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An excreted small molecule promotes *C. elegans* reproductive development and aging

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Excreted small-molecule signals can bias developmental trajectories and physiology in diverse animal species. However, the chemical identity of these signals remains largely obscure. Here we report identification of an unusual *N*-acylated glutamine derivative, nacq#1, that accelerates reproductive development and shortens lifespan in *Caenorhabditis elegans*. Produced predominantly by *C. elegans* males, nacq#1 hastens onset of sexual maturity in hermaphrodites by promoting exit from the larval dauer diapause and by accelerating late larval development. Even at picomolar concentrations, nacq#1 shortens hermaphrodite lifespan, suggesting a trade-off between reproductive investment and longevity. Acceleration of development by nacq#1 requires chemosensation and is dependent on three homologs of vertebrate steroid hormone receptors. Unlike ascaroside pheromones, which are restricted to nematodes, fatty acylated amino acid derivatives similar to nacq#1 have been reported from humans and invertebrates, suggesting that related compounds may serve signaling functions throughout metazoa.

Interorganismal small-molecule signaling has been implicated in regulating multiple aspects of animal biology[1]. In addition to shaping behaviors on relatively short timescales, chemosocial signals can also modulate gene expression programs that regulate development and physiology. These longer-lasting changes often occur in response to chemical messages from particularly salient emitters, such as potential mates and competitors. For example, *Drosophila melanogaster* males exposed to female sex pheromones showed complex physiological changes consistent with expectation of mating[2]. In mice, sexually mature males produce a small-molecule signal that induces earlier onset of the first estrus in peripubescent females[3].

Previous studies in *C. elegans* demonstrated that individuals of both sexes excrete as-yet-unidentified signals that alter development: *C. elegans* hermaphrodites develop faster on hermaphrodite-conditioned plates[4] or following exposure to trace quantities of male-excreted metabolites[5] (Fig. 1a). Accelerated progression through larval development in response to small-molecule signals from other individuals in a population appears to be in direct opposition to larval arrest of development at the dauer stage, which is triggered by harsh environmental conditions in combination with excreted pheromones called ascarosides[6–8]. The ability to modify the rate of development in response to environmental conditions may be particularly important to short-lived animals in ephemeral boom-and-bust habitats[9]. Notably, the ability to accelerate development in response to male and hermaphrodite signals appears to come at a cost—both of these treatments reduce hermaphrodite lifespan[4,10–12] (Fig. 1a), mirroring the effect of female sex pheromones on longevity of *Drosophila* males[3].

Similar effects of chemical signals on developmental time and longevity have been observed in divergent species raising the possibility that at least some of the underlying mechanisms are conserved among metazoa. However, little is known regarding the chemical nature of the signal(s) and the regulatory pathways that implement their effects. In this study, we identified an excreted small molecule, nacq#1 (**1**), that promotes sexual maturation of *C. elegans* hermaphrodites by modulating conserved signaling pathways. nacq#1 is an *N*-acylated glutamine that belongs to a class of small molecules found in diverse animal lineages, unlike the previously described ascarosides, which appear to be largely restricted to nematodes. Furthermore, we demonstrate that the effects of nacq#1 are mediated by the chemosensory system and a network of nuclear hormone receptors (NHRs). Our findings expand the repertoire of known metabolites that specifically alter development and physiology in members of the same species. Because nacq#1 is predominantly present in male excretions, our results contribute to a better understanding of the sexual dimorphism of metabolism, particularly with respect to excreted signaling compounds. Finally, the availability of a pure molecule that potently alters development and lifespan offers a new tool for the study of mechanisms that regulate development and longevity in response to social signals.

Results

Excreted metabolites accelerate development and aging. In pursuit of the specific molecule(s) that accelerate *C. elegans* development and aging, we first compared the properties of the male- and hermaphrodite-excreted products. Analyses of hermaphrodite development on plates conditioned by exometabolomes (Fig. 1b) revealed that male excretions were approximately tenfold more potent than those of hermaphrodites. Accordingly, exometabolomes from *him-5* cultures, which contain >30% males, were more potent at accelerating sexual maturation than extracts of wild-type cultures, which are >99% hermaphrodite (Fig. 1c). *daf-22* males, which are defective in peroxisomal β-oxidation (pβO), accelerate hermaphrodite development as effectively as wild-type males[5], indicating that the active compound is likely not an ascaroside pheromone, as these are pβO dependent[13,14]. Similarly, the hermaphrodite-excreted product was *daf-22*-independent (Supplementary Fig. 1 and ref. [4]). These

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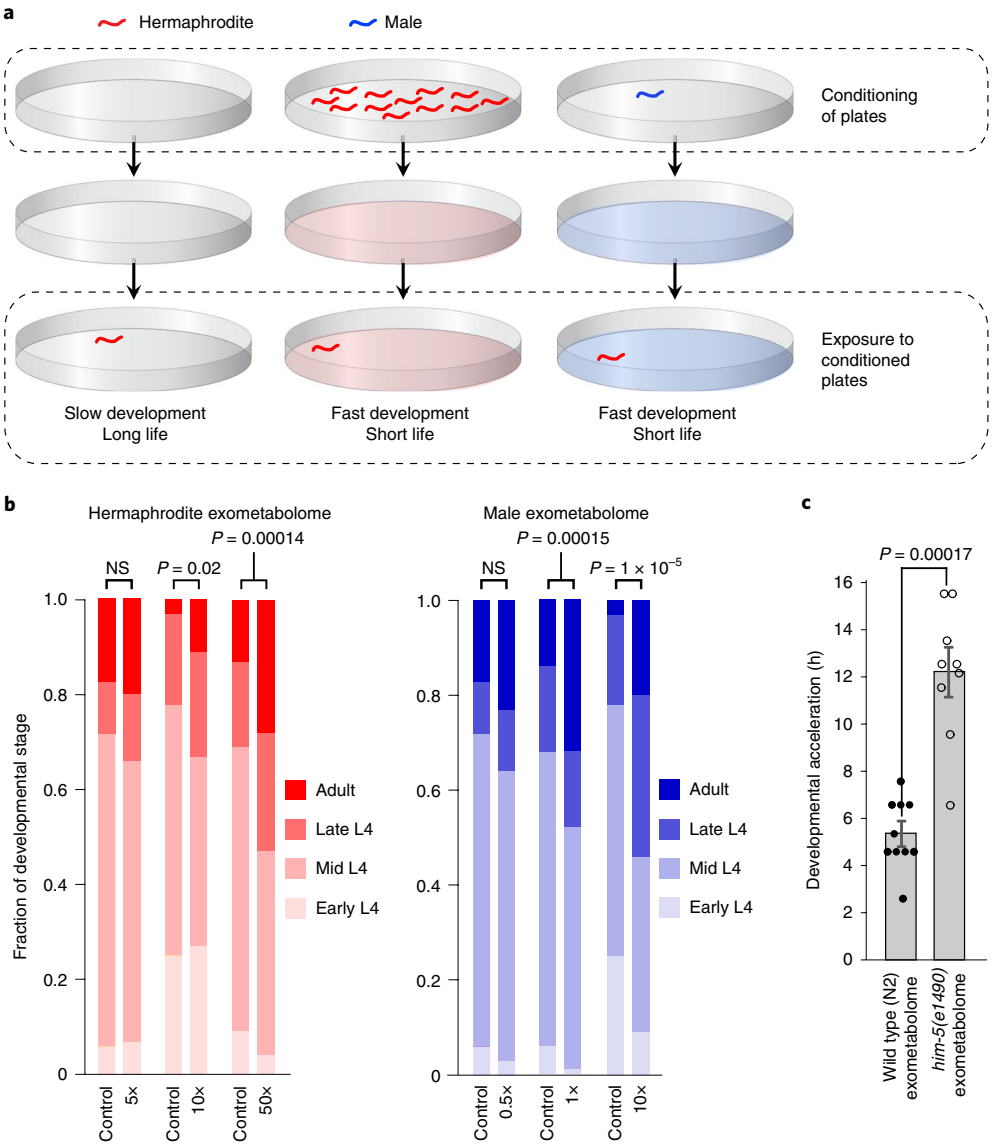

Fig. 1 | Excreted small molecules accelerate development in *C. elegans*. **a**, Schematic of preconditioning experiments: worms on plates conditioned by single males or groups of hermaphrodites develop faster and have a shorter lifespan than worms raised on non-conditioned plates[4,5,11]. **b**, Developmental progression of N2 hermaphrodites grown on plates conditioned with exometabolomes of N2 hermaphrodites or males ($n = 5$ biologically independent samples, where each replicate was one plate with ~30 animals; also see Supplementary Dataset). **c**, Developmental acceleration, measured by the onset of egg laying, in *daf-22* hermaphrodites exposed to wild-type (N2; >99% hermaphrodites) and *him-5* (~30% males) exometabolome ($n = 10$ worms for N2 and $n = 9$ worms for *him-5*). For underlying data, see Supplementary Table 2. Data are presented as mean ± s.e.m. and testing for significance was performed using a χ^2 test or two-tailed *t* test. NS, not significant.

results suggest that developmental acceleration and lifespan shortening are likely caused by a metabolite that is excreted in higher quantities by males than by hermaphrodites and does not belong to known families of *C. elegans* pheromones.

