THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2016, Vol. 71, No. 7, 882–890
doi:10.1093/gerona/glv174
Advance Access publication October 15, 2015

OXFORD

Original Article

Sex-specific Tradeoffs With Growth and Fitness Following Life-span Extension by Rapamycin in an Outcrossing Nematode, *Caenorhabditis remanei*

Martin I. Lind, Martyna K. Zwoinska, Sara Meurling, Hanne Carlsson, and Alexei A. Maklakov

Ageing Research Group, Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University, Sweden.

Address correspondence to Martin I. Lind, PhD, Department of Animal Ecology, Uppsala University, 752 36 Uppsala, Sweden. E-mail: martin.i.lind@gmail.com

Received April 29, 2015; Accepted September 14, 2015

Decision Editor: Rafael de Cabo, PhD

Abstract

Rapamycin inhibits the nutrient-sensing TOR pathway and extends life span in a wide range of organisms. Although life-span extension usually differs between the sexes, the reason for this is poorly understood. Because TOR influences growth, rapamycin likely affects life-history traits such as growth and reproduction. Sexes have different life-history strategies, and theory predicts that they will resolve the tradeoffs between growth, reproduction, and life span differently. Specifically, in taxa with female-biased sexual size dimorphism, reduced growth may have smaller effects on male fitness. We investigated the effects of juvenile, adult, or life-long rapamycin treatment on growth, reproduction, life span, and individual fitness in the outcrossing nematode *Caenorhabditis remanei*. Life-long exposure to rapamycin always resulted in the strongest response, whereas postreproductive exposure did not affect life span. Although rapamycin resulted in longer life span and smaller size in males, male individual fitness was not affected. In contrast, size and fitness were negatively affected in females, whereas life span was only extended under high rapamycin concentrations. Our results support the hypothesis that rapamycin affects key life-history traits in a sex-specific manner. We argue that the fitness cost of life-span extension will be sex specific and propose that the smaller sex generally pay less while enjoying stronger life-span increase.

Keywords: Antiaging—Evolution—Longevity

The nutrient-sensing target-of-rapamycin (TOR) is a protein kinase that is structurally and functionally conserved in all animals as a regulator of cell growth and metabolism in response to environmental cues, such as nutrient availability (1). TOR exists in two protein complexes (mTORC1 and mTORC2), and it has recently been found that mTORC1 forms a signaling network with the insulin/insulin like growth factor 1 (IGF-1) pathway (IIS) and manipulations of these signaling networks affect life span and aging across taxa (2–8). Although these findings have required ablations or genetic manipulations of the signaling networks, pharmaceutics with similar regulatory effects could increase life span in non-model organisms. Rapamycin is a drug that inhibits the TOR pathway and has been shown to extend life span in *Saccharomyces cerevisiae* yeast (9), *Caenorhabditis elegans* nematodes (10), *Drosophila melanogaster* fruit flies (11), and *Mus musculus* mice (12–14). Moreover, adult or late-life only treatment by rapamycin was shown to be sufficient to extend life span in several studies (10,12,14), despite the well-known link between early growth and life span across taxa (15–18).

However, given the level of conservation of the signaling pathways, it has been surprising to find that the effect of rapamycin is sex specific with a stronger effect on male life span in *D melanogaster* (11) and on female life span in mice (12,14). The reason behind this sex-specific effect is poorly understood. It may reflect different levels of rapamycin in blood (14), but rapamycin treatment also results in sex-specific gene expression in mice (14). Theory and empirical evidence suggest that life span often differs between the sexes because selection optimizes key life-history tradeoffs differently in males and females (19–22). Therefore, any pharmaceutical compound

that alters physiology to extend life span is likely to have different effects on other life-history traits, and on overall fitness, in each sex. Consequently, sex-specific effects of manipulating TOR using rapamycin can be expected, but the challenge is to understand the underlying tradeoffs to be able to predict the effect of rapamycin and other interventions that downregulate nutrient-sensing signaling on sex-specific life span and fitness in different taxa.

Life-history tradeoffs are often understood using the concept of resource allocation, and its role for life span has been realized for quite some time (15,23,24). The tradeoff between growth, reproduction, and life span is central to life-history evolution, although its proximate causes are debated (25). Investments in growth or early-life fecundity are often associated with a decrease in life span (16,17,26), fast growth is directly associated with high biosynthesis and metabolic rate (27) and also increased oxidative damage (28). Moreover, because resources for maintaining growth are taken away from maintenance, fast-growing individuals often invest less in protection against oxidative stress (29). In addition, mutants or ablations of the IIS/TOR pathways in *C elegans* and *D melanogaster* have resulted in increased life span (3–7) at the cost of reduced growth (5,7,30–32) and female fecundity (3,5,33). This suggests that tradeoffs could also be present at the level of conserved signaling pathways. However, the tradeoff in resource allocation could be hidden by individual differences in resource acquisition (34), and genetic manipulations of the IIS pathway suggest that these tradeoffs may sometimes be decoupled in *D melanogaster* (35) and *C elegans* (36) at least under laboratory conditions. It must be added, however, that tradeoffs between reproduction and survival may go beyond the tradeoff between fecundity and life span and include other key life-history traits such as growth rate and development time (15,16).

Taken together, these considerations suggest that life span should not be seen in isolation but as an integrated part of the life history of an organism, which includes the costs of growth and reproduction. Sexes often have different life-history strategies, and evolutionary theory predicts that males and females will resolve the classic life-history tradeoffs between growth, reproduction, and life span differently, depending upon the sex-specific relationship between size and fitness (37) and the degree of sex-specific genetic variation (38). Specifically, in taxa with female-biased sexual dimorphism in body size (females largest), reduced growth may have small effects on male fitness whereas females suffer more because of lower fecundity (39–42).

Nutrient-sensing IIS/TOR signaling influences both growth and reproduction in model systems. Nutrient-uptake mutants of *C elegans* (that have reduced IIS/TOR signaling) suffer from reduced egg-laying (5), while there is mixed evidence for an effect of rapamycin on female reproduction, from no effect in *C elegans* hermaphrodites (10) to a negative effect in *D melanogaster* females (11). Mutations in the TOR signaling pathway result in reduced growth in both *C elegans* hermaphrodites (5) and *D melanogaster* males (31), and rapamycin reduces food intake and growth of male rats (43). However, with the exception of life span (10,14,44), the previous studies have been limited to one sex only, and given the sex-specific response in life span, sex-specific responses in growth and reproduction are expected.

Here we study the effect of rapamycin administered at different stages of the life cycle—juvenile, adult, late-life, and life-long exposure—on life span of male and female nematodes, *Caenorhabditis remanei*, in conjunction with comprehensive analyses of key life-history traits, such as growth and reproduction, as well as overall individual fitness. Thus, our aim is not to uncover the genetic basis of rapamycin action but to investigate the effects of pharmaceutical downregulation of TOR on key life-history traits in both sexes. Size and reproduction are traits that commonly have sex-specific relationship with fitness (21,39,40) and are often traded off against long life (23,45). In contrast to hermaphroditic *C elegans*, *C remanei* is a closely related obligate outcrossing species with similarly pronounced sexual dimorphism in size and life span and rapid life cycle and is therefore a suitable model organism for investigating sex-specific life-history effects of rapamycin over the whole life cycle of an organism. We found that the effect of rapamycin on life span was stronger the longer the exposure time, with most effect for life-long treatment and no effect when only administered late in life. Rapamycin reduced growth in both sexes, but this growth reduction only affected female, but not male fitness. In contrast, the life-span–prolonging effect of rapamycin was most pronounced for males, the small and long-lived sex.

