

The worm that lived

Evolution of rapid aging under high extrinsic mortality revisited

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Organisms age because of the “selection shadow”—the decline of the force of natural selection with age. Seemingly straightforward corollary of this theory is the Medawar-Williams prediction, which maintains that increased extrinsic (non-aging) mortality will result in the evolution of accelerated aging and decreased longevity. Despite its centrality to modern thinking about the ultimate causes of aging, this prediction ignores the fact that mortality is often a non-random process depending on individual condition. Increased condition-dependent mortality inescapably results in increased selection for resistance against the agent of mortality. Provided that resistance to various stressors is commonly associated with increased longevity, the evolutionary outcome is no longer certain. We recently documented this experimentally by showing that populations of *Caenorhabditis remanei* evolved to live shorter under high extrinsic mortality, but only when mortality was applied haphazardly. On the contrary, when extrinsic mortality was caused by heat-shock, populations experiencing the same rate of increased mortality evolved greater longevity, notwithstanding increased “selection shadow.” Intriguingly, stress-resistant and long-lived worms were also more fecund. We discuss these results in the light of recent theoretical developments, such as condition-environment interactions and hyperfunction theory of aging.

The Trouble with Aging

Aging is a multifaceted physiological deterioration of organismal function, which

increases the probability of death over time and ultimately limits intrinsic longevity.^{1,2} While the proximate mechanisms of aging are widely debated despite several established theories,^{2,3} the ultimate evolutionary reasons are well accepted.⁴⁻⁶ Because of extrinsic (non-aging) mortality due to predation, competition, disease, abiotic hazards and accidents, the strength of natural selection declines with age.⁷ Mutations that cause the loss of vitality in late-life are shadowed from natural selection—hence the term “selection shadow”—and can accumulate in the population.⁸ In 1957, George Williams⁹ proposed the antagonistic pleiotropy version of this theory by reasoning that an allele that contributes to increased performance in early-life can spread in the population even at the cost of performance in late-life. Later, the “disposable soma” theory combined the ultimate explanatory power of antagonistic pleiotropy with proximate physiological causes of aging.⁴ Organisms undergo a constant wear-and-tear and natural selection optimizes allocation to somatic maintenance and reproduction in a way that maximizes fitness rather than longevity. Current reproduction is often worth more than intrinsic longevity and future reproduction in the unpredictable world, and high-precision repair is costly.⁵ Thus, optimal resource allocation results in imperfect repair systems and slow accumulation of damage,^{4,10} in particular molecular damage,² bringing about the deterioration and finally the demise of the organism.

The key prediction of the evolutionary theory of aging is that increased extrinsic mortality will lead to the evolution of increased intrinsic mortality and reduced

Keywords: senescence, extrinsic mortality, evolution, longevity, Medawar-Williams prediction, tradeoff, reproduction, condition-dependence, stress, heat-shock

Submitted: 01/07/13

Accepted: 01/17/13

<http://dx.doi.org/10.4161/worm.23704>

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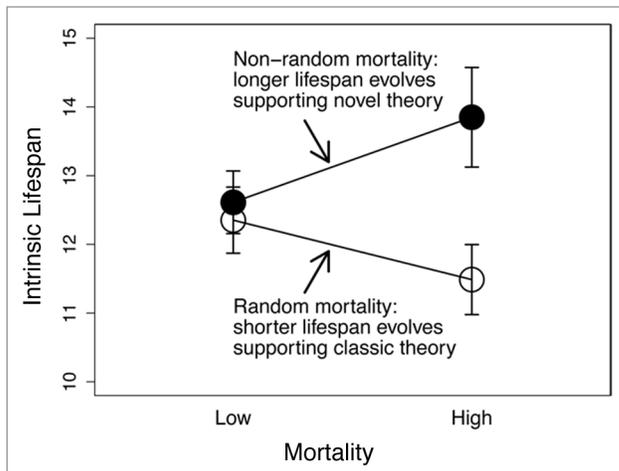


