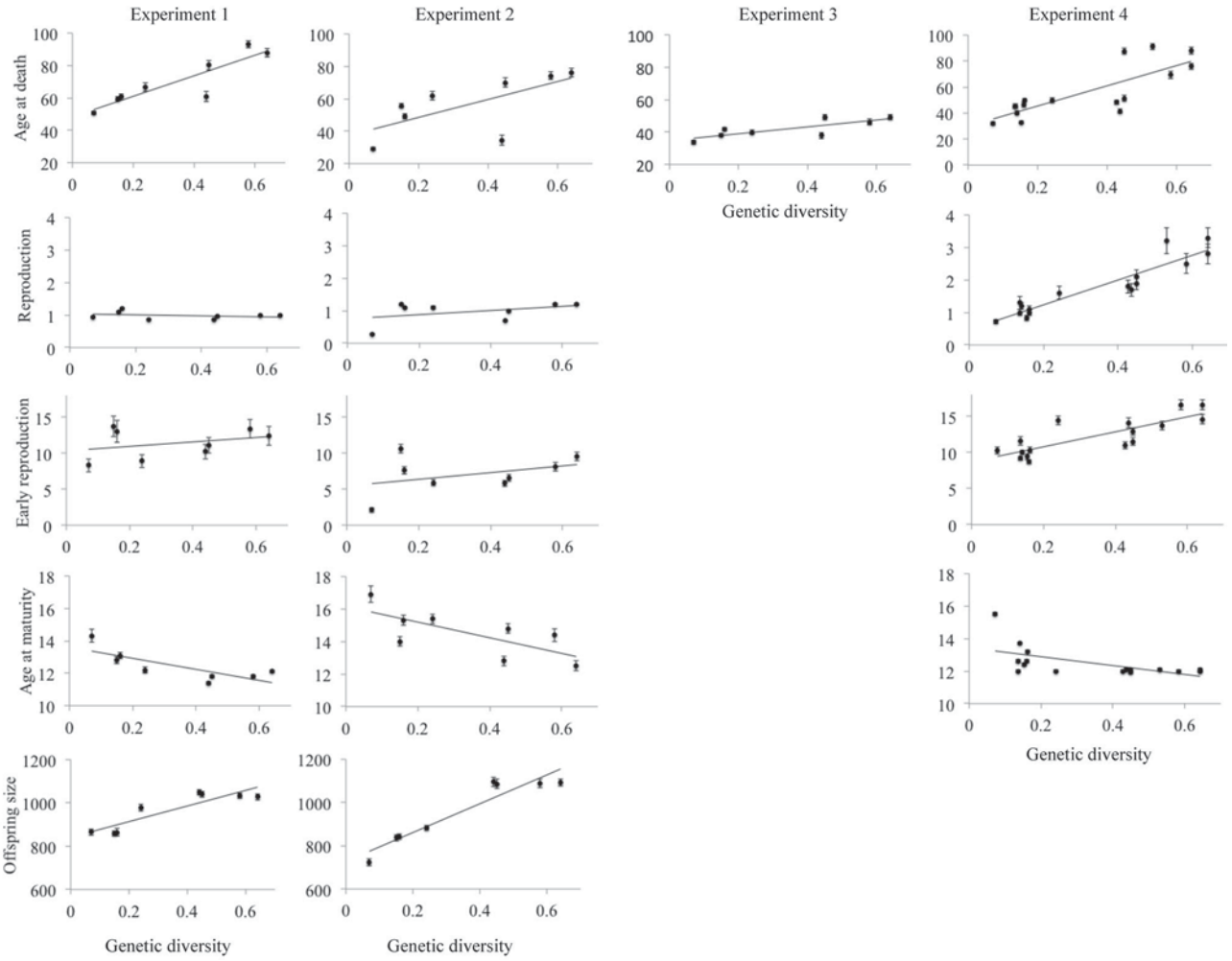

Figure 2. **Linear regression plots showing the relationship between the life-history traits and genetic diversity in experiment 1–4. Age at death, in days, average daily reproduction, in number of offspring per mother per day, early reproduction, in number of offspring during the first 20 days following onset of reproduction, age at first reproduction, in days, offspring size, in micrometer. Error bars show the standard error of the clonal means per population. For experiment 4, only the parental clones are shown. The regression lines are shown for illustrative purposes only; the statistical analyses are based on linear-mixed effect models, which adequately account for the data structure.**

in large populations: $\chi^2 = 0.92$, $df = 1$, $P = 0.681$), and the interaction between breed and genetic diversity was only marginally significant (Supplementary Table S2).

