Pristionchus pacificus: a well-rounded nematode

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Summary

Nematodes pervade Earth's biosphere and occupy innumerable ecological niches. The role of *Caenorhabditis elegans* as a model for developmental processes has encouraged us to cultivate a second nematode, *Pristionchus pacificus*, as a comparative counterpoint to address questions in development, behavior and ecology in nematode evolution. We hope that this endeavor, now more than a decade underway, will allow us to project findings onto other comparative models for biological processes. To this end, our laboratory has made an extensive genetic map and mutant screens to understand changes in developmental programs. Recently, we have been capitalizing on the whole genome sequence of *P. pacificus* to describe more thoroughly the molecular basis for these changes, as well as to better integrate our molecular knowledge with the biodiversity of *Pristionchus* species. *BioEssays* 28:651–659, 2006.
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Introduction

Most known species in the phylum Nematoda develop through a fixed number of cells by lineage-dependent differentiation, a phenomenon known as eutely. This is a virtue that facilitates comparisons among homologous cells in different species of nematodes, especially in organs such as the male sensilla and the female/hermaphrodite vulva. All nematodes also have a general vermiform body shape. Yet what nematodes lack in overall morphological variety, they make up in the plenitude of regulatory styles imposed within their lineage-dependent developmental programs, which allow them to conquer a vast range of ecological niches through changes in behavior and modes of reproduction. Although studies in *C. elegans* have been invaluable for understanding a wide range of biological phenomena, generalizations from one species about a phylum consisting of more than a million are risky.[1] In response to the need to expand the *C. elegans* model, one of us (RJS) became intrigued by the possibility of comparative studies as a post-doc in Paul Sternberg's laboratory at Caltech. *Pristionchus pacificus* was one among the many nematodes surveyed for comparative studies in vulva patterning, before emerging as our sole satellite model organism (Figs. 1 and 2).[2,3] From the start, *P. pacificus* was unique in showing interesting developmental differences to *C. elegans*, as well as in being easily manipulatable under laboratory conditions due to its high brood size, short generation time and hermaphroditic reproduction. Since then, our research group has been able to compare *C. elegans* and *P. pacificus* to pinpoint cases of changes in the evolution of not just vulva development, but also gonad morphology, sex determination, chemotaxis behavior and ecology. With the complete genome sequence of *P. pacificus* and low coverages of two sister species soon available, the future research for *P. pacificus* and its close relatives promise even greater potential.

P. pacificus belongs to the Diplogastridae family, which itself may be part of the rhabditid group that also includes the *Eurhabditis* (*Caenorhabditis* species) and *Pleiorhabditis* (*Pelodera* species) as ascertained by the analysis of three nuclear genes in the latest molecular phylogeny (Fig. 3a).[4] The estimated time since the last common ancestor of *P. pacificus* and *C. elegans* existed is 200–300 million years ago.[5] The Diplogastridae is a monophyletic group and includes about 312 species in 28 major genera.[8] A peculiarity of the Diplogastrids is that they undergo an embryonic molt from J1 to J2 before hatching, unlike the postembryonic stages of L1–L4 larvae defined for other nematodes such as *C. elegans*.[6] Thus a *P. pacificus* emerges from the egg as a J2 (Juvenile 2), which proceeds then through J3 and J4 before reaching adulthood (Figs. 1c and 2). The presence of a dorsal tooth and the lack of the pharyngeal grinder found in many eurhabditids such as the *Caenorhaditis* species are considered apomorphies common to all diplogastrids.[7] Phylogenetic relationships within the Diplogastridae were constructed based on these comparatively complex structures of the diplogastrid buccal cavity, consisting of movable teeth and exposed fangs (Fig. 3b).[8] A fascinating hypothesis was proposed for how such complicated mouthparts were accommodated into the preexisting developmental program: the diplogastrid-specific heterochronic shift of having the first juvenile instar molt within the egg allows proportionally more

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Funding agency: R.L.H. is supported by the Alexander von Humboldt Foundation.
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DOI 10.1002/bies.20404
Published online in Wiley InterScience (www.interscience.wiley.com).

