Caenorhabditis evolution in the wild

*Asher D. Cutter**

Recent research has filled many gaps about *Caenorhabditis* natural history, simultaneously exposing how much remains to be discovered. This awareness now provides means of connecting ecological and evolutionary theory with diverse biological patterns within and among species in terms of adaptation, sexual selection, breeding systems, speciation, and other phenomena. Moreover, the heralded laboratory tractability of *C. elegans*, and *Caenorhabditis* species generally, provides a powerful case study for experimental hypothesis testing about evolutionary and ecological processes to levels of detail unparalleled by most study systems. Here, I synthesize pertinent theory with what we know and suspect about *Caenorhabditis* natural history for salient features of biodiversity, phenotypes, population dynamics, and interactions within and between species. I identify topics of pressing concern to advance *Caenorhabditis* biology and to study general evolutionary processes, including the key opportunities to tackle problems in dispersal dynamics, competition, and the dimensionality of niche space.

Keywords:
adaptation; *Caenorhabditis elegans*; dispersal; evolution; sexual selection; speciation

Introduction

> "*. . .small size, by permitting animals to become specialized to the conditions offered by small diversified elements of the environmental mosaic, clearly makes possible a degree of diversity quite unknown among groups of larger organisms*." G.E. Hutchinson. 1959. Homage to Santa-Rosalia or why are there so many kinds of animals? *Am Nat* 93: 145-59.

If one knows anything about *Caenorhabditis* nematodes, it is that they are small animals. Their small size (~1 mm adults) defines just one attribute that endears them to students of laboratory biology and destines their lives to the relaxed confines of a Petri dish. However, the prolific study of *Caenorhabditis* in the laboratory since the 1970s sidelined their potential as a system also to address problems of evolution in nature. This oversight is now in the process of correction by the scientific community [1–3]. In-depth study of wild worms began in earnest only about 10 years ago from fresh collections of natural isolates [1, 4], and has since expanded to collections and analysis of thousands of cryopreserved strains for dozens of species around the world. While the discovery phase of *Caenorhabditis* natural history continues, the collective knowledge of the system has reached critical mass to transition more fully into a hypothesis-testing phase of studying evolution in the wild.

Here, I outline key points of *Caenorhabditis* biology in connection with broader themes in ecology and evolution. I aim to identify important gaps in knowledge toward further developing these organisms as models for evolution in nature, in addition to their role as a biomedical model. Excellent reviews provide accounts of *C. elegans* natural and scientific history [1, 2], so here I avoid reiterating such information by considering *Caenorhabditis* more generally and focus on recent advances, except where necessary to motivate broader issues. First, I discuss patterns of *Caenorhabditis* biodiversity and factors that define it, then develop more deeply two drivers of diversity in the wild: dispersal dynamics and trait evolution underlying adaptation. Next, I consider the evolutionary forces acting on sexual systems, including sexual selection and evolution in populations with self-fertilization. I end by touching on key issues in speciation and macroevolution. Within each section, I aim to integrate laboratory and field observations with conceptual ideas in ecology and evolution in order to synthesize information and draw attention to constructive lines of future inquiry (Box 1).

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Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada

Corresponding author:
Asher D. Cutter
E-mail: asher.cutter@utoronto.ca

Box 1

Outstanding questions about *Caenorhabditis* in the wild

- Dimensionality of niche space
  - Are the primary dimensions of niche space differentiation biotic (food, pathogens, predators, competitors) or abiotic (e.g., temperature, microhabitat chemistry)? How frequently do *Caenorhabditis* species compete with each other in nature?
  - Which factors most limit population growth (dispersal ability, resource availability, competition, abiotic conditions)? Can these limiting factors explain common versus rare and generalist versus specialist species? How does species colonization order influence community structure?
  - Which traits define species range limits? Are particular traits commonly involved in adaptive divergence between species? Is adaptation limited by mutational input?
- Dispersal dynamics: space, time, and mode
  - What is the spatial distribution of migration for different *Caenorhabditis* species (dispersal kernel)? How does it change over time (season, microhabitat resource exhaustion)? Do different scales of dispersal depend on different vectors? How does vector specialization influence dispersal dynamics over space and time?
  - What is the rate of colonization of new habitat patches and migration between habitat patches? Is colonization of fresh resources limited by dispersal or do multiple sources simultaneously colonize a given patch (migrant pool)? What role do anthropogenic sources play in dispersal and evolution?
  - How many individuals disperse to a new patch during a single colonization event (propagule size)? Does a dauer "seed bank" contribute to patch colonization?
  - What is the rate of patch turnover owing to resource exhaustion (local extinction)? What is the critical patch scale (e.g., fruit, tree, orchard, region)? Following large scale patch extinction during winter or dry seasons, what are the sources for new colonization?
  - How linked are spatial and population genetic patchiness? Do populations fundamentally violate the Wright–Fisher model? How hierarchical are population subdivisions and metapopulation dynamics (extinction-recolonization)? Is inter-demic selection an important force in evolution?
- Generation turnover and the molecular clock
  - How many generations do different *Caenorhabditis* species pass through each year? How does generation turnover vary seasonally? How do species survive and re-grow after winter? Does the molecular clock differ among species owing to different generation times per year?
- Speciation
  - How important in the accumulation of reproductive isolation are divergent adaptation, parallel adaptation to common environments in allopatry, sexual selection, and genomic conflict? What are the contributions of pre-zygotic versus post-zygotic isolation and of gene-environment interactions in speciation?
  - What traits correlate with macroevolutionary diversification (species origination, extinction)? Is speciation coupled with the origins of self-fertility?

Macro- to micro-evolutionary patterns of *Caenorhabditis* biodiversity

Scientific recognition of *Caenorhabditis* species diversity has exploded over the last two decades. From only five species widely available in laboratory culture in 1998 when *C. elegans* became the first animal to have its genome sequenced, 27 species currently appear in published literature [5] (Fig. 1), with over 20 more known but awaiting detailed study (K. Kiontke pers. comm.). A streamlined procedure for diagnosing and naming new species has led to about half of the known species enjoying Latin names (the remainder with temporary numerical identifiers) [6]. Multiple species are known from every continent, excepting Antarctica, and despite incomplete sampling in many regions of the globe (Fig. 2). As with many taxa, the tropics appear to be especially diverse (Fig. 2), as for the community of nine species known from a single site in French Guiana [7] (C. Braendle and A. Cutter pers. obs.). The major phylogenetic subgroups within the genus show geographic trends that might reflect their origins of diversification: 10 of 10 Elegans group species have been found in the Asia-Pacific (cf. just one of eight Drosophilae group species), at least five of eight Drosophilae group species are found in the Americas, and only members of the Elegans group are known to have pan-global distributions (six of ten species) (Fig. 1). Although some species certainly have biogeographically restricted range distributions (e.g., *C. sinica* in eastern Asia, *C. tropicalis*, *C. brenneri*, and *C. nigoni* in tropical latitudes), more than half are known from only one or two sampling localities, and so it remains unclear how restricted their range distributions truly are in nature and how much human activity influences contemporary distributions [8].

Genetic variation within species provides another source of biodiversity, with concerted ongoing efforts to understand the genetic architecture of variation in fitness and other traits [9]. Molecular variation within some species is diverse in the extreme [10]. *C. brenneri* populations contain the highest sequence variability of any known eukaryote [11], although the genomes of *C. remanei* and *C. sinica* also have enormous

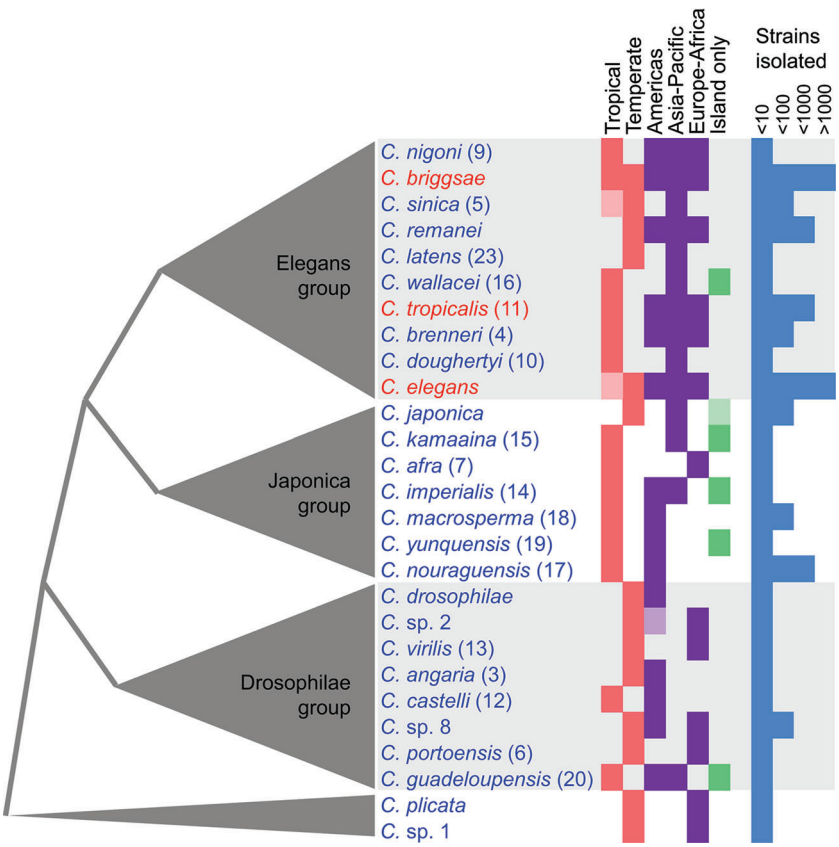

Figure 1. Mapping of *Caenorhabditis* species geographic sampling locations onto the major phylogenetic groups in the genus. All species known to have pan-global distributions occur in the Elegans group. Elegans group species often occur in the Asia-Pacific region, unlike most other *Caenorhabditis*; Drosophilae group species occur more commonly in the Americas. Phylogeny summarizes relationships from [5], with recent species names as in [6] (former numerical identifiers in parentheses). The three species with hermaphrodites are indicated with red text. Most new species discoveries, not represented in the figure, derive from single sampling localities in tropical regions.

densities of SNPs compared to most organisms [10]. Such high molecular diversity is thought to reflect enormous sizes of breeding populations, permitted by abundant bacterial food sources in rotting fruit and vegetation. Extremely large breeding populations confer interesting and powerful implications for how evolution will proceed in such systems [10, 12]. These species are obligately outbreeding, which is the ancestral and dominant mode of reproduction among *Caenorhabditis* [5]. Consequently, outbreeding reproduction in hugely abundant populations should form the point of departure for discussing evolution in these organisms, with the repeated independent origins of selfing and ecological specialization forming obvious and important features for special consideration.