Discussion

Our model confirms the verbally formulated idea that, under MA, small populations, which experience increased levels of genetic drift, are expected to evolve higher rates of ageing and shorter lifespans than large populations (Hughes 2010). A positive correlation between genetic diversity and lifespan is therefore predicted across populations. These results are reminiscent of those predicting that inbreeding depression should increase with age (Charlesworth and Hughes 1996) and that hybrid individuals between populations should show a reduced rate of ageing (Escobar et al. 2008). However, inbreeding by nonrandom mating and genetic drift in small populations have a different genetic basis (Glémin 2003), and thus our model expands on previous efforts investigating differences among populations in lifespan and ageing. Importantly, the two models of mutation action (limited time interval of action around a certain age, or indefinite action starting at a certain age) yield the same qualitative predictions that lifespan and senescence rates should be shorter (respectively faster) in small demes, or demes with low genetic diversity. The only difference is that mortality at age zero is related to deme size in the time-window but not in the lasting-effects model; however, this is largely due to our model considering exclusively mutations that affect mortality from our conventional age zero (i.e., age at first reproduction); in reality, mutations may act before this, in which case small demes would have higher initial

Table 2. Results of the linear mixed-effect models testing for a relationship between life-history traits and genetic diversity in experiments 1–4. Shown are, for each trait, the *t*-ratios, degrees of freedom (*df*), and associated *P*-values for each fixed factor in the simple as well as the full models (see methods). Note that the *df* refers to those used in the *t*-test, that is the residual *df* of the model. A star indicates a significant *P*-value. *N* indicates the total number of individuals used in the analysis (only parental clones in experiment 4).

	Experiment 1			Experiment 2			Experiment 3			Experiment 4		
	$N = 751$			$N = 563$			$N = 694$			$N = 1270$		
	t	*df*	*P*	*t*	*df*	*P*	*t*	*df*	*P*	*t*	*df*	*P*
Age at death												
Simple model												
Genetic diversity	5.4	6	0.002*	8.3	6	0.002*	3.9	6	0.008*	5.2	14	<0.001*
Full model												
Genetic diversity	4.5	4	0.011*	6.6	4	0.003*	2.6	4	0.062	2.9	12	0.013*
Latitude	1.6	4	0.175	4.2	4	0.014*	1.1	4	0.331	0.1	12	0.919
Pond size	0.8	4	0.494	2.7	4	0.053	0.2	4	0.818	0.1	12	0.381
Average daily reproduction												
Simple model												
Genetic diversity	0.9	6	0.393	1.2	6	0.289				10.0	14	<0.001*
Full model												
Genetic diversity	0.2	4	0.846	1.9	4	0.117				5.5	12	<0.001*
Latitude	−1.1	4	0.334	1.8	4	0.154				−0.1	12	0.967
Pond size	1.2	4	0.307	2.5	4	0.069				1.6	12	0.139
Early reproduction												
Simple model												
Genetic diversity	0.9	6	0.411	1.2	6	0.279				5.7	14	<0.001*
Full model												
Genetic diversity	0.7	4	0.514	0.3	4	0.621				4.1	12	0.001*
Latitude	−1.0	4	0.384	−0.2	4	0.873				0.7	12	0.519
Pond size	0.9	4	0.421	2.0	4	0.114				1.4	12	0.200
Age first reproduction												
Simple model												
Genetic diversity	−3.3	6	0.017*	−2.8	6	0.030*				−3.0	14	0.009*
Full model												
Genetic diversity	−1.1	4	0.328	−0.8	4	0.469				−1.5	12	0.152
Latitude	−1.5	4	0.199	1.2	4	0.291				−0.9	12	0.387
Pond size	1.1	4	0.315	−0.6	4	0.571				0.1	12	0.899
Offspring size												
Simple model												
Genetic diversity	5.6	6	0.001*	−9.0	6	<0.001*						
Reduced model												
Genetic diversity	1.7	4	0.161	2.0	4	0.123						
Latitude	2.8	4	0.047*	1.8	4	0.151						
Pond size	−3.0	4	0.039*	−2.7	4	0.051						

mortalities in both the time-window and lasting-effects models. This may be further accentuated by deleterious mutations affecting viability in an age-independent fashion (Kimura et al. 1963) and, in nature, modulated by differences in extrinsic mortality due to environmental rather than genetic differences among populations.