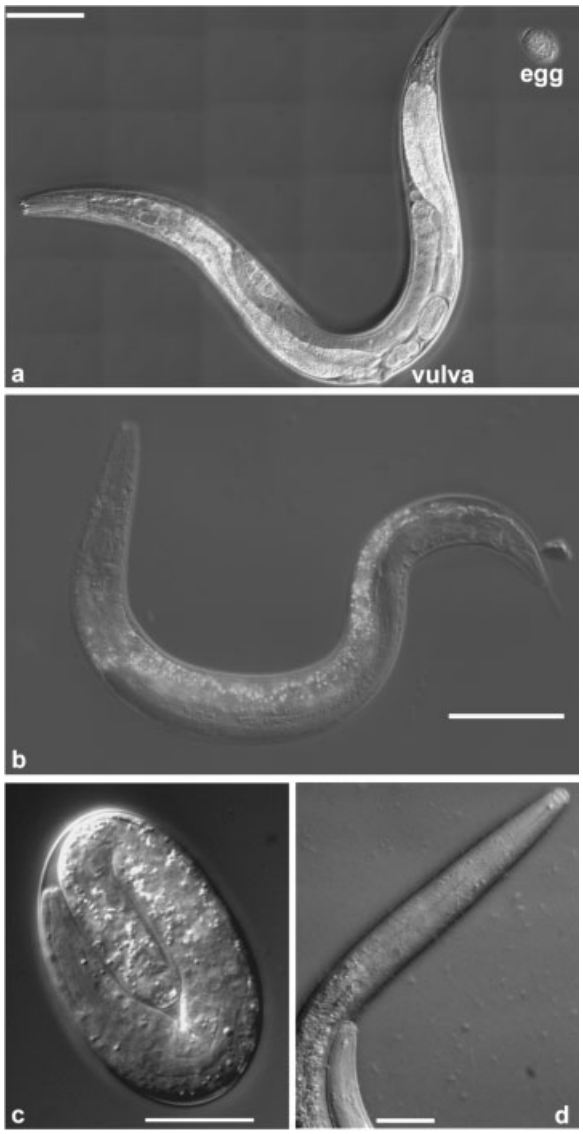

Figure 1. *Pristionchus pacificus*. **a:** An adult hermaphrodite next to an egg. **b:** A young adult male, notice the lack of prominent bursa velum in the caudal papillae. **c:** A J1 larva inside an egg. **d:** Two dauer larvae, notice the enclosed mouth openings. Scale bars represent 100 μm **(a, b)** and 20 μm **(c, d).**

time for stoma morphogenesis compared to the same processes in non-diplogastrids.[6] Hence heterochrony may be a prerequisite for morphological diversity in this evolutionary branch.

Several life history traits make *P. pacificus* an ideal animal for developmental genetics. *P. pacificus* is a self-fertilizing hermaphrodite with occasional 0.1% spontaneous males arising from X-chromosome meiotic non-disjunction.[9] Karyotypic and genetic analyses indicate the presence of six chromosomes including one sex chromosome, as is the case in *C. elegans. P. pacificus* is an omnivore but can be raised on monoxenic OP50 *E. coli* cultures on agar plates. Other assets include its short generation time of 3.5 days at 20°C under standard laboratory conditions, large brood size (200/hermaphrodite), and its ability to be cryo-preserved. *P. pacificus* is also amenable to many standard techniques, such as cell lineaging, laser ablations, mutagenesis (EMS, ENU, UV), deletion library screening and morpholino gene knockdowns. However, RNAi and transgenic protocols are still being developed. The proudest accomplishment in our toolkit is the construction of a high-density genetic linkage map consisting of 560 Single Stranded Length Polymorphic markers (SSCP), which was initially based on BAC-end and fosmid library sequences,[10,11] but is now being integrated into a whole genome assembly from the NHGRI-funded *P. pacificus* genome sequencing initiative (http://genome.wustl.edu/; www.pristionchus.org). One stark genetic difference is the increased genetic distances found in *P. pacificus* when compared to those in *C. elegans* (Table 1). We speculate that this is due to high incidences of double crossovers in *P. pacificus* in contrast to only a single crossover per homologous chromosome in *C. elegans* due to meiotic crossover interference. With regard to genomic structure, there is limited microsynteny within a contiguous 126 kb genomic region, implying extensive intrachromosomal rearrangements between *C. elegans* and *P. pacificus*.[12] However, based on sequence similarity comparisons at the global scale, five of the six *P. pacificus* chromosomes correspond to the same chromosomes in *C. elegans*—with the exceptions of *Ppa*_ChrI mapping to both *Cel*_ChrV and *Cel*_ChrX, while *Ppa*_ChrV has similarity to only *Cel*_ChrI (Table 1). Consequently, although these preliminary results foreshadow limited positional conservation between *C. elegans* and *P. pacificus* to be confirmed by whole genome analysis, the separation of gene arrangements from gene functions will elucidate the role of clusters, polycistrons and intron number on the evolution of gene functions.

