

# Pheromone sensing regulates *Caenorhabditis elegans* lifespan and stress resistance via the deacetylase SIR-2.1

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Lifespan in *Caenorhabditis elegans*, *Drosophila*, and mice is regulated by conserved signaling networks, including the insulin/insulin-like growth factor 1 (IGF-1) signaling cascade and pathways depending on sirtuins, a family of NAD<sup>+</sup>-dependent deacetylases. Small molecules such as resveratrol are of great interest because they increase lifespan in many species in a sirtuin-dependent manner. However, no endogenous small molecules that regulate lifespan via sirtuins have been identified, and the mechanisms underlying sirtuin-dependent longevity are not well understood. Here, we show that in *C. elegans*, two endogenously produced small molecules, the dauer-inducing ascarosides *ascr#2* and *ascr#3*, regulate lifespan and stress resistance through chemosensory pathways and the sirtuin SIR-2.1. Ascarosides extend adult lifespan and stress resistance without reducing fecundity or feeding rate, and these effects are reduced or abolished when nutrients are restricted. We found that ascaroside-mediated longevity is fully abolished by loss of SIR-2.1 and that the effect of *ascr#2* requires expression of the G protein-coupled receptor DAF-37 in specific chemosensory neurons. In contrast to many other lifespan-modulating factors, ascaroside-mediated lifespan increases do not require insulin signaling via the FOXO homolog DAF-16 or the insulin/IGF-1-receptor homolog DAF-2. Our study demonstrates that *C. elegans* produces specific small molecules to control adult lifespan in a sirtuin-dependent manner, supporting the hypothesis that endogenous regulation of metazoan lifespan functions, in part, via sirtuins. These findings strengthen the link between chemosensory inputs and conserved mechanisms of lifespan regulation in metazoans and suggest a model for communal lifespan regulation in *C. elegans*.

aging | chemosensation |  $\beta$  oxidation | ecology

The nematode *Caenorhabditis elegans* excretes a family of small molecules, the ascarosides, which regulate several different aspects of the life history of this model organism, including developmental timing (1–3), mate attraction (3–5), aggregation behavior (6, 7), and olfactory learning (8). These compounds are derived from the dideoxysugar ascarylose, which is linked to fatty acid-like side chains of varying lengths and, in some cases, features additional substituents derived from amino acid metabolism and other pathways (7). Originally, the ascarosides were identified as components of a population-density signal, the *C. elegans* dauer pheromone. High population density results in accumulation of the constitutively expressed ascarosides, which, in combination with additional environmental stimuli such as limited food availability or temperature stress, promote larval arrest at the dauer stage (1–3). Dauer larvae are nonfeeding and highly stress-resistant and can survive for up to 6 mo before resuming development into adults of a normal lifespan of about 2 wk (9). The signaling cascade from pheromone to dauer induction has been characterized in considerable detail. The dauer-inducing ascarosides are sensed by several different G protein-coupled receptors (GPCRs) in specific chemosensory

neurons (10–12). These are coupled to several downstream signaling cascades, including the conserved insulin/insulin-like growth factor-1 (IGF-1) and transforming growth factor (TGF)- $\beta$  signaling pathways (13–15). Mutations in dauer signaling genes often display long-lived lifespan phenotypes in adult *C. elegans* (16, 17), and homologs of dauer genes are involved in lifespan regulation of many higher organisms (13).

The strong analogies between dauer signaling and conserved aging pathways suggested that dauer pheromone components may also affect *C. elegans* adult lifespan. Previous studies have yielded conflicting evidence regarding the effects of dauer pheromone on adults (18–20); however, these studies tested for the lifespan-extending effects of the entire *C. elegans* exometabolome, which, in addition to a variety of dauer-inducing ascarosides, contains a vast assortment of other metabolites. Therefore, we investigated the effects of pure synthetic samples of dauer-inducing ascarosides on *C. elegans* adult lifespan.

## Results

***ascr#2* and *ascr#3* Extend *C. elegans* Lifespan and Stress Resistance.** We tested the effect of *ascr#2* and *ascr#3* (Fig. 1 *A* and *B*), the two most consistently produced ascarosides (1, 3), on *C. elegans* lifespan. We found that exposure to ascarosides resulted in a concentration-dependent increase of lifespan. Mean lifespan of L4-stage worms transferred onto plates containing *ascr#2* or *ascr#3* was significantly increased in a concentration-dependent manner (Fig. 1 *C* and *D* and Table S1). Lifespan of worms on plates containing 400 nM *ascr#2* or *ascr#3*, concentrations typical of high-density *C. elegans* cultures (7), was increased by 17% and 21%, respectively. Higher concentrations did not confer significantly higher lifespan gains, and mixtures of *ascr#2* and *ascr#3* extended lifespan to a similar extent as the individual compounds (Table S1 and Fig. S1).

