Dispatches

Aging: Manipulating Sex Differences

A new study reports that male, but not female, longevity evolves in response to increasing male mortality. The sex gap in longevity widens depending on the type of mortality.

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The life histories of animals are marked by two pervasive characteristics — nearly all animals reproduce sexually, and virtually all exhibit the hallmarks of physiological deterioration (i.e. senescence) with advancing age. While some animal species are hermaphroditic, many — including ourselves — are characterized by distinct sexual entities (gonochorism); individuals are either male or female, but not both. Among such species, a striking pattern has emerged; the average longevity of one sex often exceeds that of the other. And more often than not across species — from *Drosophila* fruit flies, to mice and humans [1], although with exceptions in some taxa such as birds [2] and several nematode species [3] — it seems to be the females that typically outlive the males. A new study by Chen and Maklakov [4] in this issue of *Current Biology* provides fascinating insights into why such 'sex gaps' in longevity will evolve. By altering the mortality rate of males within experimental populations of a gonochorous nematode, the authors were able to invoke evolutionary decreases or increases in male longevity, depending on whether they applied mortality randomly or in a condition-dependent manner. Remarkably, female longevity was not dragged in tow, indicating that the alleles responsible for the male responses are sex-specific in their effects.

Sex Gaps in Longevity

In humans, early-life sex-biases in longevity can be tied to differences between the sexes in exposure to a range of extrinsic environmental hazards. Males, for instance, are more likely to engage in alcohol-fuelled violence, risk-taking behaviors, and warfare relative to their female counterparts, all of which are likely linked to the male hormone testosterone [5], and all of which could be expected to lower the mean male life expectancy within any given population. However, intriguingly the sex gap in longevity appears to hold even when we attempt to factor out cases of early to mid-life mortality. For example, at birth, an Australian female can expect to live nearly four and a half years longer than a male. Yet, even if we limit analysis to only those individuals that have already survived to the age of 65, a female can still expect to live three years longer than an equivalently-aged male [6]. In experimentally tractable taxa, such as insects, individuals can be kept in benign laboratory environments, essentially free from the stresses of disease, predation and conflicts over sexual opportunities. When this is done in *Drosophila melanogaster*, females still outlive males by up to 15 percent of their lifespan [7]. These sex-differences are therefore evolved — they are encoded 'in the genes'.

Deciphering why organisms would evolve to age seems challenging enough, given that one might expect Darwinian selection to favor adaptations that prevent physiological deterioration with advancing age. The answer to this question is, however, reconcilable by evolutionary theory. Generally, the magnitude of natural selection declines with age, simply because most reproduction is done earlier in life, and this enables mutations to accumulate within the genome that exert later-life fitness effects that lead to senescence. Increasing the rate of extrinsic mortality (i.e., mortality due to environmental hazards such as predation, disease and accidents) shifts the reproductive probability distribution to younger age classes and, according to classic theory, this should result in the evolution of shorter lifespan [8,9]. But, while this logic seems compelling, the evolution of aging is not quite so simple. Experimentally applying extrinsic mortality in a condition-dependent (as opposed to random) manner can actually increase longevity because selection favors alleles that are robust ('positively pleiotropic') in their effects across age-classes (Figure 1) [10–12].

Now, to account for the evolutionary processes that would lead to the evolution of sex gaps in longevity might seem like an ever greater conundrum. For one, with the exception of certain genomic regions (such as the Y-chromosome), the sexes actually share the same genome. Two sets of hypotheses have emerged as candidates to best explain these sex gaps. The first centers on sexual asymmetries in inheritance of the X-chromosome and mitochondrial genome [13,14]. Females always transmit a copy of the X and mitochondrial genome to their offspring, while males only transmit their X to their daughters and never pass their mitochondrial DNA to their offspring. These asymmetries have implications for the evolution of aging. Because these genomic regions are primarily screened for function in females, they can theoretically accumulate mutations that exhibit sex-biased effects (i.e., male-harming, but benign or beneficial to female lifespan). These theories have been called the 'unguarded X' [13] when applied to the X-chromosome, and 'mother's curse' [15] when applied to the mitochondrial genome. The second hypothesis centers on sex-specific selection as a driver of the sex gaps. Namely, when mortality selection is applied in a sex-specific manner, evolutionary trajectories of aging may likewise be sex-specific [1,16].