A microhistory of *P. pacificus* evo-devo

The evolution of vulva development

Studies in *C. elegans* vulva patterning, like studies of segmentation in *Drosophila melanogaster*, have served as a paradigm for understanding development. In the 1980s, vulva formation received considerable attention because two of the most important developmental principles—induction and competence—could be studied at the cellular level. In particular, the induction of the epidermal vulva by a single mesodermal anchor cell was the first example of signaling across germ layers in nematodes. Furthermore, while genetic analysis in *C. elegans* has acted as a prism to separate the spectrum of genes and pathways involved in the patterning

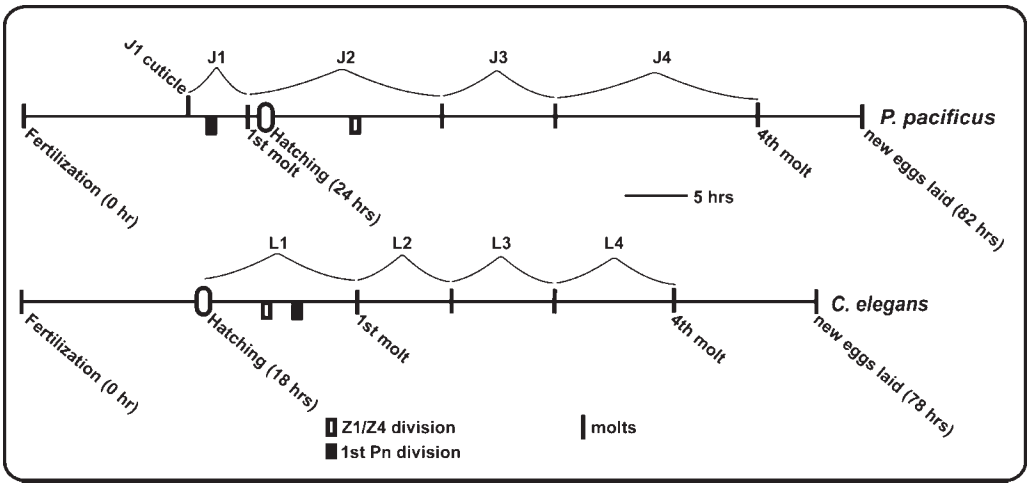

Figure 2. Timelines of life histories in *P. pacificus* and C. elegans at 20°C (adapted from Felix et al 1999 and von Lieven 2005).[6,50] In addition to the pre-hatching molt, other heterochronic differences include the timing of division of the somatic gonad precursor cells (Z1/Z4) as well as first division of the vulva precursor cells (Pn).

process, the comparative analyses to other nematode species provided the necessary perspective to view the plasticity of these processes evolving in nature. Initially, these comparisons were based on careful morphological observations of organ tissues. One would trace part of the cell lineage of the vulva precursor cells, laser ablate these critical cells and determine the consequences of these cell ablations on vulva formation.[13–15] These laser perturbations and passive observations without genetic considerations, however, are limited not only by their dependency on temporal effects and drawing correlative conclusions, but also in their ability to probe the mechanisms of change deeper than the cellular level. Therefore, the urgency to manipulate the development of another nematode at the molecular level prompted the cultivation of tools necessary to conduct genetic studies in *P. pacificus*.[9,15,16] This is in contrast to other satellite model organisms, in which expression patterns and RNA interference provide the bulk of the basis for functional comparisons. We credit the ability to conduct unbiased genetic screens for mutants defective in various aspects of vulva development for

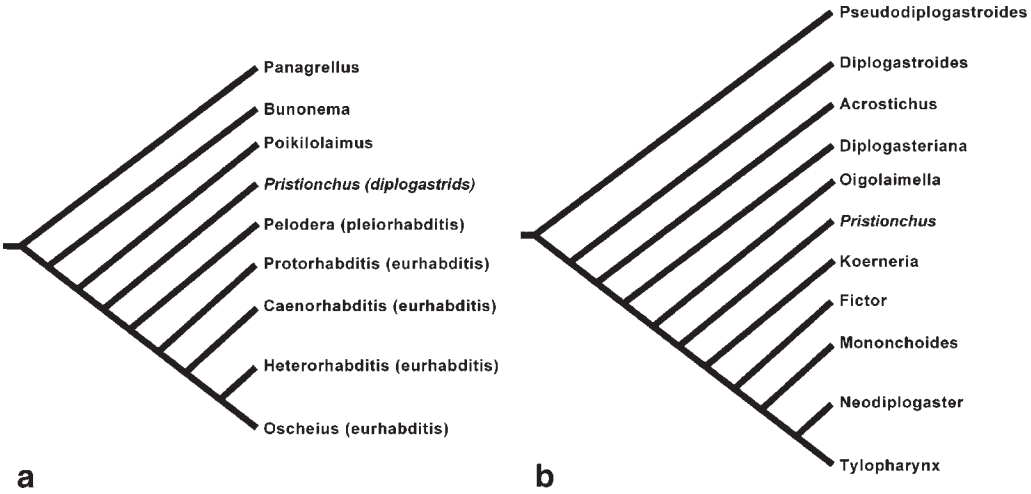

Figure 3. a: An abbreviated rhabditid phylogeny based on the analysis of molecular markers (adapted from Kiontke and Fitch 2005).[4] Names in parentheses do not have taxonomical rank. **b:** A Diplogastridae phylogeny based on morphological apomorphies of each genus. (adapted from Sudhaus and von Lieven 2003).[8]

Table 1. Comparisons of corresponding *P. pacificus* and *C. elegans* chromosomes and their total genetic distances

Number of *Ppa* SNP markers	*P. pacificus* chromosome	*C. elegans* chromosome	*Ppa*_Chr genetic distances (cM)	*Cel*_Chr genetic distances (cM)
110	I	V, X	215	50
76	II	II	168	50
78	III	III	117	50
78	IV	IV	179	50
72	V	I	162	50
65	X	X	185	50

the discoveries of fundamental molecular differences between *C. elegans* and *P. pacificus*.