Method

For all assays, we used the wild-type strain SP8 of *C remanei*, obtained from N. Timmermeyer from the Department of Biology at University of Tübingen, Germany. This strain was created by crossing three wild-type isolates (SB146, MY31, and PB206) and harbors substantial standing genetic variation for life-history traits (46). Before each assay, worms were recovered from freezing and cultivated for two generations under standard laboratory conditions (47) at 20 °C, with the addition of the antibiotics nystatin, streptomycin, and kanamycin to agar and bacterial growth medium (48). Kanamycin was added to the agar from Day 4 of adult life. Worms were feeding on the antibiotic-resistant *E coli* OP50-1 (pUC4K) obtained from J. Ewbank at the Centre d'Immunologie de Marseille-Luminy, France.

Rapamycin (LC Laboratories, Woburn, MA) was dissolved in dimethyl sulfoxide (DMSO) DMSO at 50 mg/mL and added to agar at 100 µM following the protocol for *C elegans* (10). An equal amount of DMSO was added to the control plates. For all assays, the rapamycin treatment was administrated to one or two of the different life stages: juveniles (egg to L4 larvae, 0–54 hours) and adults (54 hours to death). Thus, we had four treatment combinations: control (CC), exposure to rapamycin during juvenile stage (RC), exposure to rapamycin during adult stage (CR), and life-long exposure to rapamycin (RR).

In order to investigate whether the lack of response to female life span was caused by insufficient rapamycin concentration, or masked by the cost of mating, another life-span assay was also performed using a triple concentration of rapamycin (300 µM) dissolved in DMSO at 150 mg/mL. Finally, to test whether late-life exposure to rapamycin was enough to initiate life-span extension, a third life-span experiment was conducted, in which 300-µM rapamycin was administered from Day 12.

Life-span Assay

Life-span assays were established using 20 age-synchronized worms (10 males and 10 females) in the L4 stage (54 hours old) per replicate plate, which had been developing on either control or rapamycin (100 or 300 µM) plates since egg-laying. Worms were transferred to new plates daily, and sex ratio was adjusted to the focal sex throughout the assay. Worms were scored as dead, dead of matricide (internal hatching of eggs), or censored (escaped from agar). The 100-µM life-span assay was run in two blocks, each containing three replicate plates of each treatment and sex combination, resulting in a total of 48 plates and 480 focal worms. In the second life-span experiment,

life span was scored on control (CC) or life-long exposure to 300-µM rapamycin (RR) for both mated and virgin worms. Each plate was initially set up using 20 worms in the L4 larval stage, either 10 individuals of the target sex and 10 background worms of the opposite sex (for the mated treatment) or 20 virgins of the same sex (for the virgin treatment). Worms were transferred to new plates every day for Day 1–6 (to avoid food shortage during peak reproduction) and thereafter every second day for the remainder of the assay. The 300-µM life-span assay was run in two blocks, the first containing six replicate plates of each treatment, sex, and mating status combination, whereas the replication of virgin males was increased to 24 plates during the second block, due to massive escapes of mate-searching virgin males. This resulted in a total of 84 plates and 1,440 focal worms. In the third life-span experiment, mated worms were exposed to 300-µM rapamycin from Day 12. The experimental procedure followed that of the first life-span experiment, and was run in one block, with 10 replicate plates for each sex and treatment combination, resulting in a total of 40 plates and 400 focal worms.

Growth Assay

As for life-span assays, growth assays were established using age-synchronized worms in the L4 stage (54 hours old) that had been developing on control or 100-µM rapamycin plates since egg-laying. To estimate growth, individual worms were kept with two worms of opposite sex and photographed when moved to a new plate (control or 100-µM rapamycin), using a Lumenera Infinity2-5C digital microscope camera mounted on a Leica M165C stereomicroscope. Photographs were taken daily for Day 0–9 and then every second day. Size was measured as the cross-section area using ImageJ 1.46r (http://imagej.nih.gov/ij/). Individuals of the nontarget sex were replaced when lost. The experiment was run in two separate blocks for each sex, consisting of 12 replicate individuals per treatment and block for females and 20 replicates for males (because of male-biased dispersal from plates).

Reproduction Assay

For females, the same individuals were used for the reproduction and growth assay. Individual females with access to two males were allowed to lay eggs on a plate for approximately 24 hours, where after the hatched offspring were killed using chloroform 2 days later and counted under a microscope. The number of offspring on a plate was divided by the exact time the females had been present on the plate, and multiplied by 24:00 hours, to get a daily reproduction estimate. Reproduction was estimated daily until the death of each female.

As for females, the same males were used in the growth and reproduction assays. However, because male reproduction is limited by access to females, male age-specific reproduction was assayed by placing a single male together with eight virgin females (second day of adulthood) for 3 hours, where after the male was removed and the females were allowed to lay eggs for another 3 hours. The developing offspring were killed and counted 2 days later. This assay was performed every 3rd day, starting at Day 2, until the death of each male.

Statistical Analyses

All statistical models were implemented in *R 3.0.2*. Life span was analyzed in Cox proportional hazard models with Gaussian random effects separately for each sex using the *coxme* package for *R*. Juvenile and adult exposure to rapamycin were fitted as fixed factors and block and plate as nested random factors. The 100 and 300-µM rapamycin concentrations were analyzed in separate models, as they were conducted in separate experiments. Female life span was analyzed in two separate models, either by scoring females dying of matricide (internal hatching of eggs) as dead or alternatively censoring them. Matricide could be seen as a nematode-specific death with limited value for generalizations, but occur when females that still produce eggs are food limited, which happens if they are no longer able to move around on the agar because of aging of their locomotion system and could therefore be seen as a consequence of senescence (49).

Growth was analyzed using a three-parameter asymptotic exponential function of the form

$$\mathrm{Size}(t) = a - be^{-kt}. \quad (1)$$

The function was fitted to each individual, which gave an individual estimate of the parameters a, b, and k. We then analyzed the effect of juvenile and adult exposure to rapamycin on each parameter in separate mixed-effect models, with block as random factor. The models were implemented using the package *lme4* in R, and significance tests of main effects were performed using Wald-chi square tests in the car package. For males, we used individuals surviving to Day 10, where growth has reached an asymptote. Four males were removed from the analysis, because it was not able to fit Equation 1 to their pattern of growth. Because female shrinks from Day 5, we used data up to Day 4. However, because of shrinking, we could not obtain a meaningful estimate of the asymptote for females, therefore we also analyzed the response of the treatments on maximum size (at Day 4) in a mixed-effect model using *lme4* and car, treating block as a random factor.