How does ecology define *Caenorhabditis* biodiversity?

Which ecological factors define niche differentiation and contribute to range distributions? Some species differ in sensitivity to temperature stress [13–17], as do different phylogeographic groups within *C. briggsae* [18], so this abiotic factor surely is important in shaping species ranges. Seasonal humidity and precipitation also represent important environmental factors in population abundance [19], and heterogeneous seasonal prevalence of individuals will control how many generations a given species will pass through per year. Generation times in nature are currently just a matter of educated speculation [20, 21], and it remains unknown as to how *Caenorhabditis* overwinters in temperate latitudes and whether tropical species experience seasonality in abundances. Species can also differ in response to other abiotic stressors, such as salinity and heavy metals and lipid availability, as well as to biotic interactions with bacterial food type and dispersal vectors [3, 14, 15, 22]. Given dietary choice among bacterial species by *C. elegans* [23], sensory preference and nutritional differences among *Caenorhabditis* surely are important in defining niche breadths, but little is known about potentially distinct bacterial communities or chemical profiles of different rotting substrates that might offer opportunity for specialization or niche differentiation. Discerning the connection of these extrinsic factors to genetic responses may enlighten the vast array of *Caenorhabditis* "genes of unknown function" [24]. However, a full and general understanding of niche overlap and differentiation is lacking for *Caenorhabditis*. In particular, it will be critical to understand how ecological factors contribute to fitness differences under competitive conditions, in addition to when species are considered in isolation from one another. Despite sympatric distributions of some species, with rare exceptions [13], inter-species competition represents one of the untouched topics in *Caenorhabditis* biology.

Despite our poor knowledge generally about *Caenorhabditis* niche dimensionality, it is clear that some species have specialist or generalist life histories with respect to dispersal host vector. *C. elegans* shares with a number of other *Caenorhabditis* the trait of generalism toward invertebrate dispersal hosts, albeit not entirely promiscuous in host use [2, 5, 7]. However, the specialized relationship of *C. japonica* with the hemipteran insect *Parastrachia japonensis* provides the best understood natural history of any species [25, 26]. It is plausible that *C. drosophilae*, which appears to disperse solely on *Drosophila nigrospiracula* to new cactus rots [27], and some other less well-understood *Caenorhabditis*, also evolved specialized dispersal host relationships from a more generalist ancestral state. A better understanding of relations with dispersal vectors, including humans, are crucial to determine the key factors governing range distributions, gene flow and speciation, and metapopulation dynamics over time and space.

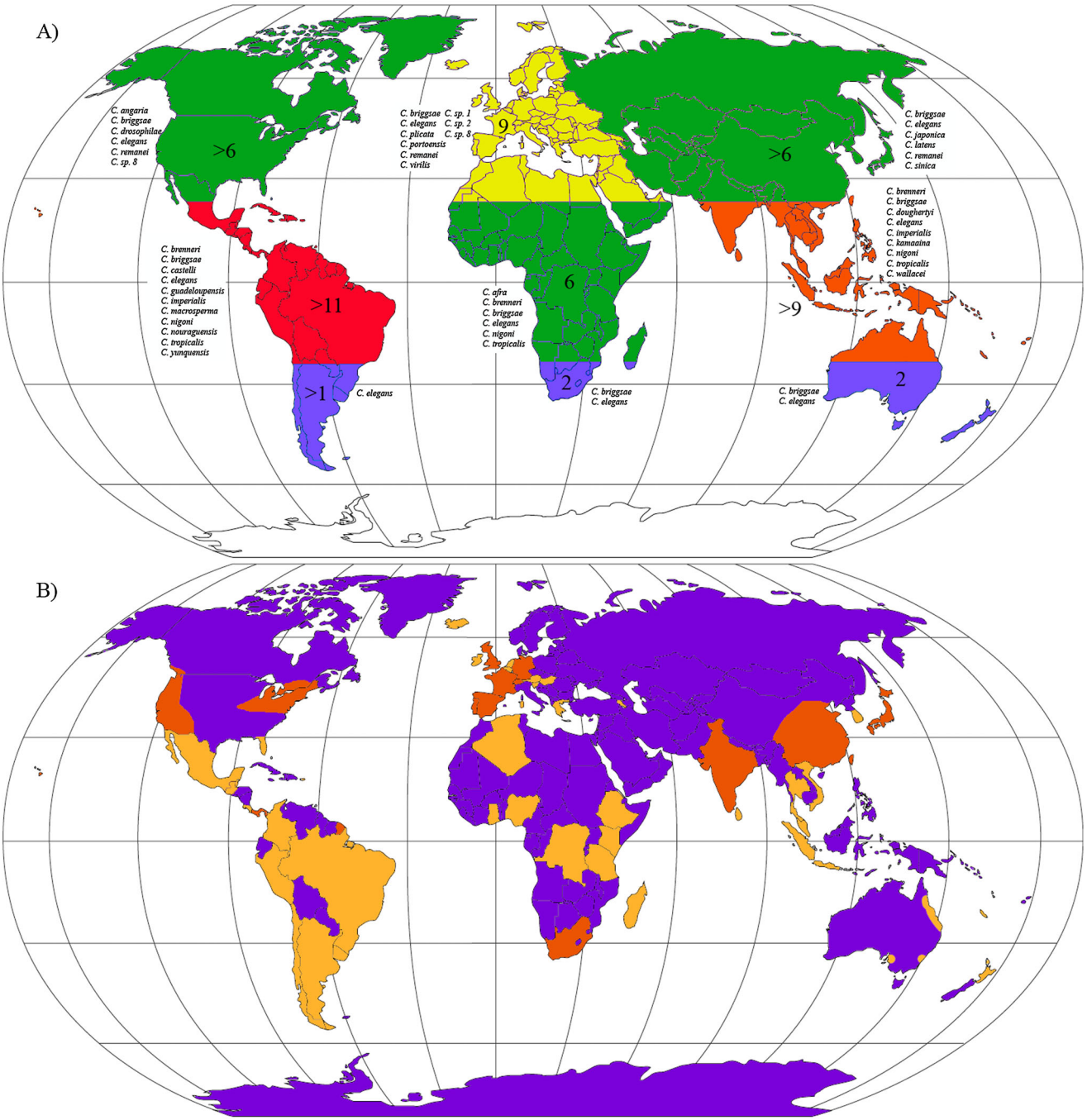

Figure 2. *Caenorhabditis* species richness (**A**) and sampling intensity (**B**) across the globe. Species known from temperate and tropical zones, roughly partitioned across the Americas, Europe-Africa, and Asia-Pacific-Australia with hotter colors representing more species (**A**). In (**B**), purple indicates no *Caenorhabditis* known from the associated region, orange represents well-sampled regions, yellow indicates regions from which *Caenorhabditis* has been reported without intensive sampling. Not visible on the map scale are the numerous samples from small oceanic islands that contribute to the species totals in (**A**); Hawaiian species are grouped with the Asia-Pacific region. Species and sampling information are summarized in [5, 7], with some more recent updates (http://worldwideworm.banshy.fr/).

How do *Caenorhabditis* disperse?

