

Nematode orphan genes are adopted by conserved regulatory networks and find a home in ecology

Melanie G Mayer and Ralf J Sommer*

¹Max Planck Institute for Developmental Biology; Tübingen, Germany

Keywords: *daf-12*, dauer formation, *dau-1*, evo-devo, intraspecific competition, nuclear hormone receptors, orphan genes, phenotypic plasticity, *Pristionchus pacificus*

© Melanie G Mayer and Ralf J Sommer

*Correspondence to: Ralf J Sommer; Email: ralf.sommer@tuebingen.mpg.de

Submitted: 07/24/2015

Accepted: 08/06/2015

<http://dx.doi.org/10.1080/21624054.2015.1082029>

This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

Comment on: Mayer MG, et al. The orphan gene *dauerless* regulates dauer development and intraspecific competition in nematodes by copy number variation. PLOS Genet 2015; 11:e100514624; <http://dx.doi.org/10.1371/journal.pgen.1005146>

Nematode dauer formation represents an essential survival and dispersal strategy and is one of a few ecologically relevant traits that can be studied in laboratory approaches. Under harsh environmental conditions, the nematode model organisms *Caenorhabditis elegans* and *Pristionchus pacificus* arrest their development and induce the formation of stress-resistant dauer larvae in response to dauer pheromones, representing a key example of phenotypic plasticity. Previous studies have indicated that in *P. pacificus*, many wild isolates show cross-preference of dauer pheromones and compete for access to a limited food source. When investigating the genetic mechanisms underlying this intraspecific competition, we recently discovered that the orphan gene *dauerless* (*dau-1*) controls dauer formation by copy number variation. Our results show that *dau-1* acts in parallel to or downstream of steroid hormone signaling but upstream of the nuclear hormone receptor *daf-12*, suggesting that DAU-1 represents a novel inhibitor of DAF-12. Phylogenetic analysis reveals that the observed copy number variation is part of a complex series of gene duplication events that occurred over short evolutionary time scales. Here, we comment on the incorporation of novel or fast-evolving genes into conserved genetic networks as a common principle for the evolution of phenotypic plasticity and intraspecific competition. We discuss the possibility that orphan genes might often function in the regulation and execution of ecologically relevant traits. Given that only few ecological processes can be studied in model organisms, the function of such genes might often go unnoticed,

explaining the large number of uncharacterized genes in model system genomes.

Introduction

Evolutionary developmental biology (evo-devo) aims for a comprehensive understanding of the evolutionary forces and the developmental processes that generate biological diversity.¹⁻³ The fact that diverse organisms have highly conserved developmental control genes but fundamentally different regulatory mechanisms for the specification of homologous structures resulting in developmental systems drift is a central finding of evo-devo research and by now, a truism of modern biology.^{4,5} However, explaining the link between genetic variation and phenotypic diversity requires a more integrative approach combining evo-devo with population genetics and evolutionary ecology.^{6,7} Over the last 2 decades, evolutionary biologists have attributed increasing importance to the influence of ecology on evolution and development, demonstrated by the growing number of studies on phenotypically plastic traits.^{8,9}

Phenotypic plasticity describes the ability of an individual organism to develop alternative forms of a trait in response to changing environmental conditions. In the last decade, phenotypic plasticity has been suggested to not only be of ecological but also evolutionary importance because it can facilitate morphological innovations that result in the origin and evolution of novel traits.⁸ Nematode dauer formation is an example of phenotypic plasticity in an ecologically relevant trait, which exists as a choice that many free-living nematodes have between direct development into

reproductive adults and indirect development into stress-resistant dauer larvae (Fig. 1A).^{10,11} The dauer stage is an alternative third larval stage, induced by a dauer pheromone, and formed under harsh environmental conditions, such as food shortage or high population density.^{12,13} Dauer formation is also considered to be of relevance for the evolution of parasitism. Many theoretical studies have discussed dauer larvae of free-living

nematodes and their morphological similarities to infective juveniles of parasitic nematodes as a preadaptation toward the evolution of parasitism.¹⁴⁻¹⁷ In this context, it is important to note that in the wild *P. pacificus* predominantly exists as dauer larvae, which live in a necromenic association with scarab beetles.^{18,19} Only after the natural death of its host, does *P. pacificus* exit the dauer stage and resume its development by feeding on bacteria,

fungi, and other organisms growing on the dead beetle. Therefore, dauer formation represents a survival and dispersal strategy, enabling individuals of a population to endure and escape unfavorable conditions. In contrast to many other nematode associations with invertebrates that are often unspecific, such as *C. elegans* with slugs and isopods,²⁰ the association of *Pristionchus* nematodes with scarab beetles is mostly species-specific, an important prerequisite for the evolution of parasitism.^{15,16}

