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A Model for Evolutionary Ecology of Disease: The Case for *Caenorhabditis* Nematodes and Their Natural Parasites

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Abstract: Many of the outstanding questions in disease ecology and evolution call for combining observation of natural host–parasite populations with experimental dissection of interactions in the field and the laboratory. The "rewilding" of model systems holds great promise for this endeavor. Here, we highlight the potential for development of the nematode *Caenorhabditis elegans* and its close relatives as a model for the study of disease ecology and evolution. This powerful laboratory model was disassociated from its natural habitat in the 1960s. Today, studies are uncovering that lost natural history, with several natural parasites described since 2008. Studies of these natural *Caenorhabditis*–parasite interactions can reap the benefits of the vast array of experimental and genetic tools developed for this laboratory model. In this review, we introduce the natural parasites of *C. elegans* characterized thus far and discuss resources available to study them, including experimental (co)evolution, cryopreservation, behavioral assays, and genomic tools. Throughout, we present avenues of research that are interesting and feasible to address with caenorhabditid nematodes and their natural parasites, ranging from the maintenance of outcrossing to the community dynamics of host-associated microbes. In combining natural relevance with the experimental power of a laboratory supermodel, these fledgling host–parasite systems can take on fundamental questions in evolutionary ecology of disease.

Key words: bacteria, *Caenorhabditis*, coevolution, evolution and ecology of infectious disease, experimental evolution, fungi, host–parasite interactions, immunology, microbiome, microsporidia, virus.

The field of infectious disease evolution and ecology seeks to understand the forces driving the spread of parasites and the severity of host–parasite interactions. Significant progress in the past 40 years has brought to the forefront many open questions in the field. In Lively et al. (2014), several prominent researchers presented a sample of these outstanding questions: (i) Does host genetic diversity limit the spread of an infectious disease? (ii) Can selection by parasites maintain genetic diversity in host populations? (iii) What are the effects of environmental variation and community context on the interaction between a host and parasite? (iv) Is the one host–one parasite framework instructive for understanding the real-world interaction of multiple hosts and multiple parasites? (v) What is the effect of a host's microbiota on the evolution and ecology of a host–parasite system? These questions highlight the importance of studying disease at both the individual and population levels. The persistence of such questions indicates that studies aimed at addressing them are not easily done.

The nature of many systems used for disease research may contribute to the difficulty in linking individual and population level outcomes, particularly in a natural context. There presently exists a divide between tractable laboratory systems, with extensive genetic and experimental tools, and field systems, where ecological and evolutionary changes play out in complex, natural settings. In predominantly laboratory-based systems, host and even parasite genetics are often well characterized (e.g., laboratory mice and rodent malaria *Plasmodium chabaudi*; the model nematode *C. elegans* and various bacterial parasites; many bacteria and their coevolving phages). Researchers can readily manipulate experimental conditions to test the effect of specific factors (e.g., habitat structure: Boots et al., 2004; transmission mode: Stewart et al., 2005; resource level: Lopez-Pascua and Buckling, 2008) on replicate individuals and populations. The degree of control we can exercise in laboratory systems renders them experimentally powerful but highly artificial. This ultimately restricts the natural relevance of results drawn from purely laboratory-based host–parasite systems.

Contrast these laboratory systems with systems that are predominantly studied in the field (e.g., the freshwater snail *Potamopyrgus antipodarum* and its sterilizing trematode *Microphallus* sp., the Soay sheep and their many parasites, and the woodland star *Lithophragma* and *Greya politella* moths). These systems have a rich ecological context, and researchers extensively characterize population dynamics by replicating field studies across time and space (Hudson et al., 1998; Coltman et al., 1999; Jousimo et al., 2014). The natural context of field systems makes them of central importance to evolutionary ecologists. It also renders them complex, such that factors of interest are continually confounded: replicate individuals and populations may differ in many ways. The dichotomy between laboratory and field is acknowledged by Lively et al. (2014). The authors proposed that one approach to answering the outstanding questions of disease evolution and ecology is the development of "new study systems. . .where natural populations can be used in laboratory experiments and/or where experimental studies can be conducted in the wild" (Lively et al., 2014: p. S5).

Bridging the divide between laboratory and field, between control and complexity, allows us to reap the benefits of both. One solution is to bring relatively tractable field systems into the laboratory. Evolutionary biologists have successfully uncovered the genetic basis of many natural host–parasite interactions by examining families or clonal lineages in the laboratory (Alexander

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and Antonovics, 1995; Lively and Dybdahl, 2000; Thrall et al., 2012; Luijckx et al., 2013). An increasingly popular approach among disease ecologists is to dissect drivers of field dynamics using theory, experimental populations, and characterization of variation among individual hosts (Borer et al., 2007; Johnson et al., 2013; Rohr et al., 2015). Here, we advocate a complementary solution: returning natural context to laboratory-based model systems. An astounding array of experimental tools and genetic resources are available for model systems. Their principle weakness is their utter isolation from nature. Accordingly, we often have a poor understanding of their natural parasites (see next section). This problem is slowly being remedied for several model systems (Mus musculus: Abolins et al., 2011; Beura et al., 2016) (*Drosophila*, rev. in: Keebaugh and Schlenke, 2014) (bacteria and phage: Gomez and Buckling, 2011; Koskella et al., 2011), including for the model nematode *C. elegans*. In the following pages, we review the history of disease research in *C. elegans* and discuss its new potential as a model for evolution and ecology of disease.

Model Organisms: The Case of the Missing Parasites

It has been said that "Diseases are like the stars. The longer you look the more you see." (Anonymous: Antonovics et al., 2011). This adage was not written with model organisms in mind. For a host species in the Brassicales, the number of associated fungal parasites increases with study effort, measured as the number of citations in Google Scholar (Fig. 1). The model plant *Arabidopsis thaliana* clearly bucks this trend. It has far fewer reported parasite species than we would expect given the study effort devoted to this host. Study effort for *A. thaliana* (citation number = 523,000) is 308-fold greater than that for the false flax *Camelina microcarpa* (citation number = 1,700). Yet, the same number of fungal parasites ($n = 14$ species) is reported for the two relatives. The missing parasites of *A. thaliana* are emblematic of the separation of major model organisms from their natural context. Parasitic taxa do not accumulate when much of the study effort is devoted to individuals reared in climate-controlled growth chambers with continual application of pesticides and herbicides.

Caenorhabditis elegans has experienced a similar history of sterile laboratory captivity. Its success as a model organism has gone hand-in-hand with the reduction of its environment. Simplification of the environment and genetic background of *C. elegans* enabled characterization of the genetic and molecular basis of mutant phenotypes. A single inbred lineage of *C. elegans* can rapidly proliferate on lawns of a single bacterial food source, *Escherichia coli*. Case in point: much research relies on the inbred strain N2, which has been reared in petri dishes on lawns of a specific strain of *E. coli*, OP50, for most of the 60+ years since its isolation from mushroom compost in Bristol, England (Sterken et al., 2015). Populations can even proliferate in axenic liquid media (Samuel et al., 2014).

