INVITED REVIEWS AND META-ANALYSES

The nematode *Pristionchus pacificus* as a model system for integrative studies in evolutionary biology

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Abstract

Comprehensive studies of evolution have historically been hampered by the division among disciplines. Now, as biology moves towards an '-omics' era, it is more important than ever to tackle the evolution of function and form by considering all those research areas involved in the regulation of phenotypes. Here, we review recent attempts to establish the nematode *Pristionchus pacificus* as a model organism that allows integrative studies of development and evo-devo, with ecology and population genetics. Originally developed for comparative study with the nematode *Caenorhabditis elegans*, *P. pacificus* provided insight into developmental pathways including dauer formation, vulva and gonad development, chemosensation, innate immunity and neurobiology. Its subsequent discovery across a wide geographic distribution in association with scarab beetles enabled its evaluation in a biogeographic context. Development of an evolutionary field station on La Réunion Island, where *P. pacificus* is present in high abundance across a number of widespread habitat types, allows examination of the microfacets of evolution – processes of natural selection, adaptation and drift among populations can now be examined in this island setting. The combination of laboratory-based functional studies with fieldwork in *P. pacificus* has the long-term prospective to provide both proximate (mechanistic) and ultimate (evolutionary and ecological) causation and might therefore help to overcome the long-term divide between major areas in biology.

Keywords: evolutionary biology, integrative studies, model organism, nematode, *Pristionchus pacificus*

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An integrative approach to evolutionary biology

Animals, plants and fungi show an astonishing diversity of form that has fascinated evolutionary biologists and ecologists ever since Darwin and Wallace. This diversity is the subject of intense studies: How did the complexity of multicellular organisms arise? What are the molecular mechanisms involved in the generation of body parts and specific morphological structures? Which evolutionary forces cause changes in phenotypes? How do changes in the genotype, initiated as changes in gene frequencies, result in morphological alterations? And, what is the role of the environment in all these processes?

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Following the elucidation of the structure of DNA, evolutionary patterns and processes have been investigated using comparative approaches that combine molecular studies with investigations at all other hierarchies of biological organization (Rieppel 1988; Harvey & Pagel 1991). By now, the detailed analysis of genes and even genomes provides fundamental insight into the molecular processes and mechanisms at work in evolution (Koonin 2011). However, the unfortunate divide between some of the major disciplines in the biological sciences has prevented a fully comprehensive analysis in evolutionary biology. Most importantly, development – the unfolding of morphology and form during the ontogeny of the individual plant or animal – was excluded from evolutionary studies in the Neo-Darwinian synthesis (Amundson 2005). As pointed out by West-Eberhard and others, evolutionary biology has

long focused on how traits (or genes) are modified or lost but has to a large extent disregarded the appearance of novelty and its underlying developmental mechanisms and principles (Schlichting & Pigliucci 1998; West-Eberhard 2003).

Several authors have argued for integrative approaches that try to tackle the evolution of function and form by considering all those research areas involved in the regulation and evolution of phenotypes (Gerhard & Kirschner 1997; Schlichting & Pigliucci 1998; Fox & Wolf 2006; Lynch 2007; Sommer 2009). Three basic research areas are involved in the study of the generation of form and biological diversity (Fig. 1).

Developmental biology and evo-devo

Morphological structures are formed throughout the ontogeny of the individual. Thus, the developmental perspective and its genetic and mechanistic basis are of ultimate importance for an understanding of phenotypic evolution. Developmental processes change during the course of evolution; hence, evolutionary developmental biology (evo-devo) can unravel the mechanistic changes in developmental processes that give rise to altered developmental and morphological structures.

Ecology

Evolution is, in part, the influence of the environment on development (Van Valen 1973); therefore, the ecological perspective on developmental processes is crucial to understand the generation of novelty over evolutionary timescales.
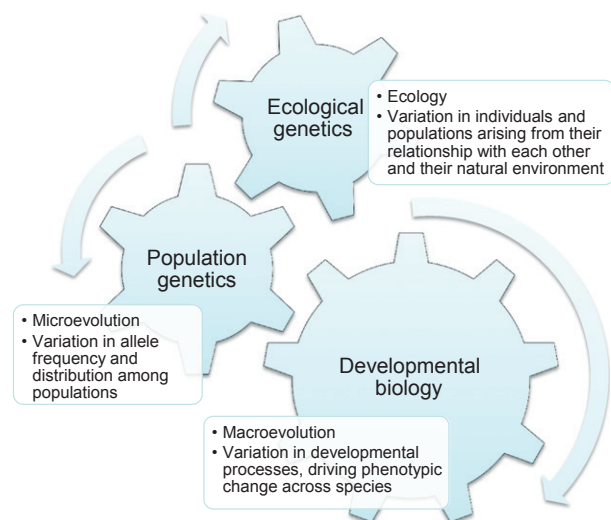
Fig. 1 Graphic to demonstrate the integrative approach that can now be applied in studies using the *Pristionchus pacificus* system.

Population genetics

Modifications and novelty arise as 'natural variation' in populations; hence, a population genetic perspective is a prerequisite for obtaining a comprehensive understanding of phenotypic change.

One of us has argued previously that a comprehensive understanding of the evolutionary forces that generate biological diversity requires integrative approaches that bring developmental biology and evo-devo closer to evolutionary theory (Sommer 2009). Such integrative studies, when performed in the same organism to study the same patterns and processes, can be integrated into a comprehensive framework. However, only a few classical 'model systems', such as yeast, *Drosophila*, mice and some plants including *Arabidopsis* meet most of these conditions required for highly integrative approaches. At the same time, organisms with interesting ecological patterns are often hard to study under laboratory conditions. Here, we review recent attempts to establish the nematode *Pristionchus pacificus* as an additional model that allows integrative studies of development and evo-devo, with ecology and population genetics.

