



Review

Cite this article: Gray JC, Cutter AD. 2014
Mainstreaming *Caenorhabditis elegans*
in experimental evolution. *Proc. R. Soc. B* **281**:
20133055.
<http://dx.doi.org/10.1098/rspb.2013.3055>

Received: 21 November 2013

Accepted: 10 December 2013

Subject Areas:

evolution, ecology

Keywords:

experimental evolution, *Caenorhabditis*,
evolution

Author for correspondence:

Asher D. Cutter

e-mail: asher.cutter@utoronto.ca

Mainstreaming *Caenorhabditis elegans* in experimental evolution

Jeremy C. Gray and Asher D. Cutter

Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2

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.

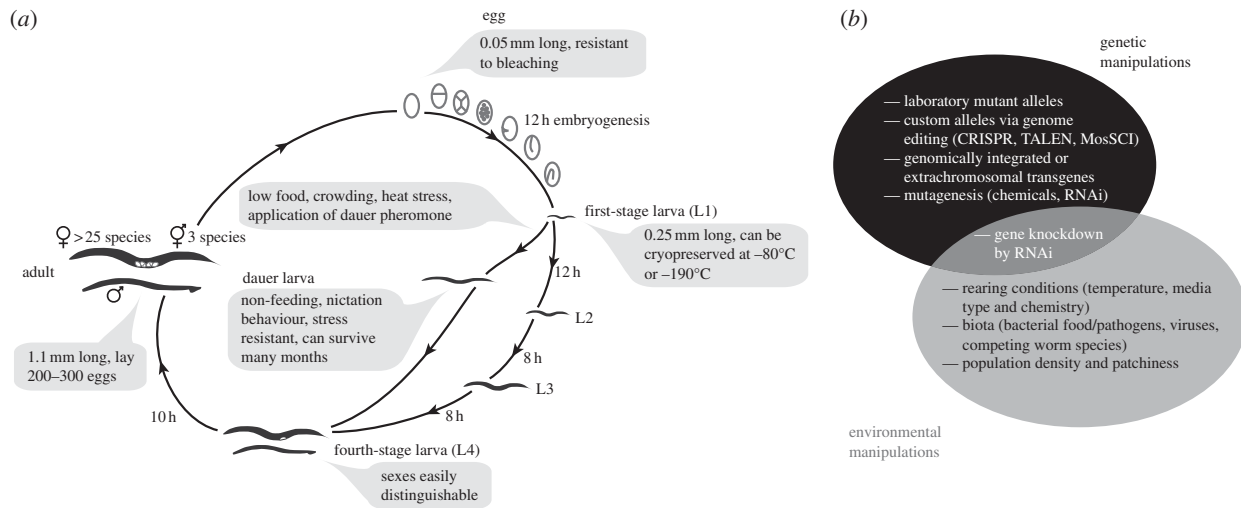


Figure 1. (a) Life cycle of *C. elegans* at 25°C, annotated with key life-history features pertinent to experimental study. (b) A schematic diagram indicating some of the many genetic and environmental manipulations possible in rearing worms. See main text for more details on methods and examples.

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; out-crossed 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

