Report

A Conserved Endocrine Mechanism Controls the Formation of Dauer and Infective Larvae in Nematodes

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Summary

Under harsh environmental conditions, *Caenorhabditis elegans* larvae undergo arrest and form dauer larvae that can attach to other animals to facilitate dispersal [1]. It has been argued that this phenomenon, called phoresy, represents an intermediate step toward parasitism [2, 3]. Indeed, parasitic nematodes invade their hosts as infective larvae, a stage that shows striking morphological similarities to dauer larvae [1]. Although the molecular regulation of dauer entry in *C. elegans* involves insulin and TGF-β signaling [4–8], studies of TGF-β orthologs in parasitic nematodes didn't provide evidence for a common origin of dauer and infective larvae [9–14]. To identify conserved regulators between *Caenorhabditis* and parasitic nematodes, we used an evolutionary approach involving *Pristionchus pacificus* as an intermediate. We show by mutational and pharmacological analysis that *Pristionchus* and *Caenorhabditis* share the dafachronic acid-DAF-12 system as the core endocrine module for dauer formation. One dafachronic acid, Δ7-DA, has a conserved role in the mammalian parasite *Strongyloides papillosus* by controlling entry into the infective stage. Application of Δ7-DA blocks formation of infective larvae and results in free-living animals. Conservation of this small molecule ligand represents a fundamental link between dauer and infective larvae and might provide a general strategy for nematode parasitism.

Results and Discussion

In *C. elegans*, pheromonal cues that indicate overcrowding, high temperature, or starvation are processed through several signaling pathways including insulin/IGF, TGFβ-like, and guanylyl cyclase pathways (Figure 1A) [4–8, 15–20]. This results in the decrease of a class of steroidal hormones, Δ4-dafachronic acids and Δ7-dafachronic acids (DAs), and shifts the nuclear hormone receptor DAF-12 from its ligand-bound form to a ligand-free form, which specifies the dauer fate [21–25]. DAF-12 is strictly required for *C. elegans* dauer development [26], and its systemic expression suggests a role in the

specification of dauer fate at the level of individual tissues [24]. Despite the wealth of knowledge of dauer formation in *C. elegans*, the extent to which this paradigm applies to other nematodes is not comprehensively investigated. Pharmacological studies in *Ancylostoma* species, parasitic nematodes related to *C. elegans* (Figure 1C), identified potential parallels in the recovery of infective and dauer larvae [27–29]. However, little is known about mechanisms involved in the specification of cell fates during the formation of the infective larvae. Furthermore, studies on the ortholog of *daf-7* in *Ancylostoma* and other parasitic nematodes indicated substantial functional divergence during infective larvae formation [8–14]. Also, dauer pheromones of several rhabditid nematodes were found to be ineffective for *C. elegans* [15]. Only in the close relative *C. briggsae* several genes were found to play a similar role as in *C. elegans* [30].

Given the complexity of the dauer regulatory network and the limited functional conservation of some dauer control genes, we used an evolutionary approach to identify candidate genes that might be shared with parasitic nematodes. Specifically, we involved a distant relative of *C. elegans*, *P. pacificus*, as an intermediate (Figure 1C). *P. pacificus* is a genetically tractable system that shares many features with *C. elegans*, such as a short generation time, easy laboratory culture, and a complete genome sequence [31]. Forward and reverse genetic analysis in *P. pacificus* and the deep position of the last common ancestor of *Pristionchus* and *Caenorhabditis* make *Pristionchus* an ideal model for exploring the evolution of developmental mechanisms [32, 33]. In the wild, *Pristionchus* species associate with beetles as dauer larvae, which resume development only after the death of the beetle when they begin feeding on microbes on the carcass [34, 35]. Thus, the dauer stage is important for the adaptation of *P. pacificus* to its ecological niche.