Identifying nacq#1 via comparative metabolomics. To determine the chemical identity of the acceleration signal, we pursued a two-pronged approach, combining comparative metabolomics with activity-guided fractionation. To identify metabolites that are excreted in greater amounts by males, we compared the exometabolomes from wild-type cultures and *him-5* cultures (Fig. 2a). High-resolution HPLC–MS using the XCMS platform[15,16] revealed more than 20 compounds that were at least threefold enriched in *him-5* cultures relative to wild-type cultures and thus represented plausible candidates for the acceleration signal (Supplementary Table 1). We then fractionated the *him-5* metabolome, using the onset of egg laying to measure developmental acceleration[4] (Supplementary Fig. 2). For this assay, we used *daf-22* worms, because this mutant does not produce ascaroside pheromones[13], which may antagonize the activity of the acceleration signal[4]. The activity-guided fractionation revealed three active fractions, which were analyzed for the presence of metabolites enriched in *him-5* cultures. The two most abundant *him-5*-enriched compounds were detected in the most robustly active fraction, fraction 13, but not in adjacent inactive fractions, fraction 12 and fraction 14 (Fig. 2b), and thus were selected as the most likely candidates for the acceleration signal. Tandem MS analysis suggested that these compounds represent two isomeric fatty acylated amino acid derivatives that do not correspond to any previously reported metabolites (Fig. 2b and Supplementary Fig. 3). Following isolation of the compounds via preparative HPLC, we

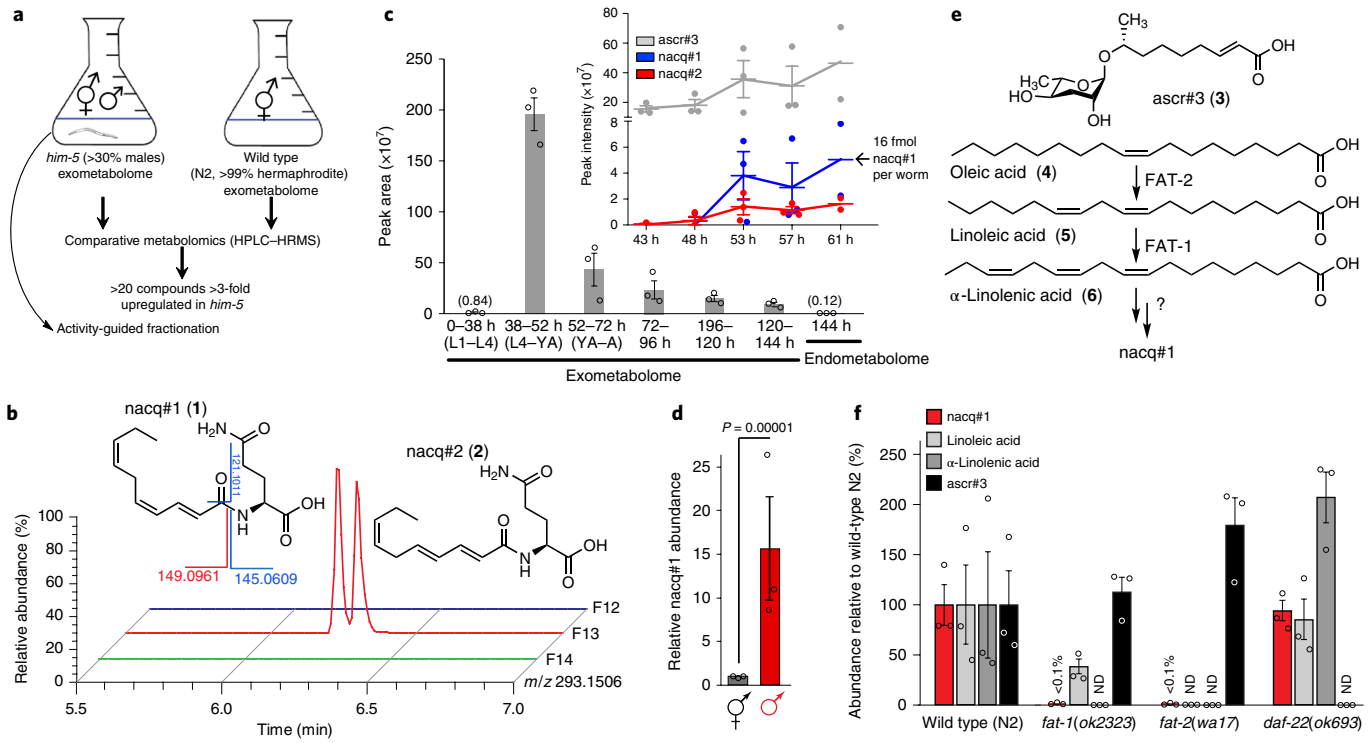

Fig. 2 | Identification of nacq#1, a signaling molecule primarily produced by males. a, Experimental setup for comparative metabolomics with male-enriched *him-5* and wild-type N2 worm cultures. **b**, Ion chromatograms comparing HPLC fractions 12, 13 and 14 for *m/z* 293.1506 revealing the presence of nacq#1 and nacq#2 in fraction 13 (this fractionation was performed once). **c**, HPLC–MS-based quantification of nacq#1 in exometabolomes collected at different time intervals, corresponding to different developmental stages. The insert shows data for cumulative excretion of nacq#1, nacq#2 and ascr#3 up to specific time points during the transition from L4 to young adult (YA). See the Methods for details ($n = 3$ biologically independent samples per condition, except for the data point at 61 h, $n = 2$). **d**, Relative amounts of nacq#1 excreted by hermaphrodites and males ($n = 3$ biologically independent samples). **e**, Chemical structures of the compounds in **f** and enzymatic roles of FAT-1 and FAT-2. **f**, HPLC–MS-based quantification of nacq#1, linoleic acid, α-linolenic acid and ascr#3 in the exometabolomes of wild-type (N2), *fat-1*(*ok2323*), *fat-2*(*wa17*) and *daf-22*(*ok693*) worms ($n = 3$ biologically independent samples). ND, not detected. Data are presented as mean ± s.e.m.

determined their structures via NMR spectroscopic analysis, which revealed *cis* and *trans* isomers of a triply unsaturated ten-carbon fatty acid attached to the amino acid glutamine. The absolute configuration of the glutamine moiety was determined using Marfey's reagent (Supplementary Fig. 4). We named the *cis* and *trans* isomers nacq#1 (*N*-acyl glutamine #1) and nacq#2 (**2**), respectively (Fig. 2b). nacq#1 isomerizes into nacq#2 under physiological conditions, suggesting that nacq#1 is the primary biosynthetic product.

HPLC–MS analysis of endometabolomes (worm body extracts) and exometabolomes showed that nacq#1 production commences around the time of the transition from L4 larva to young adult, approximately corresponding to sexual maturity, and declines shortly thereafter (Fig. 2c and Supplementary Fig. 5a,b). Moreover, similarly to dauer pheromones such as ascr#3 (**3**), nacq#1 was primarily found in the exometabolome, as would be expected for an interorganismal signaling molecule (Supplementary Fig. 5a). In line with the estimate from functional assays of total exometabolomes (Fig. 1b), we found that males excreted approximately tenfold more nacq#1 than hermaphrodites (Fig. 2d), and that this compound was about threefold more abundant in male-enriched *him-5* mutant cultures (>30% male) than in wild-type cultures (Supplementary Fig. 5c).

Given that nacq#1 incorporates an omega-3 polyunsaturated fatty acid, we asked whether its biosynthesis requires the fatty acid desaturases FAT-2 and FAT-1, which are required for the introduction of omega-6 and omega-3 double bonds in *C. elegans*, respectively (for example, in the conversion of oleic acid (**4**) to linoleic acid (**5**) and α-linolenic acid (**6**) (Fig. 2e)). nacq#1 production was largely abolished in *fat-1* and *fat-2* mutant worms (Fig. 2f), indicating that nacq#1 biosynthesis proceeds via longer-chained polyunsaturated fatty acids. Furthermore, consistent with the observation that developmental acceleration by male- and hermaphrodite-derived exometabolomes is not dependent on the ascaroside biosynthetic enzyme DAF-22 (Supplementary Fig. 1 and refs. [4,5]), nacq#1 biosynthesis was not affected by loss of *daf-22* in the wild-type (Fig. 2f) or *him-5* mutant background (Supplementary Fig. 5c). We confirmed that biosynthesis of ascaroside pheromones, for example the dauer pheromone component ascr#3, was abolished in *daf-22* mutants (Fig. 2f). Furthermore, we found that nacq#1 biosynthesis was not strongly affected by nutritional conditions (Supplementary Fig. 6).

nacq#1 accelerates development and shortens lifespan. To explore the biological properties of nacq#1, we developed a short synthesis that provided access to pure nacq#1 and related compounds (Fig. 3a). The triply unsaturated fatty acid chain in nacq#1 was derived from (3*Z*)-hexenol (**7**) and aldehyde (**8**), which after conversion of **7** to phosphonium salt (**9**), Wittig reaction and hydrolysis yielded (2*E*,4*Z*,7*Z*)-decatrienoic acid (**10**). Acid **10** was then coupled to L-glutamine *t*-butyl ester followed by acidic deprotection to furnish synthetic nacq#1 that was used for all subsequent biological experiments. First, we confirmed that synthetic nacq#1 at picomolar to low nanomolar concentrations accelerated reproductive development, by measuring the onset of egg laying (Fig. 3b and Supplementary Fig. 7a) and the age at which adult morphology was attained (Fig. 3c and Supplementary Fig. 7b,c). Higher concentrations of nacq#1 were less effective, resulting in a bell-shaped dose–response curve, as is the case for responses to other small-molecule signals in