Cross-preference of dauer pheromones and intraspecific competition

The huge advantage of working with *P. pacificus* is our extensive understanding of its natural ecology and population genetics. Since *P. pacificus* is a cosmopolitan species, many wild isolates have been collected and are available for ecological studies.^{7,21} We previously used a natural variation approach to compare various aspects of dauer formation among diverse *P. pacificus* strains.^{22,23} Our results indicate high levels of natural variation in dauer longevity, as well as in dauer formation in response to individual small molecules and dauer pheromone, the complex blend of small molecules extracted from each strain. Furthermore, we observed significant qualitative and quantitative differences in production of and response to individual small molecules. Specifically, some strains synthesize considerable amounts of a certain compound, although they do not respond to it, whereas other strains form many dauers in response to compounds they do not produce themselves.²³ Indeed, most strains show cross-preference of dauer pheromones, whereby a strain forms more dauers in response to another strain's pheromone extract than in response to its own pheromone, suggesting intraspecific competition as a previously unconsidered aspect of dauer formation.

Following these initial experiments, we developed a novel assay to directly test for competition.²³ In contrast to our previous dauer formation assays, involving the response of an individual strain to a specific small molecule or pheromone extract, the competition assay was designed to enable

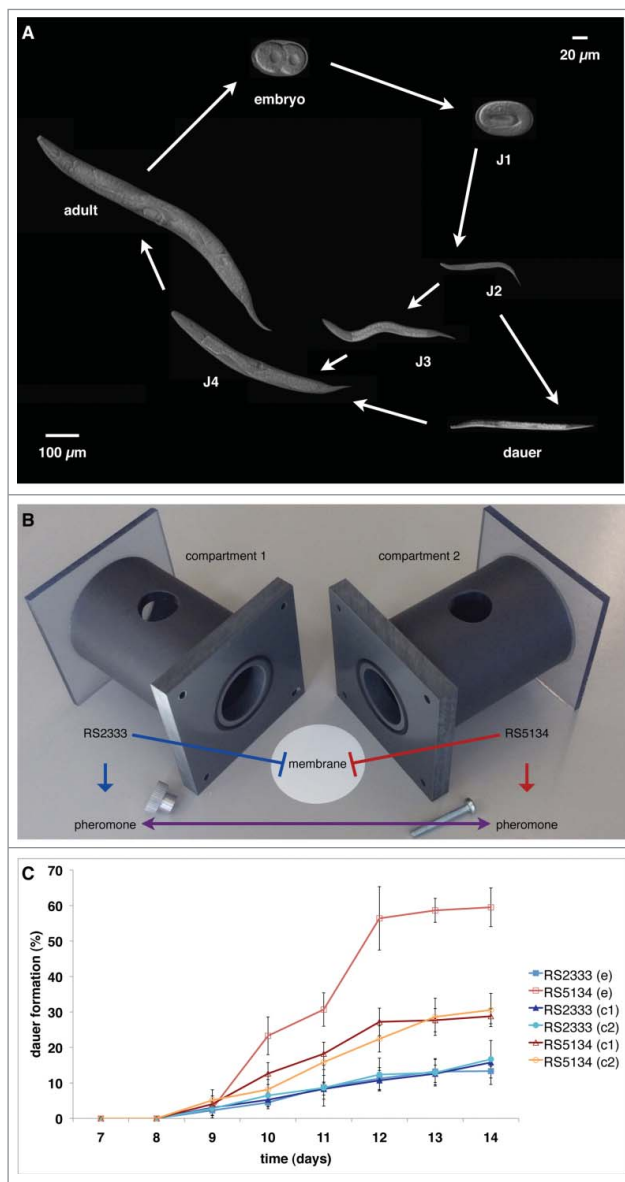


Figure 1. *P. pacificus* dauer formation and intraspecific competition. (A) *P. pacificus* life cycle. (B) Experimental design of Ussing chamber competition assay. (C) Dauer formation observed over time, showing intraspecific competition between RS2333 and RS5134. In the competition experiment (e) one compartment of the Ussing chamber contains RS2333 and the other RS5134, whereas in control experiments (c) the same strain is grown in both compartments.²⁴