Because in many animals reduced food intake [dietary restriction (DR)] results in increased lifespan, it seemed possible

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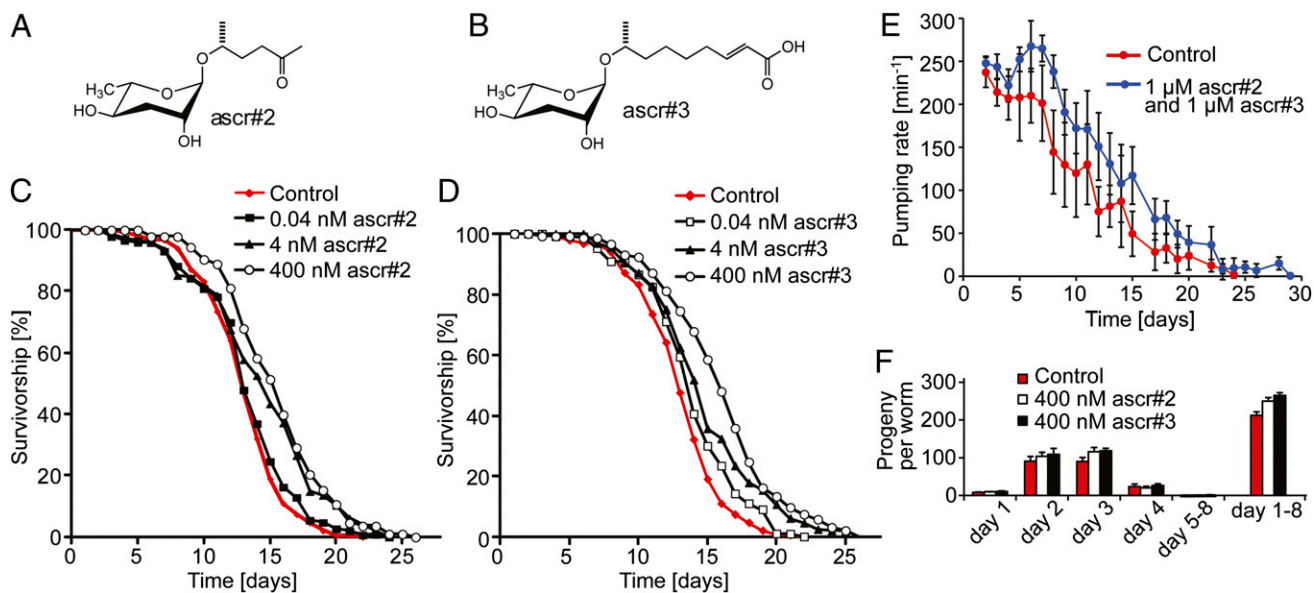
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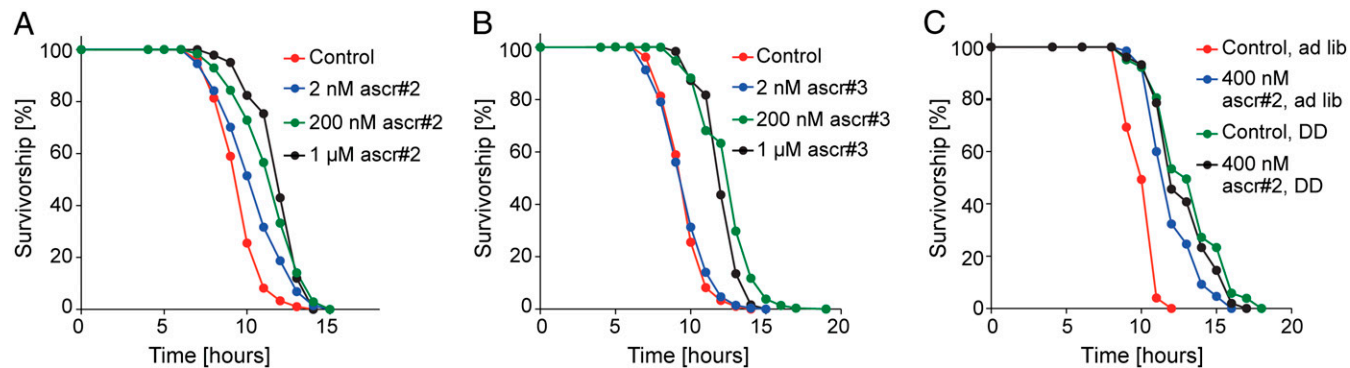
**Fig. 1.** Ascarosides increase adult lifespan in wild-type (N2) *C. elegans* in a concentration-dependent manner. (A) Chemical structure of ascr#2. (B) Chemical structure of ascr#3. (C) Survivorship of worms at 20 °C exposed to 0.04 nM ascr#2: number of worms ( $n$ ) = 147, average lifespan ( $m$ ) = 13.4 d,  $P$  = 0.253; 4 nM ascr#2:  $n$  = 137,  $m$  = 14.5 d,  $P$  < 0.0001; 400 nM ascr#2:  $n$  = 111,  $m$  = 15.1 d,  $P$  < 0.0001; and mock-treated controls:  $n$  = 155,  $m$  = 13.2 d. (D) Survivorship of worms exposed to 0.04 nM ascr#3,  $n$  = 139,  $m$  = 14.6 d,  $P$  < 0.0001; 4 nM ascr#3:  $n$  = 132,  $m$  = 14.9 d,  $P$  < 0.0001; 400 nM ascr#3:  $n$  = 134,  $m$  = 16.0 d,  $P$  < 0.0001; and mock-treated:  $n$  = 155,  $m$  = 13.2 d. (E) Pharyngeal pumping rate of worms exposed to 1  $\mu$ M ascr#2 and ascr#3 compared with mock-treated control. (Error bars, SD.) (F) Fecundity of worms exposed to 400 nM ascr#2 or ascr#3 compared with mock-treated control. (Error bars, SD.)