To delve into specifics, the nematode vulva is an egg-laying organ formed by a subset of the 12 ventral epidermal cells P(1–12).p, following cell-fate specifications and inductive signals from different pathways. In *C. elegans*, further divisions in 6 of these 12 cells are restricted by cell fusion, leaving behind 6 cells P(3–8).p, known as the vulval precursor cells (VPCs), which adopt one of three alternative cell fates (1°, 2°, 3°), with P6.p in the center adopting the 1° fate.[17,18] In contrast, the VPCs in *P. pacificus* consist of only 4 cells, P(5–8).p (Fig. 4; Table 2).[19] More importantly, the limiting of the vulval equivalence group size in *P. pacificus* utilizes apoptosis. The Hox protein *Ppa*-LIN-39 is required for the suppression of apoptosis in the presumptive VPCs.[20] The vulva defect in *Ppa-lin-39* mutants can be suppressed by mutations in the pro-apoptotic gene *Ppa-ced-3*, indicating that, unlike *Cel*-LIN-39, *Ppa*-LIN-39 has a permissive and not an instructive role in vulval induction.[21,22] Another crucial difference in vulva patterning is that, while only the anchor cell induces the vulva in *C. elegans*, the *P. pacificus* vulva is induced by continuous signals from multiple cells of the somatic gonad (Fig. 4; Table 2).[23] These two vulva induction methods are only part of a larger repertoire involving multiple steps and signal sources known in nematodes.[15,24] The mapping of vulval developmental characters such as apoptosis in the ventral cord lineage onto the current molecular phylogeny of rhabditids suggests that it is restricted to only a few distinct monophyletic groups—the diplogastrids, and the *Poikilolaimus* and *Panagrolaimus* genera.[3,19,25] Since *Poikilolaimus* seems to be basal to both the crown eurhabditids and the diplogastrids, we deem the cultivation of species in this genus for genetic studies to be pivotal in addressing the evolutionary processes involved in the use of apoptosis in the VPCs.[26]

Ppa-lin-17, a frizzled homolog, was one of the first mutations that we have positionally mapped using our genetic linkage map.[27] Since then we have also mapped four other mutations involved in vulva patterning. *lin-17* has a common function in both *C. elegans* and *P. pacificus* in the polarization of the P7.p lineage to generate a symmetrical vulva (i.e. to mirror the P5.p lineage). Mutations in *lin-17* result in a bivulva phenotype caused by the unpolarized P7.p descendents.[28,29] In *P. pacificus* however, *Ppa-lin-17* has an additional role in mediating a negative signal to prevent gonad-independent vulva differentiation (Table 2). Although the EGF–RAS–MAPK signaling pathway plays a prominent role in *C. elegans* vulva induction, as evident from numerous mutants found in this pathway (e.g. *lin-3, let-23, let-60, lin-45, mpk-1*), molecular characterization of vulva mutants in *P. pacificus* has so far revealed that the Wnt signaling pathway plays a more prominent role, since morpholino attenuations of molecules homologous to *C. elegans lin-44* (Wnt), *egl-20* (Wnt), *mig-5*

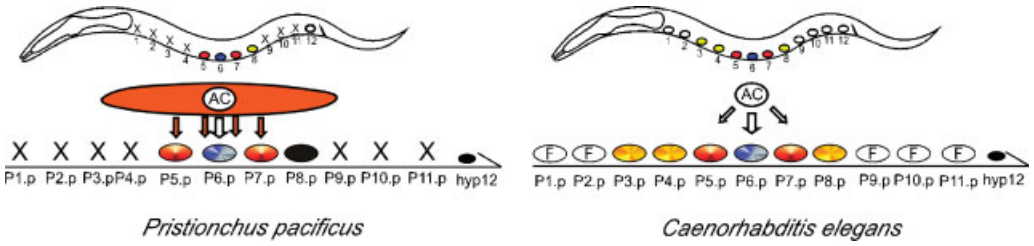

Figure 4. Comparisons of vulval precursor cell fates in *P. pacificus* and *C. elegans*. Blue-filled circles denote 1° fate, red-filled circles 2° fate, and yellow-filled circles 3° fate. P8.p has a unique 4° fate in *P. pacificus*. "X's" represent apoptotic cells while "F's" in open circles represent cell fusions. AC denotes the anchor cell. *C. elegans* requires only the anchor cell for vulva induction, whereas inductive signaling also originate from other gonadal cell in *P. pacificus*.