Reproduction was estimated as rate-sensitive individual fitness and total reproduction. Individual fitness was estimated by λ_{ind} which is a rate-sensitive measure of fitness that encompasses the timing and number of offspring, as well as survival, and is considered the most appropriate proxy of fitness for data from one generation (50). It is thus analogous to the intrinsic growth rate of a population and is estimated by solving the Euler–Lotka equation for each individual life table (45). The effect of juvenile and adult exposure to rapamycin on log λ_{ind} and total reproduction was then analyzed in separate mixed models for each sex, with block as random effect, using *lme4* and car. Five infertile males were removed from the dataset.

Results

Life Span

In our first assay, rapamycin extended life span for males but not for females (Figure 1, Supplementary Figure 1). For males, we found no interaction between juvenile and adult exposure ($\chi^2 = 1.140$, $df = 1$, $p = .286$), and in the simplified model, we found that adult exposure ($z = -2.93$, $p = .003$) and juvenile exposure ($z = -2.15$, $p = .032$) had additive effects on male life span. For females, the interaction between juvenile and adult exposure was outside significance both when females dying of matricide were included ($\chi^2 = 2.464$, $df = 1$, $p = .117$) and censored ($\chi^2 = 2.300$, $df = 1$, $p = .129$), and in the simplified models, neither juvenile (including matricide: $z = -0.44$, $p = .66$, censoring matricide: $z = -0.24$, $p = .81$) nor adult exposure (including matricide: $z = -0.92$, $p = .36$, censoring matricide: $z = -0.80$, $p = .43$) was significant on its own.

To test whether the lack of female response to rapamycin was caused by insufficient rapamycin concentration and/or hidden by the cost of mating, we also performed a second life-span experiment using a triple dose of rapamycin (300 µM) as well as including virgins (Figure 2, Supplementary Figure 1). Life-long exposure

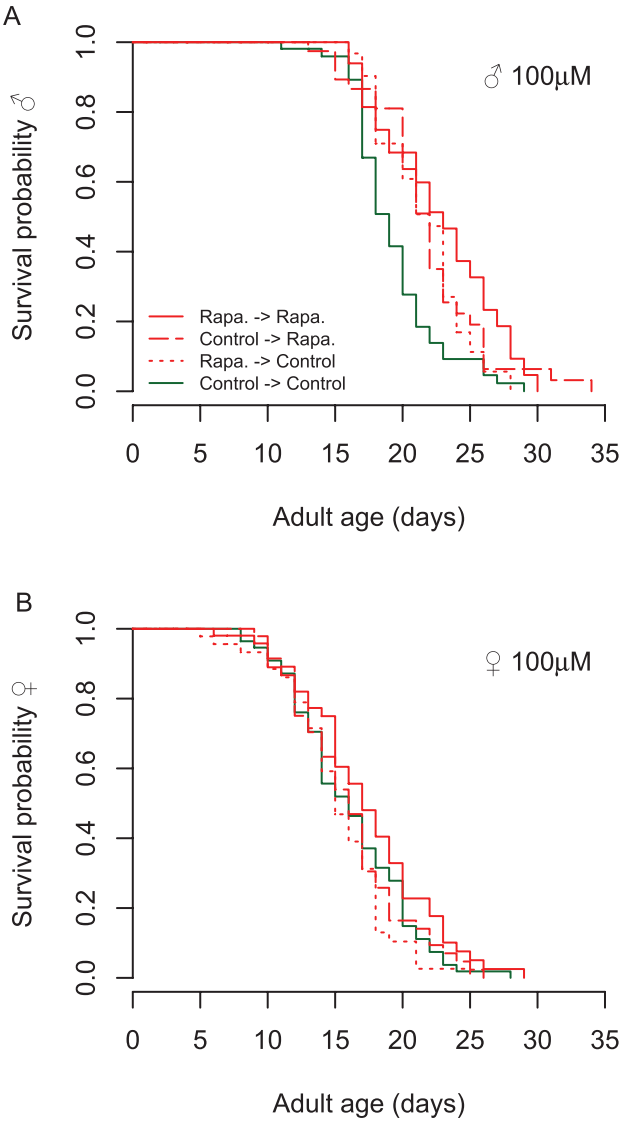

Figure 1. Survival curves for males (**A**) and females including matricide (**B**) exposed to 100-µM rapamycin. The control treatment is indicated by green solid lines, juvenile exposure to rapamycin by red dotted lines, adult exposure to rapamycin by red dashed lines, and life-long exposure to rapamycin by red solid lines.

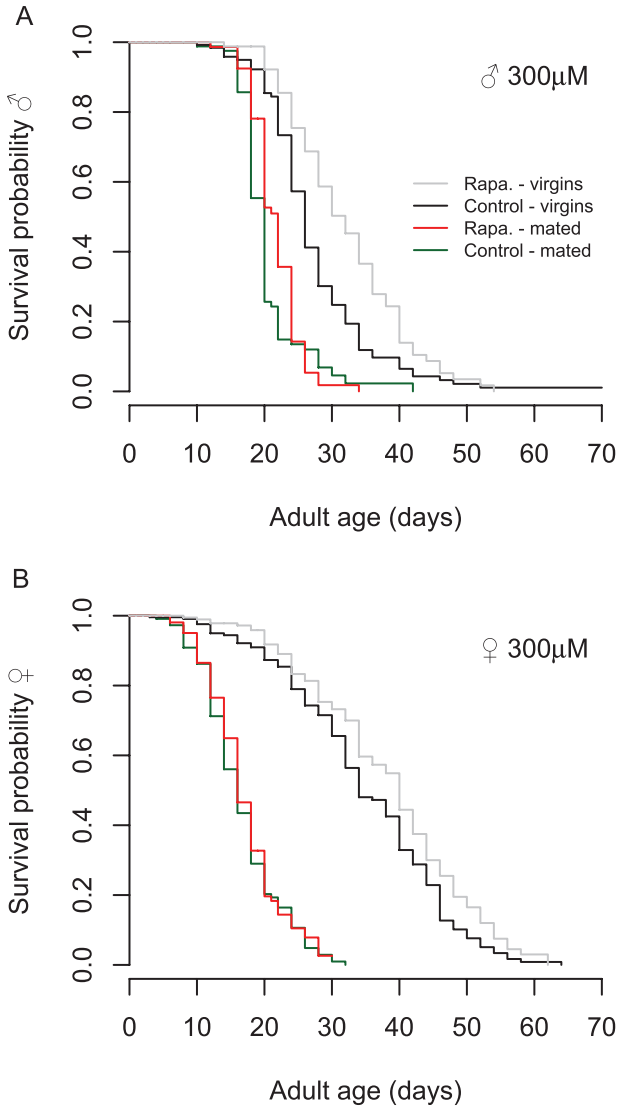

Figure 2. Survival curves for males (**A**) and females including matricide (**B**) exposed to 300-µM rapamycin (indicated by red or gray lines). Mated worms are shown in color and virgin worms in gray scale.

to 300-µM rapamycin expanded life span for both mated and virgin males (z = −2.98, p = .003, Figure 2A). Virgin males lived substantially longer than mated males (z = −8.65, p < .001), but rapamycin treatment did not interact with mating status (χ^2 = 0.918, df = 1, p = .338). For females, life-long exposure to 300-µM rapamycin resulted in life-span extension when matricide was included (z = −2.02, p = .043) but not when matricidal females were censored (z = −1.89, p = .059). The interaction between mating status and rapamycin treatment was not significant when matricide was included (χ^2 = 1.279, df = 1, p = .258) or censored (χ^2 = 1.538, df = 1, p = .215) Virgin females lived substantially longer than mated females both when matricide was included as a source of death for mated females (z = −16.96, p < .001) or censored (z = −14.81, p < .001), which resulted in a reverse of the sexual dimorphism in life span, because males are the longest lived sex when mated, but females live longer as virgins (Figure 2).