The empirical data show a clear and robust pattern of reduced lifespan in populations where genetic drift is relatively strong (i.e., populations with low genetic diversity). Consistent with the predictions derived in our model, the same populations also show an increased rate of ageing (although the negative correlation between Gompertz *b* and genetic diversity was significant only in three out of four experiments; the difference between small and large was significant in all four). It is important to note that large cohort sizes are needed to accurately estimate Gompertz *b*, and the variation among experiments in per-population estimates of this parameter are thus likely explained by the moderate cohort sizes (per population) used in these experiments. Overall, however, the

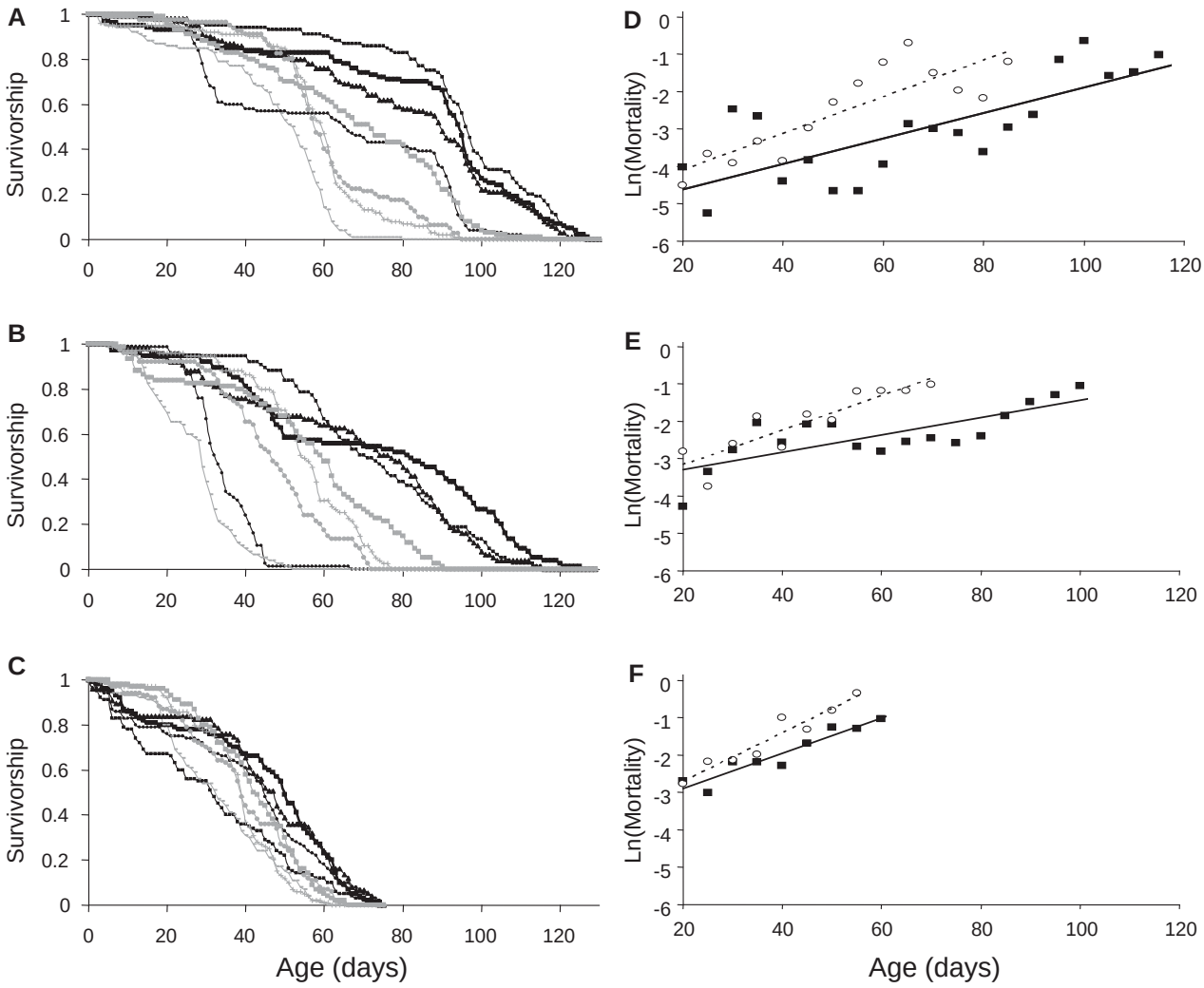

Figure 3. **Survivorship (A–C) and log mortality curves (D–F) for experiments 1–3. In the survivorship curves the black lines indicate large populations and gray lines small populations (line thickness increases with genetic diversity). The straight lines through the mortality curves are regression fits averaged across all small and across all large populations. The slope can be interpreted as Gompertz *b* and the intercept the Gompertz *a*, but the actual values of these parameters for the statistical analysis were estimated using the ML procedure. Solid lines and squares refer to large populations and the open circles and dotted lines to small populations.**

patterns were similar across four independently run experiments, hence providing strong evidence for a correlation between the level of genetic drift and the rate of ageing in *D. magna*.

In contrast, and perhaps somewhat surprisingly, we did not find consistent differences in the Gompertz *a* between small and large populations. This parameter is often interpreted as baseline mortality or age-independent mortality in Gompertz analyses of survival data (Pletcher 1999; Pletcher et al. 2000), and, as noted above, it is expected that mutations with age-independent viability effects should also accumulate in small populations via genetic drift (Kimura et al. 1963). However, the estimation of this parameter is complex because we found non-parallel linear relationships between log-mortality and age (differences in *b* among populations). As a result differences in intercepts (*a*) depend on what is considered age zero. As per convention (Jones et al. 2013), we fixed age zero for the onset of ageing at age at first reproduction. In our experiments, very little mortality occurred at this stage, thus the estimates of *a* are strongly determined by deaths that occurred much later. While the maximum-likelihood procedure for estimation of the Gompertz parameters does not suffer from systematic biases (Promislow et al. 1999), it can only provide reliable estimates of *a* if the Gompertz function adequately describes the mortality curve (and being the best fitting model of several ones tested does not guarantee this).Without wanting to interpret absence of differences in *a* among populations we observed very little mortality differences between small and large populations

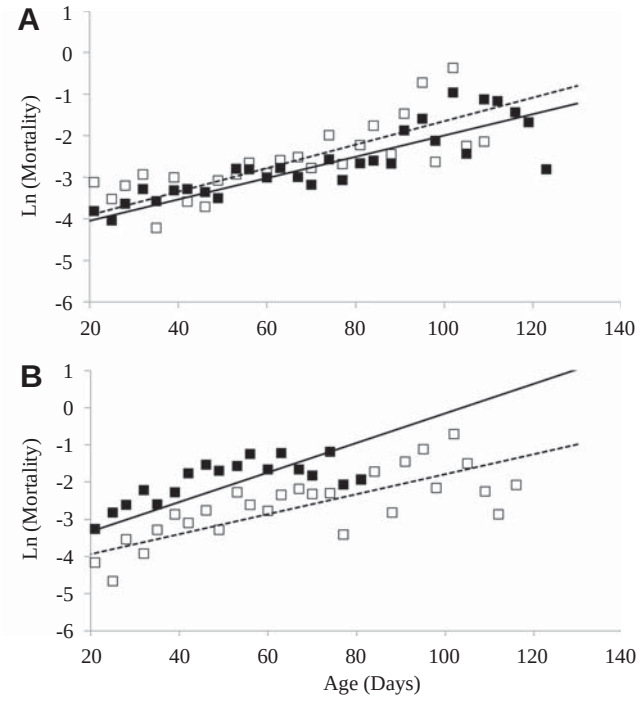

Figure 4. **Log mortality curves for parental and hybrid clones, averaged across (A) all large and (B) all small populations. The straight lines through the mortality curves are the regression fits, corresponding to the Gompertz model (these linear regression lines are purely for illustrative purposes, and that the actual values used for the statistical analysis come from the ML procedure), where the slope is the Gompertz *b* and the intercept the Gompertz *a*. The straight lines through the mortality curves are regression fits averaged across all small and across all large populations. The slope can be interpreted as Gompertz *b* and the intercept the Gompertz *a*, but the actual values of these parameters for the statistical analysis were estimated using the ML procedure. Solid lines and squares refer to large populations and the open circles and dotted lines to small populations.**

before 40 days after maturity (Fig. 3). This suggests that, in our laboratory experiments, mutations affecting mortality in an age-independent manner (or at early ages only) did not contribute to the differences in lifespan observed in our experiment. Instead, the observed patterns seem to depend on genetic variation in late mortality. A possible interpretation is that even in the populations most subject to drift, early-acting or age-independent mutations were still under too strong selection to accumulate; only mutations expressed after a certain age may have been under such a weak selection as to be affected by drift.