Table 2. Comparisons of differences in vulval development between *P. pacificus* and *C. elegans*

Vulval developmental feature	*C. elegans*	*P. pacificus*
Number of vulval precursor cells (VPCs)[19]	6	4
Fate of non-vulval VPCs[19]	Cell fusion	Programmed cell death
Vulva induction[23]	Single step (anchor cell)	Continuous (somatic gonad)
Lateral inhibition by the VPC P8.p[49]	Absent	Present
Negative signaling by Wnt[27]	Absent	Present

(Dishevelled), or *bar-1* (β-catenin) in *Ppa-lin-17* mutant background exacerbates gonad-independent vulva differentiation.[27,30] Therefore it remains to be seen whether the EGF pathway plays a redundant role in vulval induction, or is more important in developmental programs other than vulval formation in *P. pacificus*. Efforts are now underway to determine the developmental function of *P. pacificus* molecular homologs of the EGF–RAS pathway by reverse genetics such as TILLING and deletion library screening.

Sex-determination pathways

Another well-known developmental signaling pathway is sex determination. In *P. pacificus*, the first somatic sex determination component isolated was the GLI-family zinc-finger transcription factor TRA-1A.[5] *tra-1* loss-of-function mutations transform XX hermaphrodites to males in both *C. elegans* and *P. pacificus*, although the overall amino acid sequence identity outside of the conserved C2H2 zinc-finger domain is a scant 5%. Since TRA-1A is the most downstream global sex regulator in *C. elegans*, functional conservation of these TRA-1A homologs in such distantly related nematodes is therefore surprising. The impending molecular identification of more transformer mutants may answer whether or not the rapidly evolving sex determination pathways in nematodes support the retrograde evolution model.[31] Since core factors in the somatic pathway are also involved in germline sex determination as well, identifying factors in both pathways will ultimately aid in our understanding of the changes intrinsic to the nematodes' reproductive strategy. Hermaphroditism appears to have evolved independently in *C. elegans* and *C. briggsae*, and genetic analyses supports multiple components involved in these convergences.[32–34] The *Pristionchus* genus phylogeny exemplifies this vacillation between the hermaphroditic and gonochoristic reproduction modes more frequently, thus allowing additional comparisons of mating systems among closely related species (Fig. 5).

Gonad morphogenesis

The nematode gonad is another highly variable organ available for developmental and cellular dissection in a comparative context. Proper gonadogenesis ensures the differentiation of somatic tissues necessary to support not just the germ line, but also the regulation of vulva induction. Gonads in females and hermaphrodites are generally composed of two rotationally symmetrical reflexed tubular arms, while males and some female/hermaphrodite species have a single gonadal arm. Meticulous cellular and SEM analyses of the *P. pacificus* hermaphrodite gonads have uncovered numerous differences to *C. elegans*.[35] These distinctions in *P. pacificus* include the novel ventral path of distal arm migration, the more elaborate sheath cell processes along the arms, and the reduced dependence of the germ cells on somatic gonad tissues. Present studies focus on the signals responsible for the ventral migration of the *P. pacificus* gonad by forward and reverse genetic methods.

Diversity and ecology of *Pristionchus* species

True to its name, *P. pacificus* was first isolated in 1988 by a high-school student from a garden in Pasadena, California, and then subsequently also by others in Port Angeles, Washington, locations that are just breaths away from the Pacific Ocean.[9] Interestingly, this hermaphroditic member of the diplogastridae family of nematodes was never described previously in literature, although its relatively slimmer body size and hermaphroditic reproductive trait could have been easily discernable from its stockier hermaphroditic brethren, *P. maupasi*, as well as from its gonochoristic brethren, *P. lheritieri*, had any nematologist previously isolated it. To date, 11 additional natural isolates have been found throughout North America, from Hawaii to New York, as well as other parts of the world, Madagascar, China, Japan, and Poland. Despite our earnest sampling efforts, the Poland isolate represents the only one of European provenance. More curiously, although the Poland strain is indiscriminable from the California isolate even by Amplified Fragment Length Polymorphism markers (AFLP), there are several differences in their VPC competences as revealed by ablation experiments.[36] Therefore, this Poland isolate enables us to study microevolutionary changes at the cellular level, whose molecular mechanisms may ultimately be uncovered genetically by Quantitative Trait Locus analysis (QTL) when compared to the Washington strain (Zauner and Sommer, unpublished results).

