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Report

Longer Life Span Evolves under High Rates of Condition-Dependent Mortality

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Summary

Aging affects nearly all organisms, but how aging evolves is still unclear [1–5]. The central prediction of classic theory is that high extrinsic mortality leads to accelerated aging and shorter intrinsic life span [6, 7]. However, this prediction considers mortality as a random process, whereas mortality in nature is likely to be condition dependent. Therefore, the novel theory maintains that condition dependence may dramatically alter, and even reverse, the classic pattern [2–4]. We present experimental evidence for the evolution of longer life span under high condition-dependent mortality. We employed an experimental evolution design, using a nematode, *Caenorhabditis remanei*, that allowed us to disentangle the effects of mortality rate (high versus low) and mortality source (random versus condition dependent). We observed the evolution of shorter life span under high random mortality, confirming the classic prediction. In contrast, high condition-dependent mortality led to the evolution of longer life span, supporting a key role of condition dependence in the evolution of aging. This life-span extension was not the result of a trade-off with reproduction. By simultaneously corroborating the classic results [8–10] and providing the first experimental evidence for the novel theory [2–4], our study resolves apparent contradictions in the study of aging and challenges the traditional paradigm by demonstrating that condition-environment interactions dictate the evolutionary trajectory of aging.

Results and Discussion

We provide experimental evidence for the theory that aging evolves via condition-environment interactions. We show that when selection is condition dependent, high mortality rate leads to the evolution of increased longevity, defined here as mean adult life span in the absence of extrinsic mortality hazard. In order to test the evolutionary theories of aging, one must separate the effects of mortality rate (i.e., high versus low) and mortality source (random versus condition dependent). To achieve this, we quantified the evolutionary response in life span to selection under different rates of extrinsic mortality that were imposed either haphazardly or in a condition-dependent manner in replicate populations of a nematode worm, *Caenorhabditis remanei*. We anticipated that the evolution of life span would proceed according to classic prediction when extrinsic mortality rates were imposed at random. In contrast, we expected this pattern to be altered when high extrinsic mortality was imposed in a condition-dependent manner. Thus, our experiment aimed to simultaneously evaluate the relative strength of two opposing evolutionary forces that shape life span and aging: (1) combined effects of antagonistic pleiotropy and mutation accumulation due to reduction in the force of selection in late-life versus (2) positive pleiotropy due to condition dependence of mortality.

Mortality source significantly modified the evolution of life span under different mortality rates, resulting in source × rate interaction (Figure 1; $F_{1,\ 12.01} = 13.33$; $p = 0.003$). When the extrinsic mortality was applied at random, populations exposed to high mortality regimes had reduced life span compared to populations exposed to low mortality regimes, supporting the prediction of classical aging theories that high mortality leads to faster aging (Figure 1; within-model contrast: $F_{1,\ 12.20} = 5.75$; $p = 0.033$). However, the response to selection was reversed when the extrinsic mortality was condition dependent, such that high mortality rate resulted in increased life span (Figure 1; within-model contrast: $F_{1,\ 11.82,} = 7.66$; $p = 0.017$). Populations evolving under high condition-dependent mortality (HC-d) lived ~20% longer than their counterparts from high random mortality regimes (HR) despite experiencing the same level of extrinsic mortality (HC-d = 13.9 days, HR = 11.5 days; $F_{1,\ 11.82,} = 33.20$; $p < 0.0001$). We also note that the shape of the mortality curves (see Figure S1 available online) suggests that HC-d populations have better survival rates than HR populations starting from early life. We further tested for the potential trade-off between survival and reproduction by evaluating female lifetime reproductive performance. There was a significant effect of mortality rate with high-mortality populations producing more offspring, but no effect of mortality source (Figure 2).

We used increased temperature to impose condition-dependent selection in our experiment. High temperature kills, immobilizes, or slows down the worms, and only vigorous survivors were transferred to the next generation (see Supplemental Experimental Procedures for details). This source of mortality selection is appropriate for several reasons. First, *Caenorhabditis* nematodes are naturally exposed to increased temperatures and are known to be thermotolerant [11]. Second, resistance to increased temperature is associated with increased survival and immunity in nematodes [12], thereby reflecting general phenotypic condition. Third, the so-called heat shock proteins (HSPs) that are responsible for thermotolerance are, in reality, general molecular chaperones mediating an organism's defense against a broad range of biotic and abiotic stresses [13]. Fourth, it has been shown that heat shock increases the proportion of robust individuals in the population [14]. Thus, in our experiment, low condition-dependent (LC-d) mortality selected for a lower level of robustness than HC-d mortality, such that LC-d mortality populations were selected toward a lower peak in physiological performance compared with HC-d mortality populations and therefore could never reach the same high level as HC-d mortality populations.

