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Sex-specific effects of the DAF-12 steroid receptor on aging in *Caenorhabditis elegans*

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Abstract

Sex differences in longevity and aging are seen throughout the animal kingdom. These are likely to result, in part, from sex differences in endocrinology. In the nematode *C. elegans*, males are the longer-lived sex. Here we explore the possibility that sex differences in insulin/IGF-1 and steroid endocrinology contribute to this sex difference in aging, studying *C. elegans* populations in liquid culture. We report that in hermaphrodite populations, mutational loss of the DAF-12 steroid receptor affected lifespan as in previous plate culture studies: mutant longevity is suppressed in a weak *daf-2* insulin/IGF-1 receptor mutant, but enhanced in a stronger *daf-2* mutant. However, in males mutation of *daf-12* had little effect on aging in either weak or strong *daf-2* mutants. Moreover, while mutation of *daf-12* marginally reduced lifespan in *daf-2*(+) hermaphrodites, as in plate cultured populations, it did not in *daf-2*(+) males. These results could imply that in *C. elegans*, as in mammals, sex differences in steroid endocrinology contribute to sex differences in aging.

Keywords

C. elegans; aging; gender; steroid; insulin/IGF-1 signaling

INTRODUCTION

A salient feature of human aging is that women age slightly more slowly than men. In developed country this leads to a gender gap in mean lifespan of some 4 - 6 years[1]. Sex differences in aging are typical of animal species, but their biological basis remains poorly understood[2]. Many aspects of sexual dimorphism are specified by sex differences in endocrine function, and this may apply to aging too. For example, the shorter lifespan of men is thought to arise, in part, from the effects of testicular hormones (e.g. the steroid hormone testosterone) on behavior and on cardiovascular health[1]. While in many animal species, females are longer lived, in the nematode *Caenorhabditis elegans* the male is the longer lived sex, living some 20% longer than hermaphrodites[3].

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Several endocrine pathways are known to influence longevity and aging in *C. elegans*. Insulin/IGF-1 signaling (IIS) shortens lifespan[4] and mutation of the *daf-2* insulin/IGF-1 receptor gene can more than double adult lifespan[5]. Lifespan is also modulated by a bile acid-like steroid hormone which acts via the nuclear receptor DAF-12[6, 7]. The interplay between insulin-like and steroid pathways in the control of aging is complex. In adult hermaphrodites, mutational inactivation of *daf-12* slightly shortens lifespan in a *daf-2*(+) background, partially suppresses the longevity phenotype of weaker *daf-2* mutants, but enhances that of more severe *daf-2* mutants[8, 9]. Thus, the DAF-12-mediated steroid pathway can increase or decrease lifespan, depending on the genetic context.

In this study, we explore the possibility that steroid hormone biology in *C. elegans* might, like that of higher animals, be sexually dimorphic and contribute to sex differences in aging. To do this, we have directly compared the effect on lifespan in males and hermaphrodites of mutation of *daf-12*, either alone or in combination with mutations affecting *daf-2*. We report that the effects of *daf-12* on aging differ substantially between the sexes.

EXPERIMENTAL METHODS

Nematodes were raised on NGM agar seeded with *Escherichia coli* OP50 bacteria, as previously described[10]. Measurement of lifespan in *C. elegans* males is complicated by male-male interactions which shorten lifespan, and frequent escape of males from agar plates[3]. Therefore, for this study, lifespan was measured in monoxenic liquid culture with *E. coli* OP50, with animals maintained individually in microtitre wells, as previously described[11].

The three mutant alleles studied were as follows. *daf-12(m20)* results in a dauer defective phenotype, and has a stop codon near the 5′ end of the open reading frame, and therefore approximates to a null allele[6]. *daf-2(m41)* and *daf-2(e1370)* are both hypomorphic alleles resulting in constitutive formation of dauer larvae, and increased adult longevity. *m41* is a less pleiotropic (class 1) allele with a missense mutation in the Cys-rich region of the receptor, while *e1370* is a more pleiotropic (class 2) allele with a missense mutation in the kinase region of the receptor[12, 13].