Species of *Caenorhabditis* occupy ephemeral micro-habitats: microbial blooms in rotting fruit and vegetation [1, 2, 5]. This leads to inevitable extinction of a local habitat patch owing to resource exhaustion [13, 21]. Species persistence thus requires dispersal and colonization of new patches (Fig. 3). Theory predicts that such circumstances will favor the evolution of high dispersal, provided that the patches are exploited sufficiently quickly to preclude local adaptation that would favor sedentary persistence in a seemingly stable patch [28]. *Caenorhabditis* have several adaptations that appear to facilitate both of these endeavors: to stay put in a good patch

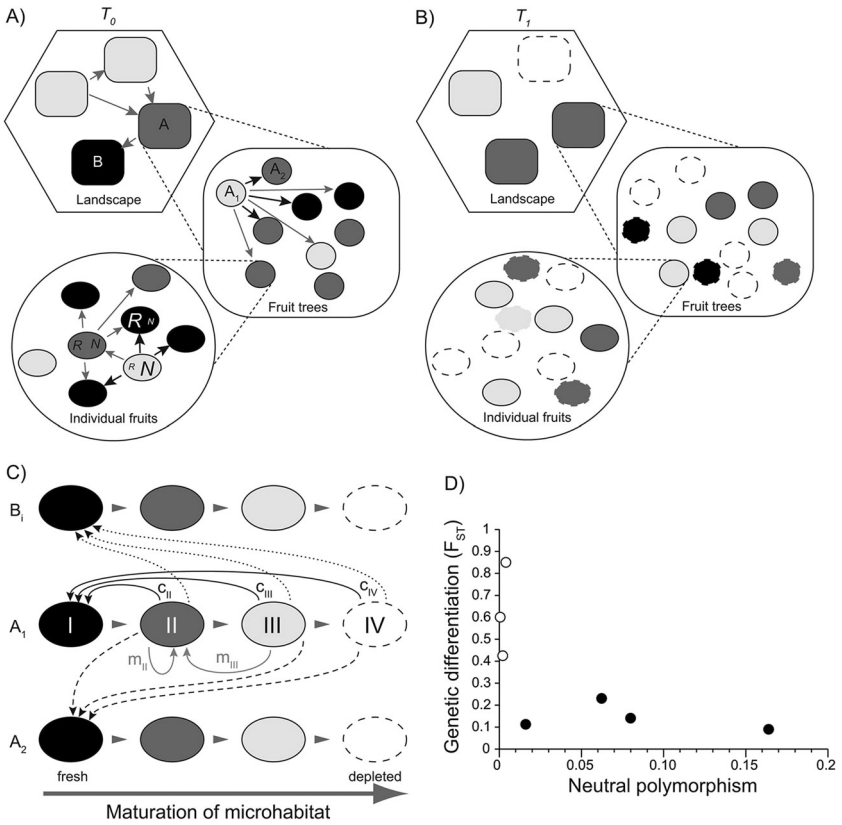

Figure 3. Population subdivision in *Caenorhabditis*. **A:** A metapopulation hypothesis for hierarchically structured populations of *Caenorhabditis* in ephemeral local resource patches from time T_0 to (**B**) time T_1. Shown are three spatial scales (individual "fruits" associated with a "tree" which occur in a cluster within a "landscape" patch), where arrows at the two smaller scales indicating dispersal from example focal patches (darker arrow indicates higher dispersal). Shading of patches indicates stage of resource depletion (black indicates unexploited resource, lighter shades indicating decreasing resource remaining, *R*, and increasing worm abundance, *N*). At T_1 in (**B**), many patches have become exhausted of resources (dashed lines, white fill) and new patches have emerged (dashed line edge, solid fill). **C:** Paths of dispersal at different scales with patch labels corresponding to labels in (**A**). Gray arrowheads indicate maturation stage of "fruit" substrate, and black arrows indicate colonization of new resources within a "tree" location (A_1 to A_1, solid lines), between tree locations (A_1 to A_2, dashed lines), or between landscape regions (A_1 to B_i, dotted lines). Migration between previously colonized patches is indicated with patch A_1 by gray arrows. Colonization (*c*) and migration rates (*m*) will depend on the degree of source patch exhaustion (e.g. $c_{IV} > c_{III} > c_{II}$ owing to differences in population size and prevalence of dauers). In a formal model, one might also weight dispersal by the abundance of patches of a given stage, and consider propagule size in dispersal. **D:** Genetic differentiation among populations within *Caenorhabditis* species, loosely corresponding to the "landscape" scale in (**A**), trends negatively with the amount of genetic diversity in those species of *Caenorhabditis* owing largely to the disparity between selfing (empty circles) and outcrossing species (filled circles; data for *C. briggsae*, *C. elegans*, *C. tropicalis*, *C. brenneri, C. japonica, C. remanei*, *C. sinica* as in [25]).

and to disperse once conditions deteriorate. Their development is phenotypically plastic in that they develop directly into reproductive adults when resources are plentiful, but pass through the diapause-like and dispersive "dauer" larval stage when starved and overcrowded [29]. First stage larva also are tolerant of the absence of food ("L1 arrest") [30], and represent the life stage that best survives cryopreservation in the laboratory. Dauer larvae are stress-tolerant and can survive for months without feeding, potentially in a vermiform "seed bank." Moreover, dauer larvae participate in nictation behavior – they climb up substrate protrusions, standing on their tails and waving, often forming large ropy and wriggling aggregates of thousands of worms – that facilitates adhesion to and transport by dispersal vectors [2, 13, 31]. Invertebrate vectors appear common, albeit with few examples of flight-capable hosts [2, 3, 26], and frugivorous vertebrates might also contribute to *Caenorhabditis* natural dispersal. More recently, commercial human agriculture likely provides a means of movement, especially for cosmopolitan species such as *C. elegans*, *C. briggsae*, and *C. brenneri*. Perhaps, in conjunction with "urban" ecological analysis [32], ancient DNA techniques could be applied to agricultural artifacts to test for incidental anthropological associations of man and worm. Dispersal in all its guises represents another major topic for which quantitative data from nature are generally lacking (Box 1).

What are the evolutionary and ecological consequences of dispersal dynamics?

A lifestyle of colonization, exploitation, and dispersal to new patches imposes a number of predictable consequences on population genetic variation, adaptation, and speciation. First, high dispersal implies a high migration propensity and extensive gene flow across a species' range. Indeed, despite some population genetic differentiation (Fig. 3), the magnitude of genetic structure within obligatorily outcrossing species can be rather modest given the tiny size of the animals and the great geographic size of their ranges [10]. Effective population sizes will often be enormous, given high abundance and high dispersal, meaning that genetic drift will be weak and species-wide natural selection will be very efficient on even extremely weakly selected mutations [10]. High gene flow across the species range, however, could hamper local adaptation to conditions specific to only a portion of their range [33]. Because local adaptation also involves a degree of inbreeding, sufficiently high migration can favor generalist strategies and preclude the evolution of locally adaptive phenotypes [33]. Similarly, genetic mixing from high migration could slow speciation, especially if adaptations evolve globally rather than locally [33]. However, should some local populations become

Box 2

Adaptive evolution in self-fertilizing species

Reproductive assurance of dispersing individuals can favor the evolution of self-fertilization [114], a mode of reproduction that has evolved repeatedly in *Caenorhabditis* [5]. Perhaps, initially, this would be most favored near range margins or in the colonization of more isolated locales, because extant outbreeding species typically suffer strong inbreeding depression that would counteract the evolution of selfing in range centers [114]. Episodes of population growth from a few founders generates substantial inbreeding between sibs of outcrossing species which could facilitate the purging of alleles causing inbreeding depression [115]. However, outcrossing species appear still to suffer strong inbreeding depression that they fail to purge in experiments [91]. Experimental evolution indicates that individual selection can favor the evolution of selfing, especially as a means of adapting to extrinsic conditions that limit outcrossing [116]. The fact that self-fertilizing species also retain males at low frequencies also raises the question of what role they might play in adaptation or population persistence. Developmentally, the origin of selfing is hypothesized to involve at least two key steps: production of sperm by the female gonad and the ability for those sperm to be activated in the absence of seminal fluid [117]. Once selfing is established as a reproductive strategy, a renewed propensity for high migration and geographic range expansion could alter the predicted patterns of population genetic variation, adaptation, and speciation for selfing species [85].

On the one hand, the lower genetic diversity, more potent role of genetic drift, and strong linkage of genomes for selfing species can limit adaptation. On the other hand, greater genetic isolation between local patches, exposure of recessive beneficial alleles to selection in homozygotes, and the effects of high reproductive skew in combination with very large census population sizes could all accelerate adaptation. Because of reduced population genetic diversity, species-wide adaptations probably more often involve selection on new beneficial mutations rather than segregating variants [118]. Because positive selection in selfers can quickly act upon new mutations regardless of dominance [93], the waiting time for new beneficial mutations might not be as strong of a constraint on adaptation in selfers as in outcrossers or diploid asexuals, and, the fixation time given any beneficial allele is faster in selfers [118]. Laboratory domestication in *C. elegans* and *C. briggsae* clearly illustrate the potential for rapid adaptation [64]. Selfing versus outcrossing plants show no general difference in rates of adaptation, however, suggesting that the positive and negative effects of breeding system on adaptation might often counterbalance each other [119].

Despite a good rationale for the potential of detrimental mutations to accumulate in selfing populations, genomic evidence is more sparse [84]. Population genomic studies have nevertheless revealed several molecular evolutionary features resulting from the transition to a selfing lifestyle [84, 85], including in *Caenorhabditis* [88]. Given the reduced genome sizes for selfing *Caenorhabditis* species that disproportionately affects male-expressed genes [120], selection might play an important role in producing "selfing syndrome" traits (e.g., traits that diminish outcrossing potential) as adaptations to selfing hermaphroditism per se, rather than simply decay owing to relaxed selection [118, 121]. The adaptive explanation for the evolution of selfing syndrome traits becomes more plausible with more recent origins of selfing [99].

shut off from genetic exchange, local adaptation would proceed very efficiently, given high genetic variation and a large population, leading to genetic divergence through fixed allelic differences. Perhaps just this process might explain some patterns of phylogeography (e.g., *C. briggsae* [18]), outbreeding depression (e.g., *C. tropicalis* [34]), cryptic speciation (e.g., the *C. remanei*/*C. latens*/JU724 complex [35]), and the possibility of many island endemic species (Fig. 1).