To assess whether these correlational patterns between lifespan, rate of ageing, and genetic diversity are indeed due to differences in the level of genetic drift, we investigated a potential alternative explanation, namely that small, genetically less diverse populations may occur in habitats that select for increased early-life performance or greater investment into reproduction, that is, habitats that select for faster life-histories. A possible trade-off between early and late-life performance (Medawar 1952) would result in increased late-life mortality and thus reduced lifespan. Two lines of additional evidence from the correlative data are inconsistent with this alternative hypothesis. First, genetic diversity tended to decrease with latitude because the four northern populations occur in smaller ponds than the four southern populations (Walser and Haag 2012). Yet, the correlation between lifespan and genetic diversity remained significant after statistically correcting for this effect of "small" versus "large" pond size, as well as after correcting for latitude, suggesting it was neither driven by a geographic correlate that selected for generally faster life histories in the north nor by variables that have been shown to correlate with *Daphnia* life-histories in (small) ponds versus (large) lakes (i.e., predation regime, temperature, food quality, and quantity; Dudycha 2003). Second, key life-history traits other than lifespan showed opposite patterns to those expected under the trade-off hypothesis. Individuals from the less diverse populations had lower daily reproductive outputs, lower early-life reproduction, started to reproduce later, and produced smaller offspring compared to individuals from the more diverse populations. Thus, the shorter-lived individuals did not invest more energy into reproduction and did not start to reproduce earlier, but rather showed reduced reproductive performance. This strongly suggests that life-histories of individuals from small populations are shaped by the deleterious effects of an increased genetic load, and that the decreased lifespan and faster ageing are not positively selected in these populations, but rather are by-products of genetic drift leading to nonadaptive or maladaptive phenotypes in lifespan, ageing, and other life-history traits.

The conclusion that differences in genetic drift rather than in selection regimes can explain the variation in lifespan among these *Daphnia* populations is further supported by the results of our experiment on hybrid vigor, which tested another prediction derived directly from MA theory (Escobar et al. 2008). The strong hybrid vigor seen in crosses between the small but not between the large populations and the fact that outcrossing between small populations resulted in an almost complete rescue phenotype for lifespan and the rate of ageing suggest that a large amount of the differences in lifespan between small and large populations can be attributed to the consequences of increased genetic drift.

The results of our correlational study and of the hybrid vigor experiment are thus predicted by MA models. Empirical support has also been found for other predictions of the MA hypothesis, in particular for the predicted increases in additive genetic variance and inbreeding depression with age as well as hybrid vigor for lifespan (Kosuda 1985; Charlesworth 1990; Hughes and Charlesworth 1994; Hughes 1995; Charlesworth and Hughes 1996; Hughes et al. 2002; Gong et al. 2006; Lesser et al. 2006; Swindell and Bouzat 2006; Borash et al. 2007; Keller et al. 2008).