Nematodes as model organisms

Nematodes, or roundworms, are an animal phylum that is best characterized by species richness, numerical abundance and ecological omnipresence (Lee 2002). Nematodes represent the largest animal phylum, with an estimated number of one to ten million species, although only ~25 000 species are described in the literature to date (Lambshead 1993). In many ecological niches, nematodes can occur in very high density, for example, in excess of one million individuals per square metre in some soil systems (Floyd *et al.* 2002). While nematodes are known from nearly all ecosystems, they are small in size, usually in the range of millimetres. Hard to see without a microscope, they are therefore largely unknown to the general public.

Nonetheless, several nematode species are used as model organisms in basic research, with *Caenorhabditis elegans* being one of the best-studied organisms in the modern life sciences. One reason for the use of *C. elegans* as a model in biomedical research is that it can be propagated indefinitely under laboratory conditions and reproduces by self-fertilization. *C. elegans* is 1 mm in length as adult and has a life cycle of only 3 days (20° C), and millions of animals can be cultured in one laboratory using *Escherichia coli* as a food source. The *C. elegans* model is best known for its systematic approaches. First, the complete cell lineage of 'the worm' was determined in the 1970s, providing the basis

for detailed investigations of embryonic and postembryonic development (Sulston & Horvitz 1977; Sulston *et al.* 1983). Adult hermaphrodites have 959 somatic cells, whereas males, although smaller than hermaphrodites, consist of 1031 somatic cells given the more elaborate neuronal structures in the tail required for mating. All these cells are systematically named and for each individual, the cell division pattern at any time in development can be anticipated, providing a unique level of precision for studies of development. Second, genetic screens for genes controlling development, cell, and neurobiological processes have often reached saturation level, that is, all genes with nonredundant essential functions have been identified (www.wormbook.org). Finally, *C. elegans* was the first metazoan to have its genome fully sequenced in 1998, followed by the application of other '-omics' technologies ever since (Gerstein 2010).

Free-living nematodes and their immediate link to ecology

Many free-living nematodes are found in terrestrial settings, often in association with arthropods or other invertebrates. Interestingly, in many of these associations, nematodes are not actively reproducing on their living host. Rather, they stay in an arrested developmental stage called dauer larva (for review see Sommer & Ogawa 2011). In general, the typical nematode undergoes embryonic development within an eggshell followed by post-embryonic development that consists of four stages, either called larval (L1–L4) or juvenile (J1–J4) stages (Fig. 2a). Many worms follow this direct life cycle under favourable food conditions. However, under unfavourable conditions, such as the absence of food, high temperature or high population density, worms enter the arrested dauer stage (Fig. 2a). Dauer larvae are
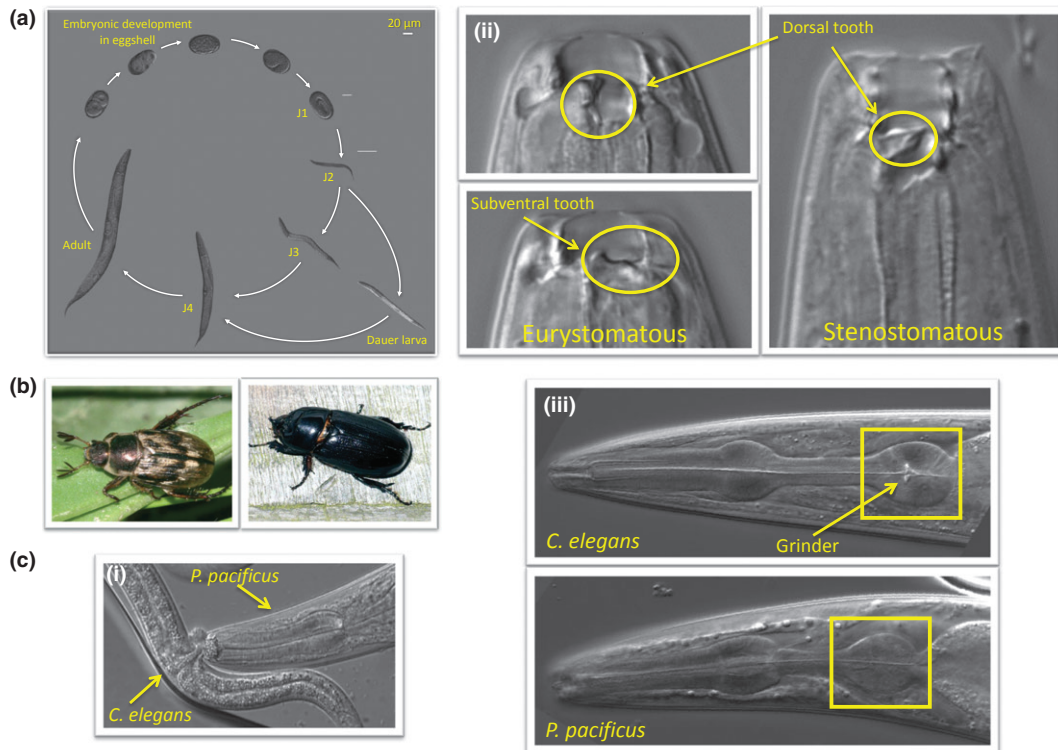
Fig. 2 (a) The life cycle of *Pristionchus pacificus*: in general, the typical nematode undergoes embryonic development within an eggshell followed by post-embryonic development that consists of four stages, either called larval (L1–L4) or juvenile (J1–J4) stages; the generation time of *P. pacificus* is approximately 4 days at 20°C, but under stressful environmental conditions, such as food shortage or high population density, J2 larvae form dauers instead of developing into J3 larvae; (b) Numerous samplings, initiated in 2004, have indicated that *Pristionchus* nematodes live in a necromenic association with scarab beetles. *P. pacificus* dauers are associated with many scarab beetles, such as *Exomala orientalis* (left), and *Oryctes borbonicus* (right). After the natural death of its host, *P. pacificus* exits the dauer stage, resumes its development, and starts feeding on microbes that grow on the beetle carcass; (c) *P. pacificus* is equipped with versatile teeth-like denticles that are unknown from *C. elegans* and other rhabditid nematodes; these structures allow predation in *P. pacificus* (i) and can occur in two alternative forms, the so-called eurystomatous (EU) and stenostomatous (ST) mouth-forms (ii); occurrence of denticles in *Pristionchus* and some other members of the Diplogastridae coincides with the loss of the grinder, a structure in the terminal bulb of Rhabditid nematodes that is crucial for the mode of bacterial feeding seen in *C. elegans* (iii).

nonfeeding but motile, representing an alternative J3 juvenile stage, which can survive for months and resume development once conditions improve. Thus, the decision for direct vs. indirect development is influenced by several ecological parameters and is of high importance for the evolutionary success of nematodes.