the observation of the dauer formation of 2 strains in a common environment in response to their naturally secreted pheromones. Using the Ussing chamber system (Fig. 1B, C), our results confirm the presence of cross-preference of dauer pheromones and reveal intraspecific competition among both allopatric and sympatric strains.^{23,24} Competitive interactions among organisms have been suggested to be associated with evolutionary arms races.²⁵ Intraspecific competition for survival and access to limited resources under changing environmental conditions represents a strong selective force driving the divergence among different populations of the same species.⁸ Strategies for surviving and competing in changing environments are well known in bacteria and involve the regulation of the expression of specific genes in response to different environmental conditions.²⁶ However, little is known about the molecular mechanisms of competitive interactions in eukaryotes.

Intraspecific competition in *P. pacificus* dauer formation probably occurs at multiple levels of dauer entry but also after dauer exit when the dauer larvae start to feed on a limited food source, such as bacteria and other organisms growing on a beetle carcass. Such competition for a limited food source may result in an evolutionary arms race regarding the dauer formation phenotypes of different *P. pacificus* strains and lead to changes in dauer pheromone production and response. Two different survival strategies may have evolved due to intraspecific competition in *P. pacificus* dauer formation. Strains with low dauer formation phenotypes, such as the *P. pacificus* reference strain RS2333 from

California, can drive other strains into early dauer formation, thereby gaining the advantage of reproducing for a longer time. In contrast, strains with high dauer formation phenotypes, such as RS5134 from Ohio, can avoid competition, overpopulation, and potential starvation by optimizing their dispersal as dauer larvae. Indeed, RS5134/Ohio is a champion of survival in the dauer stage. Under stable laboratory conditions, this strain survives for almost one year in distilled water at 8°C, in contrast to *P. pacificus* RS2333 and *C. elegans* N2, the latter of which only survives for 18 weeks.²²

A new element in the dauer regulatory network

Since RS2333/California and RS5134/Ohio show a large difference in their dauer formation phenotypes (Fig. 1C), we selected these 2 strains to investigate the molecular mechanisms that determine intraspecific competition by generating recombinant inbred lines and performing quantitative-trait-loci mapping.²⁴ We discovered that the orphan gene *dauerless* (*dau-1*) regulates dauer formation by copy number variation (CNV) (Fig. 2). A single *dau-1* copy is responsible for the high dauer formation phenotype of RS5134/Ohio, whereas 2 *dau-1* copies, resulting from a very recent gene duplication event, lead to reduced dauer formation in RS2333/California. These findings are supported by experimental studies using transgenic animals. Specifically, transgenic animals expressing multiple *dau-1* copies in either strain do not form dauer larvae at all. We detected *dau-1* expression in the CAN neurons, indicating a previously

unknown role of these neurons in dauer formation. Ablation of the CAN neurons, as well as mutations in the *dau-1* locus generated by the CRISPR/Cas9 system, causes increased dauer formation. Finally, we performed epistasis experiments to determine where *dau-1* acts in the dauer regulatory network. The key factor in the dauer regulatory network is the nuclear hormone receptor DAF-12 that acts as a developmental switch, induces dauer formation in its ligand-free form, and is conserved among many free-living and parasitic nematodes.^{27,28} Our results show that *dau-1* acts in parallel to or downstream of steroid hormone signaling but upstream of *daf-12*, suggesting that DAU-1 represents a novel inhibitor of DAF-12.

CNV, gene duplication, and orphan genes

Phylogenetic analysis reveals that the observed CNV of *dau-1* is part of a complex series of gene duplication events that occurred over short evolutionary time scales.²⁴ Gene duplication is a form of CNV and represents an important mechanism for adapting to changing environmental conditions, which has been studied extensively in bacteria.²⁹ Prevalent in a variety of organisms, gene duplication has been suggested to facilitate the evolution of novel phenotypic traits.³⁰ After a duplication event, one gene copy may retain the ancestral function, while the other copy may acquire a new function.^{30,31} Therefore, gene duplication may emerge as a common principle for the evolution of novel traits and may drive the evolution of genetic networks and the divergence among populations.^{30,32} An