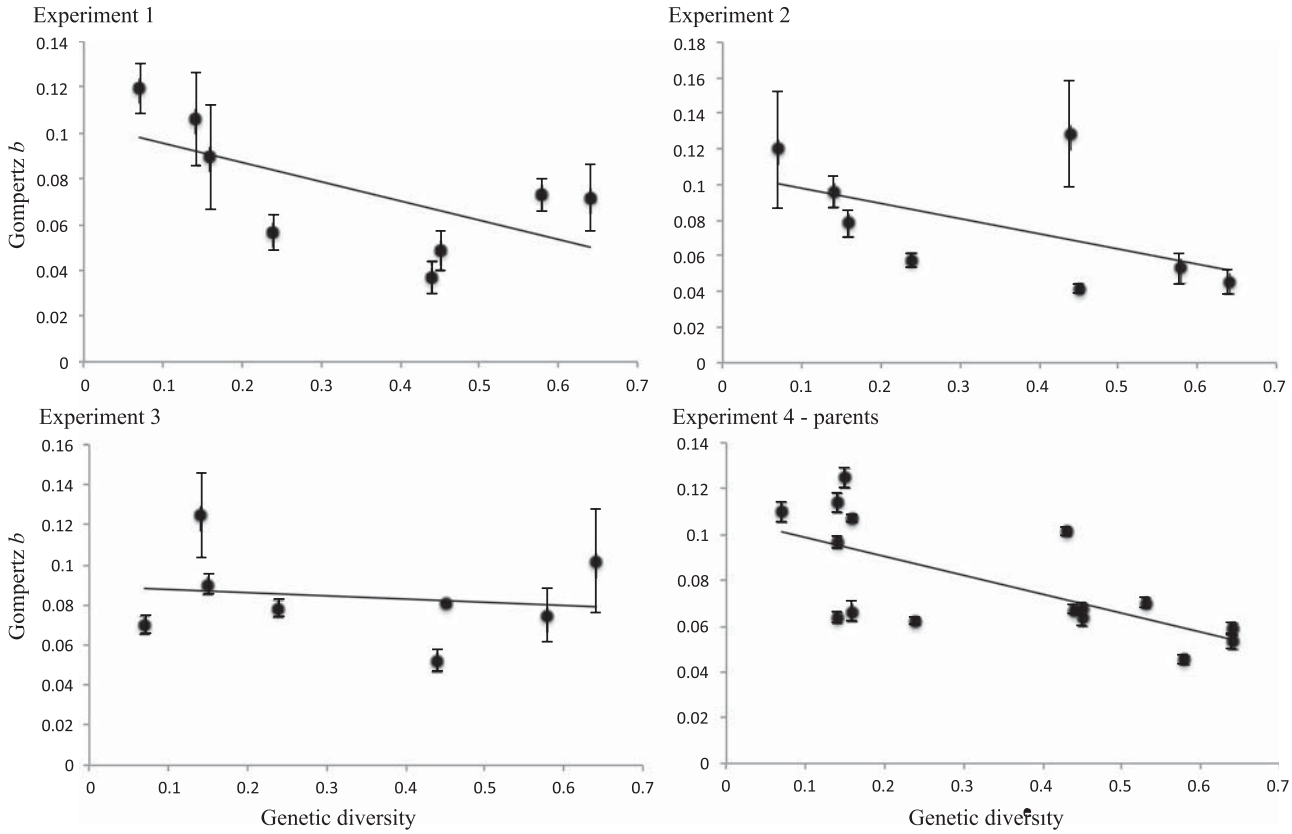

Figure 5. **Regression plots demonstrating the relationship between the Gompertz ageing parameter *b* with genetic diversity for experiments 1–4. Starting from the top left corner: Experiment 1, Experiment 2, Experiment 3, Experiment 4 (only parental clones). Error bars show the standard error of the clonal means per population.**

Yet it has been postulated that the same empirical patterns may also occur under some forms of AP (Moorad and Promislow 2009). This has led some authors to conclude that there is little empirical evidence for MA (e.g., Baudisch 2005; Danko et al. 2012) and, at present, the general consensus appears to be that AP is the predominant mechanism explaining the evolution of lifespan and ageing (Reid et al. 2003; Nussey et al. 2006; Flatt and Promislow 2007; Nussey et al. 2008; Braendle et al. 2011; but see Borash et al. 2007; Hughes 2010 for diverging opinions). Could AP thus also explain the results of our crossing experiment? The increased lifespan of hybrids between small populations indicates that different loci contributed to reduced lifespan in the various small populations and that alleles conferring short lifespan were, on average, recessive or partly recessive. It follows from standard population genetic theory that this is more likely the result of drift than of selection. Generally, alleles that are fixed by positive selection tend to be dominant, whereas deleterious alleles (which may become fixed if drift overwhelms selection) tend to be recessive (Hartl and Clark 1997; Charlesworth and Charlesworth 1999). In addition, it is likely that nearby populations would have the same alleles at loci under positive selection (only very small amounts of gene-flow are needed) but differ at loci that are predominantly influenced by drift. Thus it is highly unlikely that different, predominantly recessive alleles conferring short lifespan have been positively selected (because they confer an advantage early in life, as proposed by AP) in each of our population pairs.