Dauer vs. direct development represents a case of phenotypic plasticity as two completely different morphs can be formed with the same genomic information. Distinct from physiological (reversible) plasticity, the nonreversible phenotypic plasticity is a well-established concept in ecology and evolution (Schlichting & Pigliucci 1998; West-Eberhard 2003). However, few case studies allow detailed genetic and mechanistic inroads to this fascinating phenomenon, with *Caenorhabditis elegans* dauer formation representing one of a few systems that have been studied in great detail using mutants showing constitutive dauer formation or dauer formation defective phenotypes. Since the 1980s, the *C. elegans* research community has studied the genetic and molecular aspects of dauer formation, resulting in a tremendous understanding of this phenotypically plastic trait (Hu 2005). Building on these results, research in the last few years has explored several evolutionary and ecological patterns associated with dauer development and its importance in the wild. While some of these studies are summarized below, we refer the interested reader to more detailed accounts that highlight the importance of dauer formation as an example of phenotypic plasticity (Sommer & Ogawa 2011; Viney & Diaz 2012).

Introducing *Pristionchus pacificus*

A fundamental understanding of all types of biological processes in *Caenorhabditis elegans* has provided a platform for evolutionary studies using other nematodes in comparison (Sommer *et al.* 1996). In the last decade, our laboratory has applied this idea to the nematode *Pristionchus pacificus* (Hong & Sommer 2006). To establish *P. pacificus* as a model organism in its own right, we have produced an array of forward and reverse genetic tools, a sequenced genome and a DNA-mediated transformation system (Hong & Sommer 2006; Dieterich *et al.* 2008; Schlager *et al.* 2009). Early work in *P. pacificus* had a strong evo-devo focus, comparing several developmental pathways, for example, dauer formation (Ogawa *et al.* 2009, 2011; Sinha *et al.* 2012a), vulva development (Tian *et al.* 2008; Wang & Sommer 2011), gonad development (Rudel *et al.* 2005, 2008), innate immunity (Rae *et al.* 2008, 2010; Sinha *et al.* 2012b) and neurobiology (Bumbarger *et al.* 2013) between *P. pacificus* and *C. elegans*. The comparison of two distantly related organisms, such as these two nematodes,

provided a detailed understanding of the mechanistic changes of development during long-term evolution.

One overarching principle emerging from these studies is 'developmental systems drift', a term originally coined by True & Haag (2001) to indicate that even evolutionary stable processes show an enormous amount of drift in their underlying molecular mechanisms. While developmental systems drift seems to represent a general phenomenon in animal development, it is currently unknown to what extent these patterns are caused by adaptive and/or non-adaptive forces. Only the involvement of population genetic studies and the comparison of closely related species can provide insight into such questions.

An example of developmental systems drift is the nematode vulva, which represents an homologous organ in all studied nematodes (Kiontke *et al.* 2007; Sommer 2008). While the vulva is formed from homologous precursor cells, the regulatory mechanisms differ dramatically between species, with vulva development in *C. elegans* induced by EGF/RAS signalling and the Wnt-signalling pathway having taken over this function in *P. pacificus* (Wang & Sommer 2011). The most important take home message from these studies is that the genes encoding EGF and Wnt pathway components are conserved between *P. pacificus* and *C. elegans* (and all other studied nematodes) at the structural (sequence) level, but the interaction of their encoded protein products in functional networks has changed, resulting in novel molecular mechanisms of the regulation of development. These and similar examples of developmental systems drift provide insight into the molecular changes that drive the evolution of developmental processes. At the same time, they highlight the need for more integrative approaches that aim for an incorporation of development and evo-devo into evolutionary theory (Fig. 1) (Sommer 2009). How do developmental alterations arise in the first place and what kind of mutations is involved? Do such changes occur at the population or the species level, or only at the level of higher taxa? Are ecological changes important for the origin of developmental alterations?

Pristionchus pacificus as a model for integrative evolutionary biology

While evo-devo has been fruitful in identifying the developmental alterations that give rise to important evolutionary innovations, it cannot detect the microevolutionary variations and the potential ecological context that underlie molecular change. Therefore, questions such as those introduced above can only be addressed by taking comprehensive approaches that aim for ecological and population genetic insight. As *Pristionchus pacificus* and

Caenorhabditis elegans are only distantly related, a comparison between these two species cannot reveal the population genetic and/or ecological origin of differences. Rather, studies on *P. pacificus* ecology and population genetics need additional, more closely related reference points. Below, we summarize the attempts to move *P. pacificus* forward from a laboratory 'evo-devo' model to an organism with a phylogenetic framework that allows fieldwork.

Numerous samplings, initiated in 2004, have indicated that *Pristionchus* nematodes live in a necromenic association with scarab beetles (Fig. 2b) (Herrmann *et al.* 2006). Necromeny describes the phenomenon where a nematode associates with a living beetle (insect or other invertebrate) and then rests upon it in the dauer stage (Weller *et al.* 2010). After the beetle dies, the nematodes resume development to feed on microbes, including bacteria, fungi, protozoans and other nematodes, which come together to decompose the beetle carcass. Thus, *Pristionchus* is an omnivorous feeder that can use bacteria, protozoa, fungi and other nematodes, all of which grow on the carcass of scarab beetles, as food sources. The first specific host that was identified for *P. pacificus* is the Oriental beetle *Exomala orientalis* from Japan and the United States (Fig. 2b, left image) (Herrmann *et al.* 2007). By now, *P. pacificus* is known to have a cosmopolitan distribution that encompasses Asia, America, South Africa and the Mascareigne Islands of the Indian Ocean (Herrmann *et al.* 2010). Currently, more than 600 different *P. pacificus* strains from several continents are available in the laboratory. Thus, the *P. pacificus* system is now in a position to integrate evo-devo studies (macroevolution) with population genetics and evolutionary ecology (microevolution) to examine the contribution of natural variation and changing environments to the evolutionary process (Fig. 1).