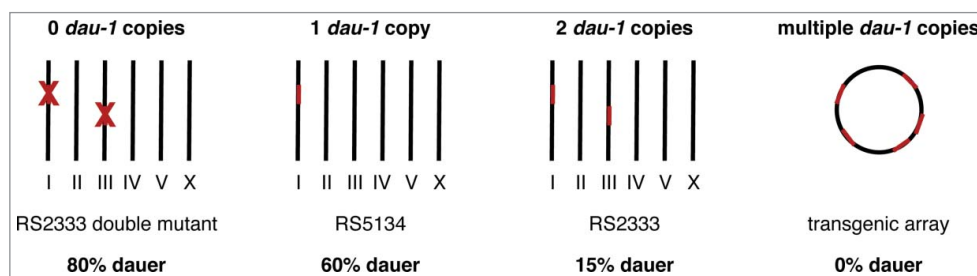


Figure 2. *dau-1* regulates dauer formation by CNV. One copy leads to high dauer formation in RS5134, 2 copies cause low dauer formation in RS2333, and the expression of multiple copies completely inhibits dauer formation in transgenic animals. In contrast, the absence of *dau-1* results in an extremely high dauer formation phenotype.²⁴

interesting aspect of gene duplication events is their ability to promote the rapid evolution of certain gene families and thus induce the formation of orphan genes.³³

Orphan genes lack significant sequence similarity to genes in other organisms and thus, they are defined as not being conserved above the genus level.³⁴ They may evolve *de novo* from previously non-coding sequences or originate from gene duplication events followed by rapid evolution.³³ Comparative genome sequencing projects have revealed a large number (10–20%) of orphan genes in all taxonomic groups, including nematodes.^{34,35} For example, in *P. pacificus*, orphan genes constitute approximately one third of the predicted 27,000 protein-coding genes.^{36,37} Interestingly, follow-up experiments have indicated that more than half of these orphan genes are transcribed.³⁷ Approximately 30% of the *P. pacificus* orphan genes can be grouped into distinct protein families that have homologues in very closely related species of the same genus but not in *C. elegans*. This suggests that these orphan genes belong to fast-evolving gene families and diverge so rapidly that no homologues can be identified at greater phylogenetic distances,³⁷ an assumption that has now been confirmed in the case of *dau-1*.

Since orphan genes are taxonomically restricted, they may have evolved to enable the adaptation to the ecology of the organism and specific environmental conditions.³⁵ For example, it has been shown that the expression of many *P. pacificus* orphan genes is upregulated in the dauer stage, suggesting a crucial role in the evolution of *P. pacificus* dauer formation, which differs in its specific functions from that of *C. elegans*.³⁸ Furthermore, proteomic and transcriptomic studies have provided strong evidence that orphan genes are expressed and of functional importance,^{37,38} yet they are not conserved. Thus, although developmental control genes and signaling pathways are often conserved throughout the animal kingdom – the truism of modern biology discussed above, a relatively large part of genes in an animal shows limited or no sequence similarity to genes in other organisms. Our work on the orphan gene *dau-1* and its role as a novel inhibitor of dauer formation therefore shows that rapidly evolving genes

are of developmental and ecological relevance. In addition, our studies suggest that the incorporation of orphan genes into conserved regulatory networks may represent a general evolutionary mechanism.

