Experimental Evolution with Caenorhabditis Nematodes

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ABSTRACT The hermaphroditic nematode Caenorhabditis elegans has been one of the primary model systems in biology since the 1970s, but only within the last two decades has this nematode also become a useful model for experimental evolution. Here, we outline the goals and major foci of experimental evolution with C. elegans and related species, such as C. briggsae and C. remanei, by discussing the principles of experimental design, and highlighting the strengths and limitations of Caenorhabditis as model systems. We then review three exemplars of Caenorhabditis experimental evolution studies, underlining representative evolution experiments that have addressed the: (1) maintenance of genetic variation; (2) role of natural selection during transitions from outcrossing to selfing, as well as the maintenance of mixed breeding modes during evolution; and (3) evolution of phenotypic plasticity and its role in adaptation to variable environments, including host–pathogen coevolution. We conclude by suggesting some future directions for which experimental evolution with Caenorhabditis would be particularly informative.

KEYWORDS adaptation; C. briggsae; C. elegans; C. remanei; domestication; experimental design; laboratory selection experiments; self-fertilization; reproduction systems; mutation accumulation; standing genetic variation; WormBook

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“With them, many important questions will be accessible to patient observers who do not fear long-term experiments.” – Emile Maupas (1900)

Over a century ago, Emile Maupas introduced the nematode Caenorhabditis elegans to the scientific community with his report on a failed experiment aimed at testing the hypothesis that continual self-fertilization (selfing) should lead to population extinction (Maupas 1900). This goal was ultimately thwarted, as after nearly 50 generations of selfing, Maupas’ C. elegans culture collapsed due to an erratic spike in temperature that led to abnormalities in development and reproduction independently of inbreeding effects. Maupas’ experimental evolution study was inspired by an ongoing debate about the long-term sustainability of selfing as a reproductive strategy (Darwin 1876), and provides a particularly telling introduction to experimental evolution: the expected outcome (extinction) was achieved, but for the “wrong” reason, as it was not a result of selfing.

Experimental Evolution (EE) has long been used as the gold standard for testing evolutionary hypotheses about natural selection and genetic drift, estimating theoretical parameters regarding standing genetic variation, such as mutation and recombination rates, and, more recently, as a means for gene discovery. The main organismal models to which EE has been applied are mice, fruit flies, yeast, and bacteria (Rose and Lauder 1996; Sclafani 1997; Garland and Rose 2009; Kassen 2014). Despite the promising start by Emile Maupas (Maupas 1900), however, it was nearly 90 years before Caenorhabditis reappeared in EE research, during which time much evolutionary theory had been mathematically formalized.

Because of its relative newcomer status in EE research, we have barely begun to tap the potential of Caenorhabditis for elucidating the patterns and processes of evolution (Gray and Cutter 2014). But, as the community of Caenorhabditis evolutionary biologists has grown—now sufficiently large to merit regular meetings and dedicated stock and databases (Supplemental Material, Table S1 in Fire S1; Carvalho et al. 2006; Haag et al. 2007; Braendle and Teotonio 2015)—so too has the array of evolutionary problems being investigated with experiments (Table 1 lists some of the studies that will be covered here).

Caenorhabditis are free-living bactiovorous roundworms with over 25 species currently being cultured in the laboratory (Kiontke et al. 2011; Felix et al. 2014), although only C. elegans, C. briggsae, and C. remanei have been utilized in EE research. A distinctive feature of this group of nematodes is that facultative selfing evolved independently from ancestral obligatory outcrossing three times (Kiontke and Fitch 2005). C. elegans, C. briggsae, and C. tropicalis have a rare androdioecious reproduction system, with hermaphrodites capable of selfing, and of outcrossing with males, but not with other hermaphrodites. Hermaphrodites from these species are developmentally similar to females of related dioecious species, except for a period during germline specification and differentiation when sperm is produced and stored in the spermatheca prior to an irreversible switch to oogenesis at adulthood. These hermaphrodites are therefore self-sperm limited and can only fertilize all of their oocytes when mated by males (Sarkar 1992; Cutter 2004). Behaviorally, hermaphrodites have lost the ancestral ability to attract males, and are generally reluctant to mate until they have depleted their own self-sperm store (Lipton et al. 2004; Chasnov et al. 2007).

Our aim with this review is to present Caenorhabditis species as excellent models for EE. We first focus on the basic principles of EE, which apply more or less to any organism, and then introduce Caenorhabditis and related resources for their use in EE. We next explore the common goals and outcomes of EE studies in sections devoted to laboratory domestication and specific EE designs that address the fundamental processes of natural selection, genetic drift, mutation, segregation, and recombination. We then review selected studies that have greatly improved our understanding of several evolutionary problems. More technical introductions are presented in Boxes and Figures, and in Supplementary Appendices. We finish with future research directions for which we believe Caenorhabditis to be particularly well-suited as model systems.