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

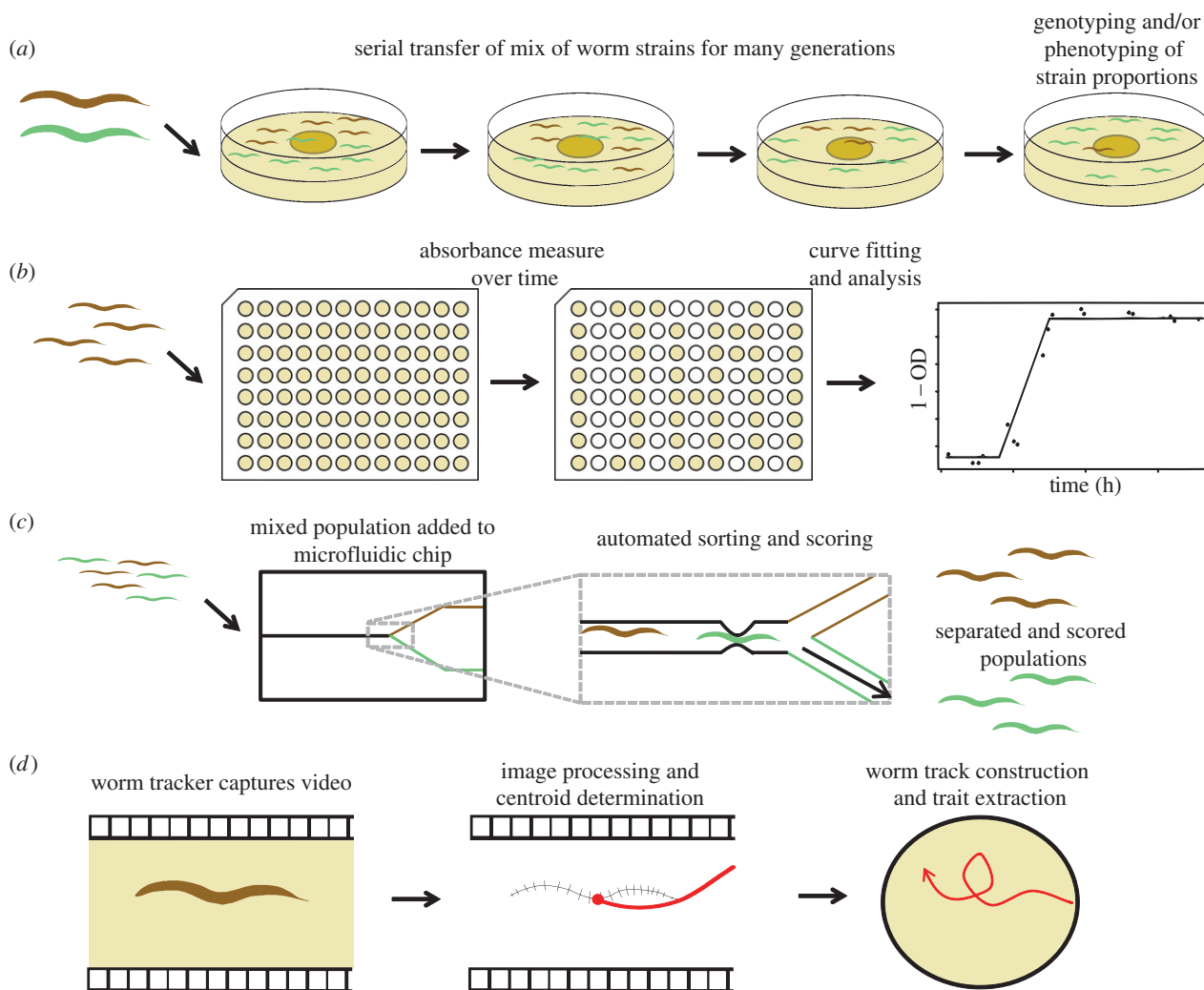


Figure 2. Example fitness and phenotyping assays in *Caenorhabditis*. (a) Competitive fitness on Petri dishes (or in liquid). Two strains of worm are inoculated on each plate and serially transferred. Change in frequency of strains is determined over time by the use of a fluorescent transgenic marker [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

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 <i>S. marsecens</i> , <i>B. thuringensis</i> , <i>P. luminscens</i>	[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
	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

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 under-exploited 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

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 <i>Drosophila</i> , and work in <i>Caenorhabditis</i> can complement this.
repeatability of evolution	<i>Caenorhabditis</i> 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 <i>C. elegans</i> 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* 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.

Funding statement. A.D.C. is supported by funds from the Natural Sciences and Engineering Research Council of Canada, the United States' National Institutes of Health, and a Canada Research Chair.