Aging, a physiological deterioration leading to reduced fertility and increased probability of death over time, reduces Darwinian fitness, yet most organisms ultimately succumb to it [1, 15]. Evolutionary theory of aging strives to explain this

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Figure 1. Evolution of Life Span under Differential Life Histories

Mean life span of worms maintained in normally reproducing subpopulations in days ± SE derived from high and low mortality rate regimes when the mortality source was either condition dependent (filled circles) or random (empty circles). There was a significant effect of sex ($F_{1,\ 12.13}$, = 51.12; $p < 0.0001$), but there were no significant interactions between sex and experimental treatments (Table S1). Therefore, the data presented here are pooled across sexes. High mortality rate resulted in significant decrease in life span compared to low mortality rate when mortality was random, but the pattern was reversed when mortality was condition dependent (see text for statistical evaluation).

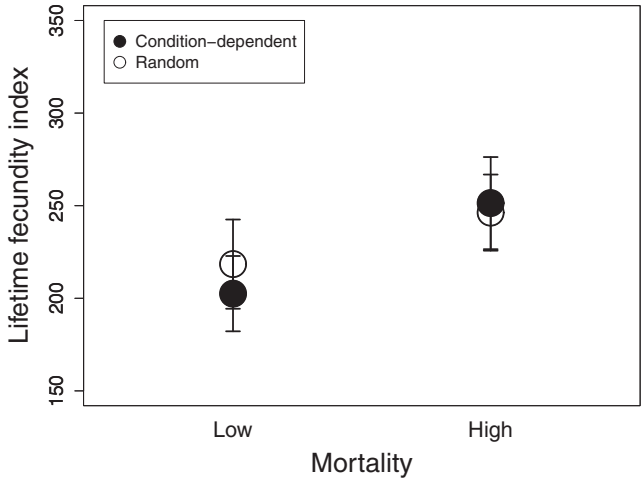

Figure 2. Evolution of Reproductive Performance under Differential Life Histories

Mean estimates of female lifetime fecundity index (number of eggs per vial ± SE) maintained in normally reproducing subpopulations derived from high and low mortality rate regimes when the mortality source was either condition dependent (filled circles) or random (empty circles). High mortality rate resulted in evolution of higher fecundity ($F_{1,\ 11.41}$, = 8.05; $p = 0.016$) irrespective of mortality source (main effect: $F_{1,\ 11.41}$, = 0.01; $p = 0.914$; interaction: $F_{1,\ 11.41}$, = 0.84; $p = 0.377$).

paradox, as well as the enormous variation in life span and aging rates across species and populations [8, 15]. The theory hinges upon the fundamental idea that the force of natural selection declines with age [6, 7]. The key prediction used for testing this theory asserts that an increase in extrinsic mortality leads to the evolution of rapid senescence and shorter intrinsic life span. This prediction, put forward by George Williams in 1957 [7], has been employed in comparative, field, and laboratory studies of aging, and most of the recent literature on the subject refers to this hypothesis [4]. Despite its importance and widespread acceptance, the empirical support for this prediction is surprisingly limited [4, 5]. The results of the comparative studies are mixed and inconclusive [1, 4, 16, 17], not the least because they often use broad ecological proxies (such as the ability to fly or climb trees) to determine the potential extrinsic hazards, and this may confound the outcome [1, 4, 18]. For example, birds and bats live longer than flightless mammals of similar body size, which has been attributed to either reduced extrinsic mortality due to reduced predation [19] or, alternatively, increased exposure to condition-dependent hazard [1, 4, 20]. Similarly, a handful of studies from natural populations have provided contrasting results, either supporting or contradicting classic prediction [4, 5]. For example, guppies (*Poecilia reticulata*) from pools with high predation lived longer compared to guppies from low-predation pools [5]. The main experimental support for the classic prediction comes from laboratory evolution studies selecting on age at reproduction or life span in model [9, 21–24] and nonmodel [25–27] organisms. A seminal paper by Stearns et al. [10] provided the most direct support for this prediction, by showing that increased extrinsic mortality did result in the predicted increase in intrinsic mortality and a shift in peak fecundity to an earlier age in populations of *Drosophila melanogaster* fruit flies.

However, laboratory evolution approaches have so far had an important limitation—mortality has been inflicted haphazardly, such that the surviving cohorts have been more or less random samples from the population. We suggest that this is rare in nature, where survivors are typically better at resisting the agents of mortality selection—predation, disease, competition, or adverse abiotic conditions [2–4, 28]. The generality of the classic prediction has been challenged by a theory suggesting that condition-environment interactions lie at the heart of the evolution of aging [3, 4]. The main argument behind this hypothesis is that increased environmental hazard (i.e., the source of increased extrinsic mortality) can select against senescence in a physiological trait that affects susceptibility to this hazard [2–4, 28]. Thus, when environmental hazard selects on the trait that is linked to a general condition, such condition-dependent selection can lead to the evolution of more robust organisms [3, 4]. The model predicts a variety of outcomes, including a scenario when high extrinsic mortality will, somewhat counterintuitively, select for decreased physiological deterioration and reduced senescence [3]. Given the diversity of contemporary theories of aging, the shortage of relevant empirical data with which to test them is hampering progress in the field [4, 5].