RESULTS AND DISCUSSION

We compared the effects on aging in both sex of mutations of *daf-12*, alone or in combination with mutation of *daf-2*. In hermaphrodites, *daf-12(m20)* alone caused a marginal, non-significant reduction in lifespan (TABLE 1, FIGURE 1). This is consistent with earlier studies using agar plates, where *daf-12* significantly shortened hermaphrodite lifespan8, 9. *daf-12* also suppressed the increased longevity (Age phenotype) of the less pleiotropic (class 1) mutant *daf-2(m41)*, but enhanced that of the more pleiotropic (class 2) mutant *daf-2(e1370)* (TABLE 1, FIGURE 1). These results also tally with earlier studies of plate cultured populations, where longevity of class 1 *daf-2* alleles was suppressed and that of class 2 *daf-2* alleles enhanced[8,9]. The only difference from earlier studies is that here *daf-12* fully suppressed *daf-2(m41)* Age, whereas previously on partial suppression was seen. However, in a subsequent test of the effect of *daf-12* on class 1 *daf-2* lifespan in liquid

culture we saw only partial suppression of Age (data not shown). Thus, the use of liquid culture rather than plate culture does not appear to markedly alter the effects on aging of mutation of *daf-12* or *daf-2*, alone or in combination.

In males, *daf-12* did not shorten lifespan but instead produced a slight increase that was significant in one trial, and almost significant in another (FIGURE 1, TABLE 1). In other trials, we also saw a significant increase in male lifespan resulting from the null allele *daf-12(rh61rh411)* (data not shown). Thus, the DAF-12 nuclear receptor weakly promotes longevity in hermaphrodites but weakly antagonises it in males.

daf-2(m41) and *daf-2(e1370)* both increased lifespan in males in liquid culture (FIGURE 1, TABLE 1). *daf-2(m41)* suppressed the sex difference in longevity, something not seen in studies of another class 1 mutant, *daf-2(m577)*, on plates[3]. By contrast, longevity was greatly increased in *daf-2(e1370)* males, as previously seen in plate cultured populations of this genotype[3]. Unexpectedly, addition of *daf-12* had only minor effects on lifespan in *daf-2* males (FIGURE 1, TABLE 1). *daf-12* marginally increased *daf-2* male lifespan, but the effects were very small compared to the effects seen in hermaphrodites. These slight increases reached statistical significance in one of two trials with *daf-2(m41)*, but were not significant for *daf-2(e1370)*.

In summary, our results show that the effects of *daf-12* on aging differ between the sexes in all three backgrounds tested: *daf-2*(+), *daf-2(m41)* and *daf-2(e1370)* (findings summarized in FIGURE 2). In a wild-type background, the DAF-12 receptor weakly promotes longevity in the hermaphrodite, but aging in the male. In a weak (class 1) *daf-2* mutant background, DAF-12 promotes longevity in hermaphrodites, but marginally promotes aging in males. In a stronger (class 2) *daf-2* mutant background, DAF-12 promotes longevity in hermaphrodites, but has little effect in males.

These findings demonstrate sexual dimorphism in the effects on aging of the DAF-12 nuclear receptor. This could be determined in any of a number of ways, including sex differences in levels or tissue localization of DAF-12 protein levels, or of cofactors that interact with DAF-12, or in production or structure of the steroid ligand that controls DAF-12 activity. Whether nematodes are, like mammals, sexually dimorphic in terms of the steroid hormones that they secrete remains to be explored.