Other models of AP, however, predict that alleles conferring short and long lifespan are maintained within populations by over dominance (this is the case if heterozygotes make the best compromise between early-life and late-life performance, i.e., if their fitness is higher than that of both alternative homozygotes). In such a scenario, the results of our study may be explained by drift leading to deviations from the optimal allele frequencies. However, as drift is by definition a random process, it would seem very difficult to explain why such deviations would systematically favour alleles conferring short lifespan in different populations, while still maintaining sufficient differentiation among populations in order to explain hybrid vigor. Nonetheless, even this rather unlikely situation would represent nonadaptive evolution of short lifespan by drift rather than adaptive divergence toward different fitness optima (the classical view of how AP might result in lifespan variation, i.e., the fast-slow life-history continuum; Jones et al. 2008).

Table 3. Results of the linear-mixed model testing for a relationship between life-history traits, breeding type ("breed": parental, outbred), and genetic diversity in experiment 4. The interaction between breed and genetic diversity tests whether hybrid vigor correlates with genetic diversity. Shown are the *t*-ratios and associated *P*-values for each fixed factor. A star indicates a significant *P*-value. Note that the *df* refers to those used in the *t*-test, that is the residual *df* of the model. The total number of individuals was $N = 1928$, distributed across 64 parental clones from 16 populations and 32 hybrid clones.

	t	*df*	*P*
Age at death			
Genetic diversity	−2.8	14	0.006*
Breed	−15.8	77	<0.001*
Breed* genetic diversity	13.9	77	<0.001*
Average daily reproduction			
Genetic diversity	−0.9	14	0.351
Breed	−30.2	77	<0.001*
Breed* genetic diversity	29.4	77	<0.001*
Early reproduction			
Genetic diversity	−1.5	14	0.128
Breed	−13.8	77	<0.001*
Breed* genetic diversity	10.9	77	<0.001*
Age first reproduction			
Genetic diversity	−2.0	14	0.041 *
Breed	10.8	77	<0.001*
Breed* genetic diversity	−7.4	77	<0.001*

In summary, it is not entirely excluded that AP contributed to the empirical patterns of variation in lifespan and ageing reported in this study. However, it appears much more parsimonious to attribute these patterns to MA. This is in line with many other empirical studies that confirmed predictions by the MA-hypothesis (see above), and, indeed it has been pointed out that explaining the results of these studies with AP models also relies on rather unlikely genetic assumptions (Hughes 2010). Hence, while it might be true that none of these studies (including ours) conclusively proves MA (Moorad and Promislow 2009), this absence of an ultimate proof should not be understood as evidence against MA. In fact, many of the empirical results in favor of AP do not constitute absolute proof of AP either (Flatt and Promislow 2007). More generally, and whatever the genetic architecture for variation in lifespan may be (AP or MA), a fundamental question is to find an evolutionary interpretation of differences in lifespan or ageing patterns among populations and species. So far, most research has focused on differences in selection pressures among populations or among species as a potential evolutionary source for such differences, including work on *Daphnia* (Dudycha 2001, 2003; Dudycha and Hassel 2013). The results of our study, however, strongly support a prominent role of genetic drift and thus nonadaptive processes in explaining this variation, at least at the within-species level.

The discussion about the genetic mechanisms underlying variation in lifespan and ageing and whether it is governed by adaptation or nonadaptive processes goes beyond a purely academic level because a strong contribution of nonadaptive processes would imply that substituting these alleles with their ancestral ones or permanently treating their effects would increase lifespan without the cost of lowered early-life performance, especially in species with relatively low effective population sizes (i.e., relatively strong drift). Reduced lifespan and faster ageing may also present a risk for endangered species, which often occur in small populations and have low genetic diversities (Allendorf and Luikart 2007). Alleviating these deleterious mortality effects late in life may represent one of the benefits of hybridizing with migrants from nearby populations (Ebert et al. 2002; Lippman and Zamir 2007). Finally, the human population has a relatively small effective population size of approximately 3000–10,000 (Takahata 1993; Tenesa et al. 2007), despite our currently large census size, pointing to strong genetic drift in our evolutionary past. Hence, it is likely that the human population has accumulated a number of mutations by drift and that these mutations continue to contribute to our ageing phenotype today (Hughes and Reynolds 2005).

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DATA ARCHIVING

The doi for our data is 10.5061/dryad.1vv8v.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

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.

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.

Figure S1. Life-history trait comparison between the two parental lines and their hybrid offspring in experiment 4.