Figure 1. Schematic illustration of the core results of our experimental evolution test of the role of extrinsic mortality in the evolution of aging. Evolution of shorter lifespan (in days) under high random mortality supported the central prediction of the classic evolutionary theory of aging. However, long-lived worms evolved when the same rate of mortality was imposed by heat-shock, i.e., mortality was not random but condition-dependent, supporting the condition-environment interaction theory of aging.

longevity.^{9,11,12} It is widely believed that this prediction enjoys overwhelming empirical support, yet a close examination suggests that much of it is correlative and allows for alternative interpretation.¹¹ For example, increased longevity of birds and bats in comparison to similar-sized terrestrial mammals have been credited to safety provided by their ability to fly. However, it is at least equally likely that their increased longevity is a by-product of a strong selection on aerobic efficiency.¹³ Strong empirical evidence in favor of Medawar-Williams prediction comes from experimental laboratory studies on *Drosophila*.⁶ In one classic study, flies were kept for many generations under varying mortality rates applied by experimenters and indeed evolved differences in intrinsic mortality rates in accordance with theory.¹² One important caveat was however that mortality rates were applied at random, while mortality in nature is likely to be non-random, because individuals in better condition are more likely to survive environmental hazards in the form of pathogens, predators, starvation or adverse abiotic conditions.^{11,14,15} Such condition-environment interactions are likely to play a key role in the evolution of aging in the wild.¹⁴

The Medawar-Williams prediction has been challenged on theoretical grounds

for quite some time but these challenges have gone largely unnoticed in the field. Yet it has been repeatedly shown that (1) extrinsic mortality can have an effect on the strength of selection only under a certain set of conditions, such as age-specific density dependence and (2) the direction of the effect is variable.^{16,17} More recently, Williams and Day¹⁴ provided the first formal model of aging evolution under condition-environment interactions. At the heart of their model lies a straightforward idea that when senescence in trait increases organism's susceptibility to an environmental hazard (e.g. predation), than increased mortality due to this hazard increases selection against senescence in this trait. More broadly, it can be argued that organisms in better condition are more likely to survive most types of extrinsic hazards such that higher condition-dependent mortality will select for organisms that are more physiologically robust and show postponed or decelerated senescence.^{15,18} It is important to note that the formal model does not necessarily predict slower aging under high condition-dependent mortality, although this is one possible scenario. Most common outcome of the model is reduced senescence early in life and increased senescence late in life.¹⁴

We directly tested for the role of condition-dependent mortality by creating four

types of replicate populations of the dioecious nematode *Caenorhabditis remanei*.¹⁵ First, we replicated the classic design by having worms evolving under either high (H) or low (L) rate of extrinsic mortality (n = 8 populations per treatment). Then we made a crucial distinction. In half of our replicate populations, mortality was imposed by heat-shock (C-d for condition-dependent)—mild for low mortality treatment or severe for high mortality treatment. In the other half of the populations, the same rate of extrinsic mortality was imposed haphazardly (R for random). After only 12 generations of selection followed by a couple of weeks of relaxed selection to attenuate environmental effects associated with selection procedure, we observed the two main patterns. First, when mortality was imposed haphazardly, worms evolved reduced longevity in accordance with textbook examples. When the same mortality rate was applied using heat-shock, high extrinsic mortality resulted in the evolution of long-lived worms (Fig. 1). These results contradict the classic prediction but support the notion that aging evolves via interactions between mortality source and individual condition. Surprisingly, our long-lived and stressed-resistant (Fig. 2) worms were also more fecund than LR controls.