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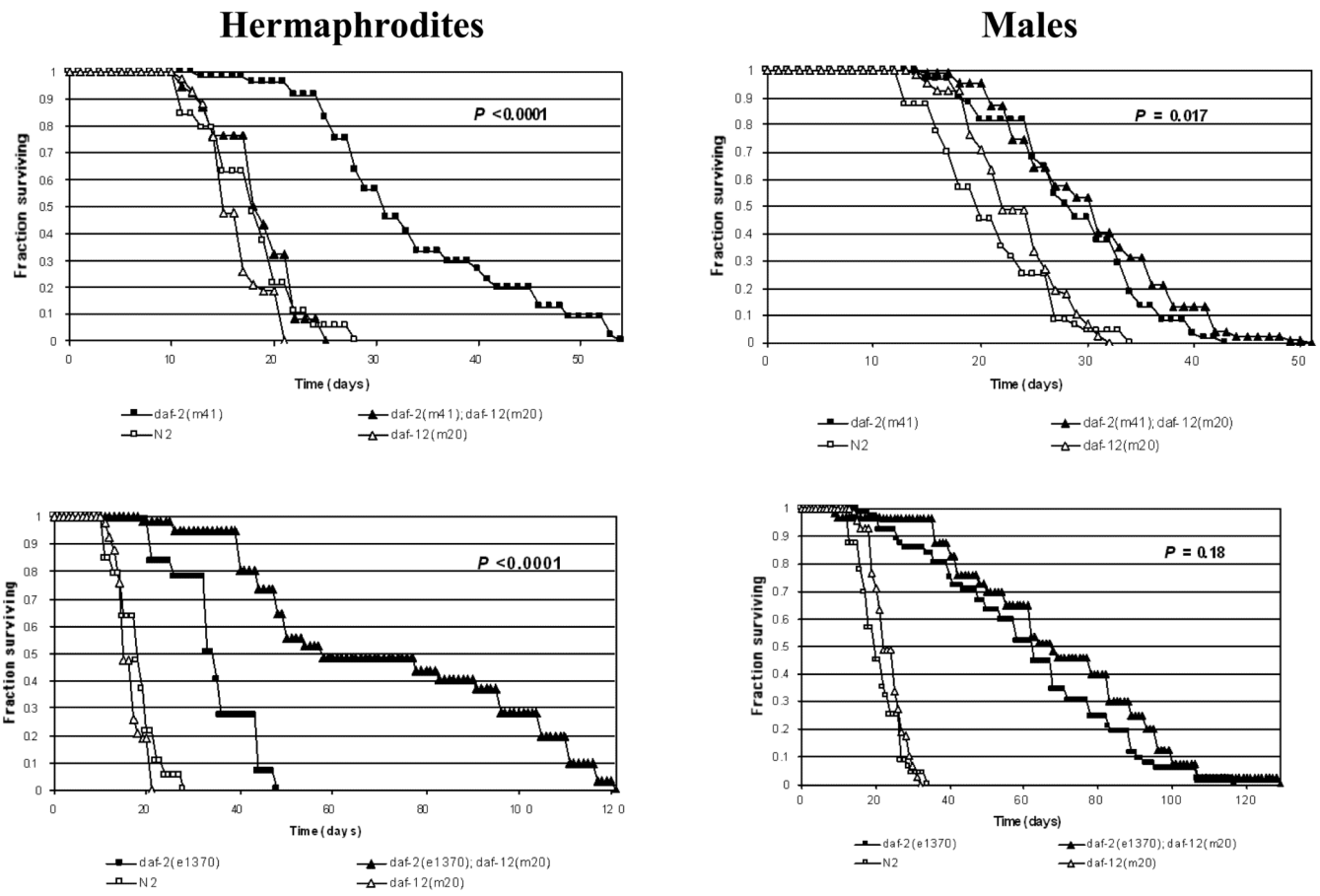

FIGURE 1.

Survival curves showing effect of *daf-12(m20)* on class 1 *daf-2(m41)* and class 2 *daf-2(e1370)* hermaphrodite and male survival (22.5°C). N2 (wild type) and *daf-12(m20)* are shown for comparison. *P* = probability that survival of *daf-2(rf)* and *daf-2(rf)*; *daf-12(m20)* differ by random chance (log rank test).