What Is Experimental Evolution?

Advantages and limitations of the experimental manipulation of evolution

EE practitioners employ laboratory or field manipulations to understand the processes that lead to, and the mechanisms underlying patterns of, genetic and/or phenotypic diversity revealed by populations across multiple generations. The basic approach is straightforward, with most experiments
Table 1 Selected studies with *Caenorhabditis* EE

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<td>Braendle et al. (2010)</td>
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<td>Population genetics</td>
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<td>Mutation accumulation</td>
<td>The rate of mutational decay of fitness in <em>C. elegans</em> is (~10^{-3}) slower than previous estimates from <em>Drosophila</em>.</td>
<td>Keightley and Caballero (1997); also see Vassilieva and Lynch (1999) and Baer et al. (2005), among others</td>
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<td>Several genes—<em>npr-1</em>, <em>glp-5</em>, <em>nph-10</em>—evolved novel alleles that encode adaptive phenotypes under laboratory conditions</td>
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being leveraged on the insights that can be provided by comparing phenotypes, and/or genotypes of populations evolved under experimenter-imposed conditions with those of an ancestor (divergence) or a control group (differentiation). Using numerical simulations, we show in Figure 1 how phenotypic responses would typically look like during EE, and in Figure 2 the statistical power to detect divergence or differentiation as a function of sample size and number of replicate populations. In Appendix 1 in File S1, we discuss some of the scaling and transformation problems in EE studies.

Because the development of evolutionary theory has far outpaced the generation of relevant data (genome-sequence data being a singular exception), using EE to confront long-standing problems has the potential to illuminate our understanding of evolution for decades to come provided a few qualifications are appreciated. The most important considerations are the potential difficulty in balancing simplicity and realism in contrived laboratory experiments (Huey and Rosenzweig 2009), the uncertainty surrounding the relevance of observations from EE for natural populations (Matos et al. 2000), and current limitations on our ability to rapidly phenotype large numbers of individuals in the relevant environment—the one in which EE was performed and where fitness can be estimated. In principle, the power of EE studies derives from the fact that they are designed, repeatable experiments; in practice, experimental design must be carefully considered to avoid unintended consequences and alternative explanations that are impossible to distinguish from the hypothesis that the experimenter originally hoped to address. The general advice for beginning students is to limit the number of variables manipulated while controlling for laboratory domestication.

Doing EE well requires careful planning and organization, and the commitment to spend at least several hours transferring individuals every few days for months, or even years, on end. Why would anyone take on such a thing when they could start today on a comparative study in which nature has already done most of the work? Since students of evolution are primarily driven by “why” questions (Mayr 1961), they will often be left wanting by results of comparative studies alone. Evolution is an inherently historical process, and so the vagaries of history are often expected to play an important role in determining evolutionary outcomes, and yet are likely to be completely opaque to us unless we can actually control and follow the structure of that history. While phylogenetic methods can be applied to infer the underlying evolutionary processes and ancestral character states (Velsenstein 2004), this approach still involves a good deal of guesswork since very different processes can plausibly lead to the same evolutionary outcome (Seroi et al. 1996; Sanderson and Huford 1996). And while the comparative perspective is vital for revealing patterns of natural diversity and generating hypotheses about the causes of those patterns, we can, in principle, eliminate much of the guesswork by directly observing evolution in controlled settings.

Advantages and limitations of Caenorhabditis for experimental evolution

Some early studies on thermal adaptation (Srun 1966a,b,c; Tower et al. 1968) and rates to lethal mutations (Rosenbluth et al. 1983; Clark et al. 1988) notwithstanding, application of EE methods with Caenorhabditis species did not begin in earnest until the 1990s with the work of Johnson and colleagues on the evolution of aging (Johnson and Hutchinson 1993; Brooks et al. 1994; Walker et al. 2000; Jenkins et al. 2004), that of LaMunyon and Ward on sexual selection (LaMunyon and Ward 1995, 1997, 1998, 1999, 2002), and that of Kightley and colleagues on mutation rates and their effects (Kightley and Caballero 1997; Davies et al. 1999; Vassilieva and Lynch 1999; Denver et al. 2000; Vassilieva et al. 2000; Szevedo et al. 2002; Peters et al. 2003). There is now a remarkably broad range of problems being addressed with Caenorhabditis EE (Table 1, and see Gray and Cutter 2014).

