Book review

Ralf J. Sommer (Editor). 2015. *Pristionchus pacificus* – a nematode model for evolutionary biology. 2015. Nematology Monographs and Perspectives, Volume 11 (Series Editors: David J. Hunt & Roland N. Perry), Koninklijke Brill, Leiden-Boston, 420 pp. ISBN13: 978 90 04 260 290

The first two chapters of the book, *Pristionchus pacificus* – a nematode model for evolutionary biology**,** describe the same story – an introduction and the early years of *P. pacificus* as a model organism. The first chapter is authored by Paul W. Sternberg, one of the founders of *Caenorhabditis elegans* research. He observed this emerging model organism from the point of view of *C. elegans* people. In this sense the chapter is properly entitled 'Why *Caenorhabditis elegans* is great and *Pristionchus pacificus* might be better'. One can imagine how strong is this concession for a *C. elegans* researcher, that 'something can be better than *Caenorhabditis*'. Occasionally this chapter contains several purely '*C.ele*gans' sections, like 'Useful features of *C. elegans*' or 'What *C. elegans* did for us'. It is always reasonable to return mentally to the origin of the 'obsession' of modern biology with this organism.

The second chapter 'Integrative evolutionary biology and mechanistic approaches in comparative biology' is authored by Ralf J. Sommer, the editor of this volume. For a Russian reader it is pleasing to see a reference to the important article of Theodosius Dobzhansky, a US evolutionary biologist of Russian origin. Ralf Sommer presents the story of *Pristionchus* in a slightly different way from that of the previous chapter. Sections bear informative and inviting titles: 'The complexity of life', 'The power of model system approaches', 'Comparative biology and a need for mechanistic approaches', 'Integrative evolutionary biology', 'Integrative evolutionary biology needs comparative approaches' *etc*. The entire chapter is constructed along this clear narrative logic. The author provides his insight into the modern situation in biology as an advent of integrative approach, combining together several main parts with contradicting basic principles: reductionist molecular biology, holistic ecology and 'evo-devo'. In his opinion the more pronounced dependence of *Pristionchus* biology on evident ecological factors (like interrelationships between this nematode and insect hosts, participation of external factors not only in the developmental patterns but even in the morphological dimorphism) makes this model organism very special.

The third chapter 'Diplogastrid systematics and phylogeny' by Natsumi Kanzaki and Robin M. Giblin-Davis links the very modern content of the book with more traditional nematology. The authors of the chapter provide a short overview of taxonomy and classification of Diplogasteromorpha and all the necessary terminology to describe the complicated diplogastrid morphology. They also recognise the advent of molecular methods into nematode systematics, considering it as a starting point of the 'integrative systematic' époque. For a traditional nematologist a list of diplogastrid genera with comments on their morphological characters will be especially helpful. Recently published paper by Susoy *at al.* (2015) is a sound basis for an analysis of diplogastrid phylogeny, by providing diagnoses and data on bionomics. The proposed phylogenetic tree is based on the combined analysis of 18S and 28S rDNA, several genes of ribosomal proteins and RNA polymerase II. The closing section 'Integrated systematics based on morphology and molecular phylogeny' is also interesting as it presents, in condensed form, the authors' vision of the problems and goals of nematode systematics in our days.