To explore evolutionarily conserved mechanisms of dauer formation, we first determined whether a pheromone(s) controls dauer formation in *P. pacificus*. We found that supernatant prepared from liquid *P. pacificus* cultures induced eggs and J2 larvae to enter the dauer stage (data not shown). Next, we tested whether *P. pacificus* and *C. elegans* employ different pheromones by preparing concentrated pheromone extracts from supernatants (Figure 2A) [36]. In plates containing *P. pacificus* pheromone extracts, *P. pacificus* animals were induced to form dauer, whereas only few *C. elegans* N2 animals went into dauer (p < 0.01). Similarly, *C. elegans* pheromone induced dauer formation in *C. elegans*, but not in *P. pacificus* (p < 0.01). Thus, *P. pacificus* and *C. elegans* use distinct dauer pheromones, a result similar to previous studies with nematodes more closely related to *C. elegans* [15].

In *C. elegans*, depletion of cholesterol from culture medium induces dauer formation [37]. This is because *C. elegans* relies on an environmental source for the precursor of the DA hormone, which through binding to DAF-12 inhibits dauer formation. Under laboratory conditions, cholesterol in the medium serves as precursor of DA. To test whether cholesterol plays a similar role in *P. pacificus* dauer formation, we examined the effect of cholesterol restriction in the dauer-entry assay. When grown on an agar plate containing

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A *C. elegans / P. pacificus*
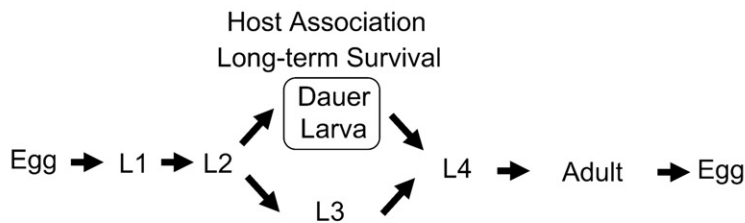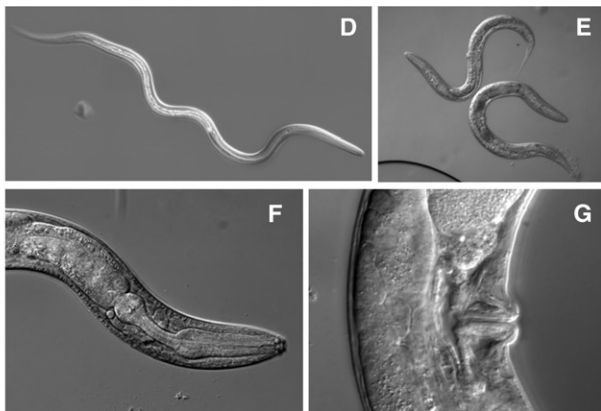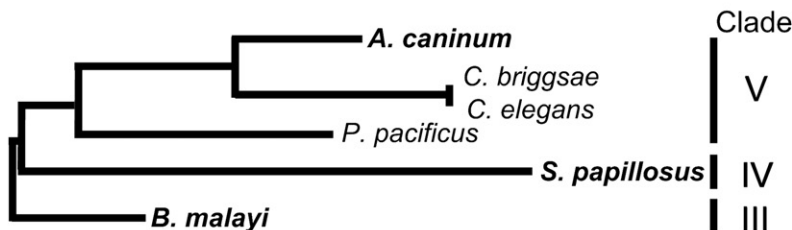

B *S. papillosus*
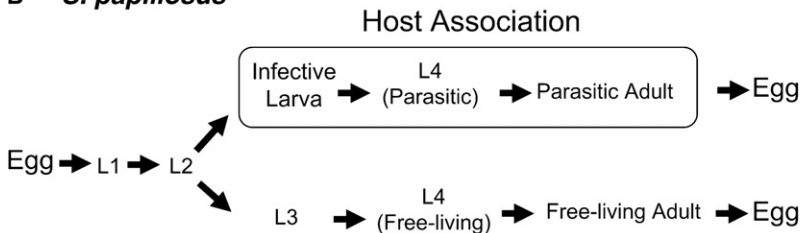
C

Figure 1. Life Cycles of Free-Living and Parasitic Nematodes

(A) Life cycles of the free-living nematodes *C. elegans* and *P. pacificus*. Under favorable conditions, animals go through direct development (L1-L4). In the laboratory, one cycle takes as little as three to four days (20°C). Under unfavorable conditions, such as high temperature, high population density, or starvation, animals can go into the arrested dauer stage. Note that the importance of the dauer larvae for host association is best known for *P. pacificus* and its beetle association.