The sampling efforts described above have also resulted in the isolation of nearly 30 *Pristionchus* species (Herrmann *et al.* 2007, 2010) providing closely related species that can also serve as reference points for *P. pacificus* ecology and population genetics. A first molecular phylogenic framework has been provided (Mayer *et al.* 2007) and is continuously up-dated with morphological descriptions of additional species (Kanzaki *et al.* 2012a, b). Of particular importance for the population genetic analysis within *P. pacificus* is the recent identification and description of what is most likely the sister species of *P. pacificus*. The sampling of stag beetles in Japan, the putative origin of *P. pacificus*, resulted in the isolation of *Pristionchus exspectatus*, a gonochoristic (male/female) species with which *P. pacificus* can form viable but sterile F1 hybrids (Herrmann *et al.* 2007; Kanzaki *et al.* 2012a). The imminently available genome sequence of

P. exspectatus and the ability to form F1 hybrids will provide an invaluable reference tool for future studies in the *P. pacificus* system.

Elucidating developmental processes in an ecological context

The identification of the necromenic association of *Pristionchus pacificus* with scarab beetles provides a unique entry point to study developmental processes in an ecological context. In particular, the development of ecologically relevant traits can be studied under these circumstances. Below, we summarize inroads into three such research areas: the development of dauer larvae, innate immunity and the formation of teeth-like denticles as an example of a morphological novelty in *P. pacificus* and its relatives.

In *Caenorhabditis elegans*, dauer formation is regulated by at least three environmental cues, that is, starvation, high temperature and high population density (for review see Hu 2005). To guarantee survival, dauer larvae also show morphological and physiological adaptations not seen in other stages (Fig. 2a). For example, dauer larvae have a closed mouth, a remodelled pharynx and a thicker cuticle, making them independent of food and more protected from environmental stresses (for review see Lee 2002; Hu 2005). In addition, *C. elegans* dauer metabolism is characterized by a low activity in enzymes of the TCA cycle, glycolytic pathway and glyoxylate cycle compared with other stages (O'Riordan & Burnell 1990). Only a few enzymes are up-regulated in the dauer stage, including several acyl-CoA synthetases, required for ß-oxidation of fatty acids. While this observation is consistent with the finding that dauer larvae contain large amounts of lipids in the intestine and hypodermis (Cassada & Russell 1975), the precise nature of dauer physiology is still not very well understood. Comparative transcriptomic studies based on whole-genome microarray experiments strongly suggest that the physiology of dauer development changes substantially during nematode evolution. Specifically, *P. pacificus* and *C. elegans* vary in their expression profiles for dauer entry and dauer exit (Sinha *et al.* 2012a). Changes in expression profiles involve genes that are not conserved at the sequence level, suggesting that novel and/or rapidly evolving genes play an important role in dauer regulation and execution. While the exact functional role of the large majority of genes and their products awaits future analysis, these results already indicate that a full understanding of a complex process such as dauer formation cannot be obtained by only studying those genes that are conserved throughout animal evolution.

Decades of work in nematology provide ample evidence that the long-lived, stress-resistant dauer stage is

highly adaptive for life under extreme conditions (for review see Lee 2002; Brown *et al.* 2011; Sommer & Ogawa 2011). Besides survival, dauer larvae are specifically adapted for dispersal, enhancing the chances of finding a new food source. Many nematode dauer larvae show a so-called nictation behaviour (originally called Winkverhalten). That is, dauer larvae stand on their tail and 'wave' to increase their chance of finding a host and being transported to a new environment (Sudhaus 2010). Although fascinated by this behaviour, nematologists are still in an early phase of understanding the genetic and molecular principles behind nictation (Brown *et al.* 2011).

As indicated above, *P. pacificus* worms are omnivorous feeders that can use bacteria, protozoa, fungi and other nematodes, all of which grow on the carcass of scarab beetles as food sources. While *Pristionchus* can also be grown indefinitely on a monoxenic diet of *E. coli* bacteria under laboratory conditions, it is thought that omnivorous feeding represents a strong advantage for these necromenic nematodes in the wild. The ability to feed on both fungi and other nematodes of similar body size to itself represent novel predatory behaviours in *P. pacificus* (Bumbarger *et al.* 2013). Interestingly, predation is achieved by another example of an evolutionary novelty: the mouth of *P. pacificus* is equipped with versatile teeth-like denticles that are unknown from *C. elegans* and other rhabditid nematodes (Bento *et al.* 2010). Phylogenetic reconstruction indicates that these teeth-like denticles represent an innovation that is restricted to the Diplogastridae family, to which *Pristionchus* belongs (Fürst v. Lieven & Sudhaus 2000). These teeth-like denticles can occur in two alternative forms, the so-called eurystomatous (EU) and stenostomatous (ST) mouth-forms (Fig. 2c). Eurystomatous animals have a claw-like dorsal tooth and a sub-ventral tooth. In contrast, the dorsal tooth of stenostomatous animals is not claw-like and the subventral tooth is absent. In addition, the buccal cavity differs between the two mouth forms in width and height. The mouth-form decision of an individual animal is taken during larval development and is irreversible (Bento *et al.* 2010). Importantly, both forms can feed on bacteria as well as be predatory. Selection for either ST or EU worms in an inbred strain for 10 generations did not change the ratio of the two mouth forms in the resulting lines (Bento *et al.* 2010). These results suggest that this novel morphological feeding structure in *P. pacificus* represents another example of phenotypic plasticity in nematodes, rather than a genetic polymorphism.

The same study also identified an endocrine signalling module consisting of the nuclear hormone receptor DAF-12 and the steroid hormone dafachronic acid to play a key role in the mouth-form decision of individual animals. The *P. pacificus* mouth dimorphism represents an exciting example of the facilitator hypothesis formulated by West-Eberhard (2003) and others, which argues that phenotypic (developmental) plasticity facilitates the evolution of morphological novelty and thereby, phenotypic diversity. Given the functional toolkit available in *P. pacificus*, this system can provide mechanistic insight into the molecular processes associated with phenotypic plasticity.

