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Review

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Mainstreaming *Caenorhabditis elegans* in experimental evolution

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Experimental evolution provides a powerful manipulative tool for probing evolutionary process and mechanism. As this approach to hypothesis testing has taken purchase in biology, so too has the number of experimental systems that use it, each with its own unique strengths and weaknesses. The depth of biological knowledge about *Caenorhabditis* nematodes, combined with their laboratory tractability, positions them well for exploiting experimental evolution in animal systems to understand deep questions in evolution and ecology, as well as in molecular genetics and systems biology. To date, *Caenorhabditis elegans* and related species have proved themselves in experimental evolution studies of the process of mutation, host–pathogen coevolution, mating system evolution and life-history theory. Yet these organisms are not broadly recognized for their utility for evolution experiments and remain underexploited. Here, we outline this experimental evolution work undertaken so far in *Caenorhabditis*, detail simple methodological tricks that can be exploited and identify research areas that are ripe for future discovery.

1. The tool of experimental evolution

Experimental evolution is the controlled study of evolutionary change as it occurs under experimenter-imposed conditions in the laboratory or field. At its most basic, experimental evolution combines two separate procedures: the multi-generation culturing of populations, and the quantification of change in those populations. Its great power is in being a broker between theory and nature. Much of evolutionary theory has been formalized since the early days of the great mathematical geneticists, and yet many aspects of theory have proved difficult to test with traditional experiments or comparative data. Experimental evolution provides a compelling methodological alternative. Despite experimental evolution being a relatively recent paradigm in evolutionary biology, it has now established itself as a powerful method for testing evolutionary theory [1,2].

The first evolution experiment, by Dallinger in 1878, described the adaptation to heat stress by 'a minute septic organism' [3]. In modern times, Lenski *et al*. [4] pioneered and popularized long-term experimental evolution with *Escherichia coli*, which has now been cultured for more than 55 000 generations [2]. This microbial beginning has since metastasized to test a broad variety of theories about the mechanisms of evolution [1,2,5].

Short generation time and laboratory tractability constitute key prerequisites for study organisms in carrying out long-term evolution in a compact period of time. Consequently, the majority of such experiments exploit microbes. Unfortunately, microbes cannot speak to the evolution of the many traits and properties that are unique to eukaryotes and metazoans, such as sexual selection, development and behaviour. Thus, key systems of study have expanded to include fruitflies, plants, fish and mice, among many others [6]. The nematode *Caenorhabditis elegans* and its relatives offer another powerful, but underexploited option for addressing ecological and evolutionary questions with experimental evolution. In this review, we synthesize the current state of what has been learned by applying experimental evolution to *C. elegans* with its potential for establishing new discoveries.

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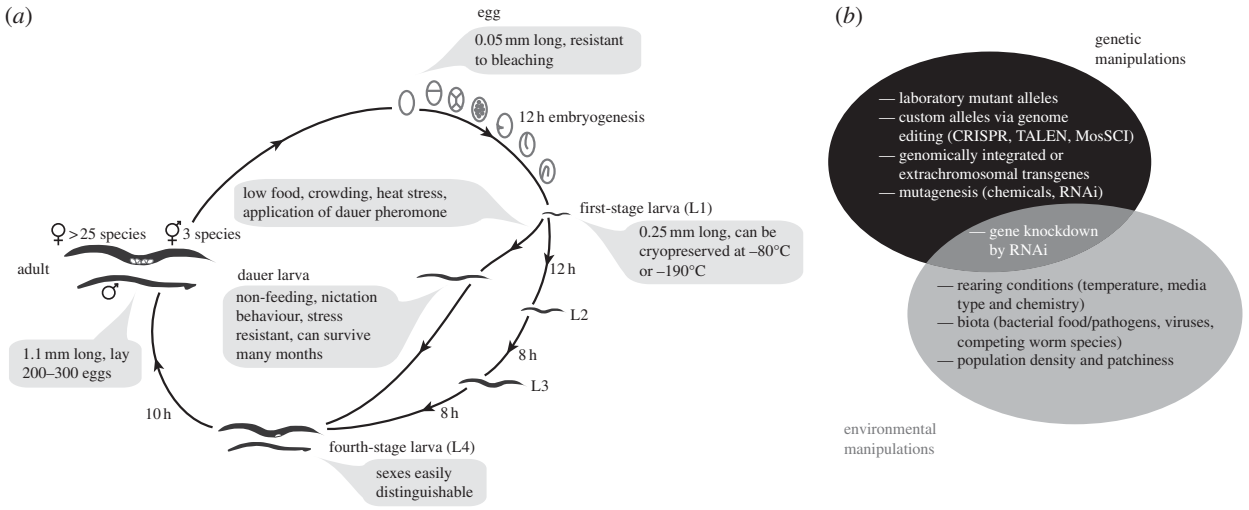