Not surprisingly, natural history and ecology have not been direct beneficiaries of scientific progress under *C. elegans*. It was only in 2008, nearly a half-century after the beginnings of *C. elegans* as a model system, that a parasite, the microsporidian *Nematocida parisii*, was described from a wild-caught individual (Troemel et al., 2008). This discovery is part of a greater effort to reconnect *C. elegans* with the decaying patches of organic matter that are now known to be its home outside the laboratory (rev. in Félix and Braendle, 2010; Cutter, 2015; Frézal and Félix, 2015). This effort holds great promise for the future of disease ecology and evolution.

Unnatural History

In spite of a lack of natural parasites, model organisms serve as powerful systems in which to study the immunological and molecular basis of infection. *Arabidopsis thaliana* is a model for studying plant parasites.

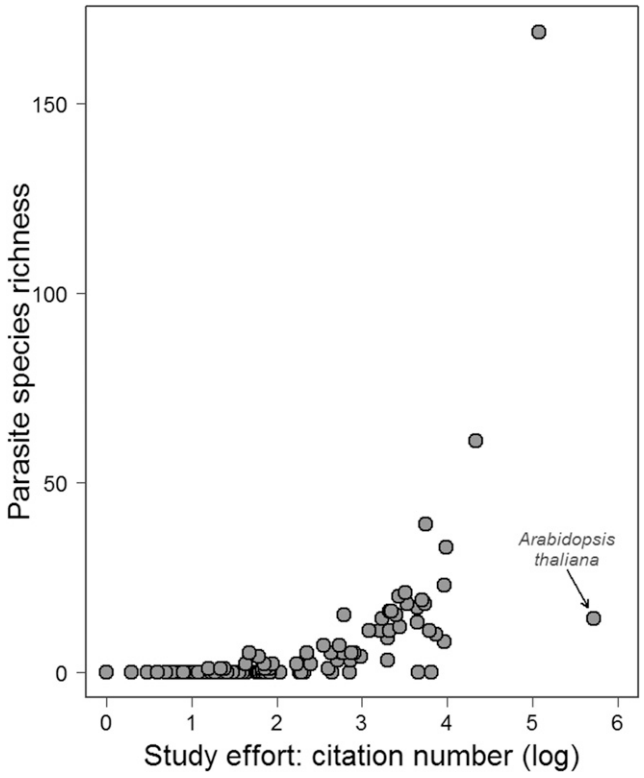

Fig. 1. Fungal parasite species richness as a function of study effort for hosts in the Brassicales. Host taxa were obtained from the Brassicales phylogeny in Beilstein et al. (2010, Fig. S1). Study effort is the number of citations obtained by copying and pasting a host's full name, in quotations, in Google Scholar. Parasite species richness is the number of fungal parasite species reported infecting a host in the USDA Fungus-Host Database (https://nt.ars-grin.gov/fungaldatabases/). There is a positive correlation between study effort (citation number, untransformed) and parasite species richness, with (Spearman rank correlation, $\rho = 0.772$, $P < 0.001$) and without ($\rho = 0.767$, $P < 0.001$) the uppermost point (*Brassica oleracea*) (tests exclude *Arabidopsis thaliana*).

Generalist crop parasites (e.g. *Pseudomonas* sp.) can infect *A. thaliana*, and mutant screens for increased susceptibility have identified components of plant immunity (reviewed in Dangl and Jones, 2001; Jones and Dangl, 2006; Piquerez et al., 2014). The *A. thaliana* genome also allowed a thorough cataloguing of parasite resistance (R) genes (Initiative, 2000). Much of our knowledge of the genetic basis of innate immunity is based on studies in *Drosophila melanogaster* (rev. in Hultmark, 1993; Hoffmann and Reichhart, 2002). Most famously, discovery of the Toll pathway in *D. melanogaster* catalyzed the characterization of mammalian Toll-like receptors (TLR), which gave new life to the study of innate immunity (Gay and Keith, 1991; Lemaitre et al., 1996; rev. in O'Neill et al., 2013). Today, this knowledge of *Drosophila* immunity facilitates the study of natural *Drosophila* parasites and natural transmission modes (rev. in Keebaugh and Schlenke, 2014). Laboratory mice are the mammalian immune model and the infection model for many parasites that infect humans, such as Ebola virus (Rasmussen et al., 2014) and *Myobacterium tuberculosis* (North, 1974; Lefford, 1975). Among many other things, mammalian TLR were functionally characterized in mice (Hoshino et al., 1999; Underhill et al., 1999; Hemmi et al., 2000; rev. in Medzhitov, 2001).

Caenorhabditis elegans is the youngest of these models. Research on infection and immunity in *C. elegans* began in 1999, when Tan et al. (1999a) showed that a single clinical isolate of *Pseudomonas aeruginosa* (PA14) infects host species that are separated from one another by many generations of evolution: laboratory mice, *A. thaliana*, and *C. elegans*. Follow-up studies made use of the tractability of *C. elegans* to identify the *P. aeruginosa* genes required for infection, many of which overlapped with those identified as necessary for infection of mice and *A. thaliana* (Mahajan-Miklos et al., 1999; Tan et al., 1999b). This work was particularly exciting because of the ease of genetic manipulation of both the host and the parasite. This group of studies suggested that the interactions of *C. elegans* with parasites that infect distant hosts (e.g. humans, crops) might provide insight into general mechanisms of infection and conserved components of host immunity.

In pursuit of this goal, researchers have exposed *C. elegans* to a variety of unnatural parasites since 1999, among them the agent of bubonic plague *Yersinia pestis* (Darby et al., 2002), the plant parasite *Agrobacterium tumefaciens* (Couillault and Ewbank, 2002), and the opportunistic human parasites *Staphylococcus aureus* (Garsin et al., 2001; Sifri et al., 2003), *Serratia marcescens* (Mallo et al., 2002), and *Cryptococcus neoformans* (Mylonakis et al., 2002). From here on out, we discuss microbial taxa that differ substantially from one another in their relationship with *C. elegans*: some kill whereas others merely cause constipation; some cannot reproduce outside of a host (obligate parasites) whereas others are opportunistic associates. We will refer to all of these diverse taxa as parasites, drawing on the general definition of parasitism as living on or in a host and causing some degree of harm (Zelmer, 1998; Lafferty and Kuris, 2002; for further thoughts on definitions, refer to Poulin, 2007; Preface, Ch. 2 of Schmid-Hempel, 2011).