The fourth chapter of the book – 'Taxonomy and natural history: the genus *Pristionchus*' by Erik J. Ragsdale, Natsumi Kanzaki and Matthias Herrmann is a logical continuation of the previous one. The authors start from the history of diplogastrid research. It is interesting to know that the first laboratory culture of *P. pacificus* was established from a soil sample taken by students in Pasadena in 1988. The authors provide sufficient information of the bionomics of *Pristionchus* species. As we can understand it now the majority of the species are in necromenic relationships with different beetles (mainly Scarabaeidae). A connection with other invertebrates (*e.g.*, diplopods) or occurrence in rich soil habitats without a stable link with invertebrates are supposed for some *Pristionchus* species. A biological connection of one *Pristionchus* species (*P. uniformis*) with the Colorado potato beetle reminds one of attempts in the former USSR to develop the biocontrol of this pest with diplogastrid nematodes. The obvious presence of nematodes in beetle cadavers was presented as a proof of the potential of this diplogastrid for pest suppression. Now we can see that was only "*post hoc ergo propter hoc*" logical error. The authors of this chapter provide a complete list of synonyms for all the *Pristionchus* binomials. The chapter includes very detailed analysis of 'light microscopy level' studies of *Pristionchus* morphology. This part will be helpful, even for experienced nematode taxonomists, as providing English names for all the main structures of rhabditids. It is interesting that the function of some morphological structures of *Pristionchus* is still obscure. For example, the lateral pores with adjacent gland are described for *Pristionchus* as also two (?) pairs of postdeirids. The function of lateral pores is still unknown. It is a pity that *Priostionchus* embryology has not been studied up to the level comparable with that of *C. elegans*. It would be very interesting to known about the embryonic origin of cells forming these lateral structures (same lineage as postdeirids?). A short section on biogeography contains one statement that can be helpful for those interested in nematode global distribution: 'human activity has dispersed nematodes across otherwise formidable barriers'. The authors provide data on the occurrence of separate clades of *Pristionchus*. It is interesting that androdioecious and gonochoristic forms demonstrate different biogeographical patterns.

The fifth chapter, 'The laboratory model: genetics, genetic mapping and transgenics' by Laura Aurilio and Jagan Srinivasan**,** opens the part of the book containing less traditional science by presenting the main features of *P. pacificus* as a model organism. The time scale on the page 122 reflects this quite short history. It is really fascinating to see how well planned and organised was the introduction of *Pristionchus* as a model organism. Mutations were discovered and mapped after mutagenesis with EMS or TVP-UV. The nomenclature of mutations was established, resembling, but not repeating, that for *C. elegans* (nomenclature of mutations enable their identification immediately as those of *P. pacificus*). Genetic studies on *P. pacificus* passed through the period of linkage maps to the publication of a draft genome. The chapter authors propose an overview of all methods now in use in *Pristionchus* studies. Nematodes are so different that some methods successfully applied in *C. elegans* studies were not productive with *P. pacificus*. For example, RNA interference did not result in expected phenotypes (it was only successful with genes *tra*-1 and *Ppa-prl*-1). *In-situ* hybridisation and immunohistochemistry are not widely used, but NGS (next-generation sequencing) is increasingly important. NGS is used for the detection of mutagen-induced sequence alterations, when sequencing results for a mutant are compared with those of the non-mutagenised reference genome. The chapter closes with several very helpful references to the genomic resources for cloning genes, gene nomenclature, genetic maintenance and sequence information of libraries and genome sequence for *P. pacificus*.

The sixth chapter 'Comparative and functional genomics' by Christian Rödelsperger and Christoph Dieterich provides the main facts about genomic studies in nematology. Starting from an overview of published nematode genomes, the chapter presents several key facts about *Pristionchus* genome. The genomes of two strains of *P. pacificus* are available now: that of the reference strain PS312 and PS1943 from Washington. From 24,000 up to 30,000 protein genes are predicted for *P. pacificus,* although up to now the existence of only 4,000 genes is supported by transcriptome analysis. In total, exons of protein-coding genes constitute about 18-21% of the assembled *P. pacificus* genome. Among the remarkable features of *P. pacificus* genome, the authors mention the reduced content of repeats. Both DNA and RNA transposons were detected in *P.pacificus*, including the proven fact of horizontal transfer of one insect transposon (Rte-1). An important contribution was made in recent years to the knowledge of miRNA genes in nematodes. Up to 362 miRNAs are described for *P. pacificus*. It was also shown that conserved families of miRNA genes exist in three rhabditid species of *Caenorhabditis*, *Pristionchus* and *Strongyloides* genera. For example, very similar miRNA participate in the dauer formation in these species. Numerous genes were detected in *Pristionchus* and other nematodes, for which no recognisable homologues were found in nematode genomes. These genes, named 'orphan genes', constitute an important part of known nematode genomes. An analysis of available genomes indicates that the number of such 'orphan' genes is not decreasing with the publication of new genomes. It means that sequencing of nematode genomes and transcriptomes is very far from saturation, and data for a few genomes are not representative for the entire phylum. Surprising evidence described in this chapter is the horizontal transfer of some genes to the nematodes from other organisms. The facts of several independent cases of horizontal transfer of cellulose genes to nematodes from fungi and molds serve as most obvious examples of horizontal gene transfer in eukaryotes.

