

1 The Genus *Pristionchus*: a Model for Phenotypic Plasticity, Predatory Behavior, Self-Recognition and Other Complex Traits

Ralf J. Sommer* and James W. Lightfoot
Max-Planck Institute for Biology, Tübingen, Germany

Introduction

Pristionchus pacificus is a soil nematode that was first described in 1996 based on an original isolate from Pasadena, California (Fig. 1.1A) (Sommer *et al.*, 1996). As a hermaphroditic species with the spontaneous formation of males, it shares many of the key characteristics that were essential for establishing *Caenorhabditis elegans* as a model organism in the first place. For example, *P. pacificus* grows well under laboratory conditions when provided with *E. coli* OP50 as food on standard agar plates. At 20°C, the generation time is about 4 days and hermaphrodites produce around 150 self-progeny, which allows about 100 generations of worms to be propagated annually in the laboratory (Fig. 1.1B). Mating of young and old adult hermaphrodites with males will result in outcrossing and worms can be frozen in liquid nitrogen indefinitely. All these features combined made *P. pacificus* a prime target as a satellite organism for studies in evolutionary developmental biology (evo-devo), which was a new discipline emerging at that time, and for a detailed comparison with *C. elegans*.

Indeed, *P. pacificus* lived up to its promise, as many of the major techniques that resulted in the success of *C. elegans* as a model system were implemented in *P. pacificus* over the years. First, forward genetics using various mutagens resulted

in the isolation of morphological and developmental mutants offering the possibility to investigate any biological process in an unbiased manner (Sommer and Sternberg, 1996; Ogawa *et al.*, 2009; Ragsdale *et al.*, 2013). This advantage is still important in the contemporary analysis of the evolution of complex traits (see below). Second, DNA-mediated transformation techniques were established, although these protocols require more sophistication than in *C. elegans* and necessitate the involvement of complex arrays (Schlager *et al.*, 2009). Third, reverse genetic tools were implemented with CRISPR-Cas9-based technologies largely outcompeting all previous methods (Witte *et al.*, 2015; Nakayama *et al.*, 2020). Fourth, a sophisticated “omics” toolkit has been developed involving genome sequencing and resequencing by single-molecule sequencing technologies (Dieterich *et al.*, 2008; Rödelsperger *et al.*, 2017), proteomics (Borchert *et al.*, 2010), RNA sequencing-based transcriptomics (Rödelsperger *et al.*, 2018), and epigenomics (Werner *et al.*, 2018a,b). All these genomic features are visualized on the websites www.pristionchus.org and www.wormbase.org, accessed 16 March 2022.

Given the strong focus on evolutionary questions, early *Pristionchus* research aimed to identify the ecological niche of these nematodes. While *Pristionchus* worms can be found in soil

*Email: Ralf.sommer@tuebingen.mpg.de

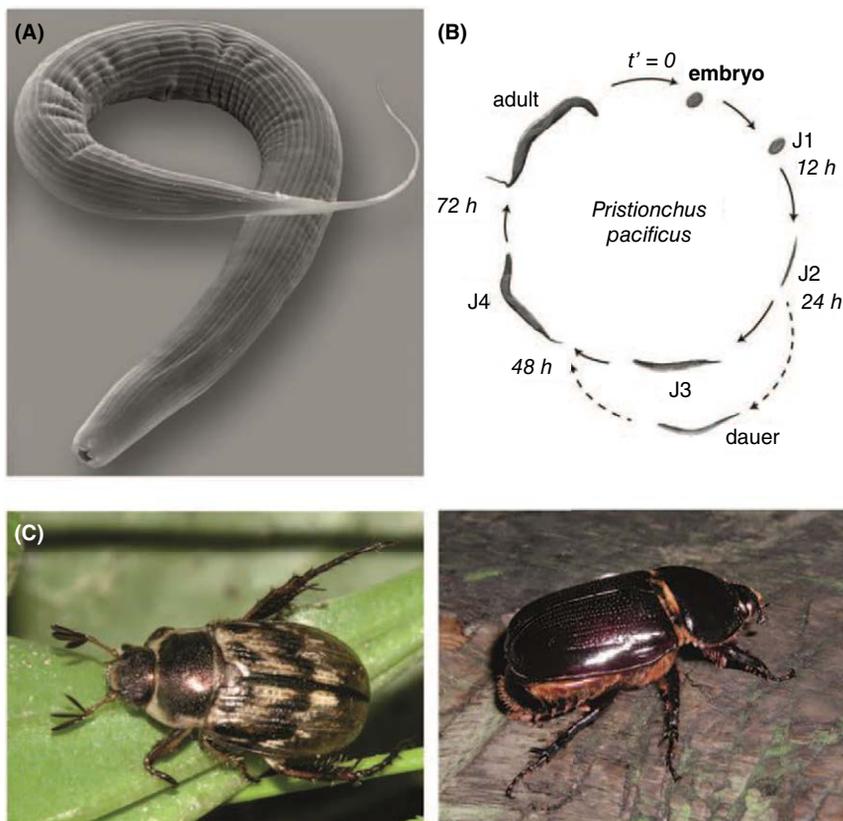


Fig. 1.1. An introduction to *Pristionchus*. **(A)** An SEM image of the main laboratory wild-type isolate of *Pristionchus pacificus*, PS312, now utilized in labs around the world. **(B)** *P. pacificus* life cycle with a generation time of roughly 4 days. Like all species of the family Diplogastridae, in *P. pacificus* the J1 stage is not free-living and remains confined to the egg. There is therefore an embryonic molt with the J1 larvae molting to the J2 stage before hatching from the egg. Redrawn from Werner *et al.* (2020). **(C)** In addition to being retrieved in soil samples, *Pristionchus* nematodes show a frequent association with beetles, including the Oriental beetle *Exomala orientalis* (left) and the rhinoceros beetle *Oryctes borbonicus* (right). Pictures courtesy of M. Herrmann.

samples from around the world, the most reliable source for *Pristionchus* nematodes is scarab beetles, including chafers, stag beetles, and dung beetles (Fig. 1.1C). This association, after being first discovered in central Europe (Herrmann *et al.*, 2006), resulted in the identification of the Oriental beetle *Exomala orientalis* in Japan as a common host of *P. pacificus* (Herrmann *et al.*, 2007). After more than a decade of intense samplings around the globe, around 50 *Pristionchus* species are available and additional host species from soil settings, such as millipedes, have been discovered (Kanzaki *et al.*, 2018). One unique feature of *Pristionchus* nematodes is the independent convergent evolution of hermaphroditism at

least seven times in this genus, allowing unique insight into genome evolution (Rödelsperger *et al.*, 2018).

Alongside these studies, *P. pacificus* has been developed into a prime model organism in various research areas from developmental genetics, population genetics, genomics, and bioinformatics to evolutionary ecology, behavioral ecology, epigenetics, and neurobiology. This approach always aimed for an integrative analysis of evolutionary biology (Sommer, 2009). The following contribution will provide a brief overview on original studies in evo-devo and *Pristionchus* phylogeny, ecology, and life history. The center of this contribution is the more recent work using

P. pacificus as a model system for complex trait formation and the evolution of novelty. This approach largely uses the unique features of *Pristionchus* nematodes, such as mouth-form plasticity, predatory behavior and self-recognition, all of which are unknown from *C. elegans* and most other nematode species.

Evo-devo Beginnings and Developmental Systems Drift

The development of *P. pacificus* as a model system for comparative studies has to be seen in the context of the evo-devo movement in the late 1980s and early 1990s. Historically, zoologists had relied on developmental processes and morphological structures to explain animal phylogeny. However, with the Neo-Darwinian synthesis the importance of development for evolutionary research and for explaining evolutionary patterns had faded away. A strong revival of evolutionary developmental biology only occurred when developmental genetics started to provide mechanistic insight into development. This was first achieved for segmentation in the early embryo of *Drosophila*, which initiated a wave of comparative studies (Patel *et al.*, 1989; Sommer and Tautz, 1993). This was later followed by investigations of various postembryonic processes in multiple insects and other arthropods (for review, see Carroll *et al.*, 2001).

In nematodes, the first comparative investigation was the complete postembryonic cell lineage analysis of *Panagrellus redivivus* (Sternberg and Horvitz, 1981, 1982), to be followed by the analysis of early embryogenesis in multiple nematodes, such as *Acrobeloides* or *Diploscapter* (for review, see Schierenberg and Sommer, 2014). When the molecular mechanisms associated with *C. elegans* vulva development were being identified, systematic comparative studies were initiated using cell lineage analysis and experimental cell ablation studies, involving around a dozen species of multiple genera and families of nematodes (Sommer and Sternberg, 1994, 1995, 1996). While some of them showed striking deviations in development relative to *C. elegans*, these species were exclusively either gonochorists, with a male–female mode of reproduction, or parthenogenetic. Thus, they missed the hermaphroditic

mode of reproduction that would eventually enable easy genetic and experimental manipulation. The early choice of *P. pacificus* as a satellite species was based on the fact that this species shared with *C. elegans* hermaphroditic reproduction and other technical features (see above), but at the same time differed in various developmental features, including vulva and gonad development (Sommer and Sternberg, 1996; Rudel *et al.*, 2005). Thus, *P. pacificus* combined the prospect of mechanistic insights with evolutionary diversification of developmental processes.

Already the description paper of *P. pacificus* as a novel species provided the proof of principle of its genetic accessibility, by describing the isolation of first *dumpy* (*Dpy*) and *uncoordinated movement* (*Unc*) mutants based on *C. elegans*-type mutagenesis experiments (Sommer *et al.*, 1996). The first systematic mutagenesis experiment for *P. pacificus* vulva development was published in the same year (Sommer and Sternberg, 1996), and the first mutant-based developmental control gene to be cloned was the Hox gene *lin-39* just 2 years later (Eizinger and Sommer, 1997). During the next decade, systematic cell ablation studies, saturation-based mutagenesis experiments combined with map-based cloning and reverse genetics provided detailed insight into the molecular mechanisms of *P. pacificus* vulva development, which are summarized for the interested reader in Fig. 1.2 (Tian *et al.*, 2008; Wang and Sommer, 2011). Together, these studies resulted in the unexpected finding that vulva development in *P. pacificus* and *C. elegans* is a prime example of what is now called “developmental systems drift”: while the vulva of these two species is a homologous organ and is formed from homologous precursor cells, the underlying molecular mechanisms and regulatory logic are fundamentally distinct. Most importantly, vulva development in *C. elegans* is induced by EGF/Ras signaling (for review, see Sternberg, 2005), whereas in *P. pacificus* it relies on Wnt signaling with several different ligands that are expressed in multiple different cells and body regions (Tian *et al.*, 2008; Wang and Sommer, 2011). Developmental systems drift was first proposed as a general principle by True and Haag (2001) largely based on the evolutionary analysis of sex determination. By now, it can be considered to represent one of the two key principles of the evolution of developmental

