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Report

Condition Dependence of Male Mortality Drives the Evolution of Sex Differences in Longevity

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Summary

Males and females age at different rates and have different life expectancies across the animal kingdom, but what causes the longevity "gender gaps" remains one of the most fiercely debated puzzles among biologists and demographers [1–7]. Classic theory predicts that the sex experiencing higher rate of extrinsic mortality evolves faster aging and reduced longevity [1]. However, condition dependence of mortality [8, 9] can counter this effect by selecting against senescence in whole-organism performance [5, 10]. Contrary to the prevailing view but in line with an emerging new theory [7–9, 11], we show that the evolution of sex difference in longevity depends on the factors that cause sex-specific mortality and cannot be predicted from the mortality rate alone. Experimental evolution in an obligately sexual roundworm, *Caenorhabditis remanei*, in which males live longer than females, reveals that sexual dimorphism in longevity erodes rapidly when the extrinsic mortality in males is increased at random. We thus experimentally demonstrate evolution of the sexual monomorphism in longevity in a sexually dimorphic organism. Strikingly, when extrinsic mortality is increased in a way that favors survival of fast-moving individuals, males evolve increased longevities, thereby widening the gender gap. Thus, sex-specific selection on whole-organism performance in males renders them less prone to the ravages of old age than females, despite higher rates of extrinsic mortality. Our results reconcile previous research with recent theoretical breakthroughs [8, 9] by showing that sexual dimorphism in longevity evolves rapidly and predictably as a result of the sex-specific interactions between environmental hazard and organism's condition.

Results and Discussion

Sex differences in longevity and aging are ubiquitous and attract wide attention from biologists and demographers alike but remain one of the unsolved problems in evolutionary biology. A prediction by George Williams [1], which guided most of the research on the evolution of sexually dimorphic life histories, was that sexual selection in males would drive the evolution of costly male traits and increased age-independent mortality, resulting in the evolution of more rapid aging in males compared to females [1, 12–16]. Conversely, in some taxa where females experience higher age-independent mortality than males, more rapid female aging is expected to evolve [17]. Despite broad multidisciplinary acceptance of this hypothesis, the empirical support is rather inconclusive [5, 16, 18, 19]. Remarkably, a direct experimental test of Williams's hypothesis that increased sex-specific mortality would result in the evolution of reduced longevity in the focal sex is still lacking. Moreover, recent theoretical work challenged the generality of Williams's prediction [8, 9, 20, 21] and emphasized that condition dependence of mortality can paradoxically result in longer lifespan under high extrinsic mortality rate, which has now been empirically confirmed [11]. The corollary is that strong sexual selection on male condition and whole-organism performance [10] can favor alleles with positively pleiotropic effects on longevity [5, 7]. However, the role of such positive pleiotropy in the evolution of sexual dimorphism in longevity has never been tested empirically.

Here, we directly address the theory that sex difference in extrinsic (age-independent) mortality shapes the evolution of longevity "gender gaps." We employ an experimental evolution approach by imposing male-limited extrinsic mortality in obligately sexually reproducing nematode *Caenorhabditis remanei*, in which males live longer than females. We simultaneously test two different mechanisms that can contribute to the evolution of sexually dimorphic life histories: (1) Williams's hypothesis that increased extrinsic (age-independent) mortality will result in the evolution of reduced longevity in the affected sex and (2) the positive pleiotropy hypothesis, suggesting that increased condition-dependent mortality (when low-condition individuals have higher risk of death) will result in increased selection on whole-organism performance and the evolution of longer lifespan in the affected sex. We provide strong experimental support for both of these hypotheses and show that gender gaps evolve rapidly and can widen or disappear in the course of 20 generations.