Second, population dynamics and evolution depend on the means as well as frequency of dispersal, in terms of the numbers of migrants that colonize new and previously occupied habitat patches and whether migrants derive from a "propagule pool" or "migrant pool" [36]. Should a given patch (e.g., a single rotting fruit) be colonized by only a small number of individuals of a given species, with little further immigration, then reproduction will amplify the few allele copies present within those founders by many orders of magnitude (in addition to the drift and inbreeding effects of the local founder event). This situation creates bottlenecks in local patches that can limit global genetic diversity and mimic coalescent models of populations having high variance in reproductive success, if there is high variance among habitat patches in subsequent successful dispersal and colonization (or extinction) [36, 37]. This effect would be more extreme in, and favor evolution of, species with self-fertilizing hermaphrodites (Box 2), but might also contribute to the lower molecular diversity of dioecious species like *C. japonica* with its specialist lifestyle that exacerbates local inbreeding [25]. Differential patch productivity also presents opportunity for interdemic selection [38], although it is not clear which traits might experience conflict in the direction of selection between levels (individual vs. group). Further, seasonal fluctuations induce some synchronization of population bottlenecks across patches [13], at least in temperate latitudes. All these effects may drive substantial and qualitative deviations from the expectations for the idealized population envisioned in models of most evolutionary theory (i.e., a Wright–Fisher population) [37, 39].

Despite the above intuition about metapopulation processes, truly, we have no hard data in hand regarding dispersal dynamics. For example, if local patch dispersal and colonization is very rapid, then the physical spatial patchiness

of resources might not imply patchiness at all with respect to species-wide demography and population genetics. Similarly, the extent to which patch colonization limits population dynamics will influence the scope for inter-species interactions, such as competition, between *Caenorhabditis*. But what is the rate of patch exhaustion relative to colonization and immigration? We know that a given patch can be colonized by multiple genetic backgrounds or species [7, 13], making worms an intriguing system to test ideas from community ecology about species coexistence [40]. These local and global processes defining the relevant extent of hierarchical nestedness in habitat extinction-recolonization dynamics have critical implications for the geographic scale at which adaptation occurs and the degree to which mutational input limits adaptation [41]. Clearly, it is crucial to establish the temporal and spatial limiting factors in migration at a range of scales to put *Caenorhabditis* evolution in the wild into a firmer theoretical context.

Which traits are important for adaptive divergence in the wild?

Two implications of large population size are that populations will contain, in absolute terms, a lot of functional variation and that natural selection can efficiently sort among the variants. But which traits are most likely to be important in organismal evolution? Because nematodes interact with environmental heterogeneity through smell and taste – to navigate, find food and mates, to avoid noxious conditions and approach preferable ones – chemosensory traits provide strong candidates for adaptive evolutionary change. To date, there has been little quantification of phenotypic variation in species other than the well-studied selfing species that have lower molecular diversity, although chemosensory variation in *C. remanei* is indeed much higher than *C. elegans* [42]. In *C. elegans*, wild alleles of chemosensory receptor genes frequently contain nonsense mutations and deletion polymorphism that could reflect turnover of functional diversity of these large multi-gene families [43], or perhaps trait degeneration. Genes in these chemoreceptor families also are thought to have experienced positive selection in their history, presumably reflecting adaptive evolution in response to their role in mediating interactions between the worm and its environment [44].

Chemosensation, and corresponding behavioral responses, also are key in interactions with pathogens [45], and coevolution of host and pathogen are predicted by theory to drive continual genetic divergence [46]. Several classes of pathogen are now known to infect *Caenorhabditis*, including viruses, bacteria, microsporidia, and fungi [1]. Although pathogen burden and selective pressure in nature still remains poorly understood, the observations of intraspecies variation and interspecies differences in pathogen sensitivity [47], rapid inter-species molecular evolution of immune-related genes [48], as well as the ease by which it can evolve experimentally [49], all implicate great potential for evolution of innate immunity in the wild. Chemical secretions also could represent an important set of traits subject to diversifying selection in nature, as they are used as cues by predators such as nematode-trapping-fungi [50], in dauer larva induction [29, 51], and in mate-finding [51–53].

The cuticle and the gut represent the main tissues that interact physically with the environment. Evolution of nematode surface coat characteristics are thought to be important in responses to the external environment [54]: cuticle-expressed proteins contribute to cold tolerance [55] and susceptibility to a range of pathogens [56], and differentially expressed collagens show distinct patterns of evolution across development [57]. Species differ in the intestine-mediated RNAi by feeding response [58], which also is important in immunity [47]. The intestine is a key route of infection [47, 56], in addition to its role in nutritional intake from appropriate microbial food, making it a likely battleground for host-pathogen coevolution and diet specialization. The vulva provides a port of entry for other pathogens [59], although it remains unknown whether sexually transmitted infections occur in *Caenorhabditis*. Clearly, the traits and molecules associated with these bodily surfaces that interact proximately with the biotic and abiotic environment of the worm form plausible key substrates for adaptive change.

Dauer larva formation is a stunning model of phenotypic plasticity, tuning animal development to growth and reproduction, or to dormancy and stress tolerance, in response to signals of environmental quality [29]. *C. elegans* shows genetic variation for the propensity to enter and exit the dauer stage and *C. remanei* also shows substantial genetic variation in stress responses [9, 60]. However, little is known about heterogeneity across taxa in sensitivity to dauer triggers, other than that, in contrast to *C. elegans*, high temperature does not trigger dauer development in *C. briggsae* [61]. Chemosensation in dauer may be critical for successful dispersal on appropriate invertebrate vectors for overwintering, as is the case in *C. japonica* [26]. Much research on the genetic pathways controlling dauer development connect intimately to studies of aging and lifespan [62]. However, given the drastically shorter lifespans of worm individuals in pseudo-natural environments, even in the absence of pathogens or predators [63], post-reproductive longevity is unlikely to be a direct target of selection in the wild.

Of course, many additional physiological and behavioral features to the few emphasized here are likely to provide the substrate of adaptive divergence among species, and among populations within species. Adaptation to laboratory conditions [64] can inadvertently inform us about trait values favored under natural conditions through comparison to the ancestral undomesticated phenotypic state. More generally, a better understanding of the key components of biotic and abiotic niche dimensionality variation across *Caenorhabditis* will prove crucial in grounding our expectations for how trait differences evolve.

A challenge to understanding the ecological importance of many of these traits is that they seem cryptic and potentially tedious to quantify in comparison to the conspicuous morphological differences for many larger organisms. After all, at first glance, the streamlined morphology of most *Caenorhabditis* is notoriously barren of obvious diagnostic anatomical differences among species [6, 8]. A *Caenorhabditis*

aficionado might smugly quip that evolution has simply found the perfect outward phenotype! But this veneer of phenotypic stasis can belie extensive change in developmental genetic pathways underlying outwardly conserved phenotypes [65], or evolutionary cell biology [66]. These properties already are being tapped to explore questions in evo-devo [29, 67, 68], but a future challenge is to integrate such ideas into an ecological context to inform our understanding of *Caenorhabditis* evolution in nature.

How intense are sexual selection and sexual conflict in nature?

Sexual selection is likely to influence the evolution of both mating behavior and gametic interactions, given promiscuous mating in outbreeding species. Moreover, trait values of shared phenotypes can differ between the sexes, leading to sexually antagonistic coevolution [69]. Hints about this process in nature are gained by contrasting phenotypes of *Caenorhabditis* species with females to those with hermaphrodites, which are thought to have adapted for reproducing almost entirely by self-fertilization. Virgin females facilitate mating by becoming stationary, unlike hermaphrodites and females that already have sperm: such individuals instead keep moving upon contact with males [70, 71]. The success of inter-species mating also varies substantially among species pairs [72], which is suggestive of divergence in mate attraction, resistance, or recognition. Virgin females secrete a potent sex pheromone, until they mate, only resuming full pheromone secretion 12–18 hours later [52]. *C. elegans* hermaphrodites do secrete compounds attractive to males [51, 53], but their lower potency [52] implies that conflict over mating traits has resolved in favor of "female" reproduction in species with hermaphrodites. These observations suggest that optimal mating rates differ between the sexes, such that selection favors constitutive mating by males but opportunistic mating by females only when sperm stores are low.

Sexual conflict in *Caenorhabditis* also may involve male-induced harm to females. Male spicules scrape and physically damage the hermaphrodite cuticle in the vicinity of the vulva [73], and mating reduces her lifespan owing in part to physiological changes induced by sensing the chemical secretions of males [74–76]. The mating structure of males provides one of the most conspicuous and rapidly evolving morphological traits in *Caenorhabditis* [5], as in many other organisms [69], implicating selection of some form in its evolution. It is unknown whether species differences in the propensity to engage in "spiral" versus "lateral" mating [5] might result from sexual conflict, male–male competition, or instead reflect differential environmental selection on mating strategies. Further evidence of conflict between the sexes is witnessed by the observation that females display sperm ejection behavior [77] and sperm are capable of migrating ectopically beyond the spermatheca which, in inter-species matings, can sterilize hermaphrodites and females and lead to premature death [72]. These effects may act as direct costs to females and contribute to differential optima in mating rates between the sexes.

