The Natural Biotic Environment of *Caenorhabditis elegans*

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ABSTRACT Organisms evolve in response to their natural environment. Consideration of natural ecological parameters are thus of key importance for our understanding of an organism's biology. Curiously, the natural ecology of the model species *Caenorhabditis elegans* has long been neglected, even though this nematode has become one of the most intensively studied models in biological research. This lack of interest changed ~10 yr ago. Since then, an increasing number of studies have focused on the nematode's natural ecology. Yet many unknowns still remain. Here, we provide an overview of the currently available information on the natural environment of *C. elegans*. We focus on the biotic environment, which is usually less predictable and thus can create high selective constraints that are likely to have had a strong impact on *C. elegans* evolution. This nematode is particularly abundant in microbe-rich environments, especially rotting plant matter such as decomposing fruits and stems. In this environment, it is part of a complex interaction network, which is particularly shaped by a species-rich microbial community. These microbes can be food, part of a beneficial gut microbiome, parasites and pathogens, and possibly competitors. *C. elegans* is additionally confronted with predators; it interacts with vector organisms that facilitate dispersal to new habitats, and also with competitors for similar food environments, including competitors from congeneric and also the same species. Full appreciation of this nematode's biology warrants further exploration of its natural environment and subsequent integration of this information into the well-established laboratory-based research approaches.

KEYWORDS WormBook; *Caenorhabditis elegans*; natural ecology; microbiome; pathogens; competition

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WHY are >40% of the genes of *Caenorhabditis elegans* still without functional annotation and >60% without a described phenotype (Petersen *et al.* 2015a)? These are surprising numbers considering the enormous amount of research performed with this nematode across almost all biological disciplines. A likely reason is that the species' natural ecology is largely neglected across these studies. These usually rely on an artificial environment consisting of agar plates supplemented with the bacterial food strain *Escherichia coli* OP50 and analysis of the canonical *C. elegans* strain N2, which shows numerous adaptations to the laboratory conditions (Sterken *et al.* 2015). Yet, the nematode's ecology has not been completely ignored. During the 20th century, a handful of studies repeatedly isolated *C. elegans* from nature and characterized specific aspects of its ecology, for example its interaction with certain food microbes (Grewal 1991a; Grewal and Wright 1992; Venette and Ferris 1998) or variation in its reproductive system (Hodgkin and Doniach 1997). The interest in *C. elegans* natural populations has especially gained momentum since 2005, when several articles on its natural distribution and population genetic characteristics were published (Barrière and Félix 2005; Haber *et al.* 2005; Sivasundar and Hey 2005; Cutter 2006). These papers were followed by an increasing number of studies on the interaction of *C. elegans* with its environment and/or certain environmental components. Now, our understanding of *C. elegans* ecology has greatly improved since the previous review by Kiontke and Sudhaus (2006), which was published at a time when the species was only known from compost heaps and garden soil.

The biotic environment is of particular importance in this context, as biotic interactions are often a major driver of evolutionary change. The biotic environment includes interactions with competitors, parasites, predators, vectors, food, associated micro-organisms, and also interactions among *C. elegans* individuals, such as those among the different sexes with potentially conflicting interests. As changes in interaction characteristics are based on randomly occurring mutations in the two involved entities and are often unpredictable, they can impose high selective pressure. This has

been particularly well-documented for interactions of host organisms with their coevolving parasites and pathogens (Woolhouse *et al.* 2002; Decaestecker *et al.* 2007; Schulte *et al.* 2010; Morran *et al.* 2011; Brockhurst *et al.* 2014; Koskella and Brockhurst 2014; King *et al.* 2016). Parasites/pathogens often show a high potential for evolutionary adaptation, because of comparatively shorter generation times, comparatively larger populations, or more flexible genomes (shaped by horizontal gene transfer). As parasites/pathogens by definition reduce host fitness and often depend on their hosts for survival and proliferation, their adaptation can impose continuously high selective constraints on their hosts (Woolhouse *et al.* 2002; Brockhurst *et al.* 2014), likely contributing to the evolution and maintenance of sex and recombination (Lively and Morran 2014). Sexual selection, based on diverging evolutionary interests of the sexes, can similarly cause ongoing cycles of adaptation and counteradaptation. The exceptionally high selective pressures produced by parasites or sexual interactions is reflected in the finding of significantly higher evolutionary rates in the genes associated with immunity (and thus parasite defense) and also sex-related genes (Ellegren and Parsch 2007; Fumagalli and Sironi 2014; Sironi *et al.* 2015; Cheng and Kirkpatrick 2016). Other biotic interactions may impose similar selective constraints, for example those involving predators or competitors (Cortez and Weitz 2014; Hiltunen and Becks 2014; Wilson 2014). Consequently, these kinds of biotic interactions are likely a key determinant in shaping the life history and underlying genome characteristics of any organism, including *C. elegans*. These changing selection dynamics are not only countered by single point mutations, but may account for the emergence of large gene families, when gene duplications allow a faster response to the selective challenge than point mutations or small insertions/deletions, as repeatedly documented in bacteria (Andersson and Hughes 2009; Pena-Miller *et al.* 2013) and suggested for some eukaryotes (Kondrashov 2012; Katju and Bergthorsson 2013; Assogba *et al.* 2016), including *C. elegans* (Farslow *et al.* 2015).

The aim of this review is to summarize our current understanding of the naturally occurring interactions of *C. elegans* with other organisms, ranging from conspecifics to interactions with other species (Figure 1). We will focus on studies that have repeatedly isolated *C. elegans* from nature and characterized its habitat, including locations in France and Northern Germany. We will additionally consider the increasing number of studies that have assessed naturally occurring biotic interactions under laboratory conditions, especially those with pathogens, food microbes, and the *C. elegans*-associated microbiome. Based on this work, we will first provide a brief overview of the characteristics of the nematode's natural habitat (*Habitats and Substrates*), followed by a summary of the vectors and invertebrate hosts that are used and/or inhabited by *C. elegans* (*Macroscopic Invertebrates as Possible Vectors or Hosts*). We will discuss in detail *C. elegans*' microbial environment, including potential food microbes, its microbiome, and also pathogens and parasites (*The Microbial Environment* and *Pathogens and Parasites*). We will provide an overview of the nematode's competitors, predators, and enemies (*Competitors and Predators*), different types of intraspecific interactions in nature (*Intraspecific Interactions*), the presence of natural genetic polymorphisms as indicators for biotic interactions (*Natural Genetic Polymorphisms as an Indication for Biotic Interactions*), and conclude by highlighting selected topics important for future research (*Perspectives*).

Habitats and Substrates

Habitat types in which C. elegans was repeatedly isolated

C. elegans appears to have a preference for humid temperate areas with a wealth of decaying vegetation. It was first sampled mostly in human-influenced habitats (compost heaps, orchards, vegetable gardens, and botanical gardens), and now also in more natural environments such as humid areas of woods and shrubland (Figure 2) (Barrière and Félix 2005, 2007; Sivasundar and Hey 2005; Kiontke *et al.* 2011; Félix and Duveau 2012; Petersen *et al.* 2014, 2015b; Frézal and Félix 2015; Cook *et al.* 2016). *C. elegans* is found on several continents (Europe, North and South Americas, Africa, Oceania, and rarely in Asia) and also on isolated islands, such as Hawaii, Madeira, Azores, and Réunion (see http://worldwideworm.banshy.fr/ for a database of *C. elegans* wild isolates with their location, habitat, and substrate type).

Note that sampling is biased toward substrates and landscapes where *C. elegans* has been previously found and also toward the geographical location of collectors. Thus, it is possible that new habitat and substrate types will be discovered in the future, especially if sampling efforts go beyond France, Germany, the UK, and the US, where most previous collections were made. Compared to other *Caenorhabditis* species such as *C. japonica* (Yoshiga *et al.* 2013; Okumura and Yoshiga 2014) or *C. drosophilae* (Kiontke 1997), the species *C. elegans* does not appear to have a highly specialized habitat nor a highly specialized biotic association with larger invertebrates. Although much remains to be discovered, *C. elegans* seems to have a more generalist lifestyle, which appears similar to that of some other *Caenorhabditis* species such as *C. briggsae* or *C. remanei* (see possible competition relationships in *Possible competitors*).

Overview of substrate types

C. elegans is most easily isolated from rotting fruits and stems, compost, and some invertebrates (see below *Macroscopic Invertebrates as Possible Vectors or Hosts*). In temperate areas, the large rotting fruits are chiefly found in human-associated gardens and orchards, and compost by definition is of anthropogenic origin. In more natural areas, rotting plant stems appear to be a very common substrate (Félix and Duveau 2012), as well as, occasionally, rotting flowers, fruits, and mushrooms (Kiontke *et al.* 2011; Félix *et al.* 2013; M.-A. Félix, unpublished data). The common feature of these substrates is that they consist of microbe-rich decomposing plant material. In contrast, *C. elegans* is rarely found in pure soil samples (except immediately adjacent to rotting fruits or stems), nor in rotting wood, leaf litter, or decomposing grass (Félix and Duveau 2012;

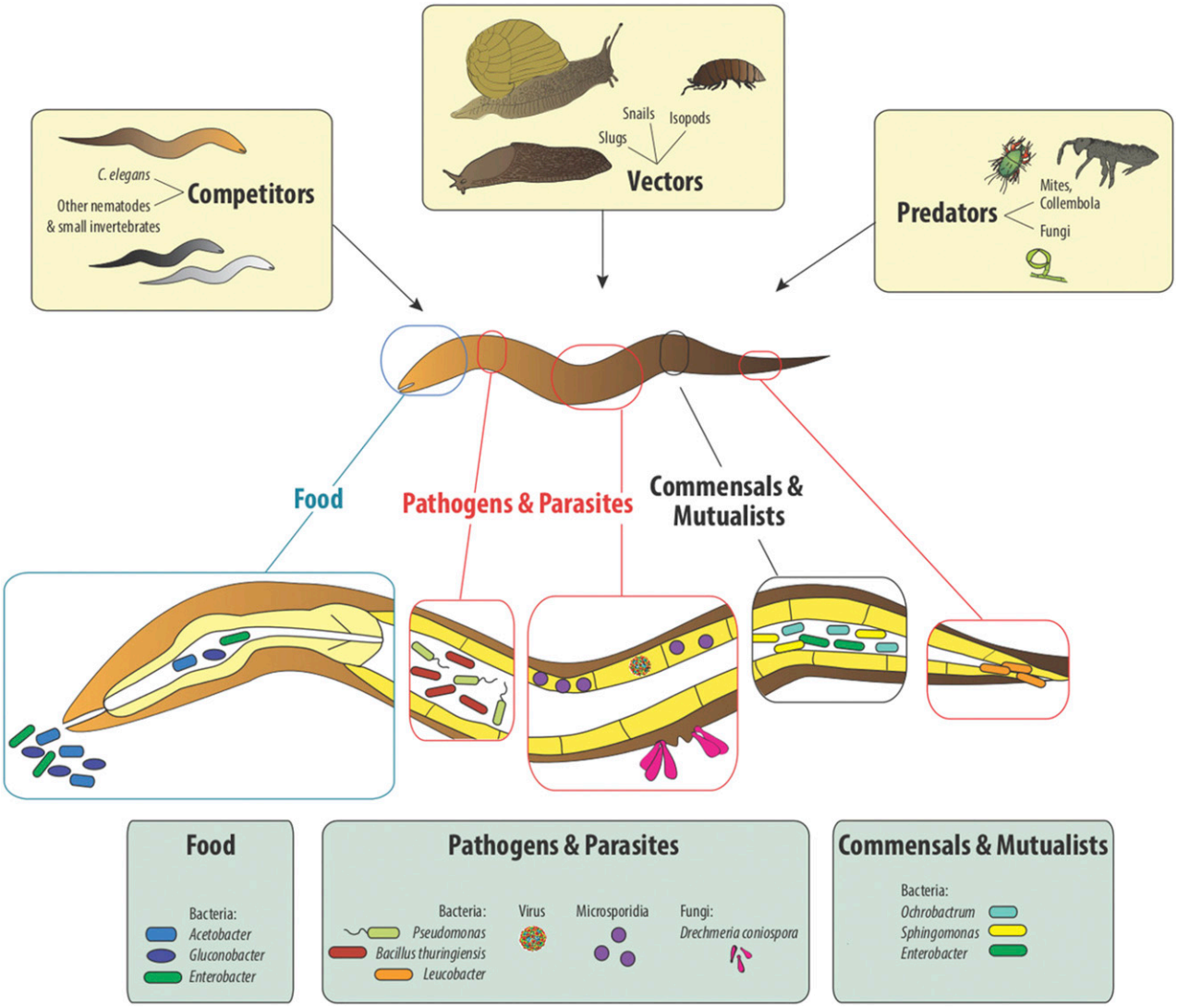

Figure 1 Overview of biotic interactions of *C. elegans*. The illustration highlights examples of different types of interactions, ranging from competition with other nematodes or invertebrates over interactions with vectors (*e.g.*, slugs, snails, or isopods), interactions with predators (*e.g.*, mites, collembola, and nematode-trapping fungi), to the diverse interactions with microorganisms. The latter include food bacteria (as examples members of the genera *Acetobacter*, *Gluconobacter*, and *Enterobacter*), then pathogens and parasites (*e.g.*, microsporidia, the Orsay virus, the fungus *D. coniospora*, and the bacteria *P. aeruginosa*, *B. thuringiensis*, and *Leucobacter* sp.), and also commensals and possibly mutualists (likely examples are members of the genera *Ochrobactrum*, *Sphingomonas*, and *Enterobacter*).

Frézal and Félix 2015). Overall, microbe-rich rotting plant matter seems to represent the original substrate for *C. elegans* in nature, while it is possible that *C. elegans* populations have additionally adapted to human environments, such as compost or some orchards, which may provide a more stable source of nutrition across the year than found in most natural habitats. *C. elegans* may, thus, possess a hemerophilous (human-associated) lifestyle, as already described for various bird species or mammals (*e.g.*, house sparrow, common pigeon, and house mouse, etc.; Marzluff *et al.* 2008). Finally, different *C. elegans* developmental stages were recently isolated from the intestines of living slugs (Petersen *et al.* 2015c), which may possibly be used as a completely different type of bacteria-rich substrate (see more details in *Macroscopic Invertebrates as Possible Vectors or Hosts*).

Life cycle of C. elegans and its biotic environment

The physical and biotic environment profoundly affects the development, physiology, and behavior of *C. elegans*, thereby influencing its life cycle. In the presence of food and at low population density, *C. elegans* develops directly from an embryo through four feeding larval stages to an adult. In contrast, in the absence of food and at high population density, *C. elegans* may arrest development at various stages, especially as a dauer larva, an alternative developmental stage that takes place following the second molting phase (Maupas 1915; Riddle and Albert 1997) (Figure 3). The environmental cues to enter the dauer stage are perceived during the L1 and L2 larval stages through sensory neurons, and include *C. elegans* density, food availability, and temperature (Bargmann and Horvitz 1991; Hu 2007;

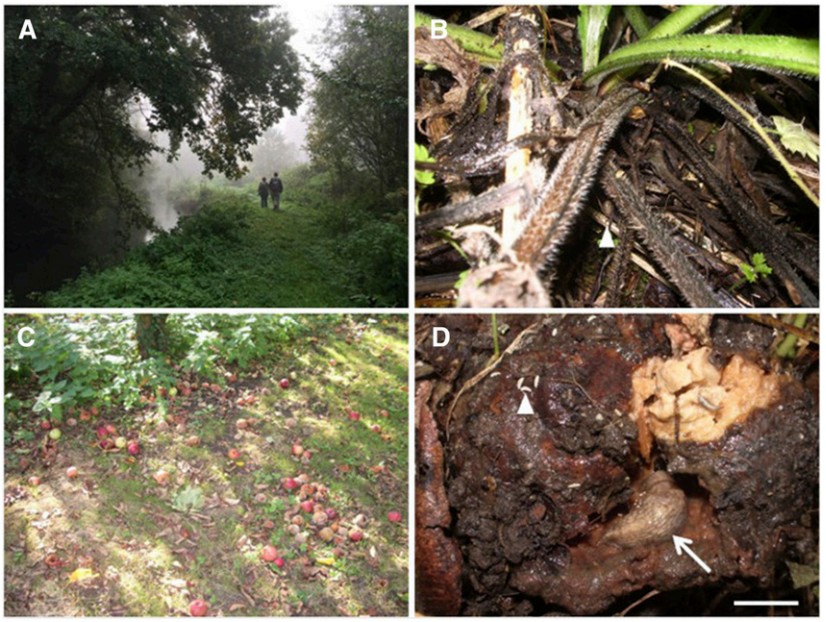

Figure 2 Representative habitats and substrates of *C. elegans*. (A) A humid temperate area. (B) Rotting stems in the habitat of (A). The arrowhead indicates an isopod. (C) An orchard. (D) Rotting fruit in the habitat of (C). The arrowhead indicates collembola. The arrow indicates a slug. Bar, 1 cm. Photograph in A courtesy of Patrick Phillips; photographs in B-D by M.-A.F.