As was the case for *C. elegans*, we did not originally know where *P. pacificus* lives in the wild. Therefore in the last couple of years, we began exploring the ecology of *Pristionchus*

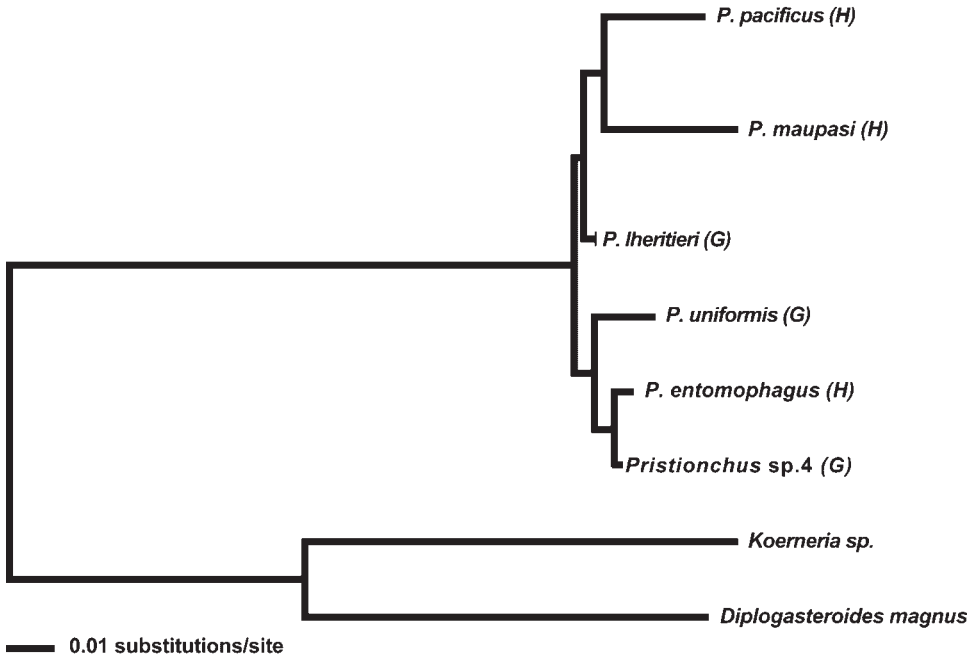

Figure 5. A *Pristionchus* maximum likelihood neighbor-joining tree constructed using the 18S SSU ribosomal gene. Each node is supported by >50 bootstrap value, with *Koerneria* and *Diplogasteroides* as outgroup species (Courtesy of W. Mayer). *Pristionchus* sp. 6 mentioned in the text is not included. ''H'' denotes hermaphroditic species while ''G'' denotes gonochoristic species.

species. We found that many *Pristionchus* species associate as dauer larvae with western European scarab beetles and disembark when the beetles are sacrificed on agar plates seeded with *E. coli*. Multiple molecular markers followed by mating tests were employed to discriminate and construct their phylogenetic relationships. In the first comprehensive study, 371 isolates belonging to six species were found, although *P. pacificus* was not among them (Herrmann, Mayer and Sommer, unpublished data). Of the six species identified, only two had scientific names with molecular entries in GenBank (*P. maupasi, P. lheritieri*), while two others were unknown (*Pristionchus* sp. 4 and sp. 6). The two remaining species were recognized as *P. entomophagus* and *P. uniformis* since their biology resemble previous descriptions (Fig. 5).[37,38] *P. entomophagus* associated mainly with dung beetles (average ~19% of *Geotrupes stercorosus*, up to 70% in certain populations) while *P. uniformis* was found chiefly with Colorado Potato Beetles (average ~16% of *Leptinotarsa decemlineata*). Subsequent sampling of North American scarab beetles also yielded both known and undescribed species, this time including several *P. pacificus* isolates (Herrmann, Mayer, and Sommer, in press).

The sheer number of the estimated nematode species on this planet has prompted nematologists to speculate that they occupy an equally diverse number of ecological niches. Species in the *Caenorhabditis* crown clade can be found predominantly in anthropogenic habitats in soil and compost samples (*C. elegans* and *C. briggsae*), in casual association with terrestrial invertebrates such as snails and isopods (*C. briggsae* and *C. remanei*), as well as in intimate relationship with specific arthropods (*C. drosophilae* on *Drosophila nigrospiracula*). Various nematode–invertebrate associations are distinguished by the apparent function of their hosts. In the case of *C. remanei*, the dauer larvae embark onto the associated animal and wait for the animal to die to resume development on the nutrient-rich decomposing cadaver, an association known as necromenic. In the case of *C. drosophilae*, the nematodes use the associated fly as a means of transportation only and do not feed on the carcass, an association known as phoretic.[39] *C. elegans* is usually considered to be phoretic, although some reports stress a necromenic association whereby the worms propagate directly on the host cadaver.[40] Despite the prevalence of *C. elegans* as a model nematode for many aspects of its biology, its natural habitat outside of human environments such as garden composts remains largely unknown.[41]