that ascaroside-mediated lifespan increases were the result of altered feeding behavior. Therefore, we monitored changes in pharyngeal pumping rate, a measure for *C. elegans* food intake, and fecundity upon ascaroside exposure (21). Pharyngeal pumping rates of ascaroside-treated worms were similar to control until day 4 of the experiments and significantly higher than control from day 5 until death (Fig. 1E), indicating that reduced food intake is not a likely cause for the observed lifespan increases. Furthermore, overall fecundity of ascaroside-treated worms was slightly higher than control (Fig. 1F).

In *C. elegans* and other organisms, environmental conditions or genetic changes that result in increased lifespan are frequently associated with enhanced resistance to stress, including heat stress and oxidative stress (22). We found that ascarosides markedly

increased survival under oxidative stress (Fig. S24) and resistance to heat stress in a concentration-dependent manner (thermotolerance at 35 °C; Fig. 2A and B and Table S2). We further measured pharyngeal pumping rates under heat stress and found that pumping rates of worms on ascaroside plates were significantly higher than on control plates (Fig. S2B). Consequently, we named the observed effect ascaroside-mediated increases of lifespan and stress resistance (AMILS).

**AMILS Depends on Nutritional Conditions.** Next, we asked whether nutritional conditions influence AMILS. For thermotolerance assays under dietary deprivation (DD) conditions, we used a protocol in which young adult worms are transferred to plates without bacteria before exposure to heat stress (23). Mean heat stress survival time under DD conditions was higher than for worms with



**Fig. 2.** Ascarosides increase thermotolerance at 35 °C in wild-type (N2) *C. elegans* in a concentration-dependent manner. (A) Survivorship of adult worms at 35 °C exposed to 2 nM, 200 nM, and 1  $\mu$ M ascr#2; 2 nM ascr#2:  $n$  = 278,  $m$  = 10.6 h,  $P$  < 0.0001; 200 nM ascr#2:  $n$  = 313,  $m$  = 11.6 h,  $P$  < 0.0001; 1  $\mu$ M ascr#2:  $n$  = 142,  $m$  = 12.1 h,  $P$  < 0.0001; and mock-treated control:  $n$  = 1062,  $m$  = 9.7 h. (B) Survivorship of adult worms at 35 °C exposed to 2 nM, 200 nM, and 1  $\mu$ M ascr#3; 2 nM ascr#3:  $n$  = 278,  $m$  = 9.8 h,  $P$  = 0.004; 200 nM ascr#3:  $n$  = 289,  $m$  = 12.6 h,  $P$  < 0.0001; 1  $\mu$ M ascr#3:  $n$  = 126,  $m$  = 12.6 h,  $P$  < 0.0001; and mock-treated control:  $n$  = 1062,  $m$  = 9.7 h. (C) Survivorship of adult worms at 35 °C exposed to 400 nM ascr#2 compared with mock-treated controls, using two different nutritional conditions [adlib (with bacteria) and DD (without bacteria)]; adlib ascr#2:  $n$  = 65,  $m$  = 12.2 h,  $P$  < 0.0001; adlib control:  $n$  = 75,  $m$  = 10.2 h; DD ascr#2:  $n$  = 103,  $m$  = 12.9 h,  $P$  = 0.145; DD control:  $n$  = 103,  $m$  = 13.3 h.

bacteria, in accordance with previous studies demonstrating increased stress resistance under these conditions (23). Notably, addition of ascarosides did not further increase thermotolerance of DD worms (Fig. 2C). Additional lifespan assays using DR conditions (24) showed a similar pattern (Fig. S3). As expected, DR control worms lived longer than non-DR controls; however, ascarosides increased lifespan of DR worms less than of non-DR worms (Fig. S3). These results show that the effect of ascarosides on *C. elegans* thermotolerance and lifespan strongly depends on nutritional conditions.