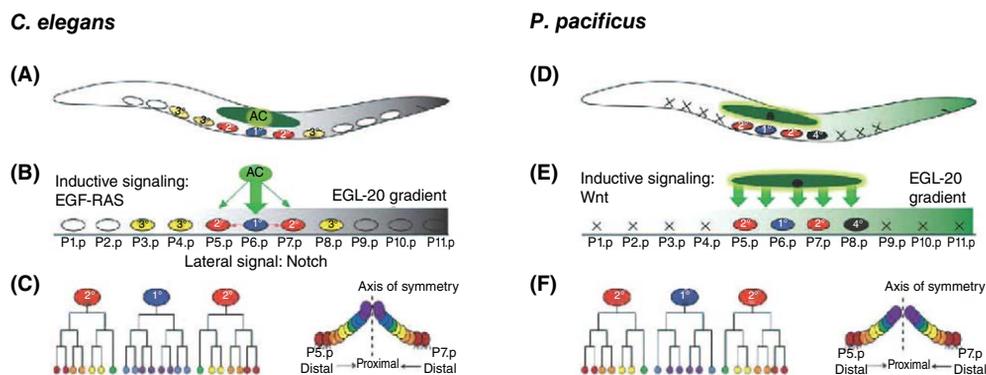


Fig. 1.2. Comparison of vulval development between *C. elegans* (left) and *P. pacificus* (right). The vulva develops from the three precursor cells P(5–7).p of the ventral epidermis (A,D). In *P. pacificus*, the anterior and posterior precursor cells P(1–4,9–11).p die of programmed cell death, whereas in *C. elegans* these cells fuse with the hypodermal syncytium hyp7. In *C. elegans*, vulva formation by P(5–7).p is induced by a signal from the gonadal anchor cell (AC) (green) and requires epidermal growth factor (EGF)-RAS signaling (green) (B). P6.p (blue) has a 1° cell fate, generates eight progeny and forms the central part of the final vulval structure (C). P(5,7).p have a 2° cell fate through *lin-12*/Notch signaling, and form seven progeny and the anterior and posterior part of the final structure, respectively. In *P. pacificus* in contrast, vulval formation is induced by Wnt signaling and requires multiple Wnt ligands, including EGL-20 from the posterior body region and Wnt ligands expressed in the gonad (green) (E). Thus, *P. pacificus* vulva induction differs from *C. elegans* in: (i) the inducing signaling pathway; (ii) number of involved ligands; and (iii) cellular basis of signal induction (B). P(5–7).p form 2°-1°-2° cell fates again and produce seven, six, and seven progeny, respectively (F). Together, the example of vulva formation indicates developmental systems drift: homologous cells form a homologous structure relying on different signaling pathways and molecular mechanisms.

processes together with *cis*-regulatory evolution. While *cis*-regulatory evolution explains the co-option of conserved developmental control genes in independent developmental processes and in multiple species and phyla (Raff, 1996; Carroll *et al.*, 2001), developmental systems drift provides a complementary perspective: even homologous structures are often regulated by non-homologous molecular mechanisms. The detailed comparison of *P. pacificus* vulva development to that of *C. elegans* helped to identify developmental systems drift as a major principle in evo-devo.

Rapid Genome Evolution and Taxonomically Restricted Genes

While the *C. elegans* genome is around 100 Mb in size and contains around 20,000 genes (Worm-Base), the original sequencing of the *P. pacificus* genome in 2005 revealed that it has a size of roughly 170 Mb and contains more than 25,000

genes (Dieterich *et al.*, 2008). This has subsequently been further refined through single molecule resequencing and improved gene annotation pipelines suggesting a total of around 28,000 genes in *P. pacificus* (Rödelsperger *et al.*, 2017, 2019). Most strikingly, only 25% of all *P. pacificus* genes share 1:1 orthologs with *C. elegans*, which includes many of the developmental control genes that are involved in developmental systems drift. In contrast, the overwhelming majority of genes have undergone more recent gene birth and death events (Markov and Sommer, 2015). Even more surprising was the discovery that around one-third of all *P. pacificus* genes have no similarity to genes in *C. elegans* or other nematodes (Dieterich *et al.*, 2008). This finding has survived the test of time, with more comprehensive nematode sequencing validating the high frequency of genes appearing to be unique within a species. Most recently, comparative genomics of eight *Pristionchus* species with two closely related outgroups revealed that gene evolution is an ongoing steady process (Prabh

et al., 2018). Thus, a substantial fraction of genes undergoes rapid evolutionary diversification or evolves *de novo*, resulting in so-called orphan or taxonomically restricted genes (for review, see Rödelsperger *et al.*, 2019).

The Phylogeny of the Genus *Pristionchus*

Finding *Pristionchus* reliably in association with scarab beetles (Herrmann *et al.*, 2006), but also other arthropods, resulted in a systematic analysis throughout the world to isolate new specimens. Fortunately, the majority of soil- and arthropod-derived *Pristionchus* species which have been discovered thus far can easily be cultured in the laboratory and can be used in crossing experiments. This has made this genus a prime model for nematode taxonomy and systematics. Indeed, for reasons that are still not fully understood, *Pristionchus* nematodes show a strong positive correlation between DNA sequence divergence of the SSU ribosomal spacer and species status. Isolates with more than 1 nucleotide divergence out of a 700 bp fragment of the SSU that is commonly used for species identification usually fail to produce progeny after crossing, indicating species status (Mayer *et al.*, 2007, 2009). In all such cases, detailed morphological and by now genomic investigations later confirmed these observations (for review and recent examples, see Ragsdale *et al.*, 2015). The most recent study of this kind described nine novel species of *Pristionchus* from mainland China after two sampling trips in Yunnan and Shaanxi provinces (Kanzaki *et al.*, 2021). One of these nine novel species is another hermaphroditic species, which represents the eighth hermaphrodite in this genus, a record for nematodes. Note that, unfortunately, the positive correlation between SSU sequence divergence and species status does not hold in other nematode genera, preventing the idea of using a strict molecular barcode as early indicator of the result of crossing experiments.

The known *Pristionchus* species fall into six “clades” or “species-groups” that show striking biogeographic features (Fig. 1.3). First, the four major clades are the *maupasi*-, the *lheritieri*-, and the *pacificus*-groups, which are largely restricted to North America, Europe, and Asia, respectively,

and the *triformis*-group (Fig. 1.3). Second, biogeographic restrictions are seen for most gonochoristic species in these groups, whereas the hermaphrodites escape these restrictions and are mostly cosmopolitan. This likely results from easier dispersal mechanisms, because single dauer larvae of hermaphrodites are more likely to successfully invade a new region by hitchhiking with insect vectors. Third, all basal species of the *fissidentatus*- and *elegans*-groups are found in Asia, which suggests that the genus has evolved in Asia (Fig. 1.3). Indeed, the outgroup to *Pristionchus*, the genus *Parapristionchus*, is also found on scarab beetles in Asia (Kanzaki *et al.*, 2012). Note that a second branch of *Pristionchus* species has evolved after a basal host switch to figs and fig wasps. While these species show an extraordinary polyphenism of up to five distinct mouth-forms, these species are not culturable in the laboratory (Susoy *et al.*, 2016). Therefore, no RNA-seq-based transcriptomics are available for any of these species; thus, they are not included in the *Pristionchus* phylogeny depicted in Fig. 1.3. Finally, it is also important to note that *Pristionchus* sampling is far from saturation and completion, as the recent studies in Taiwan and China indicate (Kanzaki *et al.*, 2018; Yoshida *et al.*, 2018; Herrmann *et al.*, 2019; Kanzaki *et al.*, unpublished observation).

Due to the independent evolution of self-fertilizing hermaphroditism from gonochoristic (male/female) ancestors at least seven times throughout the genus *Pristionchus*, it is possible to assess the impact of these mating type switches on other aspects of biology. Indeed, by investigating 11 species (six gonochorists, five hermaphrodites) it has been observed that lifespan is considerably shorter in hermaphrodites relative to closely related females (Weadick and Sommer, 2016a). Also, females outlived males, with *P. expectatus*, the sister species of *P. pacificus*, providing an extreme example as females have a maximum lifespan of 137 days and males only 76 days (Weadick and Sommer, 2016a). However, *P. expectatus* exhibits an unusual sex-specific reproductive senescence as females lose the ability to reproduce on average after 4.7 weeks, although they expressed a median lifespan of 12.3 weeks (Weadick and Sommer, 2016b). In contrast, males lost their reproductive capabilities after 6.6 weeks with a median lifespan of 7.6 weeks (Weadick and Sommer, 2016b). These

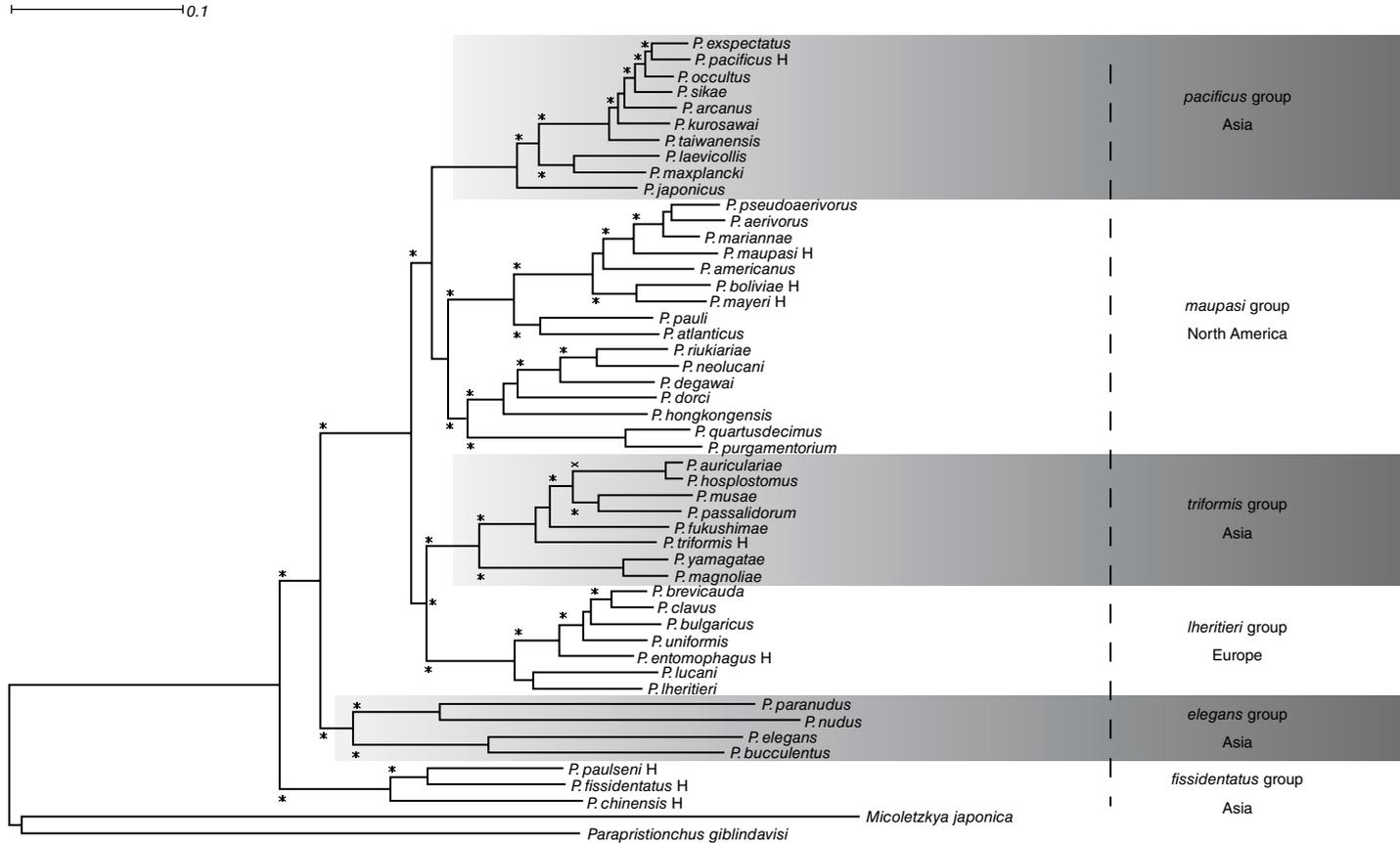


Fig. 1.3. Current phylogeny of the genus *Pristionchus* with 48 species, all of which are in living culture, and the outgroups *Paraprisionchus giblindavisi* and *Micoletzky japonica*. This maximum-likelihood tree is based on transcriptomic datasets resulting in a supramatrix of 350,000 concatenated amino acids. Six species groups or clades can be distinguished that show biogeographic restrictions. Note that only one part of the *maupasi*-group is restricted to North America. Eight species show a hermaphroditic mode of reproduction (H). All other species are gonochorists (dioecious) with males and females. Given the distribution of these hermaphroditic species, hermaphroditism has evolved at least seven times independently in this genus, a record for nematodes. Asterisks indicate strong bootstrap support. Redrawn with modifications from Kanzaki *et al.* (2021).

findings established *Pristionchus* nematodes with their parallel evolution of mating type switching as model system for sex-specific senescence and post-reproductive survival (Weadick and Sommer, 2017).