We use the term "unnatural" to designate parasite species or strains that are not known to infect *C. elegans* in nature; the pairings with *C. elegans* are constructed in the laboratory. Most of these unnatural parasites are bacterial (Fig. 2A). Their original hosts derive from a wide swath of eukaryotes, including mammals and plants. The majority (more than 60%) are of interest because they infect humans, often opportunistically. Only 16% naturally infect invertebrates (estimate based on a modification of Table 1 in Sifri et al., 2005). These parasites reduce *C. elegans* fitness via different mechanisms, including toxin-mediated killing (e.g., *Bacillus thuringiensis*) (Griffitts et al., 2001), biofilm formation (*Y. pestis*) (Darby et al., 2002), and proliferation in the intestine (*Salmonella enterica*) (Aballay et al., 2000). They vary in virulence: the longevity of infected nematodes is as short as a few hours (e.g., *Enterococcus faecium* and *P. aeruginosa*'s "fast-killing," both toxin mediated) (Tan et al., 1999a; Moy et al., 2004) and as long as a few days (e.g., *S. enterica* and *P. aeruginosa*'s "slow-killing") (Tan et al., 1999a; Aballay et al., 2000). They almost exclusively afflict the intestine, with the exception of *Yersinia* species, which form a biofilm around the mouth opening and thereby obstruct feeding (Darby et al.,

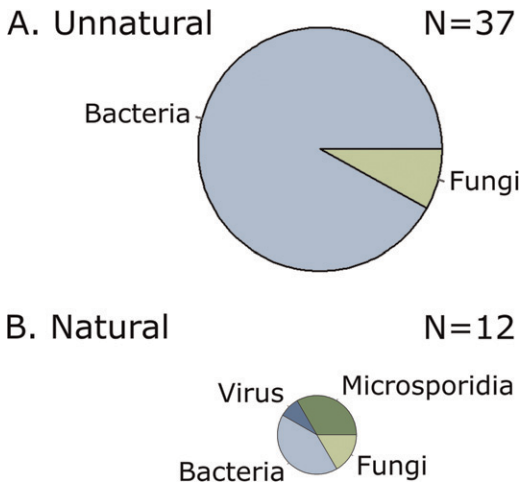

Fig. 2. Natural parasites of *Caenorhabditis elegans*. A. The taxonomic distribution of unnatural parasites of *C. elegans*. These are parasites paired with *C. elegans* in the laboratory that are not presently known to infect *C. elegans* in the wild. The parasites are identified in Sifri et al. (2005, Table 1). Unlike in Sifri et al. (2005), we did not include *Microbacterium nematophilum* and *Drechmeria coniospora* here, because we treated these as natural parasites. See our Table 1 for more details. B. The taxonomic distribution of known natural parasites of *C. elegans*. These are parasites known, or strongly suspected, to facultatively or obligately associate with *C. elegans* in the wild and depress fitness. The parasites are listed in Table 1, with descriptive details and citations. These are only the parasites that have been reported in sufficient detail to characterize the nature of their association with *C. elegans*. Further study will certainly uncover more species. The sizes of the pie charts are scaled to the number of total taxa.

2002). Many reviews cover this diversity in more detail (Aballay and Ausubel, 2002; Couillault and Ewbank, 2002; Ewbank, 2002; Sifri et al., 2005; Gravato-Nobre and Hodgkin, 2011).

Development of *C. elegans* as a Model Host

Much of the appeal of *C. elegans* as a system stems from the breadth and depth of tools available to researchers. *Caenorhabditis elegans* biology and life history is conducive to studying both populations and individuals within populations. *Caenorhabditis elegans* has a short generation time and can be maintained at large population sizes with relative ease. Therefore, multiple populations can be studied concurrently. Extensive microscopy tools and the ease of genome sequencing permit comparisons between populations, and the ability to store live *C. elegans* in the freezer allows comparison between time points. Individual phenotypes and genotypes can also be readily studied. Targeted mutagenesis and QTL mapping is a common practice in *C. elegans*, which is aided by its androdioecious mating system. Furthermore, a wide range of behavioral and life history assays exist to test individual genotypes and phenotypes. With these tools in hand, researchers can measure the fitness of specific alleles or genotypes by tracking changes in their frequencies within and between populations in real time. We highlight these experimental resources to emphasize the potential of *C. elegans* to serve as a powerful model for disease ecology and evolution. The following paragraphs discuss some of these *C. elegans* tools in detail.

High-throughput genetic screens define *C. elegans* research. In forward genetic screens, mutant individuals with increased or decreased survival after parasite exposure are used to identify the alleles underlying susceptibility (Marroquin et al., 2000; Kim et al., 2002). Surveys of gene expression after parasite exposure similarly reveal candidate immune genes (Mallo et al., 2002; O'Rourke et al., 2006; Shapira et al., 2006; Troemel et al., 2006). Reverse genetic screens can test the function of candidate genes or of *C. elegans* homologues that are known to play a role in immunity in other organisms (Aballay and Ausubel, 2001; Pujol et al., 2001). Preexisting mutants with described phenotypes enable the testing of specific hypotheses (O'Quinn et al., 2001). For example, several feeding mutants (e.g., *esp-1*, *eat-13*, *phm-2*, etc.) fail to lyse bacteria as they pass through the grinder of the host's pharynx. With these mutants, one can perform a direct test of the hypothesis that bacterial lysing by the grinder contributes to host resistance (as in Labrousse et al., 2000; Kim et al., 2002; Smith et al., 2002). Many bacterial parasites are amenable to genetic manipulation, which raises the possibility of studying interspecific genetic interactions (Mahajan-Miklos et al., 1999; Tan et al., 1999a, 1999b; Pradel et al., 2007).

From the combined power of these methods, a map of the immune system of *C. elegans*, and its genetic basis, has taken shape (several reviews: Alegado et al., 2003; Kurz and Ewbank, 2003; Millet and Ewbank, 2004; Schulenburg et al., 2004; Gravato-Nobre and Hodgkin, 2005; Ermolaeva and Schumacher, 2014). Physical barriers hinder parasite establishment. The tough, collagen-based cuticle covers all but a few openings to the worm body. Intestinal access via the mouth is guarded by the muscular pharynx and its tooth-like, chitinous grinder (Labrousse et al., 2000; Kim et al., 2002; Smith et al., 2002). Intestinal peristalsis and defecation, approximately every 45 sec, can limit the residence time of parasites in the gut (Rae et al., 2012).

Upon entry, invaders meet the molecular defenses of *C. elegans*. Gut bacteria encounter secreted proteins or peptides with antimicrobial activity (e.g., lysozymes, lipases: Mallo et al., 2002) that either have a general role in digestion or are upregulated specifically in response to infection. Four signaling pathways have well-described roles in defense against unnatural parasites: the TGF-β, p38 MAPK (PMK-1), programmed cell death, and insulin-like receptor (DAF-2) pathways. Certain of these pathways (PMK-1 and DAF-2) also contribute to stress resistance, suggesting a shared basis for *C. elegans* immune defense and resistance to environmental stressors, such as heat shock and oxidative stress (rev. in Schulenburg et al., 2004).

The mechanism through which *C. elegans* recognizes infectious agents remains uncharacterized. Ubiquitin ligase adapters are candidates for parasite recognition receptors (Thomas, 2006; Bakowski et al., 2014a; Szumowski and Troemel, 2015), but their role has yet to be fully characterized. There is growing evidence for the hypothesis that parasite recognition occurs primarily through detection of general damage to host cells and tissues (Dunbar et al., 2012; McEwan et al., 2012) (rev. in Balla and Troemel, 2013). We use the term "damage" as in Matzinger's Danger Model, where, instead of specifically recognizing foreign invaders, hosts first detect "danger" signals released by stressed and/or injured cells (Matzinger, 1994, 2002). Alternatively, the focus of immune research on unnatural, bacterial parasites may limit the present scope of our knowledge.