Darwinian Demon

Thus, as far as stress resistance and extrinsic mortality are concerned, our results are reasonably well explained by condition-environment interactions theory.¹⁴ However, this theory does not predict a positive effect of stress resistance on fecundity. On the contrary, one would expect that selection for increased stress resistance, accompanied by increased longevity in the absence of stress, would result in reduced fecundity because of the trade-off between reproduction and somatic maintenance. Nevertheless, our long-lived stress-resistant worms did not suffer a reduction in reproductive performance. In fact, HC-d females had slightly better fecundities in mid-life, which eventually resulted in higher total fecundity than control LR females,¹⁵ while HC-d males showed similar levels of reproductive success as LR males (Chen et al., in preparation).

There are several potential reasons as to why this was the case. These reasons can be broadly grouped into two main types, which we refer to as “hidden trade-off” and “beyond trade-off” hypotheses. Different forms of “hidden trade-off” hypotheses are often invoked in situations when a particular experiment produces results suggesting that there is no obvious genetic trade-off between reproduction and longevity. These hypotheses emphasize idiosyncratic nature of the results suggesting for example that selection can maximize reproduction and longevity in a particular environment at the cost of the performance of these traits in other environments. Provided that model organisms are often studied in the laboratory settings that do not resemble their ancestral environment, such arguments carry a lot of weight. We certainly believe that such scenario could potentially apply to our results and explain why there is sufficient amount of standing genetic variation for fitness.¹⁵ Another possibility is the increased strength of selection under our experimental conditions. A recent study in *Drosophila* suggested that flies possessing naturally occurring mutations in a gene encoding heat-shock protein HSP 90 suffer not only from reduced longevity and stress resistance but also from reduced fecundity and that such effects are particularly pronounced under heat-shock.¹⁹ Arguably, our selection procedure in HC-d treatment might have eliminated naturally occurring deleterious variants and resulted in improved fecundity, longevity and stress resistance. Yet naturally occurring deleterious mutations of this sort can only exist at very low frequencies, which was the case in the *Drosophila* study,¹⁹ and thus unlikely to account for rapid increase in fecundity in our experiment. Finally, animals in nature experience different sources of mortality, which may have different and interactive effects on the evolution of intrinsic longevity. For example, while some environmental stressors are positively genetically correlated with longevity, others are not.²⁰ Moreover, resistance to different stressors could involve trade-offs, further complicating the correlative evolutionary response in longevity in the natural environment.

There is however a possibility that increased stress resistance and reduced

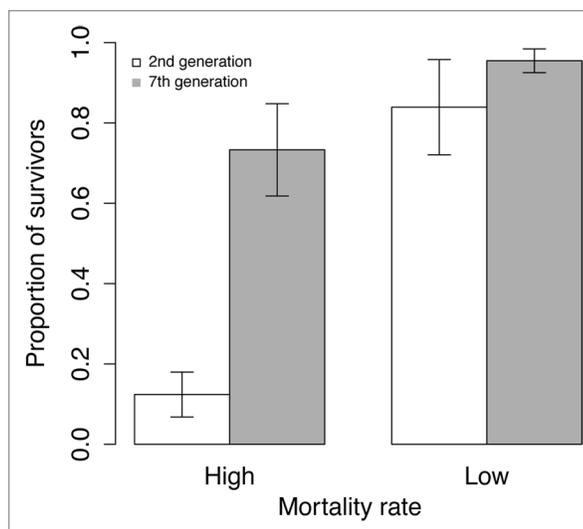


Figure 2. Rapid evolution of heat-shock resistance in populations where extrinsic mortality was applied using heat-shock (see text for details). The proportion of survivors significantly increased, in particular in high mortality rate treatment, between generations 2 and 7 (ANOVA on population means, generation: $F_{1,12} = 46.20$; $p < 0.0001$; interaction: $F_{1,12} = 8.90$; $p = 0.011$; data were arcsine-square root transformed prior to the analysis).