When we apply these criteria on *Pristionchus* species, our preliminary data support most *Pristionchus* species to be necromenic. While we have not yet ruled out associations to other arthropods or gastropods found in several cases to host *Caenorhabditis* species, we can confidently say that no *Caenorhabitis* species and begrudgingly few eurhabditids at all have been found on the beetles that harbor *Pristionchus* species. Nevertheless, continued sampling of other

invertebrates will be required. In addition, questions regarding the life histories and specificities of these associations have hardly yet been broached. Do *Pristionchus* species embark on the beetles only as dauer larvae or also as gravid hermaphrodites/females? If only as dauer larvae, are they cruisers or ambushers (both are observed in laboratory)? How do the nematodes navigate toward their target hosts? What are the general and specific host cues? These are the line of inquiries that we hope address by examining *P. pacificus* dauer larvae formation and olfaction in more detail.

Sense and sensitivity

Although both *C. elegans* and *P. pacificus* can be maintained in the laboratory on agar plates seeded with *E. coli*, it is unlikely that either of these species feed on monoxenic bacterial cultures in the wild. While *C. elegans* may be a general bacterial feeder in composts, our collective laboratory observations suggest that *P. pacificus* can additionally consume other nematodes (including its own kind), as well as fungi, such as the insect-pathogenic *Beauveria bassiana*. These observations under laboratory conditions are consistent with our sampling results showing that *Pristionchus* species spend at least part of its life cycle on beetles, where both fungi and competing nematodes occur together. One possibility for this wider range of food could be *P. pacificus*' ability to mature into two mouth forms, the broader, shallower eurystomatous form or the narrower, longer stenostomatous form (Fig. 6).[7,26] This dimorphism of the buccal cavity was also observed in *P. lheritieri* as well as two other diplogastrid genera.[7,24] However, it is yet uncertain whether the dimorphism depends on a committed developmental switch akin to dauer larvae formation, or is a stochastic process occurring during molting. In contrast to a regimented vulva development, the highly variable buccal structures represented by many members of the diplogastrids may be a dimorphism crucial for the expansion of their ecological niches. As a first step towards understanding this complex trait, we are starting to investigate the genetic and environmental factors controlling the mouth form dimorphism in *P. pacificus*.

P. pacificus' natural resistance towards *E. coli* expressing the *Bacillus thuringiensis Bt* toxin offers another example of a different natural food source from *C. elegans*.[43] Wei et al. suggested that *P. pacificus* could be resistant to the Bt toxin due to the lack of a grinder in the posterior bulb of the pharynx, since purified *Bt* crystals can lower fecundity in *P. pacificus*. Nevertheless, even the purified *Bt* toxin did not kill *P. pacificus*, which we contend represents an altogether different digestion mechanism for *P. pacificus* compared to the *Bt* susceptible nematodes, i.e. the lack of glycolipid receptors found in *C. elegans* gut.[44] Not surprisingly, electron micrographs of laboratory-raised *P. pacificus* gut show that most of the bacterial membrane coat remains intact in the gut (M. Riebesell, personal communication). Similarly, *P. pacificus*' resistance to the encapsulated fungal pathogen *Cryptococcus neoformans* may also lie with *P. pacificus*' ability to extract nutrients from these microorganisms without physically rupturing their cell membranes (R. May, pers. comm.). Further investigations into the biochemical milieu of *P. pacificus* gut will be necessary to begin understanding *P. pacificus*' resistance to several biological toxins found in soil. In contrast to its resistance to several biological toxins, *P. pacificus* could be a canary for chemical soil toxicity since *P. pacificus* is more sensitive to copper than *C. elegans* and *Panagrellus redivivus*.[45] Indeed taken all together, the stark contrast between the sensitivities of *P. pacificus* and *C. elegans* to environmental toxins reflect different adaptations to their wild habitats whence they evolved.

If *P. pacificus* eats and cohabits with different microorganisms than *C. elegans*, it follows that *P. pacificus* must also sense different volatile cues in the environment. Although