Box 3

Sexual selection on sperm

Sexual selection by sperm competition in *Caenorhabditis* involves larger sperm outcompeting smaller sperm, owing to their greater speed and ability to dominate occupation of the spermathecal sites of fertilization [72, 122, 123]. Experimentally increased sperm competition risk leads to evolution of larger male sperm, and males of species with hermaphrodites have smaller sperm than their dioecious counterparts, presumably an evolutionary response to the removal of sperm competition among males [121, 124]. Despite the seeming advantage of ever larger sperm, size trades off with other costs such as their rate of production in yielding abundant sperm in ejaculates [122, 125]. Other sperm characteristics besides size also likely influence their competitive ability [126], potentially including chemotactic guidance and oocyte surface interactions [127]. Sperm-associated genes also turnover and evolve more rapidly in sequence than do other kinds of genes [57, 120, 128], which is consistent with rapid evolution by sexual selection.

Sperm and oocytes communicate inside the reproductive tract in ways that also might be shaped by sexual selection. In *C. elegans*, the most mature oocyte secretes prostaglandins that attract sperm to the spermatheca [129]. These attractant compounds must be similar among species because sperm from inter-species matings migrate to the spermathecae [72, 123], although heterogeneity among species suggests the possibility of divergence in attractant signals or sperm responsiveness [72]. In turn, sperm cells secrete a signal protein (major sperm protein, MSP) to trigger ovulation [130]. It remains to be determined how much of this gametic communication might involve conflicts of interest, either directly or indirectly, for example, owing to evolutionary byproducts of among-male sperm competition that has led to divergent interactions of sperm and female reproductive tracts between species [72].

Given the short duration of mating encounters between males and females [78], the female reproductive tract provides a protracted arena for sexual selection owing to post-mating interactions with the ejaculate. The amoeboid sperm cells themselves are well-known subjects of sexual selection in *Caenorhabditis* (Box 3). Males of all species also deposit a mucilaginous mating plug over the vulva of their mating partner [79, 80], although it remains unresolved whether plugs might primarily inhibit mating by subsequent males (male–male competition) or save sperm and seminal fluid from passive loss or ejection from the uterus (male–female mutual benefit or male–female conflict). Relaxed selection on male mating traits in *C. elegans* could be consistent with either scenario to yield the inability of males from many wild strains to deposit a plug, owing to retrotransposon disruption of the mucin gene *plg-1* [79]. Beyond *plg-1*, few other components of the seminal fluid are

known, other than some factors controlling sperm activation [81, 82]. Given the important roles of seminal fluid constituents for other organisms in modulating fertility, female physiology and behavior, and in their involvement in sexual selection [83], understanding seminal fluid composition, function, and evolution represents an important underexploited area of *Caenorhabditis* biology.

How special is evolution in self-fertilizing species?

The triplicate independent origins of *Caenorhabditis* self-fertilization known to date motivate special consideration of evolutionary processes under selfing. This attention is particularly warranted given the intense research efforts on selfing species in worms and plants, and the key contrasts expected with respect to outbreeding species [84, 85]. Selfing intrinsically reduces heterozygosity, but this twofold effect on diversity appears miniscule in the face of empirical findings of orders of magnitude differences in genetic variability [10]. Adaptation by natural selection interacts with selfing in complex ways (Box 2). Strong genetic linkage in the genomes of selfers means that natural selection drastically reduces genetic diversity in long blocks along chromosomes [86], especially in the low-recombination centers of *C. elegans* and *C. briggsae* chromosomes [87, 88]. Such selection at linked sites disrupts even the genomic architecture of functional variation [89, 90]. The diversity-limiting effects of metapopulation dynamics and skewed reproductive distributions are more extreme for selfing species because they amplify the effects of natural selection [37] and because selfing species can successfully colonize a new patch with just a single individual [36]. In spite of such extreme possible founder events upon patch colonization, selfing *Caenorhabditis* generally suffer little to no inbreeding depression [34, 91].

Extremely high selfing rates, as seen in selfing *Caenorhabditis*, imply that the genome will largely be inherited as a linked co-evolving unit. Irregularity in gene flow and recombination facilitates local adaptation [92] and can favor selfing as an extreme form of assortative mating [33], while the rapid production of homozygotes through selfing can allow large-scale genomic rearrangements to accumulate more quickly between lineages [93]. This could foster "co-adapted gene complexes" evolving independently in different selfing populations [94], each accumulating distinct alleles. Distinct alleles fixed in subpopulations by any process, including local adaptation (Box 2), can contribute to Dobzhansky–Muller reproductive incompatibilities caused by unfavorable interaction of two genetic backgrounds [95]. These factors could expedite the accumulation of reproductive isolation between lineages and accelerate subsequent speciation [33]. Sporadic, temporally varying migration episodes might foster divergence: genetic incompatibilities accumulate during intervals of low migration and then negatively feed back to reduce effective gene flow during subsequent periods of higher migration. Relative measures of population differentiation (e.g., F_{st}) are typically greater between local populations of selfing than of outbreeding species [96], and *Caenorhabditis* is no exception (Fig. 3). Indeed, phylogeographic groups of *C. briggsae* show deep genomic divergence [88] and outbreeding depression appears to be relatively common in crosses of distinct strains within selfing *Caenorhabditis* [34, 91, 97], which could be interpreted as a precursor to formation of distinct "good species." Consequently, selfing populations are effectively more "allopatric" and amenable to accumulating differences in a way that could encourage speciation [98].

And yet, phylogenetic evidence does not support diversification and long-term persistence of selfing *Caenorhabditis* lineages [5, 99] (Figure 1). High extinction rates might limit net macroevolutionary diversification in accordance with the "evolutionary dead end" hypothesis for highly selfing lineages [85]. If migration is regular and frequent, then selfing lineages will compete directly and work against differentiation among them, eventually causing some to go extinct. Extinction from metapopulation dynamics also may constrain macroevolutionary diversification [100]. Given the lack of support for extensive deleterious mutation accumulation of selfers driving them extinct through mutational meltdown [101], in general, a greater sensitivity to extinction in the face of frequently changing environmental conditions might provide the most promising explanation for selfing as an "evolutionary dead end" [84]. In sum, a better understanding of the connection between ecological population dynamics, migration, and time would help predict and interpret patterns of evolution for selfing *Caenorhabditis* in particular.

In addition to the consequences of self-fertilization, the forces driving the origins of selfing also require explanation. We can rule out Fisher's "automatic selection" hypothesis, which can apply to plant systems [102], because *Caenorhabditis* hermaphrodites cannot inseminate one another. But do ecological pressures for reproductive assurance provide a continual force that has favored repeated convergent evolution of selfing hermaphroditism [5]? What roles do strong versus weak selfing alleles, inbreeding depression, and instantaneous versus protracted origins play in the genesis and spread of selfing species [102]? Is the low but detectable incidence of outcrossing within selfing *Caenorhabditis* key to their persistence? Research thus far has made more headway on the tractable proximate question of which mechanisms endow females with the ability to self-fertilize, showing independent developmental genetic solutions to the problem of how to make a hermaphrodite [67]. The profound inroads of developmental genetic studies into these mechanisms represent another area that could benefit further integration with evolutionary ecology theory.

On the origin of species

Speciation is the evolution of reproductive isolation between populations, and its study in *Caenorhabditis* has gained momentum only in recent years [103]. The discovery of two sister-species pairs with incomplete isolation permits genetic crossing experiments [16, 104], complementing the long tradition of *Drosophila* speciation genetics [95]. Of particular interest is elucidating the genetic and developmental

underpinnings to such universal "rules of speciation" as Haldane's rule [16, 105, 106], Darwin's corollary to Haldane's rule [35, 107], and the large-X effect [108]. So far, this work has emphasized genetically intrinsic hybrid dysfunction traits such as male sterility and embryonic inviability. Given the power of developmental biology in *Caenorhabditis*, an area ripe for profitable study is synthesis of the developmental trajectory of hybrid dysfunction with evolutionary theory [106]. Further, emerging evidence implicates roles for post-mating pre-zygotic interactions, and perhaps pre-mating isolation, in the evolution of reproductive barriers in *Caenorhabditis* [72, 106]. Evolution of traits associated with both kinds of pre-zygotic barrier likely involve sexual selection and sexual conflict [69]. A fuller understanding of speciation will require dissection of both post-zygotic and pre-zygotic mechanisms of isolation in terms of genetics, selective pressures, and the ecological-dependence that has led to species divergence.

As revealed from inter-species crosses, most *Caenorhabditis* are incapable of forming any fertile hybrids [103], and intrinsic post-zygotic dysfunction is strong even in hybrids of incompletely isolated *C. briggsae-nigoni* and *C. remanei-latens*. Examples of very recent speciation still await detection, but seem plausible, given hints of a "species complex" related to *C. remanei* and *C. latens* [35] and the rapid pace of species discovery [5, 6]. *C. briggsae* provides an intriguing possibility for considering population differentiation as a model for incipient speciation [97] and, upon further investigation, *C. tropicalis* might provide similar opportunities [34]. Outbreeding depression, divergent adaptation, and genomic congealing that separates some populations of *C. briggsae* [18, 88, 97] make it comparable to more established systems for studying incipient speciation [109]. Such work could help relate *Caenorhabditis* evolution to the extensive literature on "ecological speciation" that explicitly connects natural selection to speciation, as in organisms like stickleback fish, Darwin's finches, Caribbean anoles, *Heliconius* butterflies, and *Timema* walking sticks [109]. More broadly, we would like to understand how important adaptive divergence is in creating reproductive incompatibilities as compared to parallel or stabilizing selection that occurs independently on allopatric populations [109]. Similarly, to what extent do selfish genetic elements and genomic conflict contribute to the evolution of reproductive isolation [95, 110]? Many *Caenorhabditis* species are known from just single islands (Fig. 1), suggesting the possibility that divergence of isolated, allopatric populations could play a pivotal role in *Caenorhabditis* diversification. The issues of niche occupation and dispersal dynamics, as outlined in previous sections, in combination with selection and genotype $\times$ environment interactions clearly also must connect intimately to *Caenorhabditis* speciation in ways that we do not yet fully understand.