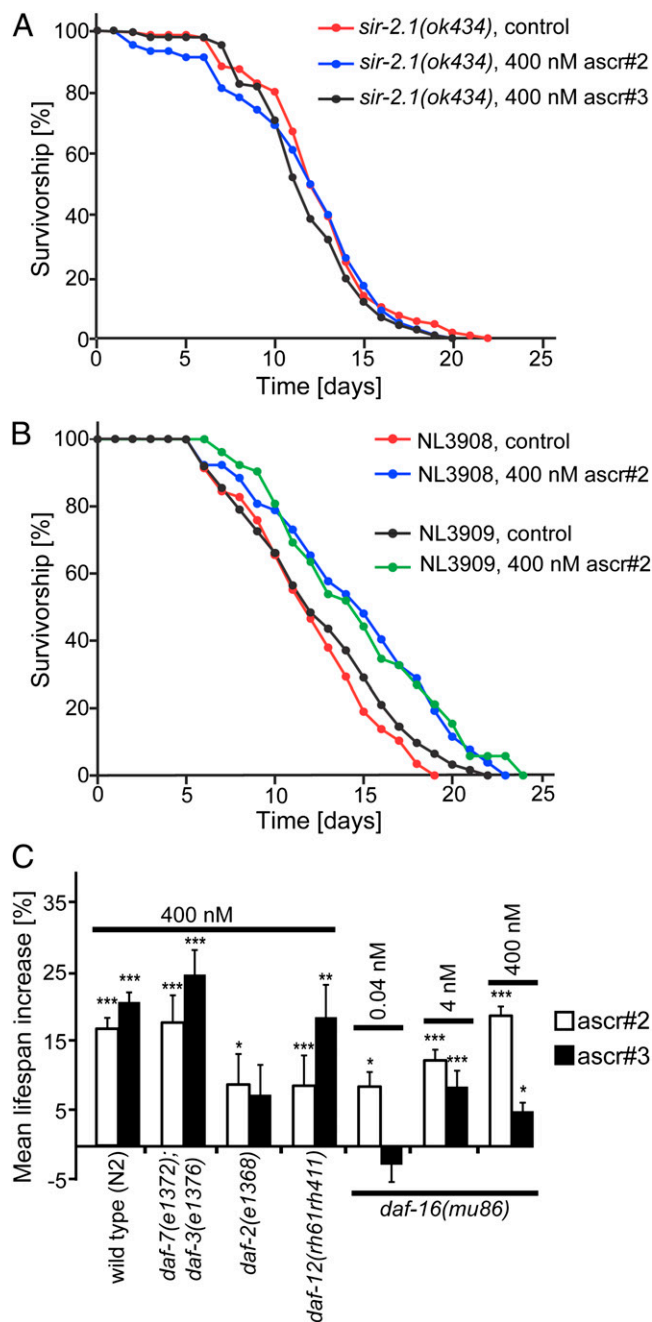
**AMILS Requires the Histone Deacetylase SIR-2.1.** To investigate the mechanism of AMILS, we probed conserved genetic pathways known to regulate lifespan in *C. elegans* and other metazoans. The observation that AMILS is reduced or abolished under starvation conditions led us to investigate a possible role of the sirtuin SIR-2.1, a NAD<sup>+</sup>-dependent histone deacetylase (25). DR, DD, and other forms of caloric restriction (CR) (26) have been shown to increase lifespan and stress resistance in many organisms, and in several cases, including yeast and mice, this effect has been found to depend on sirtuins (13, 27–32). In *C. elegans*, SIR-2.1 has been shown to be required for lifespan increases by resveratrol and other compounds (25, 33), although it is unclear to what extent SIR-2.1 is required for DR-dependent lifespan increases. For example, SIR-2.1 is not required for lifespan extension associated with DD of nonfertile worms (23), whereas lifespan increases associated with milder forms of DR appear to require this gene (13). The role of sirtuins in *C. elegans* and *Drosophila* lifespan continues to be discussed extensively (13, 34, 35).

We found that neither *ascr#2* nor *ascr#3* extend lifespan or thermotolerance in the null allele *sir-2.1(ok434)* (lifespan: Fig. 3A and Table S1; thermotolerance: Fig. S4A and B and Table S2). We then investigated the effect of *ascr#2* on an outcrossed SIR-2.1-overexpressing strain, NL3909, relative to its control, NL3908 (34, 36, 37). *ascr#2* increased lifespan of NL3909 worms to a similar extent as for NL3908 control worms (Fig. 3B and Table S1). These results indicate that SIR-2.1 is required for AMILS, but overexpression of SIR-2.1 does not significantly affect the amount of lifespan extension.

#### AMILS Is Largely Independent of Classical Dauer Signaling Pathways.

Because ascarosides induce formation of dauer larvae in *C. elegans*, we asked whether AMILS depends on conserved aging pathways involved in dauer formation, including insulin/IGF-1, TGF- $\beta$ , and nuclear hormone receptor (DAF-12) signaling (38). By testing the effect of ascarosides on the insulin/IGF-1 receptor mutant strain *daf-2(e1368)* (13), we found that both *ascr#2* and *ascr#3* further increased lifespan and thermotolerance of this long-lived mutant (Fig. 3C, Fig. S5, and Table S1). DAF-2 negatively regulates the FOXO-like transcription factor DAF-16, which is required for many lifespan- or stress resistance-enhancing mutations described for *C. elegans* (13). Ascarosides significantly increased lifespan and thermotolerance of the short-lived null allele *daf-16(mu86)* even at low concentrations (Fig. 3C, Fig. S5, and Table S1), although the observed lifespan increases were somewhat smaller than for wild type. We confirmed lifespan increase in a second *daf-16* allele, a *daf-16(mgDf50)* mutant in *sir-2.1*-overexpressing background (Fig. S6 and Table S1). AMILS, therefore, is largely independent of insulin signaling via DAF-16.