Sydney Brenner saw in *C. elegans* an ideal system for the study of the genetics and development of the nervous system and behavior. Much early research thus focused on behavioral mutants, specifically in movement (Brenner, 1974). Likely as a result of this history, there exist many tools for investigating the behavioral response of *C. elegans* to parasites. In fact, a behavioral study might be considered the first work on a parasitic interaction with *C. elegans*. Andrew and Nicholas (1976) reported that *C. elegans* was attracted to several bacterial species but actively avoided the bacterium *Bacillus megatherium*. They then demonstrated that *B. megatherium* was highly virulent, killing nematodes in ~15 min.

Today, researchers leverage similar assays to study the underlying genetics of parasite avoidance behavior. The response to the virulent parasite *S. marcescens* is particularly well characterized. *Caenorhabditis elegans* learn to

avoid lawns of *S. marcescens* after exposure (Zhang et al., 2005). Nematodes respond specifically to serrawettin (Pradel et al., 2007), an *S. marcescens* growth factor. Mutant hosts, defective in serrawettin recognition, revealed the role of two chemosensory neurons (Pradel et al., 2007) and a TLR gene (*tol-1*) (Pujol et al., 2001). Glater et al. (2014) demonstrated that natural genetic variation in the *C. elegans* response to *S. marcescens* exists and arises from the interaction of multiple loci. Penley and Morran (2017) found that *C. elegans* lineages evolved increased avoidance of *S. marcescens* during 30 generations of experimental selection for host survival after parasite exposure. For *C. elegans*, life in the wild hinges on finding bacterial food sources, so variation in bacterial discrimination likely has natural relevance. Such behavioral assays can be used in combination with feeding, mobility, and chemosensory mutants to dissect the contribution of parasite exposure to disease resistance. Ultimately, natural variation in these traits may serve to connect laboratory findings to wild interactions of *C. elegans* and its parasites.

Experimental evolution and coevolution offer yet another powerful approach for studying host–parasite interactions with *C. elegans*. Here, unnatural parasites offer a real advantage—coevolutionary interactions can be built from scratch in the laboratory, without the confounds of a prior coevolutionary history. *Caenorhabditis elegans* is well equipped for experimental (co)evolution studies: it has a short generation time, large populations can be maintained in confined spaces, and larvae survive cryogenic preservation, allowing for long-term archiving of populations. Studies have taken on major evolutionary questions with this tool in hand: the evolution of sex (Morran et al., 2009, 2011; Slowinski et al., 2016), the evolution of virulence (Gibson et al., 2015; Masri et al., 2015), and the evolution of defensive mutualisms (King et al., 2016). Box 1 discusses a few foundational studies in more detail. These studies suggest possible scenarios for the evolution of natural *C. elegans* populations in response to natural parasites.

Natural History

Of course, no number of experimental tools can make *C. elegans* a model for natural host–parasite interactions without the existence of actual natural host–parasite interactions. In the early 2000s, we still knew very little about the natural history of *C. elegans*, including its natural habitats. Extensive searching revealed that populations proliferate on decomposing plant material, including rotting fruits and stems. Here, nematodes find rich blooms of bacteria (Barrière and Félix, 2005; Sivasundar and Hey, 2005; Kiontke et al., 2011; Petersen et al., 2014). They colonize these resource patches and reach population sizes of up to 10,000 individuals. As populations become dense and their bacterial prey dwindle, an increasing number of dauer larvae appear. Starvation, heat, density, and other cues can trigger very young larvae (L1) to develop as dauers, instead of immediately developing to reproductive maturity. During this diapause stage, dauers cease feeding and seek out new resource patches. They may expedite this process by attaching onto an invertebrate host (e.g., snail and isopod) (i.e., phoresy). If a dauer larvae successfully finds a new bacterial bloom, most likely a rare event, then rapid population expansion begins anew (Félix and Duveau, 2012; Frézal and Félix, 2015; Petersen et al., 2015; Schulenburg and Felix, 2017).

The search for caenorhabditid nematodes in nature also led to the discovery of parasites that infect them (Table 1). The microsporidian *N. parisii* was the first parasite of *C. elegans* to be described (Troemel et al., 2008). This parasite diverges substantially from the prior unnatural parasites of *C. elegans*. Extracellular bacteria were the primary focus of research on unnatural parasites. Indeed, only one of many unnatural parasites tested has ever been able to penetrate the intestinal cells via the gut (*Salmonella typhimurium*—Jia et al., 2009), leading to the hypothesis that *C. elegans* intestinal cells are exceptionally well defended. On the contrary, the fungus-like *N. parisii* is a horizontally transmitted, obligate parasite that replicates exclusively within intestinal cells. *Caenorhabditis elegans* ingests the spores of *N. parisii* while feeding. A spore germinates in the intestine, rapidly discharging its polar tube and penetrating the membrane of an intestinal cell. The spore inverts its contents through this polar tube, directly into the host cytoplasm. The parasite then grows and divides (Balla et al., 2016), eventually producing more transmissible spores. These exit the cell and return to the environment via the intestine (Estes et al., 2011; Szumowski et al., 2014, 2016). Infection reduces host survival and offspring production, particularly when hosts are exposed as young larvae (L1). Host strains differ in survival and parasite load after infection, indicating genetic variation for resistance and/or tolerance. The genetic basis is somewhat complex: at least four loci contribute to genetic variation in resistance between two divergent laboratory strains, N2 and CB4856 (HW) (Balla et al., 2015).

Microsporidia appear to be the most common natural parasites of caenorhabditis. Additional sampling has isolated multiple strains of *N. parisii*, as well as new microsporidian species that infect *C. elegans* and its relatives. They vary in their host range (Zhang et al., 2016), virulence (Balla et al., 2016), and tissue tropism (Luallen et al., 2016). *Nematocida displodere* primarily replicates in the muscle and epidermis, producing large numbers of spores that are trapped within the host body. The host eventually bursts open at the vulva (Luallen et al., 2016). Of the natural parasites identified thus far, *N. parisii* and its relatives are the most intensively studied and best understood. They are now the model for intracellular infection and immunity in *C. elegans* (Troemel et al., 2008; Estes et al., 2011; Bakowski et al., 2014a; Balla et al., 2015, 2016; Botts et al., 2016; Reinke et al., 2017), and several

Box 1. Experimental (co)evolution with *Caenorhabditis elegans*.

The following studies illustrate the potential for experimental coevolution to answer fundamental questions in evolutionary biology and inform host–parasite interactions in the wild.