(B) A simplified scheme for the life cycle of the vertebrate parasite *Strongyloides papillosus*. Note that not only the third larval stage but also the fourth larval stage and the adult stage are specialized for each life style. For a more precise representation of *S. papillosus* life cycle, see Figure S2.

(C) Phylogenetic relationship of the three species used in this study (*P. pacificus*, *C. elegans*, and *S. papillosus*), with three other taxa *C. briggsae*, the hookworm *A. caninum*, and *B. malayi*. Parasitic species are indicated in bold.

(D) Overall morphology of a *S. papillosus* infective larva.

(E–G) Phenotype of Δ7-DA-treated progeny *S. papillosus* that show morphological characteristics of free-living adults. (E) shows the overall morphology of free-living adults. (F) shows a rhabdiform esophagus with a grinder in the pharynx, which is only known from free-living adults but not parasitic females. (G) shows a vulva opening in the midbody region, a feature that is also only known from free-living females.

pheromone without added cholesterol, a significantly higher number of dauer larvae were formed than on plates with added cholesterol (p < 0.01), suggesting that as in *C. elegans*, sterol hormones might regulate dauer formation in *P. pacificus* (Figure 2B).

To test whether a DA-DAF-12 system controls dauer formation, we set out to isolate dauer-formation defective (Daf-d) mutants in forward genetic screens in *P. pacificus*. To identify *Ppa-daf-12* alleles that would be epistatic to cholesterol depletion, we screened for Daf-d mutants by using liquid medium without cholesterol. Three Daf-d mutants mapped to the *Ppa-daf-12* region of the genome and molecular analysis indeed showed that they carry a molecular lesion in *Ppa-daf-12* (Figure S4 available online). *Ppa-daf-12* encodes a nuclear hormone receptor, which shows >92% and 54% amino acid sequence identity to *Cel*-DAF-12, in the DNA-binding domain (DBD) and ligand-binding domain (LBD), respectively (Figures

S4B and S4C). Three segments of the DBD are important for DNA recognition, the proximal (P), distal (D), and direct repeat (DR) boxes [38, 39]. All three segments are completely conserved, suggesting conservation of the DNA-recognition specificity. The three mutations in *Ppa-daf-12* are nonsense and splice-acceptor mutations that truncate the LBD and parts of the hinge region (Figure S4A). None of the *Ppa-daf-12* mutants formed dauer larvae on an agar plate containing dauer pheromone, indicating that *Ppa*-DAF-12 is essential for dauer entry (Figure 2C).

Next we wanted to know whether DAs, the steroid compounds that serve as small molecule ligands for the DAF-12 receptor, are also conserved between *P. pacificus* and *C. elegans*. In *C. elegans*, DAs can rescue dauer-formation constitutive (Daf-c) phenotypes resulting from mutations in multiple signaling pathways acting upstream of DAF-12 [5]. In forward genetic screens for *P. pacificus* Daf-c mutants, we isolated two incompletely penetrant and two fully penetrant mutations. These mutations map to four separate chromosomal regions (Figure S1). We tested whether Δ4- DA and Δ7-DA could rescue these *Ppa*-Daf-c mutants. Administration of Δ7-DA strongly suppressed dauer formation in all four *P. pacificus* Daf-c strains (p < 0.01; Figure 3A). Δ4-DA rescued two incompletely penetrant mutations (p < 0.01 for both strains), but did not significantly affect the two fully penetrant
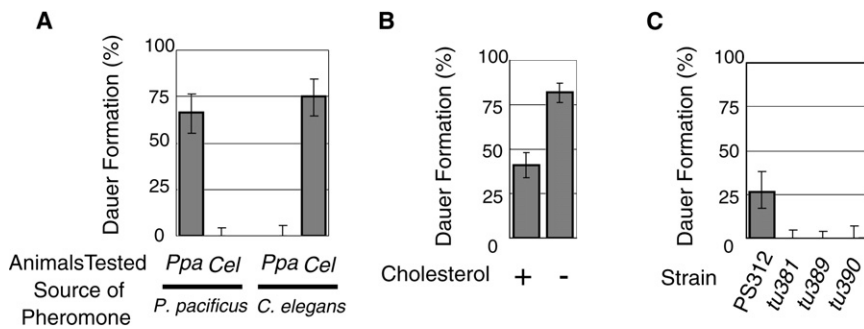
Figure 2. Dauer Formation Assays in *P. pacificus* Wild-Type and Mutant Animals