The growing size of the *Caenorhabditis* phylogeny now permits application of phylogenetic comparative methods to formally test macroevolutionary hypotheses about trait evolution and diversification rates, as commonly applied in other taxa [111]. Developing a better understanding of the traits and ecology that govern species niche space and geographic distributions will be particularly important in such analyses. Integrating population genetic properties into a phylogenetic framework for *Caenorhabditis* also may help elucidate the possible implications of molecular hyperdiversity for speciation [10, 112]. For example, hyperdiversity may complicate inference of species relationships from genealogies, as ancestral polymorphism and genomic islands of divergence can create heterogeneous phylogenetic signal across the genome [10, 112, 113].

Conclusion and outlook

After a decade of concerted natural history discovery in *Caenorhabditis*, the opportunity is now at hand to develop hypothesis-driven research programs to target ecological and evolutionary theory (Box 1). Dispersal dynamics form a glaring gap in our understanding of *Caenorhabditis* biology, and this topic touches on diverse aspects of their ecology and evolution, including scales of spatial and population genetic structure, local versus global adaptation, sexual selection and mating system evolution, range distributions and speciation, and competition. Ongoing research aims to flesh out several of these areas, but competition between species stands out as a topic in need of particular attention, which necessarily also requires a deeper understanding of the multifarious factors that characterize niche space of different species. Niche space defines the ecological distinctiveness of species, which can be integrated with experimental genetics in *Caenorhabditis* to elucidate the molecular causes of divergent traits and divergent genotypic interactions that, in nature, stifle gene flow and maintain species integrity. Traits associated with chemosensation, immunity, food exploitation, and temperature responses all provide especially promising foci for studies of divergent adaptation, but more thorough analysis is required to discover the extent of differentiated axes of niche dimensionality in terms of biotic and abiotic factors. The place that sexual selection plays in nature to foster divergence is especially tantalizing in *Caenorhabditis*, given that typically cryptic phenomena like sperm competition are amenable to experimental evolution and visualization in vivo through the window of the worm's transparent cuticle. Despite the continued need for basic knowledge about natural populations – such as new species discovery and determination of generation time turnover – the devising of manipulative experiments to test fundamental ideas in ecology and evolution represents a new frontier for understanding *Caenorhabditis* evolution in the wild.

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References

1. **Frézal L, Félix M-A.** 2015. C. elegans outside the Petri dish. *eLife* **4**: e05849.
2. **Felix MA, Braendle C.** 2010. The natural history of *Caenorhabditis elegans*. *Curr Biol* **20**: R965–R9.
3. **Kiontke K, Sudhaus W.** 2006. Ecology of Caenorhabditis species. In The *C. elegans* Research Community, ed; *WormBook*. http://www.wormbook.org
4. **Barrière A, Félix M-A.** 2014. Isolation of *C. elegans* and related nematodes. In The *C. elegans* Research Community, ed; *WormBook*: WormBook.
5. **Kiontke K, Felix M-A, Ailion M, Rockman M**, et al. 2011. A phylogeny and molecular barcodes for Caenorhabditis, with numerous new species from rotting fruits. *BMC Evol Biol* **11**: 339.
6. **Felix MA, Braendle C, Cutter AD.** 2014. A streamlined system for species diagnosis in Caenorhabditis (Nematoda: Rhabditidae) with name designations for 15 distinct biological species. *PLoS ONE* **9**: e94723.
7. **Felix M-A, Jovelin R, Ferrari C, Han S**, et al. 2013. Species richness, distribution and genetic diversity of Caenorhabditis nematodes in a remote tropical rainforest. *BMC Evol Biol* **13**: 10.
8. **Sudhaus W, Kiontke K.** 2007. Comparison of the cryptic nematode species *Caenorhabditis brenneri* sp. n. and *C. remanei* (Nematoda: Rhabditidae) with the stem species pattern of the *Caenorhabditis Elegans* group. *Zootaxa* **1456**: 45–62.
9. **Gaertner BE, Phillips PC.** 2010. *Caenorhabditis elegans* as a platform for molecular quantitative genetics and the systems biology of natural variation. *Genet Res* **92**: 331–48.
10. **Cutter AD, Jovelin R, Dey A.** 2013. Molecular hyperdiversity and evolution in very large populations. *Mol Ecol* **22**: 2074–95.
11. **Dey A, Chan CK-W, Thomas CG, Cutter AD.** 2013. Nucleotide hyperdiversity defines populations of *Caenorhabditis brenneri*. *Proc Natl Acad Sci USA* **110**: 11056–60.
12. **Lanfear R, Kokko H, Eyre-Walker A.** 2014. Population size and the rate of evolution. *Trends Ecol Evol* **29**: 33–41.
13. **Felix MA, Duveau F.** 2012. Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol* **10**: 59.
14. **Grewal PS.** 1991. Influence of bacteria and temperature on the reproduction of *Caenorhabditis elegans* (Nematoda, Rhabditidae) infesting mushrooms (*Agaricus bisporus*). *Nematologica* **37**: 72–82.
15. **Amrit FRG, Boehnisch CML, May RC.** 2010. Phenotypic covariance of longevity, immunity and stress resistance in the Caenorhabditis nematodes. *PLoS ONE* **5**: e9978.
16. **Woodruff GC, Eke O, Baird SE, Felix MA**, et al. 2010. Insights into species divergence and the evolution of hermaphroditism from fertile interspecies hybrids of Caenorhabditis nematodes. *Genetics* **186**: 997–1012.
17. **Begasse Maria L, Leaver M, Vazquez F, Grill Stephan W**, et al. 2015. Temperature dependence of cell division timing accounts for a shift in the thermal limits of *C. elegans* and *C. briggsae*. *Cell Reports* **10**: 647–53.
18. **Prasad A, Croydon-Sugarman M, Murray RL, Cutter AD.** 2011. Temperature-dependent fecundity associates with latitude in *Caenorhabditis briggsae*. *Evolution* **65**: 52–63.
19. **Petersen C, Dirksen P, Prahl S, Strathmann E**, et al. 2014. The prevalence of *Caenorhabditis elegans* across 1.5 years in selected North German locations: the importance of substrate type, abiotic parameters, and Caenorhabditis competitors. *BMC Ecol* **14**: 4.
20. **Cutter AD.** 2008. Divergence times in *Caenorhabditis* and *Drosophila* inferred from direct estimates of the neutral mutation rate. *Mol Biol Evol* **25**: 778–86.
21. **Barrière A, Félix M-A.** 2007. Temporal dynamics and linkage disequilibrium in natural *Caenorhabditis elegans* populations. *Genetics* **176**: 999–1011.
22. **Wang X, Chamberlin HM.** 2004. Evolutionary innovation of the excretory system in *Caenorhabditis elegans*. *Nat Genet* **36**: 231–2.
23. **Volkers R, Snoek L, Hubar CJvH, Coopman R**, et al. 2013. Gene-environment and protein-degradation signatures characterize genomic and phenotypic diversity in wild *Caenorhabditis elegans* populations. *BMC Biol* **11**: 93.
24. **Petersen C, Dirksen P, Schulenburg H.** 2015. Why we need more ecology for genetic models such as *C. elegans*. *Trends Genet* **31**: 120–7.
25. **Li S, Jovelin R, Yoshiga T, Tanaka R**, et al. 2014. Specialist versus generalist life histories and nucleotide diversity in Caenorhabditis nematodes. *Proc R Soc B-Biol Sci* **281**: 20132858.
26. **Okumura E, Tanaka R, Yoshiga T.** 2013. Species-specific recognition of the carrier insect by dauer larvae of the nematode *Caenorhabditis japonica*. *J Exp Biol* **216**: 568–72.
27. **Kiontke K.** 1997. Description of *Rhabditis* (*Caenorhabditis*) *drosophilae* n. sp. and *R.* (*C.*) *sonorae* n. sp. (Nematoda: Rhabditida) from saguaro cactus rot in Arizona. *Fundam Appl Nematol* **20**: 305–15.
28. **Olivieri I, Michalakis Y, Gouyon PH.** 1995. Metapopulation genetics and the evolution of dispersal. *Am Nat* **146**: 202–28.
29. **Braendle C, Milloz J, Felix MA.** 2008. Mechanisms and evolution of environmental responses in *Caenorhabditis elegans*. *Curr Top Dev Biol* **80**: 171–207.
30. **Baugh LR.** 2013. To grow or not to grow: nutritional control of development during *Caenorhabditis elegans* L1 arrest. *Genetics* **194**: 539–55.
31. **Lee H, Choi M-k, Lee D, Kim H-s**, et al. 2012. Nictation, a dispersal behavior of the nematode *Caenorhabditis elegans*, is regulated by IL2 neurons. *Nat Neurosci* **15**: 107–12.
32. **Ramalho CE, Hobbs RJ.** 2012. Time for a change: dynamic urban ecology. *Trends Ecol Evol* **27**: 179–88.
33. **Lenormand T.** 2012. From local adaptation to speciation: specialization and reinforcement. *Int J Ecol* **2012**: 11.
34. **Gimond C, Jovelin R, Han S, Ferrari C**, et al. 2013. Outbreeding depression with low genetic variation in selfing Caenorhabditis nematodes. *Evolution* **67**: 3087–101.
35. **Dey A, Jin Q, Chen Y-C, Cutter AD.** 2014. Gonad morphogenesis defects drive hybrid male sterility in asymmetric hybrid breakdown of Caenorhabditis nematodes. *Evol Dev* **16**: 362–72.
36. **Ingvarsson PK.** 2002. A metapopulation perspective on genetic diversity and differentiation in partially self-fertilizing plants. *Evolution* **56**: 2368–73.
37. **Der R, Epstein C, Plotkin JB.** 2012. The dynamics of neutral and selected alleles when the offspring distribution is skewed. *Genetics* **191**: 1331–44.
38. **Simon B, Fletcher JA, Doebeli M.** 2013. Toward a general theory of group selection. *Evolution* **67**: 1561–72.
39. **Chang H-H, Moss EL, Park DJ, Ndiaye D**, et al. 2013. Malaria life cycle intensifies both natural selection and random genetic drift. *Proc Natl Acad Sci USA* **110**: 20129–34.
40. **HilleRisLambers J, Adler PB, Harpole WS, Levine JM**, et al. 2012. Rethinking community assembly through the lens of coexistence theory. *Annu Rev Ecol Evol Syst* **43**: 227–48.
41. **Pritchard JK, Pickrell JK, Coop G.** 2010. The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. *Curr Biol* **20**: R208–R15.
42. **Jovelin R, Ajie BC, Phillips PC.** 2003. Molecular evolution and quantitative variation for chemosensory behaviour in the nematode genus Caenorhabditis. *Mol Ecol* **12**: 1325–37.
43. **Maydan J, Lorch A, Edgley M, Flibotte S**, et al. 2010. Copy number variation in the genomes of twelve natural isolates of *Caenorhabditis elegans*. *BMC Genomics* **11**: 62.
44. **Thomas JH, Kelley JL, Robertson HM, Ly K**, et al. 2005. Adaptive evolution in the SRZ chemoreceptor families of *Caenorhabditis elegans* and *Caenorhabditis briggsae*. *Proc Natl Acad Sci USA* **102**: 4476–81.
45. **Hasshoff M, Bohnisch C, Tonn D, Hasert B**, et al. 2007. The role of *Caenorhabditis elegans* insulin-like signaling in the behavioral avoidance of pathogenic *Bacillus thuringiensis*. *FASEB J* **21**: 1801–12.
46. **Obbard DJ, Gordon KHJ, Buck AH, Jiggins FM.** 2009. The evolution of RNAi as a defence against viruses and transposable elements. *Philos Trans R Soc Lond B-Biol Sci* **364**: 99–115.
47. **Felix MA, Ashe A, Piffaretti J, Wu G**, et al. 2011. Natural and experimental infection of Caenorhabditis nematodes by novel viruses related to nodaviruses. *PLoS Biol* **9**: e1000586.
48. **Schulenburg H, Boehnisch C.** 2008. Diversification and adaptive sequence evolution of Caenorhabditis lysozymes (Nematoda: Rhabditidae). *BMC Evol Biol* **8**: 114.
49. **Gray JC, Cutter AD.** 2014. Mainstreaming *Caenorhabditis elegans* in experimental evolution. *Proc R Soc Lond B-Biol Sci* **281**: 20133055.
50. **Saxena G, Dayal R, Mukerji KG.** 1987. Interaction of nematodes with nematophagous fungi: induction of trap formation, attraction and detection of attractants. *FEMS Microbiol Ecol* **45**: 319–27.
51. **Srinivasan J, Kaplan F, Ajredini R, Zachariah C**, et al. 2008. A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. *Nature* **454**: 1115–8.
52. **Chasnov JR, So WK, Chan CM, Chow KL.** 2007. The species, sex, and stage specificity of a *Caenorhabditis* sex pheromone. *Proc Natl Acad Sci USA* **104**: 6730–5.