The occurrence of the teeth-like denticles in *Pristionchus* and other members of the Diplogastridae coincides with the loss of the grinder, a structure in the terminal bulb of Rhabditid nematodes that is crucial for the mode of bacterial feeding seen in *C. elegans* (Fig. 2c). The lack of grinder means that *Pristionchus* species can ingest whole bacterial cells and disseminate living bacteria to new areas in the environment (Chantanao and Jensen 1969). At the same time, the presence or absence of a grinder must affect the nematode – bacterial interactions of *C. elegans* and *Pristionchus,* respectively, because in *P. pacificus* the gut is consistently exposed to living microbes.

Several studies over the last decade have shown that *P. pacificus* is more resistant to bacteria in general and to pathogens particularly, when compared to *C. elegans* (Wei *et al.* 2003). For example, a large array of bacteria has been shown to be associated with three different *Pristionchus* species in the wild (Rae *et al.* 2008). Furthermore, a survey of around 800 wild *Bacillus* isolates showed distinct virulence patterns in *P. pacificus* and *C. elegans*, respectively (Rae *et al.* 2010). These studies have now been extended to a system-wide analysis of the evolution of innate immunity in *C. elegans* and *P. pacificus* against four different bacterial pathogens using parallel microarray studies (Sinha *et al.* 2012b). A high level of specificity in host response to distinct pathogens was observed within and across species suggesting an unexpected level of complexity in the effectors of innate immunity. Large differences in the functional domains enriched after exposure to the same pathogen suggests that both recognition and response networks have changed substantially during nematode evolution (Sinha *et al.* 2012b). As for the example of dauer formation, many novel and/or fast evolving genes are part of the transcriptional response to pathogens in both organisms. On-going studies try to reveal the molecular mechanisms that underlie the observed specificity and complexity of nematode innate immunity.

Taken together, the three examples of dauer development, mouth form plasticity and innate immunity indicate that nematodes have evolved many distinct mechanisms to explore their environment, resulting in specific adaptations. These mechanisms involve

innovations in basic life cycle, morphology and physiology. Innovations at the level of the life cycle are even more extreme in species that have evolved to become parasites. Indeed, parasitism represents an exciting phenomenon that has evolved at least seven times independently in nematodes and the last few years have seen an expansion of genome studies that provide exciting insight into this lifestyle mode (for review see Sommer & Streit 2011).

From ecology to population genetics

The extension of the *Pristionchus.pacificus* system to encompass ecological and population genetics, involving the recently discovered sister species *P. exspectatus*, allows examination of phenotypic and genotypic evolution outside the laboratory. Microevolutionary approaches are enhanced by our recent establishment of an evolutionary field station on the Island of La Réunion, which lies off the coast of Madagascar in the Indian Ocean (Fig. 3).

Considered to be a major biodiversity hotspot (Myers *et al.* 2000; Thebaud *et al.* 2009), La Réunion is the youngest (2-3 Ma), largest (2,512 km²), steepest (up to 3,070 m a.s.l.) and most complex (both topographically and ecologically) island in the Mascareigne island chain (Strasberg *et al.* 2005). Volcanic activity, which continues to the present day, has shaped the island's rugged landscape, where short geographic distances can see dramatic altitudinal changes. Wind patterns across the island add to this diversity; climate on the north-eastern, windward side of the island is characterized by high rainfall, while the south-western, leeward side is substantially drier. Climatic variables acting upon a dynamic geological template together create a complex suite of some 19 habitat types or 'ecozones' across La Réunion (Strasberg *et al.* 2005).

In the last few years, nematode sampling across several geographic regions/ecozones and encompassing several host beetle species has identified La Réunion Island as an oasis for *P. pacificus* (*e.g.* Herrmann *et al.* 2010). Population genetic analyses to date are based on
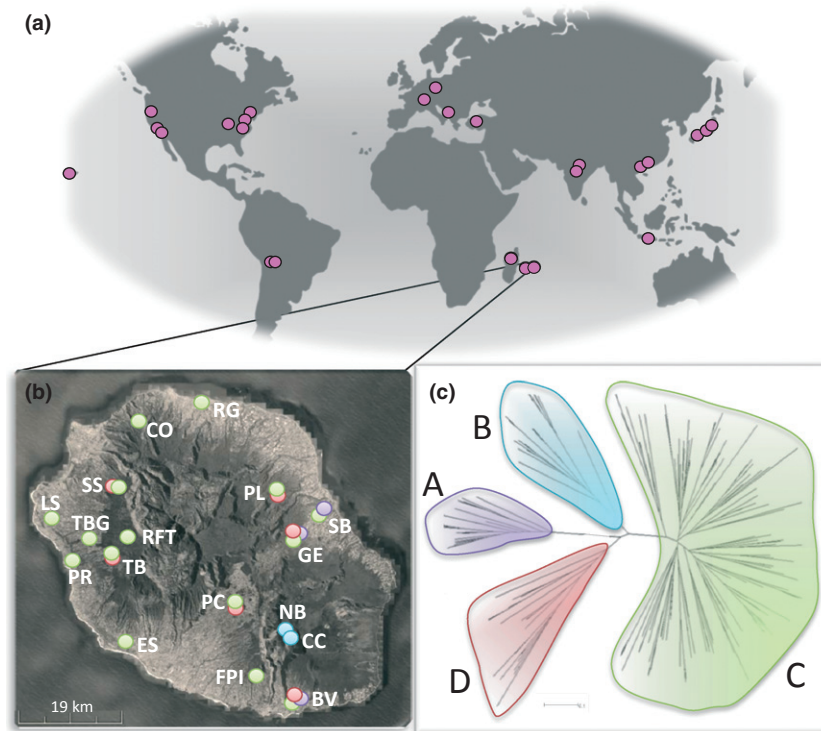
Fig. 3 Graphic to show: (a) The cosmopolitan world-wide distribution of currently-collected *Pristionchus pacificus* isolates; (b) Diversity and distribution of the four genetic lineages of *P. pacificus* sampled to date from La Réunion Island [lineages coloured purple, blue, green and red for A, B, C and D, respectively; location codes correspond to: Basse Vallée (BV), Le Cratere Commerson (CC), Colorado (CO), Etang Salé (ES), Foret du Petite Ile (FPI), Grand Etang (GE), La Saline (LS), Nez du Boeuf-Vulcano (NB), Plan de Cafrès (PC), Plaines des Lianes (PL), Petite Ravine (PR), Route Forestière des Tamarin (RFT), Roland Garros (RG), Saint Benoit (SB), San Souci (SS), Trois Bassin (TB), Trois Bassin Garden (TBG)]; (c) Neighbour-joining tree constructed in Phylip v. 3.69, using 19 microsatellite markers and 271 strains, to show the four (colour-coded) genetic lineages present in La Réunion *P. pacificus*. Microsatellite data are from Morgan *et al.* (2012).