Every 3 days, we removed 80% of the males in a focal population and replaced them with the same number of virgin young males derived from the offspring cohort of this population (n_{males} = 50 per population and $n_{females}$ = 50 per population; $n_{populations}$ = 4 per selection regime). Thus, each replicate population maintained the same population size throughout the experiment, but male mortality rate was artificially increased compared with natural conditions (see Figure S1 available online for a detailed drawing of the experimental design). Female extrinsic mortality was kept identical in both selection regimes by replacing 50% of females during each selection bout. This was done to ensure that males always had access to fertile females and to avoid population bottlenecks because female fecundity starts to decline rapidly after 3 days of age [11]. In random mortality treatment, the males were removed haphazardly, whereas in condition-dependent mortality treatment, males were first subjected to a mate search test, and the 80% that scored worst were removed from the population. To simulate condition-dependent mortality based on whole-organism performance by males in the context of reproduction, we focused on male chemotaxis during mate search, which integrates male locomotion and chemosensory ability. Whole-organism performance is broadly defined as an organism's performance in a dynamic, ecologically relevant behavior, such as locomotion [10], and mate search is a male-specific trait in *Caenorhabditis* nematodes [22]. Specifically, males and immobilized females used as a

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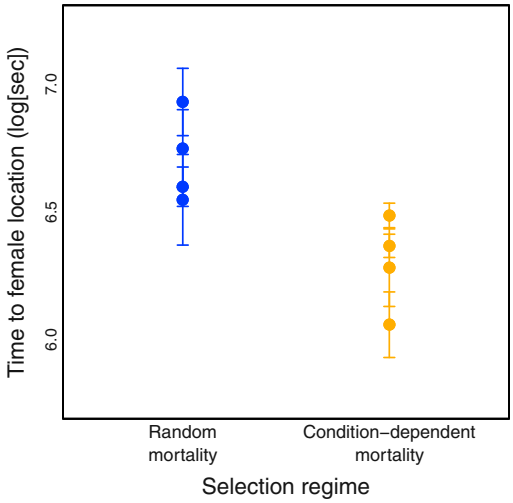

Figure 1. Evolution of Male Chemotaxis toward Females under Differential Male-Limited Extrinsic Mortality

Males from populations evolving under condition-dependent mortality (orange circles), based on the speed with which they were able to locate virgin females, indeed evolved to do so faster than males from populations evolving under random extrinsic mortality (blue circles) after 20 generation transfers. This mate search assay precisely followed the original selection procedure: 50 males from each replicate population were put on the plate, and the time it took to locate immobilized virgin females was recorded for the first ten individuals. Data represent mean values ± SE for each replicate population.

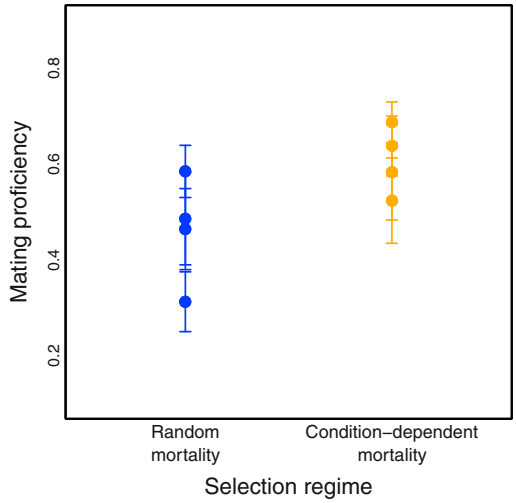

Figure 2. Evolution of Male Mating Proficiency under Differential Male-Limited Extrinsic Mortality

Males from populations evolving under condition-dependent mortality (orange circles) were more successful in mating than males from populations evolving under random extrinsic mortality (blue circles). In this assay, ten individual males from each replicate population were allowed access to eight virgin females, and mating proficiency, which was measured as the proportion of total females the male successfully mated with during 3.5 hr, was recorded. Data represent mean values ± SE for each replicate population.

pheromone source were placed at the opposing long ends of a rectangular slide of agar, and the first 20% of males that arrived at the pheromone spot were kept in the population and could continue to reproduce. Thus, although mortality rate was increased in an identical fashion in both treatments, the remaining 20% of males represented either a random (random [R] treatment) or a nonrandom (condition-dependent [C-d] treatment) subset of the initial population. Because the population was always maintained at the same population size, these 20% of remaining males from the previous generation were always competing against the 80% of newcomers. After 20 bouts of selection, we kept all populations without selection for two generations before we assayed the evolutionary response in male performance during mate search (time it took to locate females), male mating success, and longevity in both sexes. The assay of mate search performance followed the same protocol as C-d treatment (see above). Male mating success was measured as the number of females the male could successfully fertilize when presented with unlimited number of potential mates. For longevity assays, four replicates of 25 same-sex individuals (two replicate plates per sex) were created and kept with an equal number of wild-type individuals of the opposite sex. We continuously adjusted the number of wild-type worms to maintain a 1:1 sex ratio throughout the assay [11]. Longevity was measured until the last individual died.