reproduction observed in many studies is coincidental and can be uncoupled,^{2,21} which is why we refer to these explanations as “beyond-tradeoff” hypotheses. Under classic disposable soma theory, exposure to stress diverts the resources from reproduction to somatic maintenance thereby prolonging life span.^{4,10} This logic has been successfully applied to explain the prolongevity effect of dietary restriction (DR), which is characterized by increased longevity and reduced fecundity under reduced availability of nutrients.²² However, a recent study indicated that reduced fecundity under DR can be restored by supplementation of a single essential amino-acid methionine, while keeping the prolongevity effect intact.²¹ Intriguingly, longevity under DR can be reduced by a mere smell of food, rather than by direct supplementation of resources.²³ Taken together, these results indicate that the simple trade-off model may not directly apply and that manipulation of nutrient-sensing pathway may hold the key to the prolongevity effect of DR and other stressors, including heat-shock.

Hyperfunction Theory

How can we reconcile these findings with the evolutionary theory of aging and is there a link between nutrient-sensing

pathway and heat-shock? Recently, Blagosklonny has put forward a new hyperfunction theory, which aims to combine the logic of antagonistic pleiotropy with the known facts about the complex relationship between stress resistance, reproduction and longevity.^{24,25} This theory argues that aging occurs primarily as a result of developmental growth program which is continuing to run at full capacity in late-life when its effects are no longer needed and detrimental because the organism has ceased growing. Apart from putting a new light on a relationship between growth rate and aging rate,²⁶ this theory fits well with the fact that nutrient-sensing pathway target-of-rapamycin (TOR) regulates both growth and aging and mediates the effects of DR and DR-mimetics on longevity.²⁵ An extensive recent review by Gems and Partridge² suggests that this theory might fit the current knowledge about aging biology better than previous models and, therefore, has a strong potential to become a new link between ultimate and proximate theories of aging.

Is there direct evidence for the role of heat-shock in antagonizing TOR-mediated aging? On one hand, there is a well-developed argument for the connection between heat-shock and TOR.²⁷ While activation of TOR increases protein biosynthesis, which is the main scourge under hyperfunction

theory, heat-shock decreases protein biosynthesis via the action of heat-shock proteins. In essence, heat-shock mimics the inhibition of TOR signaling. It is possible that we selected for the worms with continually upregulated heat-shock response (making them heat-shock resistant) and, therefore, continually reduced biosynthesis (making them long-lived).

Domestic Worm

On the other hand, upregulation of heat-shock response in late-life can buffer the worms against mutations whose deleterious effects are also confined to late-life potentially with little cost to growth and early reproduction. In our experiment, the worms experienced heat-shock earliest on the fourth day of adulthood, which suggests that we might have selected for age-specific upregulation of stress resistance. Currently we do not have data to test this hypothesis but it would be interesting to know whether HC-d worms are more stress-resistant than controls at larval stage, as well as young adults. Another possibility is that elevated stress response is not particularly costly for the worms in the presence of abundant food supply and the virtual absence of pathogens and predators. While such explanation would render our results less applicable to aging in natural populations, it would make them more relevant to aging in humans and domestic livestock, suggesting that aging is sufficiently malleable for medical interventions in the protected environments without the large costs of reproduction.

Worms in the Wild

In nature, *Caenorhabditis* nematodes are most commonly found to be associated with rotten fruits or other plant material, an environment that features abundant life and complex ecological communities^{28,29} and is affected by a broad variety of abiotic factors. Decaying organic matter provides nutrients and habitats for microbes and small invertebrates, which can be food source, pathogens or predators of nematodes. Thus, in their natural environment, nematodes are subjected to different kinds of condition-dependent and random agents of mortality affecting aging evolution.