both microsatellite (representative of the six *P. pacificus* chromosomes; $n = 20$) and mitochondrial markers in over 200 strains (Morgan *et al.* 2012). These studies revealed that the genetic diversity in *P. pacificus* on La Réunion covers the complete known worldwide diversity of the species. Specifically, strains falls into four broad groupings (lineages 'A', 'B', 'C' and 'D'; Herrmann *et al.* 2010; Morgan *et al.* 2012), separated by a high degree of genetic distance (Fig. 3c). The finding that these La Réunion groupings characterize the global gene pool of the species is remarkable considering the islands' young age and relatively isolated location.

Model-based statistical methods have now been used to rigorously test hypotheses regarding the evolutionary history of *P. pacificus*. Divergence and demographic analyses among the four 'world' lineages present on La Réunion Island clearly indicate that the diversity of *P. pacificus* clades results from independent colonization events (McGaughran *et al.* 2013), a finding that supports earlier observations on specific beetle associations among strains of different clades (Herrmann *et al.* 2010). Furthermore, a modelling-based approximate Bayesian computation (ABC) approach provided insight into the order and timing of the establishment of *P. pacificus* populations on La Réunion (McGaughran *et al.* 2013).

At a finer scale, approximately 15 subpopulations can be retrieved from the La Réunion genetic data when applying a clustering algorithm. Such structure is in stark contrast to the low diversity and lack of structure observed in *Caenorhabditis elegans* (Zauner *et al.* 2007; Rockman & Kruglyak 2009). While these two species are similar in many respects (*e.g.* reproductive mode), they clearly have contrasting evolutionary histories.

Evolutionary history of *Pristionchus pacificus*

To begin to determine which factors have been important in defining the evolutionary and natural history of *Pristionchus pacificus*, a mutation accumulation (MA) line approach was employed (Molnar *et al.* 2011). With MA line experiments, it is possible to directly assess the minimum number of new mutations that appear in a given lineage over a specified number of generations (Lynch 2010). Such experiments are limited to a small number of model organisms, including *Caenorhabditis elegans* (Denver *et al.* 2000, 2004). The MA lines established in *P. pacificus* were first used to estimate a mitochondrial mutation rate of 7.6×10^{-8} ($\pm 2.2 \times 10^{-8}$) per site per generation, which is not statistically different from the rates derived for *C. elegans* (Denver *et al.* 2000) and *C. briggsae* (Howe *et al.* 2010). In addition, mutations in microsatellite markers have been investigated in these lines (Molnar *et al.* 2012) and recent studies

aim for a genome-wide analysis (A. M. Weller & R. J. Sommer, unpublished).

Using mutation rate estimates, multiple dating assessments suggest that lineage diversification occurred early in the evolutionary history of *P. pacificus*, with the oldest time to the most recent common ancestor estimated at 1.2×10^6 years before present (ybp) (Molnar *et al.* 2011; McGaughran *et al.* 2013). In the La Réunion context specifically, this suggests that divergence among the *P. pacificus* lineages most likely preceded the emergence and colonization of La Réunion Island.

Characterizing the colonization process in *P. pacificus* requires knowledge about the beetle host, the movement of which necessarily dictates dispersal in the nematode. On La Réunion, *P. pacificus* is found in association with several distinct scarab beetles that are known to have invaded La Réunion at different times in history and in a highly species-specific manner. For example, *Oryctes borbonicus* (Fig. 2b right image), an endemic La Réunion scarab beetle, shows the highest infestation rate for *P. pacificus* (Herrmann *et al.* 2010). This beetle most likely invaded the island early in its history and co-evolution between *P. pacificus* and *O. borbonicus* may have resulted in the enormous radiation that encompasses many strains found in *P. pacificus* clade C today (Fig. 3c), an idea that is currently being tested experimentally. In contrast, *Maladera affinis* is a beetle that is known to have invaded the island during the last few hundred years from India (Cheke & Hume 2008). Consistently, *P. pacificus* strains found on *M. affinis* fall into clade A, which is geographically restricted in its' distribution on La Réunion (Fig. 3b), but harbours a wide Asian distribution, including India, China and Japan.

The independent colonization of La Réunion by different *P. pacificus* linegaes (clades) provides a powerful system for ecological studies. It is reasonable to assume that once a lineage was established on La Réunion, ecological and geological factors have both been important in limiting dispersal and driving differentiation among populations. Indeed, first empirical studies suggest that such factors are likely to have varied across populations on La Réunion because they differ in the nature of their original beetle-host assemblages as well as in their ability to undergo host-switching events (Morgan *et al.* 2012). For example, in addition to the *O. borbonicus* association (see above), clade C populations have undergone extensive host-switching episodes, shifting to newly invaded beetle hosts of wide habitat breadth such as *Hoplochelus marginalis*, which may further account for the wider distribution of this lineage on Réunion (Morgan *et al.* 2012). Current genomic studies explore this phenomenon further, as *Hoplochelus* beetles harbor different *P. pacificus* clades in different localities on the Island.