One reason for the delay in adopting Caenorhabditis in EE is that natural intraspecific and interspecific diversity was little studied until the 2000s (Phillips 2006; Kiontke et al. 2011), at least in part because large-scale collection of natural isolates was not feasible until relox et al. (2014) discovered effective ways of targeting collections in natural habitats of rotting fruit and plant stems. This lack of knowledge limited the scope of questions that could be tackled. In particular, since most short-term evolution in sexual species is thought to occur from standing genetic variation rather than from new mutational variance (Sill 1982; Caballero and Santiago 1995; Matuszewski et al. 2015), the highly inbred and genetically depauperate C. elegans laboratory strains that were available at the time were initially perceived as being of little use for testing evolutionary theory.

We now understand that natural populations of C. elegans are inbred due to selving, and that outcrossing is rare (Chasnov and Chow 2002; Stewart and Phillips 2002; Barriere and Felix 2005; Teotonio et al. 2006). Selective sweeps and background selection appear to dominate its population genetics (Cutter and Payseur 2003; Rockman et al. 2010; Andersen et al. 2012; Thomas et al. 2015), resulting in poor gene diversity and extremely strong linkage disequilibrium (Gräuslein et al. 2002; Sivasundar and Hey 2003; Barriere and Relix 2005; Iaber et al. 2005; Cutter 2006; Thompson et al. 2015). Related outcrossing species display extensive natural variation, however (Cutter et al. 2013; Dey et al. 2013; Cutter 2015), with C. brenneri being perhaps the most genetically diverse eukaryote described so far (Dey et al. 2013). And now, populations of C. elegans constructed via the hybridization of wild isolates have been developed for the specific purpose of studying evolution from standing genetic variation (Table S1 in File S1 lists a few of the resources for EE available in C. elegans).

Many of the same features that make the natural population genetics of Caenorhabditis somewhat odd also make it ideally suited for laboratory approaches to studying
evolutionary questions (Frezal and Felix 2015; Petersen et al. 2015). For example, questions about the interaction between reproduction system and standing genetic variation can be nicely matched with the choice of experimental design (e.g., manipulating sex determination in C. elegans or using natural populations of the highly polymorphic C. remanei as experimental starting points). Of course, the real power here is the ability to capitalize on decades of hard work on the genetics and functional biology of C. elegans that has been provided by the broader C. elegans research community (Corsi et al. 2015).

Because of these advantages, Caenorhabditis are now arguably the best metazoan models for the experimental study of evolution (Gray and Cutter 2014). Although worms cannot match the short generation times or large population sizes of microbes such as yeast or bacteria commonly employed for EE (Bell 1997; Kassen 2014), relatively long-term evolution is feasible with C. elegans; e.g., lineages have been cultured under selection for up to 400 generations (Matsuba et al. 2012; Katju et al. 2015), and maintained under different reproductive systems for up to 200 generations (Theologidis et al. 2011).

Like many microbes, Caenorhabditis can be easily and reliably cryopreserved. Cryogenics allows one to halt evolution of ancestral stocks and permits accurate evaluation of repeatability and parallelism of evolution (Oenner et al. 2010; Estes et al. 2011). Not only does the ability to cryopreserve stocks allow direct comparisons between evolved and ancestral states, it means that an experiment can live on after the initial period of evolution and new features of the experimental system can be investigated—a major advantage over other metazoan systems such as Drosophila.

But the feature of androdioecious Caenorhabditis that is unique among metazoan experimental systems is that sex determination can be genetically manipulated, allowing researchers to obtain populations with variable ratios of males, females, and hermaphrodites (Isaag et al. 2002; Valdi et al. 2009; Seadell et al. 2011), and thus to achieve different degrees of selfing and outcrossing. This has allowed the role of segregation and recombination in evolution to be tested independently of confounding environmental factors (Cutter 2005; Morrison et al. 2009; Baer et al. 2010; Chelo and Teotónio 2013; Theologidis et al. 2014). In contrast, for microbes such as the yeast Saccharomyces cerevisiae (Goddard et al. 2005; McDonald et al. 2016), and the green alga Chlamydomonas reinhardtii (Colegrave 2002; Lachapelle and Bell 2012), or metazoans like the rotifer Brachionus calyciflorus (Seeks and Agrawal 2010; Uijckx et al. 2017), manipulation of sexuality usually involves distinct life-histories imposed by different environments.
During EE depending on how selection and genetic drift in differentiation requires that replicate populations within each treatment show random effects due to genetic drift and uncontrolled selection. When generations and if linear or nonlinear trajectories are expected (see Figure 1). Sampling several time points during EE will generally increase power, although it is often unclear how to model for trait autocorrelations across necessary to be con.