After 20 bouts of selection, males from the C-d treatment that were selected on the basis of mate search test were indeed better able to locate females than males from the R treatment ($F_{1,5}$ = 11.453, p = 0.020, Figure 1) and had higher mating success when confronted with several virgin females in a separate assay (X^2 = 4.765, p = 0.029, Figure 2), suggesting that the condition-dependent treatment resulted in adaptation. Thus, we created replicate populations that evolved under an identical rate of extrinsic male mortality but differed in the direction of sex-specific selection on males, resulting in divergent evolution of male reproductive ability. We then employed a "time machine" approach to compare sex-specific longevities in the evolved populations after 20 bouts of selection to their own ancestral populations cryopreserved at generation 1 (Figure 3; note that evolved populations were also cryopreserved prior to the assays). The time machine approach allows contemporaneous longevity assays in the evolved and ancestral populations but prevents the ancestral population from evolving, thereby providing perhaps the ideal form of control in experimental evolution [23].

Whereas female longevity did not evolve and remained the same in both treatments, male longevity evolved in the opposing directions in R and C-d populations (selection regime * history * sex interaction: $F_{1,12}$ = 8.408, p = 0.013; Figure 3; Table 1). In accordance with Williams's prediction, R males that experienced increased rates of sex-specific mortality evolved reduced longevity (within-model contrast: t = 2.22, p = 0.037; Figure 3), and this effect was so pronounced that sexual dimorphism in longevity, a pervasive biological feature not only of *C. remanei* but also of *Caenorhadbitis* nematodes in general [17], was completely eroded. Essentially, increase in male-limited mortality resulted in the evolution of sexual monomorphism in longevity in a species that naturally exhibits sexual dimorphism in this trait.

Nevertheless, in accordance with the positive pleiotropy hypothesis, when mortality rate was increased in a condition-dependent fashion, males evolved increased lifespan (within-model contrast: t = 2.83, p = 0.009; Figure 3), thereby widening the longevity gap between the sexes.

Despite strong interest in sex differences in longevity in the fields of human biodemography and evolutionary biology, our understanding of this phenomenon is very incomplete [5–7, 24–26]. The prediction derived from classic evolutionary theory

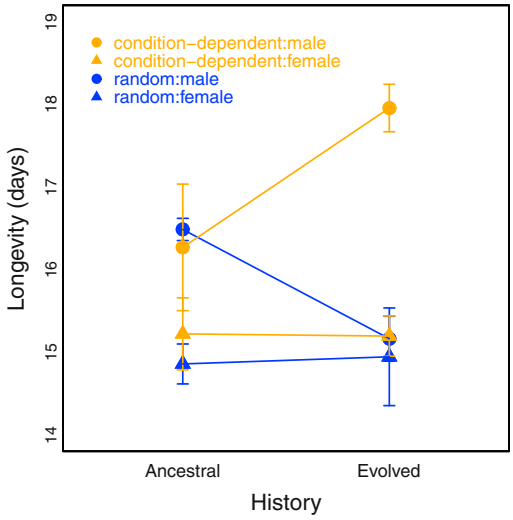

Figure 3. Evolution of Sexual Dimorphism and Monomorphism in Longevity under Differential Male-Limited Extrinsic Mortality