References

- Buckling A, MacLean RC, Brockhurst MA, Colegrave N. 2009 The beagle in a bottle. *Nature* **457**, 824–829. (doi:10.1038/nature07892)
- Kawecki TJ, Lenski RE, Ebert D, Hollis B, Olivieri I, Whitlock MC. 2012 Experimental evolution. *Trends Ecol. Evol.* **27**, 547–560. (doi:10.1016/j.tree.2012.06.001)
- Dallinger WH. 1878 On the life-history of a minute septic organism: with an account of experiments made to determine its thermal death point. *Proc. R. Soc. Lond.* **27**, 332–350. (doi:10.1098/rspl.1878.0055)
- Lenski RE, Rose MR, Simpson SC, Tadler SC. 1991 Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000

- generations. *Am. Nat.* **138**, 1315–1341. (doi:10.1086/285289)
5. Garland TJ, Rose MR. 2009 *Experimental evolution: concepts, methods, and applications of selection experiments*. Berkeley, CA: University of California Press.
 6. Bell G. 2008 *Selection: the mechanism of evolution*, 2nd edn. Oxford, UK: Oxford University Press.
 7. Brenner S. 1974 The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.
 8. *C. elegans* Sequencing Consortium. 1998 Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018. (doi:10.1126/science.282.5396.2012)
 9. Sulston JE, Horvitz HR. 1977 Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110–156. (doi:10.1016/0012-1606(77)90158-0)
 10. White JG, Southgate E, Thomson JN, Brenner S. 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond. B* **314**, 1–340. (doi:10.1098/rstb.1986.0056)
 11. Timmons L, Fire A. 1998 Specific interference by ingested dsRNA. *Nature* **395**, 854–854. (doi:10.1038/27579)
 12. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. 1994 Green fluorescent protein as a marker for gene expression. *Science* **263**, 802–805. (doi:10.1126/science.8303295)
 13. Stiernagle T. 2006 Maintenance of *C. elegans*. *WormBook*, 11 February. (doi:10.1895/wormbook.1.101.1)
 14. Félix M-A, Braendle C. 2010 The natural history of *Caenorhabditis elegans*. *Curr. Biol.* **20**, R965–R969. (doi:10.1016/j.cub.2010.09.050)
 15. Wood WB. 1988 *The nematode Caenorhabditis elegans*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
 16. Anderson JL, Morran LT, Phillips PC. 2010 Outcrossing and the maintenance of males within *C. elegans* populations. *J. Hered.* **101**, S62–S74. (doi:10.1093/jhered/esq003)
 17. Ward S, Carrel JS. 1979 Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Dev. Biol.* **73**, 304–321. (doi:10.1016/0012-1606(79)90069-1)
 18. Murray RL, Cutter AD. 2011 Experimental evolution of sperm count in protandrous self-fertilizing hermaphrodites. *J. Exp. Biol.* **214**, 1740–1747. (doi:10.1242/jeb.053181)
 19. Teotónio H, Carvalho S, Manoel D, Roque M, Chelo IM. 2012 Evolution of outcrossing in experimental populations of *Caenorhabditis elegans*. *PLoS ONE* **7**, e35811. (doi:10.1371/journal.pone.0035811)
 20. Chen H, Maklakov AA. 2012 Longer life span evolves under high rates of condition-dependent mortality. *Curr. Biol.* **22**, 2140–2143. (doi:10.1016/j.cub.2012.09.021)