Fielenbach and Antebi 2008; Neal *et al.* 2015). *C. elegans* density operates through the secretion of ascarosides that are sensed by amphid neurons (Ludewig and Schroeder 2013). The bacteria-derived chemicals that regulate dauer entry are also sensed by amphid neurons but their chemical nature is still undefined, even for the artificial *E. coli* food provided to *C. elegans* in the laboratory. A recent study purified Nicotinamide Adenine Dinucleotide (NAD^+) from a dauer exit-inducing fraction of *E. coli* and showed that it could induce serotonin signaling in specific sensory neurons, thus promoting dauer exit (Sze *et al.* 2000; Zhang 2004; Mylenko *et al.* 2016). Whether the same cue (*i.e.*, its absence) acts in dauer entry is so far unclear. The effect of other more naturally encountered bacteria has hardly been studied (Jensen *et al.* 2010). Environmental cues regulating dauer entry vary among *C. elegans* wild isolates, perhaps reflecting the various encountered ecological conditions (Viney *et al.* 2003; Harvey *et al.* 2008; Diaz *et al.* 2014; Diaz and Viney 2015).

The dauer larva is the resistant dispersal stage that may be able to colonize new patches of food. The dauer has a closed mouth and a particularly resistant cuticle, does not feed, and yet is behaviorally active. It acts as a dispersal stage, either through its own locomotion or by hitchhiking on a larger invertebrate (isopod, gastropod, etc., see *Macroscopic Invertebrates as Possible Vectors or Hosts*). Dauer exit is an important decision that is also regulated by environmental cues (in particular, presence of bacteria). One or a few dauer larvae may start developing in a favorable environment, producing a proliferating population without any dauers (Félix and Duveau 2012; Figure 3). After population expansion, resource exhaustion, and density increase, the young larvae enter the dauer stage and the cycle starts again.

C. elegans, thus, adopts a boom-and-bust life cycle that is strongly dependent on its environment (Félix and Braendle 2010; Félix and Duveau 2012; Cutter 2015; Frézal and Félix 2015). Patches of rotting plant material enable fast *C. elegans* population growth with direct development, while resource exhaustion leads to entry into the dauer stage and migration toward a new resource patch. This life cycle is characteristic of many (but not all) members of the Rhabditidae family, which serves as an indicator of richness of soil (Yeates and Bongers 1999; Yeates 2003). Thus, through its effect on *C. elegans* development, the biotic environment has a key influence on population dynamics. Yet, to date, it is still unclear which exact environmental parameters are most influential. Here, the microbial environment is likely to be most important, as discussed in more detail below (*The Microbial Environment*).

Macroscopic Invertebrates as Possible Vectors or Hosts

Vectors or hosts: types of association

A few selected studies have assessed the association of *C. elegans* with other invertebrate species. Here, association is defined as the presence of *C. elegans* on or inside of the other animal, indicating a more intimate relationship between the two species. The focus of these studies has been on macroscopic invertebrates, especially insects, crustaceans, spiders, millipedes, chilopods, and molluscs. The common assumption is that *C. elegans* can use these comparatively larger invertebrates as vectors to move between locations, as do other species of rhabditid nematodes (Völk 1950; Kiontke and Sudhaus 2006). This assumption is particularly supported by the fact that dauer larvae appear to actively search for vectors for dispersal. The behavior shown has been termed nictation, whereby the dauer larvae stand on their tail, wave their body in the air, and easily attach themselves to any passing object, such as a larger animal (Lee *et al.* 2011). Several dauers can even jointly form a column and nictate as a group, possibly enhancing the likelihood of getting into contact with a vector (Figure 3) (Félix and Braendle 2010; Félix and Duveau 2012). *C. elegans*, especially

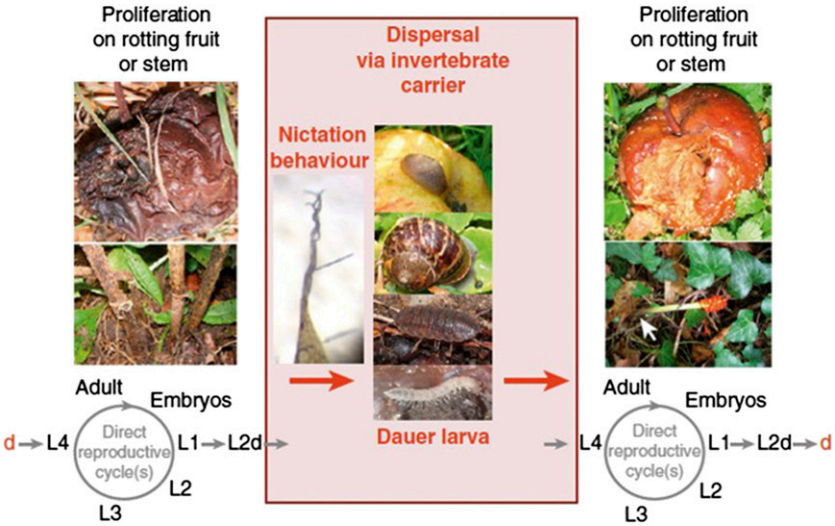

Figure 3 The life cycle of *C. elegans* and the influence of biotic associations. *C. elegans* eats bacteria and grows in various types of bacteria-rich rotting plant material. Dauer larvae are induced by bacterial food depletion, high *C. elegans* density, and high temperature. Dauer larvae may actively disperse to colonize new food sources through their own locomotion. In addition, their nictation behavior may allow them to attach to carriers, such as slugs, snails, isopods, or myriapods, until a new food source is encountered, where development resumes. Reproduced from Félix and Braendle (2010) with permission from Elsevier. d, dauer larva; L1–L4, larval stages; L2d, pre-dauer larva in the L2 stage.

when in the dauer stage, are likely able to attach to all macroscopic invertebrates that share the same habitat. Whether they may be taken away by any passing animal or whether any specificity exists is unclear.

The type of interaction with the invertebrate animals to which *C. elegans* physically associates is also not always clear. This is mainly due to the study approach, which is based on collecting individual invertebrates in the wild, bringing them to the laboratory, and checking for the presence of *C. elegans* in or on the other animal (Barrière and Félix 2007; Félix and Duveau 2012; Petersen *et al.* 2015c). Such associations may not only be explained by a vector-type association. Purely random relationships are conceivable, and it cannot yet be excluded that some form of parasitism or necromeny (*i.e.*, feeding on the decomposing host after it dies) underlies the association (Kiontke and Sudhaus 2006). More specific relationships with invertebrate hosts are known, for example for the nematode *Pristionchus pacificus*, which in part lives in association with scarab beetles (Sommer and McGaughran 2013), or *C. japonica*, which shows a phoretic interaction with the bug *Parastrachia japonensis* (Yoshiga *et al.* 2013; Okumura and Yoshiga 2014). In the case of *C. elegans*, more details on the specificity of the interaction would now require specially designed studies, such as life history assays on the host or collection of dead hosts from the wild (beyond one anecdotal report in Barrière and Félix 2007). In addition, larger vertebrate animals interact with known *C. elegans* substrates such as rotting fruits, for example small rodents, certain bird species, and humans. Therefore, these could also act as hosts or vectors, but are not yet part of the available data.

Overview of vectors

Early studies found *C. elegans* and its relatives, such as *C. briggsae* and *C. remanei* (Baird 1999), in association with isopods, snails, slugs, and myriapods (Kiontke and Sudhaus 2006). A systematic study of snails in California revealed a high number of associations with *C. elegans* (Caswell-Chen 2005). A survey in Scotland isolated *C. elegans* from isopods such as *Porcellio scaber* and *Po. spinicornis* (Cutter 2006). In France, *C. elegans* was repeatedly isolated from various isopod taxa, slugs (including small slugs identified as *Deroceras*), and snails (including genera *Helix* and *Pomatia*) (Barrière and Félix 2005, 2007; Félix and Duveau 2012). A comprehensive survey in North Germany found *C. elegans* in >10% of collected slugs (mainly of the genus *Arion*, especially *Arion lusitanicus*, and occasionally *Limax maximus*), isopods (*e.g.*, *Po. scaber*, *Oniscus asellus*, and *Armadillidium vulgare*), and also chilopods (Figure 4, A and B), while not with any of the assayed species of insects and spiders (Petersen *et al.* 2015c). The invertebrates were particularly prone to harbor *C. elegans* if collected on compost or rotting fruits, reaching a prevalence of up to or even above 30%. The association with these animals rather than insects or spiders, also present on compost or rotting plants, may be influenced by humidity (Petersen *et al.* 2014, 2015c). As most *C. elegans* stages are highly sensitive to dehydration (*e.g.*, Erkut *et al.* 2011), they are likely able to better survive on animals that provide a more humid environment, such as isopods and the various molluscs.

The peculiar relationship with slugs

The association with slugs appears peculiar. In the North German locations, slugs harbored *C. elegans* in habitat areas with apparently little rotting plant matter from which the nematodes could have been taken up (*e.g.*, in some parks; Petersen *et al.* 2015c). This may suggest that slugs are able to harbor *C. elegans* for longer time periods. Detailed characterization of different body parts revealed a significantly higher preponderance of C. *elegans* in the slugs' intestines, whereas they were only rarely found on the head, tail, or middle part of the body (Figure 4C; Petersen *et al.* 2015c). Although the majority of worms in the intestines were dauers, some non-dauers were also repeatedly isolated, suggesting survival of other stages in slug intestines and possibly their reproduction in this environment. An experimental assessment of the interaction demonstrated that some *C. elegans* individuals are able to pass the slug's radula, enter the gut alive, transit through the intestine, and be released to the environment with the slug's feces. Different *C. elegans* stages (including L4, adults, and

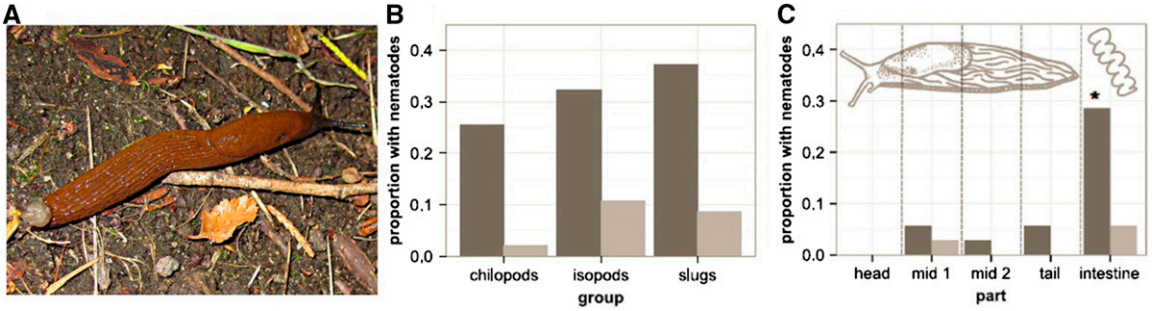

Figure 4 Association of *C. elegans* with invertebrates, especially slugs. (A) Slug of the genus *Arion*, often found to harbor *C. elegans* in North Germany. (B) Proportion of different taxa associated with *C. elegans* (dark gray bars) or *C. remanei* (light gray) in North Germany, based on a screen of a total of 51 chilopods, 93 isopods, and 35 slugs carried out between July and September 2013. (C) Localization of *C. elegans* (dark gray) and *C. remanei* (light gray) in different slug body parts, based on 35 tested slugs in 2013 in North Germany, highlighting a high abundance of *C. elegans* in slug intestines. Photograph in A courtesy of C. Petersen; B and C are from Petersen *et al.* (2015c).

dauers) succeeded in entering the gut. When slugs were exposed to nematode cultures for only 3 hr, their feces still contained worms after 30 hr (Petersen *et al.* 2015c). The particular association of *C. elegans* with slugs in North German locations is consistent with the findings from French locations, where slugs were found containing non-dauer stages, most likely present in the slug's intestines (Félix and Duveau 2012). These results also confirmed previous reports, which focused on parasitic nematodes, yet repeatedly found *C. elegans* or the congeneric *C. briggsae* inside of slugs from Africa and Europe (Mengert 1953; Ross *et al.* 2010, 2012). Altogether, these findings suggest that *C. elegans* may use slug intestines as a microbe-rich habitat for proliferation. It is even possible that *C. elegans* may thereby harm slugs. The exact relationship between *C. elegans* and slugs clearly deserves further investigation. A similar kind of relationship is further conceivable for snail species, which in some cases were also found to harbor *C. elegans* feeding stages (Félix and Duveau 2012).

The Microbial Environment

Overview of the diverse interactions with microorganisms

Microorganisms are of key importance for *C. elegans* biology. This species proliferates on decomposing substrates that contain a high density of microorganisms, especially of bacteria (Barrière and Félix 2005, 2007; Félix and Duveau 2012; Berg *et al.* 2016a; Dirksen *et al.* 2016; Samuel *et al.* 2016). These bacteria in the environment can have diverse interactions with *C. elegans*: they may directly serve as food, they may process substrate material to make it accessible for the nematode as food, they can be part of the worm's associated microbiome in its gut or body surface, and they may be pathogens and parasites with harmful effects (Figure 1). Microbial communities can be highly dynamic with rapidly changing compositions over space and time. Even within microbial lineages, changes can be fast, as mutations are often abundant due to usually large population sizes, frequent horizontal gene transfer, and the fact that favorable variants can spread rapidly due to the microorganism's comparatively short generation times. As a consequence of these fast evolutionary dynamics, the microbial environment can impose continuously high selective pressures on *C. elegans*. The nature and type of interaction of *C. elegans* with possible food microbes has been described for selected microorganisms since the 1990s (*e.g.*, Grewal 1991a; Grewal and Wright 1992; Venette and Ferris 1998), followed by additional work focused on few bacterial taxa (Avery and Shtonda 2003; Shtonda and Avery 2006; Coolon *et al.* 2009). The first natural pathogens and parasites of *C. elegans* were described in 2008 and 2011 (Troemel *et al.* 2008; Félix *et al.* 2011) (reviewed in *Pathogens and Parasites*). More systematic analyses of the *C. elegans* natural microbial environment were only published in 2016, and these addressed either the microbial composition of *C. elegans* substrates (Samuel *et al.* 2016), the native microbiome associated with wild-caught animals (Dirksen *et al.* 2016), or the microbiome of the canonical N2 strain exposed to soil under experimental conditions (Berg *et al.* 2016a). A recent meta-analysis assessed the differences and similarities among these three first systematic studies on the *C. elegans* microbiome (Zhang *et al.* 2017). Below, we summarize the findings and add information from the earlier studies, whenever appropriate. In a subsequent section, we will separately cover *C. elegans*' parasites and pathogens, which are also part of its microbial environment, yet produce a specific type of relationship based on antagonistic interactions.

The general natural microbial environment of C. elegans

The bacterial environment of *C. elegans*, defined as the bacterial composition of the various substrates where it can be found (*e.g.*, rotting fruit, etc.), was systematically analyzed by Samuel *et al.* (2016) via high-throughput sequencing of a bacterial 16S ribosomal DNA (rDNA) PCR fragment. These methods are only semiquantitative as there are numerous biases in the PCR amplification of different bacterial groups; yet, they still provide a first overview of bacterial community composition. The recent meta-analysis demonstrated that this composition differed from that associated directly with *C. elegans* nematodes (Zhang *et al.* 2017) (see also below). In the different substrate samples, sequences from the bacterial phyla *Proteobacteria* (mostly α- and γ-*Proteobacteria*), *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* were most common. At the family and genus levels, the

most common representatives were: in the γ-*Proteobacteria*, the families *Enterobacteriaceae* (*e.g.*, genus *Enterobacter*), *Pseudomonaceae* (*Pseudomonas*), and *Xanthomonadaceae* (*Stenotrophomonas*); in the α-*Proteobacteria*: *Acetobacteriaceae* (*Acetobacter*, *Gluconobacter*, and *Acetogluconobacter*, common in fruits); in the *Bacteroidetes*: *Flavobacteriaceae* (*Flavobacterium* and *Wautersiella*) and *Sphingobacteriaceae* (*Sphingobacterium*); in the *Firmicutes*: *Lactobacillaceae* (*Lactobacillus*), *Streptococcaceae* (*Lactococcus*), and *Leuconostocaceae* (*Leuconostoc*); and *Actinobacteria*, such as *Microbacteriaceae*, which were commonly found, but in low amounts.