(A) Dauer formation of *P. pacificus* PS312 (*Ppa*) and *C. elegans* N2 (*Cel*) on agar plates containing either *P. pacificus* or *C. elegans* pheromone.

(B) Cholesterol restriction enhances dauer formation in *P. pacificus* PS312. Dauer formation was tested on pheromone plates (as in [B]) in the presence (5 μg/ml) or absence of added cholesterol.

(C) Daf-d phenotypes of the three *Ppa-daf-12* alleles, *tu381*, *tu389*, and *tu390*. Dauer formation of wild-type (PS312) and mutant animals was tested on agar plates containing the same amount of pheromone extract. Error bars denote 95% confidence intervals. Note that the experiments described in (A)–(C) were performed with different batches and different amounts of pheromone. See Table S1 for number of animals tested.

mutations (p = 0.11, and p = 0.56 for *Ppa-dfc-3* and *Ppa-dfc-4*, respectively). These results suggest that DA/DAF-12 is a conserved endocrine module and represents a candidate for conservation in parasitic species.

Although many nematodes are obligate parasites with limited access for experimental studies, some species retain a free-living life cycle in addition to the parasitic life cycle. One such example is *Strongyloides papillosus*, a parasite of ruminants that can be cultured in rabbits under laboratory conditions [40]. Phylogenetically, *Strongyloides* is an outgroup to *Caenorhabditis* and *Pristionchus* and represents a clade IV nematode according to the phylogeny of Blaxter et al., whereas *Caenorhabditis*, *Pristionchus*, and the hookworm *Ancylostoma* are all clade V nematodes (Figure 1C) [32, 33]. *S. papillosus* has a complex life history in which parthenogenetic females of *S. papillosus* in the small intestine of the host produce eggs that are released with the feces (Figure 1B). These eggs can either become infective female larvae (Figure 1D) developing into parasitic females or become free-living males and females, which produce offspring that become parasites and that are all females [41, 42].

To test whether DAs control infective larvae formation of *S. papillosus*, we collected young larvae from feces of rabbits infected with parasitic females. When we grew these larvae on agar plates containing Δ4-DA or Δ7-DA, we observed a complete suppression of the formation of infective larvae by Δ7-DA (p < 0.01) (Figure 3B). In contrast, the same concentration of Δ4-DA did not show any significant effect (p = 0.90). These results indicate that Δ7-DA can completely inhibit the development of parasitic females from feces-derived larvae under laboratory conditions.

Next we determined whether the progeny of free-living adults, all of which usually form infective larvae, could be prevented from developing into infective larvae. We found that administration of Δ7-DA completely prevented infective larvae formation (p < 0.01) (Figure 3C). Instead, the Δ7-DA-treated animals developed into individuals with a rhabdiform esophagus characteristic of free-living animals and a midbody vulva characteristic of adult females (Figures 1E–1G). No males were found in the Δ7-DA-treated progeny of free-living adults. However, female adults obtained by the Δ7-DA treatment could mate with the male progeny of parasitic females and laid eggs that again developed to a free-living adult upon treatment with Δ7-DA (data not shown). These results suggest that Δ7-DA negatively regulates the formation of infective larvae in *S. papillosus* and that exogenous administration of Δ7-DA redirects the development to an additional free-living life cycle.