 21. Kiontke KC, Félix M-A, Ailion M, Rockman MV, Braendle C, Pénigault J-B, Fitch DH. 2011 A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. *BMC Evol. Biol.* **11**, 339. (doi:10.1186/1471-2148-11-339)
 22. Denver DR *et al.* 2009 A genome-wide view of *Caenorhabditis elegans* base-substitution mutation processes. *Proc. Natl Acad. Sci. USA* **106**, 16 310–16 314. (doi:10.1073/pnas.0904895106)
 23. Denver DR, Wilhelm LJ, Howe DK, Gafner K, Dolan PC, Baer CF. 2012 Variation in base-substitution mutation in experimental and natural lineages of *Caenorhabditis* nematodes. *Genome Biol. Evol.* **4**, 513–522. (doi:10.1093/gbe/evs028)
 24. Andersen EC, Gerke JP, Shapiro JA, Crissman JR, Ghosh R, Bloom JS, Félix M-A, Kruglyak L. 2012 Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *Nat. Genet.* **44**, 285–290. (doi:10.1038/ng.1050)
 25. Félix M-A, Jovelín R, Ferrari C, Han S, Cho YR, Andersen EC, Cutter AD, Braendle C. 2013 Species richness, distribution and genetic diversity of *Caenorhabditis* nematodes in a remote tropical rainforest. *BMC Evol. Biol.* **13**, 10. (doi:10.1186/1471-2148-13-10)
 26. Husson SJ. 2012 Keeping track of worm trackers. *WormBook*, 10 September. (doi:10.1895/wormbook.1.156.1)
 27. Frøkjær-Jensen C. 2013 Exciting prospects for precise engineering of *Caenorhabditis elegans* genomes with CRISPR/Cas9. *Genetics* **195**, 635–642. (doi:10.1534/genetics.113.156521)
 28. Thompson O *et al.* 2013 The million mutation project: a new approach to genetics in *Caenorhabditis elegans*. *Genome Res.* **23**, 1749–1762. (doi:10.1101/gr.157651.113)
 29. Schulte RD, Makus C, Hasert B, Michiels NK, Schulenburg H. 2010 Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. *Proc. Natl Acad. Sci. USA* **107**, 7359–7364. (doi:10.1073/pnas.1003113107)
 30. Chelo IM, Teotónio H. 2013 The opportunity for balancing selection in experimental populations of *Caenorhabditis elegans*. *Evolution* **67**, 142–156. (doi:10.1111/j.1558-5646.2012.01744.x)
 31. Cutter AD, Jovelín R, Dey A. 2013 Molecular hyperdiversity and evolution in very large populations. *Mol. Ecol.* **22**, 2074–2095. (doi:10.1111/mec.12281)
 32. Duveau F, Félix M-A. 2012 Role of pleiotropy in the evolution of a cryptic developmental variation in *Caenorhabditis elegans*. *PLoS Biol.* **10**, e1001230. (doi:10.1371/journal.pbio.1001230)
 33. Gloria-Soria A, Azevedo RBR. 2008 *npr-1* regulates foraging and dispersal strategies in *Caenorhabditis elegans*. *Curr. Biol.* **18**, 1694–1699. (doi:10.1016/j.cub.2008.09.043)
 34. LaMunyon CW, Ward S. 2002 Evolution of larger sperm in response to experimentally increased sperm competition in *Caenorhabditis elegans*. *Proc. R. Soc. Lond. B* **269**, 1125–1128. (doi:10.1098/rspb.2002.1996)
 35. Elvin M, Snoek LB, Frejno M, Klemstein U, Kammenga JE, Poulin GB. 2011 A fitness assay for comparing RNAi effects across multiple *C. elegans* genotypes. *BMC Genomics* **12**, 510. (doi:10.1186/1471-2148-12-510)