Exposure to 300-µM rapamycin from Day 12 of adult life did not significantly extend life span in males (z = −1.64, p = .100) or females, regardless of whether matricide was included as a mortality source (z = −0.26, p = .790) or censored (z = 0.58, p = .580; Figure 3, Supplementary Figure 1).

Growth

Adult exposure to rapamycin resulted in a smaller adult size in both males and females (Figure 4). When fitting a three-parameter asymptotic exponential growth model for males, we found that adult (but not juvenile) exposure to rapamycin resulted in a lower coefficient a (asymptote, maximal size; juv. exp.: χ^2 = 2.061, df = 1, p = . 151; adult exp.: χ^2 = 31.978, df = 1, p < .001; juv. × adult exp.: χ^2 = 0.131, df = 1, p = .717) and coefficient b (initial growth; juv. exp.: χ^2 = 0. 045, df = 1, p = . 832; adult exp.: χ^2 = 13.400, df = 1, p < .001; juv. × adult exp.: χ^2 = 0.030, df = 1, p = . 863) but did not affect the parameter k (rate of increase; juv. exp.: χ^2 = 0.048, df = 1, p = . 460; adult exp.: χ^2 = 0.008, df = 1, p = . 896; juv. × adult exp.: χ^2 = 1.156, df = 1, p = . 282).

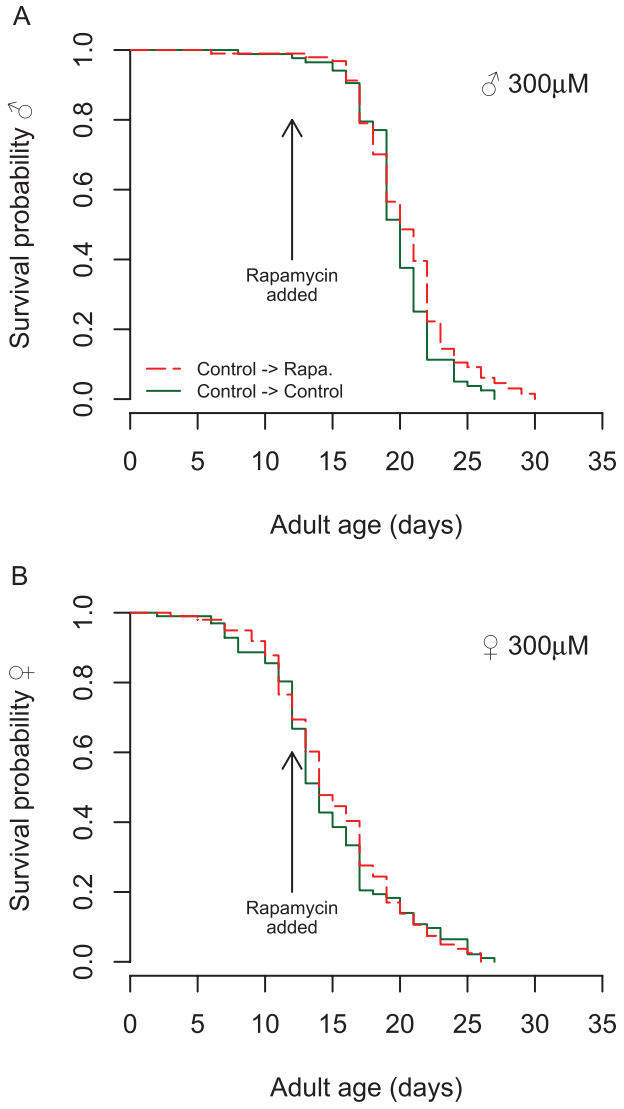

Figure 3. Survival curves for mated males (**A**) and females including matricide (**B**) exposed to 300-µM rapamycin from Day 12 of adult life (indicated by red dashed lines) or control conditions (green lines).

It was not possible to fit a growth function for females, because male-induced shrinking of females (51) occurred before the growth models predicted that the females had reached their maximum size (model output is presented in Supplementary Table 1). However, when analyzing the effect of rapamycin at the age of maximal female size (Day 4, see Figure 4), we found a strong negative effect of adult treatment of rapamycin ($\chi^2 = 3.051$, $df = 1$, $p < .001$), reducing the size of females from the RR treatment with 7% compared with control females. No effect was found by juvenile treatment of rapamycin ($\chi^2 = 0.005$, $df = 1$, $p = .945$) or their interaction ($\chi^2 = 0.314$, $df = 1$, $p = .575$).

Reproduction

The effect of rapamycin on reproduction was sex specific. No significant effect of rapamycin on either individual fitness λ_{ind} or total fecundity was found for males, whereas both juvenile and adult exposure to rapamycin significantly reduced female λ_{ind} but not total lifetime fecundity (Figure 5).

Male λ_{ind} was not affected by juvenile ($\chi^2 = 0.190$, $df = 1$, $p = .63$) or adult ($\chi^2 = 0.022$, $df = 1$, $p = .884$) exposure to rapamycin, neither was their interaction ($\chi^2 = 0.219$, $df = 1$, $p = .640$). The same was true for total fecundity, where neither juvenile ($\chi^2 = 0.867$, $df = 1$, $p = .352$) nor adult ($\chi^2 = 0.090$, $df = 1$, $p = .148$) exposure or their interaction ($\chi^2 = 0.188$, $df = 1$, $p = .665$) had any effect (Figure 5A, C, Supplementary Figure 2).

Female individual fitness λ_{ind} was negatively affected by both juvenile ($\chi^2 = 9.304$, $df = 1$, $p = .002$) and adult ($\chi^2 = 1.253$, $df = 1$, $p = .001$) exposure to rapamycin. The effect of juvenile and adult exposure was however not additive, as indicated by the fact that a model containing this interaction was retained in the model simplification process ($\chi^2 = 3.888$, $df = 1$, $p = .049$), although the interaction was borderline significant when the model was refitted using restricted maximum likelihood ($\chi^2 = 3.831$, $df = 1$, $p = .050$). In contrast, female total fecundity was not significantly affected by juvenile ($\chi^2 = 1.430$, $df = 1$, $p = .232$) or adult ($\chi^2 = 2.046$, $df = 1$, $p = .153$) exposure to rapamycin, or by their interaction ($\chi^2 = 1.416$, $df = 1$, $p = .234$) (Figure 5B, D, Supplementary Figure 3).