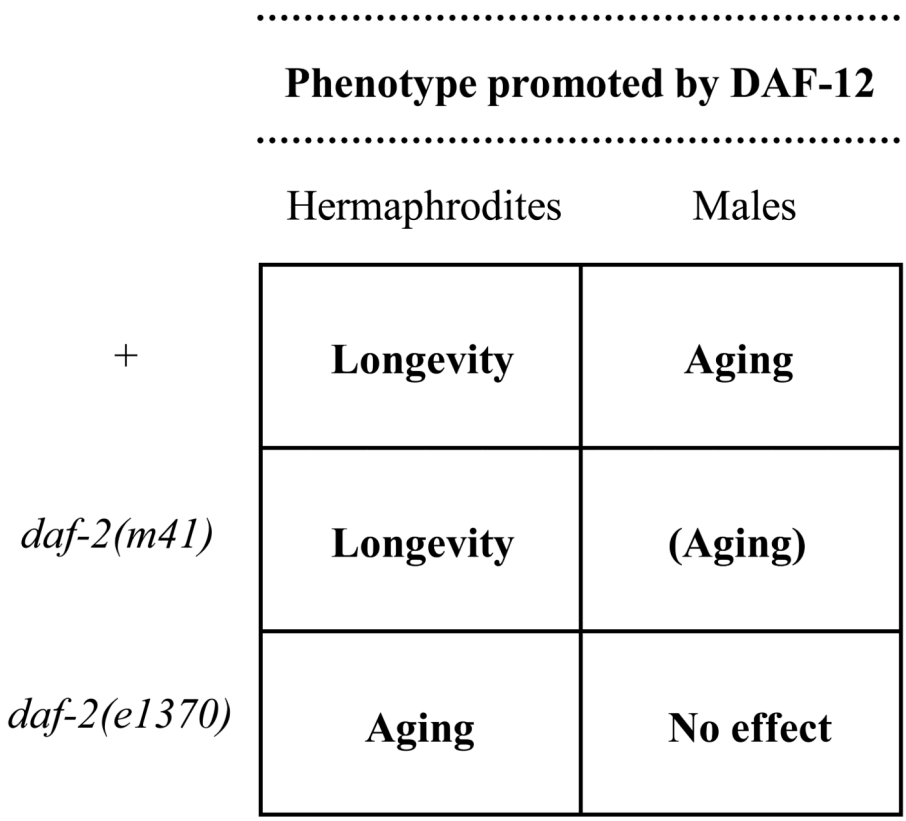

FIGURE 2.
Summary of the inferred effects of the DAF-12 steroid receptor on longevity and aging. In all three genetic contexts, the effects of mutation of *daf-12* on lifespan differs between males and hermaphrodites.

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TABLE 1

Effect of *daf-12* on lifespan in both sexes and in *daf-2*(+) and *daf-2* mutant backgrounds

Genotype/gender	Median lifespan (days) ± 95% C.I.	% effect of *daf-12* on median	Maximum lifespan (days)	% effect of *daf-12* on maximum	N*	*p* †
+ H[1]	17.7 (19.0, 16.5)	----	23.0	----	165 (200)	----
+ M[2]	19.8 (21.3, 18.3)	----	29.0	----	135 (211)	----
daf-12(m20) H	16.0 (17.5, 15.0)	−10	20.0	−13	88 (151)	0.56
daf-12(m20) M	23.5 (24.5, 22.1)	+ 19	29.5	+2	131 (152)	0.07, 0.0001
daf-2(m41) H	31.8 (36.0, 28.8)	----	51.5	----	44 (80)	----
daf-2(m41) M	29.0 (32.0, 26.5)	----	41.5	----	59 (80)	----
daf-2(m41); *daf-12* H ‡	18.0 (20.0, 18.0)	−43	22.0	−57	37 (80)	<0.0001
daf-2(m41); *daf-12* M	30.0 (33.5, 28.0)	+3	44.0	+6	87 (90)	0.017, 0.74
daf-2(e1370) H	29.3 (32.0, 27.3)	----	45.0	----	124 (332)	----
daf-2(e1370) M	53.0 (56.0, 50.8)	----	105.0	----	209 (262)	----
daf-2(e1370); *daf-12* H ‡	54.0 (75.0, 43.5)	+84	113.5	+ 152	30 (72)	<0.0001
daf-2(e1370); *daf-12* M ‡	64.0 (83.0, 55.0)	+21	124.0	+ 18	40 (72)	0.18

[1] Hermaphrodite

[2] Male

* Senescent deaths (starting population)

† Probability that survival curves of a strain with and without *daf-12(m20)* differ by random chance (log rank test). Multiple significance values represent results from trials performed at separate times.

‡ Results from only one replicate, due to bacterial contamination in two other replicates. Trials performed at 22.5°C.