Figure 1. (*a*) Life cycle of *C. elegans* at 25°C, annotated with key life-history features pertinent to experimental study. (*b*) A schematic diagram indicating some of the many genetic and environmental manipulations possible in rearing worms. See main text for more details on methods and examples.

2. Virtues and vices of the *Caenorhabditis elegans* experimental system

Caenorhabditis elegans is one of the supermodels of modern biology. Since its debut in this capacity in 1974 [7], *C. elegans* became the first metazoan to have its genome sequenced [8], its complete cellular developmental pathway has been mapped [9] and its neural connection networks determined [10]. This focus of study has led to the invention and application of many molecular experimental methods, further accelerating *C. elegans* investigations; for example, RNA interference by feeding [11] and green-fluorescent protein expression reporters [12]. Thousands of laboratory mutants with a common genetic background confer experimentally useful phenotypes, each of which is publicly available and can be cryopreserved indefinitely [13]. With the wealth of knowledge and tools gleaned over nearly four decades of study, as well as intriguing organismal biology in its own right, the worm has now expanded into more diverse areas of biology, including evolution and ecology [14].

In the laboratory, the *C. elegans* life cycle can be as short as 50 h, with a single egg hatching into a larva that undergoes four moults before maturing into the adult hermaphrodite that lays around 300 self-fertilized eggs over a period of about a week (figure 1) [15]. *C. elegans*'s lifespan averages two weeks, but crowding and starvation induce a diapause-like 'dauer' stage, in which worms stop feeding and can live for several months. First-stage larvae survive cryopreservation in a glycerol solution, permitting indefinite storage of populations or isogenic strains. Worms grow readily to their adult length of approximately 1 mm when reared on a diet of *E. coli* or on many other bacterial species, or even in an axenic medium. Populations may be reared in liquid culture, on an agar substrate in Petri dishes or in three-dimensional environments.

Caenorhabditis elegans is androdioecious, meaning that self-fertilizing hermaphrodites and rare males comprise natural and laboratory populations. Sex determination is chromosomal, with males being haploid for the X chromosome, but diploid for the five autosomes (hermaphrodites are diploid for all chromosomes). In N2, the commonly used reference strain, males occur in populations at the frequency at which they are expected to arise through X-chromosome non-disjunction in meiosis, although alternative genetic backgrounds and environmental conditions yield males with greater abundance [16]. When mated to a male, a hermaphrodite's sperm stores are supplemented by the male, so that she lays more eggs; outcrossed egg production occurs first owing to precedence of the larger male sperm in fertilization [17,18]. A drawback to this highly selfing sexual system for many purposes is that populations will inbreed at a high rate, creating extensive linkage disequilibrium and diminished heterozygosity in genetically variable populations. This issue can be circumvented by standard genetic modifications that transform hermaphrodites into females [19] or by using one of the many related species of *Caenorhabditis* that outcross obligatorily [20].

Indeed, *C. elegans* is no longer the only player for research in the *Caenorhabditis* genus, which contains at least 26 species in laboratory culture [21]. These other members of the genus have very similar life cycles to *C. elegans* (figure 1). Twelve of these species have had their genomes sequenced (see http://www.nematodes.org/nematodegenomes), permitting comparative methods to analyse development and genetics. The relatively compact size of *Caenorhabditis* genomes (100–150 Mb, approx. 25% of which is coding genes, approx. 17% repetitive DNA) also makes genome sequencing of experimental evolution populations a viable strategy with high-throughput sequencing [22,23]. Only *Caenorhabditis briggsae*, *C.* sp. 11 and *C. elegans* have self-fertilizing hermaphrodites. All other species in the genus share the ancestral gonochoristic (dioecious) reproductive habit with a 1 : 1 ratio of males and females, allowing standard crosses and experimental designs comparable with flies and beetles. Despite the many species, ecological understanding of this genus, as for many model organisms, is still in its infancy [14]. Nevertheless, cryopreserved strain collections from diverse populations around the world and numerous phylogeographic analyses provide the basis for relating experimental evolution to natural variation [24,25]. Ongoing advances in automation, image processing and microfluidics makes possible high-throughput studies for many traits [26]. Chemosensory, behavioural and fitness traits are particularly amenable to such automation. However, the streamlined morphology of all *Caenorhabditis* means that evolutionary study of form is a challenge in these organisms, excepting relatively subtle developmental phenotypes. The simplicity of form