AMILS is also maintained in the null allele of another key component of dauer signaling, the nuclear hormone receptor DAF-12 (Fig. 3C and Table S1) (39). To determine a possible role of TGF- $\beta$  signaling, we tested the effect of ascarosides on lifespan and thermotolerance of a *daf-7;daf-3* double mutant strain. *daf-7* and *daf-3* encode the TGF- $\beta$  ligand and a co-SMAD protein that functions as a transcriptional regulator downstream of *daf-7*, respectively (40). *daf-7(e1372);daf-3(e1376)* double mutant worms responded to ascarosides similarly to wild type (Fig. 3C, Fig. S5,



**Fig. 3.** AMILS requires SIR-2.1 but not classical dauer pathway genes. (A) Survivorship of *sir-2.1(ok434)* mutant worms exposed to 400 nM *ascr#2* or *ascr#3*; *ascr#2*:  $n = 100$ ,  $m = 11.8$  d; *ascr#3*:  $n = 119$ ,  $m = 11.9$  d; mock-treated control:  $n = 109$ ,  $m = 12.5$  d. (B) Survivorship of the *sir-2.1* overexpressor NL3909 and its control NL3908 treated with *ascr#2* 400 nM; NL3909 *ascr#2*:  $n = 178$ ,  $m = 13.7$  d,  $P = 0.002$ ; NL3909 mock-treated control:  $n = 174$ ,  $m = 12.4$  d; NL3908 *ascr#2*:  $n = 111$ ,  $m = 15.2$  d,  $P < 0.0001$ ; NL3908 mock-treated control:  $n = 125$ ,  $m = 12.0$  d. (C) Mean lifespan extension for mutant strains of genes involved in dauer induction. (Error bars, SEM for highlighted experiments.) For full data, see Table S1. \*\*\* $P < 0.0001$ ; \*\* $P < 0.001$ ; \* $P < 0.05$ .

and Table S1). We conclude that AMILS functions largely independently from the main dauer signaling pathways, requiring SIR-2.1 but not insulin/IGF-1 or TGF- $\beta$  signaling.

**Sensory Neurons and a GPCR Mediate *ascr#2*-Dependent Lifespan Extension.** Next, we asked whether AMILS is mediated by chemosensory neurons and receptors that have been shown to

participate in ascaroside sensing (4, 6, 10–12). For these investigations, we focused on *ascr#2* for which we had recently described a highly specific GPCR, DAF-37 (12). DAF-37 expression in the ASI chemosensory neurons is required for dauer formation in response to *ascr#2*, whereas the behavioral phenotypes of *ascr#2* depend on DAF-37 expression in the ASK neurons. *ascr#2* directly binds to DAF-37, and it was further shown that *daf-37* null worms are defective in their dauer and behavioral responses only to *ascr#2* but not to other ascarosides (12). We found that *ascr#2* does not increase lifespan of *daf-37* null worms (Fig. 4A), which could be rescued by *daf-37* cDNA under its own promoter (Fig. 4B). In addition, *ascr#2*-dependent lifespan increase was fully abolished in worms lacking the ASK neurons (Fig. 4C) because of cell-specific expression of mammalian caspase in the developing neurons. *ascr#2*-induced lifespan increase was also rescued by *daf-37* cDNA driven by the ASK-selective *srbc-64* promoter (Fig. 4D). In contrast, *ascr#2* treatment of worms expressing *daf-37* cDNA under the ASI-specific *gpa-4* promoter resulted in variable results, whereby one experiment showed no change in lifespan compared with control and two subsequent experiments showed a small but significant lifespan decrease (Fig. S7 and Table S1). These results indicate that *daf-37* expression in the ASK neurons is sufficient for *ascr#2*-dependent lifespan increase, in contrast to the dauer-inducing effects of *ascr#2*, which require expression in ASI (12).