Discussion

We investigated the effects of juvenile and adult treatment with the TOR signaling antagonist rapamycin on growth, reproduction, life span, and individual fitness in the outcrossing nematode *C remanei*. Although rapamycin treatment resulted in a smaller adult size of both sexes, we found substantial fitness cost of rapamycin treatment for females but not for males. In contrast, the life-span–prolonging effect of rapamycin was stronger for males than for females. Thus, the fitness cost of rapamycin was only present in females, the sex with the smallest life-span increase. The effect of rapamycin on life span was stronger the earlier in life rapamycin treatment was initiated, with no detectable effect when administered only in late life.

TOR is a conserved signaling pathway that controls growth and metabolism with input from environmental cues (1). Genetic manipulations of this pathway have extended life span in model organisms (4,6). TOR also seems to be involved in natural life-span variation in *D melanogaster* (52), and recently, the drug rapamycin has proven an effective way of downregulating TOR without the need of genetic manipulations, and thus is applicable also to non-model organisms, potentially including humans (53). Although rapamycin was initially believed to bind specifically to mTORC1 (1), recent evidence suggests that rapamycin also acts by binding to mTORC2 (54). Rapamycin has been shown to extend life span in *C cerevisiae* (9), *C elegans* (10), *D melanogaster* (11), and *M musculus* (12–14), and in agreement with these studies, we show that rapamycin treatment also extends life span in *C remanei*.

It has been proposed that rapamycin mainly affects aging when administrated late in life (1), but this prediction, to the best of our knowledge, has never been tested. Although most studies have focused on the effects of late-life (12,14) or adult (10) treatment by rapamycin, we also investigated the effect of juvenile rapamycin treatment in a full-factorial design allowing us to disentangle potential effects of rapamycin treatment at different stages of the life cycle. We found that the effect of rapamycin on life span was stronger when administered during the adult than during the juvenile phase, but on the other hand, juvenile exposure to rapamycin also had a significant additive effect on male life span and the combined juvenile and adult treatment always resulted in longest life span, suggesting that downregulation of TOR signaling in juveniles is also important for life-span extension. This latter result suggests that reduced nutrient-sensing signaling in the

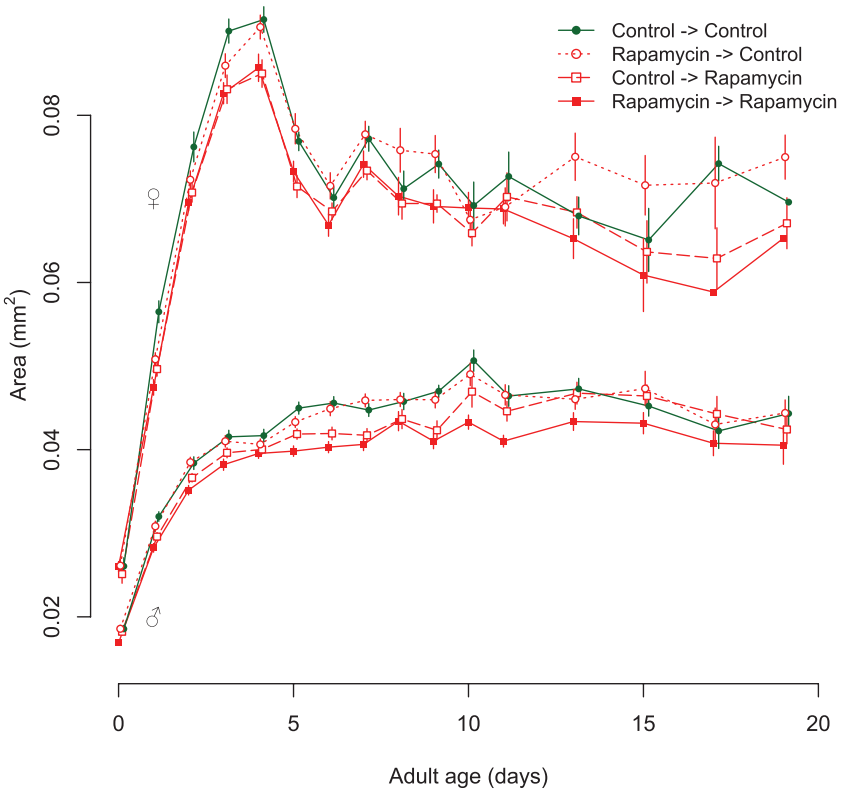

Figure 4. Age-specific size (treatment mean ± *SE*) for males and females from control treatment (closed green circles), juvenile exposure (open red circles), adult exposure (open red squares), and life-long exposure (closed red squares) to 100-μM rapamycin.

juvenile phase has positive carryover effects on life span manifested late in life and that rapamycin treatment of adults could not fully compensate for lack of exposure in early life. Moreover, when rapamycin was administered only in late life [from Day 12, corresponding to the late-life treatment in mice (12,14)] in a follow-up experiment, we found no detectable effect on life span, despite higher replication.

We also found the effect of rapamycin on life span to be sex specific, with a stronger effect in males, whereas the effect on female life span was only detected under high rapamycin concentrations. Together with findings in *D melanogaster* (11) and mice (14), these results suggest that sex-specific effects of rapamycin on life span are to be expected, although which sex is most affected differs among species. Rapamycin has the strongest effect on the long-lived sex in *C remanei* (males) and in mouse (females) (14) but on the short-lived sex in *D melanogaster* (males) (11). Thus, rapamycin does not preferentially extend life span of any particular sex, nor does it mostly extend the life span of the short-lived sex. Instead, rapamycin seems to prolong life of the smaller sex. For further insight in the direction of the sex-specific effects of rapamycin across taxa, it is necessary to consider the effect across a suite of life-history traits, which often have sex-specific optima. Life-history theory maintains that resources are allocated between growth, reproduction, and somatic maintenance (15,23,24). Although allocation tradeoffs can be difficult to detect whether individuals also differ in resource acquisition (34), empirical studies suggest that selection on growth results in a correlated response in reduced life span (55) and that high growth correlates with short life span both within (26,56,57) and between (18) taxa. Using genetic manipulations, it has also been shown that the IIS/TOR pathways affect these traits in concert in *C elegans* and *D melanogaster*, so that reduced IIS/TOR signaling results in increased life span (3–7) at the cost of reduced growth (5,7,30–32,43) and female fecundity (3,5,33), suggesting common underlying molecular pathways. However, sexes often have different reproductive strategies, resulting in different life histories (19,21,22). This is often expressed as sexual size dimorphism, which corresponds to different relationships between size and fitness in males and females. Whereas male-biased size dimorphism (larger males than females) predominates in birds and mammals, female-biased size dimorphism is prevalent in all other animal groups (40). In species with female-biased size dimorphism, the size–fitness relationship is stronger in females, because size translates into fecundity (37,39–42), and, therefore, reduced female size is also predicted to reduce female fitness. This agrees with our findings in *C remanei*, where rapamycin-driven reduction in adult body size (reduced growth rate) of both sexes results in reduced female, but not male, fitness. Females are substantially larger than males, have higher growth rate, and longer development time until maturation, therefore theory (37) suggests that *C remanei* females are selected for size-dependent fecundity, while male fitness is to a lesser degree depending upon body size. Our results suggest that rapamycin affects the allocation between life-history traits in a sex-specific manner. To the best of our knowledge, no other study to date has measured the effect of rapamycin on growth of both sexes or on male fitness, but we note that male growth of rats is reduced by rapamycin (43) and also that female reproduction is lowered by genetic manipulation of insulin signaling (3,33) and by rapamycin in *D melanogaster* (11). Because reduced TOR signaling by genetic manipulations reduces growth in *D melanogaster* (31) and *D melanogaster* shows a female-biased sexual size dimorphism (58) with female size positively related to fecundity (42), the effect of rapamycin on fecundity in *D melanogaster* could be mediated by reduced growth. Generally,