When focusing on rotting apples in a given orchard and separating the apples according to *C. elegans* population state (*e.g.*, dauer *vs.* proliferating), a significant difference in bacterial composition was found, perhaps in part reflecting the degree of rotting of these apples (Figure 5A). *C. elegans* tended to be found proliferating in apples with a simpler microbiome, enriched in *Acetobacteriaceae* (*Acetobacter* and *Gluconobacter*) and poor in *Pseudomonas*, *Stenotrophomonas*, *Flavobacterium*, *Chryseobacterium*, *Xanthomonas*, and *Sphingomonas*. Overall, this trend matches the effect of representative genera when tested in the laboratory, as seen below. Note that *Acetobacteriaceae* and *Lactobacillae* are dominant in the gut microbiota of *Drosophila* fruit flies (Corby-Harris *et al.* 2007; Wong *et al.* 2013, 2015).

To test the effect on *C. elegans* of these naturally associated bacteria, a culture collection of 565 bacteria was established from the diverse samples. Representatives of the main genera found by 16S rDNA genotyping can be cultured relatively easily in the laboratory. These isolates were tested individually for their effect on *C. elegans* growth and on the induction of stress and immune reporter genes (Samuel *et al.* 2016). Overall, some genera tended to have most representatives being beneficial (*e.g.*, *Gluconobacter* and *Enterobacter*), while others tended to be detrimental and induce expression of the reporters (*Xanthomonas*, *Chryseobacterium*, *Stenotrophomonas*, and *Aeromonas*) (Figure 5B). Over the 111 *Pseudomonas* isolates, the whole spectrum of effects was found, with an overall tendency toward a detrimental effect. When *C. elegans* growth was tested in the presence of two bacteria (one detrimental and one beneficial), in some cases, the beneficial bacterial strain could rescue the detrimental one (better than *E. coli* could) and in other cases, a small proportion of the detrimental strain was already pathogenic. Finally, more complex mixtures of 18–24 bacterial strains mimicking good or bad growth environments could reconstitute good or bad *C. elegans* growth conditions (Samuel *et al.* 2016). Overall, from the pattern of natural associations and the dissection of the effect of individual bacteria or combinations of bacteria, this study provides an insight into the external bacterial environment of *C. elegans*.

The worm's microbiome

We will now turn to the bacteria that are physically associated with *C. elegans*, mostly in the gut and some on the cuticle surface. Most *C. elegans* researchers only know this worm with an empty gut and a neat cuticle, devoid of any microorganisms. This is a consequence of using the N2 reference strain and culturing it monoaxenically on *E. coli* OP50. A routine laboratory protocol, bleaching (Stiernagle 2006), is frequently used to remove bacterial contaminants from *C. elegans* cultures and synchronize nematode populations. Bleaching efficiently kills all microorganisms and *C. elegans* stages except embryos that are protected by their eggshell. Indeed, so far there is no indication of a vertically transmitted symbiont in *C. elegans* (except for genome-encoded retrotransposon sequences that can assemble capsids in the germline of some isolates; Dennis *et al.* 2012). The bleaching protocol thus produces germ-free animals. The nematodes are then routinely combined with the *E. coli* OP50 strain that is used as food added on the culture plates.

In contrast, nematodes isolated from their natural substrates often contain a vast number of microorganisms in their intestines and body surface (Félix and Braendle 2010; Félix and Duveau 2012). Here, we use the word "microbiome" to refer to these microorganisms that are physically associated with *C. elegans* individuals. Two main studies characterized the native *C. elegans*-associated microbiome, using slightly different approaches. One of these focused on animals directly isolated from nature (Dirksen *et al.* 2016), whereas the other exposed the canonical laboratory strain N2 to defined substrates under controlled conditions, followed by microbiome characterizations (Berg *et al.* 2016a).

In the first case, natural strains of *C. elegans* and two congeneric species, *C. briggsae* and *C. remanei*, were obtained from various locations in France, one in Portugal, and two main locations in Northern Germany (Dirksen *et al.* 2016). Two types of samples were analyzed, both by 16S rDNA genotyping. On the one hand, the bacteria associated with single or few worms were characterized directly after their isolation from the substrates. On the other hand, *Caenorhabditis* populations were allowed to proliferate with their native microbiome under laboratory conditions for at least 2 weeks after original isolation (a new environmental challenge as the laboratory conditions are clearly different from the natural environment), followed by microbiome analysis. Substrate samples were also assessed as controls for many of the isolated individuals. In spite of the differences in processing protocols and collection sites of the samples from France/Portugal and those from Germany, the analysis revealed significant similarities in microbiome composition among the various *C. elegans* samples and, at the same time, significant differences of these to the microbiomes of the corresponding substrate and of the congeneric *C. remanei* (Dirksen *et al.* 2016) (Figure 6A). Dominant taxonomic groups that are physically associated with *C. elegans* are *Proteobacteria*, especially members of the *Enterobacteriaceae* and those of the genera *Pseudomonas*, *Stenotrophomonas*, *Ochrobactrum*, and *Sphingomonas*. This result strongly suggests that *C. elegans* possesses a microbiome that is distinct from its direct environment.

The colonization of *C. elegans* could be reconstituted in the laboratory after establishment of a collection of associated bacteria isolated from crushed *C. elegans*. Some bacteria are able to persist in the nematode gut over long time periods, especially *Ochrobactrum* isolates (Troemel *et al.* 2008; Dirksen *et al.* 2016), possibly indicating a more intimate

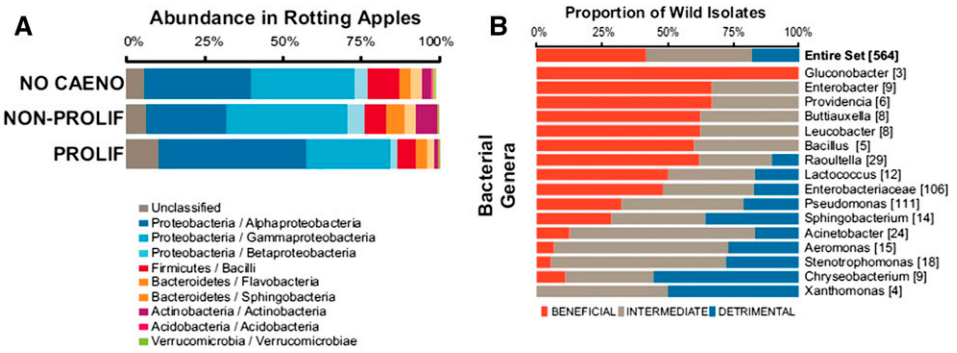

Figure 5 The natural microbial environment of *C. elegans*. (A) Relative abundance of bacterial taxa (indicated by color) in rotting apples containing either no worms (indicated by NO CAENO), nonproliferating worm populations (*e.g.*, consisting of dauer stages; NON-PROLIF), or proliferating populations of *C. elegans* (PROLIF). (B) Proportion of bacterial strains of a particular genus from the natural environment of *C. elegans*, which either have positive (orange color), intermediate (gray), or detrimental effect (blue) on worm life history. Both figures from Samuel *et al.* (2016).

relationship. In some experiments, *C. elegans* was cultured on plates seeded with mixes of bacteria and the *C. elegans*-associated microbiome was then compared to the plate microbiome by 16S rDNA genotyping. These experiments confirmed that the *C. elegans* microbiome is indeed distinct from that of its environment and that both *C. elegans* wild genotype and developmental stage have an effect on the composition of the associated microbial community. The latter result suggests that some specificity of colonization takes place. This may be a consequence of genetic adaptation of *C. elegans* to its microbial environment, possibly based on its behavioral choice, grinder properties, gut environment, or defecation efficacy. Alternatively, it may be determined by the ability of the various bacteria to invade and establish themselves in different *C. elegans* microenvironments, also determined by the genetic composition and biology of the animal. It is similarly possible that such variations result from a combination of host and bacteria properties. In this context, it is worth noting that the survey of the native microbiome did not reveal a specific bacterial species or strain (or set of strains) found across a larger number of *C. elegans* samples. The range of individual bacterial strains or operational taxonomic units (OTUs) varies across samples; the high similarities are only found at higher taxonomic level. This observation is inconsistent with the idea of coevolving host and microbe lineages, but it could instead be explained by host-mediated selection of favorable bacteria, which would then generate a host-specific microbiome with an important influence on host fitness (Bordenstein and Theis 2015; Douglas and Werren 2016). Our findings for *C. elegans* are thus consistent with the characteristics of the microbiome of other animal species, for example the house mouse or the fruitfly *Drosophila melanogaster* (Chandler *et al.* 2011; McCafferty *et al.* 2013; Wong *et al.* 2013).

The second microbiome analysis of *C. elegans* was based on an experimental approach (Berg *et al.* 2016a). Germ-free populations of the laboratory strain N2 were transferred to standardized soil samples supplemented with plant matter including various fruits. The animals were thus allowed to "collect" their preferred microbes. They were subsequently reisolated, followed by characterization of their microbial composition and that of the corresponding substrates. Consistent with the above study (Dirksen *et al.* 2016), the results demonstrated a worm-specific microbiome that was distinct from the corresponding substrates and that was highly similar, even if assembled from different substrates (*e.g.*, characterized by different fruits added to the soil). Microbial composition was influenced by temperature in a host-dependent manner. Bacteria that were generally enriched in these worms again included members of the *Enterobacteriaceae*, *Pseudomonaceae*, and *Xanthomonadacea*e (which contains the genus *Stenotrophomonas*), but also other taxa such as *Sphingobacteriaceae* or *Rhizobiaceae* (Berg *et al.* 2016a). Representatives of the commonly identified taxonomic groups, especially the three first families, may thus be part of the core microbiome of *C. elegans*. However, as in the study by Dirksen *et al.* (2016), no single bacterial strain or OTU was systematically associated with *C. elegans*. In analogy with many other hosts, it is therefore likely that *C. elegans* is preferentially colonized by a range of bacterial taxa, whose presence is influenced by the environment, the colonization ability of individual bacterial strains, and/or selection by the host [see also Shapira (2016)].

The recent meta-analysis, which compared the first three systematic microbial characterizations, confirmed that the composition of bacterial communities from *C. elegans* is significantly different to that of the corresponding substrates (Zhang *et al.* 2017). Importantly, *C. elegans*-associated communities from the two very distinct study approaches are highly similar, including enrichment of eight particular families across the two studies, such as *Enterobacteriaceae*, *Pseudomonaceae*, *Xanthomonadacea*e, *Comamonadaceae*, *Sphingomonadaceae*, *Sphingobacteriaceae*, *Weeksellaceae*, and *Flavobacteriaceae* (Zhang *et al.* 2017). In spite of these recent advances, it is yet unclear how stable the *C. elegans*-associated microbiome really is, either during the lifetime of individuals or across host generations. It is possible that the microbial community can be maintained in natural *C. elegans* populations, at least temporarily, via some form of vertical transmission. In principle, such vertical transmission may occur through eggs (*i.e.*, transovarial transmission, so far not seen in *C. elegans*), through transfer to the developing offspring in the uterus (enhanced when larvae hatch inside their mothers, an essential feature for symbiont transmission in *Heterorhabditis*) (Ciche *et al.* 2008; Griffin 2012), or via the sharing of the environment between parent and

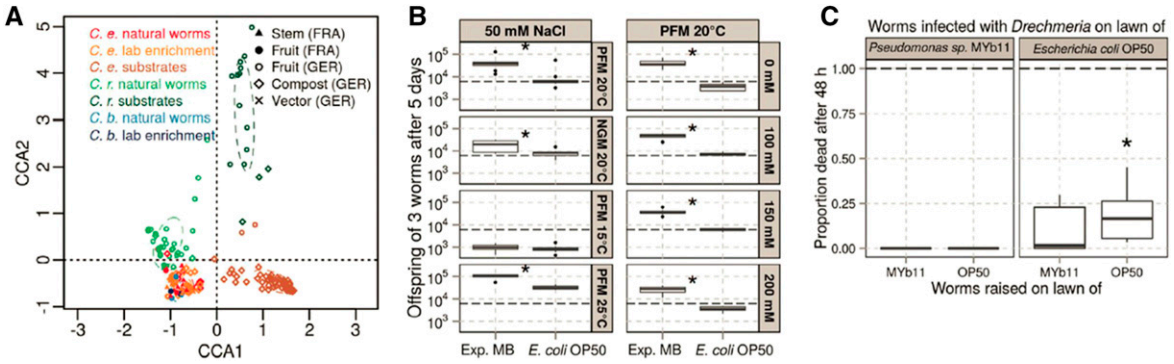

Figure 6 The native microbiome of *C. elegans*. (A) Canonical correspondence analysis of the microbiome of *C. elegans* (*C.e.*), *C. remanei* (*C.r.*), *C. briggsae* (*C.b.*), and corresponding substrate samples from France (FRA) and Germany (GER). Worm samples included nematodes, which were analyzed directly after their isolation from the wild (natural worms) and after proliferation in the laboratory for ~2 weeks (lab enrichment). The first and second axes separate the substrate samples from the worms (filled symbols in the bottom left corner), highlighting a characteristic microbiome of worms. (B) An experimental microbiome of a mixture of 14 bacterial strains (Exp. MB) enhances population growth relative to *E. coli* OP50 (see horizontal axes) under stress conditions such as nutrient-poor media or high temperatures (left panels), and also different salt conditions (right columns). Asterisks indicate a significant difference between Exp. MB and the *E. coli* OP50 control. (C) The *Pseudomonas* strain MYb11 protects nematodes from infection with the fungal pathogen *D. coniospora*, both when worms are raised on MYb11 (horizontal axis) and when infection takes place in the presence of MYb11 (left panel). The asterisk indicates significant difference in survival of worms grown under the tested conditions in the presence of the fungus versus the same conditions without fungus. All figures from Dirksen *et al.* (2016).

offspring (*e.g.*, Douglas 2010). Alternatively, the worm's microbiome is unstable across generations and determined by the changing environmental microbial community. The composition of the gut community then depends on the interaction between both host and microbe properties: on the host side on the foraging and feeding behaviors and the properties of the gut lumen; and on the microbe side, on the ability to pass the pharyngeal grinder and to persist in the gut without the population being entirely digested or expelled live by defecation. Interestingly, the recent meta-analysis highlighted that, even though substrate and nematode microbial communities are significantly different, they are still related. Taxa abundant across *C. elegans* samples are often abundant across the substrate samples (Zhang *et al.* 2017). This finding is consistent with the idea that the nematode microbiome is specifically assembled from the microbes available in the environment.

The recent studies also provided insights into the possible range of effects of the bacteria found in the environment or associated with *C. elegans*. Many of these bacterial strains are able to sustain *C. elegans* population growth, as measured as increases in population size over 5 days (Dirksen *et al.* 2016), developmental rate, and body size (Samuel *et al.* 2016), and some of them do it better than *E. coli* OP50. These fitness improvements were consistently expressed under different environmental conditions, including high osmolarity or various temperatures. Interestingly, on peptone-free agar media that could not sustain bacterial growth, naturally associated bacteria helped *C. elegans* to grow better in comparison to *E. coli*, likely in a mutual interaction where *C. elegans* provided an environment for bacterial population growth, from which *C. elegans* in turn obtained some food (Figure 6B) (Dirksen *et al.* 2016).

In contrast, some bacteria isolated from the natural environment could not sustain *C. elegans* growth (Samuel *et al.* 2016). Others induced expression of *C. elegans* genes usually activated by other stresses or pathogens, and at least some of these bacteria appeared pathogenic, because their effect could not be rescued by good food (Liu *et al.* 2014; Samuel *et al.* 2016). While some bacteria from the natural environment induced a mitochondrial stress response (measured with a *hsp-6p*::*GFP* reporter), other bacteria (*Pseudomonas* species) were able to suppress the mitochondrial stress response induced upon mitochondrial activity disruption by the *Streptomyces* toxin antimycin, perhaps indicating a counterdefense mechanism (Liu *et al.* 2014). Interestingly, certain bacterial taxa appear to have immune-protective effects, either through direct interaction with pathogens or indirectly through stimulation of host responses (Figure 6C). These immune-protective bacteria include members of the genera *Pseudomonas*, *Gluconobacter*, *Providencia*, and *Enterobacter* (Montalvo-Katz *et al.* 2013; Berg *et al.* 2016b; Dirksen *et al.* 2016; Samuel *et al.* 2016). The results complement the recent demonstration of the selective benefit of an immune-protective *Enterococcus faecalis* strain during experimental evolution with *C. elegans* and pathogenic *Staphylococcus aureus* (Ford *et al.* 2016; King *et al.* 2016). It is additionally possible that the associated microorganisms provide an advantage to *C. elegans* by shaping its natural environment, for example by eliminating harmful microbes and/or processing environmental substances and thereby making them accessible as nutrients for the nematode. These and other possible effects of the microbiome remain to be characterized and represent an exciting challenge for future research.