Maintenance of sex

Outcrossing, or biparental sex is a less-efficient reproductive strategy than selfing or asexual cloning (Maynard Smith, 1971; Williams, 1971; Maynard Smith, 1978). Yet, outcrossing is ubiquitous in nature. Thus arises the paradox of sex. *Caenorhabditis elegans* hermaphrodites predominantly self in the laboratory, but they can outcross with rare males (Chasnov and Chow, 2002; Stewart and Phillips, 2002). Morran et al. (2009, 2011) leveraged this mixed mating strategy to test a fundamental prediction of the Red Queen hypothesis: selection imposed by coevolving parasites favors outcrossing over self-fertilization. The Red Queen argues that coevolving parasites confer a selective advantage on outcrossed offspring, thus maintaining sex (Jaenike, 1978; Hamilton, 1980; Bell, 1982; Hamilton et al., 1990). Initial work showed that the frequency of *C. elegans* males increased in populations as they adapted to a nonevolving population of the bacterial parasite *Serratia marcescens*. Male frequency then declined, suggesting that outcrossing was only transiently favored during the brief period of adaptation (Morran et al., 2009). In a later study, Morran et al. (2011) applied experimental coevolution and demonstrated that male frequency increased, and remained at high levels, only when host populations were passaged with coevolving populations of *S. marcescens*. Slowinksi et al. (2016) then demonstrated that coevolving parasites can maintain obligately outcrossing host populations by preventing the invasion of hosts capable of self-fertilization. Thus, in support of the Red Queen, antagonistic coevolution can maintain outcrossing in *C. elegans*. These results raise the question: Why are males so rare in nature? Males are typically observed to be <1% of a sample, although experimental coevolution produced male frequencies close to 50%. One testable hypothesis is that *C. elegans* can escape coevolving parasites in the wild via dispersal in the dauer life stage (Ladle et al., 1993; Wilson and Sherman, 2010; Wilson and Sherman, 2013).

Parasite adaptation and virulence

The power of experimental coevolution lies in comparing treatments where both partners can evolve and reciprocally adapt against treatments in which one of the partners is held static, preventing coevolution (Brockhurst and Koskella, 2013). Masri et al. (2015) showed how this approach reveals the process of parasite adaptation. They selected for increased infectivity and virulence of the parasite *Bacillus thuringiensis* against populations of *C. elegans* that were evolving simultaneously or held static. Virulence remained high when coevolution was possible. In contrast, when host populations were held static, most of the parasite populations lost killing ability and were deemed extinct. The authors also included a control treatment in which *B. thuringiensis* was maintained in a free-living state. Virulence against *C. elegans* declined steeply in this control treatment. Selection on free-living growth apparently favored biofilm production, which may trade-off against within-host virulence. Consistent with this idea, biofilm production declined in potentially coevolving parasite populations. Overall, parasite populations diverged significantly from the ancestor and differed substantially between treatments after only 12 generations of selection. Potentially coevolved parasite populations were dominated by a virulent genotype associated with multiple nematocidal toxin genes. This genotype was nearly absent from control parasite populations and less frequent in populations selected against a static host. In the wild, *C. elegans* encounters bacteria that likely proliferate in both a free-living and host-associated state (Félix and Duveau, 2012; Samuel et al., 2016). Masri et al. (2015) outlines the process of bacterial adaptation to the host and the trade-offs inherent to that process. Do intermittent interactions with nematodes exert selection on bacteria in nature? Can trade-offs between free-living and within-host growth maintain polymorphism in bacterial populations?

Mutualism–parasitism continuum and the evolution of virulence

Host–microbe interactions range from parasitism to mutualism. Does coevolution contribute to shifts along this gradient? Directly answering this question requires an artificial system where selection can be carefully directed. Accordingly, Gibson et al. (2015) selected for a less antagonistic interaction between the laboratory associates *C. elegans* and *S. marcescens*. They passaged only hosts and/or parasites that formed a sustained association, one in which an infecting parasite was maintained in the host and the host survived long enough to reproduce. This selection was possible because the bright red of *S. marcescens* is clearly visible in transparent *C. elegans*. With different passaging schemes, the authors selected for reduced antagonism with and without the possibility of coevolution. Comparisons of infected host fecundity at generation 20 revealed that reduced antagonism evolved only when coevolution was possible. Selection for reduced antagonism on the host alone or the parasite alone did not lead to any shift in the nature of the host–parasite interaction. Within potentially coevolving host and parasite lines, Gibson et al. (2015) found local adaptation: reduced antagonism was only evident between sympatric (i.e., potentially coevolving) pairs. Thus, the degree of antagonism in this host–parasite interaction hinged on the genetic interaction of host and parasite. These findings underpin the significance of coevolutionary history to the evolution of species interactions. Do natural populations of *C. elegans* hosts coevolve with their local parasites? How do these interactions shape the evolution of virulence?

genomes are available (Cuomo et al., 2012; Bakowski et al., 2014b). Nonetheless, there remain many basic questions about the evolutionary ecology of this interaction and much potential for it to develop as a natural disease system.

Next came the Orsay virus, an RNA virus that is distantly related to nodaviruses (Felix et al., 2011). Like the microsporidian parasites, these are transmitted horizontally (Felix et al., 2011) and replicate predominantly in intestinal cells (Franz et al., 2014). The closely related LeBlanc and Santeuil viruses were isolated from wild-caught *Caenorhabditis briggsae* (Felix et al., 2011; Franz et al., 2012), which is often found in sympatry with *C. elegans* (Félix and Duveau, 2012). These viruses show host species specificity: the Orsay virus cannot infect *C. briggsae*, nor can the Santeuil virus infect *C. elegans* (Felix et al., 2011). Before the discovery of these viruses, researchers hypothesized that viruses could not replicate in *C. elegans* cells, perhaps because of its strong RNAi response (rev. in Gravato-Nobre and Hodgkin, 2005, 2011). In the laboratory, replication of unnatural viruses was obtained only under highly artificial conditions (Flock house virus—Lu et al., 2005) (vesicular stomatitis virus—Schott et al., 2005; Wilkins et al., 2005). Although the Orsay virus reveals that *C. elegans* is not immune to viral

TABLE 1. Natural parasites of *Caenorhabditis elegans*. The list comprises parasites that are confirmed or strongly suspected to infect *C. elegans* and related hosts in the wild. Parasites are lethal to their hosts unless otherwise indicated.