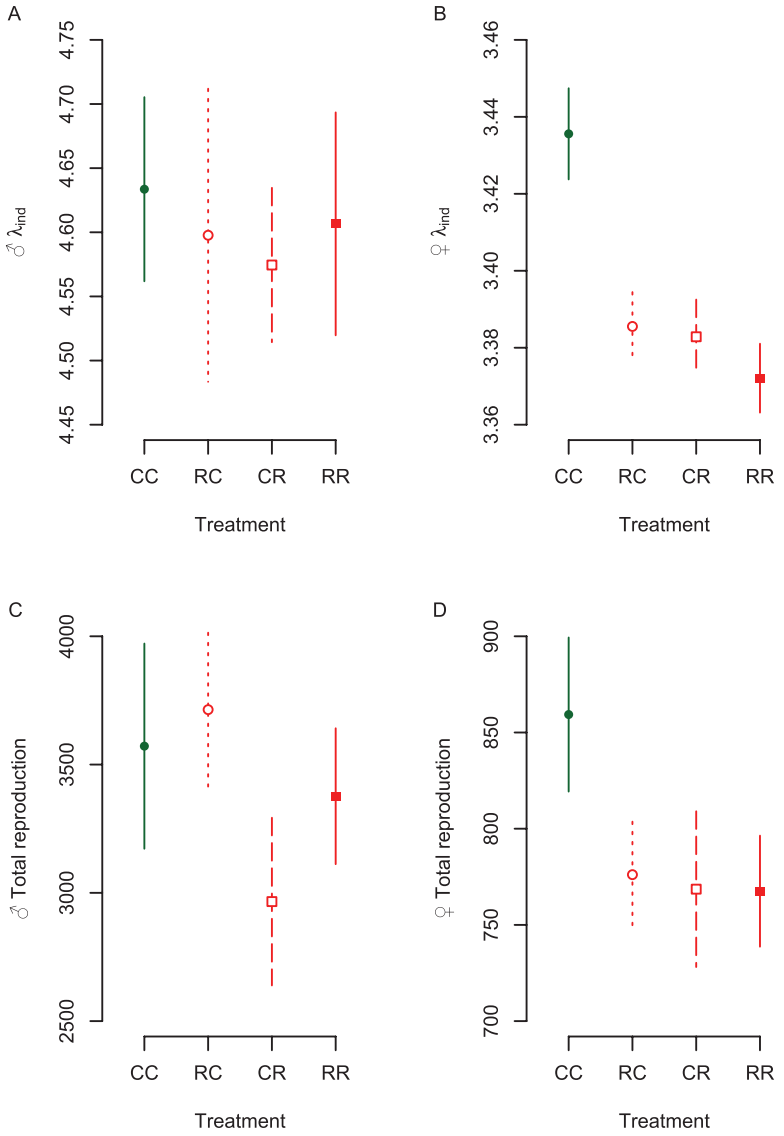

Figure 5. Treatment mean (± *SE*) of individual fitness λ_{ind} (**A**, **B**) and total reproduction (**C**, **D**) for males (**A**, **C**) and females (**B**, **D**). CC indicates control, RC is juvenile exposure, CR is adult exposure, and RR is life-long exposure to 100-μM rapamycin.

we argue that the cost of life-span extension via downregulation of nutrient-sensing pathways will always be sex specific, but the direction of the effect will depend on the relationship between growth and fitness within each sex. Because female-biased size dimorphism is prevalent in most animal taxa (40), we expect our findings of female-biased fitness costs to be widespread, while examples of male-biased costs are more likely to be found among birds and mammals with male-biased sexual size dimorphism. Interestingly, rapamycin (13,14) as well as genetic knockdown of IGF-1 in the IIS pathway (8) has a stronger effect on female life span in mice, a species where males are polygynous territory holders and thus the larger sex, and rapamycin also reduces growth in male rats (43). In summary, for *C remanei*, *D melanogaster*, and mice, the effect of rapamycin on life span is stronger in the smaller sex, where the relationship between size and fecundity is weakest. The fitness cost seems however to be paid by the largest sex. Thus, knowledge of the sex-specific reproductive strategy, and therefore the tradeoffs between life-history traits, can potentially give insight into the sex-specific response to rapamycin and other manipulations of TOR signaling. We propose that the sex-specific relationship between size and fitness is a candidate explanation for this pattern. This finding is not fully compatible with the hypothesis that growth drives aging (15), because growth is affected in both sexes but life span mostly in males, but is in line with earlier work suggesting that in *Caenorhabditis* nematodes the relationship between growth and aging is not causal, although both are likely pleiotropically affected by nutrient-sensing signaling (7). The proximate mechanism by which rapamycin causes larger longevity effect in the smaller sex is currently unknown, but it is possible that body size per se (eg, different cell size in *C remanei*), growth rate, and development time all play important roles. Elucidating the relative importance of these factors constitutes a fruitful area for future research.

Another potential explanation for the larger effect of rapamycin in males is that life span is determined by different factors in the sexes. Female *Caenorhabditis* nematodes pay a substantial cost of mating, where male sperm and seminal fluid alter female physiology by manipulating germline proliferation through the hormone receptor

DAF-12 and the transcription factor DAF-16, resulting in fat loss and shrinking, and ultimately death of the female (51). This female-biased cost of mating is illustrated both by the observed shrinking of females from Day 5 of adult life and especially by our finding that removal of mating opportunities reverses the sexual dimorphism in life span, so that females is the short-lived sex when mated but the long-lived when virgins. Therefore, we also investigated whether the lack of rapamycin-mediated life-span increase for mated females could be masked by the cost of mating. The interaction between mating status and rapamycin treatment for females was not significant, arguing against this hypothesis. Although visual examination of the data suggests that this hypothesis may be worth pursuing with more replication, the potential effect is still much smaller than the strong effect of rapamycin on life span of both mated and virgin males. Thus, the higher cost of mating in females cannot fully explain the sex-specific effect of rapamycin on life span.