The Life History and Ecology of *P. pacificus*

P. pacificus is a nearly cosmopolitan species that exhibits a huge genetic diversity with four major lineages as revealed by mitochondrial and microsatellite markers (Zauner *et al.*, 2007; Herrmann *et al.*, 2010). Note that Europe is the only continent where *P. pacificus* is not frequently found with only four isolates available. In contrast, samplings in North America, Bolivia, New Caledonia, South Africa, and several countries in Asia resulted in a huge collection of *P. pacificus* strains. Additionally, the first exploration of an island system revealed that the Mascarene island of La Réunion harbors *P. pacificus* in high abundance and on multiple beetle hosts (Herrmann *et al.*, 2010). La Réunion is the youngest (2–3 Ma) of the Mascarene islands and is known as a major biodiversity hotspot (Myers *et al.*, 2000; Thébaud *et al.*, 2009). Surprisingly, the genetic diversity of *P. pacificus* on La Réunion island mimics that of the rest of the world, arguing for multiple independent invasions, most likely in conjunction with different beetle hosts (Morgan *et al.*, 2012). These originally unexpected findings provided unique research opportunities in two different directions. First, intense population genetic studies coupled with mutation accumulation lines experiments revealed a nuclear mutation rate of $1.4\text{--}2.6 \times 10^{-9}$ and a diversity of the four major *P. pacificus* lineages of 4.3×10^6 generations (Molnar *et al.*, 2011; McGaughran *et al.*, 2013; Weller *et al.*, 2014). This divergence likely preceded the colonization of La Réunion island. Indeed, further studies of the different *P. pacificus* lineages on the island revealed complex demographic patterns also shaped by independent migrations (Morgan *et al.*, 2012; McGaughran *et al.*, 2013, 2016). Such migrations included recent events between La Réunion and the neighboring island Mauritius, which with 10–12 Ma is substantially older (Morgan *et al.*,

2014). Second, the geological, geographic, and ecological diversity of La Réunion with its extreme altitude and temperature differences allowed further investigations of how environmental factors influence population structure (McGaughran and Sommer, 2014). Not surprisingly, climatic variables are the most important factors influencing the genetic structure of *P. pacificus* on La Réunion. Additionally, the distinct beetle hosts with their uneven and often very local distribution strongly determine the distribution of *P. pacificus* lineages (Morgan *et al.*, 2012; McGaughran *et al.*, 2013). For example, one of the four *P. pacificus* lineages is restricted to high-altitude locations and largely to the endemic stag beetle *Amneidus godefroyi*. Detailed analysis of this association revealed a strong adaptation to the low oxygen concentrations at high altitudes resulting in oxygen-induced social behaviors (Moreno *et al.*, 2016). Taken together, these studies highlight the complex interplay between *P. pacificus* population structure, abiotic environmental factors, and beetle host biology. In particular, beetle host biology requires intense consideration to fully understand the novel complex traits seen in *P. pacificus*.

While the reliable association of *Pristionchus* worms with scarab beetles and some other soil arthropods has allowed the isolation and characterization of a large number of species in the relatively short time period of only 15 years, this type of association complicates the full understanding of the biology and the life cycle of *Pristionchus*. Adult scarab beetles, in particular chafers and stag beetles, are well known to the general public. However, their life cycle is complex, long-lasting and in large parts inaccessible. Many species have life cycles of several years with only the short-lived adult stage living above ground. After feeding and mating in trees, females go back into the soil (e.g., chafers) or dead wood (e.g., stag beetles), where they lay their eggs. After hatching, grubs spend years in the soil feeding on plant roots and debris before pupation. In many species, after hatching the young adults overwinter one more time before flying in spring; for example, the European cockchafer *Melolontha melolontha* has a 4-year life cycle, but animals are visible for only around 2 weeks as adults.

Through the association of *Pristionchus* with scarab beetles, a further layer of complexity is apparent as nematode beetle infestation rates vary between locality, year, and species (Herrmann *et al.*, 2006). It is, therefore, particularly difficult to perform empirical studies on the ecology of *P. pacificus*. In order to overcome this shortcoming, much empirical work has focused on the rhinoceros beetle *Oryctes borbonicus*, native to La Réunion (Herrmann *et al.*, 2010; Morgan *et al.*, 2012). *O. borbonicus* is known to have a *P. pacificus* infestation rate of roughly 90% on one particular meadow (Herrmann *et al.*, 2010). This unique prerequisite has facilitated experimental investigations by killing wild-caught beetles and burying them in specifically designed cages. These ongoing studies have resulted in a number of surprising findings. First, *P. pacificus* is exclusively found in the arrested dauer stage on the living beetle (Herrmann *et al.*, 2010; Meyer *et al.*, 2017). Second, dauer larvae of *P. pacificus* exit 1 week after the beetle's death to start feeding on the developing microbes at the carcass (Meyer *et al.*, 2017). Note that other nematodes also present on the rhinoceros beetle usually exit from the dauer stage prior to *P. pacificus*. Third, the microbiome of the nematode is largely identical to that of the living beetles but not of the surrounding soil (Meyer *et al.*, 2017). Fourth, recent studies, using larger cages with 27 sub-compartments and longer investigation periods of up to 3 months, revealed a biphasic boom-and-bust life cycle of *P. pacificus* centered around the beetle carcass (Renahan *et al.*, 2021). Reproducing animals are found only on the carcass, whereas dauer larvae disperse. Fifth, two peaks of dauer larvae formation have been observed on the carcass at 4 and 8 weeks after killing the insect, indicating an unexpected biphasic cycle. Interestingly, these cycles anti-correlate with bacterial abundance and with the abundance of reproducing nematodes. Finally, dauer larvae disperse surprisingly fast reaching the edge of the 15 × 15 × 15 cm cage after 6 weeks (Renahan *et al.*, 2021). While these experiments have helped clarify some of the basic aspects of the *P. pacificus* life cycle in association with a scarab beetle, many facets remain unknown, with current studies attempting to address these issues using increasingly complex experimental set-ups. However, these studies on the *P. pacificus* life cycle in

association with *O. borbonicus* provide a useful context for the evaluation of the complex traits to be discussed below.

Developmental Plasticity and Predatory Feeding

Mouth-form plasticity in *P. pacificus*

The most striking feature of *P. pacificus* is the plasticity of its stoma resulting in two alternative mouth-forms associated with omnivorous feeding strategies. The so-called “stenostomatous” (St) form has a narrow buccal cavity with a single dorsal tooth resulting in a strict bacterial feeding morph (Fig. 1.4A). Alternatively, the “eurystomatous” (Eu) form has a wide buccal cavity with a dorsal and a right subventral tooth permitting these animals additionally to feed on fungi and other nematodes (Fig. 1.4B). No intermediate mouth-forms exist and adult animals are fixed and can no longer switch their acquired mouth-form fate. The formation of these two alternative morphs is a prime example of developmental (phenotypic) plasticity, the ability of an organism to form distinct phenotypes under different environmental conditions. Developmental plasticity is an important concept in developmental biology and evolutionary biology, but empirical studies have long been scarce largely because controlling and manipulating plastic traits is difficult under laboratory conditions. During the past two decades, theoretical studies have pointed towards the importance of developmental plasticity for the evolution of novelty and as a previously neglected concept and mechanism of evolution (Pigliucci, 2001; West-Eberhard, 2003). As a result, experimental studies on various examples of plasticity have been initiated in the past decade. These studies not only highlight the significance of plasticity but also provide mechanistic insight into the underlying molecular, phylogenetic, and evolutionary processes (for recent review, see Sommer, 2020). Importantly, *P. pacificus*, with its hermaphroditic mode of reproduction and thus clonal propagation, is a powerful model to study developmental plasticity and the environmental influence on developmental decision making, as will be discussed below.

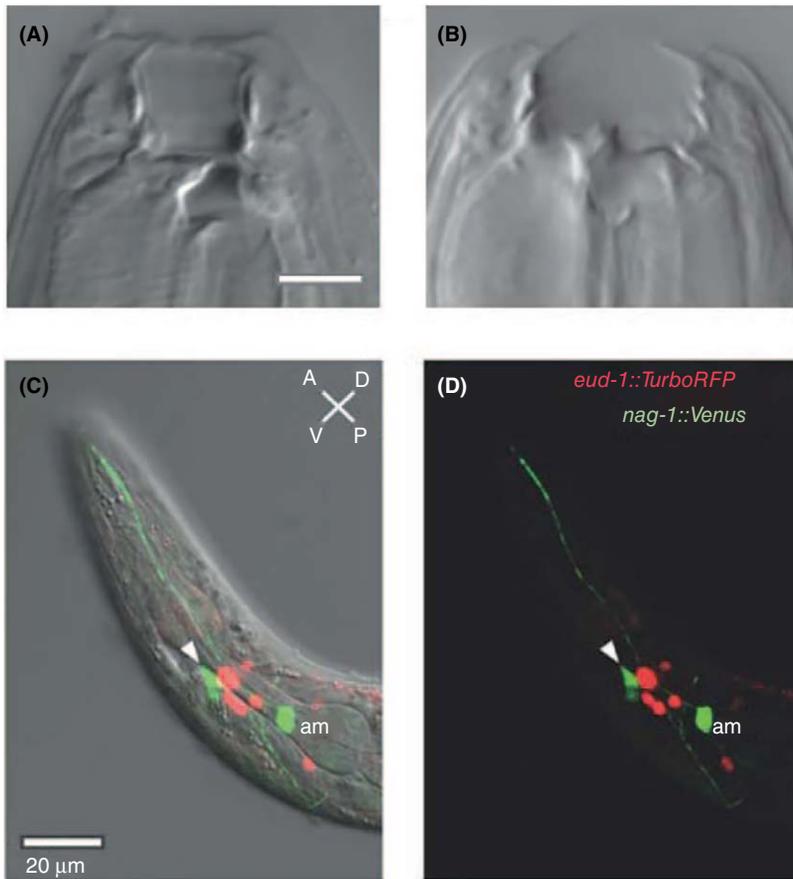


Fig. 1.4. Mouth-form plasticity in *P. pacificus*. **(A)** Stenostomatous morph animals are narrow-mouthed with a single tooth and are bacterial feeders. **(B)** Animals that acquire the alternative Eurystomatous morph have a wide buccal cavity with a dorsal and a right subventral tooth expanding their dietary range to include feeding on fungi and other nematodes. **(C)** The gene regulatory network behind the mouth-form decision is well characterized with several genes including *eud-1* and *nag-1* expressed in discrete sensory neurons. Redrawn from Sieriebriennikov *et al.* (2018).

Genetic basis and environmental influence

Most *P. pacificus* isolates form a stable strain-specific ratio of Eu:St animals independent of the phenotype of the mother. For example, the wild-type reference strain PS312 from California has a 70–90% Eu ratio. This ratio is found in progeny of both Eu or St mothers of the PS312 genotype. The mouth-form ratio of any given strain can be altered by environmental and genetic factors allowing specific manipulation and thus the elucidation of underlying molecular mechanisms. First, a simple shift of culture conditions

can reverse the mouth-form ratio of *P. pacificus* PS312. While growth on standard nematode growth medium (NGM) agar plates results in the 70–90% Eu ratio mentioned above, liquid culture conditions using the same *Escherichia coli* food source establishes a 90% St phenotype (Werner *et al.*, 2017). Note that variation in liquid culture medium, rotation speed, and other abiotic factors can result in any desirable mouth-form ratio (Werner *et al.*, 2017). Thus, the *P. pacificus* mouth-form plasticity can be very easily manipulated, making it a prime candidate for plasticity research.