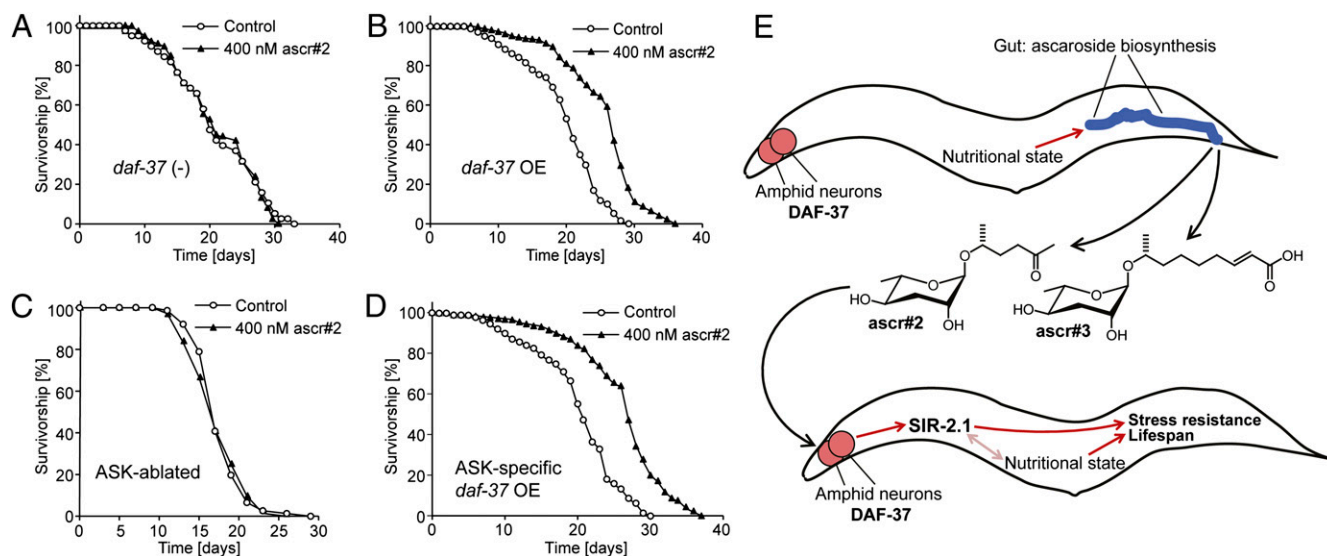
## Discussion

We demonstrate here that sensing of excreted small molecules regulates *C. elegans* lifespan and stress resistance via pathways that require the sirtuin SIR-2.1. Ascarosides increase adult lifespan at physiological concentrations typical of high-density *C. elegans* cultures, without reducing fecundity or feeding rate. In contrast to many other metazoan examples for life extension, AMILS does not require insulin signaling via DAF-16. Furthermore, AMILS does not depend on DAF-12, which is required for germ-line-

dependent modulation of *C. elegans* lifespan (41). These observations starkly differentiate AMILS from dauer formation.

Previous studies suggested a complex role for *sir-2.1* in *C. elegans* lifespan regulation (13, 20). There is increasing evidence that sirtuin overexpression increases lifespan across model systems (37, 42), although, in some cases, this effect may not be as robust as initially reported (34, 35, 43). In addition, genetic studies of *sir-2.1*-deletion strains suggest that *sir-2.1* influences lifespan in a *daf-16*-independent manner as part of the CR pathway (44–46). Recent work connecting sirtuins and longevity in mouse affirmed a conserved role for sirtuins in lifespan regulation (47). With regard to sirtuin dependence, AMILS resembles the lifespan-increasing effects of exogenous small molecules such as resveratrol. Comparatively high concentrations of resveratrol (10–100  $\mu$ M) increase lifespan in *C. elegans* and *Drosophila*, and this effect has been shown to require SIR-2.1 in *C. elegans* and its homolog Sir2 in *Drosophila* (25). Similar to resveratrol-mediated lifespan increases, AMILS does not carry any apparent cost of reproduction, is reduced or abolished when nutrients are restricted, and does not require *daf-16*. It has been suggested that resveratrol increases metazoan lifespan via pathways related to CR (25); however, the mechanisms of resveratrol-mediated lifespan increases are not well understood (13), and it has been suggested that the relatively weak lifespan-increasing effects of resveratrol in *C. elegans* could merely reflect activation of detoxification mechanisms (35). Given that AMILS requires much lower (compared with resveratrol) concentrations of compounds that are endogenously produced and perceived by highly specific membrane receptors, it is unlikely that detoxification plays a role for the lifespan-mediating effects of ascarosides.

In dauer formation, the ascarosides act through several amphid neurons (4, 48), and six GPCRs have been associated with ascaroside-mediated dauer induction (10–12). We recently showed that DAF-37 is required specifically for sensing of *ascr#2* and that DAF-37 expression in the ASK neurons is required for the behavioral phenotypes of *ascr#2*, whereas DAF-37 expression



**Fig. 4.** ASK neurons and the GPCR DAF-37 are required for lifespan extension via *ascr#2*. (A) Survivorship of *daf-37* (-) mutant worms exposed to 400 nM *ascr#2* ( $n = 42$ ,  $m = 20.9$  d), compared with mock-treated controls ( $n = 38$ ,  $m = 21.3$  d). (B) Survivorship of *daf-37*; *mIs41*[*daf-37p::cMyc::daf-37*] (*daf-37* OE) worms exposed to 400 nM *ascr#2* ( $n = 56$ ,  $m = 25.5$  d,  $P < 0.0001$ ), compared with mock-treated controls ( $n = 77$ ,  $m = 19.8$  d). (C) Survivorship of worms with genetically ablated ASK neurons exposed to 400 nM *ascr#2* ( $n = 79$ ,  $m = 18.8$  d), compared with mock-treated controls ( $n = 74$ ,  $m = 19.0$  d). (D) Survivorship of *daf-37*; *unc-119*; *mEx188*[*srbc-64p::daf-37*; *unc-119(+)*] (ASK neuron-specific *daf-37* OE in *daf-37* null background) worms exposed to 400 nM *ascr#2* ( $n = 60$ ,  $m = 26.2$  d,  $P < 0.0001$ ), compared with mock-treated controls ( $n = 85$ ,  $m = 20.0$  d). (E) Model for communal lifespan regulation via pheromone signaling. *C. elegans* constitutively produce *ascr#2* and *ascr#3*, resulting in population density-dependent ascaroside concentrations. Chemosensation of the ascarosides integrated with environmental and nutritional cues results in activation of longevity and/or stress-resistance genes in a SIR-2.1-dependent manner.

in the ASI neurons is required for *ascr#2*-mediated dauer induction (12). Here, we show that DAF-37 is also required for *ascr#2*-mediated lifespan extension and that DAF-37 expression in the ASK neurons is sufficient for this phenotype, further distinguishing AMILS from dauer. Therefore, AMILS provides a direct example for a lifespan-regulatory mechanism based on chemosensation of an endogenously produced small-molecule signal.