Sexes commonly have different optimal values for key life-history traits, such as rate of growth, rate of reproduction, and life span (19,21), but the evolution of sex-specific optima in these traits via sexually antagonistic selection is constrained by the shared genetic machinery (59) resulting in intralocus sexual conflict (60). Sexually antagonistic selection has been put forward as one of the key explanatory frameworks for the maintenance of genetic variation in traits closely related to fitness in general (60,61), and life span in particular (22,62), although the sexes also harbor substantial sex-specific genetic variation for life span (38,63). Alleles that reduce TOR signaling would increase male life span without fitness cost, but selection on females is predicted to hamper the evolution of increased male life span because of the cost to female fitness. Therefore, sexually antagonistic selection may contribute to the maintenance of genetic variation for sex-specific response to life-span–extending pharmaceuticals.

Although our finding of reduced female fecundity agrees with similar findings in *D melanogaster* (11), rapamycin did not alter the fecundity of self-fertilized *C elegans* hermaphrodites (10). However, *C elegans* hermaphrodites are sperm limited if not mated (64) and can increase their fecundity dramatically by mating with males. Because female *C remanei* were continuously mated in our experiment, it may explain the different effects on female fecundity between our studies. The fact that only rate-sensitive individual fitness λ_{ind} but not total fecundity was significantly affected by rapamycin in our study highlights the importance of the first days of reproduction for individual fitness.

The key finding of this study is that the fitness cost of life-span extension by rapamycin differs between the sexes, and the sex with smaller increase in life span suffered larger fitness cost. We propose that the magnitude of the effect depends on the sex-specific relationship between growth and fitness. More specifically, we suggest that life-span–extending interventions that target pathways affecting nutrient-sensing signaling and biosynthesis may have stronger effect on smaller sex, where the relationship between size and fecundity is weakest. Further work is needed to test the taxonomic generality, as well as physiological and molecular underpinnings of this effect.

Supplementary Material

Please visit the article online at http://gerontologist.oxfordjournals.org/ to view supplementary material.

Funding

This work was supported by the Swedish Research Council (C0636601) and by the Evolutionary Biology Centre, Uppsala University to M.I.L. A.A.M. was supported by the European Research Council Starting Grant-2010 (St-G 2010 AGINGSEXDIFF 260885) and by the Swedish Research Council (621-2013-4828).

Acknowledgments

We thank Johan Andersson and Tuuli Larva for their help in the laboratory.

References

1. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell*. 2006;124:471–484. doi:10.1016/j.cell.2006.01.016
2. Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of ageing and age-related disease. *Nature*. 2013;493:338–345. doi:10.1038/nature11861
3. Broughton SJ, Piper MD, Ikeya T, et al. Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc Natl Acad Sci U S A*. 2005;102:3105–3110. doi:10.1073/pnas.0405775102
4. Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr Biol*. 2004;14:885–890. doi:10.1016/j.cub.2004.03.059
5. Meissner B, Boll M, Daniel H, Baumeister R. Deletion of the intestinal peptide transporter affects insulin and TOR signaling in *Caenorhabditis elegans*. *J Biol Chem*. 2004;279:36739–36745. doi:10.1074/jbc.M403415200
6. Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Müller F. Genetics: Influence of TOR kinase on lifespan in *C. elegans*. *Nature*. 2003;426(6967):620. doi:10.1038/426620a
7. McCulloch D, Gems D. Body size, insulin/IGF signaling and aging in the nematode *Caenorhabditis elegans*. *Exp Gerontol*. 2003;38:129–136. doi:10.1016/S0531-5565(02)00147-X
8. Holzenberger M, Dupont J, Ducos B, et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature*. 2003;421:182–187. doi:10.1038/nature01298
9. Powers RW 3rd, Kaeberlein M, Caldwell SD, Kennedy BK, Fields S. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev*. 2006;20:174–184. doi:10.1101/gad.1381406
10. Robida-Stubbs S, Glover-Cutter K, Lamming DW, et al. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metab*. 2012;15:713–724. doi:10.1016/j.cmet.2012.04.007
11. Bjedov I, Toivonen JM, Kerr F, et al. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab*. 2010;11:35–46. doi:10.1016/j.cmet.2009.11.010
12. Harrison DE, Strong R, Sharp ZD, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. 2009;460:392–395. doi:10.1038/nature08221
13. Miller RA, Harrison DE, Astle CM, et al. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *J Gerontol A Biol Sci Med Sci*. 2011;66:191–201. doi:10.1093/gerona/glq178
14. Miller RA, Harrison DE, Astle CM, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell*. 2014;13:468–477. doi:10.1111/acel.12194
15. Metcalfe NB, Monaghan P. Growth versus lifespan: perspectives from evolutionary ecology. *Exp Gerontol*. 2003;38:935–940. doi: 10.1016/S0531-5565(03)00159-1
16. Lee W-S, Monaghan P, Metcalfe NB. Experimental demonstration of the growth rate–lifespan trade-off. *Proc R Soc B Biol Sci*. 2013;280(1752):20122370. doi:10.1098/rspb.2012.2370
17. Rollo CD. Growth negatively impacts the life span of mammals. *Evol Dev*. 2002;4:55–61. doi:10.1046/j.1525-142x.2002.01053.x
18. de Magalhães JP, Costa J, Church GM. An analysis of the relationship between metabolism, developmental schedules, and longevity using phylogenetic independent contrasts. *J Gerontol A Biol Sci Med Sci*. 2007;62(2):149–160.
19. Trivers R. Parental investment and sexual selection. In: Campbell B, ed. *Sexual Selection and the Descent of Man*. Chicago, IL: Aldine-Atherton; 1972:136–179.
20. Promislow D. Mate choice, sexual conflict, and evolution of senescence. *Behav Genet*. 2003;33:191–201. doi:10.1023/A:1022562103669