Conclusions

Ever since the *C. elegans* genome was sequenced as the first metazoan representative,³⁹ researchers have been puzzled by the following overarching pattern. While a small number of conserved genes was identified as developmental control genes that are shared between *C. elegans*, *Drosophila*, and several vertebrate models, the vast majority of genes has no associated function and limited or no sequence similarity to genes in other taxonomic groups. This observation, when combined with the results of developmental genetics at large, resulted in an ascertainment bias. While developmental geneticists identified the same (homologous) genes over and over again, most genes found in genome sequencing projects are not conserved and often novel.³¹ As a result, we are clueless about the function of the majority of genes, even in some of the best-studied model organisms of the life sciences, such as *C. elegans*.⁴⁰ Do all of these genes have any function at all or are they just rapidly evolving units of the genome that come and go? To answer these questions, we have to appreciate that the highly successful model system approach has one important downside: the ecology and the environment, in which organisms normally live and evolve, is not considered. Rather, we study them in (sterile) laboratory environments. Could it be that many orphan genes have functions in the environment? These questions will only begin to be addressed in future studies. However, the story of *P. pacificus dau-1* tells us that orphan genes can find a home in ecology and are incorporated into preexisting regulatory networks.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Raff RA. The shape of life: genes, development, and the evolution of animal form. Chicago: The University of Chicago Press 1996. 544.
- Gerhart J, Kirschner M. The theory of facilitated variation. PNAS 2007; 104:8582–89; PMID:17494755; <http://dx.doi.org/10.1073/pnas.0701035104>
- Carroll SB. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 2008; 134:25–36; PMID:18614008; <http://dx.doi.org/10.1016/j.cell.2008.06.030>
- True JR, Haag ES. Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 2001; 3:109–19; PMID:11341673; <http://dx.doi.org/10.1046/j.1525-142x.2001.003002109.x>
- Wang X, Sommer RJ. Antagonism of LIN-17/Frizzled and LIN-18/Ryk in nematode vulva induction reveals evolutionary alterations in core developmental pathways. PLOS Biol 2011; 9:e1001110; PMID:21814488; <http://dx.doi.org/10.1371/journal.pbio.1001110>
- Sommer RJ. The future of evo-devo: model systems and evolutionary theory. Nat Rev Genet 2009; 10:416–22; PMID:19369972
- Sommer RJ, McGaughan, A. The nematode *Pristionchus pacificus* as a model system for integrative studies in evolutionary biology. Mol Ecol 2013; 22:2380–93; PMID:23530614; <http://dx.doi.org/10.1111/mec.12286>
- West-Eberhard MJ. Developmental Plasticity and Evolution. Oxford: Oxford University Press 2003. 816.
- Gilbert SF, Epel D. Ecological developmental biology: integrating epigenetics, medicine and evolution. Sunderland (MA): Sinauer Associates Inc 2009. 480.
- Fielenbach N, Antebi A. C. elegans dauer formation and the molecular basis of plasticity. Genes Dev 2008; 22:2149–65; PMID:18708575; <http://dx.doi.org/10.1101/gad.1701508>
- Sommer RJ, Ogawa A. Hormone signaling and phenotypic plasticity in nematode development and evolution. Curr Biol 2011; 21:R758–66; PMID:21959166; <http://dx.doi.org/10.1016/j.cub.2011.06.034>
- Cassada RC, Russell RL. The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol 1975; 46:326–42; PMID:1183723; [http://dx.doi.org/10.1016/0012-1606\(75\)90109-8](http://dx.doi.org/10.1016/0012-1606(75)90109-8)
- Golden JW, Riddle DL. A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science 1982; 218:578–80; PMID:6896933; <http://dx.doi.org/10.1126/science.6896933>
- Osche G. Die Präadaptation freilebender Nematoden an den Parasitismus. Zool Anz 1956; 19:391–96.
- Poulin R. Evolutionary ecology of parasites. 2nd ed. Princeton (NJ): Princeton University Press 2007. 332.
- Dieterich C, Sommer RJ. How to become a parasite - lessons from the genomes of nematodes. Trends Genet 2009; 25:203–9; PMID:19361881; <http://dx.doi.org/10.1016/j.tig.2009.03.006>
- Sudhaus W. Preadaptive plateau in Rhabditida (Nematoda) allowed the repeated evolution of zooparasites, with an outlook on evolution of life cycles within Spiroscarida. Palaeodiversity 2010; 3:117–30.
- Herrmann M, Mayer WE, Sommer RJ. Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. Zoology 2006; 109:96–108; PMID:16616467; <http://dx.doi.org/10.1016/j.zool.2006.03.001>
- Ragsdale EJ. *Pristionchus pacificus*: a nematode model for comparative and evolutionary biology. Leiden, Netherlands: Brill 2015. Chapter 11, Mouth dimorphism and the evolution of novelty and diversity. 301–329.
- Petersen C, Hermann RJ, Barg MC, Schalkowski R, Dirksen P, Barbosa C, Schulenburg H. Travelling at a slug's pace: possible invertebrate vectors of *Caenorhabditis* nematodes. BMC Ecol 2015; 15:19; PMID:26170141; <http://dx.doi.org/10.1186/s12898-015-0050-z>