Extensive modelling work has detected signals of recent population expansion on La Réunion (all in the period 59,000–125,000 ybp), and a suggestion that *P. pacificus* populations can undergo rapid increases in local effective population size after contraction events (McGaughran *et al.* 2013). This work points to the potential success of *P. pacificus*, and perhaps hermaphroditic species in general, in recovering population size following disturbance (Herrmann *et al.* 2010; McGaughran *et al.* 2013), especially in cases where avenues of host switching can be exploited to promote range expansion. Taken together, combining an island nematode system with highly complex genetic structure provides a unique opportunity to unite ecology and population genetics to begin to disentangle the intricacies of the evolutionary pathway of *P. pacificus*.

The relationship between selection and neutrality in *Pristionchus pacificus*

The ability of *Pristionchus pacificus* to tolerate a wide variety of environments and its codispersal with a variety of beetle species make it a good model species for investigating the complex effects of environmental, ecological and geological factors on local adaptation and genotypic evolution. Isolation among lineages following differentially timed foundation (from an already diverse gene pool; see above) may have resulted in a suite of phenotypic and genotypic differences as discrete populations diverge in adaptive traits and/or host specificity (*e.g.* Roman & Darling 2007; Dlugosch & Parker 2008). This reasoning can be tested against a neutral null hypothesis. On Réunion, adaptive divergence has been seen in birds (Mila *et al.* 2010) and insects (Paupy *et al.* 2001; Morlais *et al.* 2005), recapitulating the general trend of rapid genetic and phenotypic diversification in association with ecological shifts seen in other island systems (*e.g.* Jordan *et al.* 2005; Kleindorfer *et al.* 2006; Lawton-Rauh *et al.* 2007; McGaughran *et al.* 2010; Mathys & Lockwood 2011). Defining natural variation in phenotypic traits in an evolutionary context is an on-going aim of our research group (Hong *et al.* 2008; Mayer & Sommer 2011). But, in particular, how does *P. pacificus* align with this island paradigm where niche variability can facilitate adaptive divergence in association with distinct local environments?

Some signatures in the *P. pacificus* La Réunion data suggest that local adaptation may be driving differences among populations at the broad regional scale. For example, an east/west partition identified in the population structure on La Réunion (Morgan *et al.* 2012) could reflect a pattern of poor adaptation among eastern lineages to the arid western climate, and/or better adaptation among the western-distributed clade C

strains. Other examples point towards additional layers of complexity. For example, clade B strains, found exclusively on La Réunion (Fig. 3), form a genetically distinct group because of their association with the endemic beetle *Amneides godefroyi*. In strict association with these beetles, clade B strains show a local adaptation to the cooler conditions that characterize high-altitude locations at which the beetle is found. Carrying out detailed genotype × environment analyses might lead to identification of the mechanistic forces underlying the incipient adaptive and/or nonadaptive processes but depends heavily on genomic approaches. In our final paragraph below, we summarize *P. pacificus* genomics and some unexpected findings in nematode genomes in general.

Pristionchus pacificus genomics

Whole-genome sequencing data provide a unique framework for studying the ecology and population genetics of any organism. The *Pristionchus pacificus* genome was sequenced in 2005 and 2006, resulting in a full assembly and annotation (Dieterich *et al.* 2008). By now, other omics platforms have been added (Borchert *et al.* 2010; Sinha *et al.* 2012b) and regular updates can be obtained online at www.pristionchus.org. In the context of multiple other nematode genome sequencing projects, a comparative genomics approach can provide useful insight into the evolution and ecology of life styles by comparing distinct free-living nematodes such as *P. pacificus* and *Caenorhabditis elegans* with parasites of plants, animals and humans. While this topic is outside the area of this review, we refer the interested reader to other relevant review articles (*e.g.* Sommer & Streit 2011).

The analysis of the *P. pacificus* genome provided three unexpected findings that are useful for the ecology of the organism. First, the *P. pacificus* genome is substantially larger than the one of *C. elegans* (Fig. 4a) and contains more than 26 000 predicted protein coding genes (Dieterich *et al.* 2008). A large amount of these gene predictions represent genes with sequences not conserved above the genus level. Such genes have been called 'pioneer' or 'orphan' genes by different authors and in the case of *P. pacificus* they constitute up to one-third of all gene predictions (Rödelsperger *et al.* 2013). It is important to note that this phenomenon is not specific to *Pristionchus* and denser genome sequencing efforts in nematodes indicate the existence of large amounts of orphan genes in all studied species (Rödelsperger *et al.* 2013). While these genes are not conserved in sequence, transcriptomic (Sinha *et al.* 2012a, b) and proteomic (Borchert *et al.* 2010) studies provide strong evidence for their expression and suggest functional importance, which has yet to be identified.
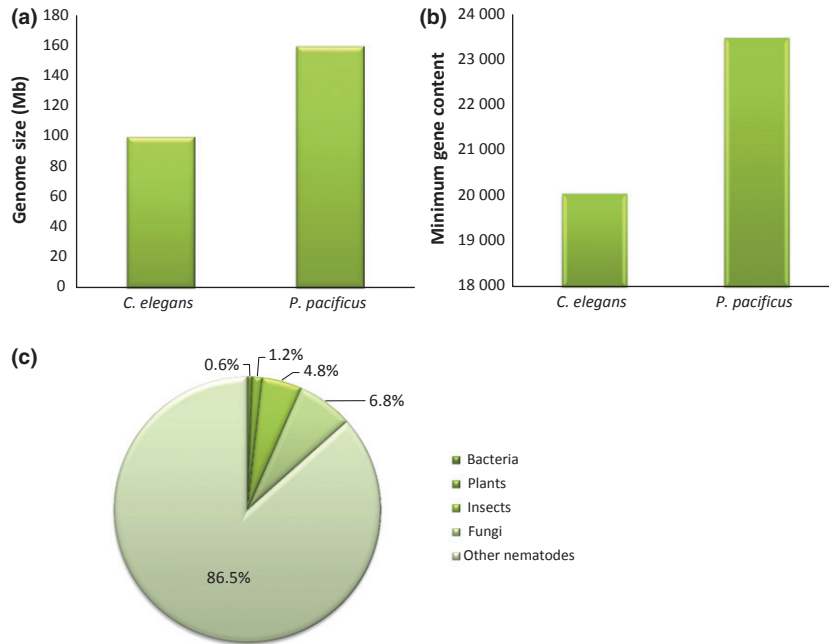
Fig. 4 (a) The *Pristionchus pacificus* genome is substantially larger than the one of *Caenorhabditis elegans* and (b) contains many more predicted genes. (c) Many unrelated *P. pacificus* genes have been acquired by horizontal gene transfer (HGT) from distinct donor organisms: the pie chart shows the distribution of predicted taxonomic groups based on GC-normalized relative codon frequency profiles for the 9,217 genes in *P. pacificus* that have no homologues; codon usage profiles of *P. pacificus* genes were compared with the average genome-wide profiles of 71 species and assigned to the species with the closest Euclidean distance in these profiles (Rödelsperger & Sommer 2011).