53. **Leighton DHW, Choe A, Wu SY, Sternberg PW.** 2014. Communication between oocytes and somatic cells regulates volatile pheromone production in Caenorhabditis elegans. *Proc Natl Acad Sci USA* **111**: 17905–10.
54. **Blaxter ML, Page AP, Rudin W, Maizels RM.** 1992. Nematode surface coats: actively evading immunity. *Parasitol Today* **8**: 243–7.
55. **Svensk E, Stahlman M, Andersson CH, Johansson M**, et al. 2013. PAQR-2 regulates fatty acid desaturation during cold adaptation in *C. elegans*. *PLoS Genet* **9**: e1003801.
56. **Gravato-Nobre MJ, Hodgkin J.** 2005. *Caenorhabditis elegans* as a model for innate immunity to pathogens. *Cell Microbiol* **7**: 741–51.
57. **Cutter AD, Ward S.** 2005. Sexual and temporal dynamics of molecular evolution in *C. elegans* development. *Mol Biol Evol* **22**: 178–88.
58. **Nuez I, Felix MA.** 2012. Evolution of susceptibility to ingested double-stranded RNAs in Caenorhabditis nematodes. *PLoS ONE* **7**: e29811.
59. **Jansson H-B.** 1994. Adhesion of conidia of *Drechmeria coniospora* to *Caenorhabditis elegans* wild type and mutants. *J Nematol* **26**: 430–5.
60. **Reynolds RM, Phillips PC.** 2013. Natural variation for lifespan and stress response in the nematode *Caenorhabditis remanei*. *PLoS ONE* **8**: e58212.
61. **Inoue T, Ailion M, Poon S, Kim HK**, et al. 2007. Genetic analysis of dauer formation in *Caenorhabditis briggsae*. *Genetics* **177**: 809–18.
62. **Narasimhan SD, Yen K, Tissenbaum HA.** 2009. Converging pathways in lifespan regulation. *Curr Biol* **19**: R657–R66.
63. **Van Voorhies WA, Fuchs J, Thomas S.** 2005. The longevity of *Caenorhabditis elegans* in soil. *Biol Lett* **1**: 247–9.
64. **Sterken MG, Snoek LB, Kammenga JE, Andersen EC.** 2015. The laboratory domestication of *Caenorhabditis elegans*. *Trends Genet* **31**: 224–31.
65. **Haag ES, Chamberlin H, Coghlan A, Fitch DH**, et al. 2007. Caenorhabditis evolution: if they all look alike, you aren't looking hard enough. *Trends Genet* **23**: 101–4.
66. **Farhadifar R, Baer Charles F, Valfort A-C, Andersen Erik C**, et al. 2015. Scaling, selection, and evolutionary dynamics of the mitotic spindle. *Curr Biol* **25**: 732–40.
67. **Ellis RE, Lin SY.** 2014. The evolutionary origins and consequences of self-fertility in nematodes. *F1000Prime Rep* **6**: 62.
68. **Félix M-A.** 2012. Evolution in developmental phenotype space. *Curr Opin Genet Dev* **22**: 593–9.
69. **Chapman T, Arnqvist G, Bangham J, Rowe L.** 2003. Sexual conflict. *Trends Ecol Evol* **18**: 41–7.
70. **Garcia LR, LeBoeuf B, Koo P.** 2007. Diversity in mating behavior of hermaphroditic and male-female *Caenorhabditis* nematodes. *Genetics* **175**: 1761–71.
71. **Bahrami AK, Zhang Y.** 2013. When females produce sperm: genetics of *C. elegans* hermaphrodite reproductive choice. *G3 (Bethesda)* **3**: 1851–9.
72. **Ting JJ, Woodruff GC, Leung G, Shin N-R**, et al. 2014. Intense sperm-mediated sexual conflict promotes gametic isolation in Caenorhabditis nematodes. *PLoS Biol* **12**: e1001915.
73. **Woodruff GC, Knauss CM, Maugel TK, Haag ES.** 2014. Mating damages the cuticle of *C. elegans* hermaphrodites. *PLoS ONE* **9**: e104456.
74. **Maures TJ, Booth LN, Benayoun BA, Izrayelit Y**, et al. 2014. Males shorten the life span of *C. elegans* hermaphrodites via secreted compounds. *Science* **343**: 541–4.
75. **Shi C, Murphy CT.** 2014. Mating induces shrinking and death in Caenorhabditis mothers. *Science* **343**: 536–40.
76. **Diaz SA, Haydon DT, Lindstrom J.** 2010. Sperm-limited fecundity and polyandry-induced mortality in female nematodes *Caenorhabditis remanei*. *Biol J Linn Soc* **99**: 362–9.
77. **Kleemann GA, Basolo AL.** 2007. Facultative decrease in mating resistance in hermaphroditic *Caenorhabditis elegans* with self-sperm depletion. *Anim Behav* **74**: 1339–47.
78. **Chasnov JR, Chow KL.** 2002. Why are there males in the hermaphroditic species *Caenorhabditis elegans*? *Genetics* **160**: 983–94.
79. **Palopoli MF, Rockman MV, Tinmaung A, Ramsay C**, et al. 2008. Molecular basis of the copulatory plug polymorphism in *Caenorhabditis elegans*. *Nature* **454**: 1019–22.
80. **Timmermeyer N, Gerlach T, Guempel C, Knoche J**, et al. 2010. The function of copulatory plugs in *Caenorhabditis remanei*: hints for female benefits. *Front Zool* **7**: 28.
81. **Smith JR, Stanfield GM.** 2011. TRY-5 is a sperm-activating protease in *Caenorhabditis elegans* seminal fluid. *PLoS Genet* **7**: e1002375.
82. **Wei Q, Zhao Y, Guo Y, Stomel J**, et al. 2014. Co-option of alternate sperm activation programs in the evolution of self-fertile nematodes. *Nat Comm* **5**: 5888.
83. **Perry JC, Sirot L, Wigby S.** 2013. The seminal symphony: how to compose an ejaculate. *Trends Ecol Evol* **28**: 414–22.
84. **Glemin S, Galtier N.** 2012. Genome evolution in outcrossing versus selfing versus asexual species. *Meth Mol Biol* **855**: 311–35.
85. **Wright SI, Kalisz S, Slotte T.** 2013. Evolutionary consequences of self-fertilization in plants. *Proc Biol Sci* **280**: 20130133.
86. **Cutter AD, Payseur BA.** 2013. Genomic signatures of selection at linked sites: unifying the disparity among species. *Nat Rev Genet* **14**: 262–74.
87. **Andersen EC, Gerke JP, Shapiro JA, Crissman JR**, et al. 2012. Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *Nat Genet* **44**: 285–90.
88. **Thomas CG, Wang W, Jovelin R, Ghosh R**, et al. 2015. Full-genome evolutionary histories of selfing, splitting and selection in Caenorhabditis. *Genome Res* **25**: 667–78.
89. **Rockman MV, Skrovanek SS, Kruglyak L.** 2010. Selection at linked sites shapes heritable phenotypic variation in *C. elegans*. *Science* **330**: 372–6.
90. **Denver DR, Morris K, Streelman JT, Kim SK**, et al. 2005. The transcriptional consequences of mutation and natural selection in *Caenorhabditis elegans*. *Nat Genet* **37**: 544–8.
91. **Dolgin ES, Charlesworth B, Baird SE, Cutter AD.** 2007. Inbreeding and outbreeding depression in *Caenorhabditis* nematodes. *Evolution* **61**: 1339–52.
92. **Rice SH, Papadopoulos A.** 2009. Evolution with stochastic fitness and stochastic migration. *PLoS ONE* **4**: e7130.
93. **Charlesworth B.** 1992. Evolutionary rates in partially self-fertilizing species. *Am Nat* **140**: 126–48.
94. **Templeton AR.** 1986. Coadaptation and outbreeding depression. In Soule M, ed; *Conservation Biology: The Science of Scarcity and Diversity*. Sunderland, MA: Sinauer Associates. p. 105–16.
95. **Presgraves DC.** 2010. The molecular evolutionary basis of species formation. *Nat Rev Genet* **11**: 175–80.
96. **Charlesworth D, Pannell J.** 2001. Mating systems and population genetics structure in light of coalescent theory. In Silvertown J, Antonovics J, eds; *Integrating Ecology and Evolution in a Spatial Context*. Oxford: Blackwell Publishers. p. 73–95.
97. **Baird SE, Stonesifer R.** 2012. Reproductive isolation in *Caenorhabditis briggsae*: dysgenic interactions between maternal- and zygotic-effect loci result in a delayed development phenotype. *Worm* **1**: 189–95.
98. **Levin DA.** 1971. The origin of reproductive isolating mechanisms in flowering plants. *Taxon* **20**: 91–113.
99. **Cutter AD, Wasmuth JD, Washington NL.** 2008. Patterns of molecular evolution in Caenorhabditis preclude ancient origins of selfing. *Genetics* **178**: 2093–104.
100. **Gavrilets S, Acton R, Gravner J.** 2000. Dynamics of speciation and diversification in a metapopulation. *Evolution* **54**: 1493–501.
101. **Loewe L, Cutter AD.** 2008. On the potential for extinction by Muller's ratchet in *Caenorhabditis elegans*. *BMC Evol Biol* **8**: 125.
102. **Barrett SCH, Arunkumar R, Wright SI.** 2014. The demography and population genomics of evolutionary transitions to self-fertilization in plants. *Philos Trans R Soc Lond B-Biol Sci* **369**: 20130344.
103. **Baird SE, Seibert SR.** 2013. Reproductive isolation in the Elegans-Group of Caenorhabditis. *Nat Sci* **5**: 18–25.
104. **Dey A, Jeon Y, Wang G-X, Cutter AD.** 2012. Global population genetic structure of *Caenorhabditis remanei* reveals incipient speciation. *Genetics* **191**: 1257–69.
105. **Baird SE.** 2002. Haldane's rule by sexual transformation in Caenorhabditis. *Genetics* **161**: 1349–53.
106. **Bundus JD, Alaei R, Cutter AD.** 2015. Gametic selection, developmental trajectories and extrinsic heterogeneity in Haldane's rule. *Evolution*, in press.
107. **Kozlowska JL, Ahmad AR, Jahesh E, Cutter AD.** 2012. Genetic variation for post-zygotic reproductive isolation between *Caenorhabditis briggsae* and *Caenorhabditis* sp. 9. *Evolution* **66**: 1180–95.
108. **Bi Y, Ren X, Yan C, Shao J**, et al. 2015. A genome-wide hybrid incompatibility landscape between *Caenorhabditis briggsae* and *C. nigoni*. *PLoS Genet* **11**: e1004993.
109. **Schluter D.** 2009. Evidence for ecological speciation and its alternative. *Science* **323**: 737–41.
110. **Crespi B, Nosil P.** 2013. Conflictual speciation: species formation via genomic conflict. *Trends Ecol Evol* **28**: 48–57.
111. **Symonds ME, Blomberg S.** 2014. A primer on phylogenetic generalised least squares. In Garamszegi LZ, ed; *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*. Berlin Heidelberg: Springer. p. 105–30.
112. **Cutter AD.** 2013. Integrating phylogenetics, phylogeography and population genetics through genomes and evolutionary theory. *Mol Phylogen Evol* **69**: 1172–85.