Ambient temperature has profound effects on survival and general physiological condition in nematodes.³⁰ In our paper, we demonstrated that increased temperature, as the source of condition-dependent mortality, can lead to the evolution of prolonged lifespan and increased fecundity in *C. remanei*. We anticipate that other species of nematodes will show similar responses to increased temperature, at least with respect to longevity, because heat-shock proteins are responsible for general stress resistance. However, *Caenorhabditis* species have different geographical distribution, which may play a role in shaping the genetic correlation between thermotolerance and life-history traits. Some species (e.g., *C. briggsae*) are globally distributed, while others (e.g., *C. elegans* and *C. remanei*) are found in temperate regions.³¹ In *C. elegans*, isolates with different innate thermal preferences maximize their fitness through different routes - some isolates prefer temperatures at which they can achieve higher lifetime reproductive success, while others prefer temperatures that favor faster population growth rate.³² Besides, tropical strains of *C. briggsae* showed greater reproductive fitness when cultured in high temperature, while the opposite was found in temperate strains.³³ It would be interesting to explore how adaptation to local temperature affects the response in fitness to increased or decreased temperature.

In *Caenorhabditis* nematodes and other animals, immunity and aging share a common regulatory pathway, such that expression of the regulatory factor can increase immunity and simultaneously prolong lifespan.³⁰ Nematodes in the wild are constantly in risk of infection, as microbes are not only the source of food but also potential pathogens. Indeed, fresh isolates of nematodes often show indications of infection.²⁸ Moreover, while one main effect of pathogenic microbes is diminished lifespan,³⁴ longer-lived nematode species show increased immunity,³⁵ which suggests an important role of mortality risk imposed by pathogens in the evolution of aging. Bronikowski and Promislow suggested that pathogen-induced mortality is particularly interesting in the context of aging evolution under condition-dependence because the organisms

that survived the infection are likely to be more resistant to future attacks. Another promising direction is the interaction between viral pathogens and RNA silencing system in nematodes. *Caenorhabditis* species differ in their sensitivity to environmental RNA,³⁶ a defense mechanism against virus infection.^{37,38} Moreover, different wild isolates of *C. elegans* show different susceptibility to infection by their natural viral pathogens.³⁸ These results indicate a heterogeneous infection risk by viral pathogens in different geographical locations, which may lead to the evolution of different immune mechanisms in *Caenorhabditis* nematodes. Interestingly, while *C. briggsae* is susceptible to natural viral pathogen,³⁸ it is insensitive to environmental RNA,³⁶ suggesting the underlying immune mechanism may be more complex than currently understood.

Predation pressure is yet another potentially important source of condition-dependent mortality. In guppies, populations evolved under high predation pressure show longer lifespan and better performance in predator-evasion behavior early in life compared with populations from low predation sites.³⁹ In *Caenorhabditis* nematodes, however, the significance of predation pressure is largely overlooked. The natural habitat of nematodes is shared by diverse groups of organisms, particularly small invertebrates, many of which (e.g., mites, flatworms and tardigrades) are potential predators of nematodes.^{28,29,40-44} For example, mites that prey on nematodes swallow them like spaghetti^{28,45} and can induce extremely high extrinsic mortality that eventually can lead to extinction of experimental population of nematodes.⁴⁴ Such sources of mortality are likely to be random with respect to nematode condition, but only detailed studies of nematode ecology in semi-natural microcosms will be able to confirm this.

Conclusion

The Medawar-Williams prediction that high extrinsic mortality results in the evolution of rapid aging has guided research in the field for many decades.¹¹ Yet, given the theoretical challenges to this hypothesis, we concur with the previous authors

that it is only surprising that findings contrary to this prediction have only now started to appear.¹⁸ Recent theoretical^{14,16} and empirical^{15,28} advances suggest that the evolution of aging in response to high extrinsic mortality is complex and likely to proceed in different directions. Several potential scenarios are possible depending on age-specificity of density-dependence and on the interactions between the source of extrinsic mortality and individual condition. Our experimental results suggest that evolution of long-lived, stress-resistant and fecund worms is possible under high extrinsic mortality imposed by heat-shock. We are currently investigating whether heat-shock resistance is age-specific and whether the growth rate was slowed down in these populations. Future research in this direction should focus on elucidating the generality of these findings by using different sources of mortality (e.g., pathogens, predators) and on testing the performance of the selected populations under the more natural conditions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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