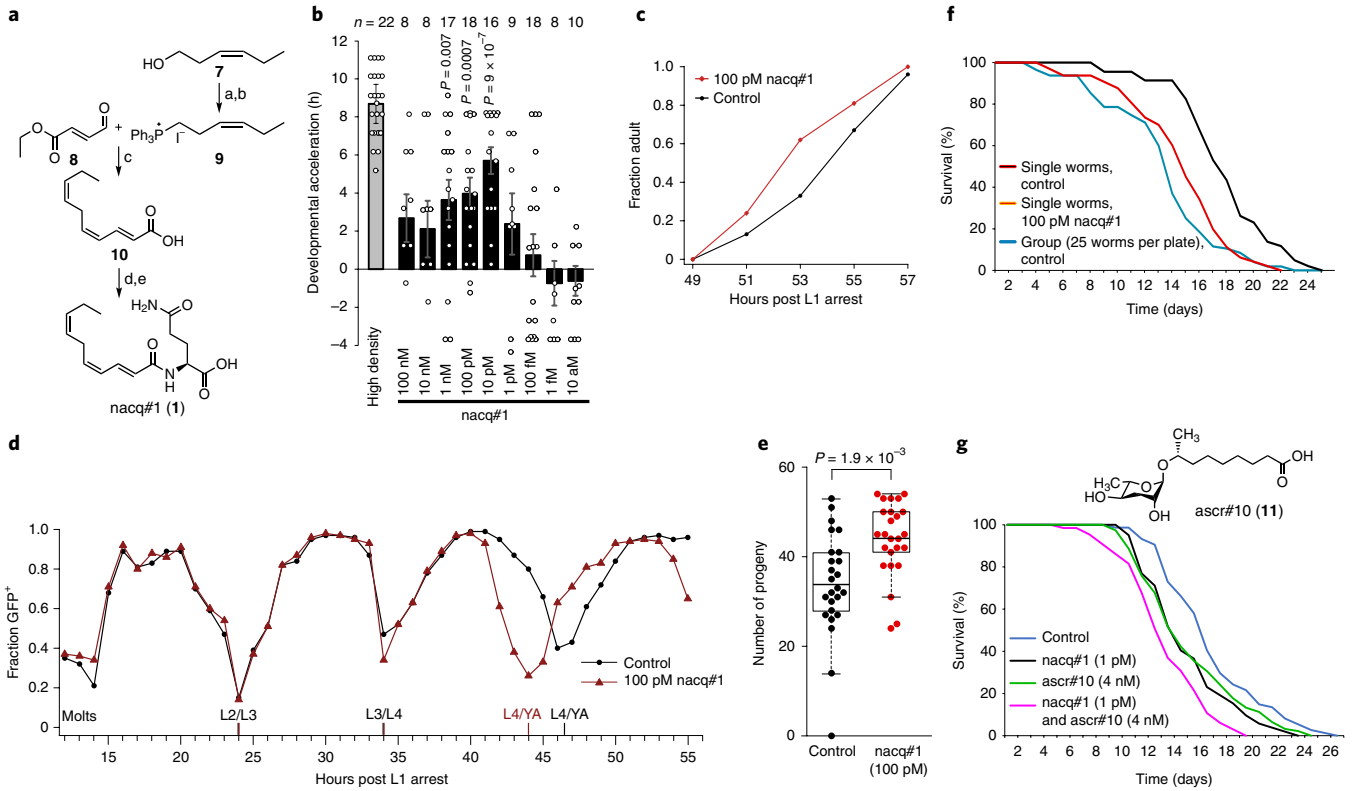

Fig. 3 | Biological properties of nacq#1. a, Synthesis of nacq#1 and related molecules. **b**, Developmental acceleration assays measuring the onset of egg laying in *daf-22* worms exposed to nacq#1 (numbers of worms tested for each condition as indicated; Supplementary Table 5). **c**, Singled wild-type hermaphrodites reach adulthood faster on plates conditioned with nacq#1 than animals on control plates ($n = 25$ (control) and $n = 21$ (nacq#1) worms per time point). Also see Supplementary Fig. 7c. **d**, Fractions of GFP$^+$ *mlt-10*::*GFP* animals developing on plates containing 100 pM nacq#1 as compared to controls ($n = 99$ worms, 13–24 and 36–47 h (control); $n = 98$ worms, 25–35, 48–59 h (control); $n = 101$ worms, 13–24, 36–47 h (nacq#1 treated); and $n = 95$ worms, 25–35, 48–59 h (nacq#1 treated)). Boundaries between larval stages are designated at GFP expression minima. **e**, Progeny production during the first day of reproduction. Each dot represents the offspring of one parent ($n = 25$ hermaphrodite parents). **f**, Survival of groups of hermaphrodites (25 worms per plate; blue) and singled hermaphrodites (1 worm per plate) in the presence (red) or absence (black) of nacq#1. Assays were repeated three times for grouped worms and six times for singled worms. See Supplementary Table 6 for the total number of worms. **g**, Survival of singled hermaphrodites on plates containing nacq#1, ascr#10 or both compounds. Assays were repeated five times. Also see Supplementary Fig. 10 and Supplementary Tables 6–9. Data are presented as mean ± s.e.m. and statistical analysis was performed using two-tailed *t* tests.

C. elegans[17,18] and other species[19]. Hermaphrodites produced on the order of 16 femtomoles per individual of nacq#1 during the transition from L4 to young adult, corresponding to accumulation of picomolar concentrations of nacq#1 on conditioned plates (Fig. 2c), which is consistent with the range of active concentrations observed for synthetic nacq#1. In contrast, neither nacq#2 nor the free fatty acid (nacq#1 without glutamine) was active at any of the tested concentrations (Supplementary Fig. 8). Similarly, the most abundant male-enriched ascaroside, ascr#10 (**11**) (ref. [20]), did not by itself accelerate larval development in hermaphrodites, nor did it synergize with nacq#1 (Supplementary Fig. 9). Male-excreted compounds shorten the last larval stage (L4) (ref. [5]), and we found that synthetic nacq#1 also specifically accelerated this stage of development (Fig. 3d). Given the relatively modest extent of acceleration—approximately 2–3 h or approximately 3–5% of the total egg-to-egg developmental time—we tested whether there was any impact on the onset of reproduction. Hermaphrodites raised in the presence of nacq#1 on average produced roughly 30% more offspring during the first day of egg laying (Fig. 3e), which is a notable advantage for a species with a fast boom-and-bust life cycle[21].

Next, we asked whether nacq#1 could also explain the observation that the presence of males[10–12] and, under some conditions, high population density[4] shorten the lifespan of hermaphrodites. Previous studies had shown that the lifespan-shortening effect of the male exometabolome is at least partly dependent on the ascaroside biosynthetic enzyme DAF-22 (refs. [11,12]), implicating as one factor the ascaroside ascr#10, which is excreted at much higher levels by males than by hermaphrodites[20]. To test whether nacq#1 affects hermaphrodite lifespan, we used a single-worm assay that largely prevented the potentially confounding exposure of test animals to excreted metabolites from other worms. We found that exposure to picomolar concentrations of nacq#1 significantly reduced lifespan from 16.3 d to 13.6 d (Fig. 3f), similarly to the lifespan shortening observed for hermaphrodites exposed to male exometabolome[11]. In contrast, nacq#1 had little effect in lifespan assays conducted at higher population density.

Because males produce much more nacq#1 than hermaphrodites (Fig. 2d), our results suggest that individual males and hermaphrodites at high population density produce sufficient quantities of nacq#1 (and possibly additional substances) to shorten hermaphrodite lifespan. However, singled hermaphrodites and groups of hermaphrodites have a similar lifespan in aging assays set up with worms as young adults[4] or L4 larvae[12,22]. Combined with our finding that nacq#1 is excreted primarily during the transition from the L4 larval stage to young adults (Fig. 2c), these results indicate that lifespan is determined not only by population density during adulthood, but also by the social conditions and associated exposure to nacq#1 and other pheromones experienced by larvae between hatching and the onset of adulthood.

Next we assessed the effects of nacq#1 and ascr#10, both of which are produced in larger quantities by males, on hermaphrodite lifespan. We confirmed that ascr#10 shortens hermaphrodite lifespan, and found that the lifespan-shortening effects of ascr#10 and nacq#1 were additive at low concentrations (Fig. 3g and Supplementary Fig. 10), indicating that they may act via partly independent molecular mechanisms. Notably, nacq#1 shortened hermaphrodite lifespan significantly at concentrations as low as 1 pM, to a degree that was similar to the lifespan-shortening effect of ascr#10 at 4 nM. Because nacq#1 is active at lower concentrations than ascr#10, and given that males excrete similar amounts of ascr#10 (ref. [20]) and nacq#1, the effect of nacq#1 on hermaphrodite lifespan may outweigh that of ascr#10 under natural conditions; however, ascarosides[23] are chemically much more stable than nacq#1, and the spatial and temporal deposition of these compounds by males may differ.