Detecting divergence is much easier than differentiation, and that a moderate to high number of replicate populations and high sample sizes are employing frequentist statistical modeling, taking replicate populations as a

Here, we consider how the number of replicate populations and sample size affect the power to detect responses to selection on a quantitative trait when reproduction occurs exclusively by selfing. We rely on the numerical simulation model presented in http://datadryad.org/, doi: 10.5061/dryad.bq08n; the reader can explore it to generate trait value trajectories and power curves as a function of standing and mutational genetic variance, population size and number of offspring, truncation and Gaussian selection, sample sizes, etc. The analysis we show is based on the simulated evolutionary trajectories of Figure 1. Individual fitness was defined as: \[ w = \exp\left(- \frac{\text{offspring}_\text{trait_value} - \text{optimum}}{2 \times \text{mutational variance}}\right) \] (Figure 1, right plot), with the new fixed phenotypic optimum set to \(-1\ SD\) from the ancestral phenotypic distribution, and an intensity resulting in an initial linear selection gradient of 0.15. These are realistic values for natural populations (Kingsolver et al. 2001), but in the laboratory selection may be more substantial. The trait value of each hermaphrodite in the simulations is defined by its breeding value plus a stochastic component, assumed both to follow Gaussian distributions. Each hermaphrodite produced a fixed number of 10 offspring, with each one of them being represented in the next generation with a probability given by the fitness function, while keeping population size constant at 1000 individuals. This number was the approximately the effective population size of EE done by Teotónio et al. (2012), employing nonoverlapping and constant adult densities of 10^4 (Chelo and Teotónio 2013). Many studies, however, employ overlapping generations, and do not test for the stability of age-structure during EE. Even though densities may be large in these studies, it is unclear which effective population sizes are realized (Box 1). For all plots, segregation/mutational heritability was of \~0.15\% at each generation, in line with Caenorhabditis NA estimates, e.g., (Salomon et al. 2009). But note that trait heritability will change during EE depending on how selection and genetic drift influence standing levels of genetic variation. Once EE is done, testing for divergence or differentiation requires that replicate populations within each treatment show random effects due to genetic drift and uncontrolled selection. When employing frequentist statistical modeling, taking replicate populations as a fixed factor is incorrect since the degrees of freedom over which the significance of evolutionary responses are tested will be inflated. Using a linear mixed effects model, we tested for differentiation of unselected and selected populations after 50 generations by sampling 10 or 50 individuals from each of 2, 5, or 15 replicate populations. This model assumes that the heterogeneity among replicates is the same between treatments, which may not be true. Left panels show the probability of detecting differentiation (the cumulative density distribution of the simulations) as a function of significance level, for a trait that has a starting heritability of 5\% (scenario 1 in Figure 1) or 30\% (scenario 2 in Figure 1). Right panels show similar power curves when testing for divergence, where EE populations at generation 50 are compared to the ancestral state. The null hypothesis is that there is no divergence or differentiation (the identity dashed line). This analysis shows that detecting divergence is much easier than differentiation, and that a moderate to high number of replicate populations and high sample sizes are necessary to be confident that divergence or differentiation are due to selection (power of at least 80\% at the significance level of 0.05; vertical line). Sampling several time points during EE will generally increase power, although it is often unclear how to model for trait autocorrelations across generations and if linear or nonlinear trajectories are expected (see Figure 1).

Goals, Outcomes, and Interpretation of Experimental Evolution

Domestication to laboratory conditions

When s. brener decided to embark on a new research program based on studying a simple metazoan organism, he selected a C. elegans strain, N2, which had been propagated in the laboratory for perhaps thousands of generations (weber et al. 2010; sterken et al. 2015; Nigon and Felix 2016). Unbeknownst to him, this strain had already undergone extensive adaptation to laboratory conditions and probably became even more specifically adapted to what are now the standard C. elegans handling conditions (stiernagle 1999). It is now clear that the N2 strain is quite distinct from natural isolates of C. elegans for a variety of developmental, physiological, and behavioral traits. For example, specific alleles of npr-1, glb-5, and nath-10 are only found in N2 and have wide-ranging effects (versson et al. 2009; mcGrath et al. 2011;
 genomes have been published thus far (Andersen et al. 2011). Adaptation to the high-density growth conditions experienced in liquid culture rendered individuals resistant to the pheromone-induced dauer larval formation observed in N2 and several wild C. elegans isolates—a response shown to result from deletions in two pheromone receptor genes: srg-36 and srg-37. Comparison to a closely related C. elegans laboratory strain and an isolate of C. briggsae, both of which had experienced multiple generations in high-density liquid culture, revealed the same loss of resistance due to deletions in identical or a paralogous gene(s), respectively. This long-term laboratory selection has also modified allocation among N2’s life-history traits via modification of chromatin state by the NURF chromatin remodeling complex (Iarge et al. 2016).