The next chapter, 'Small-molecule signalling: encoding biological information in chemical structures' by Frank C. Schroeder from Cornell University, is something very new for an old-fashioned zoologist. Surely one can suspect that some hidden chemical processes are running in any living creature, but this chapter demonstrates visible and very sound progress in this field achieved in studies on *Pristionchus*. The starting lines of this chapter contain an expression: “almost any chemical structure an organic chemist could imagine may conceivably exist in nature”. Some of such compounds not only exist but also play a leading role in information transfer inside the organisms. The author is speaking about biogenic small molecules (BSM), which probably arise as a part of the metabolome but then evolving into inner signals responsible for an array of biological phenomena. It is mentioned in the chapter that despite such obvious role, the metabolomes of even model organisms remained unexplored during decades of intensive investigations on these organisms. One familiar with the history of *C. elegans* studies can remember the milestone on this way – the publication of Golden and Riddle (1982) which demonstrated the participation of ‘dauer pheromone’ in the formation of this special stage of *C. elegans* life cycle. Since then the studies of this pheromone revealed the participation of nuclear hormone receptor DAF-12 in the downstream regulation of this chemical signal. An important part of ‘dauer pheromone’ is a mixture of glycosides (ascarosydes). Such molecules consist of dideoxysugar ascarylose and a variety of fatty acid side chains. It was shown that different ascarosydes mediate different events in *C. elegans* morphogenesis and behaviour, and in this way pass information about overall physiological state of the worm. The author describes the advent of ‘comparative metabolomics’ as an approach that circumvents the need of pure compound isolation by focusing on comparison of high-resolution spectroscopic datasets. Such an approach is described in the next subchapter ‘The *P. pacificus* metabolome: adventures in structure space’, which depicts the participation of BSM in the formation of two types of stomatal cavity in *P. pacificus*. Just to reflect the complexity of such research,we can mention that more than 5,000 unknown metabolites were detected in *P. pacificus*. Some of these are presumably building blocks, from which ‘informative’ molecules will be constructed. In many cases an active compound is a molecule containing carbohydrate, amino acid and nucleoside parts. If in *C. elegans* such molecules contain solely ascarylose, in *P. pacificus* two sugars play important roles – the aforementioned ascarylose and L-paratose (paratoside). This latter was found before only in bacteria. It was also shown (see the section ‘Modular biosynthesis is selective’) that the nematode is not producing a random array of molecules, but directed synthesis of few informative compounds occurs. A comparison of dauer-inducing pheromones between the *P. pacificus* strains demonstrated that pheromones of one strain can induce the dauer formation in another strain even more effectively. It means that some strains can force other strains in premature formation of dauers just to exclude the neighbours from competition for limited food resources. The fascinating mechanism of nuclear hormone receptor DAF-12 is described in this chapter. Specific ligands are connecting with DAF-12, which initiates the transcription of let-7-family microRNAs mir-84 and mir-241. This transcription initiates the normal reproductive development of the nematode. If ligand biosynthesis is inhibited, unliganded DAF-12 connects with DIN-1 co-repressor and prevent transcription, which leads to the reproductive path of development.