Antagonism of nacq#1 and dauer-inducing ascarosides. To gain insight into the mechanisms underlying the effects of nacq#1 on *C. elegans* development and lifespan, we investigated the interaction of nacq#1 with a subset of ascaroside pheromones, which are known to divert development into the dauer diapause and extend lifespan, including ascr#2 (**12**) and ascr#3 (refs. [12,24–26]) (Fig. 4a). Dauer larvae are adapted to long-term survival under adverse environmental conditions and have been used extensively as a model to study conserved mechanisms of developmental plasticity and aging[27–29]. When encountering conditions conducive to growth and reproduction, dauer larvae resume development into reproductive adults. Our results suggest that nacq#1 and dauer-inducing ascarosides have generally opposing effects on reproductive development. First, nacq#1 hastens the onset of reproduction (Fig. 3b), whereas ascaroside pheromones promote developmental arrest. Second, nacq#1 and high population density[4] (Fig. 3b and Supplementary Fig. 1) accelerate development more strongly in ascaroside-deficient *daf-22* mutants than in wild-type worms. Addition of dauer-inducing ascarosides reduced developmental acceleration by nacq#1, confirming that these ascarosides antagonize the effects of nacq#1 (Fig. 4b).

Whereas ascarosides are abundantly produced by larvae and adult worms[30], nacq#1 production was transient, peaking around sexual maturation (Fig. 2c), suggesting that it may serve as a signal of conditions that support reproductive development and promote exit from the dauer stage. To test this idea, we used *daf-2*/insulin receptor and *daf-7*/TGF-β mutants, which constitutively form dauer larvae at non-permissive temperatures[28,31]. nacq#1 promoted dauer exit in *daf-7*(*m62*) and *daf-2*(*e1368*) mutants (Fig. 4c,d) and also counteracted the suppression of dauer exit by ascarosides in wild-type animals (Fig. 4e). In contrast, nacq#1 did not significantly affect ascaroside-triggered dauer entry (Supplementary Fig. 11), indicating that it primarily serves as a dauer-exit pheromone. These results show that nacq#1 and ascarosides exert mutually opposing effects on development.

nacq#1 requires NHR-dependent and chemosensory pathways. Ascarosides trigger dauer development primarily via downregulation of insulin and TGF-β signaling, which abolishes the biosynthesis of steroidal ligands of DAF-12, a homolog of mammalian steroid hormone receptors[31–33]. Unbound DAF-12 then binds to its co-repressor DIN-1/SHARP, causing developmental arrest at the dauer stage[34]. We asked whether acceleration of development by nacq#1 depends on insulin and TGF-β signaling and tested mutants of the FOXO transcription factor DAF-16, the target of the DAF-2 receptor, and a TGF-β ligand, DAF-7. We found that developmental acceleration was abolished with both *daf-16* mutant alleles, whereas *daf-7* mutants behaved similarly to the wild type (N2) (Fig. 5a). Next, we tested mutants of DAF-12 and its two paralogs, NHR-8 and NHR-48. Whereas NHR-8 has been shown to regulate steroid hormone biosynthesis and thus link steroid hormone signaling and lifespan[35,36], little is known about the role of NHR-48 in development. We found that developmental acceleration by nacq#1 was abolished in null mutants of all three NHRs (Fig. 5b and Supplementary Fig. 12a,b). To remove possible confounding interference from ascaroside pheromones, we also tested double-mutant combinations of *daf-22* with

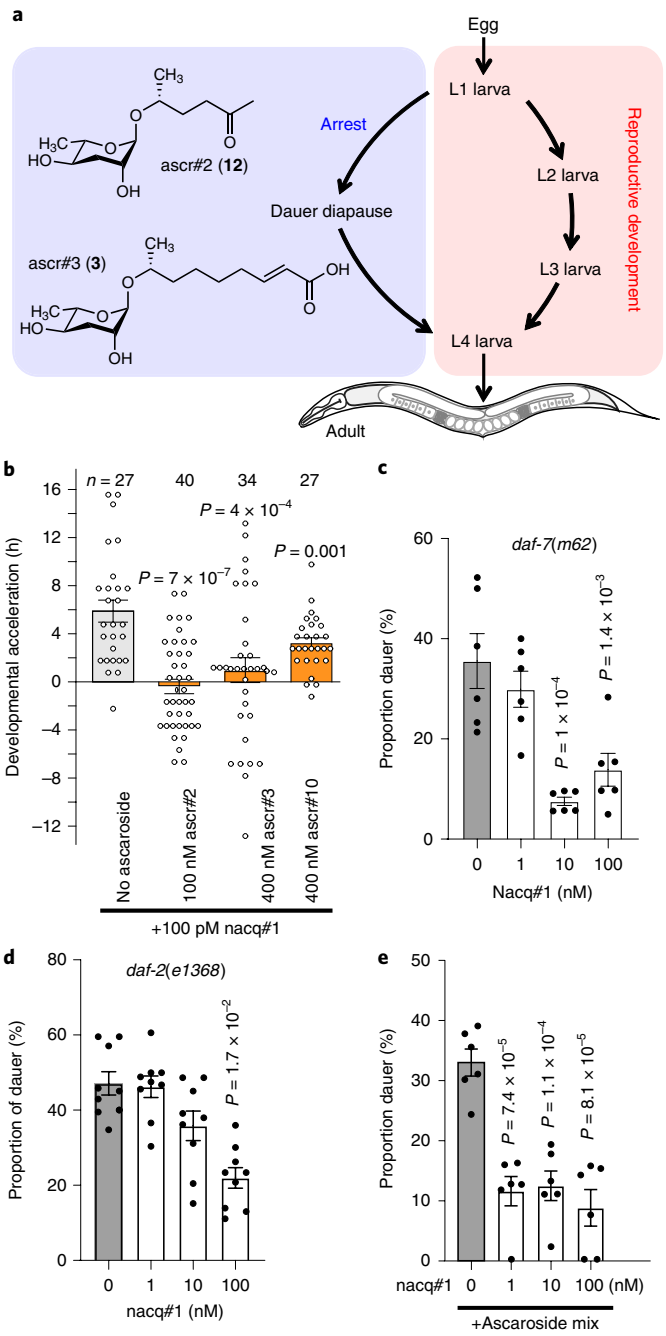

Fig. 4 | nacq#1 and ascarosides are mutually antagonistic signals. **a**, Ascarosides, for example ascr#2 and ascr#3, induce dauer diapause, a stress-resistant alternate larval stage. **b**, Ascarosides counteract the developmental acceleration of *daf-22* mutants treated with nacq#1 (numbers of worms tested for each condition are indicated). See also Supplementary Table 11. **c**,**d**, nacq#1 promotes exit from the dauer stage in *daf-7* (**c**) and *daf-2* (**d**) mutants ($n = 6$ (*daf-7*) and $n = 9$ (*daf-2*) biologically independent samples). **e**, nacq#1 counteracts the effects of ascarosides in dauer exit ($n = 6$ biologically independent samples). For dauer data, see Supplementary Table 12. Data are presented as mean ± s.e.m. and statistical analysis was performed using two-tailed *t* tests.