Food: C. elegans' prey

Finding out what *C. elegans* eats in natural conditions is not a simple task. In laboratory conditions, *C. elegans* is fed *E. coli* OP50 and the N2 reference strain is able to grind *E. coli* efficiently through its pharyngeal grinder (Avery and You 2012). Nutrients are then imported into the intestinal cells from the

gut lumen. In natural conditions, *C. elegans* is likely to eat mainly bacteria. Some small eukaryotes (such as yeasts) and perhaps already processed material may additionally be taken up (see below). We first focus on bacteria. From the above studies, in natural settings we know which bacteria may be found in *C. elegans*' immediate environment and which are present in its gut in a form that allows 16S rDNA sequencing (Berg *et al.* 2016a,b; Dirksen *et al.* 2016; Samuel *et al.* 2016). Two questions arise. Is the 16S rDNA in the gut of *C. elegans* that of the food or mostly that of live bacteria that will not be digested? Can bacteria that survive the pharyngeal grinder of the nematode be later digested in the gut? The DNA of digested bacteria is probably rapidly degraded in the gut and metabolized, so the food may even appear as those bacterial sequences that are more abundant in the substrates than in the corresponding nematodes (Dirksen *et al.* 2016). One particular candidate group that is highly abundant in rotting fruits (maybe not in other substrates) but less so in the nematode are the *Acetobacteriaceae*, which were also a good indicator of colonization of an apple by *C. elegans* (Dirksen *et al.* 2016; Samuel *et al.* 2016). As mentioned above, most of the isolated strains of environmental or associated bacteria may serve as food and support growth of *C. elegans*. Those that do not are generally pathogens rather than bad food *per se*, as they affect *C. elegans* population growth, even in the presence of a palatable bacterial species (Samuel *et al.* 2016).

The relationship of *C. elegans* to food around it has been studied in various ways, but so far not using the recently isolated bacteria naturally found with *C. elegans*. The type of bacteria that was offered as food was found to influence the growth rate of *C. elegans* populations (Grewal 1991a; Venette and Ferris 1998). Food availability affects behavior, including progeny production (Goranson *et al.* 2005), egg-laying, and bagging (Chen and Caswell-Chen 2004). Using a set of soil bacteria, Darby and Herman (2014) showed that in a mixture of bacteria, *C. elegans* grows as fast as the best available prey allows. Smaller size bacteria tend to be better food (Avery and Shtonda 2003). When given the choice between different soil bacteria (Avery and Shtonda 2003), *C. elegans* is able to learn to discriminate the food on which it grows best (Shtonda and Avery 2006): in a behavioral assay with patches of different bacteria, *C. elegans* tends to dwell on patches of good food and leave patches of worse food. In natural conditions, this is likely to be relevant, because the substrate is not a well-stirred bacterial mix and instead is structured with bacterial patches, *i.e.*, colonies forming from single bacterial cells.

Nutritional requirements of *C. elegans* have been studied in efforts to devise a chemically defined medium on which *C. elegans* can grow (Lu and Goetsch 1993; Perelman and Lu 2000; Szewczyk *et al.* 2003; Balachandar and Lu 2005; Xiong and Lu 2008; Zhao and Lu 2011). In addition to standard requirements for salts, amino acids, sugar, nucleotides, and various specific compounds/vitamins, *C. elegans* is defective in heme synthesis and must import it from bacteria (Hieb *et al.* 1970; Rao *et al.* 2005). In modern standard culture conditions, the heme is provided by *E. coli*, but in axenic conditions, heme must be provided together with a carrier protein (Buecher *et al.* 1970; Vanfleteren 1974). The rate of *C. elegans* growth and reproduction seems to be dependent on metabolically active bacteria or possibly a heat-labile nonsoluble component of live bacteria (Lenaerts *et al.* 2008). In the absence of such components, the nematodes reproduce more slowly and display an increased life span that is mediated by elevated activity of the Foxo transcription factor DAF-16, as in the dietary restriction response (Szewczyk *et al.* 2006; Lenaerts *et al.* 2008). This may suggest that *C. elegans* is able to express different healthy life histories in response to bacterial availability (Szewczyk *et al.* 2006). The growth conditions and physiological state of bacteria thus, in turn, affect *C. elegans* development and physiology.

Different bacteria vary in the nutritional supplies they provide (Watson and Walhout 2014; Yilmaz and Walhout 2014). Vitamin B12, a coenzyme required for breakdown of the short-chain fatty acid propionate (CH_3CH_2CO) and the methionine/S-adenosylmethionine cycle, is only synthesized by some species of bacteria. *E. coli* OP50 is a poor provider of vitamin B12 and *C. elegans* individuals were found to grow faster on a better provider such as *Comamonas aquatica* DA1877 (MacNeil *et al.* 2013; Watson *et al.* 2014). When grown on *E. coli* OP50, *C. elegans* activates the transcription of metabolic enzymes in a shunt pathway of propionate breakdown (Watson *et al.* 2016). Whether *E. coli* uses a fermentative or a respiratory metabolism further appears to matter for *C. elegans* longevity (Saiki *et al.* 2008). Other examples of nutrients that differ between bacteria concern the amount of dietary folate (Virk *et al.* 2012, 2016), tryptophan (Gracida and Eckmann 2013), or nitric oxide (Gusarov *et al.* 2013).

Bacteria do not provide all nutrients required for *C. elegans*, and specifically this nematode requires an external sterol source (Hieb and Rothstein 1968; Lu *et al.* 1977). In the standard laboratory medium, cholesterol is added as the sterol source. Where does *C. elegans* get its sterols in natural settings? One possible source of sterols is fungi, another being the degraded plant tissue itself. Fungal cells or cell walls are sometimes observed in the gut of wild-caught *C. elegans* (Félix and Duveau 2012). Therefore, it is possible that this nematode specifically takes up fungal cells (mostly unicellular yeast forms) and/or plant material and/or material from other animals (*e.g.*, dead and decomposing animals found in rotting fruits or compost) to satisfy its nutritional needs.

In this context, it may be speculated that access to these environmental nutrients may be mediated by bacterial members of the nematode's microbiome. In analogy to the gut microbiomes of termites (Brune 2014; Peterson and Scharf 2016) or ruminants (Krause *et al.* 2013), these bacteria may express the relevant enzymes to process environmental substances, which the worm itself cannot digest directly. The associated bacteria may thus fulfill a twofold function by directly serving as food and by indirectly providing access to environmental nutrients through metabolizing and further processing the available components. The latter ability and the relevance for nematode viability and fitness clearly deserve further study in the future.

C. elegans as a disperser

Besides potentially providing a substrate for bacterial growth, *C. elegans* may also disperse micro-organisms. It is so far unclear whether dauer larvae may carry bacteria, but feeding stages certainly do. Studies of dispersal by *C. elegans* have been performed using different experimental approaches. *C. elegans* can be found in the rich compost of mushroom farms and in this context may affect growth of the commercial mushroom *Agaricus bisporus* (Grewal 1991b), in part but not only because it spreads bacteria, such as *Pseudomonas tolaasii* (Grewal 1991c). Moreover, in microcosm experiments, *C. elegans* was found to mediate the spread of different bacteria (*e.g.*, specific strains of *E. coli* and *Salmonella newport*) to new substrates such as compost or plant material (Kenney *et al.* 2005, 2006; Anderson *et al.* 2006). Similar laboratory-based experiments demonstrated that *C. elegans* can enhance the dispersal of pathogenic *P. aeruginosa* (Diaz and Restif 2014) and of a bacteriophage of *P. syringae* (possibly with its host bacterium) (Dennehy *et al.* 2006). A recent study demonstrated nematode-mediated dispersal of *E. coli*, which resulted in a growth advantage for the bacteria on the new substrate patches and, most impressively, a subsequent increase in population growth of *C. elegans* (Thutupalli *et al.* 2017). In this experiment, the bacteria likely dispersed mostly by attaching to the animal's cuticle, as *srf-3* cuticle mutants did not disperse *E. coli*. Because of the fitness advantage for the nematode, the phenomenon was termed "farming" (Thutupalli *et al.* 2017). However, this term is misleading because it implies specific adaptations that allow *C. elegans* to disperse and initiate new *E. coli* colonies. To date, it cannot be excluded that bacterial dispersal is simply a by-product of normal *C. elegans* foraging behavior instead of the result of past adaptive evolution specific for this trait.

Another dispersal system uses the artificial pairing of two model organisms, *Dictyostelium discoideum* and *C. elegans*. These interactions may possibly occur in nature, as various slime molds are found in the same samples as *C. elegans*. *C. elegans* adults (although not necessarily the larvae; H. Schulenburg, unpublished data) can feed on the amoebae but not on the aggregated stages of *Dictyostelium*. Instead, on the aggregated stage, *C. elegans* helps by dispersing spores that survive the animal's gut (Kessin *et al.* 1996). Finally, dauers crawl up the fruiting bodies and nictate, helping in their own dispersal. This constitutes an interesting example of the web of relationships that *C. elegans* could have with associated organisms.

Pathogens and Parasites

Overview of the diversity of microbial antagonists

We will now turn to the microbes that entertain an antagonistic relationship with *C. elegans*. Pathogens are defined as harming *C. elegans*, while parasites are defined as taking advantage of it. Both types of relationship usually coexist for a given interaction. Moreover, a given organism can be pathogenic in one condition and beneficial in another. Note that, in immunity studies, longevity assays are often used, yet they are not necessarily informative concerning pathogenicity. For example, a particular microorganism may shorten the *C. elegans* postreproductive life span, but may not really matter to the nematode (or its evolution) if it does not affect the animal's fitness in a given environment. The consequences of pathogens are thus not always visible in reduced life span, but rather reduced offspring production. Indeed, in exponentially growing *C. elegans* populations, a slight slowing down of progeny production and/or brood size decrease should have a much stronger effect than decreased longevity (Hodgkin and Barnes 1991) [see below in *Viruses* and the competition experiment with a *C. elegans* virus in Ashe *et al.* (2013)].

Harmful effects of pathogens may be caused by toxic substances, which are known to be produced by various *C. elegans* pathogens, including both bacteria and fungi (Griffitts and Aroian 2005; Cezairliyan *et al.* 2013; Kirienko *et al.* 2013; Li and Zhang 2016). The pathogen may induce harm by disrupting cellular integrity or cellular and physiological homeostasis. For a parasite, it is additionally necessary that it can benefit from the interaction, generally by invading and replicating in the nematode body. Below, we provide an overview of naturally associated pathogens and parasites, ranging from fungal taxa, microsporidia, viruses, and oomycetes to bacterial pathogens.

Fungal pathogens

For nematode-harming fungi, a rather arbitrary frontier between predation and pathogens/parasites is usually set depending on the size of the relevant fungal life stage relative to the worm: hyphae for predators (*Competitors and Predators*) and spores for pathogens/parasites (here). In the latter case, spores attack worms and subsequently grow into hyphae inside the nematode. To date, two types of fungal pathogens have been found to infect *C. elegans* and other rhabditids in nature: *Drechmeria* and *Harposporium* (Félix and Duveau 2012). Most extensively studied is the interaction between *C. elegans* and *Drechmeria coniospora*. Spores of this fungus attach to the nematode cuticle, mostly around the mouth and the vulva, pierce it, and hyphae start invading the nematode, killing it within 2–4 days (Jansson 1994; Pujol *et al.* 2001). The hyphae then pierce out again and, once emerged, start budding spores along their axis (Figure 7). A single infected animal can yield thousands of spores and an infected *C. elegans* culture can be completely killed under laboratory culture conditions. *C. elegans* responds to *Drechmeria* infection by the secretion of antimicrobial peptides and other cellular responses that ameliorate its survival and reproduction capacity. The signaling pathways and effectors for the response have been extensively studied using *C. elegans* genetics and have become a model for the complexity of invertebrate immune responses [reviewed in Kim and Ewbank (2015)]. The genome of *D. coniospora* has been recently assembled and annotated (Lebrigand *et al.* 2016), providing a valuable resource for studying the genetics of interacting pathogen and host molecules.

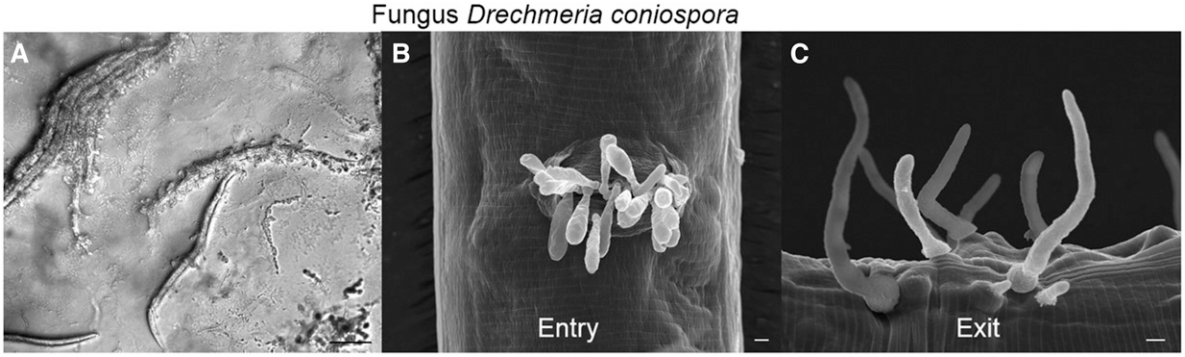

Figure 7 Infection of *C. elegans* by the fungus *D. coniospora*. (A) Infected *C. elegans* larvae on an agar plate. In these last stages of infection, fungal hyphae bearing spores exit from the dead nematodes. Bar, 100 μm. (B and C) Scanning electron microscopy. Bar, 2 μm. (B) Adhesion of *D. coniospora* spores to the vulva (they also adhere preferentially to the mouth periphery). (C) Exit of the fungus after infection. Pictures by M.-A.F.

In contrast to spores of *Drechmeria*, those of *Harposporium* enter through ingestion (at least for most species of this genus) (Esser and El-Gholl 1992). The intestinal eptihelium is thus the first tissue to be attacked by the growing fungus. The transcription/RNA turnover response to infections of various pathogens depends greatly on the infected tissue rather than on the type of pathogen (Engelmann *et al.* 2011).

Microsporidia

Microsporidia were the first parasites found in wild-caught *C. elegans* (Troemel *et al.* 2008). These close relatives of fungi are obligate intracellular parasites. They can be visualized inside the host cells by Nomarski microscopy. The most visible stage is the spore stage, when they resemble rod-shaped bacteria such as *E. coli* [indeed, they were mistakenly dubbed intracellular bacteria in Barrière and Félix (2005)]. Four species of microsporidia have been found in *C. elegans* so far, all placed in a new genus called *Nematocida* (Troemel *et al.* 2008; Luallen *et al.* 2016; G. Zhang *et al.* 2016; Reinke *et al.* 2017). The first and most common one, *Nematocida parisii*, and its close relatives *N. ironsii* and *N. ausubeli*, infect *C. elegans*' intestinal cells and are horizontally transmitted through ingestion and defecation (Figure 8). Microsporidia spores contain a characteristic "polar tube," which is discharged in some specific environments such as the *C. elegans* gut and injects the microsporidian DNA into the host cell. DNA replication and nuclear divisions ensue in a syncytial meront stage inside the host cell. The microsporidia can spread laterally from intestinal cell to intestinal cell (Balla *et al.* 2016). The meronts then secrete an envelope, cellularize (sporont stage), and start maturing in spores with a polar tube and its anchoring disc. The mature spores are surrounded by an additional membrane and routed through a vesicular pathway, recruiting RAB-11 and an actin coat, until they exit to the gut lumen (Szumowski *et al.* 2014, 2016). One infected animal may yield over 1000 spores.