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and behaviour may therefore be viewed as a drawback or as a benefit, depending on the focal question.

The molecular methods available to *C. elegans* researchers are unparalleled. Reversible gene knockdown by RNAi is straightforward in *C. elegans* by simply introducing or removing a plasmid into the food bacteria [11], and RNAi libraries are publicly available to target most genes in the genome. Publicly available gene deletion strains are plentiful, and recent work on TALENs and CRISPR/Cas9 has allowed targeted genome editing to engineer particular alleles in *Caenorhabditis* [27]. These molecular tools offer powerful means of interrogating the outcomes of experimental evolution in subsequent experiments.

3. Experimental evolution paradigms applied to *Caenorhabditis elegans*

Evolution, by definition, requires genetic change over time. Thus, there must be a source of genetic diversity for populations to undergo this change. In experimental evolution, genetic diversity is generally obtained in one of three ways: evolution from standing natural genetic variation, competition experiments between defined alternative alleles or evolution from new mutational input (naturally arising, or artificially elevated). Here, we describe these approaches as applied to *Caenorhabditis* and then point to specific example applications in the next section.

The large number of wild isolates of *C. elegans* available to researchers provides a cross-section of natural variation in the species as starting material with known genome sequence [24,28]. In order to generate diversity for selection to act upon quickly, an arbitrary number of strains can be crossed using a careful design to reduce linkage [19], to be used as a starting population [16,29]; established strain resources are available that were derived in this way [19,30]. Outbreeding species of *Caenorhabditis* harbour even greater genetic variation in traits and DNA, with *Caenorhabditis brenneri* having the highest molecular variation known for any eukaryote [31].

The starting genetic variation is more specific in experiments in which the effect of a single gene is quantified. To do this, an allele of a single gene (e.g. a knockout or gain of function laboratory mutation) with an interesting phenotypic effect can be tested in a common genetic background or introgressed into multiple backgrounds [18,32–34]. The strains can then be allowed to evolve together (figure 2). This approach allows testing of hypotheses about differences in fitness owing to single alleles under alternative controlled environmental conditions, but requires the genetic construction of appropriate tester strains.

It is possible to wait for the slow response to *de novo* mutations from an isogenic ancestor [38], and this is exactly the approach taken by mutation accumulation (MA) experiments to measure the mutation process itself [39]. Alternatively, initially isogenic experimental populations can be stocked with new mutations by chemical mutagenesis [40–43] or with genetics, such as from a knockout of a mismatch repair gene [44,45]. RNAi knockdown of DNA repair genes provides an inducible means of introducing new mutations with an endogenous mutational spectrum [46]. These mutagenic approaches introduce many more new mutations than would occur naturally, allowing evolution to proceed more quickly.

4. Current contributions from *Caenorhabditis elegans* experimental evolution

Experimental evolution studies in *Caenorhabditis* have touched on diverse areas of biology. This work offers improved understandings of general problems in evolution, as well as of particulars about *C. elegans* biology. The first evolution experiment carried out with *C. elegans*, in fact, pre-dates its inception as a modern biological model [38]. Below, we discuss some of the key topics for which *C. elegans* experimental evolution has been important, including host–pathogen coevolution, fundamental mutational properties, mating systems and life-history theory.

(a) Pathogenesis, coevolution and ecological microcosms

To nematode worms, bacteria are simply food, but can be pathogenic upon ingestion. With the amenability of both microbes and worms to experimental evolution, coevolution of host–pathogen dynamics is a natural extension to pursue. Species interactions, environmental structure and multi-species systems are classic areas of theoretical study, and notoriously difficult to investigate from a microbial perspective. *C. elegans*, as a motile metazoan, allows a convenient inroad for these topics.