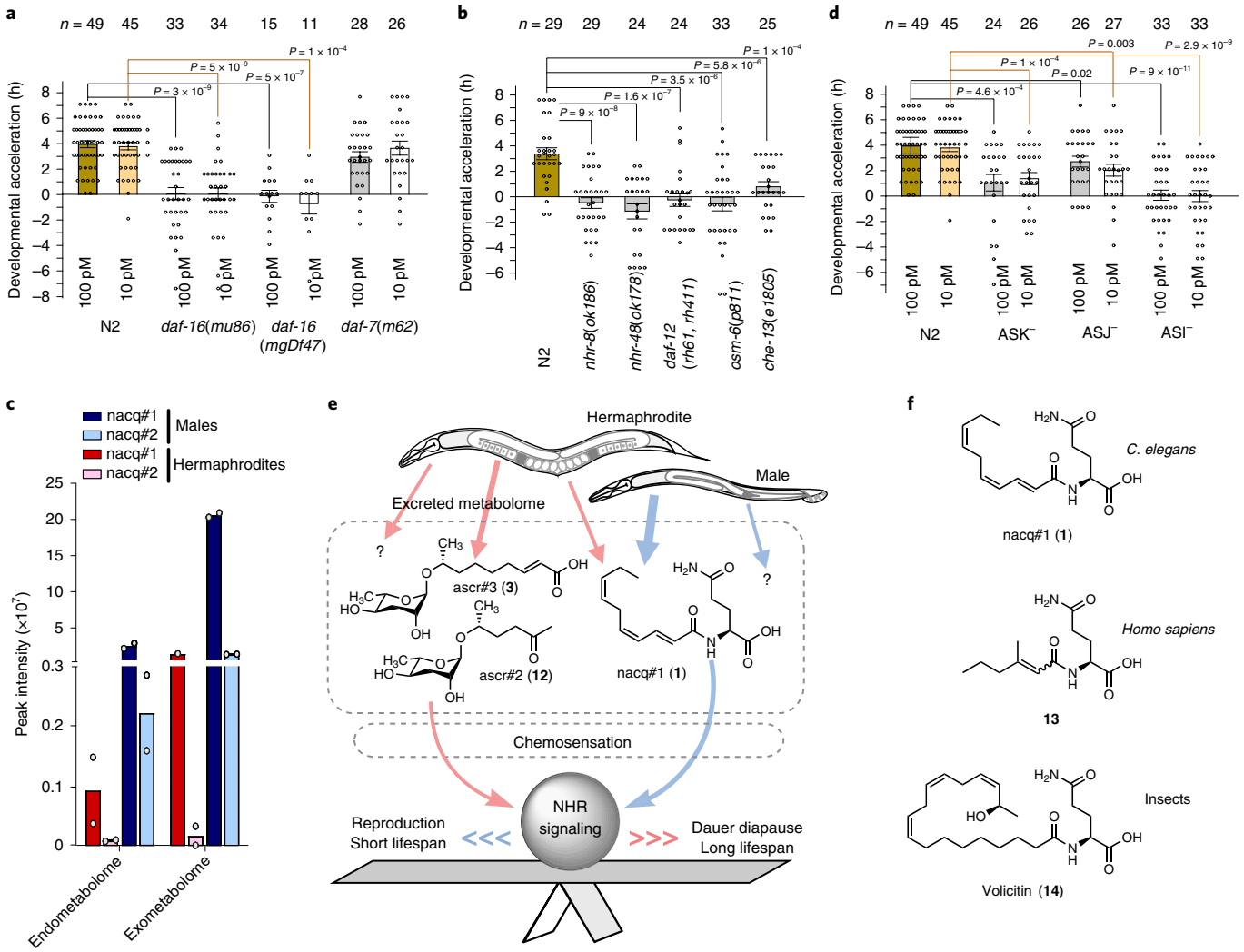

Fig. 5 | nacq#1 signals via conserved signaling pathways. a, Acceleration of development by the indicated concentrations of nacq#1 in wild-type individuals and *daf-16* and *daf-7* mutants (numbers of worms tested for each condition are indicated). **b**, Acceleration of development by nacq#1 (100 pM) in wild-type individuals and *nhr-8*, *nhr-48*, *daf-12*, *osm-6* and *che-13* mutants (numbers of worms tested for each condition are indicated). **c**, HPLC–MS-based quantification of nacq#1 in the exo- and endometabolomes of *C. briggsae*, collected after the worms reached the young adult stage ($n = 2$ biologically independent samples). **d**, Acceleration of development at the indicated concentrations of nacq#1 in the wild type (N2) and after genetic ablation of ASI, ASK or ASJ chemosensory neurons (numbers of worms tested for each condition are indicated). **e**, Competing small-molecule signals regulate development via NHR signaling. **f**, Chemical structures of *N*-acyl amino acids similar to nacq#1 identified from other phyla. See Supplementary Tables 13–15 for data presented in **a**, **b** and **d**. Data are presented as mean ± s.e.m. and statistical analysis was performed using two-tailed *t* tests.

nhr-8, *nhr-48* and *daf-12*, which confirmed that these NHRs are required for developmental acceleration by nacq#1 (Supplementary Fig. 12a). Consistent with a model in which nacq#1 engages conserved development pathways, we detected this compound (and nacq#2) in *Caenorhabditis briggsae* (Fig. 5c) and other *C. elegans* relatives (Supplementary Fig. 13).

As an interorganismal signaling molecule, nacq#1 could serve as a ligand for one of the required NHRs or, alternatively, could be perceived via chemosensation. Chemosensory neurons regulate entry into and exit from dauer diapause[37,38] and modulate lifespan[39,40], and have been implicated in the lifespan-shortening effects of male excretions[11]. Whereas cell culture luciferase assays showed no evidence of activation of NHR-8 and NHR-48 by nacq#1 (Supplementary Fig. 14), developmental acceleration by nacq#1 was abolished or strongly reduced in *osm-6* and *che-13* mutants, which are defective in processing of chemosensory signals (Fig. 5b and Supplementary Fig. 12c,d). In addition, developmental acceleration by nacq#1 is abolished or reduced in worms lacking chemosensory neurons implicated in dauer regulation[38,41] (abolished after ASI ablation and reduced after ablation of ASK and ASJ; Fig. 5d). Although the exact mechanism of nacq#1 perception remains to be elucidated, our results indicate that nacq#1 is perceived via chemosensory circuits that trigger downstream signaling pathways targeting conserved steroid hormone receptors.

Discussion

Males and, to a lesser extent, hermaphrodites excrete a small-molecule signal, nacq#1, that antagonizes diapause and accelerates development, hastening sexual maturation, but at the expense of a reduced lifespan (Fig. 5e). Because of the associated costs, reproductive commitment may be singularly attuned to signals from potential mates and competitors. In natural habitats, *C. elegans* are often found as developmentally arrested, dispersing dauer larvae[9]. A small molecule excreted upon reaching sexual maturity, nacq#1, may indicate that sufficient resources are available to exit the dauer stage and resume reproductive development. nacq#1 further

accelerates sexual maturation in larvae that are already developing into adults. This latter role of nacq#1, predominantly produced by males, is reminiscent of the signal produced by sexually mature male mice that induces earlier onset of the first estrus in peripubescent females[3]. These parallels suggest that signals excreted primarily by males modulate similar developmental processes in divergent animal lineages.

Downstream of chemosensation, nacq#1 regulates development via transcription factors of the NR1 subfamily[42], which includes steroid hormone receptors such as the vitamin D receptor and liver X receptor that coordinate vertebrate development[43]. Our results suggest that the antagonism between nacq#1 and dauer-inducing ascarosides may result from differential regulation of the three NR1 family members in *C. elegans*, as the effects of these ascarosides on development primarily depend on DAF-12, whereas acceleration of development by nacq#1 also strictly requires NHR-8 and NHR-48 (Fig. 5b and Supplementary Fig. 12a,b). Correspondingly, dauer-inducing ascarosides and the dauer-exit-promoting nacq#1 have opposing effects on lifespan: whereas dauer-inducing ascarosides extend lifespan[26], nacq#1 reduces longevity (Fig. 3f), consistent with a tradeoff between reproductive investment and somatic maintenance[44,45]. Two additional points deserve to be mentioned. First, whereas some ascarosides extend lifespan, others such as ascr#10 shorten it, likely via mechanisms that are at least somewhat independent from those that mediate the activity of nacq#1. Second, although the nacq#1-induced shortening of the last larval stage by 2–3 h could represent the beginning of a faster rate of aging that ultimately leads to a shortened lifespan, these two processes may also be mechanistically decoupled. Overall, our findings present a picture of a pull–push system of small-molecule signals that fine-tune development and longevity in response to changing social environments by modulating conserved gene regulatory networks (Fig. 5e).

The chemical structure of nacq#1 suggests parsimonious use of simple building blocks from primary metabolism—the amino acid glutamine and an unusual triply unsaturated ten-carbon fatty acid that could be derived from canonical omega-3 fatty acids, for example eicosapentaenoic or linolenic acid. Notably, unusual fatty acylated glutamines have been reported from multiple animal phyla. For example, a compound featuring an uncommon branched acyl moiety, N-3-methyl-2-hexenoylglutamine (**13**), occurs in human sweat[46] (Fig. 5f). It is unclear whether these human metabolites serve a biological function, as may be suggested by the otherwise uncommon fatty acid moieties. In addition, amino acid derivatives of long-chain fatty acids have been implicated in intra- and interorganismal signaling in vertebrates[47,48] and invertebrates[49,50], for example volicitin (**14**). Our discovery of nacq#1 as a regulator of development and lifespan in *C. elegans* suggests that related small molecules may serve signaling functions throughout metazoa.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41589-019-0321-7.

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Author contributions

I.R. and F.C.S. supervised the study. A.H.L., A.B.A., I.R. and F.C.S. designed experiments. A.H.L., A.B.A., E.Z.A., P.R.R., D.C.P., R.N.B., P.G. and O.P. performed chemical and biological experiments. R.N.B. and Y.K.Z. performed syntheses. I.R. and F.C.S. wrote the paper with input from the other authors.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Nematode and bacterial strains. Unless indicated otherwise, worms were maintained on nematode growth medium (NGM) 6-cm Petri dish plates with *E. coli* OP50 (http://www.wormbook.org/methods)[51]. The following *C. elegans* strains were used: wild-type Bristol N2, FCS1 *daf-22*(*ok693*), DR476 *daf-22*(*m130*), AA86 *daf-12*(*rh61*, *rh411*), PR811 *osm-6*(*p811*), SP1734 *osm-6*(*m511*), CB3323 *che-13*(*e1805*), AE501 *nhr-8*(*ok186*), *nhr-8*(*ok186*); *daf-12*(*rh61*, *rh411*), AA107 *nhr-48*(*ok178*), DR466 *him-5*(*e1490*), CB467 *him-5*(*e1467*), CF1038 *daf-16*(*mu86*), GR1352 *daf-16*(*mgDf47*), GR1395 *mgIs49*[*mlt-10*::*GFP-pest*;*ttx-1*::*GFP*], DR62 *daf-7*(*m62*) and DR1572 *daf-2*(*e1368*), RB1795 *fat-1*(*ok2323*), BX26 *fat-2*(*wa17*), ASI$^-$ PY7505 *oyIs84*[*Pgpa-4*::*ced-3*(*p17*), *Pgcy-27*::*ced-3*(*p15*), *Pgcy-27*::*gfp*, *Punc-122*::*dsRed*], ASK$^-$ PS6025 *qrIs2*[*sra-9*::*mCasp1*], ASJ$^-$ ZD762 *mgIs40*[*daf-28p*::*nls-GFP*]; *jxEx100*[*trx-1*::*ICE+ofm-1*::*gfp*], FCS23 [*nhr-48*(*ok178*); *daf-22*(*ok693*)], FCS24 [*nhr-8*(*ok186*);*daf-22*(*ok693*)] and FCS30 [*daf-12*(*rh61*, *rh411*); *daf-22*)*ok693*)] were generated by crossing FCS1 *daf-22*(*ok693*) males with AE501 *nhr-8*(*ok186*), AA107 *nhr-48*(*ok178*) or AA86 *daf-12*(*rh61*, *rh411*) hermaphrodites, respectively. Genotypes were confirmed using PCR. In addition, we used AF16 *C. briggsae*, NKZ2 *Caenorhabditis inopinata* and CB5161 *Caenorhabditis brenneri*.