N. parisii slows down progeny production and severely reduces *C. elegans* progeny number. If the animals are infected at the L1 stage, death of the animal may occur after ~3–5 days (Troemel *et al.* 2008; Balla *et al.* 2015). As with many intestinal pathogens, an obvious consequence of microsporidian infection is a severe shrinkage of intestinal cells, including of their storage granules and microvilli. Thus, the animals become pale under the dissecting microscope and likely display a reduced input of nutrients and metabolic activity. *N. parisii* infection results in upregulation of SCF (Skp/Cullin/F-box containing) ubiquitin-ligase subunits and ubiquitinylation of the pathogen, which may target the microsporidia to degradation by autophagy (Bakowski *et al.* 2014b). Individuals of some wild isolates of *C. elegans* are able to fully clear the infection: if inoculated with spores as young larvae, they can be infected intracellularly by *N. parisii* and then clear it entirely from their body. The genetic basis for natural variation in this process is being studied (Balla *et al.* 2015). Interestingly, the microsporidian proteins that are exposed to the host cytoplasm or nucleus tend to bear signal peptides or transmembrane domains and belong to large and/or fast-evolving, species-specific gene families (Reinke *et al.* 2017). Further details on the etiology and genetics of *C. elegans*–microsporidia interactions have been nicely summarized elsewhere (Balla and Troemel 2013; Bakowski *et al.* 2014a; Szumowski and Troemel 2015).

The ability to infect other nematode species varies among the microsporidia species. *N. parisii* and *N. ausubeli* both appear to infect *C. elegans*, *C. briggsae*, and other wild-caught *Caenorhabditis* species of the *Elegans* group. *N. parisii* is not able to infect *Oscheius tipulae* in the laboratory (G. Zhang *et al.* 2016). Conversely, microsporidia infecting *O. tipulae*, a very common rhabditid nematode species, cannot infect *C. elegans*. Individual microsporidia taxa may thus be able to infect closely related species, but not necessarily all rhabditids. A third *Nematocida* species with a different tissue tropism has been found recently in a wild-caught *C. elegans*, and named *N. displodere* (Luallen *et al.* 2016). This species proliferates mostly in the epidermis, as well as muscles and neurons. The polar tube in the spore is long and may directly reach the epidermis from the gut lumen. Spores are then released from the epidermis and other tissues via bursting of the animal at the vulva, thus also killing the host.

Viruses

The discovery of viruses infecting *C. elegans* came with the observation of wild-caught *C. elegans* and *C. briggsae* animals with

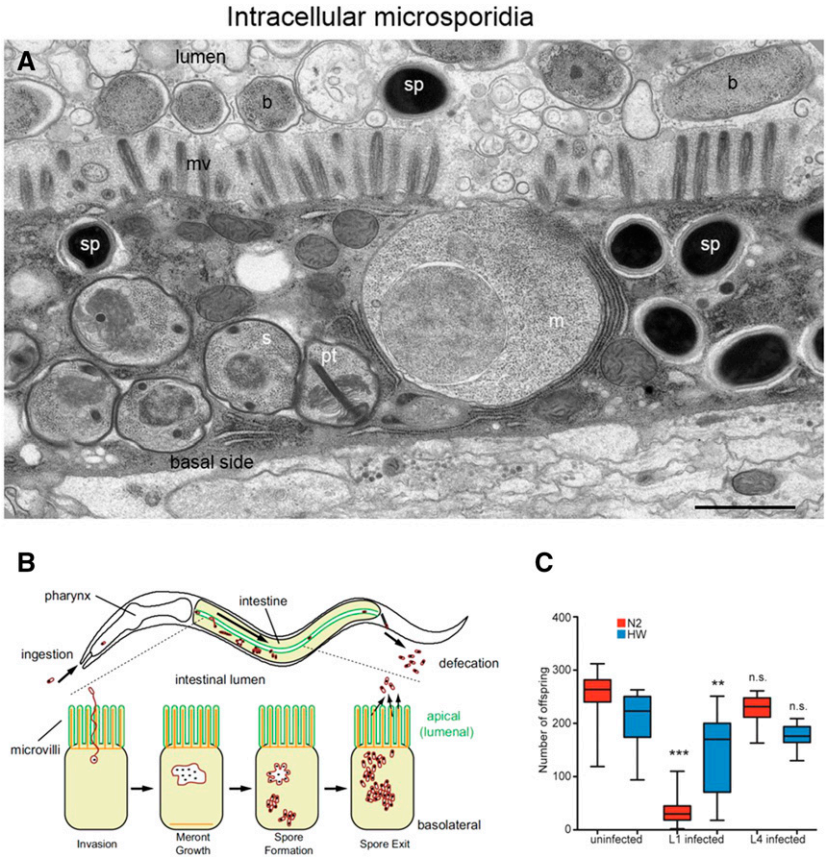

Figure 8 Microsporidian infections of *C. elegans*. (A) Transmission electron microscopy of *N. ausubeli* infecting *C. elegans*. The different developmental stages of the microsporidia are visible within a host intestinal cell: m, meront; s, sporont; sp, spore; pt, polar tube. Also visible are b, bacteria; mv, microvilli. Bar, 1 μm. (B) Schematic depiction of the life cycle of *N. parisii* in *C. elegans*. (C) Effect of *Nematocida* infection on *C. elegans* brood size. Red bars: N2 strain. Blue bars: CB4856 strain, from Hawaii (HW). The CB4856 strain is able to actively clear infection when infected early in the L1 stage. (A) Courtesy of M. Sachse and G. Zhang. (B) Reproduced from Szumowski *et al.* (2014). (C) Reproduced from Balla *et al.* (2015).

intestinal cell abnormalities, yet without visible pathogens, by Nomarski microscopy. These symptoms could be cured by bleaching and reinfection experiments after 0.2 μm filtration yielded the same cellular symptoms. These experiments led to the discovery of a group of nematode viruses related to fish and arthropod nodaviruses (Félix *et al.* 2011; Franz *et al.* 2012). These three viruses are transmitted horizontally and all infect intestinal cells (Félix *et al.* 2011; Franz *et al.* 2014) (Figure 9). Their genome is composed of two positive-strand RNA molecules: RNA1 encoding a RNA-dependent RNA polymerase and RNA2, a capsid with a facultative N-terminal δ domain translated by facultative ribosomal frameshifting at the first stop codon (Félix *et al.* 2011; Jiang *et al.* 2014) (Figure 9C). These viruses are species-specific, with the Orsay virus able to infect *C. elegans* but not *C. briggsae*, and conversely for the Santeuil and Le Blanc viruses (Félix *et al.* 2011; M.-A. Félix, T. Bélicard, G. Brésard and L. Frézal, unpublished data). The Orsay virus has, so far, only been found on rare occasions in *C. elegans* isolates of the region around Paris (L. Frézal and M.-A. Félix, unpublished data). These viruses all cause the same symptoms in the host intestinal cells, which progressively lose internal structures such as cytoplasmic granule content and nuclei, and fuse to each other. At the level of the organism, these viral infections are detrimental to population growth by slowing down progeny production and slightly lowering the brood size, without a detectable effect on worm longevity (Ashe *et al.* 2013) (Figure 9, D and E).

The replication cycle of RNA viruses includes a double-stranded stage, to which *C. elegans* may respond by initiating a small RNA cascade that degrades the viral RNAs (Félix *et al.* 2011; Ashe *et al.* 2013). However, wild isolates of *C. elegans* differ widely in their sensitivity toward the Orsay virus: after laboratory infection, a wide range of viral load is observed, with some isolates being fully unable to sustain viral replication. A genome-wide association study pointed to a single main locus explaining most of the species' phenotypic variation in viral replication, identified as a deletion polymorphism in the *drh-1* resistance gene. Figure 9, panels D and E, shows the rescue of the wild isolate JU1580 by an intact *drh-1*(*N2*) transgene. The DRH-1 protein is a homolog of the vertebrate RIG-I family, which starts the transcriptional interferon response in mammals. In *C. elegans*, DRH-1 instead starts the small RNA antiviral response (Ashe *et al.* 2013). That the derived allele is a deletion in a resistance gene is odd in terms of evolution of host–pathogen interactions. One possible explanation is that this conditional deleterious variant hitchhiked on another variant due to the low level of outcrossing and the high level of linkage disequilibrium in *C. elegans* (Ashe *et al.* 2013).

Oomycetes

Oomycetes were previously classified with Fungi because of similarities in their life cycle, but are now known to belong to the clade of Heterokonts, together with brown algae and

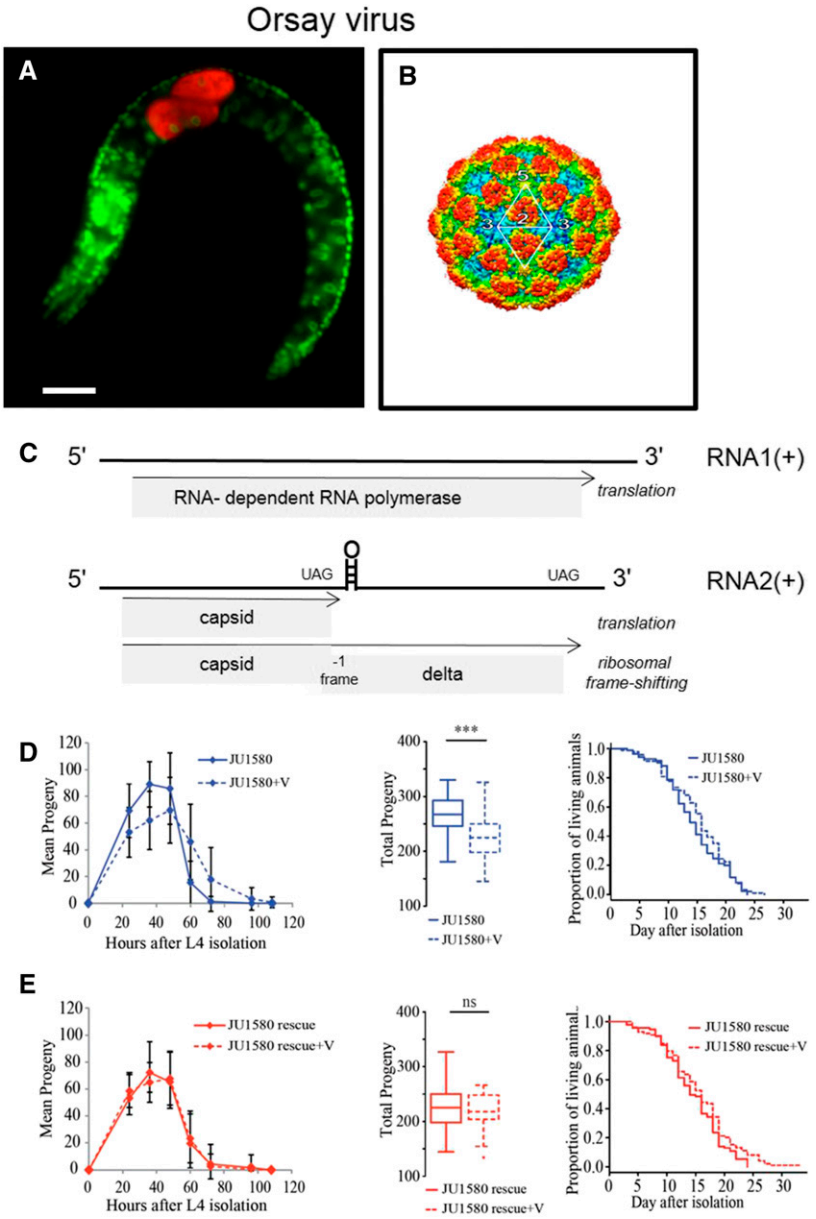

Figure 9 Orsay virus infection in *C. elegans*. (A) Fluorescent *in situ* hybridization of the Orsay virus in *C. elegans* JU1580 using a probe against RNA2 (red). DAPI is in green. Bar, 20 μm. Picture courtesy of L. Frézal. (B) Cryo-electron microscopy structure of capsid assembly (Guo *et al.* 2014). (C) Structure of the Orsay virus, with the facultative ribosomal frameshifting in the translation of RNA2 (Jiang *et al.* 2014). (D) Infection by the Orsay virus has an effect on progeny production but not longevity. (E) Rescue of the wild isolate JU1580 (carrying a *drh-1* deletion) with a *drh-1*(*N2*) transgene. Panels from left to right: dynamics of progeny production, total brood size, and survival curves (n = 40 animals for brood size, n =130 for longevity). ***$P < 0.001$. (D and E) Reprinted from Ashe *et al.* (2013).

diatoms (Beakes *et al.* 2012). Oomycetes include mostly plant-infecting species, such as *Phytophthora infestans*, which causes the potato blight disease. Oomycetes were previously found infecting other rhabditid nematodes (Maupas 1915). Their life cycle was studied morphologically (Beakes *et al.* 2012), but as for trapping fungi the authors did not pay much attention to the nematode species being infected, presumably because of the low host specificity. *C. elegans* was recently found infected with an oomycete of the genus *Myzocytiopsis* (M.-A. Félix, unpublished results), and this now allows the study of oomycete–*C. elegans* molecular interactions (M. Barkoulas, personal communication) (Figure 10).

Bacterial antagonists

Overview of bacterial pathogens: Despite the fact that *C. elegans* immune defenses must have evolved in the context of naturally encountered bacteria, the first focus has been to study *C. elegans* interaction with human pathogenic bacteria rather than natural pathogens of *C. elegans* itself (Darby 2005; Kim and Ewbank 2015). Several of these bacteria are nevertheless likely to be relevant in the natural context, for example *P. aeruginosa*, *Bacillus thuringiensis*, and *Serratia marcescens*, for which there is some indication for coexistence with *C. elegans* and which we describe in more detail below. We left out some of the other previously studied opportunistic human pathogens, for which it is possible, yet currently less clear, that they interact with *C. elegans* in the field, for example *Burkholderia pseudomallei* (*e.g.*, Day and Sifri 2012; Lee *et al.* 2013; Lim *et al.* 2016).

Note that the natural coexistence of pathogens and *C. elegans* is difficult to uncover, in contrast to the natural interaction with mutualists or commensals. Because of the

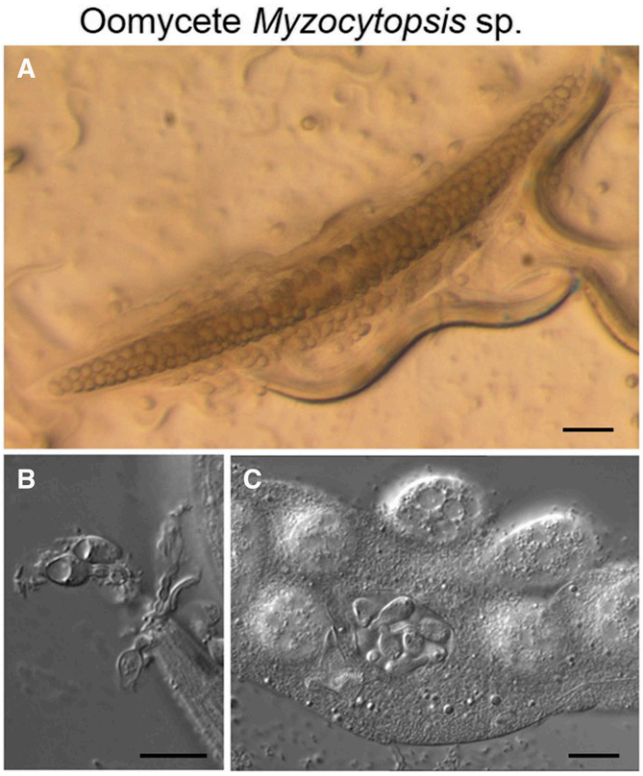

Figure 10 Infection of *C. elegans* by the oomycete *Myzocytopsis* sp. (A) Infected *C. elegans* adult on an agar plate. In this last stage of infection, the oomycete forms in the host body spherical multinucleate structures called sporangia. Bar, 100 μm. (B and C) Nomarski microscopy. Bar, 10 μm. (B) Adhesion of *Myzocytopsis* spores to the *C. elegans* mouth (they may adhere elsewhere on the cuticle as well). (C) Formation of spores in the sporangia. Only the cuticle and grinder of *C. elegans* remain visible. Pictures by M.-A.F.

antagonistic nature of the interaction, the animals are often paralyzed or even dissolved before they can be isolated and identified using the common *C. elegans* isolation protocols. Therefore, it is likely that the taxa that have so far been found are biased toward those mildly pathogenic for *C. elegans*. Many more pathogens are likely to be discovered in the future. Nevertheless, there is little doubt that pathogens have had a strong impact on *C. elegans* evolution. Although the species possesses a comparatively simple immune system that lacks specialized immune cells, it is still based on complex, interconnected immunity pathways and comprises defenses in the form of behavior, physical barrier, and physiology. Thus, *C. elegans* has become an important model system for studying the genetics of the innate immune system [reviewed in Irazoqui *et al.* (2010), Pukkila-Worley and Ausubel (2012), and Kim and Ewbank (2015)] as well as behavioral responses to pathogens. The latter include direct avoidance behaviors, reduced oral uptake of pathogens, and a learning response upon first encounter of pathogens, leading to enhanced avoidance upon secondary encounters [reviewed in Schulenburg and Ewbank (2007), Zhang (2008), and Meisel and Kim (2014)].