Different *Caenorhabditis* strains and species vary in their susceptibility to bacterial pathogens, including *Serratia marcescens* [47] and *Bacillus thuringiensis* [29]. This fact has been exploited to test models of host–pathogen dynamics by tracking coevolution between worm host and bacterial pathogen, and then quantifying responses to selection imposed on each of them. For example, trade-offs evolve between worm growth rates and resistance to pathogens [29,48], local adaptation occurs between pathogens and hosts [49,50], and outcrossing sex increases during host–pathogen coevolution [51–53]. With the recent discovery of viruses that infect natural populations of *C. elegans* and *C. briggsae* [54], viral coevolution experiments provide a further dimension to test these and related hypotheses.

In addition to coevolution, multi-species experiments can test ecologically motivated questions as well. A tri-trophic microcosm comprising *C. elegans*, *Pseudomonas syringae* and phage $\Phi 6$ has been developed to study simple ecosystem interactions [55]. Dispersal is an ecologically important trait, and a system has been developed and modelled in *C. elegans* to describe competition between strains that differ in dispersal rates under different ecological scenarios, made possible by virtue of specific alleles that affect motility and fertility [56,57]. By competing in a patchy environment, the experiments showed that environmental variation can favour the evolution of increased dispersal tendencies and that alternative dispersal strategies can coexist under intermediate rates of environmental disturbance [56,57]. Fragmented environments have also been used to test hypotheses about balancing selection for the maintenance of genetic variation within a population, specifically associated with genetic control of feeding strategies [33]. While Petri dishes provide a relatively uniform environment, imposing physical barriers to dispersal has allowed study of dispersal propensity [52] and construction of artificial dirt permits worms to perform behaviour in a more realistic three-dimensional environment

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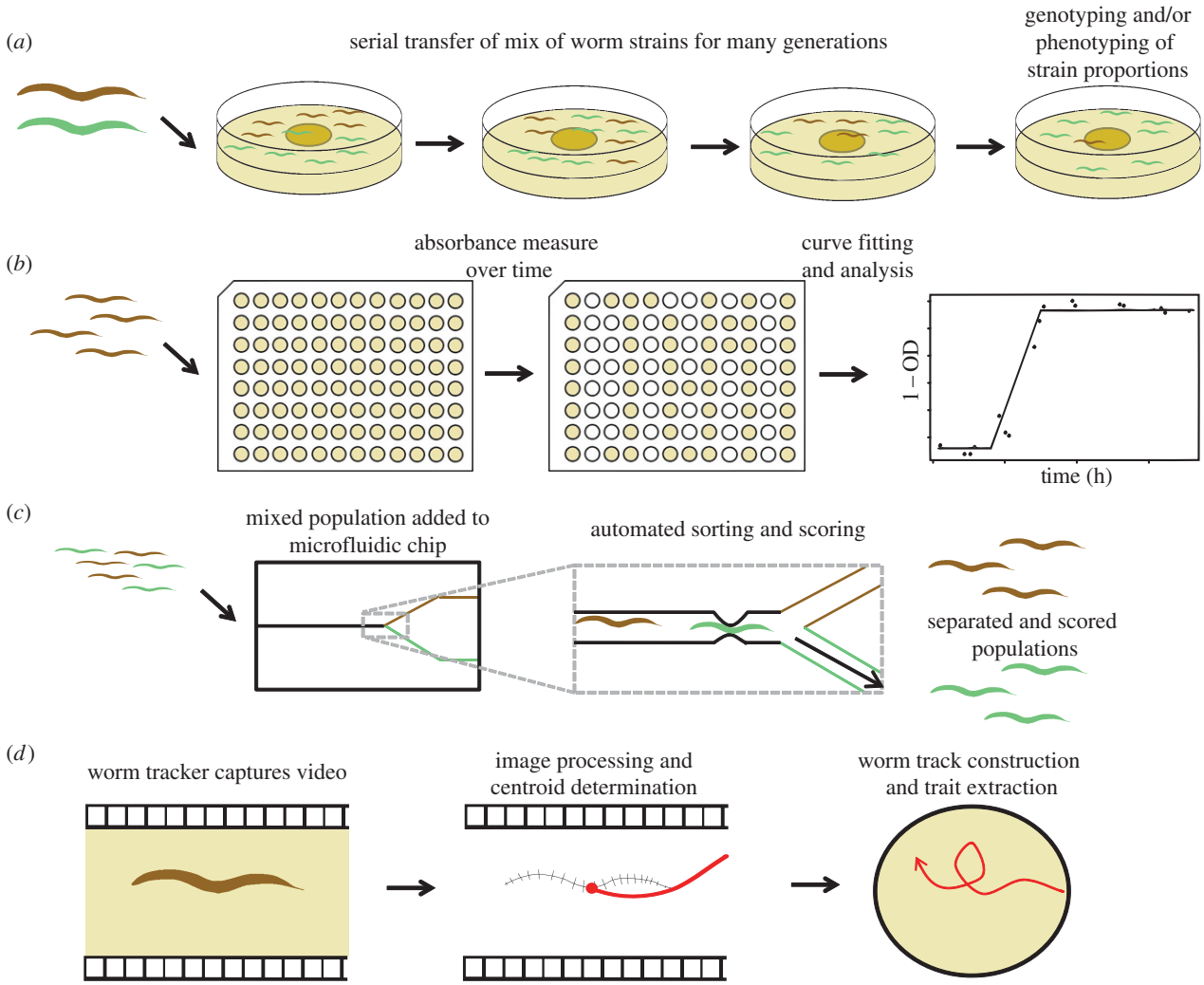