Another unexpected discovery was made when observing a peculiar behavioural reaction of *P. pacificus* – the formation of so called ‘dauer towers’. Several thousand juveniles aggregate into clumps that form miniature towers. Sometimes the height of towers reach 1 cm. In was obvious that tower formation is facilitated by an oily compound covering all the structure. Chemical analysis revealed that this oily substance is a wax ester (nematoil). The ester is a molecule consisting of 30-carbon fatty acid and 30-carbon alcohol (really two identical chains). The author ends this chapter with an important supposition. Unlike plants and microorganisms, metazoans use as building parts for BSM the blocks derived from conserved primary metabolism. It seems possible that similar types of modular small-molecule signals are produced by other animals.

The next chapter ‘Population genetics and the La Réunion case study’ by Angela McGaughran and Katy Morgan returns us to more traditional types of biological studies. The chapter contains the description of molecular-phylogenetic studies of *P. pacificus* strains isolated on an island in the Indian Ocean, La Réunion, and neighbouring Mascarene islands. The chapter contains detailed description of diversity and distribution of *Pristionchus* on the island, evolutionary history and demography of nematode populations. Environmental conditions on the island are analysed and several ecological zones are defined. Four main lineages of *P. pacificus* were discovered in the island, which demonstrate some level of geographical segregation. Unlike *C. elegans*, an enormous diversity and differentiation was demonstrated for *P. pacificus*. The introduction of Mutation Accumulation (MA) experiments into nematode studies makes it possible to estimate the minimum number of new mutations that appear in a given lineage over the specified number of generations. Such an approach was able to demonstrate that divergence of four mitochondrial lineages on La Réunion preceded the colonization of the island. Considering the demography, the authors mention that if lineages A, C and D demonstrate some level of overlap and rare admixture/recombination event the lineage B (strain from central volcanic plateau) is well separated from the others. The fact that lineages remain distinct suggests that some degree of reproductive isolation exists.

The ninth chapter with quite a complex title: 'Evo-devo and developmental systems drift: an evolving paradigm in organ formation and tissue coordination, vulva and gonad development in *Pristionchus pacificus*' by David Rudel presents *P pacificus* as a convenient model for developmental studies. As with *C. elegans*, vulva and gonad formation are the most striking examples of the intricate cell-to-cell communications during morphogenesis. The fates of ventral precursor cells in *C. elegans* revealed a sophisticated cellular mechanism, which result in the construction of such multi-cell and multi-layered structure as the vulva. The expression of *Hox* gene in the cells only competent for vulva formation is a main regulator of such ability. Two homeodomain proteins regulate the anterior and posterior border of vulval equivalence group (*i.e.*, a group of cells that can produce the vulva). Another mechanism is an induction of vulva formation. Special ligands are expressed and sent to the cellular surface of vulvar precursors by the gonad. The same vulva formation is a process of cell divisions with strictly specified orientations. For a classical nematologist the differences between *C. elegans* and *P. pacificus* are especially interesting. Laser-ablation experiments enable understanding of the interconnection between separate morphogenesis events. For example, ablation of the vulva prevents normal growth of gonad reproductive zone. The ablation of Z1 and Z4 cells (somatic gonad precursors) results in the loss of germ line. The role of distal tip cell of gonad is also discussed in this chapter. Between several important conclusion from this overview of vulva and gonad formation studies in two model nematode author makes one that is very important for understanding nematode evolution: continuous induction of vulva in *P. pacificus* (*i.e.*, several events) may be less derived than the single-cell short-duration event of vulva formation in *C. elegans*.

Because of space limitation, detailed analysis of the next two chapters cannot be provided. These two describe intricate mechanisms of the dauer formation and dauer-specific behaviours (Chapter 10 by Akira Ogawa and Federico Brown) and mouth dimorphism in *P. pacificus* (Chapter 11 by Eric Ragsdale). These chapters present these phenomena in detail.