Second, population density has a strong influence on mouth-form ratios and already early studies identified associated small molecules, such as the diascaroside *dasc#1* (Bose *et al.*, 2012). More recent work revealed that these population-based interactions are largely cross-generational, as *dasc#1* and other small molecules are mostly secreted by adult animals (Werner *et al.*, 2018a,b). These cross-generational interactions are likely of relevance in the wild, potentially influencing mouth-form ratios in developing progeny to better adapt to shifting environments. It is important to note that these mouth-form plasticity features show striking similarities with the regulation of dauer development, the plasticity of nematode life cycles that has been discussed in greater detail elsewhere (Sommer and Mayer, 2015).

Third, relatively constant mouth-form ratios of *P. pacificus* on agar plates allow unbiased forward genetic screens for the isolation of mutants with mouth-form deficient phenotypes. The first such genetic screen resulted in the isolation of *eud-1*, a *eurystomatous-form* defective strain that only forms St animals (Ragsdale *et al.*, 2013). *eud-1* is an unusual gene, which encodes a sulfatase. Mutants in *eud-1* are dominant so that hermaphrodites with a single mutant copy are already preferentially St. Such *eud-1* mutants are *loss-of-function*, a rare genetic phenomenon, already suggesting extreme dose sensitivity. Indeed, overexpression of *eud-1* in a *eud-1* mutant background can completely revert the all-St mutant phenotype into an all-Eu phenotype (Ragsdale *et al.*, 2013). Further, *eud-1* is located on the X chromosome; therefore, males with their XO genotype are preferentially St. However, overexpression of *eud-1* in males will result in all-Eu males indicating dose-dependence (Ragsdale *et al.*, 2013). Taken together, all these experiments indicate that *eud-1* is a classic genetic switch gene, as it is known from developmental genetic studies in multiple model organisms, including *C. elegans*. Most importantly, however, *eud-1* with its characteristics confirms a long-standing prediction of evolutionary theory and plasticity theory, namely that phenotypic plasticity relies on developmental switch genes that can sense the environment and subsequently direct alternative developmental decisions (West-Eberhard, 2003; Ragsdale *et al.*, 2013). While it was striking

that already the first genetic screen for *P. pacificus* mouth-form mutants identified such a switch gene, later research indicated that the situation is far more complicated, as indicated below.

***eud-1* and the gene regulatory network**

Subsequent investigations revealed that *eud-1* is a complex genetic locus. First, it contains an antisense transcript that acts to promote *eud-1* expression itself (Serobyán *et al.*, 2016). Second, *eud-1* is part of a multigene locus that contains two pairs of duplicated genes in a tandem inverted configuration (Sieriebriennikov *et al.*, 2018). The other gene pair, *nag-1* and *nag-2*, encode for *N*-acetyl-glucosaminidases, which in humans act on extracellular matrix proteins similar to sulfatases. Interestingly, mutants in *nag-1* and *nag-2* have a phenotype opposite to *eud-1*, resulting in all-Eu animals under all culture conditions. Most importantly, *eud-1*, *nag-1*, and *nag-2* are expressed in different sensory neurons (Fig. 1.4C). This finding further supports the notion that these genes are involved in sensing the environment and that the environmentally induced mouth-form switch is a network with modular organization.

This switch network not only senses the environment, but also directs a gene regulatory network (GRN), which finally executes one of the two alternative mouth-form phenotypes. More recent genetic studies have identified large parts of this GRN of mouth-form plasticity in *P. pacificus* (Kieninger *et al.*, 2016; Bui and Ragsdale, 2019; Sieriebriennikov *et al.*, 2020). Two conserved nuclear hormone receptors, *nhr-1* and *nhr-40*, are involved in transmitting environmental information to mouth-form decision-making processes (Kieninger *et al.*, 2016; Sieriebriennikov *et al.*, 2020). Interestingly, the nuclear hormone receptors NHR-40 and NHR-1 have only a small number of common targets, which show unexpected evolutionary patterns. All of these common targets are fast-evolving genes that have no 1:1 orthologs in *C. elegans*. In contrast, *nhr-1* and *nhr-40* themselves share 1:1 orthologs between *P. pacificus* and *C. elegans*, although, in general, NHRs evolve extremely rapidly (Sieriebriennikov *et al.*, 2020).

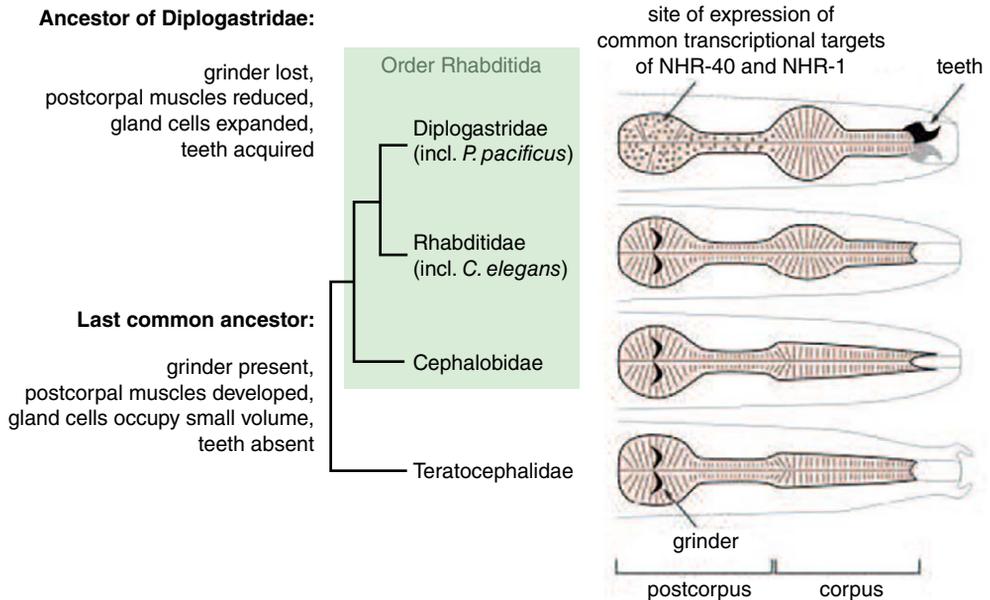


Fig. 1.5. Evolution of pharynx morphology in the order Rhabditida. The ancestral character state includes the presence of a grinder in the terminal bulb of the pharynx. In the Diplogastridae, the grinder has been lost and, concomitantly, muscle cells were reduced, gland cells expanded, and teeth are formed as an evolutionary novelty (top image). Redrawn from Sieriebriennikov *et al.* (2020).

The most remarkable aspect about the targets of NHR-40 and NHR-1 is their common expression in the single gland cell g1D (Sieriebriennikov *et al.*, 2020). Importantly, the gland cell g1D is the site of a major evolutionary innovation in *P. pacificus* and the whole Diplogastridae family (Riebesell and Sommer, 2017). Phylogenetic reconstruction indicates that the outgroup Teratocephalidae and the rhabditid families Cephalobidae and Rhabditidae represent the ancestral character state of mouth-associated characters, with: (i) a grinder; and (ii) five pharyngeal muscle cells; but (iii) no teeth (Fig. 1.5). In contrast, species of the Diplogastridae have: (i) no grinder; (ii) concomitantly gained teeth; and (iii) accompaniment of the loss of the grinder by the reduction of muscle cells in the postcorpus. This reduction of postcorpus muscle cells resulted in the expansion of three gland cells, g1D, g1VL, and g1VR, one in each sector of the trilaterally symmetrical pharynx (Fig. 1.5) (Riebesell and Sommer, 2017). It has been speculated that the functional remodeling of g1D, in which the target genes of NHR-40 and

NHR-1 are expressed and which, intriguingly, empties through the tip of the large dorsal tooth, may be a prerequisite for the formation of teeth and the evolution of predation (Sieriebriennikov *et al.*, 2020). Taken together, major morphological changes in the g1D gland cell accompanied the evolution of teeth, mouth-form plasticity, and predation, linking rapid gene turnover with morphological innovations and suggesting that the origin of feeding plasticity involved novelty at the level of genes, cells, and behavior.

Mouth-form plasticity and evolutionary novelty

What is the evolutionary significance of mouth-form plasticity in *P. pacificus*? Theoretical studies by Mary Jane West-Eberhard had already in 1989 suggested that developmental plasticity is a crucial step in the evolution of novel and complex traits (West-Eberhard, 1989). While one would think that the origin

of novelty has been a central question in evolutionary biology, this is actually not so. Mainstream evolutionary biology focuses largely on adaptive processes and on the ways in which genes are propagated. In contrast, the origin of novel traits in evolutionary lineages has found little attention. West-Eberhard's original proposal was heavily expanded in a monograph in 2003, which represents one of the major milestones in modern evolutionary biology (West-Eberhard, 2003). In a nutshell, she proposed three major predictions for the origin and evolution of novel and complex traits. First, novelty often starts as environmentally sensitive and developmentally plastic traits. Today, this phenomenon is also referred to as "Plasticity first evolution" or "Flexible stem hypothesis" (Gibert, 2017; Levis and Pfennig, 2019). Second, environmental sensitivity requires developmental switch genes that can integrate environmental information and finally direct alternative decision-making processes. The *eud-1* developmental switch described above was the first experimental confirmation of this important prediction (Ragsdale *et al.*, 2013). Finally, traits will not stay plastic indefinitely. Instead, environmental dependence will become genetically encoded in a process called "canalization" or "assimilation", a theory first proposed by Waddington (1975). Examples of plasticity, like the one discussed here for *P. pacificus*, can move the plasticity research field from theory to experiment and can address important challenges, which eventually will prove the significance of this phenomenon for evolution (Sommer, 2020).

Convergent evolution of predation in nematodes and organismal consequences

Finally, it should be briefly noted that *Pristionchus* and other diplogastrid nematodes are not the only group of predatory nematodes. Instead, predation has evolved multiple times by convergent evolution and is known mostly from soil but also from aquatic nematodes. One group, the Mononchida, is even exclusively predatory (Ahmad and Jairajpuri, 2010). In general, predatory

nematodes are found most frequently in undisturbed environments and many of these predators feed on other bacterivorous nematodes; however, other invertebrates can be preyed upon as well. Mechanistically, predatory nematodes can be piercers or ingesters, as originally classified by Yeates *et al.* (1993). These different mechanisms and the convergent evolution of predation have resulted in multiple independent morphological adaptations. Therefore, the nematode head and the buccal cavity in particular have been subject to enormous evolutionary innovations, often complicating proper morphological interpretations that remain controversial. While a certain consensus has been reached in the past decades (Decraemer *et al.*, 2014), it should be noted that major uncertainties about the developmental origin and the structural composition of the nematode head remain, which is of particular importance for predatory species (Wright, 1987).

Unfortunately, most predacious nematodes cannot easily be cultured in the lab and so the studies described above for *P. pacificus* are largely unparalleled in any other predatory nematodes. Also, it is currently unknown if, when, and how often predation requires plasticity in the associated feeding structures. While current knowledge suggests that most predatory nematodes are non-plastic, this notion might result from study biases. For example, a recent investigation by Kanzaki and co-workers revealed that *Bursaphelenchus sinensis* exhibits a feeding dimorphism similar to *P. pacificus* with one predatory form that went previously unnoticed (Kanzaki *et al.*, 2019). Indeed, follow-up investigations revealed that the predatory morph of this species is similar to the morphology of *Berntsenus* Massey 1974, which thereby becomes a junior synonym of *Bursaphelenchus* Fuchs 1937 (Kanzaki and Giblin-Davis, 2020). Thus, many biological aspects associated with predation in nematodes remain to be investigated.