Species	Infection site	Obligate?	Phylogenetic context	Citation
Microsporidia				
Nematocida parisii	Intestinal cells	Yes	Zhang et al. (2016) describe many species of *Nematocida* infecting rhabditid nematodes. They also describe two new genera of microsporidia infecting nematodes of the genus *Oscheius*.	Troemel et al. (2008)
Nematocida ausubeli	Intestinal cells	Yes		Troemel et al. (2008), Zhang et al. (2016)
Nematocida ironsii	Intestinal cells	Yes		Reinke et al. (2017)
Nematocida displodere	Systemic: primarily muscle and epidermis	Yes		Luallen et al. (2016)
Viruses				
Orsay virus	Intestinal cells	Yes	*Caenorhabditis briggsae* is infected by close relatives, the LeBlanc and Santeuil viruses (Felix et al., 2011; Franz et al., 2012, 2014). All these viruses are distantly related to nodaviruses.	Felix et al. (2011)
Fungi				
Harposporium sp.	Systemic: nematophagous members of the genus establish in the intestine or on the cuticle, then produce hyphae that invade the entire body	Likely: endoparasitic species can grow outside the host, but there is no evidence that they do so in nature	Species of *Harposporium* are found infecting nematodes of many genera, including *Acrobeles*, *Panagrellus*, *Bunonema*, and *Aphelenchoices* (Esser and El-Gholl, 1992).	Félix and Duveau (2012)
Drechmeria coniospora	Systemic: adheres to the cuticle, frequently around the mouth, then hyphae invade the entire body	Yes	A model fungal parasite of *C. elegans*. *Drechmeria coniospora* was identified as a natural parasite after it was found infecting *C. briggsae* in the wild (Félix and Duveau, 2012). It can infect a broad range of nematode species (Jansson and Nordbring-Hertz, 1983), suggesting that it likely infects *C. elegans* naturally.	Jansson et al. (1985), Jansson (1994), Félix and Duveau (2012)
Bacteria				
Leucobacter musarum japonicus[a]	Rectal, postanal	Likely facultative[b]	This species is very similar to a related coryneform bacteria, *Microbacterium nematophilum*, which infects laboratory cultures of *C. elegans* (Hodgkin et al., 2000). *Leucobacter musarum* subsp. *musarum* and a related species (*Leucobacter celer*) were found naturally infecting an unidentified *Caenorhabditis* sp. (Hodgkin et al., 2013; Clark and Hodgkin, 2015).	Hodgkin et al. (2013)
Elizabethkingia sp.[c]	Systemic: dissolves nematode cuticle	Likely facultative[b]		Félix and Duveau (2012)
Chryseobacterium sp.[a,b]	Not described	Likely facultative[b]	This genus is closely related to *Elizabethkingia* (Bernardet et al., 2006)	Samuel et al. (2016)
Serratia sp.[c]	Not described	Likely facultative[b]	*Serratia* species are facultative parasites of humans, plants, and invertebrates (corals, nematodes, and insects) and occasionally mutualists or various hosts (plants, nematodes, and aphids) (Petersen and Tisa, 2013). *Serratia marcescens* infection of *C. elegans* is commonly studied in the laboratory, al though this species is not known to cause infection in the wild.	Samuel et al. (2016)

(*Continued*)

TABLE 1. Continued.

Species	Infection site	Obligate?	Phylogenetic context	Citation
Pseudomonas sp.[c]	Not described	Likely facultative[b]	*Pseudomonas* species are widespread in the environment and cause infection in a diversity of hosts, including plants and humans. Particular strains of *P. aeruginosa* infect *C. elegans* in the laboratory.	Samuel et al. (2016)

[a] Nonlethal. Slows development rate.
[b] Unknown, but other members of genus are commonly found free-living in the environment.
[c] Reported as the most detrimental to *C. elegans* among the bacterial associates observed in collections of Félix and Duveau (2012) and Samuel et al. (2016). Thus, this is not an exhaustive list of the bacterial parasites of *C. elegans*.

infection, RNAi does in fact contribute to *C. elegans* resistance to this natural virus. Genetic variation in resistance may stem in part from variation in the robustness of the RNAi response of different host lineages (Felix et al., 2011). Sterken et al. (2014) also reported increased resistance in the offspring of parents exposed to Orsay virus. This phenomenon was absent in RNAi-defective mutants, suggesting that inherited RNAi confers transgenerational resistance. When infected intracellularly with virus or microsporidia, *C. elegans* hosts upregulate an overlapping set of loci. The resulting gene expression patterns are quite distinct from those associated with unnatural, extracellular infection (Bakowski et al., 2014a; Reddy et al., 2017).

Caenorhabditis elegans encounters fungi and bacteria in the wild as well. Jansson et al. (1985) and later Jansson (1994) described the laboratory infection of *C. elegans* with the fungus *Drechmeria coniospora*. The fungus adheres to the nematode's cuticle, then hyphae grow through the openings in the cuticle for the sensory neurons (Jansson and Nordbring-Hertz, 1983; Jansson et al., 1984). *Drechmeria coniospora* is a generalist parasite of nematodes (Jansson and Nordbring-Hertz, 1983; Jansson et al., 1985), but it was not known to naturally infect caenorhabditids until a report by Félix and Duveau (2012) of infection in *C. briggsae*. They also reported a similar infection with a fungus of the genus *Harposporium*.

Recent study of the natural bacterial associates of *C. elegans* focuses on the microbiome as a whole, rather than specifically on parasites (see Box 2 for a review of this work). This approach fits well with the natural ecology of *C. elegans*: it ingests a diversity of free-living bacteria that vary in their value as a food source and in their potential for parasitism. Among those bacteria that are parasitic, facultative parasites likely outnumber obligate ones. A few isolated bacteria stand out as likely parasites: strains of *Elizabethkingia* (Félix and Duveau, 2012), *Chryseobacterium*, *Serratia*, and *Pseudomonas* (Samuel et al., 2016) slow development and reduce survival of *C. elegans*. *Leucobacter musarum japonicus* colonizes the rectum of *C. elegans*, causing constipation and slowed development (Hodgkin et al., 2013; Clark and Hodgkin, 2015). This infection resembles that of the related *Microbacterium nematophilum*, which first contaminated *C. elegans* laboratory cultures in 1986. It causes the Dar phenotype (deformed anal region), which was initially thought to be a new mutant phenotype until *M. nematophilum* was described in 2000 (Hodgkin et al., 2000).

For caenorhabditids, the evolutionary ecology of disease remains very much in its infancy. The vast number of resources for nematode research will accelerate progress, as will the potential for large-scale field sampling. Within a few hours, thousands of nematodes might emerge from a small sample of rotting vegetation (Barrière and Félix, 2006; Félix and Duveau, 2012). The limited dispersal of *C. elegans* means that many sites and multiple spatial scales can be rapidly sampled with minimal travel. The same sites can be sampled through time (Barriere and Félix, 2007; Richaud et al., 2017), although the ephemeral nature of individual resources patches makes this challenging at the finest spatial scales. Thousands of field-sampled individuals can give rise to inbred or outbred lineages that can be archived at −80°C and revived later. A transparent host allows for direct visualization of internal parasites in many cases. Identification of host and parasite taxa may require molecular verification (host: Barrière and Félix, 2006). Many options also exist for reducing contamination (fungal, bacterial, etc.) of nematode field samples, which is widespread (Stiernagle, 2006). A wealth of information, protocols, and other resources are available online at sites such as WormBook and WormBase (for more, see Antoshechkin and Sternberg, 2007). Most importantly, the research carried out thus far indicates that disease is among the important obstacles faced by wild *C. elegans* and provides sufficient natural history to begin asking interesting questions.

Open Questions in Ecology and Evolution of Natural *C. elegans*–Parasite Interactions

Many persistent questions in disease ecology and evolution lie at the interface of ecology and genetics: Why do we see more parasites in some places versus others? How do environmental factors change the evolution of host populations in response to parasites? Do host-associated microbes underlie host resistance to parasites, and how might that shape the evolutionary trajectory of a host–parasite interaction? What limits the host range of parasites, and which changes underscore host shifts? These questions ultimately require a merging of disease ecology and

Box 2. The natural microbiome of *Caenorhabditis elegans*.