Consistently, we could amplify cDNA sequences encoding putative *Spa-daf-12* from the transcripts of young progeny of parasitic and free-living adults by RT-PCR (Figures S4B and S4C and Figure 3D).

The functional conservation of the small molecule ligand Δ7-DA has several evolutionary and practical implications. First, DA-DAF-12 represents an endocrine-signaling module of a kind that is a common feature in the regulation of adaptive phenotypic plasticity [43, 44]. Endocrine signaling was suggested as a useful mechanism for controlling phenotypic plasticity because it allows organisms to modulate gene expression in response to environmental fluctuation in a fast and systemic manner. In addition, susceptibility of endocrine systems to both environmental and genetic perturbation may produce phenotypic variations, facilitating evolutionary change. Although many studies suggested the conservation of developmental regulation by steroid hormones within insects and within vertebrates [44], no such conserved mechanism has been known to exist in nematodes. This study provides evidence for a conserved steroid signaling in nematodes and builds a platform on which to study the evolution of life histories and its genetic and environmental regulation.

Second, the similar effect of Δ7-DA on *C. elegans* [21], *P. pacificus*, and *S. papillosus* supports a common origin of dauer and infective larvae in these species and leads to hypotheses on the evolution of parasitism. Nematode parasitism has evolved multiple times from free-living ancestors [33]. Parasitic nematodes often infect their hosts as specialized infective larvae and their morphological similarities to dauer larvae led to the suggestion that infective larvae might have evolved from dauer larvae of free-living species [1–3]. The conservation of Δ7-DA function suggests that the dauer and infective larvae indeed share a common regulatory basis for cell-fate specification and is consistent with the notion that infective larvae evolved from dauer larvae. The evolution of the parasitic life cycle in *S. papillosus* involved the diversification of the postdauer development from the post-L3 development to produce highly specialized parasitic and free-living adults, respectively (Figure 1B). We hypothesize that the gradual evolution of parasitism has been facilitated by phenotypic plasticity [44], thereby enabling the retention of a bacteria-feeding free-living life cycle while already shaping an alternative feeding strategy. Many other parasitic nematodes infect their hosts as dauer-like arrested larvae [1]. In most cases, development to such infective larvae is obligatory. Although the regulatory mechanisms and origin of infective larvae in these species need to be verified individually, specialization of postdauer
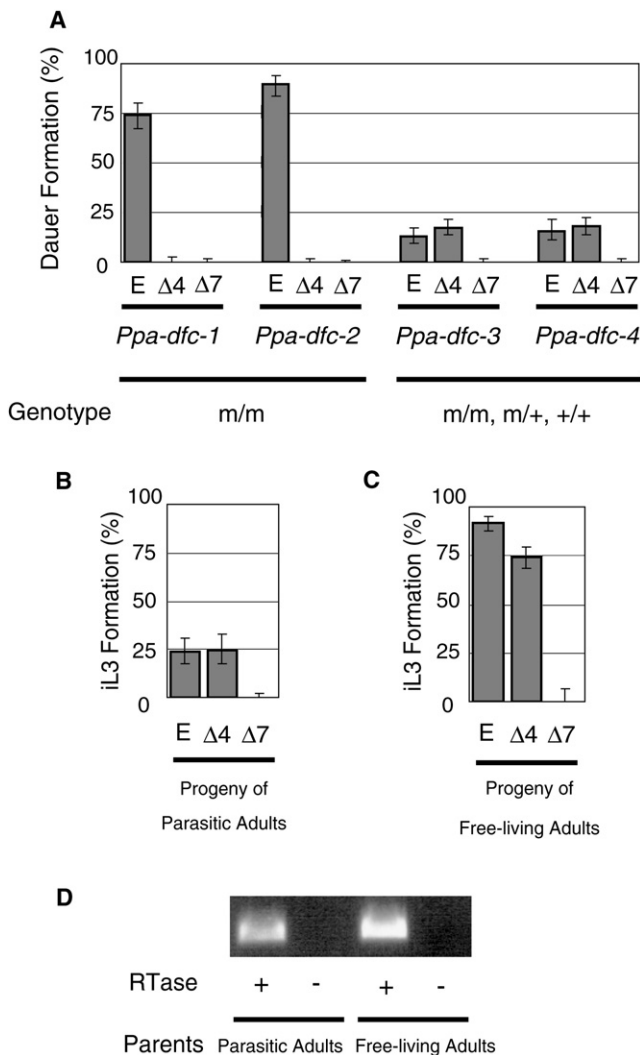
Figure 3. Assays for Dauer Formation and Infective Juvenile Formation