Sex-specific Selection

While hypotheses based on sexually asymmetric gene transmission have received theoretical and empirical substantiation [7,14,17], the hypothesis based on sex-specific selection is similarly compelling. Indeed, the Chen and Maklakov study [4] points to a pervasive role for sex-specific selection. Previously, these same authors [11] had demonstrated that

trajectories of longevity evolution in the nematode *Caenorhabditis remanei* were contingent on the source of mortality applied. Increases in randomly-applied mortality led to reduced longevity consistent with classic predictions, while condition-dependence led to enhanced longevity [11]. With that knowledge, the authors again set out to increase mortality rates across experimental populations of these nematodes in much the same way, but this time with an intriguing twist; mortality selection was applied only to males. And while the source of condition-dependent mortality used previously had been a sex-general thermal stress [11], this time the authors applied it to a sex-specific male trait putatively under sexual selection; chemotaxis while mate searching, promoting the survival of the fastest males that responded to a female sex pheromone. The results were striking. Evolutionary responses of longevity were completely male-specific, increasing under condition-dependent mortality, decreasing under random mortality. Furthermore, because female longevity did not respond to male mortality selection, the sex gap in longevity could effectively be abolished, or accentuated, according to the source of mortality.

Much now remains to be done to verify the generality of this result. First, it will be important to determine the relative contributions of sex-specific random versus condition-dependent extrinsic mortality to driving sex gaps in longevity in natural populations. Second, the contribution of sex-specific selection in driving these gaps needs to be formally compared to that of non-adaptive mechanisms attributable to sexual asymmetry in gene transmission. Furthermore, the results, while fascinating, paradoxically harness a species exhibiting an atypical pattern, because in these worms the males generally outlive the females [3]. Proof-of-concept in a species exhibiting the typical pattern of 'females outliving males' will be the next step. One can also extend the authors' lines of reasoning to predict that any population evolving under high sexual selection on males could evolve longer male lifespan than counterparts evolving under monogamous conditions. In this respect, many experimentally-evolved resources have already been developed, in which the premise of the authors' predictions might be tested.

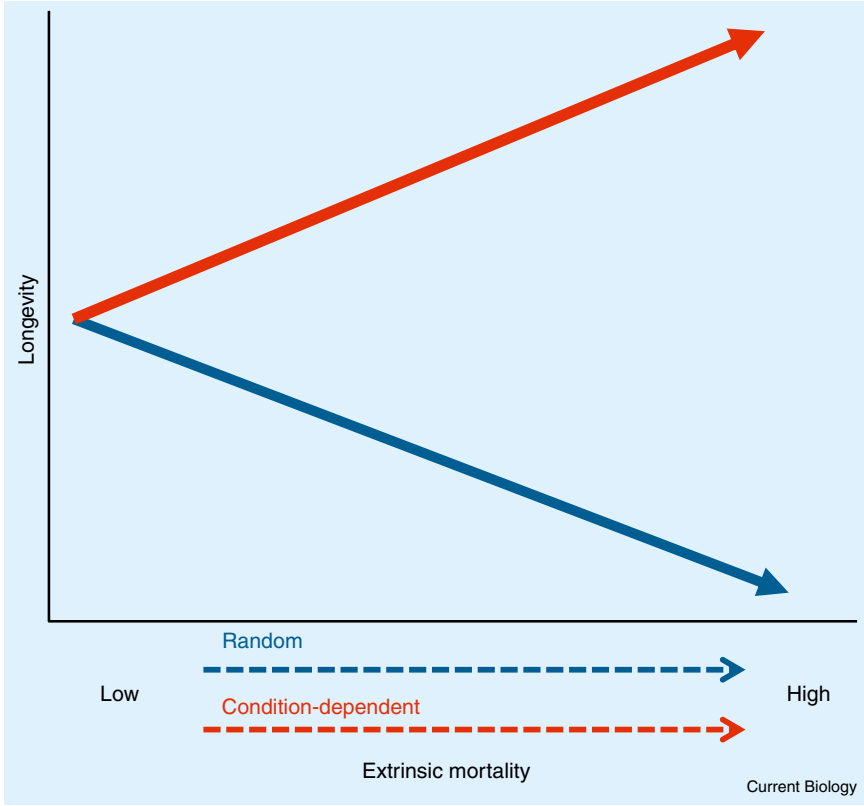

Figure 1. Extrinsic mortality and the evolution of longevity.

When extrinsic mortality increases in a random manner in a population, such that all individuals within that population are equally vulnerable, this should result in the evolution of reduced longevity. This scenario is depicted by the trajectory of the blue arrow, and consistent with classic evolutionary predictions. However, when mortality is applied in a condition-dependent manner, such that better-conditioned individuals are more likely to survive, then the opposite trajectory of longevity evolution can ensue; as depicted by the red arrow.