From Behavior to Neuroscience

Predatory behaviors

Alongside the mouth-form plasticity and the detailed ecology associated with *Pristionchus*, these

nematodes also show diverse behaviors not observed in other more commonly studied nematode species. Several of these behaviors are now being explored in earnest in order to understand the genetic, molecular, and neurobiological processes behind these behaviors and, importantly, how they may have evolved. The most obvious and dramatic of these behaviors is the previously mentioned predatory feeding (Fig. 1.6A), which depends on the development of the enlarged and paired teeth found in the Eu mouth-form to kill the larvae of other nematodes (Wilecki *et al.*, 2015). While only the dorsal tooth is moveable during predation, together the teeth structures are capable of puncturing through the cuticle of other nematode larvae with a high efficiency. As an interesting side note, in *Pristionchus* species the killing behavior appears to be entirely suppressed in the St mouth-forms; however, they do still scavenge on any available carcasses found in their vicinity (Wilecki *et al.*, 2015). Further, while predation has been observed in all *Pristionchus* Eu animals, the majority of studies have thus far concentrated on *P. pacificus*, which is the focus of the predatory descriptions below.

Upon contact with a potential prey larva, *P. pacificus* undergoes a feeding mode switch from a bacterial feeding mode involving rapid pumping of the pharynx and a stationary tooth, to a predatory feeding mode consisting of slower pharyngeal pumping and activation of the dorsal tooth in a 1:1 ratio with the pharyngeal action. Subsequent experiments have demonstrated that the predatory feeding mode in *P. pacificus* can be recapitulated in the absence of prey by the exogenous addition of the neurotransmitter serotonin and there appears to be an extra pair of serotonergic neurons in its pharynx in comparison to *C. elegans* (Fig. 1.6B) (Wilecki *et al.*, 2015). The additional function of serotonin and its importance in predatory feeding has been further emphasized through studies of mutating enzymes responsible for serotonin biosynthesis and through neuron ablation experiments. In both the serotonin-deficient mutants and in animals with ablated serotonergic neurons, the synchronicity of the pharyngeal pumping and tooth action is disrupted, which in turn also reduces the killing efficiency (Okumura *et al.*, 2017). Therefore, these studies have

begun to hint at some of the molecular and neurobiological mechanisms behind the predatory behavior with divergence and acquisition of additional functions in pathways activated through serotonin.

A further question at the heart of the predatory behaviors is understanding how *P. pacificus* senses its prey. This requires both the identification of the specific prey-sensing mechanism such as potential receptors involved and, equally importantly, the identification of the neuronal circuits that facilitate this behavior. Similar to the whole-animal connectome available for *C. elegans*, a neuronal wiring diagram consisting of the pharyngeal (Bumbarger *et al.*, 2013) and head sensory neurons (Fig. 1.6C) (Hong *et al.*, 2019) now exists for *P. pacificus*. Interestingly, these neurons show a remarkable degree of conservation in cell number and frequently cell body position between *P. pacificus* and *C. elegans*. There is, however, a clear divergence in synaptic connectivity and, in the case of the head sensory neurons, also structural differences. This is particularly apparent in the amphid neurons, which in *P. pacificus* lack the wing-shaped cilia morphology found in *C. elegans* (Hong *et al.*, 2019). Despite these differences, 1:1 homology has been assigned between *P. pacificus* and *C. elegans* pharyngeal and head sensory neurons with minimal ambiguity. Therefore, investigation of these circuits will help to enable identification of those neurons essential for predation and how they may differ from other non-predatory nematodes.

Some clues to the identification of the sensory neurons involved in the prey-recognition circuits have recently come to light through mutations affecting the growth and function of the cilia. Similar to observations in *C. elegans*, in *P. pacificus* many of the head sensory neurons also possess cilia at the end of their dendritic processes and these structures have been shown to be involved in environmental perception. Interestingly mutants defective in cilia formation, while still capable of some degree of predation, appear defective for efficient prey detection, as the frequency of prey biting is strongly reduced (Moreno *et al.*, 2019). Coinciding with this, these mutants also show abnormal mouth-form ratios, reinforcing the importance of environmental sensing for correct mouth-form development.

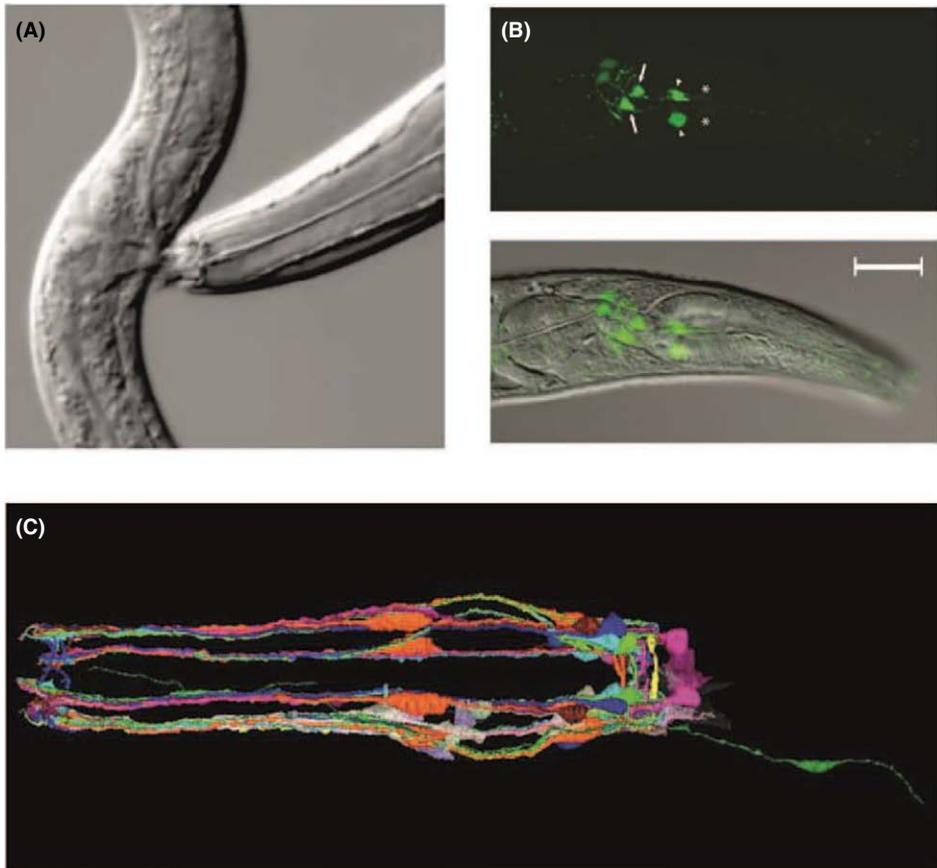


Fig. 1.6. Neurobiology of predation. **(A)** *Pristionchus* animals which develop the Eurystomatous mouth-form are voracious predators, capable of killing and feeding upon the larvae of other nematodes. In this image, a *C. elegans* larvae (left nematode) has fallen prey to a *P. pacificus* predator (right nematode). **(B)** The neurobiology behind the predatory feeding behavior is currently being elucidated, with serotonin playing a key role in regulating the predatory tooth action. Using anti-serotonin antibodies in *P. pacificus*, neurons probably corresponding to NSM (arrowheads), ADF (arrows) and I1 (asterisks) can be detected. Redrawn from Wilecki *et al.* (2015). **(C)** Connectomes provide a powerful tool for dissecting the circuits behind behaviors. Using TEM sections, the connectome for both the pharynx and the head sensory circuit (shown here) is available for *P. pacificus*, revealing distinct morphological structures and synaptic connections from *C. elegans*.

Environmental sensing and its associated behaviors

While predatory feeding offers one of the most striking and obvious behavioral differences between *Pristionchus* species and many other nematodes, there are several other intriguing behavioral distinctions. Indeed, *Pristionchus* demonstrates sophisticated environmental sensing mechanisms, which are likely adapted to the specific environments in which these species are found. Firstly,

Pristionchus displays remarkable chemosensory abilities and particularly strong attraction to the volatile pheromones associated with certain beetle species (Hong and Sommer, 2006; Hong *et al.*, 2008). It is therefore logical to assume that this chemical attraction probably facilitates the host-seeking in *Pristionchus*, aiding in the colonization and dispersal to new environments (Cinkornpumin *et al.*, 2014). Second, in a similar manner to the insect host-seeking behaviors, *Pristionchus* also finds specific bacteria attractive

or repellent. In chemotaxis experiments utilizing *P. pacificus* and bacteria found naturally associated with it, many of the most repellent bacteria were also toxic or pathogenic. In contrast, bacteria that were strongly attractive were frequently sources of high nutrition, indicating a far-ranging ability to ascertain environmental cues and generate an appropriate behavioral response (Rae *et al.*, 2008; Akduman *et al.*, 2018). Furthermore, it has been subsequently demonstrated that several of these bacteria are also capable of modulating the predatory feeding behaviors, with the production of vitamin B₁₂ by some of these bacterial species being one of the key influences increasing *P. pacificus* killing behaviors (Akduman *et al.*, 2020). Finally, experiments have also been carried out on the perception of other environmental stimuli in *P. pacificus*, in particular the occurrence of oxygen-induced social behaviors already mentioned above. In *C. elegans* the majority of wild isolates display clumping and bordering behaviors, which is thought to help avoid hypoxia under laboratory conditions. Contrary to this, most wild *P. pacificus* isolates demonstrate solitary behaviors indicating a distinct evolutionary history, genetic regulation, and sensory mechanism behind this behavior (Moreno *et al.*, 2016). Thus, *Pristionchus* shows a sensory and behavioral identity, which differs from other nematodes.

Self-recognition and Cannibalism

With contact between *Pristionchus* and other nematode larvae eliciting such an aggressive response, and as predation is so prevalent in the *Pristionchus* genus, a fundamental question has arisen from these behaviors, namely: do *Pristionchus* also kill and cannibalize their own progeny or do they perhaps have a self-recognition system to avoid this costly outcome? A recent study tested this by analyzing several *Pristionchus* species, which were fed upon larvae of either other nematode species or their own self-progeny. In all of the *Pristionchus* species assessed, it was clear that while predators showed frequent killing behavior towards other nematode species, there was no killing of self-progeny, indicating the presence of a robust self-recognition system

(Lightfoot *et al.*, 2019). This finding was all the more significant because, while thousands of researchers all around the world study many aspects of biology in *C. elegans* and other nematodes, this was the first reported example of an organismal self-recognition behavior described in nematodes.

Further experiments on mixed prey cultures with *P. pacificus* predators demonstrated that the self-recognition system requires direct nose contact with larvae and hinted at a cuticle-bound self-recognition signal while ruling out any significant role for small signaling molecules. Additionally, and perhaps most importantly, it was shown that not only are *P. pacificus* capable of distinguishing between other nematode species, but also in fact the self-recognition system shows an incredible degree of specificity, as these nematodes are also capable of distinguishing and killing other closely related *P. pacificus* strains. These results paved the way for subsequent genetic crosses and mapping experiments between different strains of *P. pacificus*, which kill one another, and from this a single gene was identified encoding one of the major components of the self-recognition signal. This gene was named *self-1* for self-recognition defective and in the main *P. pacificus* laboratory strain PS312 it was shown to encode for a small peptide of only 63 amino acids in length. Through further analysis, it was demonstrated that SELF-1 also included a hypervariable region in which it was commonly found to contain differences between diverse strains. Targeted CRISPR-Cas9 experiments reinforced the importance of this domain in generating the self-recognition specificity as even subtle amino acid changes including a Serine > Isoleucine and Isoleucine > Leucine mutation within this region are enough to disrupt the self-recognition system. Finally, through a transcriptional reporter, the SELF-1 small peptide was observed to be expressed in all the hypodermal cells throughout all life cycle stages.