(A) Effect of DAs on dauer formation on four *P. pacificus* Daf-c strains. Mutant animals were grown in the presence of ethanol (E, control), Δ4-DA (Δ4, 250 nM), or Δ7-DA (Δ7, 250 nM). For two fully penetrant mutants *Ppa-dfc-3(tu393)* and *Ppa-dfc-4(tu394)*, mixed populations of homozygous, heterozygous, and wild-type eggs, which are F1 and F2 progeny of single heterozygous hermaphrodites, were tested. Therefore, less than 25% of animals are expected to be mutant. *Ppa-dfc-1(tu391)* and *Ppa-dfc-2(tu392)* were tested as homozygous animals.

(B) Infective third-stage larvae (iL3) formation of the progeny of *S. papillosus* parasitic females in the presence of ethanol (E, control), Δ4-DA (Δ4, 250 nM), or Δ7-DA (Δ7, 250 nM).

(C) iL3 formation of the progeny of *S. papillosus* free-living adults in the presence of ethanol (E, control), Δ4-DA (Δ4, 250 nM), or Δ7-DA (Δ7, 250 nM). Note that the non-iL3s in the plates with ethanol and Δ4-DA were small larvae that were presumably younger than the third-stage-arrested larvae. The majority of non-iL3s in the plate with Δ7-DA were females as shown in Figure 1E. Error bars denote 95% confidence intervals. See Table S1 for the number of animals tested.

(D) RT-PCR amplification of a partial cDNA of *Spa-daf-12*. RNA was prepared from young progeny of parasitic and free-living adults. Reverse transcriptase (RTase) was omitted in the negative control. The identity of the amplified fragments was verified by DNA sequencing (data not shown).

development to produce parasitic morphs and the subsequent loss of ancestral free-living life cycle could serve as a useful working hypothesis for the evolution of parasitism. Finally, our data suggest that a DAF-12 ligand with a 3-keto-5α-7-ene

(Δ7-DA) rather than a 3-keto-4-ene (Δ4-DA) ring structure [21] might be more strongly conserved. The deep conservation of this small molecule ligand has obvious pharmacological implications for the prevention of problems caused by nematodes.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, three figures, and one table and can be found with this article online at http://www.current-biology.com/supplemental/S0960-9822(08)01623-0.