 36. Ramani AK, Chuluunbaatar T, Verster AJ, Na H, Vu V, Pelte N, Wannissorn N, Jiao A, Fraser AG. 2012 The majority of animal genes are required for wild-type fitness. *Cell* **148**, 792–802. (doi:10.1016/j.cell.2012.01.019)
 37. Shi W, Wen H, Lin B, Qin J. 2011 Microfluidic platform for the study of *Caenorhabditis elegans*. *Top. Curr. Chem.* **304**, 323–338. (doi:10.1007/128_2011_145)
 38. Brun J. 1965 Genetic adaptation of *Caenorhabditis elegans* (Nematoda) to high temperatures. *Science* **150**, 1467–1467. (doi:10.1126/science.150.3702.1467)
 39. Baer CF, Miyamoto MM, Denver DR. 2007 Mutation rate variation in multicellular eukaryotes: causes and consequences. *Nat. Rev. Genet.* **8**, 619–631. (doi:10.1038/nrg2158)
 40. Flibotte S *et al.* 2010 Whole-genome profiling of mutagenesis in *Caenorhabditis elegans*. *Genetics* **185**, 431–441. (doi:10.1534/genetics.110.116616)
 41. Manoel D, Carvalho S, Phillips PC, Teotónio H. 2007 Selection against males in *Caenorhabditis elegans* under two mutational treatments. *Proc. R. Soc. B* **274**, 417–424. (doi:10.1098/rspb.2006.3739)
 42. Morran LT, Parmenter MD, Phillips PC. 2009 Mutation load and rapid adaptation favour outcrossing over self-fertilization. *Nature* **462**, 350–352. (doi:10.1038/nature08496)
 43. Morran LT, Ohdera AH, Phillips PC. 2010 Purging deleterious mutations under self fertilization: paradoxical recovery in fitness with increasing mutation rate in *Caenorhabditis elegans*. *PLoS ONE* **5**, e14473. (doi:10.1371/journal.pone.0014473)
 44. Cutter AD. 2005 Mutation and the experimental evolution of outcrossing in *Caenorhabditis elegans*. *J. Evol. Biol.* **18**, 27–34. (doi:10.1111/j.1420-9101.2004.00804.x)
 45. Estes S, Phillips PC, Denver DR, Thomas WK, Lynch M. 2004 Mutation accumulation in populations of varying size: the distribution of mutational effects for fitness correlates in *Caenorhabditis elegans*. *Genetics* **166**, 1269–1279. (doi:10.1534/genetics.166.3.1269)
 46. Pothof J, van Haften G, Thijsen K, Kamath RS, Fraser AG, Ahringer J, Plasterk RHA, Tijsterman M. 2003 Identification of genes that protect the *C. elegans* genome against mutations by genome-wide RNAi. *Gene Dev.* **17**, 443–448. (doi:10.1101/gad.1060703)
 47. Schulenburg H, Ewbank JJ. 2004 Diversity and specificity in the interaction between *Caenorhabditis elegans* and the pathogen *Serratia marcescens*. *BMC Evol. Biol.* **4**, 49. (doi:10.1186/1471-2148-4-49)
 48. Schulte RD, Hasert B, Makus C, Michiels NK, Schulenburg H. 2012 Increased responsiveness in feeding behaviour of *Caenorhabditis elegans* after experimental coevolution with its microparasite *Bacillus thuringiensis*. *Biol. Lett.* **8**, 234–236. (doi:10.1098/rsbl.2011.0684)
 49. Schulte RD, Makus C, Hasert B, Michiels NK, Schulenburg H. 2011 Host-parasite local adaptation after experimental coevolution of *Caenorhabditis elegans* and its microparasite *Bacillus thuringiensis*.