21. Bonduriansky R, Maklakov A, Zajitschek F, Brooks R. Sexual selection, sexual conflict and the evolution of ageing and life span. *Funct Ecol*. 2008;22(3):443–453. doi:10.1111/j.1365-2435.2008.01417.x
22. Maklakov AA, Lummaa V. Evolution of sex differences in lifespan and aging: causes and constraints. *Bioessays*. 2013;35:717–724. doi:10.1002/bies.201300021
23. Charnov EL, Turner TF, Winemiller KO. Reproductive constraints and the evolution of life histories with indeterminate growth. *Proc Natl Acad Sci U S A*. 2001;98:9460–9464. doi:10.1073/pnas.161294498
24. Lee WS, Metcalfe NB, Monaghan P, Mangel M. A comparison of dynamic-state-dependent models of the trade-off between growth, damage, and reproduction. *Am Nat*. 2011;178:774–786. doi:10.1086/662671
25. Stearns SC. Does impressive progress on understanding mechanisms advance life history theory? In: Flatt T, Heyland A, eds. *Mechanisms of Life History Evolution*. New York, NY: Oxford University Press; 2011:364–374.
26. Kraus C, Pavard S, Promislow DE. The size-life span trade-off decomposed: why large dogs die young. *Am Nat*. 2013;181:492–505. doi:10.1086/669665
27. Criscuolo F, Monaghan P, Nasir L, Metcalfe NB. Early nutrition and phenotypic development: "catch-up" growth leads to elevated metabolic rate in adulthood. *Proc R Soc B Biol Sci*. 2008;275(1642):1565–1570. doi:10.1098/rspb.2008.0148
28. Nussey DH, Pemberton JM, Pilkington JG, Blount JD. Life history correlates of oxidative damage in a free-living mammal population. *Funct Ecol*. 2009;23(4):809–817. doi:10.1111/j.1365-2435.2009.01555.x
29. Robert KA, Bronikowski AM. Evolution of senescence in nature: physiological evolution in populations of garter snake with divergent life histories. *Am Nat*. 2010;175:147–159. doi:10.1086/649595
30. Oldham S, Böhni R, Stocker H, Brogiolo W, Hafen E. Genetic control of size in Drosophila. *Phil Trans R Soc Lond B Biol Sci*. 2000;355:945–952. doi:10.1098/rstb.2000.0630
31. Colombani J, Raisin S, Pantalacci S, Radimerski T, Montagne J, Léopold P. A nutrient sensor mechanism controls *Drosophila* growth. *Cell*. 2003;114:739–749. doi:10.1016/S0092-8674(03)00713-X
32. Soukas AA, Kane EA, Carr CE, Melo JA, Ruvkun G. Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in *Caenorhabditis elegans*. *Genes Dev*. 2009;23:496–511. doi:10.1101/gad.1775409
33. LaFever L, Drummond-Barbosa D. Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*. *Science*. 2005;309:1071–1073. doi:10.1126/science.1111410
34. van Noordwijk AJ, de Jong G. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat*. 1986;128(1):137–142. doi:10.1086/284547
35. Clancy DJ, Gems D, Harshman LG, et al. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science*. 2001;292:104–106. doi:10.1126/science.1057991
36. Dillin A, Crawford DK, Kenyon C. Timing requirements for insulin/IGF-1 signaling in *C. elegans*. *Science*. 2002;298:830–834. doi:10.1126/science.1074240
37. Arak A. Sexual dimorphism in body size: a model and a test. *Evolution* 1988;42(4):820. doi:10.2307/2408874
38. Lehtovaara A, Schielzeth H, Flis I, Friberg U. Heritability of life span is largely sex limited in *Drosophila*. *Am Nat*. 2013;182:653–665. doi:10.1086/673296
39. Andersson M. *Sexual Selection*. Princeton, NJ: Princeton University Press; 1994.
40. Fairbairn DJ. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annu Rev Ecol Syst*. 1997;28:659–687.
41. Head G. Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (class Araneae). *Evolution*. 1995;49(4):776–781. doi:10.2307/2410330
42. Reeve JP, Fairbairn DJ. Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity* 1999;83(6):697–706. doi:10.1046/j.1365-2540.1999.00616.x
43. Carter CS, Khamiss D, Matheny M, et al. Rapamycin versus intermittent feeding: dissociable effects on physiological and behavioral outcomes when initiated early and late in life. *J Gerontol A Biol Sci Med Sci*. 2015:glu238. doi:10.1093/gerona/glu238
44. Gems D, Riddle DL. Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics*. 2000;154:1597–1610.
45. Stearns SC. *The Evolution of Life Histories*. New York, NY: Oxford University Press; 1992.
46. Chen HY, Maklakov AA. Longer life span evolves under high rates of condition-dependent mortality. *Curr Biol*. 2012;22:2140–2143. doi:10.1016/j.cub.2012.09.021
47. Stiernagle T. Maintenance of C. elegans. In: The C. elegans Research Community, ed. *WormBook: The Online Review of C elegans Biology*. 2006. doi:10.1895/wormbook.1.101.1
48. Lionaki E, Tavernarakis N. Assessing aging and senescent decline in *Caenorhabditis elegans*: cohort survival analysis. In: Galluzzi L, Vitale I, Kepp O, Kroemer G, eds. *Cell Senescence. Methods in Molecular Biology*. New York, NY: Humana Press; 2013:473–484.
49. McCulloch D, Gems D. Evolution of male longevity bias in nematodes. *Aging Cell*. 2003;2:165–173. doi:10.1046/j.1474-9728.2003.00047.x
50. Brommer JE, Merilä J, Kokko H. Reproductive timing and individual fitness. *Ecol Lett*. 2002;5(6):802–810. doi:10.1046/j.1461-0248.2002.00369.x
51. Shi C, Murphy CT. Mating induces shrinking and death in *Caenorhabditis* mothers. *Science*. 2014;343:536–540. doi:10.1126/science.1242958
52. Ivanov DK, Escott-Price V, Ziehm M, et al. Longevity GWAS using the Drosophila genetic reference panel. *J Gerontol A Biol Sci Med Sci*. 2015:glv047. doi:10.1093/gerona/glv047
53. Evans DS, Kapahi P, Hsueh WC, Kockel L. TOR signaling never gets old: aging, longevity and TORC1 activity. *Ageing Res Rev*. 2011;10:225–237. doi:10.1016/j.arr.2010.04.001
54. Lamming DW, Ye L, Katajisto P, et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science*. 2012;335:1638–1643. doi:10.1126/science.1215135
55. Eklund J, Bradford GE. Longevity and lifetime body weight in mice selected for rapid growth. *Nature* 1977;265(5589):48–49. doi:10.1038/265048b0
56. Olsson M, Shine R. Growth to death in lizards. *Evolution*. 2002;56:1867–1870. doi:10.1111/j.0014-3820.2002.tb00202.x
57. Lints FA, Soliman MH. Growth rate and longevity in *Drosophila melanogaster* and *Tribolium castaneum*. *Nature*. 1977;266:624–625. doi:10.1038/266624a0
58. David JR, Moreteau B, Gauthier JP, Pétavy G, Stockel A, Imasheva AG. Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. *Genet. Sel. Evol*. 1994;26(3):229. doi:10.1186/1297-9686-26-3-229
59. Griffin RM, Dean R, Grace JL, Rydén P, Friberg U. The shared genome is a pervasive constraint on the evolution of sex-biased gene expression. *Mol Biol Evol*. 2013;30:2168–2176. doi:10.1093/molbev/mst121
60. Bonduriansky R, Chenoweth SF. Intralocus sexual conflict. *Trends Ecol Evol*. 2009;24:280–288. doi:10.1016/j.tree.2008.12.005
61. Rice WR, Chippindale AK. Intersexual ontogenetic conflict. *J Evol Biol*. 2001;14(5):685–693. doi:10.1046/j.1420-9101.2001.00319.x
62. Berg EC, Maklakov AA. Sexes suffer from suboptimal lifespan because of genetic conflict in a seed beetle. *Proc R Soc B Biol Sci*. 2012;279(1745):4296–4302. doi:10.1098/rspb.2012.1345
63. Chen HY, Maklakov AA. Condition dependence of male mortality drives the evolution of sex differences in longevity. *Curr Biol*. 2014;24:2423–2427. doi:10.1016/j.cub.2014.08.055
64. Ward S, Carrel JS. Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Dev Biol*. 1979;73:304–321. doi:10.1016/0012-1606(79)90069-1