References

1. Lee D.L., ed. (2002). The Biology of Nematodes (New York: Taylor and Francis).
2. Anderson, R.C. (1984). The origins of zooparasitic nematodes. Canadian Journal of Zoology *62*, 317–328.
3. Poulin, R. (2007). Evolutionary Ecology of Parasites, Second Edition (Princeton: Princeton University Press).
4. Estevez, M., Attisano, L., Wrana, J.L., Albert, P.S., Massague, J., and Riddle, D.L. (1993). The Daf-4 gene encodes a bone morphogenetic protein-receptor controlling *C. elegans* dauer larva development. Nature *365*, 644–649.
5. Hu, P.J. (2007). Dauer. In WormBook, The *C. elegans* Research Community, ed. 10.1895/wormbook.1.144.1, http://www.wormbook.org.
6. Morris, J.Z., Tissenbaum, H.A., and Ruvkun, G. (1996). A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature *382*, 536–539.
7. Kimura, K.D., Tissenbaum, H.A., Liu, Y.X., and Ruvkun, G. (1997). *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science *277*, 942–946.
8. Ren, P., Lim, C.S., Johnsen, R., Albert, P.S., Pilgrim, D., and Riddle, D.L. (1996). Control of *C. elegans* larval development by neuronal expression of a TGF-beta homolog. Science *274*, 1389–1391.
9. Viney, M.E., Thompson, F.J., and Crook, M. (2005). TGF-beta and the evolution of nematode parasitism. Int. J. Parasitol. *35*, 1473–1475.
10. Brand, A.M., Varghese, G., Majewski, W., and Hawdon, J.M. (2005). Identification of a DAF-7 ortholog from the hookworm *Ancylostoma caninum*. Int. J. Parasitol. *35*, 1489–1498.
11. Crook, M., Thompson, F.J., Grant, W.N., and Viney, M.E. (2005). *daf-7* and the development of *Strongyloides ratti* and *Parastrongyloides trichosuri*. Mol. Biochem. Parasitol. *139*, 213–223.
12. Freitas, T.C., and Arasu, P. (2005). Cloning and characterisation of genes encoding two transforming growth factor-beta-like ligands from the hookworm, *Ancylostoma caninum*. Int. J. Parasitol. *35*, 1477–1487.
13. Gomez-Escobar, N., Gregory, W.F., and Maizels, R.M. (2000). Identification of *tgh-2*, a filarial nematode homolog of *Caenorhabditis elegans daf-7* and human transforming growth factor beta, expressed in microfilarial and adult stages of *Brugia malayi*. Infect. Immun. *68*, 6402–6410.
14. Massey, H.C., Castelletto, M.L., Bhopale, V.M., Schad, G.A., and Lok, J.B. (2005). *Sst-tgh-1* from *Strongyloides stercoralis* encodes a proposed ortholog of *daf-7* in *Caenorhabditis elegans*. Mol. Biochem. Parasitol. *142*, 116–120.
15. Golden, J.W., and Riddle, D.L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science *218*, 578–580.

16. Golden, J.W., and Riddle, D.L. (1984). The *Caenorhabditis elegans* dauer larva: Developmental effects of pheromone, food, and temperature. Dev. Biol. *102*, 368–378.

17. Riddle, D.L., Swanson, M.M., and Albert, P.S. (1981). Interacting genes in nematode dauer larva formation. Nature *290*, 668–671.

18. Birnby, D.A., Link, E.M., Vowels, J.J., Tian, H., Colacurcio, P.L., and Thomas, J.H. (2000). A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. Genetics *155*, 85–104.

19. Butcher, R.A., Fujita, M., Schroeder, F.C., and Clardy, J. (2007). Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. Nat. Chem. Biol. *3*, 420–422.

20. Jeong, P.Y., Jung, M., Yim, Y.H., Kim, H., Park, M., Hong, E., Lee, W., Kim, Y.H., Kim, K., and Paik, Y.K. (2005). Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. Nature *433*, 541–545.

21. Motola, D.L., Cummins, C.L., Rottiers, V., Sharma, K.K., Li, T., Li, Y., Suino-Powell, K., Xu, H.E., Auchus, R.J., Antebi, A., and Mangelsdorf, D.J. (2006). Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. Cell *124*, 1209–1223.

22. Giroux, S., and Corey, E.J. (2007). Stereocontrolled synthesis of dafachronic acid A, the ligand for the DAF-12 nuclear receptor of *Caenorhabditis elegans*. J. Am. Chem. Soc. *129*, 9866–9867.

23. Antebi, A., Culotti, J.G., and Hedgecock, E.M. (1998). daf-12 regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. Development *125*, 1191–1205.

24. Antebi, A., Yeh, W.H., Tait, D., Hedgecock, E.M., and Riddle, D.L. (2000). daf-12 encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. Genes Dev. *14*, 1512–1527.

25. Snow, M.I., and Larsen, P.L. (2000). Structure and expression of *daf-12*: A nuclear hormone receptor with three isoforms that are involved in development and aging in Caenorhabditis elegans. Biochim. Biophys. Acta *1494*, 104–116.