One important consequence of these results is that researchers who study the so-called “standard,” “wild-type,” or “reference” strain of C. elegans should recognize the possibility that the biological processes of interest could have been perturbed by adaptation to laboratory conditions. If nothing else, arguments regarding the putatively adaptive nature of a given discovery should be tempered until they can be verified with other natural isolates (cutter 2015; irrezal and relicx 2015; vetersen et al. 2015). The genetic differentiation generated by long-term selection could be used to identify the genetic changes underlying the response to selection, yielding insights into the functional basis of the response. However, because these particular domestication studies were not specifically designed for this purpose, they cannot be used to test specific evolutionary hypotheses in the way that replicated and controlled designs can be. While the capacity for parallel adaptive response revealed by the study of McGrath et al. (2011) is intriguing, it is still unclear whether this process (not to mention the particular genes or alleles involved) is relevant to evolution beyond the laboratory.

Two additional aspects of laboratory domestication potentially confound the interpretation of responses to a given experimental treatment, especially when evolution occurs from standing genetic variation instead of mutational input. First, laboratory conditions are a novel environment to which only a few genotypes will, by chance, be either very well adapted or very maladapted (Service and Rose 1985). Second, for populations maintained even at relatively large sizes, inbreeding depression will generate positive genetic correlations among fitness components (sose 1984). An example of the latter problem is given by the study of carvalho et al. (2014) who conducted EE using the populations with standing genetic variation of teotónio et al. (2012) and found that, after 100 generations at stable and intermediate selfing rates, hermaphrodite early-life fertility and lifespan increased when they were selfed, but not when they were outcrossed. This result is seemingly at odds with theory on the evolution of aging, which predicts a fitness trade-off between early and late life-history (williams 1957; Hamilton 1966). However, inbreeding depression may in this case explain the positive genetic correlation between fitness components simply because more recessive alleles are expressed under selfing than outcrossing. Thus, care must be taken to avoid misinterpreting consequences of domestication as selective responses to applied experimental treatments.

Natural selection and genetic drift

The central goal of evolutionary biology is to understand the evolution of adaptive traits—the organismal features that enhance survival and/or reproduction. Caenorhabditis offers an attractive model for performing rigorous experimental tests of theory describing rates of adaptation in terms of the fundamental processes of mutation, segregation, recombination, and natural selection. EE approaches can serve both as a source of potentially causal variants (e.g., mutations captured from sequencing of experimentally evolved lines), and as a method for characterizing their phenotypic consequences and evolutionary dynamics (see Table 2 for types of EE approaches). The latter is usually achieved by measuring fitness, and fitness component, responses in the environment(s) where evolution took place. The appropriate measure of fitness, however, depends on whether generations are overlapping or nonoverlapping, if reproduction is continuous or discrete, if there is density-dependence, and if environments are temporally or spatially variable (ssof 2008). While many of the parameters can be controlled by researchers in evolution experiments, some cannot, and fitness proxies must be found. A review of the conceptual underpinnings of defining fitness and fitness components in the context of EE would require a book-length treatise (sueller 2010; chevin 2011). We briefly review current EE methods for measuring natural selection and genetic drift in Figure 3, and, in Box 1, we expand about how population size may determine the efficiency of selection.

Competition experiments (CEs) are the best approach for measuring natural selection (Figure 3 and Table 2). CEs are a class of evolution experiments in which individuals of different identifiable genotypes are allowed to evolve in head-to-head competition with each other. Competition is implicit in most evolution experiments; however, CEs are distinct in that investigators control the starting frequencies of the different types and the “trait” of interest is the change in frequency of these types across generations. They are primarily employed to understand how selection determines the frequency dynamics of alternative alleles or genotypes that are of a priori interest (Appendix 2 in File S1). Promisingly, CEs are
Table 2 Types of EE