113. **Cruickshank TE, Hahn MW.** 2014. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Mol Ecol* **23**: 3133–57.
114. **Pannell JR, Barrett SCH.** 1998. Baker's law revisited: reproductive assurance in a metapopulation. *Evolution* **52**: 657–68.
115. **Whitlock MC.** 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. *Evolution* **46**: 608–15.
116. **Theologidis I, Chelo IM, Goy C, Teotonio H.** 2014. Reproductive assurance drives transitions to self-fertilization in experimental *Caenorhabditis elegans*. *BMC Biol* **12**: 93.
117. **Baldi C, Cho S, Ellis RE.** 2009. Mutations in two independent pathways are sufficient to create hermaphroditic nematodes. *Science* **326**: 1002–5.
118. **Glemin S, Ronfort J.** 2013. Adaptation and maladaptation in selfing and outcrossing species: new mutations versus standing variation. *Evolution* **67**: 225–40.
119. **Hereford J.** 2010. Does selfing or outcrossing promote local adaptation? *Am J Bot* **97**: 298–302.
120. **Thomas CG, Li RH, Smith HE, Woodruff GC**, et al. 2012. Simplification and desexualization of gene expression in self-fertile nematodes. *Curr Biol* **22**: 2167–72.
121. **Cutter AD.** 2008. Reproductive evolution: symptom of a selfing syndrome. *Curr Biol* **18**: R1056–R8.
122. **LaMunyon CW, Ward S.** 1998. Larger sperm outcompete smaller sperm in the nematode *Caenorhabditis elegans*. *Proc R Soc Lond B-Biol Sci* **265**: 1997–2002.
123. **Hill KL, L'Hernault SW.** 2001. Analyses of reproductive interactions that occur after heterospecific matings within the genus Caenorhabditis. *Dev Biol* **232**: 105–14.
124. **Palopoli MF, Peden C, Woo C, Akiha K**, et al. 2015. Natural and experimental evolution of sexual conflict within Caenorhabditis nematodes. *BMC Evol Biol* **15**: 93.
125. **Murray RL, Kozlowska JL, Cutter AD.** 2011. Heritable determinants of male fertilization success in the nematode *Caenorhabditis elegans*. *BMC Evol Biol* **11**: 99.
126. **Hansen JM, Chavez DR, Stanfield GM.** 2015. COMP-1 promotes competitive advantage of nematode sperm. *eLife* **4**: e05423.
127. **Maruyama R, Velarde NV, Klancer R, Gordon S**, et al. 2007. EGG-3 regulates cell-surface and cortex rearrangements during egg activation in *Caenorhabditis elegans*. *Curr Biol* **17**: 1555–60.
128. **Artieri CG, Haerty W, Gupta BP, Singh RS.** 2008. Sexual selection and maintenance of sex: evidence from comparisons of rates of genomic accumulation of mutations and divergence of sex-related genes in sexual and hermaphroditic species of Caenorhabditis. *Mol Biol Evol* **25**: 972–9.
129. **Hoang HD, Prasain JK, Dorand D, Miller MA.** 2013. A heterogeneous mixture of F-series prostaglandins promotes sperm guidance in the *Caenorhabditis elegans* reproductive tract. *PLoS Genet* **9**: e1003271.
130. **Miller MA, Nguyen VQ, Lee M-H, Kosinski M**, et al. 2001. A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. *Science* **291**: 2144–7.