- Proc. R. Soc. B **278**, 2832–2839. (doi:10.1098/rspb.2011.0019)
50. Schulte RD, Makus C, Schulenburg H. 2013 Host-parasite coevolution favours parasite genetic diversity and horizontal gene transfer. *J. Evol. Biol.* **26**, 1836–1840. (doi:10.1111/jeb.12174)
 51. Masri L, Schulte RD, Timmermeyer N, Thanisch S, Crummenerl LL, Jansen G, Michiels NK, Schulenburg H. 2013 Sex differences in host defence interfere with parasite-mediated selection for outcrossing during host–parasite coevolution. *Ecol. Lett.* **16**, 461–468. (doi:10.1111/ele.12068)
 52. Morran LT, Schmidt OG, Gelarden IA, Parrish RC, Lively CM. 2011 Running with the red queen: host–parasite coevolution selects for biparental sex. *Science* **333**, 216–218. (doi:10.1126/science.1206360)
 53. Morran LT, Parrish RC, Gelarden IA, Lively CM. 2013 Temporal dynamics of outcrossing and host mortality rates in host–pathogen experimental coevolution. *Evolution* **67**, 1860–1868. (doi:10.1111/evo.12007)
 54. Félix M-A *et al.* 2011 Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to nodaviruses. *PLoS Biol.* **9**, e1000586. (doi:10.1371/journal.pbio.1000586)
 55. Dennehy JJ, Friedenber NA, Yang YW, Turner PE. 2006 Bacteriophage migration via nematode vectors: host–parasite–consumer interactions in laboratory microcosms. *Appl. Environ. Microbiol.* **72**, 1974–1979. (doi:10.1128/AEM.72.3.1974-1979.2006)
 56. Friedenber NA. 2003 Determinism in a transient assemblage: the roles of dispersal and local competition. *Am. Nat.* **162**, 586–596. (doi:10.1086/378782)
 57. Friedenber NA. 2003 Experimental evolution of dispersal in spatiotemporally variable microcosms. *Ecol. Lett.* **6**, 953–959. (doi:10.1046/j.1461-0248.2003.00524.x)
 58. Lockery SR *et al.* 2008 Artificial dirt: microfluidic substrates for nematode neurobiology and behavior. *J. Neurophysiol.* **99**, 3136–3143. (doi:10.1152/jn.91327.2007)
 59. Keightley PD, Caballero A. 1997 Genomic mutation rates for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **94**, 3823–3827. (doi:10.1073/pnas.94.8.3823)
 60. Sicard M, Hering S, Schulte R, Gaudriault S, Schulenburg H. 2006 The effect of *Photorhabdus luminescens* (Enterobacteriaceae) on the survival, development, reproduction and behaviour of *Caenorhabditis elegans* (Nematoda: Rhabditidae). *Environ. Microbiol.* **9**, 12–25. (doi:10.1111/j.1462-2920.2006.01099.x)
 61. Ajie BC, Estes S, Lynch M, Phillips PC. 2005 Behavioral degradation under mutation accumulation in *Caenorhabditis elegans*. *Genetics* **170**, 655–660. (doi:10.1534/genetics.104.040014)
 62. Azevedo RBR, Keightley PD, Laurén-Määttä C, Vassilieva LL, Lynch M, Leroi AM. 2002 Spontaneous mutational variation for body size in *Caenorhabditis elegans*. *Genetics* **162**, 755–765.
 63. Estes S, Ajie BC, Lynch M, Phillips PC. 2005 Spontaneous mutational correlations for life-history, morphological and behavioral characters in *Caenorhabditis elegans*. *Genetics* **170**, 645–653. (doi:10.1534/genetics.104.040022)
 64. Vassilieva LL, Lynch M. 1999 The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. *Genetics* **151**, 119–129.
 65. Joyner-Matos J, Hicks KA, Cousins D, Keller M, Denver DR, Baer CF, Estes S. 2013 Evolution of a higher intracellular oxidizing environment in *Caenorhabditis elegans* under relaxed selection. *PLoS ONE* **8**, e65604. (doi:10.1371/journal.pone.0065604)
 66. Estes S, Lynch M. 2003 Rapid fitness recovery in mutationally degraded lines of *Caenorhabditis elegans*. *Evolution* **57**, 1022–1030.
 67. Denver DR, Morris K, Lynch M, Thomas WK. 2004 High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature* **430**, 679–682. (doi:10.1038/nature02697)
 68. Baer CF, Joyner-Matos J, Ostrow D, Grigaltchik V, Salomon MP, Upadhyay A. 2010 Rapid decline in fitness of mutation accumulation lines of gonochoristic (outcrossing) *Caenorhabditis* nematodes. *Evolution* **64**, 3242–3253. (doi:10.1111/j.1558-5646.2010.01061.x)
 69. Baer CF *et al.* 2005 Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. *Proc. Natl Acad. Sci. USA* **102**, 5785–5790. (doi:10.1073/pnas.0406056102)
 70. Baer CF, Phillips N, Ostrow D, Avalos A, Blanton D, Boggs A, Keller T, Levy L, Mezerhane E. 2006 Cumulative effects of spontaneous mutations for fitness in *Caenorhabditis*: role of genotype, environment and stress. *Genetics* **174**, 1387–1395. (doi:10.1534/genetics.106.061200)