<table>
<thead>
<tr>
<th>Experiment type</th>
<th>Goal(s)</th>
<th>Design features</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial selection</td>
<td>(i) Genetic architecture of specific trait(s), (ii) domestication</td>
<td>Experimenter defines fitness as a function of the value of the trait(s) of interest</td>
<td>Response to selection of the trait of interest; indirect responses of correlated traits</td>
</tr>
<tr>
<td>Laboratory natural selection</td>
<td>(i) Evolution of genetic systems, (ii) genetic architecture of the response to natural selection on fitness in a defined context</td>
<td>Experimenter imposes selective milieu; nature decides what the relevant traits are</td>
<td>The multivariate phenotype, genome-wide allele frequencies in SNPs, CNVs, etc.</td>
</tr>
<tr>
<td>Competition experiments</td>
<td>(i) Fitness of specific genotypes in a defined context, (ii) find the loci of adaptation</td>
<td>Experimenter defines starting frequencies of different identifiable genotypes, perhaps associated with phenotypes; nature selects among them</td>
<td>Derived allele (or genotype) frequencies, associated with phenotype frequencies</td>
</tr>
<tr>
<td>Reverse evolution</td>
<td>Test for nonadditive gene interactions: (i) Compensation of mutational-degraded genotypes, (ii) natural selection erases history</td>
<td>Evolved population allowed to re-evolve under ancestral conditions</td>
<td>Some measure of phenotype or fitness</td>
</tr>
<tr>
<td>Invasion experiments</td>
<td>(i) Test for transitions in character state, (ii) measure genetic drift independently of selection</td>
<td>Rare genotypes introduced into a population; Highly replicated</td>
<td>Proportion of invasions that go extinct are observed</td>
</tr>
<tr>
<td>Inbreeding experiments</td>
<td>Dominance and epistasis as revealed by inbreeding and outbreeding depression</td>
<td>Inbred individuals (typically offspring of self-mating or sib-mating) are compared to outcrossed individuals</td>
<td>Some measure of phenotype or fitness, lineage survival during inbreeding</td>
</tr>
<tr>
<td>Mutation accumulation</td>
<td>Rate, spectrum, and distribution of mutational effects</td>
<td>Replicate populations derived from a known ancestor are maintained under minimal selection</td>
<td>Some measure of phenotype or fitness; molecular mutations measured by sequencing</td>
</tr>
</tbody>
</table>

beginning to be used for gene discovery and understanding the genetic basis of adaptive traits in so-called “evolve and resequence” experiments (Weber et al. 2010; Long et al. 2015). For this purpose, in C. elegans, one can readily think of competing subsets of existing recombinant inbred panels (Table S1 in File S1).

A related form of CE is an “invasion experiment,” in which a focal genotype is tested for its ability to invade a resident population of alternative type(s) (Table 2). In one form of an invasion experiment, novel genotypes are introduced at low frequencies to test the evolutionary stability of the “resident” genotype, such as the ability of outcrossing populations to resist the invasion of a mutation that leads to self-reproduction (Theologidis et al. 2014; Slowinski et al. 2016) and vice-versa (Wegewitz et al. 2009). In invasion experiments, competition can also be maximally restricted so that the different genotypes have independent growth rate dynamics. By controlling the initial frequency of “invaders” and population sizes, such experiments can be used to test theoretical predictions regarding the role of genetic drift in determining evolutionary outcomes (Figure 3). For example, Chelo et al. (2013b) introduced a fixed number of mutants into wildtype C. elegans populations of 1000 individuals, finding that these new mutants were frequently lost even when adaptive, and, conversely, tended to linger within populations at frequencies higher than the deterministic expectation when they had deleterious effects, in keeping with the classical theory of stochastic population genetics (Haldane 1927; Kimura 1957).

When we think of a selection experiment, we usually imagine shifting a population to a new environment. We might call such approaches “forward EE.” Some of the most powerful evolutionary studies in C. elegans have taken the opposite approach—“reverse EE”—by initially perturbing the genetics of the population and then observing how they respond to their ancestral environment, or to environments that allow the recovery of ancestral states by selection. In this fashion, one can assess if and how selection overrides evolutionary history (Estes and Teotónio 2009). For example, following ~240 generations of accumulation of deleterious mutations under relaxed selection, populations of C. elegans can recover ancestral fitness within only 60 generations when selection is reimposed (Estes and Lynch 2003; Estes et al. 2011). Interestingly, the rate of recovery was strongly dependent on the specific combination of mutations present within the genetic background before recovery was initiated. Careful examination of one of the ancestral lineages via whole-genome sequencing in oenver et al. (2010) suggested that the fitness recovery to ancestral levels resulted in part from the evolution of compensatory epistatic interactions, albeit with some caveats (Box 2).

Much of our understanding of the genetics of complex and quantitative traits comes from artificial selection experiments, particularly those whose aim is to improve plant yield and animal production in agriculture (Lynch and Walsh 1998). Artificial selection has also been commonly used in EE research, mainly to test hypotheses about the genetic basis of the trait under investigation and estimate quantitative...
genetic parameters such as heritabilities and genetic correlations. Artificial selection involves the intentional breeding of individuals with particular trait values, which may or may not have been otherwise favored by natural selection (Figure 4). The primary distinction here is that in artificial selection experiments, the investigator knows the target of selection, while in “natural selection in the laboratory” experiments, any feature of the organism that increases fitness within that environment is expected to increase in frequency.