The next chapter '*Pristionchus pacificus* olfaction' by Ray L. Hong describes experiments demonstrating the attraction or avoidance by *C. elegans* and *P. pacificus* of some volatile compounds. Two strains of *P. pacificus* were used for comparative experiments. Reaction of both strains to beta-caryophyllene (a volatile compound released by plants attacked by herbivorous insects) was similar – the nematodes were attracted by this compound. At the same time the reaction of two strains of *P. pacificus* against insect-associated compounds was significantly different. Two compounds (ZTDO – Z-7-tetradecen-2-one and ETDA – E-11-tetradecenyl acetate) were mainly used in experiments. The reaction against these compounds by *P. pacificus* was found to be sensitive to the addition of exogenous cGMP. The author indicates that the behavioural reaction of *P. pacificus* strains against volatile compounds is tightly connected with their host preferences. A quite unexpected practical outcome of this research is the possibility reported in the section 'Olfaction profiles reflect host preferences'. It was found that by the reaction to only three odours (linalool, phenol and ZTDO) one can discriminate among different species and even some strains of *Pristionchus*.

The chapter 13 'Anatomy and connectivity in the pharyngeal nervous system' by Dan Bumbarger and Metta Riebesell is about the *P. pacificus* nervous system with special attention to the nervous network in the pharynx. Detailed description of nervous system of this nematode leads to the phylogenetic comparison, leading to the important conclusion : 'ancestral lineages of nematodes may have had more complex nervous systems than those commonly studied'.

The final chapter of the book 'Bacterial interactions and the innate immune system' by Amit Sinha and Robbie Rae covers a quite unexpected part of *Pristionchus* life, as it deals with bacteria naturally associated with *Pristionchus*. The chapter starts from a general overview of nematode-bacterial interactions: from symbiotic bacteria of entomopathogenic nematodes up to *Wolbachia* symbiosis. Still, the chapter is mainly directed toward the ability of nematodes to resist bacterial infections. The study of innate immunity of *C. elegans* has developed successfully during last decade, and demonstrated the use of different lectins, lysozymes and antimicrobial compounds by this nematode. *Pristionchus* nematodes are living in the environment populated with different bacteria and also need some defensive mechanisms. The authors studied the bacteria of the *Pristionchus* habitats and discovered numerous representatives of *Bacillus*, *Pseudomonas* and *Serratia*. It was demonstrated also that, unlike *C. elegans*, *P. pacificus* was resistant toward *Pseudomonas aeruginosa* and *Serratia aureus*. After the sequencing of *P. pacificus* genome it was obvious that there were many more genes involved with detoxification of xenobiotic compounds than in *C. elegans*. Recently two new toxins (so called Cry toxins) were found in *Bacillus thuringiensis*, which were involved in nematicidal activity against *C. elegans*. As the majority of *Bacillus* strains isolated by authors in Europe supported the development of both *C. elegans* and *P. pacificus* on agar plates, an experimentation scheme was developed for the screening of nematodes isolates. These two nematodes were fed with four bacteria: *Serratia marescens*, *Xenorhabdus ematophila*, *Staphylococcus aureus* and *B. thuringiensis*. Then the transcriptional response of nematodes was studied. It was shown that gene expression profiles of *C. elegans* and *P. pacificus* in response to the same pathogenic bacteria were different. For example, *P. pacificus* is resistant to *P. aeruginosa*, *S. aureus* and *B. thuringiensis*, whereas *C. elegans* is not. As in *C. elegans* the lifespan and resistance to bacterial pathogens of *P. pacificus* can be extended when germline cells are ablated with laser beam. Authors conclude that a wider study of *P. pacificus* strains is needed to identify the loci responsible for resistance to bacteria.

This book is an impressive collection of facts and ideas neatly packed into quite condensed texts. It is a breath-taking demonstration of the power of modern biology. Some elements of the global ‘*Pristionchus pacificus*’ project are still in the reach for even modestly equipped laboratories. Thus, the ‘La Réunion case’ is a good example of how the study of intra-specific groups of nematodes can reveal a complicated history of colonisations and adaptation. Such books serve as special messengers for the scientific community, provoking thought for further research.

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