21. Morgan K, McCaughran A, Villate L, Herrmann M, Witte H, Bartelmes G, Rochat J, Sommer RJ. Multi locus analysis of *Pristionchus pacificus* on La Réunion Island reveals an evolutionary history shaped by multiple introductions, constrained dispersal events and rare out-crossing. *Mol Ecol* 2012; 21:250-66; PMID:22126624; <http://dx.doi.org/10.1111/j.1365-294X.2011.05382.x>
22. Mayer MG, Sommer RJ. Natural variation in *Pristionchus pacificus* dauer formation reveals cross-preference rather than self-preference of nematode dauer pheromones. *Proc R Soc B* 2011; 278:2784-90; PMID:21307052; <http://dx.doi.org/10.1098/rspb.2010.2760>
23. Bose N, Meyer JM, Yim JJ, Mayer MG, Markov GV, Ogawa A, Schroeder FC, Sommer RJ. Natural variation in dauer pheromone production and sensing supports intraspecific competition in nematodes. *Curr Biol* 2014; 24:1536-41; PMID:24980503; <http://dx.doi.org/10.1016/j.cub.2014.05.045>
24. Mayer MG, Rödelsperger C, Witte H, Riebesell M, Sommer RJ. The orphan gene dauerless regulates dauer development and intraspecific competition in nematodes by copy number variation. *PLoS Genet* 2015; 11:e100514624; <http://dx.doi.org/10.1371/journal.pgen.1005146>
25. Dawkins R, Krebs JR. Arms races between and within species. *Proc R Soc B* 1979; 205:489-511; <http://dx.doi.org/10.1098/rspb.1979.0081>
26. Dubnau D, Losick R. Bistability in bacteria. *Mol Microbiol* 2006; 61:564-72; PMID:16879639; <http://dx.doi.org/10.1111/j.1365-2958.2006.05249.x>
27. Ogawa A, Streit A, Antebi A, Sommer RJ. A conserved endocrine mechanism controls the formation of dauer and infective larvae in nematodes. *Curr Biol* 2009; 19:67-71; PMID:19110431; <http://dx.doi.org/10.1016/j.cub.2008.11.063>
28. Antebi A. Nuclear receptor signal transduction. *Worm-Book. The C. elegans Research Community* 2015
29. Andersson DI, Hughes D. Gene amplification and adaptive evolution in bacteria. *Ann Rev Genet* 2009; 43:167-95; PMID:19686082; <http://dx.doi.org/10.1146/annurev-genet-102108-134805>
30. Zhang J. Evolution by gene duplication: an update. *Trends Ecol Evol* 2003; 18:292-98; [http://dx.doi.org/10.1016/S0169-5347\(03\)00033-8](http://dx.doi.org/10.1016/S0169-5347(03)00033-8)
31. Hoekstra HE, Coyne JA. The locus of evolution: evo devo and the genetics of adaptation. *Evolution* 2007; 61:995-1016; PMID:17492956; <http://dx.doi.org/10.1111/j.1558-5646.2007.00105.x>
32. Ohno S. Evolution by gene duplication. New York: Springer-Verlag 1970. 160.
33. Tautz D, Domazet-Lošo T. The evolutionary origin of orphan genes. *Nat Rev Genet* 2011; 12:692-702; PMID:21878963; <http://dx.doi.org/10.1038/nrg3053>
34. Rödelsperger C, Streit A, Sommer RJ. Structure, function and evolution of the nematode genome. In: eLS. Chichester: John Wiley & Sons Ltd 2013. <http://dx.doi.org/10.1002/9780470015902.a0024603>
35. Khalutrin K, Hemmrich G, Fraune S, Augustin R, Bosch TCG. More than just orphans: are taxonomically restricted genes important in evolution? *Trends Genet* 2009; 25:404-13; PMID:19716618; <http://dx.doi.org/10.1016/j.tig.2009.07.006>
36. Dieterich C, Clifton SW, Schuster LN, Chinwalla A, Delehaunty K, Dinkelacker I, Fulton L, Fulton R, Godfrey J, Minx P et al. The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. *Nat Genet* 2008; 40:1193-98; PMID:18806794; <http://dx.doi.org/10.1038/ng.227>
37. Borchert N, Dieterich C, Krug K, Schütz W, Jung S, Nordheim A, Sommer RJ, Macek B. Proteogenomics of *Pristionchus pacificus* reveals distinct proteome structure of nematode models. *Genome Res* 2010; 20:837-46; PMID:20237107; <http://dx.doi.org/10.1101/gr.103119.109>
38. Sinha A, Sommer RJ, Dieterich C. Divergent gene expression in the conserved dauer stage of the nematodes *Pristionchus pacificus* and *Caenorhabditis elegans*. *BMC Genomics* 2012; 13:254-70; PMID:22712530; <http://dx.doi.org/10.1186/1471-2164-13-254>
39. C. elegans Sequencing Consortium. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 1998; 282:2012-18; PMID:9851916; <http://dx.doi.org/10.1126/science.282.5396.2012>
40. Sternberg PW. *Pristionchus pacificus*: a nematode model for comparative and evolutionary biology. Leiden, Netherlands: Brill 2015. Chapter 1, Why *Caenorhabditis elegans* is great and *Pristionchus pacificus* might be better. 1–17.