Males evolving under increased random mortality (blue circles) evolved reduced longevities, whereas males evolving under increased condition-dependent mortality (orange circles) evolved increased longevities, compared with their ancestral state (see Results and Discussion and Table 1 for statistical evaluation). Female lifespan (blue triangles indicate random mortality; orange triangles indicate condition-dependent mortality) did not evolve. Data represent mean longevity ± SE for males and females from four replicate populations evolving under random or condition-dependent mortality, measured contemporaneously in ancestral and evolved (20 generation transfers) populations.

that increased extrinsic mortality in one sex (usually males) should lead to the evolution of reduced longevity in this sex has been supported by some [15, 16] but contradicted by other [18, 19, 27] studies, whereas the definitive experimental test based on the manipulation of extrinsic sex-specific mortality has not been performed until now. At the same time, recent theoretical developments and empirical studies in the evolutionary biology of aging cast a new light onto the idea that males may be selected to be potentially longer-lived than females despite experiencing higher extrinsic mortality [5, 7]. The key point of the new theory is that the source of extrinsic mortality matters [7–9, 20, 21, 28, 29]. When the risk of death depends on the physiological condition of the individual, increased mortality increases the strength of selection, which can result in the evolution of increased longevity because of the positive pleiotropy between whole-organism performance and longevity. Therefore, if we want to predict the evolutionary response in longevity following increase in extrinsic mortality rate in a particular sex, we have to consider the source of mortality. Thus, far from simply complicating the matter of sexual dimorphism in longevity, inclusion of these new ideas may help in reconciling classic theory with the contrasting empirical results (e.g., [18, 19, 27]) and thereby facilitate our understanding of this multifaceted phenomenon.

These results provide a clear illustration of how variation in sex-limited mortality affects the evolution of longevity and shapes sexual dimorphism in this trait. Williams's original insight is agreeably upheld by the demonstration of the evolution of monomorphism in longevity as a result of increased extrinsic mortality in the longer-lived sex. However, in stark contrast to Williams's prediction but in full agreement with the positive pleiotropy hypothesis, we show that

Table 1. The Full GLMM of the Effects of Selection Regime, Experimental History, and Sex on Lifespan

	F Ratio	Prob > F
Selection regime	$F_{1,12} = 0.3733$	0.5526
History	$F_{1,12} = 0.0211$	0.8868
Sex	$F_{1,12} = 9.1431$	0.0106
Selection regime * history	$F_{1,12} = 0.0180$	0.8955
Selection regime * sex	$F_{1,12} = 0.5810$	0.4607
History * sex	$F_{1,12} = 3.4241$	0.0890
Selection regime * history * sex	$F_{1,12} = 8.4075$	0.0133

condition-dependent mortality increases male longevity despite increased extrinsic mortality and, consequently, generates increased sexual dimorphism.

Although we originally predicted that an increase in male-limited extrinsic mortality would either reduce or amplify sexual dimorphism depending on the nature of the mortality source, we anticipated that female longevity would also evolve, albeit to a lesser degree, because of the intersexual genetic correlation for this life history trait [30–32]. However, in line with recent findings in other model organisms [33, 34], our results suggest that there is a substantial amount of sex-specific genetic variation for longevity facilitating rapid evolution of sexual dimorphism in life history in response to sex-specific selection.

Evolution of sex differences in longevity has been suggested to stem from selection on females leading to increased mutation load and reduced longevity in males because of asymmetric inheritance of sex chromosomes ("unguarded X") [2, 3, 12] and mitochondrial genomes ("mother's curse") [3, 35]. Although the mother's curse hypothesis has enjoyed experimental support [36], and mtDNA mutations are likely to increase variance in male aging, neither of these two hypotheses is likely to provide a general explanation for the evolution of sex differences in longevity and aging because males live longer than females in many taxa [7, 12, 37, 38]. Our results show that selection on males, and in a broad sense, sex-limited selection in either sex, is sufficient to generate sexual dimorphism in aging and longevity, whereas the interaction between the rate and the source of mortality (i.e., whether mortality is random or condition dependent, with respect to whole-organism performance) can determine which sex will live the longest.