**Mutation and standing genetic variation**

The constant pressure of new mutations, most of which will be detrimental to their carriers, is a likely contributor to the origin and evolution of many biological features (sexual reproduction, genetic incompatibility, and genome architectures), and is thought by some to be the primary driver of long-term evolutionary patterns (Lynch et al. 2006; Jones et al. 2007). Accurate measures of the rates, molecular spectra, and distributions of fitness effects of mutations are thus critical for many applications of evolutionary theory, including inferring evolutionary relationships, testing for selection on molecular sequence, estimating effective population size from standing levels of neutral genetic variation, and parameterizing population genetic models (Lynch et al. 1999).

To accurately characterize the mutational process, an unbiased sample of spontaneous mutations is needed; however, the standing allelic variation is a biased sample because it has been previously screened by selection. What is needed is a way to neutralize selection to the maximum extent possible so that new mutants, despite their fitness effects, can be fixed within replicate populations (Figure 5). This is the basic principle underlying the method of “mutation-accumulation” (MA). An MA experiment is, in essence, EE stood on its head: instead of experimentally investigating what evolves when a particular selective regime is imposed, the question of interest is: what evolves when selection is removed? The basic principles of MA are outlined in Appendix 3 in File S1, as
are some general findings about the mutational process in *Caenorhabditis*.

Although mutation is crucial for long-term evolution, in sexually reproducing organisms, short-term evolution on the order of tens of generations mostly occurs from standing genetic variation. Explicit tests of theoretical expectations regarding the relative timeframes where mutation and/or standing genetic variation are important are mostly missing.
Box 2 Some caveats in EE

The studies of S. Estes, D. Denver and colleagues (Estes and Lynch 2003; Denver et al. 2010; Estes et al. 2011) were useful in terms of revealing the capacity for rapid, repeatable responses to a population genetic environment at phenotypic and DNA sequence levels, but it is instructive to consider some limitations of this work, and how they may be overcome in future Caenorhabditis EE research. First, the authors’ inability to genetically isolate candidate mutations meant that they could not fully substantiate their claim that reverse evolution of fitness was due to compensatory mutation, or characterize the exact nature of any deleterious-adaptive mutational interactions. Second, the Denver et al. (2010) study could only detect single base pair changes in the nuclear genome; the contribution of other mutation types or mitochondrial DNA sequence changes to the evolutionary response was thus unknown. Although detecting genome rearrangements can still prove technically challenging, we can now readily survey other mutation types, like structural and copy-number variants (Farslow et al. 2015), and, with the high sequence coverage achievable for mitochondrial DNA, evaluate evolution of heteroplasmatic mutations (e.g., Wernick et al. 2016). Lastly, a shortcoming of Estes et al. (2011) study was that fitness of the evolved lines was quantified under standard, benign laboratory conditions rather than in the high-density, competitive conditions under which evolution had occurred; this left room for lingering questions regarding how genotype-by-environment, maternal, or other transgenerational effects may have contributed to the rapid response. A better approach would have been to assess fitness changes using competitive head-to-head assays wherein evolved lines are competed against a GFP-marked strain after culturing of populations in a “common garden” for a few generations (Figure 2 and Figure 3).

However, not only in Caenorhabditis but also in other model organisms.

Evolution from standing genetic variation can occur through the sorting of extant genotypes by segregation or through a combination of segregation and recombination between existing genotypes. For Caenorhabditis EE, the population genetic consequences of selfing vs. outcrossing should be considered as breeding mode is expected to influence the degree of effective segregation and recombination (Appendix 4 in File S1). In general, selfing reduces effective population sizes, particularly if background selection of deleterious recessive alleles is present, potentiating genetic drift and the loss of neutral diversity. When considering multiple loci, however, neutral diversity can be maintained under selfing by hitchhiking with (positive) selected alleles and/or because of “identity disequilibrium” generated by genotypic correlations. Understanding selection under selfing is highly difficult but we do know that it fundamentally depends on the degree of dominance within loci and epistasis between loci.