Nematodes are a stark reminder that most bilaterians are little more than tubes with genitalia. *Caenorhabditis elegans* is a 20-cell intestine, squeezed against a gonad, all sealed within a transparent cuticle. It seems natural to recruit *C. elegans* for study of the gut microbiome (Cabreiro and Gems, 2013). The gut microbiome appears impossibly complex at times. *Caenorhabditis elegans* makes it feasible to reduce and manage that complexity. Larvae are sterile at birth, and populations can develop in axenic culture. A simple sterilization protocol allows populations to be disassociated from their microbial communities and reassociated via feeding (Stiernagle, 2006). Transparency makes it possible to localize and image microbes within the gut using fluorescent tags (as in Wiles et al., 2016). Yet, with all these tools, there was no known natural microbiota to apply them to until very recently. In the laboratory, *C. elegans* is typically reared on a monoxenic culture of *Escherichia coli*. Other microbes are removed with a bleach wash, which eggs of *C. elegans* can survive (Stiernagle, 2006). The near absence of microbes in and on *C. elegans* cannot reflect the natural state of these animals, which live and feed on microbial blooms (Félix and Duveau, 2012; Frézal and Félix, 2015). A few recent studies have set out to address this striking gap in our ecological knowledge.

By constructing seminatural microcosms in the laboratory, Berg et al. (2016) showed that *C. elegans* does host a diverse community of bacteria when reared in a complex environment. The authors raised *C. elegans* on microbially rich soils. Sterile larvae acquired gut microbes, and the gut community was consistently distinct from and less diverse than the soil microbial community from which it was derived. Nematodes raised on distinct soil types with distinct communities hosted similar microbes at similar abundances. Thus, the host environment sorted the external microbial community, producing a characteristic *C. elegans* gut community. Dirksen et al. (2016) reported similar results from *C. elegans* sampled from the wild. The nematode gut community was distinct from that of the substrates from which populations were collected. Enriched families resembled those of Berg et al. (2016) (e.g., Enterbacteriaceae and Pseudomonadaceae). The communities of individual worms appeared more distinct from one another than in Berg et al. (2016). This may be due to a greater diversity of substrate types sampled (invertebrates as well as compost and rotting fruit) or to the genetic diversity of the field-collected hosts (Berg et al., 2016 used a single genotype).

Particular natural bacteria and bacterial communities foster *C. elegans* population growth (Dirksen et al., 2016; Samuel et al., 2016). Samuel et al. (2016) reared *C. elegans* populations on monoxenic cultures of 565 different bacterial isolates collected from rotting apples. They deemed 78% of these strains beneficial for population growth and 22% detrimental, based on reduced population growth and upregulation of stress- or pathogen-linked genes. These microcosm data were consistent with field patterns: large, proliferating populations of *C. elegans* were collected from rotten apples that were enriched in beneficial taxa, including Enterobacteriaceae, and lacking pathogenic taxa. Individual worms reached larger body sizes on experimental communities designed to resemble those field communities that supported proliferating populations vs. nonproliferating populations. This microbiome research has thus provided critical insight into a long-lost piece of *C. elegans* natural history: where populations are found and why.

Defensive mutualisms are an exciting avenue for *C. elegans* disease and microbiome research. King et al. (2016) used *C. elegans* as a vessel for studying the interaction between bacteria that can co-colonize the nematode gut. In just a few generations of experimental evolution, the mildly virulent *Enterococcus faecalis* rapidly evolved to defend *C. elegans* against the highly virulent co-colonizer, *Staphylococcus aureus*. The novel defensive microbe, *E. faecalis*, suppressed *S. aureus* growth in part through the evolution of increased superoxide production, resulting in increased host survival. Two subsequent studies explored coevolution between the two bacterial species (Ford et al., 2016a, 2016b). There are now several reports of antagonistic interactions between bacteria that mitigate infection and promote *C. elegans* longevity in the laboratory (Ikeda et al., 2007; Kim and Mylonakis, 2012) (rev. in Clark and Hodgkin, 2014). Recently, studies have investigated similar interactions using natural microbes (Montalvo-Katz et al., 2013; Samuel et al., 2016). For example, Dirksen et al. (2016) found that three strains of *Pseudomonas* isolated from nematode gut communities can reduce growth of various fungi and reduce mortality associated with *Drechmeria coniospora* infection.

epidemiology with evolution and genetics. Yet such multilateral approaches can be challenging in disease research. We find *C. elegans* and its natural parasites particularly promising in this respect: environmental variables, host genetics, and often even parasite genetics can all be surveyed in the field and manipulated in the laboratory. The relative simplicity of *C. elegans* and our deep knowledge of its biology render such endeavors tractable. Experimental evolution and epidemics in the laboratory can be matched against field patterns and models to test hypotheses. In addition, few natural disease systems have the genetic resources of *C. elegans*. With them, we can directly assess the complex intersection of genetics and the environment that lies at the heart of disease ecology and evolution.

Here, we highlight a few open questions in the ecology and evolution of nematode disease. These echo the themes of the five open questions that we highlighted in the Introduction (Lively et al., 2014) and demonstrate the utility of caenorhabditids for pioneering progress in disease ecology and evolution. In some cases, we discuss potential avenues for tackling these questions to model the power of hypothesis testing in a natural disease system that also has extensive genetic and experimental tools.

Do coevolving parasites maintain outcrossing?

The topic of mating system evolution continually arises with the caenorhabditids, and with nematodes more in general. Mating system varies throughout the entire phylum, from obligate outcrossing to selfing to parthenogenesis (Bell, 1982; Denver et al., 2011; Gibson and Fuentes, 2015). Within the caenorhabditids, selfing has evolved multiple times from the ancestral state of obligate outcrossing (Kiontke et al., 2004; Kiontke and Fitch, 2005; Cutter et al., 2008). Within the selfing taxa, their mixed mating strategy generates variation in outcrossing rate: males are typically rare, consistent with observations of rare outcrossing in nature (Haber et al., 2004; Barrière and Félix, 2005, 2007; Cutter, 2006; Rockman and Kruglyak, 2009), but they can increase dramatically in frequency in response to various laboratory interventions (Cutter, 2005; Manoel et al., 2007; Lopes et al., 2008; Morran et al., 2009, 2011; Masri et al., 2013)

(rev. in Anderson et al., 2010). These interventions seem naturally relevant: they include mutation, adaptation to a novel environment, and antagonistic coevolution, the last of which is unique in sustaining high frequencies of males through time (Box 1). Yet, males are vanishingly rare in nature.

Focusing on coevolving parasites, the absence of males in the wild suggests a few possibilities, including (i) *C. elegans* escapes coevolving parasites altogether, perhaps because host populations are so ephemeral; (ii) Selection by coevolving parasites is too weak of a force to maintain outcrossing in the face of its costs; or (iii) Outcrossing may be favored only at very particular times (e.g. epidemic peaks) and places (e.g. coevolutionary hotspots). Prior experimental research in the laboratory has shaped these hypotheses (Morran et al., 2011; Slowinski et al., 2016), which can now be tested in field populations. The original studies of wild-caught *C. elegans* addressed the question of outcrossing (Barrière and Félix, 2005, 2007), and it will likely continue to be a central focus of field research in *C. elegans*.