 71. Matsuba C, Ostrow DG, Salomon MP, Tolani A, Baer CF. 2013 Temperature, stress and spontaneous mutation in *Caenorhabditis briggsae* and *Caenorhabditis elegans*. *Biol. Lett.* **9**, 20120334. (doi:10.1098/rsbl.2012.0334)
 72. Chasnov JR, Chow KL. 2002 Why are there males in the hermaphroditic species *Caenorhabditis elegans*? *Genetics* **160**, 983–994.
 73. Wegewitz V, Schulenburg H, Streit A. 2008 Experimental insight into the proximate causes of male persistence variation among two strains of the androdioecious *Caenorhabditis elegans* (Nematoda). *BMC Ecol.* **8**, 12. (doi:10.1186/1472-6785-8-12)
 74. Lopes PC, Sucena É, Santos ME, Magalhães S. 2008 Rapid experimental evolution of pesticide resistance in *C. elegans* entails no costs and affects the mating system. *PLoS ONE* **3**, e3741. (doi:10.1371/journal.pone.0003741)
 75. Dolgin ES, Charlesworth B, Baird SE, Cutter AD. 2007 Inbreeding and outbreeding depression in *Caenorhabditis nematodes*. *Evolution* **61**, 1339–1352. (doi:10.1111/j.1558-5646.2007.00118.x)
 76. LaMunyon CW, Ward S. 1999 Evolution of sperm size in nematodes: sperm competition favours larger sperm. *Proc. R. Soc. Lond. B* **266**, 263–267. (doi:10.1098/rspb.1999.0631)
 77. Chandler CH, Chadderdon GE, Phillips PC, Dworkin I, Janzen FJ. 2012 Experimental evolution of the *Caenorhabditis elegans* sex determination pathway. *Evolution* **66**, 82–93. (doi:10.1111/j.1558-5646.2011.01420.x)
 78. Walker DW, McColl G, Jenkins NL, Harris J, Lithgow GJ. 2000 Natural selection: evolution of lifespan in *C. elegans*. *Nature* **405**, 296–297. (doi:10.1038/35012693)
 79. Anderson JL, Reynolds RM, Morran LT, Tolman-Thompson J, Phillips PC. 2011 Experimental evolution reveals antagonistic pleiotropy in reproductive timing but not life span in *Caenorhabditis elegans*. *J. Gerontol. Ser. A, Biol. Sci. Med. Sci.* **66**, 1300–1308. (doi:10.1093/gerona/66.11.1300)
 80. Denver DR, Howe DK, Wilhelm LJ, Palmer CA, Anderson JL, Stein KC, Phillips PC, Estes S. 2010 Selective sweeps and parallel mutation in the adaptive recovery from deleterious mutation in *Caenorhabditis elegans*. *Genome Res.* **20**, 1663–1671. (doi:10.1101/gr.108191.110)
 81. Hodgkin J. 2002 One lucky XX male: isolation of the first *Caenorhabditis elegans* sex-determination mutants. *Genetics* **162**, 1501–1504.
 82. Janzen FJ, Phillips PC. 2006 Exploring the evolution of environmental sex determination, especially in reptiles. *J. Evol. Biol.* **19**, 1775–1784. (doi:10.1111/j.1420-9101.2006.01138.x)
 83. Bell G. 1982 *The masterpiece of nature: the evolution and genetics of sexuality*. London, UK: Croom Helm.
 84. Goddard MR. 2007 Why bother with sex? Answers from experiments with yeast and other organisms. In *Sex in fungi: molecular determination and evolutionary implications* (ed. JKJ Heitman, JW Kronstad, JW Taylor, LA Casselton), pp. 489–506. Washington, DC: ASM Press.
 85. Barrière A, Félix M-A. 2005 High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Curr. Biol.* **15**, 1176–1184. (doi:10.1016/j.cub.2005.06.022)
 86. Barrière A, Félix M-A. 2007 Temporal dynamics and linkage disequilibrium in natural *Caenorhabditis elegans* populations. *Genetics* **176**, 999–1011. (doi:10.1534/genetics.106.067223)
 87. Cutter AD, Avilés L, Ward S. 2003 The proximate determinants of sex ratio in *C. elegans* populations. *Genet. Res.* **81**, 91–102. (doi:10.1017/S001667230300613X)
 88. Stewart AD, Phillips PC. 2002 Selection and maintenance of androdioecy in *Caenorhabditis elegans*. *Genetics* **160**, 975–982.
 89. Garcia LR, LeBeouf B, Koo P. 2007 Diversity in mating behavior of hermaphroditic and male–female *Caenorhabditis nematodes*. *Genetics* **175**, 1761–1771. (doi:10.1534/genetics.106.068304)
 90. Teotónio H, Manoel D, Phillips PC. 2006 Genetic variation for outcrossing among *Caenorhabditis elegans* isolates. *Evolution* **60**, 1300–1305.
 91. Agrawal AF. 2006 Evolution of sex: why do organisms shuffle their genotypes? *Curr. Biol.* **16**, R696–R704. (doi:10.1016/j.cub.2006.07.063)
 92. Morran LT, Cappy BJ, Anderson JL, Phillips PC. 2009 Sexual partners for the stressed: facultative