Figure 2. Example fitness and phenotyping assays in *Caenorhabditis*. (*a*) Competitive fitness on Petri dishes (or in liquid). Two strains of worm are inoculated on each plate and serially transferred. Change in frequency of strains is determined over time by the use of a fluorescent transgenic marker [18,32]. (*b*) Non-competitive liquid fitness assays. Worms are inoculated into wells seeded with bacterial food in high-throughput microtitre plates. Absorbance change is measured over time and growth parameters determined for each well [35,36]. Alternatively, worms in liquid culture can be put through a biosorter to count, measure and sort the animals. (*c*) Microfluidic devices have been designed for worm sorting and phenotyping [37]. For example, worms are added to a microfluidic chip and stopped at a junction. Worms can be automatically scored for length, or sorted by fluorescence or another trait ranging from chemotaxis to fecundity [37]. (*d*) Animal behaviour also can be assessed in relatively high-throughput assays. For example, videos of worm movement when added to a plate with a chemoattractant or environmental gradient permit worm-tracking equipment or software [26], coupled with image processing, to determine and track the skeleton of the worm. Worm trajectories can then be quantified in terms of speed, curvature and other features. (Online version in colour.)

[58]. The inclusion of multiple species and environmental heterogeneity into experimental designs suggests tantalizing opportunities to test theories about predator–prey dynamics, competition, biodiversity and ecosystem function.

(b) Understanding mutation

One of the most commonly undertaken applications in *C. elegans* experimental evolution has been for MA. This work has yielded great progress into characterizing the fundamental mutational parameters that underlie evolutionary theory to describe the rate, fitness effects, biases and types of mutation [39].

Keightley & Caballero [59] started MA in *C. elegans* by repeatedly bottlenecking to a single individual for 60 generations. They found that reproductive output declined by 0.03% per generation. Recent studies have expanded to use whole-genome sequencing of strains to quantify mutation rates directly after more than 300 generations of MA [22,23], as well as permutations on the MA scheme, including manipulations of mutation rate, the per generation bottleneck size, environmental conditions, natural genetic background of strain founders and focal species (table 1).