26. Vowels, J.J., and Thomas, J.H. (1992). Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics *130*, 105–123.

27. Brand, A., and Hawdon, J.M. (2004). Phosphoinositide-3-OH-kinase inhibitor LY294002 prevents activation of *Ancylostoma caninum* and *Ancylostoma ceylanicum* third-stage infective larvae. Int. J. Parasitol. *34*, 909–914.

28. Hawdon, J.M., and Datu, B. (2003). The second messenger cyclic GMP mediates activation in *Ancylostoma caninum* infective larvae. Int. J. Parasitol. *33*, 787–793.

29. Tissenbaum, H.A., Hawdon, J., Perregaux, M., Hotez, P., Guarente, L., and Ruvkun, G. (2000). A common muscarinic pathway for diapause recovery in the distantly related nematode species *Caenorhabditis elegans* and *Ancylostoma caninum*. Proc. Natl. Acad. Sci. USA *97*, 460–465.

30. Inoue, T., Ailion, M., Poon, S., Kim, H.K., Thomas, J.H., and Sternberg, P.W. (2007). Genetic analysis of dauer formation in *Caenorhabditis briggsae*. Genetics *177*, 809–818.

31. Hong, R.L., and Sommer, R.J. (2006). *Pristionchus pacificus*: A well-rounded nematode. Bioessays *28*, 651–659.

32. Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., et al. (1998). A molecular evolutionary framework for the phylum Nematoda. Nature *392*, 71–75.

33. Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., and Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown Clades. Mol. Biol. Evol. *23*, 1792–1800.

34. Dieterich, C., Clifton, S.W., Schuster, L.N., Chinwalla, A., Delehaunty, K., Dinkelacker, I., Fulton, L., Fulton, R., Godfrey, J., Minx, P., et al. (2008). The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. Nat. Genet. *40*, 1193–1198.

35. Herrmann, M., Mayer, W.E., Hong, R.L., Kienle, S., Minasaki, R., and Sommer, R.J. (2007). The nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) is associated with the oriental beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan. Zoolog. Sci. *24*, 883–889.

36. Kawano, T., Kataoka, N., Abe, S., Ohtani, M., Honda, Y., Honda, S., and Kimura, Y. (2005). Lifespan extending activity of substances secreted by the nematode *Caenorhabditis elegans* that include the dauer-inducing pheromone. Biosci. Biotechnol. Biochem. *69*, 2479–2481.

37. Matyash, V., Entchev, E.V., Mende, F., Wilsch-Brauninger, M., Thiele, C., Schmidt, A.W., Knolker, H.J., Ward, S., and Kurzchalia, T.V. (2004). Sterol-derived hormone(s) controls entry into diapause in *Caenorhabditis elegans* by consecutive activation of DAF-12 and DAF-16. PLoS Biol. *2*, e280.

38. Perlmann, T., Rangarajan, P.N., Umesono, K., and Evans, R.M. (1993). Determinants for selective RAR and TR recognition of direct repeat HREs. Genes Dev. *7*, 1411–1422.

39. Umesono, K., and Evans, R.M. (1989). Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell *57*, 1139–1146.

40. Eberhardt, A.G., Mayer, W.E., and Streit, A. (2007). The free-living generation of the nematode *Strongyloides papillosus* undergoes sexual reproduction. Int. J. Parasitol. *37*, 989–1000.

41. Streit, A. (2008). Reproduction in *Strongyloides* (Nematoda): A life between sex and parthenogenesis. Parasitology *135*, 285–294.

42. Viney, M.E., and Lok, J.B. (2007). *Strongyloides* spp. In WormBook, The *C. elegans* Research Community, ed. 10.1895/wormbook.1.141.1, http://www.wormbook.org.

43. Nijhout, H.F. (2003). Development and evolution of adaptive polyphenisms. Evol. Dev. *5*, 9–18.

44. West-Eberhard, M.J. (2003). Developmental Plasticity and Evolution (New York: Oxford University Press).