The identification of *self-1* as a major part of the self-recognition signaling machinery in *P. pacificus* is a significant first step to our understanding of these behaviors in nematodes. However, *self-1* mutants show no receptor phenotype; thus, *self-1* is unlikely to be the only component in this complex behavioral mechanism. As such, the identification of any receptor

responsible for the detection of SELF-1 will be pivotal for future studies and additionally it will be important to determine whether SELF-1 is the only signal involved. Furthermore, while self-recognition behaviors are ubiquitous across the natural world and found in creatures as diverse as rotifers (Gilbert, 1976) and tunicates (De Tomaso *et al.*, 2005) to frogs (Villinger and Waldman, 2008) and octopuses (Nesher *et al.*, 2014), organisms with the necessary molecular tools required to dissect these behaviors are few and far between. Thus, the self-recognition behaviors in *Pristionchus* establish it as an emerging and potent biological system capable of providing a mechanistic understanding of self-recognition and delivering new insights into how these behaviors are regulated and how they may have evolved.

Complex Small Molecule Chemistry

Mouth-form plasticity, predatory feeding behavior, and self-recognition represent spectacular examples of *Pristionchus* biology that show the diversity of evolutionary innovations to be found in this particular nematode taxon. Associated with these key innovations are a number of chemical novelties resulting in an unexpected and extreme diversity of small molecule structures. So far, three groups of previously unknown nematode metabolites have been characterized in *P. pacificus* and its relatives. First, unlike the dauer larvae of other nematodes, *P. pacificus* and other diplogastrid dauers swim on water (Ogawa *et al.*, 2009; Penkov *et al.*, 2014). This observation suggested the presence of a hydrophobic substance that would prevent them from sinking to the bottom of a water column. Indeed, subsequent chemical investigations revealed a wax-type lipid that through chemical characterization and NRM analysis was shown to be an ester with a mass-to-charge (m/z) ratio of 866.7677 and the formula $C_{60}H_{120}O_2$ (Fig. 1.7) (Penkov *et al.*, 2014). This molecule was named “nematoil” and represents one of the largest known waxes in the animal kingdom. While the biosynthesis of nematoil and its secretion to the body surface are currently unknown, the wax allows the dauer larvae to stick together in larger groups.

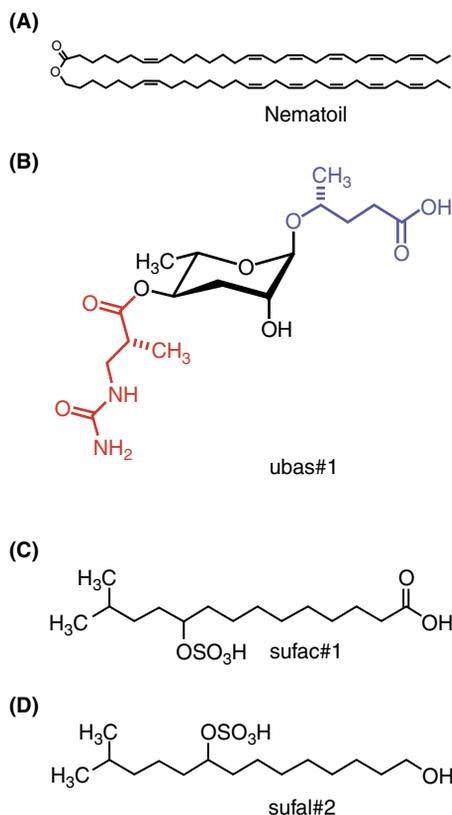


Fig. 1.7. Chemical innovation in *P. pacificus*. Structures of nematoil (A), which is produced by dauer larvae and allows them to swim on water. Ubas#1 (B) is one of several complex NDMMs involved in cross-generational population density-dependent dauer induction. Sufac#1 (C) and Sufal#2 (D) are two representatives of sulfolipids in *P. pacificus*. (Courtesy of F.C. Schroeder.)

Indeed, large so-called “dauer towers” are seen on old agar plates when many animals have entered the dauer stage. Penkov and co-workers have speculated that these structures might be helpful in the wild for dauer larvae to find an appropriate host (Penkov *et al.*, 2014).

Second, detailed analysis of lipid chemistry in *P. pacificus* identified a large number of sulphated lipids, unknown from *C. elegans* and other nematodes (Fig. 1.7) (Liu *et al.*, 2018). Liu and co-workers found that *C. elegans* when exposed to these *P. pacificus* sulfolipids will initiate an immediate escape behavior. Thus, a predator-evoked signal consisting of sulfated lipids induces a

response behavior in potential prey. However, it is currently unknown if only *C. elegans* or also other potential prey of *P. pacificus* will initiate similar escape behaviors. It is interesting to note that the large number and amount of sulfated lipids produced by *P. pacificus* coincides with a strong increase in sulfatases and sulfotransferase encoded in the *P. pacificus* genome. Relative to *C. elegans*, *P. pacificus* has roughly three times as many genes encoding for these enzymes (Dieterich *et al.*, 2008).

Finally, nematode derived modular metabolites (NDMMs) that were first characterized in *C. elegans* and shown to regulate many life history traits, including dauer induction (for review, see Schroeder, 2015), are also known from *P. pacificus*. Indeed, dauer development and mouth-form plasticity are controlled by population density relying on specific NDMMs. However, the NDMMs characterized in *P. pacificus* showed an unexpected diversity of molecular building blocks including amino acid, nucleoside, and fatty acid modules (Bose *et al.*, 2012; Yim *et al.*, 2015). For example, ubas#1 was one of the first of these NDMMs to be synthesized and subsequently demonstrated to regulate dauer induction (Fig. 1.7) (Bose *et al.*, 2012). Most recent studies indicated that these NDMMs undergo extremely rapid evolution. Specifically, the analysis of around 30 *Pristionchus* species revealed strong species-specific patterns of NDMMs, with many of the most complex NDMMs being found in only two of the four clades of *Pristionchus* (Dong *et al.*, 2020). In contrast, basal *Pristionchus* species and other diplogastrids do not form many of the complex NDMMs known from *P. pacificus*. These surprising findings suggest rapid evolutionary innovations in small molecule chemistry (Dong *et al.*, 2020). Note that, similar to other groups of organisms, including plants and insects, the majority of natural products synthesized by these nematodes awaits functional characterization. However, a handful of NDMMs has been functionally characterized in *P. pacificus* revealing roles in dauer formation and mouth-form development (Bento *et al.*, 2010; Bose *et al.*, 2012, 2014) and similarly, the biosynthetic pathways involved in the formation of these complex NDMMs is beginning to be investigated (Markov *et al.*, 2016; Falcke *et al.*, 2018). Taken together, *P. pacificus* combines major innovations

and novelty in biology and chemistry and might represent a rewarding system for further chemical investigations.

Conclusions and Outlook

The development of *P. pacificus* as a model system for integrative evolutionary biology encompassing parallel investigations in evo-devo, population genetics, and ecology was initiated in the 1990s by the senior author of this contribution as a research fellow with Paul W. Sternberg at the California Institute of Technology in Pasadena, California (USA). By that time, the undescribed *P. pacificus* strain PS312 in the Sternberg lab collection drew immediate recognition due to the many parallels it shared with *C. elegans*, in particular with regard to its growth and propagation in the lab. Additionally, the comprehensive taxonomy and systematics of nematodes by Andrassy (1984) indicated that *Pristionchus* belongs to a nematode family with exciting associations with a diversity of insects and other arthropods. While the family of Diplogastridae was clearly understudied relative to the Rhabditidae at the time, studies initiated in the late 1990s would reveal that, in contrast to the rhabditids, the diplogastrids represent a monophyletic taxon (Holterman *et al.*, 2006; von Lieven and Sudhaus, 2000). Subsequent and parallel investigations by the laboratories of James Baldwin (Riverside), Robin Giblin-Davis (Fort Lauderdale), Natsumi Kanzaki (Kyoto), our own one, and those of others have provided a wealth of knowledge on these excitingly diverse nematodes, their spectacular associations and nearly never-ending evolutionary innovations. While a monograph published in 2015 gave a detailed overview about the knowledge on *P. pacificus* at the time (Sommer, 2015), this contribution focuses on the more recent research on complex and novel traits.

The examples of mouth-form plasticity, predatory behavior, and self-recognition, together with beetle host finding and associated neuronal circuits, represent a rich collection of conserved and divergent patterns and processes in animal biology. Furthermore, with its major advancements in omics approaches, biochemistry,

reverse genetics through CRISPR-Cas mediated tools, and sophisticated laboratory culturing set-ups, *P. pacificus* has an almost unrivaled tool kit with which to obtain a molecular-level comprehension of these biological events. However, a full understanding of the range of morphological, physiological, and behavioral adaptations of *P. pacificus* will require detailed comparative

studies involving the diversity of around 50 *Pristionchus* species currently available as living or frozen cultures and the more than 1 500 *P. pacificus* isolates. Therefore, with these ongoing investigations and future work exploring *P. pacificus* and its relatives, this genus of nematodes will undoubtedly be contributing to many more significant discoveries in the future.

References

- Ahmad, W. and Jairajpuri, M.S. (2010) *Mononchida, the Predatory Nematodes*. Brill, Leiden.
- Akduman, N., Rödelsperger, C. and Sommer, R.J. (2018) Culture-based analysis of *Pristionchus*-associated microbiota from beetles and figs for studying nematode-bacterial interactions. *PLoS ONE* 13, e0198018.
- Akduman, N., Lightfoot, J.W., Röseler, W., Witte, H., Lo, W.-S. *et al.* (2020) Bacterial diet-derived vitamin B12 enhances nematode predation and surplus-killing. *The ISME Journal* 14, 1494–1507. doi: 10.1038/s41396-020-0626-2
- Andrassy, I. (1984) *Klasse Nematoda*. Gustav Fischer Verlag, Stuttgart.
- Bento, G., Ogawa, A. and Sommer, R.J. (2010) Co-option of the endocrine signaling module Dafachronic Acid-DAF-12 in nematode evolution. *Nature* 466, 494–497.
- Borchert, N., Dieterich, C., Krug, K., Schütz, W., Jung, S. *et al.* (2010) Proteogenomics of *Pristionchus pacificus* reveals distinct proteome structure of nematode models. *Genome Research* 20, 837–846.
- Bose, N., Ogawa, A., von Reuss, S.H., Yim, J.J., Ragsdale, E.J. *et al.* (2012) Complex small molecular architectures regulate phenotypic plasticity in a nematode. *Angewandte Chemie* 51, 12438–12443.
- Bose, N., Meyer, J.M., Yim, J.J., Mayer, M.G., Markov, G.V. *et al.* (2014) Natural variation in dauer pheromone production and sensing supports intraspecific competition in nematodes. *Current Biology* 24, 1536–1541.
- Bui, L.T. and Ragsdale, E.J. (2019) Multiple plasticity regulators reveal targets specifying an induced predatory form in nematodes. *Molecular Biology and Evolution* 36, 2387–2399.
- Bumbarger, D.J., Riebesell, M., Rödelsperger, C. and Sommer, R.J. (2013) System-wide circuit reorganization underlying behavioral differences between predatory and bacterial feeding nematodes. *Cell* 152, 109–119.
- Carroll, S.B., Grenier, J.K. and Weatherbee, S.D. (2001) *From DNA to Diversity*. Blackwell Science, Oxford, UK.
- Cinkornpumin, J.K., Wisidagama, D.R., Rapoport, V., Go, J.L., Dieterich, C. *et al.* (2014) A host beetle pheromone regulates development and behavior in the nematode *Pristionchus pacificus*. *eLife* 3, e03229.
- De Tomaso, A.W., Nyholm, S.V., Palmeri, K.J., Ishizuka, K.J., Ludington, W.B. *et al.* (2005) Isolation and characterization of a protochordate histocompatibility locus. *Nature* 438(7067), 454–459. doi: 10.1038/nature04150.
- Decreamer, W., Coomans, A. and Baldwin, J. (2014) Morphology of Nematoda. In: Schmidt-Rhaesa, A. (ed.) *Handbook of Zoology: Gastrotricha, Cycloneuralia and Gnathifera. Vol. 2: Nematoda*. De Gruyter, Berlin, pp. 1–60.
- Dieterich, C., Clifton, S.W., Schuster, L.N., Chinwalla, A., Delehaunty, K. *et al.* (2008) The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. *Nature Genetics* 40, 1193–1198.
- Dong, C., Weadick, C.J., Truffault, V. and Sommer, R.J. (2020) *Convergent evolution of small molecule pheromones in Pristionchus nematodes*. *eLife* 9, e55687. doi: 10.7554/eLife.55687
- Eizinger, A. and Sommer, R.J. (1997) The homeotic gene *lin-39* and the evolution of nematode epidermal cell fates. *Science* 278, 452–455.
- Falcke, J.M., Bose, N., Artyukhin, A.B., Rödelsperger, C., Markov, G.V. *et al.* (2018) Linking genomic and metabolomic natural variation uncovers nematode pheromone biosynthesis. *Cell Chemical Biology* 25, 1–10.

- Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M. and Tsai, L.-H. (2007) Recovery of learning and memory is associated with chromatin remodelling. *Nature* 447, 178–182.
- Gibert, J.M. (2017) The flexible stem hypothesis: evidence from genetic data. *Development Genes & Evolution* 227, 297–307.
- Gilbert, J.J. (1976) Sex-specific cannibalism in the rotifer *Asplanchna sieboldi*. *Science* 194(4266), 730–732. doi: 10.1126/science.982038
- Herrmann, M., Mayer, W.E. and Sommer, R.J. (2006) Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in western Europe. *Zoology* 109, 96–108.
- 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. *Zoological Science* 24, 883–889.
- Herrmann, M., Kienle, S., Rochat, J., Mayer, W.E. and Sommer, R.J. (2010) Haplotype diversity of the nematode *Pristionchus pacificus* on Réunion in the Indian Ocean suggests multiple independent invasions. *Biological Journal of the Linnean Society* 100, 170–179.
- Herrmann, M., Kanzaki, N., Weiler, C., Yoshida, K., Rödelsperger, C. and Sommer, R.J. (2019) Two new species of *Pristionchus* (Nematoda: Diplogastridae) include the gonochoristic sister species of *P. fissidentatus*. *Journal of Nematology* 51, e2019–e2024.
- Holterman, M., Van Der Wurff, A., Van Den Elsen, S., Van Megen, H., Bongers, T. *et al.* (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution towards crown clades. *Molecular Biology and Evolution* 23, 1792–1800.
- Hong, R.L. and Sommer, R.J. (2006) Chemoattraction in *Pristionchus* nematodes and implication for insect host recognition. *Current Biology* 16, 2359–2365.
- Hong, R.L., Svatoš, A., Herrmann, M. and Sommer, R.J. (2008) The species-specific recognition of beetle cues by *Pristionchus maupasi*. *Evolution & Development* 10, 273–279.
- Hong, R.L., Riebesell, M., Bumbarger, D.J., Cook, S.J., Carstensen, H.R. *et al.* (2019) Evolution of neuronal anatomy and circuitry in highly divergent nematode species. *eLIFE* 8, e47155.
- Kanzaki, N. and Giblin-Davis, R.M. (2020) The genus *Berntsenu* Massey 1974 is a junior synonym of *Bursaphelenchus* Fuchs, 1937. *Nematology* 22, 677–695. doi: 10.1163/15685411-00003332
- Kanzaki, N., Ragsdale, E.J., Herrmann, M., Röseler, W. and Sommer, R.J. (2012) *Parapristionchus giblindavisi* n. gen, n. sp. (Rhabditida: Diplogastridae) isolated from stag beetles (Coleoptera: Lucanidae) in Japan. *Nematology* 14, 933–947.
- Kanzaki, N., Herrmann, M., Yoshida, K., Weiler, C., Rödelsperger, C. and Sommer, R.J. (2018) Samplings of millipedes in Japan and scarab beetles in Hongkong result in five new species of *Pristionchus* (Nematoda: Diplogastridae). *Journal of Nematology* 50, 587–610.
- Kanzaki, N., Ekino, T. and Giblin-Davis, R.M. (2019) Feeding dimorphism in a mycophagous nematode, *Bursaphelenchus sinensis*. *Scientific Reports* 9, 13956. doi: 10.1038/s41598-019-50462-z
- Kanzaki, N., Herrmann, M., Weiler, C., Röseler, W., Theska, T. *et al.* (2021) Nine new *Pristionchus* (Nematoda: Diplogastridae) species from China. *Zootaxa* 4943, 1–66.
- Kieninger, M.R., Ivers, N.A., Rödelsperger, C., Markov, G.V., Sommer, R.J. and Ragsdale, E.J. (2016) The nuclear hormone receptor NHR-40 acts downstream of the sulfatase EUD-1 as part of a developmental plasticity switch in *Pristionchus*. *Current Biology* 26, 2174–2179. doi: 10.1016/j.cub.2016.06.018
- Levis, N.A. and Pfennig, D.W. (2019) *Evolution of phenotypic plasticity and gene expression during character displacement*. In: *eLS*. John Wiley and Sons, Chichester, UK. doi: 10.1002/9780470015902.a0028159.
- Lightfoot, J.W., Wilecki, M., Rödelsperger, C., Moreno, E., Susoy, V. *et al.* (2019) Small peptide mediated nematode self-recognition prevents cannibalism. *Science* 364, 86–89.
- Liu, Z., Kariya, M.J., Chute, C.D., Pribadi, A.K., Leinwand, S.G. *et al.* (2018) Predator-secreted sulfolipids induce defensive responses in *C. elegans*. *Nature Communication* 9, 1128.
- Markov, G. and Sommer, R.J. (2015) The same or not the same: lineage-specific gene expansions and homology relationship in multigene families in nematodes. *Journal of Molecular Evolution* 80, 18–36.
- Markov, G.V., Meyer, J.M., Panda, O., Artyukhin, A.B., Claassen, M. *et al.* (2016) Functional conservation and divergence of *daf-22* paralogs in *P. pacificus* dauer development. *Molecular Biology and Evolution* 33, 2506–2514.
- Mayer, W., Herrmann, M. and Sommer, R.J. (2007) Phylogeny of the nematode genus *Pristionchus* and implications for biodiversity, biogeography and the evolution of hermaphroditism. *BMC Evolutionary Biology* 7, 104.

- Mayer, W., Herrmann, M. and Sommer, R.J. (2009) Molecular phylogeny of beetle associated diplogastrid nematodes suggests host switching rather than nematode–beetle coevolution. *BMC Evolutionary Biology* 9, 212.
- McGaughran, A. and Sommer, R.J. (2014) Natural variation in cold tolerance in the nematode *Pristionchus pacificus* and the role of genotype and environment. *Biology Open* 3, 832–838.
- McGaughran, A., Morgan, K. and Sommer, R.J. (2013) Unraveling the evolutionary history of the nematode *Pristionchus pacificus*: from lineage diversification to island colonization. *Ecology & Evolution* 3, 667–675.
- McGaughran, A., Rödelsperger, C., Grimm, D.G., Meyer, J.M., Moreno, E. *et al.* (2016) Genome profiles of diversification and genotype–phenotype association in island nematode lineages. *Molecular Biology and Evolution* 33, 2257–2272.
- Meyer, J.M., Baskaran, P., Quast, C., Susoy, V., Rödelsperger, C. *et al.* (2017) Succession and dynamics of *Pristionchus* nematodes and their microbiome during decomposition of *Oryctes borbonicus* on La Réunion Island. *Environmental Microbiology* 19, 1476–1489.
- Molnar, R., Bartelmes, G., Dinkelacker, I., Witte, H. and Sommer, R.J. (2011) Mutation rates and intra-specific divergence of the mitochondrial genome of *Pristionchus pacificus*. *Molecular Biology and Evolution* 28, 2317–2326.
- Moreno, E., McGaughran, A., Rödelsperger, C., Zimmer, M. and Sommer, R.J. (2016) Oxygen-induced social behaviors in *Pristionchus pacificus* have a distinct evolutionary history and genetic regulation than *Caenorhabditis elegans*. *Proceedings of the Royal Society B* 283, 20152263.
- Moreno, E., Lightfoot, J.W., Lenuzzi, M. and Sommer, R.J. (2019) Cilia drive developmental plasticity and are essential for efficient prey detection in predatory nematodes. *Proceedings of the Royal Society B* 286, 1343.
- Morgan, K., McGaughran, A., Villate, L., Herrmann, M., Witte, H. *et al.* (2012) 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. *Molecular Ecology* 21, 250–266.
- Morgan, K., McGaughran, A., Ganeshan, S., Herrmann, M. and Sommer, R.J. (2014) Landscape and oceanic barriers shape dispersal and population structure in the island nematode *Pristionchus pacificus*. *Biological Journal of the Linnean Society* 112, 1–15.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. and Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.
- Nakayama, K., Ishita, Y., Chihara, T. and Okumura, M. (2020) Screening for CRISPR/Cas-induced mutations using a co-injection marker in the nematode *Pristionchus pacificus*. *Development Genes & Evolution* 230, 257–264.
- Nesher, N., Levy, G., Grasso, F.W. and Hochner, B. (2014) Self-recognition mechanism between skin and suckers prevents octopus arms from interfering with each other. *Current Biology* 24(11), 1271–1275. doi: 10.1016/j.cub.2014.04.024
- Ogawa, A., Streit, A., Antebi, A. and Sommer, R.J. (2009) A conserved endocrine mechanism controls the formation of dauer and infective larvae in nematodes. *Current Biology* 19, 67–71.
- Okumura, M., Wilecki, M. and Sommer, R.J. (2017) Serotonin drives predatory feeding behavior via synchronous feeding rhythms in the nematode *Pristionchus pacificus*. *G3 (Genes Genomes Genetics)* 7, 3745–3755.
- Patel, N.H., Martin-Blanco, E., Coleman, K.G., Poole, S.J., Ellis, M.C. *et al.* (1989) Expression of engrailed proteins in arthropods, annelids, and chordates. *Cell* 58, 955–968.
- Peleg, S., Sananbenesi, F., Zovolis, A., Burkhardt, S., Bahari-Javan, S. *et al.* (2010) Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science* 328, 753–756.
- Penkov, S., Ogawa, A., Schmidt, U., Tate, D., Zagoriy, V. *et al.* (2014) A wax ester promotes collective host finding in the nematode *Pristionchus pacificus*. *Nature Chemical Biology* 10, 281–285.
- Pigliucci, M. (2001) *Phenotypic Plasticity: Beyond Nature and Nurture: Syntheses in Ecology and Evolution*. Johns Hopkins University Press, Baltimore, Maryland.
- Prabh, N., Roeseler, W., Witte, H., Eberhardt, G., Sommer, R.J. and Rödelsperger, C. (2018) Deep taxon sampling reveals the evolutionary dynamics of novel gene families in the *Pristionchus* genome. *Genome Research* 28, 1664–1674.
- Rae, R., Riebesell, M., Dinkelacker, I., Wang, Q., Herrmann, M. *et al.* (2008) Isolation of naturally associated bacteria of necromenic *Pristionchus* nematodes and fitness consequences. *Journal of Experimental Biology* 211, 1927–1936.