In addition to the accumulation of new mutations, some studies have explored the clearance of and interactions between mutations. For example, experiments using different population sizes confirmed theory that smaller populations fix more detrimental mutations [45]. The dynamics of recovery from MA have been explored, showing that epistatic compensatory mutations dominate [80]. Investigation of the clearance of mutations at differing mutation rates has found that an increase in mutation rate can lead to a paradoxical increase in fitness [43]. Some theory predicts gonochoristic species to have higher mutation rates than selfing species, and results appear to support this [68]. Different androdioecious species also have been tested for mutation rate differences, although the conclusions regarding species differences vary depending on how mutation rate is measured [23,69]. Further work with MA should focus on the

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Table 1. Major hypotheses tested with experimental evolution in *Caenorhabditis*.

topic	key question or idea	finding	references
coevolution	is there genetic diversity for pathogen resistance?	found for resistance to *S. marsecens*, *B. thuringensis*, *P. luminscens*	[29,47,60]
	does evolution of resistance to pathogens have trade-offs?	an increase in resistance, but reductions in growth and feeding rate	[29,48]
	does coevolution and local adaptation occur between host and pathogen?	populations showed higher resistance to their own pathogens and genetic diversity between populations increased	[49,50]
	red queen hypothesis	outcrossed sex allowed faster adaptation to parasites	[51–53]
population structure and ecosystems	can ecosystems be constructed?	three species interactions and dependencies	[55]
	is dispersal beneficial in varying environments?	dispersal is beneficial under random extinction, can be regulated by a single gene and can be selected for	[33,42,56,57]
effects and accumulation of mutations	how do traits evolve with mutation accumulation (MA)?	fitness, body size, behaviour, oxidizing state and other traits degrade	[59,61–65]
	does fitness recover after MA?	restoring selection, or greatly increasing mutation rate leads to fitness recovery	[43,66]
	what is the rate and spectrum of new mutations?	many mutations identified after 396 generations of MA by genome sequencing	[22,23,67]
	do mutation properties differ among genetic backgrounds, species or environments?	different strains, species and conditions do or do not have differing mutation profiles	[23,68–71]
mating systems	are males evolutionary relics?	males reduce in frequency under the lack of selection, depending on the strain and genetic background	[16,41,72,73]
	does outcrossing sex promote removal of detrimental mutations?	male frequencies increased under higher mutational loads	[41,44]
	does outcrossing sex accelerate adaptation?	male frequencies increased under directional selection	[42,51,52,74]
	does outcrossing sex help retain heterozygosity?	no difference between reproductive modes, balancing selection dominates	[30]
	do inbreeding and outbreeding depression depend on reproductive mode?	inbreeders showed outbreeding depression and vice versa	[75]
	how does sexual selection by sperm competition evolve?	competition led to larger sperm and restored male sexual function	[34,76,77]
life history	does increased lifespan have pleiotropic costs?	fewer offspring for longer lived worms in one study, but not in another	[78,79]
	is individual vigour linked to lifespan?	selection for good condition worms led to longer lifespans	[20]
	how does selection affect reproductive life-history trade-offs?	selection between faster generation times and offspring number changed the trade-off	[18]

distribution of selective effects of mutations, and the relative rate of beneficial and detrimental mutations.

(c) Mating systems

One especially appealing aspect of *Caenorhabditis* biology is the existence of distinct mating systems among species, and the ability to manipulate mating and sex determination systems in *C. elegans* using both genetics and exogenous treatments (e.g. RNAi, temperature) [81,82]. The evolution of sex is a long-standing problem in evolutionary biology [83], having attracted many experimental evolution studies in a variety of organisms [84]. While *Caenorhabditis* experiments cannot contrast sex versus asex, studies have investigated the related problem of selfing versus outcrossing [16]. This work also has attempted to explain the incidence of males in populations, which relates directly to the frequency of outcrossed reproduction, and the effect of those outcrossing males on adaptation.

Why do functional males still occur in *C. elegans*, given that they are not strictly necessary for reproduction? Males

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are rare in natural collections [85,86], and both modelling and multi-generation experiments based on the standard N2 laboratory strain indicate rapid loss of males from populations, suggesting that they are not necessary for population survival [72,86–88]. However, the males of the N2 strain seem to have particularly low sexual vigour compared with other wild isolates and males of other species [73,89,90]. Experimental evolution under elevated mutation rates, either endogenously via mismatch repair mutants or exogenously via chemical mutagenesis, results in males and outcrossing persisting for longer durations within populations over time [41,44]. Moreover, different genetic backgrounds allow greater male persistence and outcrossing across generations [16,41].

Theory also predicts that outcrossing sex will accelerate the rate of adaptation under directional selection [91]. Experimental evolution using starvation stress [92], directional selection [42,74] and coevolution with pathogens [51,52] all showed that outcrossing sex was favoured over selfing under these conditions. Experiments with alternative reproductive modes also suggest a role for balancing selection maintaining variation in an experimental setting [30].