The naturally coexisting pathogens may infect *C. elegans* either through the cuticle or the gut. Below, we will start with several naturally associated bacteria that are likely to infect the worm through the gut, followed by cuticle-attaching pathogens, and then an overview of the nematode's interaction with *P. aeruginosa*, *B. thuringiensis* and *S. marcescens*.

Bacterial pathogens naturally associated with C. elegans. Live bacteria in the *C. elegans* gut may be beneficial in some cases, when the bacteria do not overproliferate. However, microscopic observations of wild-caught *C. elegans* indicate that naturally associated bacteria may proliferate so much that the gut lumen is filled with a large number of bacteria, leading to a substantial enlargement of the lumen at the expense of the worm's intestinal cells. In this case, the nematode appears compromised in its ability to feed and process nutrients, leading to reduced progeny production (Félix and Duveau 2012). Many bacteria that were isolated in the microbiota studies are likely to accumulate in the gut. Of those tested for their effect on *C. elegans*, some strains strongly diminish brood size and are thus potential pathogens, for example some (not all) members of *Pseudomonas* (MYb193), *Microbacterium* (MYb45 and MYb50), *Bacillus* (MYb78 and MYb56), *Chryseobacterium* (MYb7 and MYb120), *Arthrobacter* (MYb27), *Rhodococcus* (MYb53), *Leuconostoc* (MYb83), and *Sphingobacterium* (MYb181 and MYb210) (Dirksen *et al.* 2016). Moreover, specific strains of *Chryseobacterium* (JUb44), *Serratia* (JUb9), and *Pseudomonas* (GRb427) slowed down the growth of *C. elegans* individuals considerably (Samuel *et al.* 2016). Another pathogenic bacterium that was isolated with *C. elegans* is *Chryseobacterium* (or *Elizabethkingia*) sp. JUb129, a member of family *Flavobacteriaceae* in the Bacteroidetes phylum (Félix and Duveau 2012). This bacterium is able to kill *C. elegans* within a day and seems to consume it completely, including the cuticle (Félix and Duveau 2012). It is so far unclear how this infection starts, whether through the gut or through the cuticle. More work is required to determine its interaction with *C. elegans*.

Cuticle-attaching bacteria were first studied by J. Hodgkin's laboratory, making use of an infection occurring repeatedly in laboratory cultures. *Microbacterium nematophilum* bacteria attach to the cuticle next to the rectum (potentially a good place to get food) and proliferate (Hodgkin *et al.* 2000). They induce a local swelling of the anal region and harm the animal. They show some specificity among rhabditids; in *C. briggsae* they also adhere to the vulval region, following the expression pattern of the *bus-1* gene encoding a membrane O-acyltransferase (Gravato-Nobre and Hodgkin 2008). Genetic screens for mutants with an altered response to infection uncovered a number of genes involved in cuticle composition (Gravato-Nobre 2005; Yook and Hodgkin 2007; Gravato-Nobre *et al.* 2011). Although *M. nematophilum* was not found associated with *C. elegans* in natural settings so far, other bacteria of the same genus are common in the worm's microbiome (although their effect is yet unclear; H. Schulenburg, unpublished data; Dirksen *et al.* 2016), and bacteria attaching to the cuticle and sometimes affecting locomotion are often found on wild-caught *C. elegans* animals (Figure 11; M.-A. Félix and H. Schulenburg, unpublished data).

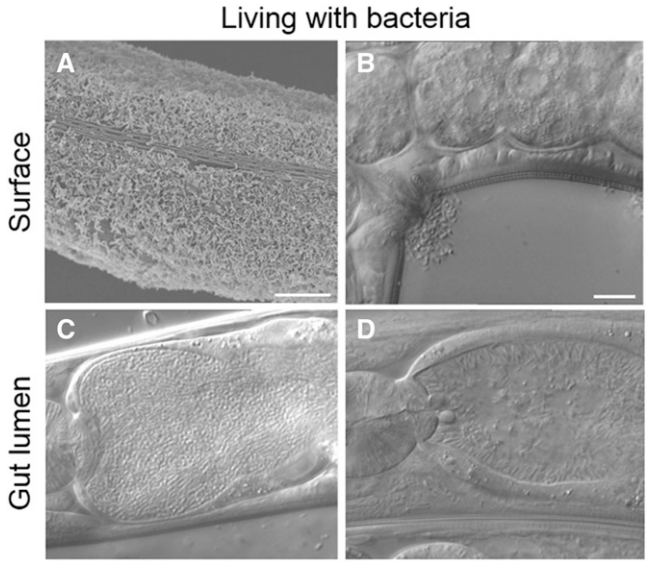

Figure 11 Types of physical interaction of *C. elegans* with bacteria (other than food). (A) Scanning electron microscopy picture of *C. elegans* covered with *Leucobacter celer* CBX151. Bar, 10 μm. (B–D) Nomarski pictures of unidentified bacteria with their wild *C. elegans* associate: adhering to the vulva of *C. elegans* (B), proliferating in the intestinal lumen of a L4 larva (C), and adhering to the apical intestinal border (D). Bar, 10 μm. Pictures by M.-A.F.

Three other bacteria found associated with *C. elegans* or *C. tropicalis* in nature were identified as *Leucobacter* spp., in the same family *Microbacteriaceae* (bacterial phylum Actinobacteria) as *M. nematophilum* (Hodgkin *et al.* 2013). Two of them, *Leucobacter musarum* CBX152/Verde 2 and CBX130, induce rectal swelling similar to *M. nematophilum* (Hodgkin *et al.* 2013; Clark and Hodgkin 2015). The third one, *L. celer* CBX151/Verde 1, coats the whole surface of the nematode (Figure 11A). Remarkably, in liquid culture, it induces the aggregation of *C. elegans* individuals through their tail (worm star formation) and rapidly kills them within a day (Hodgkin *et al.* 2013; Clark and Hodgkin 2014). The pathogenic mechanism appears to result from a physical injury of the animal's tail and subsequent invasion. The nematodes may escape via autotomy of the tail, which can heal, resulting in a sometimes partially fertile worm with a posterior body truncation. Strikingly, this lethal bacterium in liquid culture rescues on agar plate surfaces the lethality caused by *L. musarum* (CBX152/Verde 2 and CBX130) (Hodgkin *et al.* 2013). Mutant animals that are resistant to *M. nematophilum* and *L. musarum* due to a change in cuticular composition are hypersensitive to *L. celer*, suggesting a tradeoff in resistance between the two bacteria (Hodgkin *et al.* 2013). Members of genera *Microbacterium* and *Leucobacter* have been found in studies of the microbiota associated with *C. elegans* (see above), which suggests that they are relevant for *C. elegans* in the wild. However, it is not entirely clear which of the medium-dependent effects of these bacteria are most relevant in natural *C. elegans* populations. Newly infested plant matter or compost may still provide a rather intact substrate surface, thus resembling solid agar plates. Increased decomposition of plant material, especially fruits, may yield a liquid environment similar to the liquid lab medium, although usually with higher viscosity. Simulation of the most relevant natural medium conditions under laboratory conditions still represents a particular challenge for future research.

P. aeruginosa as a likely natural pathogen: Another likely natural pathogen of *C. elegans* is the Gram-negative bacterium *P. aeruginosa*. This bacterium was reported to coexist with *C. elegans* in compost collected from a mushroom farm in the UK (Grewal 1991a). Subsequent experimental analysis revealed that the bacterial isolate led to reduced worm population growth (Grewal 1991a). Almost a decade later (Darby *et al.* 1999; Mahajan-Miklos *et al.* 1999; Tan *et al.* 1999a,b), *C. elegans* was exposed to clinical isolates of this pathogen species, demonstrating the bacteria's ability for infecting and killing the worms. Three main types of killing dynamics were identified, which were dependent on the exact composition of the medium: (i) fast toxin-mediated killing on high osmolarity agar plates (Darby *et al.* 1999; Mahajan-Miklos *et al.* 1999; Cezairliyan *et al.* 2013); (ii) slow killing through bacterial accumulation in the gut and expression of various virulence factors on low osmolarity agar plates (Tan *et al.* 1999a,b; Feinbaum *et al.* 2012), and (iii) iron-dependent, hypoxia-mediated killing in liquid medium (Kirienko *et al.* 2013, 2015). This model infection system was explored in numerous studies to dissect the molecular basis of the nematode's immune defense system. It revealed the ability of *C. elegans* to behaviorally avoid this pathogen, either upon direct contact, mediated through pathogen secondary metabolites and a neuroendocrine host response based on G protein-coupled chemoreceptor and TGF-β signaling (Meisel and Kim 2014; Meisel *et al.* 2014), through the neuropeptide receptor gene *npr-1* (Styer *et al.* 2008; Reddy *et al.* 2009; Chang *et al.* 2011; Nakad *et al.* 2016), or upon a learned avoidance response mediated by serotonin and TGF-β signaling (Zhang *et al.* 2005; Zhang 2008; Zhang and Zhang 2012). Moreover, *C. elegans* can respond by activating its immune system, especially through the p38 MAPK pathway, the GATA transcription factor ELT-2, the insulin-like signaling cascade, and the hypoxia response (Kim *et al.* 2002; Shapira *et al.* 2006; Evans *et al.* 2008b; Kawli and Tan 2008; Kirienko *et al.* 2013). In-depth genetic analysis of the response to this pathogen permitted characterization of the complex signaling network underlying invertebrate immunity (Irazoqui *et al.* 2010; Pukkila-Worley and Ausubel 2012; Kim and Ewbank 2015).

The commonly used *P. aeruginosa* strain PA14 is able to interfere with the nematode's defense mechanisms, in particular with the insulin-like signaling cascade, thereby increasing its ability to infect (Evans *et al.* 2008a). Secreted *Pseudomonas* compounds, such as acylated homoserine lactones, also attract *C. elegans* (Beale *et al.* 2006). Such an attraction response has been found toward other pathogens. For example, *C. elegans* is initially attracted to pathogenic

S. marcescens, which it subsequently avoids (Pradel *et al.* 2007) (see also below *S. marcescens as a likely natural pathogen*). The nematode also responds positively to specific volatile organic compounds, such as 2-heptanone, of *B. nematocida* (Niu *et al.* 2010; C. Zhang *et al.* 2016). These observations may suggest a manipulative behavior of the bacteria, a "trojan horse" mechanism (Niu *et al.* 2010), that could facilitate successful infection of the host. In general, they support the idea that pathogen-mediated manipulation of the host has evolved repeatedly and should subsequently lead to increased selection on the host to counteradapt (Schmid-Hempel 2008).

Another study was based on an evolution experiment, which favored the emergence of infectious *P. aeruginosa* varieties that supported proliferating *C. elegans* populations (Jansen *et al.* 2015). This selection regime caused the evolving bacteria to lose virulence while maintaining their ability to accumulate inside worms. Pathogens thus evolved a commensal and perhaps mutualistic phenotype within few generations, highlighting that changes along the parasite–mutualist continuum can happen fast in response to appropriate selective constraints (Jansen *et al.* 2015). None of the recent studies used *P. aeruginosa* strains that coexist with *C. elegans* in nature and are thus likely shaping worm life history. Similarly, only a single study has yet assessed the response of a range of natural *C. elegans* isolates to this pathogen (using one of the clinical isolates of *P. aeruginosa*). QTL analysis of *C. elegans* strains in this study revealed that variation in avoidance behavior mapped to a genomic region in the middle of chromosome IV (Andersen *et al.* 2012).

B. thuringiensis as a likely natural pathogen: Another possibly natural pathogen of *C. elegans* is the Gram-positive spore-producing *B. thuringiensis*. This species is defined by plasmids that encode crystal toxins. Single strains usually contain only one to few toxin genes, which in turn determine specificity of the pathogen toward various insect and nematode hosts, including *C. elegans* (Vilas-Boas *et al.* 2007). Nematode infection begins with the oral uptake of the spores and their associated crystallized toxins. The toxins are solubilized in the nematode gut, proteolytically activated, and then specifically bind to sugars on intestinal cells, which leads to formation of pores in these host cells. Intestinal damage through the pore-forming toxins leads to a currently not yet well-understood change in milieu in the gut, which causes germination of the spores, followed by proliferation of vegetative cells. This process ultimately destroys all worm tissues. The animal is filled more or less completely by bacterial cells, which then start producing new spores and associated crystal toxins (Griffitts and Aroian 2005; Nielsen-LeRoux *et al.* 2012) (Figure 12). A nonpathogenic strain of this species was co-isolated with *C. elegans* from nature (M.-A. Félix and H. Schulenburg, unpublished data). The interaction of *C. elegans* with a variety of *B. thuringiensis* strains and specific toxins was analyzed in detail. The animals can behaviorally avoid this bacterium, a behavior mediated at least to some

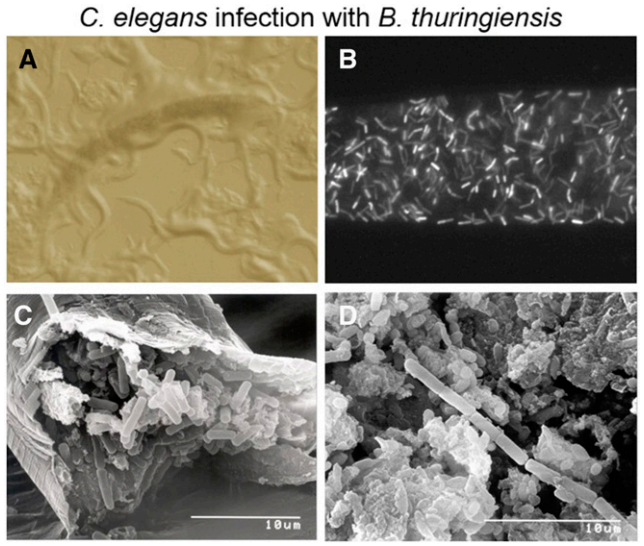

Figure 12 Infection of *C. elegans* infected with *B. thuringiensis*. (A) *C. elegans* killed by an infection with *B. thuringiensis* on an agar plate; the nematode is already disintegrating and only spores of the pathogen remain (picture courtesy of Rebecca Schulte). (B) Vegetative cells of *B. thuringiensis* inside a killed worm (picture courtesy of A. Papkou). (C) and (D) Electron micrograph of a killed infected nematode, containing mainly vegetative cells of the pathogen inside of its body. Picture in (C) from Schulte *et al.* (2010), and picture in (D) by H.S.

extent through the insulin-like signaling cascade and the neuropeptide receptor gene *npr-1* (Hasshoff *et al.* 2007; Nakad *et al.* 2016). Glycolipids on intestinal cells act as receptors for the crystal toxin Cry5B (Griffitts *et al.* 2005; Barrows *et al.* 2007), while ASP-1-mediated necrosis enhances susceptibility to the toxin Cry6Aa (F. Zhang *et al.* 2016). The physiological immune response is influenced by p38 and Jun N-terminal kinase (JNK) MAPK signaling, the hypoxia pathway, and insulin-like signaling (Huffman *et al.* 2004; Hasshoff *et al.* 2007; Bellier *et al.* 2009; Kao *et al.* 2011; Yang *et al.* 2015). Immune effectors include several lysozymes and caenopores (Boehnisch *et al.* 2011; Hoeckendorf and Leippe 2012; Hoeckendorf *et al.* 2012). Some micro RNAs additionally influence the response to the highly virulent *B. thuringiensis* strain DB27 (Iatsenko *et al.* 2013).