Second, one mechanism that seems to play an important role in gene expansion in *P. pacificus* is the existence of lineage-specific gene duplications that have resulted in a dramatic increase in gene copy numbers. Examples of gene family expansions include the cytochrome P450 enzyme-encoding genes, which have 198 copies in *P. pacificus* but only 67 in *C. elegans*, or the ABC transporter, glycosyltransferase, and sulfotransferase families (Dieterich *et al.* 2008). It is interesting to note that all of these genes encode for potential detoxification enzymes, which are of particular importance in the beetle ecosystem in which *P. pacificus* and related nematodes are found. Therefore, the expansion of the detoxification machinery provides interesting hints at how genomic changes may be initiated as adaptations to a current environment.

A third unexpected example was the identification of many *P. pacificus* genes that have been acquired by horizontal gene transfer (HGT) (Dieterich *et al.* 2008; Rödelsperger & Sommer 2011). Bioinformatic studies revealed that *P. pacificus* has acquired multiple, unrelated genes from distinct donor organisms (Fig. 4b). For example, *P. pacificus* was found to possess cellulase genes that are most similar in sequence to those of unicellular eukaryotic microbes, such as slime moulds (Dieterich *et al.* 2008). Surprisingly, *P. pacificus* strains show

cellulase activity even when grown on standard *E. coli* (Dieterich *et al.* 2008; Schuster & Sommer 2012). The *P. pacificus* cellulases belong to the GHF5 family and phylogenetic transcriptomic studies using 454 sequencing indicated that seven tested *Pristionchus* species all contained cellulases in their genome, which in all cases were strictly associated with cellulase activity (Mayer *et al.* 2011). This study also showed that the original acquisition of cellulase genes in *Pristionchus* was followed by multiple gene duplications in association with high gene turnover.

The analysis of codon usage patterns in HGT-acquired and orphan genes of *P. pacificus* using GC-normalized relative codon frequencies as a measure revealed that their codon usage is clearly distinct from 'classical' nematode genes (Rödelsperger & Sommer 2011). This atypical codon usage was further evaluated using the codon usage profiles of 71 organisms, revealing a most significant enrichment in insect-like codon usage. This finding suggests that the tight association of *Pristionchus* nematodes with beetles might have resulted in HGT of genetic material (Rödelsperger & Sommer 2011). The most abundant gene family among these genes are non-LTR retrotransposons, which can be speculated to serve as carriers of foreign genetic material. Indeed, a significant clustering tendency of orphan

genes in the vicinity of retrotransposons has been observed (Rödelsperger & Sommer 2011), inspiring follow-up studies to investigate a potential mechanistic relationship in more detail.

Finally, it should be noted that HGT into nematodes is not restricted to *P. pacificus* and its relatives. Instead, it is found in multiple plant- and animal-parasitic species (for review see, Sommer & Streit 2011). Thus, HGT represents a phenomenon that is shared among diverse nematodes and may represent a common mechanism that underlies ecological diversification in these species (Sommer & Streit 2011).

Conclusion

In this article, we have reviewed the status of the nematode *Pristionchus pacificus* as a model system for integrative studies of development and evo-devo with ecology and population genetics (*e.g.* Bento *et al.* 2010; Mayer & Sommer 2011; Morgan *et al.* 2012). This development will be a useful addition to those organisms that currently allow such integrative studies. The combination of laboratory-based functional studies with fieldwork, as possible in *P. pacificus*, has the long-term prospective to provide both proximate and ultimate causation and might therefore help to overcome the long-term divide between major areas in biology.

While the studies summarized above provide a paradigm for the integrative analysis of evolutionary phenomena, single studies so far concentrate either on the genetic and developmental analysis of biological processes or the population genetic perspective. Our future research aims to combine these efforts by investigating ecologically relevant traits, such as the mouth dimorphism introduced above, simultaneously from a genetic and natural variation perspective. Such studies can than reveal how similar or different the genetic mechanisms are at work in distinct strains. They can also provide insight into the involvement of adaptive and/or non-adaptive forces. Whole-genome data sets available for multiple *P. pacificus* strains represent an essential framework for the fine-scale examination of evolutionary patterns and processes. Moving forward, we expect to see the *P. pacificus* system contribute further exciting developments to evolutionary biology.

Outlook and perspective

Knowledge in evolutionary biology and ecology often culminates in the conclusion that all types of biological pattern represent historical singularities (Gayon *et al.* 2004; Amundson 2005; Thompson 2005). Nonetheless, general conclusions are necessary to address bigger questions. The need for integrative studies that combine laboratory research and fieldwork with genomics as an overarching tool represents a phenomenon of general significance. Therefore, studying the genetics and development of ecologically relevant traits and comparing the molecular mechanisms at work with those acting at the population (micro-evolutionary) level and across species (macroevolution), is one research direction of general importance for evolution and ecology. Given that any 'species' forms part of a historical continuum, there is a clear need for several such study systems that fulfil all necessary technical requirements. We hope that the example of *P. pacificus* acts as catalyst for the development of additional study systems. The establishment of this integrative research endeavour from the description of the species (Sommer *et al.* 1996) to the interdisciplinary analysis of development, population genetics and ecology is a testimony to the possibility of generating and developing new model organisms.

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