The differing reproductive strategies among closely related species of *Caenorhabditis* allow comparative insights into reproductive behaviour. For example, wild isolates of *Caenorhabditis remanei*, a gonochoristic species, show strong inbreeding depression (and a lack of purging of deleterious mutations) when propagated over 13 generations, whereas the highly selfing *C. elegans* and *C.* sp. 11 yield outbreeding depression consistent with the presence of 'coadapted gene complexes' [75,93].

Sexual selection also is a long-standing area of study in evolutionary biology, and *C. elegans* provides a prime underexploited system for investigation. One study explored sperm competition: in *C. elegans*, large sperm are competitively superior [76]. When *C. elegans* populations were forced to reproduce by outcrossing, which imposed male–male sperm competition, selection appears to have driven the evolution of larger males and males making larger sperm [34,94]. Natural genetic variation exists for a variety of mating traits, upon which experimental selection pressures could act [18,90]. The sex determination pathway of *C. elegans* is well understood [95], and mutations in this pathway can produce intersex individuals. Worms which were intersex owing to mutations in *tra-2* and *xol-1* re-evolved high levels of sexual dimorphism in response to selection over the course of 50 generations, shedding light into the developmental evolution of sexual dimorphism [77]. Although species differ in attractiveness to female mating pheromones and mating propensity [89], many aspects of worm mating remain to be studied from an evolutionary perspective.

(d) Ageing and life history

Caenorhabditis elegans has become a model system for ageing research [96], and this is a fruitful target for selection experiments. Lifespan has long been predicted to show a trade-off between long life and faster reproduction [97], and much work attempts to determine whether this prediction holds true generally. Worms are particularly attractive for this, as they show ageing and senescence, unlike microbes, but have life cycles on the order of weeks (rather than years, as is common in many animals).

A strain of *C. elegans* carrying an allele of the *age-1* gene—known to increase lifespan, but also leading to increased dauer formation—showed no cost of increased lifespan under benign conditions [78]. However, upon cyclical starvation, the long-lived worms rapidly declined in frequency in experimental populations owing to dauer formation, indicating that there was an antagonistic pleiotropic effect to the longer lifespan conferred by *age-1*. In other work, lifespan and reproduction were again probed by selecting for early offspring [79]. As expected, early offspring numbers increased and late offspring decreased. Yet lifespan did not show a corresponding decline, casting doubt on the idea of antagonistic pleiotropy between early- and late-acting genetic effects. Some recent theory posits that while high mortality will select for a decrease in lifespan, if mortality is condition-dependent, then longer lifespans will evolve [98]. To test this, *C. remanei* populations were subjected to condition-dependent selection, with worms dying either randomly or following heat stress, which preferentially kills low-condition worms [20]. The populations subjected to random mortality evolved a reduced lifespan, as expected from classic theory, but those with condition-dependent mortality evolved longer lifespans, consistent with the updated theory. The wealth of data on the molecular mechanisms of ageing in the worm offers a possibility of uniting both new and long-standing theory with evolutionary process and molecular function.

In *C. elegans* hermaphrodites, reproduction is often sperm-limited [17]. This is due to sperm being produced before a switch to oocyte production, leading to a trade-off between number of sperm produced and the earliest time at which fertilized eggs can be laid. Mutations known to influence sperm number have been competed in order to test theory about this fitness trade-off between early reproduction and total lifetime reproduction [18,99]. These examples illustrate the broad range of life-history trade-offs that are tractable for study by experimental evolution in the worm.

5. Prospects

Experimental evolution research in *C. elegans* has just scratched the surface of what is possible. *Caenorhabditis elegans* is an enviable research model in many respects, with the benefits of short life cycle and laboratory amenability combined with the trappings of a higher eukaryote, giving it a superb potential as an experimental system for evolutionary studies. Here, we highlight a few areas that are ripe for interrogation, or in need of development (table 2), in addition to the suite of topics commonly addressed by experimental evolution in other organisms [2].