However, such extension of predictions comes with caveats. For one, male-specific sexual selection could also increase the male extrinsic mortality rate in a random fashion (e.g. increasing exposure to some predators regardless of male condition), or act to increase mortality of the most sexually competitive males in a population (e.g. if these males have higher exposure to sexually-transmitted diseases). Thus, the ecological dynamics of a given species, or population, will need to be carefully considered when formulating predictions. The inconvenient truth is that the evolutionary dynamics of natural populations are rarely simple. A combination of random and condition-dependent mortality pressures — both sex-general and sex-specific in effects — combined with other mechanisms, such as unguarded X [13] and mother's curse [15], are likely to interact to shape the evolution of sex gaps in longevity. Despite the inherent complexity, encouraging support for the predictions under discussion here comes from recent studies in crickets (*Teleogryllus commodus*) [18] and lemurs (*Microcebus murinus*) [19].

Finally, the evolutionary responses observed by Chen and Maklakov [4] should apply to mortality selection on either of the sexes. That is, if increased sex-specific mortality is applied to females, as opposed to males, we should be able to widen and close sex gaps in longevity in much the same way the authors achieved when selecting specifically on males. Much as before, Chen and Maklakov [4] have provided us with a fascinating roadmap to further explore the evolution of longevity. Their ingenious insights into sex gaps in nematode longevity also serve to reinforce how much remains to be done if we are to achieve a full

understanding of the evolution of aging in nature.

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Microtubule Recognition: A Curvy Attraction

While many proteins specifically associate with microtubule ends, the mechanisms underlying these associations remain largely undetermined. A new study demonstrates that doublecortin may localize to microtubule tips through preferential binding to regions of microtubule curvature.

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Neuronal growth, particularly in the developing brain, requires the efficient transport of materials from the cell body through the growing axon [1]. An extensive cytoskeletal network assists in this transportation, composed largely of tracked bundles of directed microtubules [2]. Among the regulators of this system, doublecortin (DCX) plays an essential role in microtubule maintenance early in neuronal migration and differentiation. Mutations in DCX cause double cortex syndrome or lissencephaly, which manifest as epilepsy and mental retardation and are characterized by 'smooth brain' — a lack of gyri in the cerebral cortex [3–5]. These genetic mutations in DCX are concentrated in two domains with ubiquitin-like folds, designated N-DC and C-DC. Not surprisingly, cryogenic electron microscopy of a reconstituted system has shown that at least N-DC can bind directly with the microtubule components α- and β-tubulin [6]. While it has been shown that DCX colocalizes and cosediments with tubulin *in vivo*, [5], tracks the growing ends of microtubules [7], and binds cooperatively to microtubule ends containing 13 protofilaments [6–8], the recognition mechanism of DCX for microtubules has yet to be fully elucidated. In this issue of *Current Biology*, Bechstedt *et al.* provide evidence for a DCX recognition mechanism driven by the structural curvature of microtubules [9].

Currently, limited information is available for the underlying end recognition mechanisms of microtubule tip-associated proteins. High-resolution EM and crystal structures of kinesin-13 [10] and Stu2p [11] bound to α- and β-tubulin display a shear in the orientation of tubulin dimers which is not present in structures of tubulin alone, hinting that these proteins may associate with curved regions of protofilaments. Of the microtubule end-binding proteins characterized physiologically, end-binding protein 1 (EB1) has been shown to selectively recognize the γ-phosphate state of β-tubulin [12]. Since GTP hydrolysis primarily occurs at the growing ends of microtubules, EB1 is effectively localized to polymerizing tips where it functions to increase microtubule nucleation, catastrophes and rescues [12,13]. While EB1 makes direct contact with the nucleotide-binding third helix of β-tubulin, structural studies of DCX do not show a β-tubulin contact in this region, implicating a recognition mechanism independent of nucleotide state [12].

In the current study, Bechstedt *et al.* utilized a single-molecule fluorescence microscopy assay to observe DCX recognition at microtubule ends [9]. The authors first found that DCX and EB1 have distinct kinetic behaviors, quantified in these assays as 'comets', where the length of a microtubule that fluoresces from a bound protein correlates with the protein concentration at the microtubule end. While EB1 comets elongate with an increase in the microtubule nucleation rate from a higher concentration of tubulin, DCX comets remain constant for all growth rates observed (Figure 1A,B). Thus, the DCX binding site at microtubule ends does not change as a function of polymerization rate. EB1 comets have also been observed to shrink upon increases in EB1 concentration