- Raff, R.A. (1996) *The Shape of Life*. Chicago University Press, Chicago.
- Ragsdale, E.J., Müller, M.R., Rödelberger, C. and Sommer, R.J. (2013) A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell* 155, 922–933.
- Ragsdale, E.J., Kanzaki, N. and Herrmann, M. (2015) Taxonomy and natural history: the genus *Pristionchus*. In: Sommer, R.J. (ed.) *Pristionchus pacificus*. A Nematode Model for Comparative and Evolutionary Biology. Brill, Leiden, pp. 121–140.
- Renahan, T., Lo, W.-S., Werner, M.S., Herrmann, M., Rochat, J. and R.J. Sommer (2021) Nematode bi-phasic ‘boom and bust’ dynamics are dependent on host bacterial load while linking dauer and mouth-form polyphenisms. *Environmental Microbiology*, 23, 5102–5113.
- Riebesell, M. and Sommer, R.J. (2017) Three-dimensional reconstruction of the pharyngeal gland cells in the predatory nematode *Pristionchus pacificus*. *Journal of Morphology* 278, 1656–1666.
- Rödelberger, C., Meyer, J.M., Prabh, N., Lanz, C., Bemm, F. and Sommer, R.J. (2017) Single-molecule sequencing reveals the chromosome-scale genomic architecture of the nematode model organism *Pristionchus pacificus*. *Cell Reports* 21, 834–844.
- Rödelberger, C., Roseler, W., Prabh, N., Yoshida, K., Weiler, C. et al. (2018) Phylotranscriptomics of *Pristionchus* nematodes reveals parallel gene loss in six hermaphroditic lineages. *Current Biology* 28, 3123–3127.
- Rödelberger, C., Prabh, N. and Sommer, R.J. (2019) Young gene origin and deep taxon phylogenomics – opportunities and challenges. *Trends in Genetics* 35, 914–922.
- Rudel, D., Riebesell, M. and Sommer, R.J. (2005) Gonadogenesis in *Pristionchus pacificus* and organ evolution: development, adult morphology and cell/cell interactions in the hermaphrodite gonad. *Developmental Biology* 277, 200–221.
- Schierenberg, E. and Sommer, R.J. (2014) Development and reproduction in nematodes. In: Schmidt-Rhaesa, A. (ed.) *Handbook of Zoology: Gastrotricha, Cycloneuralia and Gnathifera. Vol. 2: Nematoda*. De Gruyter, Berlin, pp. 61–108.
- Schlager, B., Wang, X., Braach, G. and Sommer, R.J. (2009) Molecular cloning of a dominant roller mutant and establishment of DNA-mediated transformation in the nematode model *Pristionchus pacificus*. *Genesis* 47, 300–304.
- Schroeder, F.C. (2015) Modular assembly of primary metabolic building blocks: a chemical language in *C. elegans*. *Chemistry & Biology* 22, 7–16.
- Seroby, V., Xiao, H., Namdeo, S., Rödelberger, C., Sieriebriennikov, B. et al. (2016) Chromatin remodeling and antisense-mediate up-regulation of the developmental switch gene *eud-1* control predatory feeding plasticity. *Nature Communications* 7, 12337.
- Sieriebriennikov, B., Prabh, N., Dardiry, M., Witte, H., Röseler, W. et al. (2018) A developmental switch generating phenotypic plasticity is a part of a conserved multi-gene locus. *Cell Report* 23, 2835–2843.
- Sieriebriennikov, B., Sun, S., Lightfoot, J.W., Witte, H., Moreno, E. et al. (2020) Conserved hormone-receptors controlling a novel plastic trait target fast-evolving genes expressed in a single cell. *PLoS Genetics* 16, e1008687.
- Sommer, R.J. (2009) The future of evo-devo: model systems and evolutionary theory. *Nature Review Genetics* 10, 416–422.
- Sommer, R.J. (ed.) (2015) *Pristionchus pacificus – a Nematode Model for Comparative and Evolutionary Biology*. Brill, Leiden.
- Sommer, R.J. (2020) Phenotypic plasticity: from theory and genetics to current and future challenges. *Genetics* 215, 1–13.
- Sommer, R.J. and Mayer, M.G. (2015) Towards a synthesis of developmental biology with evolutionary theory and ecology. *Annual Review Cell & Developmental Biology* 31, 453–471.
- Sommer, R.J. and Sternberg, P.W. (1994) Changes of induction and competence during the evolution of vulva development in nematodes. *Science* 265, 114–118.
- Sommer, R.J. and Sternberg, P.W. (1995) Evolution of cell lineage and pattern formation in the vulva equivalence group of rhabditid nematodes. *Developmental Biology* 167, 61–74.
- Sommer, R.J. and Sternberg, P.W. (1996) Apoptosis limits the size of the vulval equivalence group in *Pristionchus pacificus*: a genetic analysis. *Current Biology* 6, 52–59.
- Sommer, R.J. and Tautz, D. (1993) Involvement of an orthologue of the *Drosophila* pair-rule gene *hairy* in segment formation of the short germ band embryo of *Tribolium* (Coleoptera). *Nature* 361, 448–450.
- Sommer, R.J., Carta, L.K., Kim, S.Y. and Sternberg, P.W. (1996) Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda, Diplogastridae). *Fundamental and Applied Nematology* 19, 511–521.

- Sternberg, P.W. (2005) Vulval development. In: *C. elegans* Research Community (ed.) *WormBook: The Online Review of C. elegans Biology*, WormBook, Pasadena, California. doi: 10.1895/wormbook.1.6.1
- Sternberg, P.W. and Horvitz, H.R. (1981) Gonadal cell lineages of the nematode *Panagrellus redivivus* and implications for evolution by modification of cell lineage. *Developmental Biology* 88, 147–166.
- Sternberg, P.W. and Horvitz, H.R. (1982) Postembryonic non-gonadal cell lineages of the nematode *Panagrellus redivivus*: description and comparison with those of *Caenorhabditis elegans*. *Developmental Biology* 93, 181–205.
- Susoy, V., Herrmann, M., Kanzaki, N., Kruger, M., Nguyen, C.N. *et al.* (2016) Large-scale diversification without genetic isolation in nematode symbionts of figs. *Science Advances* 2, e1501031.
- Thébaud, C., Warren, B.H., Strasberg, D. and Cheke, A. (2009) Mascarene islands, biology. In: Gillespie, R.G. and Clague, D.A. (eds) *Encyclopedia of Islands*. University of California Press, San Diego, California.
- Tian, H., Schlager, B., Xiao, H. and Sommer, R.J. (2008) Wnt signaling induces vulva development in the nematode *Pristionchus pacificus*. *Current Biology* 18, 142–146.
- True, J. and Haag, E.S. (2001) Developmental systems drift and flexibility in evolutionary trajectories. *Evolution and Development* 3, 109–119.
- Urban, I., Kerimoglu, C., Sakib, M.S., Wang, H., Benito, E. *et al.* (2019) TIP60/KAT5 is required for neuronal viability in hippocampal CA1. *Scientific Report* 9, 16173.
- Villinger, J. and Waldman, B. (2008) Self-referent MHC type matching in frog tadpoles. *Proceedings of the Royal Society B: Biological Sciences* 275(1639), 1225–1230. doi: 10.1098/rspb.2008.0022.
- von Lieven, A.F. and Sudhaus, W. (2000) Comparative and functional morphology of the buccal cavity of Diplogastrina (Nematoda) and a first outline of the phylogeny of this taxon. *Journal of Zoological Systematics & Evolutionary Research* 38, 37–63.
- Waddington, C.H. (1975) *The Evolution of an Evolutionist*. Cornell University Press, New York.
- Wang, X. and Sommer, R.J. (2011) Antagonism of LIN-17/Frizzled and LIN-18/Ryk in nematode vulva induction reveals evolutionary alterations in core developmental pathways. *PLoS Biology* 9, e1001110.
- Weadick, C.J. and Sommer, R.J. (2016a) Unexpected sex-specific post-reproductive lifespan in the free-living nematode *Pristionchus exspectatus*. *Evolution & Development* 18, 297–307.
- Weadick, C.J. and Sommer, R.J. (2016b) Mating system transitions drive lifespan evolution in *Pristionchus* nematodes. *American Naturalist* 187, 517–529.
- Weadick, C.J. and Sommer, R.J. (2017) Interspecific hybrids and the genetics of lifespan in *Pristionchus* nematodes. *Journal of Evolutionary Biology* 30, 650–657.
- Weller, A., Rödelberger, C., Eberhardt, G., Molnar, R.I. and Sommer, R.J. (2014) Opposing forces of A/T-biased mutations and G/C-biased gene conversions shape the genome of the nematode *Pristionchus pacificus*. *Genetics* 196, 1145–1152.
- Werner, M., Sieriebriennikov, B., Loschko, T., Namdeo, S., Lenuzzi, M. *et al.* (2017) Environmental influence on *Pristionchus pacificus* mouth-form through different culture methods. *Scientific Reports* 7, 7207.
- Werner, M.S., Sieriebriennikov, B., Prabh, N., Loschko, T., Lanz, C. and Sommer, R.J. (2018a) Young genes have distinct gene structure, epigenetic profiles, and transcription start site regulation. *Genome Research* 28, 1675–1687.
- Werner, M.S., Claassen, M.H., Renahan, T., Dardiry, M. and Sommer, R.J. (2018b) A vital dye method in nematodes reveals age is a critical parameter in density-dependent plasticity. *iScience* 10, 123–134.
- West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annual Review Ecology and Systematics* 20, 249–278.
- West-Eberhard, M.J. (2003) *Developmental Plasticity and Evolution*. Oxford University Press, Oxford, UK.
- Wilecki, M., Lightfoot, J.W., Susoy, V. and Sommer, R.J. (2015) Predatory feeding behavior in *Pristionchus* nematodes is dependent on a phenotypic plasticity and induced by serotonin. *Journal of Experimental Biology* 218, 1306–1313.
- Witte, H., Moreno, E., Rödelberger, C., Kim, J., Kim, J.-S. (2015) Gene inactivation using the CRISPR/Cas9 system in the nematode *Pristionchus pacificus*. *Development, Genes & Evolution* 225, 55–62.
- Wright, K.A. (1987) The nematode's cuticle – its surface and the epidermis: function, homology, analogy – a current consensus. *Journal of Parasitology* 73, 1077–1083.
- Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. and Georgieva, S.A. (1993) Feeding habits in soil nematode families and genera – an outline for soil ecologists. *Journal of Nematology* 25, 315–331.
- Yim, J., Bose, N., Meyer, J.M., Sommer, R.J. and Schroeder, F.C. (2015) Nematode signaling molecules derived from multimodular assembly of primary metabolic building blocks. *Organic Letters* 17, 1648–1651.

-
- Yoshida, K., Herrmann, M., Kanzaki, N., Weiler, C., Rödelsperger, C. and Sommer, R.J. (2018) Two new species of *Pristionchus* (Nematoda: Diplogastridae) from Taiwan and the definition of the *pacificus* species-complex *sensu stricto*. *Journal of Nematology* 50, 355–368.
- Zauner, H., Mayer, W.E., Herrmann, M., Weller, A., Erwig, M. and Sommer, R.J. (2007) Distinct patterns of genetic variation in *Pristionchus pacificus* and *Caenorhabditis elegans*, two partially selfing nematodes with cosmopolitan distribution. *Molecular Ecology* 16, 1267–1280.