Time-of-development assays based on morphological criteria. Two different protocols were used to measure time of development on the basis of morphological criteria[5]. In the first experimental setup (shown in Fig. 1b and Supplementary Figs. 5b, 7b and 12b,c), small populations of worms were tested. Synchronized cultures of L1 larvae were prepared by hypochlorite treatment of gravid hermaphrodites[52]. After treatment, the liberated eggs were allowed to hatch in M9 buffer overnight. The following morning, the resulting population of arrested L1 larvae was deposited onto the prepared (for example, metabolome-conditioned) plates at a density of 20–40 larvae per plate. After 48 h at 20 °C, the plates were placed at 4 °C until examination under a dissecting microscope.

In the second protocol (shown in Fig. 3c and Supplementary Figs. 7c and 9), L1 larvae were singled to either control or conditioned plates just after release from arrest as previously described[5]. These hermaphrodites were monitored every 2 h starting at 49 h after release from arrest until they had reached adulthood. The numbers of their progeny were counted 72 h after release from L1 arrest (as shown in Fig. 3e). Developmental staging was based on vulval and gonadal morphology[53]. All assays were repeated at least three times, unless noted otherwise. For source data, see the Supplementary Dataset.

To condition plates with exometabolome preparations (Fig. 1b), mating plates of males and hermaphrodites were pooled, and the resulting population was subjected to hypochlorite treatment to produce a synchronized mixed-sex culture. Separate age-matched populations of males and hermaphrodites were segregated at the L4 stage. The following day, worm exometabolome preparations were collected using a previously described method[20] with some modification. One hundred worms, either males or hermaphrodites, were picked into 100 µl of water in 200-µl PCR tubes. Worms were incubated for 24 h at 21 °C with shaking at 220 r.p.m. The liquid was filtered over sterile cotton to exclude the worms. The exometabolome was applied to NGM plates in 20-µl drops including 5 µl of OP50, which overlaid and dried each drop, until the desired amount of exometabolome was reached. Eggs or L1 larvae that were not filtered out by the cotton were manually removed. Plates were refrigerated until use. For data shown in Fig. 1b, see the Supplementary Dataset.

To condition plates with live animals, a 2-h timed egg lay was performed with mated hermaphrodites. L3 plates were conditioned by ten worms that were between the ages of 38 and 47 h post egg lay (just before the L2/L3 molt until just before the L3/L4 molt). When the larvae were added to the plates, it was not easy to determine their sex. Therefore, the larvae were kept after removal from the plates and their sex was determined. Every plate had at least four of ten larvae that eventually developed into males. L4 plates were conditioned by ten worms that were between the ages of 48 and 58 h post egg lay (just after the L3/L4 molt and just before the L4/adult molt). At this stage of development, it was possible to determine the sex of the larvae and each plate was conditioned with five L4 males and five L4 hermaphrodites. For data shown in Supplementary Fig. 5b, see the Supplementary Dataset.

To condition with nacq#1, nacq#1 was added to OP50 overnight culture to a final volume of 20 µl and spotted on the plate to achieve the indicated concentrations. For data shown in Fig. 3c and Supplementary Figs. 7b and 12b,c, see the Supplementary Dataset. To condition with ascr#10, 2.2 pg of ascr#10 (the amount known to induce behavioral and germline effects) was spread evenly over the surface of the agar plate with a glass rod. The plates were kept at 20 °C overnight to allow the ascaroside solution to absorb into the agar and seeded with a 5-µl spot of a 1:10 dilution of OP50 overnight culture.

Generation of *mlt-10* molting curves. The data for the molting curves were generated using the protocol described previously[54] and relied on monitoring the level of GFP in *mlt-10*::*GFP* animals[55]. Two experiments were started 12 h apart, allowing observations of GFP expression throughout the four larval stages. In each experiment, a population of *mlt-10*::*GFP* animals was synchronized by hypochlorite bleaching as described above. Approximately 30 L1 larvae from this population were transferred to either control plates seeded with 20 µl OP50 or treatment plates, which were seeded with 20 µl OP50 containing 3 pg of nacq#1. Each of the two experiments consisted of three control (~90 animals) and three treatment (~90 animals) plates. Animals were examined every hour and scored for GFP fluorescence on a Leica MZ16F stereomicroscope. For data shown in Fig. 3d, see the Supplementary Dataset.

Developmental acceleration assays with exometabolome samples and activity-guided fractionation. Developmental acceleration assays, measuring the time point of the first egg lay[4], were performed using *daf-22*(*ok693*) hermaphrodites. Exometabolome samples from N2, *him-5*(*e1490*) and *him-5*(*e1467*) worms, prepared as described[13], were assayed undiluted or diluted 1:10, 1:100 and 1:1,000 in 100% methanol. One hundred microliters of fraction, dilution or pure methanol (as a control) was then spread on 3.5-cm NGM plates, and plates were dried for 30 min, seeded with 35 µl OP50 *E. coli* bacteria and incubated at 20 °C overnight. These plates were then used in acceleration assays, measuring the time point of first egg laying, as described[4]. Developmental acceleration (in hours) was calculated as the time difference between the time of the first egg lay on untreated ISO plates (plates with a single worm) minus the time of the first egg lay on conditioned ISO (or high-density (HD)) plates. Assays were repeated at least three times, with 10–15 ISO plates used for assaying each metabolome sample.

To identify compounds that accelerated development, *him-5*(*e1490*) exometabolome samples were fractionated by preparative reverse-phase HPLC using an Agilent Zorbax Eclipse XDB-C8 column (9.4 × 250 mm, 5 µm particle diameter) and 0.1% aqueous formic acid–acetonitrile solvent gradient at a flow rate of 3.6 ml min^{-1}. Fractions were evaporated to dryness in vacuo and resuspended in 2 ml of 2% ethanol–water solution. One hundred microliters of each fraction (and 2% ethanol–water control) was spread on 3.5-cm plates, dried for 30 min, seeded with 35 µl OP50 *E. coli* bacteria and incubated for 24 h. Plates were tested for acceleration of development of isolated (one worm per plate) *daf-22*(*ok693*) hermaphrodites, as described above. Assays were repeated three times, using 5–15 ISO plates for each fraction. For data shown in Fig. 1c and Supplementary Fig. 2, see Supplementary Tables 2 and 4, respectively.

Time-of-development assays based on first egg lay. For synchronization, 50–100 worms (N2 or mutants, as specified) were allowed to lay eggs for 1 h on NGM plates seeded with OP50 *E. coli* bacteria, adult worms were removed and the resulting F_1 generation was grown for 72 h at 20 °C. From those plates, 50–70 worms were transferred to a fresh 6-cm NGM plate (100 µl fresh bacteria were spread onto the center of a plate and grown overnight at 23 °C to produce a bacterial lawn of approximately 2 cm in diameter). Worms were allowed to lay eggs for 1–2 h and either single eggs (for ISO plates) or 50–70 eggs (for HD plates) were transferred using a worm pick to fresh 3.5-cm plates containing 4 ml NGM agar, seeded with 35 µl of freshly grown OP50 bacteria. The time point of egg transfer was defined as the start time of the experiment[4]. After 59 h at 20 °C, 10–20 worms from each condition (ISO and HD) were isolated by individual transfer onto fresh 3.5-cm NGM plates seeded with OP50 *E. coli* bacteria. Animals were scored for egg laying beginning at 60 h and then every hour. Animals were scored using a Leica DM 5500B microscope and a Q Imaging 200R camera. All assays were repeated at least three times, unless noted otherwise in the Supplementary Tables. For data shown in Supplementary Fig. 1, see Supplementary Table 3.

High-resolution mass spectrometry. Liquid chromatography was performed on a Dionex 3000 UPLC coupled with a Thermo Q-Exactive high-resolution mass spectrometer. HPLC separation was achieved using an Agilent Zorbax Eclipse XDB-C18 (2.1 × 150 mm 1.8 µm particle size) maintained at 40 °C. Chromatographic conditions started with an isocratic gradient of 5% water with 0.1% formic acid and 95% acetonitrile until 1.5 min and then increasing linearly to achieve 100% acetonitrile at 13.5 min. ascr#3, nacq#1 and nacq#2 were quantified from data in ESI by integrating the peaks of their respective ion-extracted chromatograms (<2 ppm) at the previously standard-verified retention times. Data are shown in Fig. 2b and Supplementary Fig. 3.