(a) Sexual selection and behavioural evolution

Tests of ideas about sexual selection remain largely unexplored in *Caenorhabditis*, despite the sperm competition work described above. Worms have many compelling features for investigating sexual selection and sexual conflict: sperm size differences in sperm competition, mating plugs, mating pheromones, plastic re-mating propensities. Mating is one of the most cognitively demanding procedures undertaken by male worms [105], and elucidating and modifying the mate recognition and decision systems by evolution would allow a fascinating insight into behaviour. Worms perform both learned and stereotyped behavioural responses, albeit simple from an anthropocentric view [106]. The complete neural network has been mapped, and worm behaviour on a

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Table 2. Topics in need of development using *Caenorhabditis* experimental evolution.

topic	comments
speciation	The recently discovered species in the genus that can be hybridized in the laboratory offer the opportunity for an experimental insight into speciation [100–103]. Laboratory experiments selecting for reproductive isolation and reinforcement have a long heritage in *Drosophila*, and work in *Caenorhabditis* can complement this.
repeatability of evolution	*Caenorhabditis* is a large genus and a large number of species allows a comparative approach. While recent studies have tested the repeatability of evolution [104], this has occurred in a single ancestral genotype. Analysis of the genetic change during adaptation in multiple closely related species would provide a fascinating insight into evolution in different genetic backgrounds. Comparative experimental evolution work on genomic mutation rates [23] and sexual systems [72,75,77] also will be extremely valuable. The diverse reproductive strategies have already been exploited for mutation studies, and adaptive evolution is an obvious next direction to explore in detail.
ecological theory	Host–parasite coevolution is, and will be, a prosperous area for *C. elegans* experimental evolution [49,52]. But this is just one possible ecosystem. The literature on ecosystem dynamics and predator prey interactions is vast, and the systems developed with nematodes offer a way to empirically test some of these models. Promising topics include dispersal, predator–prey/consumer–resource dynamics, environmental variability and maintenance of biodiversity.

two-dimensional plane (agar plates) offers a simple environment in which to quantify behavioural evolution via automated video image processing. In addition, there are many relevant *C. elegans* manipulative genetic tricks, and the transparent cuticle allows direct visualization of the reproductive tract contents [107], permitting a diversity of options for experimental evolution and follow-up study of molecular mechanism about evolutionary responses.

(b) Genotype–phenotype mapping

A persistent goal in evolutionary genetics is the mapping of natural phenotypic differences to their genetic causes. Methods such as bulk segregants analysis or X-QTL combine selection and evolution with genotyping, allowing the elucidation of this link. The recent construction and genome sequencing of more than 2000 mutagenized and wild strains of *C. elegans* provides a compelling substrate for experimental evolution and for connecting genotype to phenotype [28]. Until recent development of automated high-throughput phenotyping [35,36], fitness assays have not been as easy or as powerful as in many microbial systems. As these techniques are refined, more individuals and populations can be assayed for fitness, allowing better detection of phenotypic trait differences, fitness effects, adaptive trajectories and connection to real-world ecologies.

(c) Integration with systems biology

The developmental genetics of *C. elegans* has been exceptionally well characterized from traditional molecular genetic approaches. The work of Chandler *et al*. [77] shows the promise of evolutionary inroads into molecular and systems biology. By altering one or a few genes, and then allowing compensatory mutations to evolve through experimental evolution, one can generate targets for subsequent molecular genetic analysis. In addition to addressing intriguing hypotheses about the evolution of development and genetic networks, this approach provides a complementary method to quantitative genetics (e.g. QTL mapping, GWAS) and standard forward and reverse genetics (e.g. mutagenesis screens) to understand gene–phenotype mapping and molecular mechanisms.

6. Concluding remarks

Recent years have seen increasing adoption of *Caenorhabditis* in experimental evolution, but it is not yet mainstream. So far, the major thrusts in *C. elegans* experimental evolution have targeted understanding mutational properties, host–pathogen coevolution and mating system evolution. Given the experimental tractability and toolkit conferred on *C. elegans* by decades of development for biomedical research, and the compelling details of its organismal biology, the *Caenorhabditis* system is primed to address topical issues throughout the disciplines of evolution and ecology with experimental evolution (tables 1 and 2). The promise of high-throughput phenotyping and genome sequencing of experimental populations, coupled with multi-species systems, can rapidly help connect evolutionary process and mechanism.

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