The interaction between *C. elegans* and *B. thuringiensis* was established as a model for experimental evolution, to study the dynamics of host–pathogen coevolution. Using genetically diverse host populations, *C. elegans* was shown in three fully independent evolution experiments to be able to adapt rapidly and in a highly specific manner to the continuously evolving pathogen challenge (Schulte *et al.* 2010, 2011, 2012, Masri *et al.* 2013, 2015; H. Schulenburg, unpublished data). At the same time, *B. thuringiensis* adapted to the host in a similarly specific manner (Schulte *et al.* 2010, 2011; Masri *et al.* 2015), apparently through changes in the copy number of a toxin-encoding plasmid and possibly also additional virulence factors (Masri *et al.* 2015). Taken together, these studies highlight that the interaction with *B. thuringiensis* has the potential to impose high selection

pressure on *C. elegans*, possibly also in nature. However, such dynamics have not yet been studied in a more natural context.

S. marcescens as a likely natural pathogen: The Gram-negative opportunistic pathogen *S. marcescens* is common in diverse environments, and an isolate showing the ability to swarm was previously found together with *C. elegans* in a compost sample in France (M.-A. Félix, unpublished results) (Pradel *et al.* 2007). Moreover, natural *C. elegans* strains produce highly genotype-specific interactions with natural *S. marcescens* isolates (Schulenburg and Ewbank 2004), possibly suggesting reciprocal coadaptations between these two antagonists. Therefore, it is likely that *C. elegans* interacts with this pathogen in nature and is able to respond to it in a highly specific form. This idea is supported by several evolution experiments under controlled conditions in the laboratory, under which the two antagonists were allowed to coadapt to each other (*i.e.*, both host and pathogen could reciprocally adapt to the other) or only one was allowed to adapt to a nonchanging partner (*e.g.*, *C. elegans* was allowed to adapt, while the same *S. marcescens* strain was newly added at each transfer step from a frozen stock culture, and vice versa). These experiments highlighted that both are able to specifically adapt to the antagonist and that the high selective constraints imposed by the continuous need to coadapt favor increased outcrossing rates in *C. elegans* (Morran *et al.* 2009, 2011, 2013, 2014; Slowinski *et al.* 2016).

The interaction between *C. elegans* and a specific *S. marcescens* isolate was further analyzed at the genetic and genomic level. These studies revealed that the inducible immune response is mediated by a TGF-β pathway and possibly a GATA transcription factor (Mallo *et al.* 2002; W. Yang *et al.* 2016), while resistance is influenced by lysozymes and C type lectins (Mallo *et al.* 2002; Miltsch *et al.* 2014). Most impressively, *C. elegans* is able to specifically recognize a surfactant from *S. marcescens* and then respond through an avoidance behavior, mediated by G protein signaling and the Toll-like receptor TOL-1 (Pujol *et al.* 2001; Pradel *et al.* 2007). TOL-1 acts by influencing the development and functioning of specific chemosensory neurons, the BAG neurons, which are involved in the surveillance of bacterial metabolism and the initiation of a pathogen avoidance response (Brandt and Ringstad 2015). *C. elegans* isolates show variation in their behavioral response to *S. marcescens*, which can be mapped to three main QTL, although the exact underlying genetic changes and molecular processes are yet unknown (Glater *et al.* 2014). It is of particular interest to find out to what extent such behavioral and physiological responses are expressed in natural *C. elegans* isolates toward coexisting *S. marcescens* varieties.

Competitors and Predators

Possible competitors

The competitors of *C. elegans* have been little studied. Other bacteriovorous nematodes can be found in the same samples, including other species of *Caenorhabditis*. *C. briggsae* was the *Caenorhabditis* species found most often in the same fruit or stem in surveys in France (Félix and Duveau 2012). In an apple orchard (Orsay), C. *briggsae* and *C. elegans* have overlapping but distinct seasonal distributions; while *C. briggsae* dominates in summer and early fall, *C. elegans* thrives in late fall. This seasonal distribution fits the species temperature preference as tested through competitions in the laboratory (Félix and Duveau 2012). However, an overlap occurred in the fall, and many samples in rotting stems in woods also contained both species in feeding stages, indicating possible competition (Félix and Duveau 2012). In contrast, in the surveys in Germany *C. briggsae* was rare, while *C. remanei* was most abundant in fruits and *C. elegans* in compost (Petersen *et al.* 2014). For the two latter species, humidity was a much stronger predictor for nematode presence than temperature (Petersen *et al.* 2014), opposite to the findings from France. To date, it is unclear why. It is similarly unknown why *C. briggsae* is apparently rare in German locations, while *C. remanei* shows low abundance in France. Moreover, it is also unclear whether the differential distribution of *C. elegans* and *C. remanei* in the German locations (compost *vs.* apples, respectively) is due to competitive exclusion. The feeding stages of other bacteriovorous nematode genera are also well-represented in the same samples containing *C. elegans*, such as members of the genera *Oscheius*, *Pristionchus*, *Panagrellus* (in fruits only), *Panagrolaimus*, *Mesorhabditis*, and various other rhabditids. Competition for live bacteria as a food source may also come from amoebas, slime molds, ciliates, and other small invertebrates (Félix and Duveau 2012).

A curious aspect of the competition with congeners is that *Caenorhabditis* nematodes readily mate with the opposite sex of closely related species under controlled laboratory conditions. These interspecies matings come with two types of cost. On the one hand, energetic resources that are invested in mating behavior, gamete production, and embryo development are wasted because hybrid offspring is usually not viable (Baird *et al.* 1992; Baird and Yen 2000; Hill and L'Hernault 2001). On the other hand, mating with a closely related species has a sterilizing effect on the production of self-fertilized offspring, resulting in significantly reduced progeny numbers in the case of *C. elegans* (Ting *et al.* 2014). The sterilization effect may result from the absence of coadapted antagonist gene(s) in the opposite sex, which is/are present in intraspecific combinations, thus preventing sterilization. As a nonexclusive alternative explanation, it is possible that the sterilization effect evolved to control population size of interspecific competitors (Ting *et al.* 2014). Whatever the cause, the effect is likely relevant in natural populations of *C. elegans*, which was found to coexist with congeners in single pieces of rotting plant material, fruit, and compost (Félix and Duveau 2012; Petersen *et al.* 2014).

Overview of possible predators

Predators feeding on *C. elegans* in natural settings have similarly been poorly studied. *C. elegans* moves forward rapidly when touched on the tail and backward when touched on the

head (Croll 1975; Chalfie and Sulston 1981; Goodman 2006). This behavior could be an adaptive avoidance response to any of these predators (Pirri and Alkema 2012) and may indicate frequent interactions of *C. elegans* with such predatory antagonists. While observing predation of *C. elegans* in the wild context is a challenge, testing whether an organism preys on *C. elegans* under artificial laboratory conditions is not necessarily relevant. A first requirement for relevance of an interaction is the cooccurrence of predator and prey in the same samples. One approach, not implemented so far for putative *C. elegans* predators, is to analyze the gut content of wild-caught predators (Read *et al.* 2006). The closest situation to a natural setting where preying on *C. elegans* was observed is in freshly collected substrate samples placed on a Petri dish with nutrient agar and brought under a dissecting microscope. In this context, the most common and obvious predators are trapping fungi. Various mites are also found in the same samples and at least one species (tentatively identified as *Sancassania* sp.; Félix and Braendle 2010) has been observed to eat *C. elegans*, swallowing it like spaghetti (Figure 13A). Nematode-eating collembola have also been observed in samples with *C. elegans*. Whether there is any specificity in the nematode species that these small arthropods eat is unclear. Note that predators can also potentially act as vectors if *C. elegans* avoids being eaten.

Besides studies in wild-derived microcosms, predation has been studied in the laboratory by exposing *C. elegans* or another small nematode to a candidate predator, although this approach does not test whether the interaction is ecologically relevant. Under these artificial conditions, *C. elegans* can be eaten by the collembola *Folsomia candida* (Lee and Widden 1996) or the nematode *P. pacificus* (Serobyan *et al.* 2014). In *Pristionchus* species, different adult mouth forms develop depending on the environment (Serobyan *et al.* 2014). In dire conditions, the mouth develops with strong teeth that enable the adults to prey on other nematodes. Feeding stages of *C. elegans* and *Pristionchus* species (other than *P. pacificus*) share the rotting vegetal matter environment (Félix and Duveau 2012), but the mouth form of the *Pristionchus* animals has not been assayed. Other nematodes such as mononchs (*e.g.*, *Prionchulus* spp.) are specialized predators (*e.g.*, Mikola and Sulkova 2001), but have not been noted so far in samples with *Caenorhabditis*.

Nematode-trapping fungi as predators of C. elegans

Nematode-trapping fungi are diverse and overall the best studied predators of small nematodes like *C. elegans* (Drechsler 1941; Gray 1987; Zhang and Hyde 2016). Nematode-trapping fungi have been frequently observed in samples with *C. elegans* (M.-A. Félix, unpublished results, see Figure 13B), but have not been characterized nor their impact on *C. elegans* populations assessed. Most of the work on nematode-trapping fungi has been carried out using other bacteriovorous nematodes or parasitic nematodes, against which they serve as biological control agents. Most nematode-trapping fungi belong to a clade in Orbiliomycetes (Li

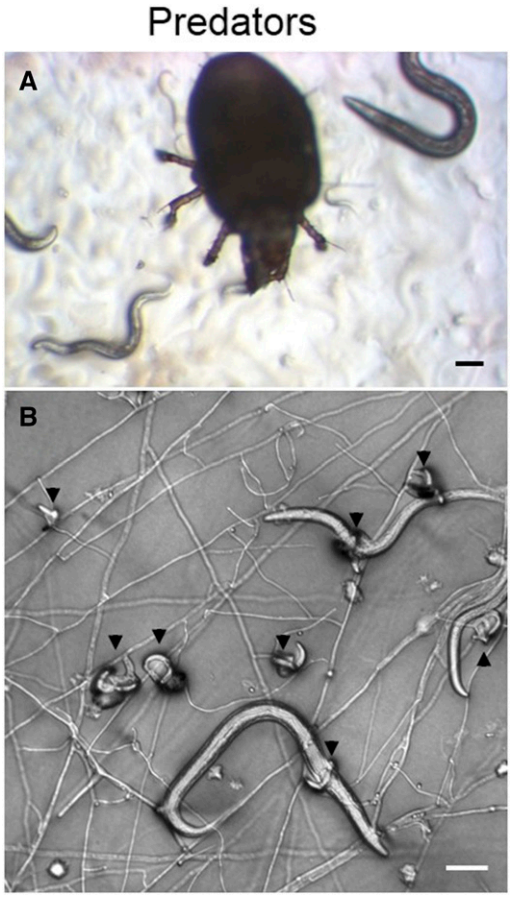

Figure 13 Predators of *C. elegans*. (A) *Sancassania* mite. (B) *C. elegans* larvae trapped by a fungus. Arrowheads designate traps. Bar, 100 μm in (A) and 20 μm in (B). Pictures by M.-A.F.

et al. 2005). These fungi are facultative predators and may also live as saprophages. Nematode traps are specialized derivatives of fungal hyphae, in the form of adhesive knobs (*e.g.*, *Monacrosporium haptotylum*; Ahren 2005), adhesive loops or networks (*e.g.*, *Arthrobotrys oligospora*), or circular rings that constrict upon entry of a nematode (*e.g.*, *Drechslerella doedycoides*; Drechsler 1941; Zhang and Hyde 2016). The fungi secrete proteases that are able to digest the nematode cuticle (J. Yang *et al.* 2016). Due to the size of its traps, *D. doedycoides* mostly traps the young larval stages of *C. elegans*. Some *C. elegans* larvae escape using active mechanosensation and suppression of lateral head movements while backing (Maguire *et al.* 2011). The fungal traps can develop constitutively or be induced by the presence of nematodes (Drechsler 1941; Zhang and Hyde 2016). For example, the traps of *A. oligospora* are induced upon sensing of ascarosides (Xie *et al.* 2010; Hsueh *et al.* 2013), chemicals produced by *C. elegans* that also act in dauer induction (as mentioned above) and male attraction behavior (Ludewig and Schroeder 2013). Thus, the fungus is capable of using the intraspecific communication signals of *C. elegans* to induce trap formation, where and when worms appear abundant.

Intraspecific Interactions

Overview of different types of intraspecific interactions and their importance

The biotic environment of *C. elegans* individuals is not only shaped by interactions with other species, but also by interaction with members of its species. Indeed, these interactions occur frequently and may be associated with highly selective dynamics. Two types of conspecific interaction can be particularly important. On the one hand, competition occurs within growing populations, especially when density increases and nutrient availability decreases. On the other hand, interactions among hermaphrodites and males occur during mating. These interactions are likely in part shaped by conflicting evolutionary interests. Fitness in females and possibly *C. elegans* hermaphrodites is enhanced by the availability and choice of high-quality mates, whereas that of males is driven by the number of matings (*i.e.*, Bateman's principle; Arnold 1994). To date, we lack data on the relative importance of these two types of interaction for *C. elegans* in nature. Yet, as explained below, several lines of evidence suggest them to be influential.

Intraspecific competition and its resolution through C. elegans pheromones

As detailed above (*Life cycle of C. elegans and its biotic environment*), *C. elegans* in nature is likely subject to a boom-and-bust life cycle (Frézal and Félix 2015). One or a few dauer larvae are assumed to colonize a new substrate. If conditions are favorable, rapid population growth ensues, until food depletion, crowding, and possibly other factors induce entry of the young larvae into the dauer stage. The increase in population density can be very dramatic, likely reaching up to >100,000 worms/g of substrate (Félix and Duveau 2012; Frézal and Félix 2015). During population growth and especially once higher densities are reached, individuals are likely to compete with each other for resources, particularly food microbes, but also for space and possibly mating opportunities (if males are available). Intraspecific competition can occur either within the same genotype or between distinct genotypes, as substrates were reported to host either a single or several genetically distinct lineages (Barrière and Félix 2007; Frézal and Félix 2015; Petersen *et al.* 2015b). In principle, coexistence of several genotypes may lead to expression of more aggressive behaviors, whereas competition within the same genotype may be shaped by kin selection and could result in cooperative behaviors (Hamilton 1963; Bourke 2014).

The dynamics of intraspecific interactions remain to be characterized in natural *C. elegans* populations. Substantial variation can be detected among natural *C. elegans* isolates in their propensity to enter the dauer stage under fixed pheromone and population size conditions and in growing populations (Viney *et al.* 2003; Green *et al.* 2013; Diaz *et al.* 2014). Moreover, different natural *C. elegans* strains vary both in the composition of the dauer pheromone they produce and in their response to the dauer pheromone of conspecifics. The result is in an intricate matrix of dauer-promoting and dauer-repressing effects of the pheromones produced and perceived by the various natural isolates (Diaz *et al.* 2014). This finding may suggest the presence of differences among strains in resolving competition through this alternate life stage. It is even conceivable that the dauer pheromone is used to induce dauer formation in competing genotypes and to remove competitors from the shared environment (Diaz *et al.* 2014), in agreement with the recent observations made for strains of the nematode species *P. pacificus* (Bose *et al.* 2014; Mayer *et al.* 2015; Sommer and Mayer 2015). As the dauer stage neither requires food nor mates, competition at these two levels is automatically removed in a population of dauers. The low levels of dauer formation that are seen in some *C. elegans* isolates might have evolved as a defense against manipulation by competitors. Further work is needed to distinguish this possibility from other ecological and evolutionary scenarios.

The *C. elegans* pheromone does not only influence dauer formation, it can also affect foraging behavior. One component of the pheromone, the ascaroside icas#9, can suppress foraging activity (Greene *et al.* 2016b). The response to icas#9 varies among natural *C. elegans* isolates due to polymorphisms in two interacting G protein-coupled chemoreceptor genes, *srx-43* and *srx-44* (Greene *et al.* 2016a,b). These two genes are subject to balancing selection in natural *C. elegans* populations (Greene *et al.* 2016a,b), possibly indicating the presence of alternative foraging strategies in response to food availability and population density. Thus, these alternative strategies may also contribute to resolve intraspecific competition. It is yet unclear to what extent natural food bacteria similarly influence alternative foraging behaviors in this context.