Determination of the absolute configuration of nacq#1. Individual samples (100 µl) of 50 µM solutions of nacq#1, L-glutamine and D-glutamine in methanol were evaporated in glass vials in vacuo and hydrolyzed with 200 µl of 6N HCl at 90 °C overnight. The reaction was then evaporated in vacuo and the residue was dissolved in 100 µl of water. In addition, 50 µM stock solutions of the four amino acids L-glutamic acid, D-glutamic acid, L-glutamine and D-glutamine were prepared in water. One hundred microliters of 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (L-FDAA, Marfey's reagent; 1% wt/vol in acetone) and 20 µl of 1M $NaHCO_3$ were added to 50 µl of the hydrolyzed sample of nacq#1 or the amino acid standards. After heating in a water bath at 37 °C for 1 h, reactions were quenched by adding 10 µl of 2N HCl. The reactions of the nacq#1 sample and amino acid standards were subjected to LC–MS analysis without further dilution (Micromass Quattro Ultima mass spectrometer coupled to Agilent 1100 HPLC; column: Agilent Zorbax Eclipse XDB-C18, 4.6 × 250 mm, 5 µm; injection 2 µl) using a linear gradient of water with 0.1% formic acid and acetonitrile (starting at 5% acetonitrile for 5 min, then ramping to 100% acetonitrile over 32 min; 1.0 ml min^{-1} flow rate; detection in the negative ionization mode). Analysis of glutamine and glutamic

acid standards indicated that complete conversion of glutamine to glutamic acid took place during the acid hydrolysis step for both nacq#1 and glutamine. Data are shown in Supplementary Fig. 4.

Isolation and purification of nacq#1 and nacq#2. Medium from *C. elegans* mixed-stage culture (total volume 7 l, total number of worms approximately 3×10^7) was lyophilized and extracted with methanol. Dried methanol extract was loaded onto Celite and fractionated by medium pressure reverse-phase chromatography (86 g C18 Combiflash RediSep column). A 0.1% aqueous acetic acid–acetonitrile solvent gradient was used at a flow rate of 60 ml min^{-1}, starting with an acetonitrile content of 0% for 10 min, which was increased to 100% over a period of 82 min. After assaying fractions by UPLC–MS, the relevant fractions were combined, solvent was removed and the residue was further fractionated by preparative reverse-phase HPLC using an Agilent Zorbax Eclipse XDB-C8 column (9.4×250 mm, 5 µm particle diameter) and 0.1% aqueous formic acid–acetonitrile solvent gradient at a flow rate of 3.6 ml min^{-1}. All fractions were assayed by LC–MS to confirm correct fractionation. Solvent from the fractions containing nacq#1 and nacq#2 was removed in vacuo and the residue was subjected to the second round of preparative HPLC on an ACE 5 C18-AR column (10×250 mm) using the same solvent gradient, which yielded a sample for NMR analysis (CD_3OD, Bruker AVANCE III HD, 800 MHz). Approximately 30 µg of nacq#1 and nacq#2 was isolated from 7 l of *C. elegans* medium. Chemical structures are shown in Fig. 2b.

Relative amounts of nacq#1 in *C. elegans* males and hermaphrodites. Worms were picked into three 1.5-ml HPLC vials containing 100 µl of M9 medium with the following numbers of males and hermaphrodites: (a) 80 N2 L4 males; (b) 80 N2 L4 hermaphrodites and (c) 40 N2 L4 males and 40 N2 L4 hermaphrodites. The vials were then incubated at 22 °C while shaking at 220 r.p.m. At 12 h and 24 h, the vials were centrifuged, and a 25-µl aliquot of the supernatant medium was removed and mixed with an equal volume of methanol to precipitate proteins. This mixture was then centrifuged and 1 µl of the supernatant was analyzed by LC–HRMS. Data are shown in Fig. 2d.

Quantification of ascr#3, nacq#1 and nacq#2 during development. Worms were synchronized by treating gravid adults with alkaline hypochlorite solution and eggs were washed three times in M9. Eggs were then arrested in S-complete overnight to produce arrested L1 larvae. Arrested L1 larvae were seeded at a density of 4×10^5 animals per 300 ml of S-complete solution supplemented with concentrated OP50. Animals were then grown in liquid culture and collected at the following timings: 12 h for L1 larvae, 24 h for L2 larvae, 32 h for L3 larvae, 40 h for mid-L4 larvae and adults were collected at 58 h. Worms were visually inspected before collection to confirm the developmental stage. In a separate experiment, animals were grown for 43 h (mid-L4), 48 h, 53 h, 57 h and 61 h. Pellets and medium were extracted as described, using 10 ml of methanol. Worms were separated from medium by centrifugation and persistent OP50 was pelleted before collection of medium for analysis. After lyophilization, worm pellets were homogenized in methanol using a glass homogenizer. Medium and pellets were then extracted in 20 ml of pure methanol for 16 h with stirring. The methanol extract was separated from insoluble materials by centrifugation, dried in vacuo and resuspended in 250 µl of methanol for LC–MS analysis. Note that, in this experiment, secreted compounds accumulate in the exometabolome from the L1 stage up until the collection time point. Data are shown in the insert of Fig. 2c and Supplementary Fig. 5a.

Estimation of nacq#1 production by hermaphrodites. To quantify the amount of nacq#1 produced by *C. elegans* hermaphrodites, we first generated serial dilutions of synthetic nacq#1 standard in *fat-1* mutant metabolome extract (to account for ion suppression effects) for analysis via LC–MS, using the conditions described above. From these data, a standard curve was generated after integrating the peak representing the nacq#1 molecular ion (293.15068 in ESI), which was used to calculate nacq#1 concentrations in the exometabolome samples obtained at 57 h and 61 h in the time-course experiment described above. This analysis indicated that one hermaphrodite produces about 16 fmol during development through 61 h. Correspondingly, nacq#1 concentrations on a 6-cm plate (assuming a volume of 10 ml) with 20 hermaphrodites would be expected to reach 32 pM during development. Data are shown in Fig. 2c.

Quantification of nacq#1 production during different time windows in adulthood. Synchronized L1 N2 larvae (prepared as above) were seeded at a density of 1×10^6 animals per 33 ml of S-complete solution supplemented with concentrated OP50. Animals were then grown in liquid culture, settled and 25 ml of the culture medium was frozen after 38 h, corresponding to the L4 stage. The worms were then rinsed two times in M9, rinsed one time in S-complete medium and growth was continued in S-complete medium supplemented with concentrated OP50. The process was repeated, and medium was collected after 52 h (at which time the worms were young adults), 72 h, 96 h, 120 h and 144 h for adults. After 144 h, worms were pelleted and rinsed two times in M9 and one time in MilliQ H_2O. After lyophilization, worm pellets were homogenized with a tissue grinder. Medium and pellets were extracted in 13 ml of pure ethanol for 16 h with stirring. The ethanol extracts were separated from insoluble materials by centrifugation, dried in vacuo and resuspended in 80 µl of ethanol for LC–MS analysis as above. Data are shown in Fig. 2c.

Quantification of linoleic acid, α-linolenic acid, nacq#1 and ascr#3 in fat mutants. Synchronized L1 larvae (prepared as above) were seeded at a density of 1×10^6 animals per 33 ml of S-complete solution supplemented with concentrated OP50. Animals were then grown in liquid culture and gravid adults were collected after 60 h. Worms were visually inspected before collection to confirm the developmental stage. Worms were separated from medium by centrifugation and persistent OP50 was pelleted before collection of medium for analysis. After lyophilization, the medium was extracted in 13 ml of pure ethanol for 16 h with stirring. The ethanol extract was separated from insoluble materials by centrifugation, dried in vacuo and resuspended in 160 µl of ethanol for LC–MS analysis as above. Data are shown in Fig. 2f.

Quantification of ascr#3, nacq#1 and nacq#2 in different bacterial dilutions. Worms were synchronized by treating gravid adults with alkaline hypochlorite solution and eggs were washed three times in M9 and allowed to arrest as L1 larvae overnight in M9. Approximately 7,000 animals per plate were then transferred to 10-cm NGM plates with 2.5 ml of OP50 previously diluted in S-basal medium to the indicated concentrations. Final colony-forming units (c.f.u.) were confirmed after the final dilutions were made. The animals were allowed to reach the gravid adult stage, collected by centrifugation and washed with S-basal medium before extraction. After lyophilization, worm pellets were homogenized in methanol using a glass homogenizer. These were then extracted in 10 ml of methanol, and the extract was separated from insoluble material by centrifugation, dried in vacuo and resuspended in 100 µl of methanol for LC–MS analysis as above. Data are shown in Supplementary Fig. 6.