What's the best defense?

When confronted with a parasite, the resulting fitness of an individual host is determined by a series of three steps: 1. contact between host and parasite; 2. establishment and proliferation of an infection; and 3. the maintenance (or not) of fitness in the face of that infection. Host avoidance reduces the probability of contact. Host resistance reduces the probability that an infection establishes and limits its proliferation once established. Tolerance refers to a host's ability to maintain fitness at a given parasite load. Which of these approaches is the most important to a given host? Does a parasite select for increased avoidance, resistance, tolerance, or all three? Are these outcomes dependent on environmental factors? The answers to these questions determine how we predict parasites to spread and evolve (Roy and Kirchner, 2000; Miller et al., 2006; Boots, 2008; Råberg et al., 2009). For example, if hosts evolve tolerance, we predict a parasite to be far more prevalent in a host population than if hosts evolve resistance.

We may be able to unwind this entanglement of traits using *C. elegans* and its natural parasites. Avoidance of parasites is important for *C. elegans*. It is well characterized in response to many unnatural bacterial parasites, and many simple assays exist to quantify the degree to which avoidance can explain the outcome of a host–parasite interaction (Andrew and Nicholas, 1976; Zhang et al., 2005; Pradel et al., 2007; Reddy et al., 2009; Glater et al., 2014) (rev. in Schulenburg and Ewbank, 2007; Meisel and Kim, 2014). Existing knowledge of innate immunity gives significant insight into the mechanistic and genetic basis of host resistance (reviewed earlier). Moreover, microscopy and quantitative polymerase chain reaction are commonly used to track increases or decreases in parasite loads from the very beginning of an infection (as in Sterken et al., 2014; Balla et al., 2015). These tools can also be leveraged to measure tolerance by relating parasite loads to host fecundity, which takes just a few days to fully assay. With experimental evolution, one might examine the genetic or environmental context that favors the evolution of one strategy over others. *Caenorhabditis elegans* and its natural parasites may prove extremely valuable for testing key predictions generated by theory. Specifically, this system could link field data and empirical tests with theoretical predictions at the levels of both populations and individuals, providing insight into both ecological and population genomic dynamics of disease.

Are some lineages sicker, and why?

We have thus far neglected an important change that natural sampling has brought to *C. elegans* research: host diversity. Historically, *C. elegans* research focused almost exclusively on the inbred, laboratory-adapted strain N2. Now, 1769 natural isolates are described and made publicly available through the Nematode Wild Isolate Collection (worldwideworm.banshy.fr). The *C. elegans* Natural Diversity Resource provides ecological and genomic data for 249 natural isolates (Cook et al., 2017). These isolates are phenotypically and genetically quite distinct from N2 (Sterken et al., 2015). They have already facilitated screens of natural genetic variation in ecologically relevant traits, including dispersal behavior (Lee et al., 2017), chemoreception (Greene et al., 2016), and habitat (Evans et al., 2017). For the field of evolutionary ecology of disease, such a wealth of well-characterized natural isolates could prove invaluable in mapping the genetic basis of natural variation in host–parasite interactions (Samuel et al., 2016).

Moreover, *C. elegans* no longer stands alone in the *Caenorhabditis* genus. The genus has recently grown to include approximately 50 members (Kiontke et al., 2011; Félix et al., 2014; Slos et al., 2017). More than 40 are maintained in laboratory cultures, and strains of at least 32 species are available for order through the *Caenorhabditis* Genetics Center. Genome sequencing is in progress for most of these taxa (evolution.wormbase.org). WormBase provides genomic resources for five species, and status updates for 27 species are available at Caenorhabditis.org. Whole genome sequences have been completed and published for several species, such as *C. briggsae* (Stein et al., 2003), *angaria* (Mortazavi et al., 2010), *remanei* (Fierst et al., 2015), and *monodelphis* (Slos et al., 2017). Many of the experimental and genetic tools developed for *C. elegans* are applicable to this growing diversity of congeners.

Within- and between-species variation in the genus as a whole lends itself to comparative studies of disease. Species within the genus differ in fundamental ways such as mating system (Kiontke and Fitch, 2005; Kiontke et al., 2011), genome size (Fierst et al., 2015), genetic variation (Thomas and Wilson, 1991; Graustein

et al., 2002; Jovelin et al., 2003), life span (McCulloch and Gems, 2003), ecology (Kiontke and Sudhaus, 2006), and body size (Woodruff et al., 2017). These different taxa nonetheless share many of the same types of parasites (Felix et al., 2011; Zhang et al., 2016). We can thus ask about the impact of genotypic and phenotypic variation in hosts on disease spread, e.g., does parasite epidemiology differ between selfing species (*C. elegans*, *briggsae*, *tropicalis*) and obligately outcrossing species (e.g., *C. remanei*, *brenneri*, *japonica*, *drosophilae*)? Does variation in life span, within and between species, correspond to different investment in immunity (Amrit et al., 2010)?

Sympatry of host species suggests a community ecology approach (Félix and Duveau, 2012): Does increasing diversity of host species in a community limit disease spread? Generalist parasites seem to be shared between sympatric taxa, notably *C. elegans* and *C. briggsae* (Félix and Duveau, 2012; Zhang et al., 2016). This observation raises the question: Do hosts differ in their suitability as hosts of generalist parasites (as in Zhang et al., 2016)? Again, experimental tools can pull apart confounding variables in field data and differentiate cause from effect.

Limitations

There are of course many questions in disease ecology and evolution for which the supermodel *C. elegans* and its relatives are not suitable. Species of *Caenorhabditis* uniformly exhibit the high growth rates and large offspring numbers of r-selected species. Short-lived, highly fecund hosts may be biased toward managing parasites by avoidance and investment in early reproduction. *Caenorhabditis*–parasite interactions may thus give limited insight into host–parasite interaction in long-lived hosts, which likely rely more on robust immune systems that promote resistance and tolerance (for further discussion, see Medzhitov and Janeway, 1997; Miller et al., 2007). Relatedly, the resource patches on which *C. elegans* proliferate are transient, and thus hosts may interact with parasites over the course of just a few generations. Evolutionarily, these brief interactions likely unfold in a very different way than sustained, stable host–parasite interactions. Last, there are realms of disease research that are not relevant for *C. elegans*, e.g., adaptive immunity or vertical and sexual transmission (at least for now—no parasites are presently known with these transmission modes).

Conclusion

Study of the caenorhabditids and their respective natural parasites can advance the field of evolutionary ecology of disease by merging the tools of the laboratory with field sampling at the levels of the population and the individual. These systems are in a unique position to address outstanding questions in disease biology that invoke the interplay between ecological processes and evolutionary change. In response to the call by Lively et al. (2014) for natural disease systems, we advocate for caenorhabditid nematodes and their natural parasites as novel systems to address critical questions in the field of disease ecology and evolution.

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