Male–hermaphrodite interactions

Another influential type of intraspecific interaction is found among the two sexes. *C. elegans* has an androdioecious reproductive system, consisting of sequential XX hermaphrodites and X0 males. Hermaphrodites can reproduce either by selfing or through mating with the males. Males can be produced by hermaphrodites as a consequence of X chromosome nondisjunction or as crossprogeny of a male and a hermaphrodite. Male frequencies in natural *C. elegans* strains were shown to be influenced by the rate of X chromosome nondisjunction and the mating efficiency of the males (Hodgkin and Doniach 1997; Wegewitz *et al.* 2008; Anderson *et al.* 2010), leading to significant variation in male abundance among *C. elegans* isolates and environments, ranging from 0.1% up to >30% males stably maintained in the populations (Hodgkin and Doniach 1997; Teotónio *et al.* 2006; Wegewitz *et al.* 2008; Anderson *et al.* 2010). Additional factors such as hermaphrodite receptivity may further contribute to variation in the proportion of males. Surprisingly, males are rare in nature, showing an abundance that is

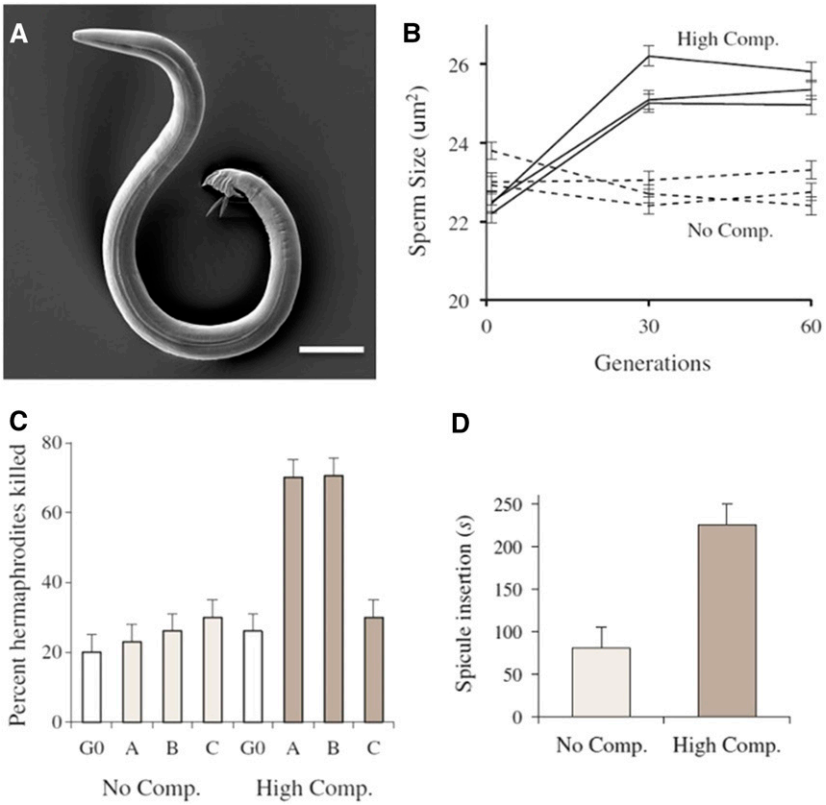

Figure 14 Male–male sexual competition enhances male competitiveness and male-induced harm. (A) Scanning electron micrograph of male *C. elegans* (Bar, 50 μm; picture by H.S. and A. Thomas). Sixty generations of experimental evolution under high male–male competition (indicated by High Comp., with three replicate lines called ABC) leads to increased sperm size (B) and thus male sperm competitiveness; to longer spicule insertion time (D) and thus mating duration; and also to increased killing of mated hermaphrodites (C). Graphs from Palopoli *et al.* (2015).

compatible with the X chromosome nondisjunction rate (Barrière and Félix 2005, 2007; Teotónio *et al.* 2006; Félix and Duveau 2012; Petersen *et al.* 2014). Nevertheless, matings do occur in the wild, leading to the occasional presence of heterozygote individuals and genetic exchange (Barrière and Félix 2005, 2007; Haber *et al.* 2005; Sivasundar and Hey 2005; Cutter *et al.* 2009; Andersen *et al.* 2012; Félix and Duveau 2012; Petersen *et al.* 2015b).

Laboratory-based evolution experiments demonstrated that mating and outcrossing can provide a fitness advantage in the presence of coevolving pathogens, such as *S. marcescens* or *B. thuringiensis* (Morran *et al.* 2009, 2011; Schulte *et al.* 2010; Masri *et al.* 2013), and other novel environments (Teotónio *et al.* 2012; Carvalho *et al.* 2014). Moreover, analysis of small molecular metabolites produced by *C. elegans* suggests a modular and flexible assembly of molecular signals that not only function as regulators of development, life span, and dauer formation (see above), but also aggregation and male–hermaphrodite interactions (*i.e.*, sex pheromone; Ludewig and Schroeder 2013; Schroeder 2015; Dong *et al.* 2016). Laboratory evolution experiments additionally showed that male–male competition can lead to strong sexual selection and cause changes in the competitiveness of male sperm (Figure 14) (LaMunyon and Ward 2002; Anderson *et al.* 2010; Palopoli *et al.* 2015). Interactions of hermaphrodites with males can lead to reduced life span and offspring production of hermaphrodites (Gems and Riddle 1996; Wegewitz *et al.* 2008; Shi and Murphy 2014; Palopoli *et al.* 2015), apparently through mechanical damage of the cuticle (Woodruff *et al.* 2014) or via secreted harmful compounds (Maures *et al.* 2014) that can decrease the number of germline progenitor cells, fat storage, or somatic stress resistance (Shi and Murphy 2014; Aprison and Ruvinsky 2016). These costs of mating or contact with males are possibly a consequence of sexual selection, which can favor the production of manipulative and damaging substances by males to increase male reproductive rate (Arnqvist and Rowe 2005). Such sexual selection is likely to act more strongly in gonochoristic *Caenorhabditis* species, as indicated for example in *C. remanei* male–female interactions (Garcia *et al.* 2007; Palopoli *et al.* 2015) and through comparison of sex-linked gene expression patterns between gonochoristic and androdioecious nematodes (Thomas *et al.* 2012). Yet, even for *C. elegans*, it remains an exciting challenge to find out how frequent potentially conflicting male–hermaphrodite interactions really occur in nature and to what extent they have shaped *C. elegans* life history evolution.

Natural Genetic Polymorphisms as an Indication for Biotic Interactions

Many biotic interactions impose high selection on the involved organisms. Such selective dynamics are likely to leave traces in the organism's genomes. In particular, selective sweeps result in reduced variation in the locus/loci under selection. As they are usually caused by locally restricted interactions (*e.g.*, a pathogen highly abundant at a specific site), they should additionally cause geographic variation. Overdominant

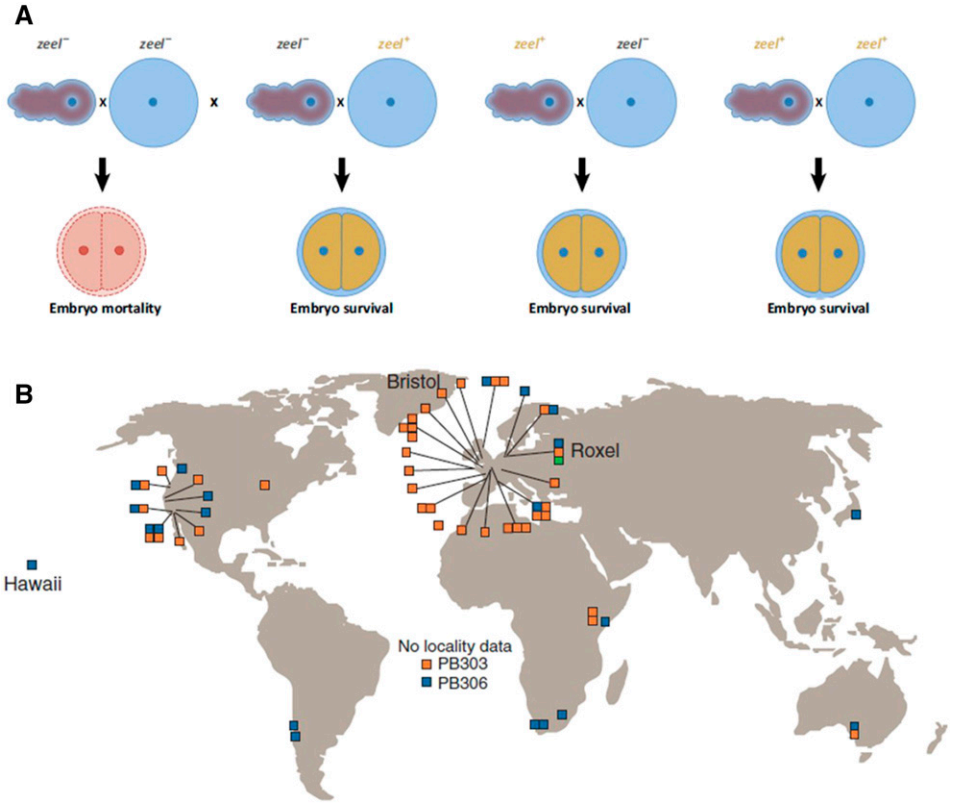

Figure 15 Natural genetic polymorphism for mating incompatibility. (A) Overview of the compatibilities defined by presence of the paternally expressed toxin PEEL-1 in the sperm (indicated by purple color) and rescue through zygotic expression of the antidote ZEEL-1 (indicated in yellow), which may be obtained through the father and/or mother genome. Absence of PEEL-1 (not shown) always results in zygote survival. (B) Worldwide distribution of the two compatibility types defined through activity (blue) or inactivity (orange) of the two genes. In one case (green dot), *zeel-1* is active, but there is not paternal killing through *peel-1*. (A) From Petersen *et al.* (2015a) with permission from Elsevier and (B) from Seidel *et al.* (2008), reprinted with permission from AAAS.

selection and other forms of balancing selection can produce variation locally and possibly globally. These types of selective dynamics are likely associated with host–parasite coevolutionary interactions (Woolhouse *et al.* 2002; Schulenburg *et al.* 2009) and may also result from other types of interaction, especially if antagonistic or unpredictable (Brockhurst *et al.* 2014). Natural genetic polymorphisms and the functional characterization of the underlying loci may thus point to important biotic interactions.

In *C. elegans*, such natural polymorphisms have been studied for a variety of traits. As expected, phenotypic polymorphisms were found for the interaction with different microbes, including microsporidian parasites (see *Microsporidia*; Figure 8C; Balla *et al.* 2015), pathogenic bacteria such as *P. aeruginosa* (*P. aeruginosa as a likely natural pathogen*; Styer *et al.* 2008; Reddy *et al.* 2009; Chang *et al.* 2011; Andersen *et al.* 2012), *S. marcescens* (*S. marcescens as a likely natural pathogen*; Glater *et al.* 2014), *B. thuringiensis* (*B. thuringiensis as a likely natural pathogen*; Volkers *et al.* 2013; Nakad *et al.* 2016), and also food bacteria (Bendesky *et al.* 2011; Volkers *et al.* 2013). Importantly, the genetic dissection of such polymorphisms can lead to the discovery of novel molecular mechanisms, as demonstrated and explained above for the interaction with the Orsay virus (*e.g.*, discovery of the *drh-1* resistance gene; *Viruses*; Ashe *et al.* 2013). It may also indicate a yet unrecognized interaction with a pathogen. The study by Ghosh *et al.* (2012) identified a long-term polymorphism in the chloride channel subunit gene *glc-1* that explains resistance to the antihelminthic avermectin. Such antihelminthic compounds are produced by bacteria of the genus *Streptomyces* (Burg *et al.* 1979), which may coexist with *C. elegans* in nature. Thus, the observed balancing selection on this gene may result from repeated exposure of the nematode to avermectin (*e.g.*, applied in agriculture) or from an ongoing interaction between *C. elegans* and *Streptomyces* pathogens. Another polymorphism was detected toward a different *Streptomyces* antihelminthic compound in Andersen *et al.* (2012).

Other genetic polymorphisms could be linked to *C. elegans* reproductive behavior and may emphasize the particular selective dynamics associated with intersexual interactions. Intriguingly, a natural polymorphism was found at two interacting genes, *peel-1* and *zeel-1*, which determine mating compatibility of *C. elegans* in the wild (Seidel *et al.* 2008, 2011). These two genes define a toxin–antitoxin system at the same genetic locus. The gene *peel-1* encodes the toxin, a transmembrane protein expressed in sperm and delivered to the embryo, which it kills at the twofold stage in the absence of an antidote (Figure 15). The gene *zeel-1* is transiently expressed in the embryo. Its transmembrane domain can suppress the killing effect of PEEL-1 (Seidel *et al.* 2011). The polymorphism concerns the absence or presence of these two linked genes. It is yet unclear which selective dynamics determine maintenance of the polymorphism in nature. Another case is the variation in genes involved in copulatory plug formation, such as *plg-1* (Palopoli *et al.* 2008) and *plep-1* (Noble *et al.* 2015), possibly as a consequence of male–male competition in this predominantly selfing species.

Yet other natural polymorphisms were linked to pheromone perception (Greene *et al.* 2016a,b) and, thus, possibly to competition within the species *C. elegans*, and also between *C. elegans* and closely related species (see also above *Intraspecific competition and its resolution through C. elegans pheromones*). Interestingly, the characterized QTL consisted of polymorphic G protein-coupled chemoreceptors (Greene *et al.* 2016a,b). These chemoreceptors are likely to perceive and subsequently mediate the response to environmental signals, for example from conspecifics (above) or also pathogens (Zugasti *et al.* 2014). They are part of the largest gene superfamily in *C. elegans*, containing >1300 genes (Thomas and Robertson 2008). Several chemoreceptor families are subject to adaptive sequence evolution (Thomas *et al.* 2005) and substantial copy number variations (Volkers *et al.* 2013). These polymorphisms may be a consequence of past natural selection on these genes as central mediators of the interaction with the environment.

Perspectives

We are only scratching the surface of the complex biotic interactions between *C. elegans* and other organisms in nature. Since these interactions have shaped *C. elegans* along its evolutionary history, they are pivotal for an in-depth characterization of *C. elegans* biology and for understanding the evolutionary maintenance of its many genes. Thus, we are in dire need of additional studies on the natural ecology of *C. elegans*. Especially neglected fields are the interactions with enemies, competitors, and also hosts and vectors. Is *C. elegans* really exclusively free-living or has it evolved a semiparasitic and/or necromenic lifestyle, at least temporarily, *e.g.*, as indicated with slugs (see above)? Do the well-characterized phenotypes and molecular processes in development, neurobiology, or cell biology, all intensively studied under artificial laboratory conditions, behave similarly when examined under more natural conditions? And are such processes and phenotypes then still influenced by mutations in the same set of genes? How do we best analyze such environmental contributions under laboratory conditions? Are the natural conditions best approximated with the help of two-dimensional solid agar media, three-dimensional liquid cultures, or some type of viscous medium?

The microbial environment should be of central importance, because it is highly dynamic in nature and is a required interaction of *C. elegans* with its environment through feeding. The recent studies provided a glimpse at the possible diversity of microbial interactors, ranging from possible mutualists to commensals and pathogens. Yet, to date, it is not entirely clear to what extent *C. elegans* is associated with specific taxa over longer time periods, allowing for repeated reciprocal coevolutionary adaptations. Alternatively, the animal may be inhabited by specific microbes only for short time periods, whereby the presence of these short-term visitors may be a consequence of selection by *C. elegans*, or the colonization ability of the microbes, or a combination thereof. Moreover, it is not clear which of the taxa only act as food or indeed represent commensals or even mutualists. Are some bacteria both food and also mutualists, because *C. elegans* carries them along to new habitats, where they process nutrients available in rotten fruits and then allow the worms to use them by feeding on them? Are pathogens exclusively detrimental or can they also serve as food or commensals under specific conditions? What is the influence of the large variety of microbes and their combination on nematode biology, particularly regarding dauer entry and exit? What is the diversity of viruses and phages associated with the worm's microbiome, and what are their effect on *C. elegans* life history? Beyond the example of the viruses, is there a strong specificity in the biotic interactions of *C. elegans* compared to those of congeners such as *C. briggsae* and *C. remanei*, and among *C. elegans* genotypes? These are some of the many questions that need to be addressed in future research.

Intraspecific interactions can be highly antagonistic (*e.g.*, sexual conflict or competition for resources) and could have resulted in selection dynamics that have shaped *C. elegans*' biology, but how common are such competitive interactions among *C. elegans* genotypes in nature? Does the cooccurrence of different genotypes result in competitive interactions, for example mediated by the dauer pheromone? To what extent is this influenced by resource availability or the level of relatedness among genotypes, as predicted by theory? How common are sexual interactions and do these associate with antagonistic sexual conflict? Are these intraspecific interactions and conflicts mainly mediated by ascarosides or similar small molecules? What is the role of the many sperm-associated proteins of unknown function? These represent highly promising foci for future research programs on *C. elegans* ecology.

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