Identification of nacq#1 in *C. briggsae*, *C. brenneri* and *C. inopinata*. For preparation of *C. briggsae* male and hermaphrodite exo- and endometabolome samples, mating plates were subjected to hypochlorite treatment to obtain age-matched populations. At about 45 h after release from L1, 200 (counts per trial) hermaphrodites and, separately, 200 males were picked onto sparse lawn plates (50 µl of a 1:100 dilution of OP50 overnight spread with a glass rod to form a lawn). The plates were left at room temperature until all of the L4 larvae had molted into adults. Adults were washed off the plates with M9 buffer into 15-ml conical tubes. The tubes were incubated at room temperature with shaking at 220 r.p.m. for 24 h. After 24 h, the contents of the tubes were pooled and placed in a clinical centrifuge. The tube was spun at 1,625*g* for 1 min. The supernatant was drawn off, leaving a small pellet of worms in the original tube, and placed in a second conical tube. All tubes were immediately frozen in liquid nitrogen and stored at −80 °C until testing. *C. brenneri* and *C. inopinata* exometabolome samples were prepared analogously from mixed populations and analyzed by HPLC–MS as described above. Data are shown in Fig. 5c and Supplementary Fig. 13.

Developmental acceleration assays with nacq#1, nacq#2 and (2*E*,4*Z*,7*Z*)-deca-2,4,7-trienoic acid. To test pure compounds, 3.5-cm NGM plates were prepared as follows: 30 µg of nacq#1 or nacq#2 was dissolved in 2 ml of 100% ethanol, providing 50 µM stock solutions. Stock solutions were further diluted in water to give 40 nM, 4 nM and 40 pM solutions. Control solutions for mock treatment had corresponding amounts of ethanol added. One hundred microliters of compound solution or mock solution was distributed onto the surface of a 3.5-cm plate containing 4 ml of NGM agar (1:40 dilution), resulting in final plate concentrations of 1 nM, 100 pM and 1 pM, respectively. For the experiments shown in Fig. 4b, plates additionally contained the indicated concentrations of ascarosides. Plates were dried with open lids for 30 min and seeded with 35 µl freshly grown OP50 bacteria. Bacterial lawns were allowed to grow overnight. Developmental acceleration was assessed by measuring the time point of first egg laying, as described above. Developmental acceleration (in hours) was calculated as the time difference between the time of the first egg lay on untreated ISO plates minus the time of the first egg lay on conditioned ISO (or HD) plates. For the data shown in Figs. 3b, 4b and 5, see Supplementary Tables 6, 12 and 14–16; for the data shown in Supplementary Figs. 7a, 8 and 12, see Supplementary Tables 11, 14 and 15.

Lifespan assays with nacq#1. Fifty to sixty hermaphrodite N2 worms from synchronized plates were placed onto 6-cm NGM plates with fresh *E. coli* OP50 bacteria and allowed to lay eggs for 1 h. From these plates, eggs were transferred onto experimental plates containing nacq#1 (at a concentration of 1 nM, 100 pM, 10 pM or 1 pM) and/or ascr#10 (at a concentration of 400 nM or 4 nM) at densities of one egg per plate or, for some assays, 10 or 25 eggs per plate. ascr#10 concentrations were chosen on the basis of an initial screen of a wider concentration range (Supplementary Table 10). Starting on day three, worms were transferred daily onto fresh experimental plates until cessation of egg laying (around day 8) and every 3 d after that, until the experiment was completed. Animals were scored as dead if they failed to respond to a tap on the head and tail with a platinum wire. Worms with internal hatching, exploders or animals that crawled off the plate were excluded. For the data shown in Fig. 3f,g and Supplementary Fig. 10, see Supplementary Tables 7–9.

Dauer assays. Eggs from DR62 *daf-7*(*m62*) or DR1572 *daf-2*(*e1368*) were obtained by picking adult hermaphrodites to a fresh NGM plate and allowing egg laying to occur for 2 h. Dauers were obtained by allowing the eggs to develop at 25 °C for 3 d. Dauers were then selected by 1% SDS treatment for 5 min and transferred to assay plates containing nacq#1. Survival was scored after treating the animals with 1% SDS for 5 min. For dauer assays with wild-type worms, dauer induction was achieved by placing synchronized eggs on NGM plates containing an ascaroside blend of 2 µM equimolar amounts of ascr#2, ascr#3, ascr#5 and ascr#8, and allowing them to develop at 26 °C for 3 d. Before assay setup, dauers were selected with SDS as described above. Worms were allowed to stay on assay plates for 2.5 h and scored after SDS selection as live/dead. For the data shown in Fig. 4c–e and Supplementary Fig. 11, see Supplementary Table 13.

Luciferase assay for nacq#1-mediated NHR activation. To determine whether nacq#1 activates transcription by steroid hormone receptor homologs in *C. elegans*, N-terminally Flag-tagged chimeric expression constructs encoding NHR-8 and NHR-48, consisting of the DAF-12 DNA-binding domain (DBD) (DAF-$12_{1–499}$) and the respective ligand-binding domain (LBD) (NHR-$8_{91–560,}$ NHR-$48_{163–817}$) were created. HEK293T cells were seeded at 0.8×10^6 cells per well in 96-well plates and transfected (per well) with 30 ng Flag-tagged NHR expression vector, 30 ng Flag-tagged DIN-1e, 30 ng *mir-241p*-driven luciferase reporter and 5 ng β-galactosidase expression vector (used as a transfection normalization control) using the calcium phosphate precipitate method. Ethanol (negative control) or ethanol solutions of compounds (nacq#1 or dafacronic acid (Δ^7- DA)) were added 8 h after transfection at a final concentration of 100 nM, and the luciferase and β-galactosidase activities were measured by a Synergy 2 BioTek luminometer with Gen5 1.11 software 16 h after addition of the compound. Data are represented as luciferase relative luminometer units (RLU), normalized to β-galactosidase activity. Data shown are representative of four replicates and are presented as mean ± s.d. Significance values are from a two-tailed paired Student's *t* test. Data are shown in Supplementary Fig. 14.

Synthesis and NMR spectroscopy data of nacq#1 and nacq#2. For synthetic procedures, see the Supplementary Note.

Statistics. Different statistical tests were used to validate the data obtained from the different experiments. Two-tailed unpaired Student's *t* tests were used for the data in Figs. 1c, 3b, 4 and 5, and Supplementary Figs. 1, 2, 7a, 8, 11 and 12a,d. Two-tailed paired Student's *t* test was used for the data shown in Supplementary Fig. 14. Significant differences in time of development (in the experimental paradigm shown in Fig. 1b and similar) were computed using a χ^2 test.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The authors declare that the data supporting the findings of this study are available within the article and its supplementary information files.

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☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Xcalibur (version 4.1.31.9) from Thermo Fisher Scientific was used to acquire and analyze high resolution HPLC-MS data; Luciferase and beta-galactosidase activities were measured by a Synergy 2 BioTek luminometer with Gen5 1.11 software
Data analysis	Chi square calculations were done in R. MS Excel (2010, 2013, and 2016 versions) and Graphpad PRISM 7 was used to plot and perform statistical analysis.

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Life sciences study design

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Sample size	Sample sizes were chosen based on previous experience for each experiment. No statistical methods were used to predetermine sample size.
Data exclusions	No data were arbitrarily excluded.
Replication	Experiments were performed in several independent replicates (usually three or more) as indicated, at different times. Several experiments were replicated in two different laboratories, and all attempts at replication were successful
Randomization	Age synchronized populations of animals were produced and assigned randomly to experimental or control groups.
Blinding	Some, but not all experiments were performed blinded. Assessment of differences between control and treated animals relied on objective, predetermined criteria, reducing the need for blinding which was not practical in some cases. Developmental acceleration assays and lifespan assays were performed blinded.

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Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T - Invitrogen (now ThermoScientific)
Authentication	Cell line was not authenticated
Mycoplasma contamination	Cell line was not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	None of the used cell lines are listed in the ICLAC database.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	The following C. elegans strains were used: Wild type Bristol N2, FCS1 daf-22(ok693), DR476 daf-22(m130), AA86 daf-12(rh61 rh411), PR811 osm-6 (p811), SP1734 osm-6(m511), CB3323 che-13(e1805), AE501 nhr-8(ok186), nhr-8(ok186);daf-12 (rh61 rh411), AA107 nhr-48(ok178), DR466 him-5(e1490), CB467 him-5(e1467), CF1038 daf-16(mu86), GR1352 daf-16(mgDf47), DR62 daf-7(m62) and DR1572 daf-2(e1368), RB1795 fat-1(ok2323), BX26 fat-2 (wa17), ASI(-) PY7505 oyIs84[Pgpa-4::ced-3(p17), Pgcy-27::ced-3(p15), Pgcy-27::gfp, Punc-122::dsRed], ASK(-) PS6025 qrIs2[sra-9::mCasp1], ASJ(-) ZD762,mgIs40[daf-28p::nls-GFP]; jxEx100[trx-1::ICE + ofm-1::gfp], FCS23 [nhr-48(ok178); daf-22(ok693)], FCS24 [nhr-8(ok186); daf-22(ok693)], and FCS30 [daf-12(rh61, rh411); daf-22)ok693)] were generated by crossing FCS1 daf-22(ok693) males with AE501nhr-8(ok186), AA107 nhr-48(ok178) or AA86 daf-12(rh61, rh411) hermaphrodites, respectively. Genotypes were confirmed using PCR. In addition, we used AF16 C. briggsae, NKZ2 C. inopinata, and CB5161 C. brenneri. Animals of all ages - egg, larval stages, and adult were used in the developmental and aging assays. Age of the used animals varied between 0 and 27 days.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field collected samples were used in this study

Ethics oversight	Ethics oversight is not required for work with soil nematodes.

Note that full information on the approval of the study protocol must also be provided in the manuscript.