Our results corroborate the hypothesis that condition dependence alters the evolution of aging in response to extrinsic mortality. The general pattern of life-history evolution under random mortality was in line with the classic trade-off—high mortality resulted in the evolution of increased fecundity but reduced survival. However, condition-dependent selection reversed the trajectory of life-span evolution. It is notable that the evolutionary response reported here was so strong that life span actually increased (Figure 1) under high mortality. Our study is thus in line with the idea that condition-dependent selection results in the evolution of more "robust" organisms that experience slower physiological decline with age [3]. The increase in longevity was achieved without a trade-off

with reproduction, because high mortality lines enjoyed the same high levels of lifetime reproductive performance under both random and condition-dependent selection (Figure 2). This does not imply that evolution of increased life span under high condition-dependent mortality did not involve life-history trade-offs. For example, it is possible that a trade-off between survival and reproduction may be manifest under different environmental conditions or that increased survival may trade off with other aspects of the phenotype, for example larval survival. Furthermore, the trade-off between life span and reproduction may be sex-specific, with increased life span correlated positively with fitness in females but negatively in males [29], providing exciting avenues for future research on the role of sexually antagonistic selection in aging.

The evolutionary theory of aging has moved beyond the classic prediction [2, 3, 28, 30–33], but the empirical studies have fallen behind, and there is little data with which to evaluate the relative merit of the new hypotheses [4, 5]. Our study reveals that evolution of aging is tightly linked to the source of mortality and that condition-dependent selection can even reverse the classic scenario, resulting in increased longevity under higher mortality rates. The main strength of our study is the replication of the earlier findings [8–10, 21, 23, 26, 27, 34] combined with support for the new theory [3, 4], which allows for integration of different results into a more general picture of aging evolution. Because the evolution of aging in response to trait-specific mortality likely depends on covariation between different traits, condition, and fitness, we suggest that future studies should focus on testing the effect of different types of predation [5], disease, and abiotic factors on age-specific life histories in a variety of model and nonmodel organisms. Ideally, such studies should control for evolution under random mortality, although this may not be feasible in natural populations.

Experimental Procedures

C. remanei wild-type strain SP8 was maintained under standard laboratory conditions [35, 36]. The experimental populations evolved for 12 generations under different mortality rates (high, mean ± SE: 85.5 ± 0.05% dead per transfer; low, 28.6 ± 0.16% dead per transfer) imposed by different mortality sources (random or condition dependent) in a 2 × 2 design, resulting in four life-history regimes (four replicate populations per regime): (1) high random mortality (HR); (2) low random mortality (LR); (3) high condition-dependent (HC-d) mortality imposed by exposure to increased temperature; and (4) low condition-dependent (LC-d) mortality imposed by exposure to increased temperature. Such 2 × 2 design is particularly powerful because the four selection regimes control for each other. The first and second regimes (HR and LR) enabled us to test the classic prediction of accelerated aging under increased mortality. Here, the populations are experiencing divergent selection on early-life performance and therefore control for each other [25, 37]. At the same time, these two regimes both served as controls for the third and fourth regimes, which directly test the effect of increased extrinsic mortality imposed by an environmental hazard in a condition-dependent manner on the evolution of intrinsic life span and actuarial aging. The strength of this design is that it eliminates the need to compare the selection regimes with the ancestral population, which would be impossible since ancestral population evolved under different density and effective population size. Essentially, LR population is the one most closely resembling the ancestral state yet kept under the same conditions as populations in the other three selection regimes, making it possible to use it as a reference population. It was crucial to maintain the same mortality rates across condition-dependent and random regimes. Therefore, we first imposed different rates of condition-dependent mortality on HC-d and LC-d populations and then imposed corresponding rates of random mortality on HR and LR populations, so that HC-d and HR populations, for instance, differed only in the source of mortality, but their mortality rates matched with each other (see Supplemental Experimental Procedures).

Life-Span and Reproductive-Fitness Assays

Life-span assay and reproductive-fitness assay were conducted after 12 generations of selection followed by 2 generations without selection to remove potential environmental effects. In both assays, focal worms derived from experimental evolution treatments were maintained as normally reproducing subpopulations with the corresponding number of background "tester" worms of the opposite sex from SP8 source population, of which the number of individuals was adjusted throughout to maintain 1:1 sex ratio in all vials at all times. Life span of focal worms from experimental populations was measured separately for each sex in different replicate assays (10 worms per vial, n = 3 vials per population per sex). Reproductive fitness was estimated by females' lifetime fecundity (see Supplemental Experimental Procedures).

Statistical Analyses

The data were analyzed using general linear mixed models with restricted maximum likelihood approach with mortality rate, mortality source, and sex as fixed factors and population nested within mortality rate and mortality source as a random factor. We also included population × sex interaction as a random term. The life-span data were log transformed prior to the analyses.

Supplemental Information

Supplemental Information includes one table, two figures, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.09.021.

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