Genetic and small-molecule screens have previously suggested a connection between GPCR signaling and lifespan in *Drosophila* (49, 50). In these studies, GPCR mutation or inhibition of downstream signaling components extended fly lifespan, but it is unclear whether chemosensation played a role. Notably, GPCRs have also been implicated in many age-related diseases (26). In Fig. 4E, we present a simplified model for AMILS in *C. elegans*. As products of peroxisomal  $\beta$ -oxidation and other primary metabolic pathways (7), ascarosides integrate input from the nutritional state of the producing animal. The primary site of ascaroside biosynthesis appears to be the worm gut (51), and it seems likely that ascarosides are also excreted via the gut. In the perceiving worm, GPCRs in the amphid chemosensory neurons trigger downstream signaling cascades promoting longevity and stress resistance via SIR-2.1, additionally integrating information about the perceiving animal's nutritional state. In essence, AMILS may represent a model for communal mechanism for lifespan regulation (Fig. 4E).

Our study highlights the significance of siruin-dependent signaling pathways for endogenous lifespan regulation, and ascarosides may serve as useful tools to investigate the mechanisms of siruin-dependent longevity, which remain poorly understood (27). The ascarosides are derived from highly conserved peroxisomal fatty acid  $\beta$ -oxidation, and their biosynthesis is regulated, in part, by nutritional state (7), suggesting a link between nutritional state, fat metabolism, and siruin-dependent lifespan regulation. Furthermore, analysis of the adaptive significance of siruin-dependent lifespan regulation via ascarosides in *C. elegans* may lead to a better understanding of the evolutionary forces that determine lifespan and may inspire a search for endogenous small molecules that promote siruin-dependent longevity in other metazoans.

## Materials and Methods

**Worm Strains.** The following *C. elegans* strains were obtained from the Caenorhabditis Genetics Center: wild type (N2), *daf-2(e1368)*, *daf-16(mu86)*, *sir-2.1(ok434)*, *daf-12(rh61rh411)*. The strain *daf-7(e1372);daf-3(e1376)* was the kind gift of Paul Sternberg (Caltech, Pasadena, CA), and the low-copy SIR-2.1-overexpressing strain NL3909 *pkl1642(unc-119[+] sir-2.1[+])*, and its control NL3908 *pkl1641(unc-119[+])*, as well as a *daf-16(mgDf50);NL3909*, were kind gifts of Sylvia Lee (Cornell University, Washington, DC). For genetic ablation of the ASK neuron, we used the transgenic strain PS6025 *qrls2[sra-9::mCasp1]*, which expresses mammalian caspase in the ASK neuron under the *sra-9* promoter [this strain was a kind gift of Tokumitsu Wakabayashi (Iwate University, Iwate, Japan)]. The following *daf-37* overexpressor and mutant strains were created as described earlier: *daf-37(DR2585)* (*daf-37* null mutant), *daf-37; mls41[daf-37p::daf-37,rol-6(su1006)]* (*daf-37* overexpressor in *daf-37* mutant background), *daf-37; unc-119; mEx188[srbc-64p::daf-37,unc-119(+)]* (ASK-specific *daf-37* overexpressor in *daf-37* mutant background), and *daf-37;unc-119;mEx187[gpa-4p::daf-37,unc-119(+)]* (ASI-specific *daf-37* overexpressor in *daf-37* mutant background) (12). Nematode stocks were maintained on nematode growth medium (NGM) (52) plates with added bacteria (*Escherichia coli* strain OP50) at 20 °C ([www.wormbook.org](http://www.wormbook.org)), unless indicated otherwise.

**Plate Preparation.** Ascarosides *ascr#2* and *ascr#3* were synthesized as described previously (3). For preparation of ascaroside-containing NGM plates, *ascr#2* and *ascr#3* were dissolved in 100% ethanol producing 10.6 mM (*ascr#2*) and 7.6 mM (*ascr#3*) stock solutions, which were further diluted with water to yield stock solutions of micromolar and nanomolar concentrations, as needed. Controls for mock treatment had corresponding amounts of ethanol added. Plates were allowed to dry for 24 h. For the heat stress and lifespan assays, 6-cm diameter Petri dish plates were prepared using noble agar-based NGM prepared according to [www.wormbook.org](http://www.wormbook.org). Minimal growth medium NGM without peptone ("pep", for DR studies) was

prepared as described for NGM plates but without adding peptone. Two hundred microliters of ascaroside solutions or mock solutions were spread on the surface of NGM plates. After drying overnight, 60  $\mu$ L of bacteria (*E. coli* OP50 pellet), freshly grown at 37 °C in LB media, was spread onto the plates, and plates were incubated for 24 h at 23.5 °C. For thermotolerance assays, plates were exposed to UV radiation for 1 h to kill the bacteria before putting worms on the plates.