The study of sex differences in longevity has two main goals: (1) to increase our understanding of how evolution contributed to the variation in longevity between males and females across the animal kingdom and (2) to inform the biomedical research community on the likely evolutionary basis of longevity gender gap in humans. Although these results help to build a general framework for understanding the evolution of sexual dimorphism in age-specific life histories, we believe they also have important implications for human aging. Biodemographers studying aging in contemporary human populations have been recently confronted with a "health-survival paradox" [6]: men are healthier than women, particularly in late ages, but die younger [6, 25, 26, 39, 40]. Recent developments in the evolutionary theory of aging and the results of this study may help resolve the paradox: stronger selection on males may render them less prone to the ravages of old age than females, in spite of males having higher rates of extrinsic mortality.

Experimental Procedures

Nematode Strain and Experimental Evolution

C. remanei wild-type strain SP8, provided by N. Timmermeyer from the Department of Biology, University of Tuebingen, was used in this study.

This strain is a cross between three wild-type isolates (SB146, MY31, and PB206) and has been shown to harbor a substantial amount of standing genetic variation for life history traits [11]. This population has been maintained for a sufficient number of generations to create recombinant genotypes [11]. All worms were maintained under standard laboratory conditions [41].

The procedure of experimental evolution is summarized in Figure S1. To investigate the evolutionary responses of sex differences in lifespan, we created two experimental evolution regimes with identical rates (80% per every 3 days) but different sources (C-d or R) of male-limited extrinsic mortality. Based on previous work, this rate of extrinsic mortality was expected to be sufficient to cause the evolution of longevity [11]. At the beginning of the experimental evolution, eight populations of *C. remanei* SP8, each consisting of 100 individuals (n_{male} = 50 and n_{female} = 50) synchronized immediately before the adult stage, were established using the standard methodology [41]. The populations were then randomly assigned to either C-d regime or R regime ($n_{population}$ = 4 per selection regime). All populations were left for 2 days to reproduce after being established. On the third day, the original 100 individuals had all become adults, whereas the progeny they produced during the previous 2 days were at the last larval stage. This allowed us to isolate the focal males and to a separate them on a nematode growth medium (NGM) plate, where they had no access to females for 24 hr until the selection took place, to standardize their mating status. On the next day, these males were subjected to either condition-dependent mortality or random mortality that they were assigned to (see below). Female mortality was maintained at the identical rate and applied in the same manner in both selection treatments by replacing 50% of females in the population every 3 days. Female fecundity in this population declines rapidly after 3 days of age [11], and, for this reason, we did not expect this procedure to result in strong selection on female longevity. Our results indicate that female longevity did not evolve and remained the same in both selection regimes, confirming that our experimental procedure neither resulted in any appreciable selection on female longevity nor differed between the selection regimes in any way.

Male-limited condition-dependent mortality was based on whole-organism performance by males in the context of reproduction. We focused on male chemotaxis during mate search, which integrates male locomotion and chemosensory ability. Experimental methods and configuration were modified from those published by Chasnov et al. [22]. Selection was performed on a 2 × 5 cm slide of NGM placed in the center of a 9 cm Petri dish. Immediately before the assay, two drops of 10–15 μl M9 buffer, separated by 3 cm, were placed at the opposite long end of the NGM slide. Twenty mature SP8 females were transferred to one side of the M9 drops and were paralyzed by applying 1.5 μl of 50 mM sodium azide. Then, the 50 males were transferred to the empty M9 drop on the opposite side and were confined in the drop. After all the males were transferred, superfluous M9 was removed by a glass Pasteur to release them all at once. Each replicate was observed for a maximum of 30 min, and the first ten males that arrived closest to the females were selected and transferred to a new NGM plate for the next generation. In the R regime, ten random males were transferred to the next generation. To restore the population size, we added a random sample of the adequate number of nematodes (i.e., 40 males and 25 females) from the corresponding progeny cohorts from the same population, which had already developed into adults by the day of selection.

The resultant populations after 20 generation transfers of experimental evolution (evolved [E] populations) and their respective ancestral populations from generation 1 (ancestral [A] populations) were cryopreserved at −80°C [41]. In the following assays, all populations were thawed and kept without selection for 1 week (two generations) before standard synchronization was applied. This means that all populations experienced four generations of relaxed selection (two before freezing and two after thawing) prior to experimental assays, which is more than is commonly used by most labs to ameliorate the potential parental effects (in most experimental evolution studies, only two generations of relaxed selection are used to save time). All experiments were in line with ethical regulations at Uppsala University.