- outcrossing in the self-fertilizing nematode *Caenorhabditis elegans*. *Evolution* **63**, 1473–1482. (doi:10.1111/j.1558-5646.2009.00652.x)
93. Gimond C, Jovelin R, Han S, Ferrari C, Cutter AD, Braendle C. 2013 Outbreeding depression with low genetic variation in selfing *Caenorhabditis* nematodes. *Evolution* **67**, 3087–3101. (doi:10.1111/evo.12203)
 94. LaMunyon CW, Bouban O, Cutter AD. 2007 Postcopulatory sexual selection reduces genetic diversity in experimental populations of *Caenorhabditis elegans*. *J. Hered.* **98**, 67–72. (doi:10.1093/jhered/esl052)
 95. Ellis RE. 2008 Sex determination in the *Caenorhabditis elegans* germ line. *Curr. Top. Dev. Biol.* **83**, 41–64. (doi:10.1016/S0070-2153(08)00402-X)
 96. Antebi A. 2007 Genetics of aging in *Caenorhabditis elegans*. *PLoS Genet.* **3**, e129. (doi:10.1371/journal.pgen.0030129)
 97. Williams GC. 1957 Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**, 398–411. (doi:10.2307/2406060)
 98. Williams PD, Day T, Fletcher Q, Rowe L. 2006 The shaping of senescence in the wild. *Trends Ecol. Evol.* **21**, 458–463. (doi:10.1016/j.tree.2006.05.008)
 99. Cutter AD. 2004 Sperm-limited fecundity in nematodes: how many sperm are enough? *Evolution* **58**, 651–655.
 100. Woodruff GC, Eke O, Baird SE, Félix M-A, Haag ES. 2010 Insights into species divergence and the evolution of hermaphroditism from fertile interspecies hybrids of *Caenorhabditis* nematodes. *Genetics* **186**, 997–1012. (doi:10.1534/genetics.110.120550)
 101. Kozłowska JL, Ahmad AR, Jahesh E, Cutter AD. 2011 Genetic variation for postzygotic reproductive isolation between *Caenorhabditis briggsae* and *Caenorhabditis* sp. 9. *Evolution* **66**, 1180–1195. (doi:10.1111/j.1558-5646.2011.01514.x)
 102. Yan C, Bi Y, Yin D, Zhao Z. 2012 A method for rapid and simultaneous mapping of genetic loci and introgression sizes in nematode species. *PLoS ONE* **7**, e43770. (doi:10.1371/journal.pone.0043770)
 103. Dey A, Jeon Y, Wang G-X, Cutter AD. 2012 Global population genetic structure of *Caenorhabditis remanei* reveals incipient speciation. *Genetics* **191**, 1257–1269. (doi:10.1534/genetics.112.140418)
 104. Herron MD, Doebeli M. 2013 Parallel evolutionary dynamics of adaptive diversification in *Escherichia coli*. *PLoS Biol.* **11**, e1001490. (doi:10.1371/journal.pbio.1001490)
 105. Jarrell TA, Wang Y, Bloniarz AE, Brittin CA, Xu M, Thomson JN, Albertson DG, Hall DH, Emmons SW. 2012 The connectome of a decision-making neural network. *Science* **337**, 437–444. (doi:10.1126/science.1221762)
 106. Bendsky A, Bargmann CI. 2011 Genetic contributions to behavioural diversity at the gene–environment interface. *Nat. Rev. Genet.* **12**, 809–820. (doi:10.1038/nrg3065)
 107. Smith JR, Stanfield GM. 2011 TRY-5 is a sperm-activating protease in *Caenorhabditis elegans* seminal fluid. *PLoS Genet.* **7**, e1002375. (doi:10.1371/journal.pgen.1002375)