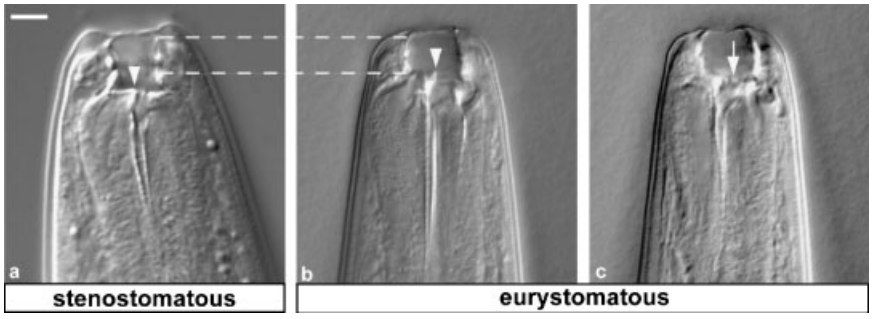

Figure 6. *P. pacificus* mouth dimorphisms in adult hermaphrodites. Dorsal is left. **a:** stenostomatous mouth form with a slightly longer buccal cavity (compared the distance between the parallel dashed lines and the width of the buccal cavity) and a flint-like dorsal tooth (arrow head). **b–c:** eurystomatous mouth form with a broader buccal cavity on the same individual at different focal planes. Notice a claw-like dorsal tooth (**b**, arrow head), and an adjacent row of denticles (**c**, arrow). Scale bar represents 5 μm in all panels.

C. elegans can sense a wide range of compounds over several magnitude differences in concentration,[46] the connection between their compounds sensitivity and ecological niche have not been investigated. In contrast, we have begun to test a similar wide array of purified compounds in their attractiveness to *P. pacificus* and have discovered both qualitative and quantitative differences when compared to *C. elegans*. Compounds attractive to both species are only attractive within 100-fold concentration ranges for *P. pacificus*, unlike the 10,000-fold range often encountered for *C. elegans* attractants. Furthermore, *P. pacificus* is keenest on volatile compounds implicated for plant–insect or insect–insect communication, most of which are not attractive to *C. elegans*. We hope that our analyses of *P. pacificus* chemosensory mutants through forward genetic screens will elucidate the molecular basis for attaining new senses towards environmental compounds in the context of ecology and evolution.

Whole genome initiatives and more

If organisms are simply ephemeral media for the expression of their genomes, which in turn are adaptive solutions to their environments, then comparing genomes at the molecular level can shed some light on the ecological evolution of these organisms. In favor of this agenda, we live in a fortunate era of whole genome sequencing (WGS) of various nematodes. This current bonanza includes the draft genomes of vertebrate parasites *Haemonchus contortus* (Sanger), *Brugia malayi* (Sanger), and *Trichinella spiralis* (WashU), the entomopathic parasite *Heterorhabditis bacteriophora* (WashU), and the plant parasite *Meloidogyne hapla* (USDA and NSF). *Caenorhabditis* species such as *Caenorhabditis japonica* (WashU), *Caenorhabditis* sp. c.f. PB2801, *Caenorhabditis remanei* (WashU) are also being sequenced to compare with the already completed genomes of *C. elegans* and *C. briggsae*.[47] Earlier studies of more than 250,000 ESTs from 30 nematode species other than *C. elegans* indicated a very diverse transcriptome, with 45% of genes from each species lacking a non-nematode homolog and 23% of the genes unique to the species sampled.[48] Having whet our appetite, we expect the WGS to reveal even more secrets of nematode genome diversity.

Concurrent with the other genome initiatives, a high-quality 4–8× coverage of the WGS of the *P. pacificus* California reference strain is nearly complete (http://genome.wustl.edu/). These data alone will accelerate the assembly of BAC contigs in our existing physical map used for positional mapping. To complement this effort, the Max Planck Society has commissioned additional 1–2× coverages of the *P. pacificus* Washington mapping strain, along with two related hermaphroditic species *P. maupasi* and *P. entomophagus*. The Washington strain sequence contains an estimated 3% SNPs to expedite the design of mapping markers, while the sequences of the sister species should provide valuable information on *cis*-regulatory elements and gene organization. Finally, we have ~12,500 ESTs to support many of the ensuing gene predictions from the genome. In addition to the priority issues such as gene content and rearrangements, we hope to further glean from the WGS *Pristionchus*-specific genes that may underlie adaptations in necromeny and pathogen resistance. Taken altogether, these genome comparisons on both species and phyla level will allow us to address not just questions particular to nematodes, such as the evolution of parasitism and nematode-specific genes, but also questions about the evolution of genome size, lateral gene transfer and chromosome structures, which are relevant to all eukaryotes.

Conclusion

In retrospect, the choice of *P. pacificus* was foremost on account of its developmental differences to eurhabditids and its suitability as a molecular genetic model. In the 10 years that our laboratory has worked on *P. pacificus*, we have focused on vulva and gonad development, as well as sex determination. With the advent of the *P. pacificus* genomic era, the investment in the genetic linkage map is coming to full fruition and lending confidence for engaging in more challenging topics. We hope that studies in *P. pacificus* may serve as a crucible for understanding not just developmental evolution, but also animal behavior and ecology, topics that we are just beginning to investigate using the model genus *Pristionchus*.

Acknowledgments

We would like to thank W. Mayer and M. Herrmann for sharing unpublished results, C. Dieterich for whole genome analysis, and D. Rudel and the anonymous reviewers for critical reading of this manuscript.

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