Response to Experimental Evolution

To evaluate male response to selection, we measured male ability to locate females (in a female location assay) and male mating success (in a mating proficiency assay) in the eight evolved populations.

The female location assay was performed on two consecutive days with balanced block design, where each block had two EC-d and two ER populations. For each population, we created a cohort consisting of 50 pairs of males and females that were synchronized at the last larval stage. As in the experimental evolution procedure, the cohorts were left for 2 days after being established, and on the third day, focal males were isolated to a separate NGM plate without access to females. The assay was performed on the fourth day, and the procedure and configuration of the assay were identical to those used during the experimental evolution. Each replicate was observed for 30 min maximum, and the average time that the first ten males took to arrive at the female location was calculated. The data were log transformed prior to statistical analyses.

In the mating proficiency assay, ten virgin males synchronized at the last larval stage were isolated from each of the eight E populations. These males were kept with no access to females to ensure virginity. On the fourth day, the assay was performed on a 9 cm NGM plate seeded with 1 mL *E. coli* and marked with two spots separated by 3 cm (i.e., the same distance as in the experimental evolution). At the beginning of the assay, eight virgin SP8 females were transferred to one of the two spots, and following that, one focal male was transferred to the opposite spot. The nematodes were left for 3.5 hr to mate, and each female was then isolated to an individual 3 cm NGM plate seeded with 100 μl *E. coli* OP50. Replicates in which males were not found were excluded from the data. The next day, the number of females that the focal male successfully fertilized was determined by the presence of eggs on individual plates. Females that were found missing were excluded from the replicate (one female missing in five replicates and two females missing in two replicates out of the 80 total replicates). Mating proficiency was estimated as the proportion of mated females in the sample and analyzed using binomial error distribution (see below).

Longevity Evolution

Longevity of males and females was measured contemporaneously in the E and A (cryopreserved) populations in both selection regimes. Each of the 16 total populations (four populations per selection regime [R or C-d] per evolutionary history [A or E]; four replicates of 25 same-sex nematode cohorts [two replicates per sex]) that were synchronized at the last larval stage were established using standard methodology [41] at the beginning of the assay. The focal individuals derived from experimental populations were kept with the corresponding number of background individuals of the opposite sex derived from SP8 populations. Background individuals were sexually mature young adults (normally between 1 day old and 3 days old) to ensure that focal individuals had access to fertile mating partners. Lifespan of the focal individuals was recorded every 3 days. Individuals that failed to respond to gentle prodding by a soft picker were considered dead. Alive individuals, together with corresponding number of background individuals, were transferred to fresh NGM plates to avoid mixing with progeny. If background individuals were found dead or missing, new individuals of the background sex would be replenished from SP8 populations. The measurement continued until the last individual died.

Statistical Analysis

All analyses were performed using the R programming language (http://www.r-project.org), with the help of lme4 and nlme packages. Longevity data were analyzed using a restricted maximum log likelihood approach in a generalized linear mixed-effects model (GLMM), with sex (male or female), mortality source (C-d or R), and experimental history (A or E) fitted as fixed effects, population nested within mortality source, and experimental history included as a random effect. We also used within-model contrasts to compare longevity of males with different experimental history following the initial discovery of significant three-way interaction among sex, selection regime, and evolutionary history. For the female location assay, the mortality source was fitted as a fixed effect, and the population nested in block was fitted as a random effect.

For the mating proficiency assay, we used a GLMM with mortality source as a fixed effect and population as a random effect. To account for missing females, we weighted the model by the number of females paired (6–8). Because the response variable was proportional and had a binomial error distribution, we used logit link in the model to transform the data. Laplace approximation was used to estimate the likelihood of the parameters. The significance of the fixed effect was estimated by comparing models with or without the effect using a likelihood ratio test.

Supplemental Information

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.08.055.

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