**Lifespan Assays.** For lifespan assays, worm strains were thawed from frozen stock and grown for at least two generations on well-fed conditions. Six-centimeter NGM plates containing *ascr#2*, *ascr#3*, 1:1 mixtures of *ascr#2* and *ascr#3*, and mock-treated control plates were used. Late L4-stage worms were picked from synchronized NGM plates and transferred (25–30 worms per plate) to experimental plates at 20 °C. Table S1 contains full lifespan data. Survival was monitored by scoring for touch-provoked movement every other day, in some cases every day. Live worms were transferred every other day to remove progeny until day 10. Day 0 corresponds to the time of transfer of the worms at the L4 stage. All experiments, except the experiment using 0.04 nM either *ascr#2* and *ascr#3* (Fig. 1), were carried out at least twice at two different times, started at least 1 wk apart. For testing the effect of ascarosides on *C. elegans* adult lifespan without peptone supplementation, NGM without peptone plates were used. For some lifespan experiments (Table S1), 2'-deoxy-5-fluorouridine (FUdR) was used. The SPSS version 19 (IBM) statistical analysis package was used for all lifespan and thermotolerance statistics. *P* values were calculated using the log-rank (Mantel–Cox) method.

**Lifespan Assays Using FUdR.** FUdR (Sigma) was added to a final concentration of 4  $\mu$ g/mL after media were autoclaved. Ascaroside and control solutions were added as described above. Late L4-stage worms were picked from synchronized NGM plates and transferred (30 worms per plate) to control plates or plates containing ascarosides that also contained FUdR.

**Thermotolerance Assays.** Six-centimeter NGM plates containing *ascr#2*, *ascr#3*, 1:1 mixtures of *ascr#2* and *ascr#3*, and mock-treated control plates were used. Experimental plates were prepared using noble agar-based NGM (52). *E. coli* OP50 was UV-killed before placing worms on plates. Late L4-stage worms were picked from synchronized NGM plates and transferred (15–25 worms per plate) to experimental plates. After completion of development to adults at 20 °C (14–20 h, depending on the strain), plates were incubated at 35 °C. Survival was scored hourly, beginning after 5–8 h of heat exposure, by checking for touch-provoked movement until all worms had died. At least four plates (usually five) for each ascaroside concentration were used, in addition to six control plates, and all experiments were carried out at least twice at two different times. Thermotolerance assays reported in Table S2 were performed as above, except that experimental plates with live *E. coli* OP50 were used instead of UV-killed bacteria. The SPSS version 19 (IBM) statistical analysis package was used for all thermotolerance statistics. *P* values were calculated using the log-rank (Mantel–Cox) method.

**Thermotolerance Assays with DD.** Late stage-L4 worms were picked from synchronized NGM plates and placed on experimental plates. After completion of development to adults at 20 °C (16 h), worms were transferred to "adlib" or "DD" plates; adlib plates: NGM plates with bacteria containing 400 nM *ascr#2* or control; DD plates: NGM plates without bacteria containing 400 nM *ascr#2* or control, additionally treated with 200  $\mu$ L of 5 mg/mL streptomycin sulfate and penicillin solution (23). Immediately following worm transfer, adlib or DD plates were exposed to 35 °C. Survival was monitored as described above. The SPSS version 19 (IBM) statistical analysis package was used for all thermotolerance statistics. *P* values were calculated using the log-rank (Mantel–Cox) method.

**Oxidative Stress (H<sub>2</sub>O<sub>2</sub>) Resistance Assay.** Synchronized L4-stage worms were placed on NGM plates (mock-treated control or containing various concentrations *ascr#2* and *ascr#3*) with bacteria (OP50) at 20 °C. After reaching young adult stage (12–16 h), worms were transferred to NGM plates (mock-treated control or containing various concentrations *ascr#2* or *ascr#3*) with bacteria and containing 0.6 mM H<sub>2</sub>O<sub>2</sub>. Survival was scored after 6 h at 20 °C.

**Brood Size Measurement.** To determine the average brood size per worm, living eggs and L1 progeny were counted on the plates used for the lifespan experiments described above. Worms were transferred 8–10 times over the course of the experiments. The number of progeny per worm was determined by dividing the total number of eggs and L1 worms counted per plate by the

number of live worms transferred. Five to six plates per condition (ascaroside-treated and control) with 20–30 worms per plate were counted.

**Measurement of Pharyngeal Pumping Rates.** Pumping of worms was counted over 1-min intervals by eye. Ten worms per condition and time point in the lifespan assays were scored, beginning at day 1 of the experiment and continued daily until all worms had died. In the thermotolerance assays, pharyngeal pumping rates of at least five worms were scored every 30 min starting 8 h after placing the worms in the incubator.

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