To address the problems of evolution from standing genetic variation, EE with C. remanei and C. elegans have employed hybrid ancestors constructed from crosses of several wild isolates (LaMunyon and Ward 2002; Anderson et al. 2010; Schulte et al. 2010; Chen and Maklakov 2012; Teotónio et al. 2012; Sikkink et al. 2014b; Palopoli et al. 2015). The dioecious C. remanei is highly polymorphic, with an average per locus nucleotide diversity on the order of 5% (Cutter et al. 2006), and, despite the potential for outbreeding depression during crosses among these isolates (Dolgin et al. 2007), the resulting hybrid strains retain plenty of genetic variation available for selection during EE (Chen and Maklakov 2012; Sikkink et al. 2014b, 2015). The same is true for C. elegans hybrid ancestors (Anderson et al. 2011; Masri et al. 2013; Carvalho et al. 2014), despite displaying average polymorphism levels of only 0.2% (Cutter 2006). Recently, however, sequencing of the Hawaiian CB4856 strain genome revealed that polymorphism in C. elegans can be quite high, reaching 16% and possibly higher in some genomic regions (Thompson et al. 2015). It is perhaps not surprising then that EE responses in C. elegans starting from hybrid populations are comparable to those of obligate outcrossing species. Nonetheless, these C. elegans hybrid populations have been shown to lose much of their initial polymorphism via purging due to outbreeding depression during strain construction (Teotónio et al. 2012; Chelo et al. 2013a).

It is common to consider the evolutionary response of individual phenotypic traits (e.g., “heat tolerance”) to selection, but organisms are, of course, not merely collections of atomized traits. Multivariate evolutionary responses will depend on how pleiotropy, linkage disequilibrium, and inbreeding/assortative mating have shaped genetic variances and covariances among traits [summarized in the genetic variance-covariance matrix G; (Lande 1980; Phillips and McGuigan 2006)]. Because of its highly selfing sexual system, resulting in strong linkage and inbreeding, multivariate evolution is expected to be more constrained in C. elegans than in C. remanei. In the hybrid C. elegans populations of Teotónio et al. (2012), 100 generations of EE under 50% of partial selfing or 100% of obligate outcrossing similarly reduced the initially high linkage disequilibrium to background levels for genetic distances ~1 cm (on a F2 map scale, where each chromosome is 50 cm). Most evolution appeared to result from single-locus selection with few signs of reduced heterozygosity signaling large-scale sweeps (Chelo and Teotónio 2013). But, depending on chromosomal location, and because recombination rates are not monotonic along the chromosomes (Rockman and Kruglyak 2009), this
small amount of linkage still means that hundreds to possibly thousands of alleles at different loci will tend to be inherited together, and have the potential to generate significant genetic correlations among traits.

Just as the fitness effects of new mutations can be characterized by means of an MA experiment, the fitness effects of standing genetic variants can be assessed by means of an “inbreeding experiment.” As with MA, several lineages are derived by selfing or extreme inbreeding with the goal of reducing heterozygosity and fixing alternative alleles within each lineage in a neutral fashion. A reduction of fitness among inbred lineages relative to the ancestral outbred population indicates inbreeding depression, and deviations from expected levels of homozygosity at marker (neutral) loci can reveal if inbreeding depression is due to strongly deleterious recessive alleles that are purged during the inbreeding experiment (Dolgin et al. 2007; Ebel and Phillips 2016). In contrast, deleterious recessive alleles in repulsion linkage disequilibrium (“associative overdominance”) or truly overdominant alleles can be maintained through balancing selection during inbreeding (Mukai et al. 1964; Ohta and Kimura 1970; Ohta 1971), and provide an alternative source of inbreeding depression. Distinguishing between these two latter alternatives is, however, notoriously difficult (Chelo et al. 2013a).

Data availability


Exemplars of Caenorhabditis Experimental Evolution

Maintenance of genetic variation

One of the most enduring questions in evolutionary biology is: Why so much genetic variation? The discussion surrounding that question will be familiar to most readers of GENETICS; it suffices to say that the poles of the issue are “Mutation + Drift” at one end, and “Balancing Selection” at the other. In recent decades, the debate has centered on variation at the molecular level, but arguments long antedates the molecular era. (Dobzhansky 1937; Lewontin 1974; Kimura 1983; Charlesworth 2015).

With respect to the understanding of molecular variation, EE, including in C. elegans, has made a critical contribution by providing (nearly) unbiased estimates of the rate and spectrum of spontaneous mutation unencumbered by selection (Appendix 3 in File S1). Direct estimates of the per-nucleotide, per-generation mutation rate, \( \mu \), in model organisms provide a critical reality-check on indirect estimates of \( \mu \) from sites putatively free from natural selection (e.g., processed pseudogenes) because many tests of non-neutral molecular evolution assume there is a class of neutrally evolving sites that can serve as a reference (Kondrashov and Crow 1993; Cutter and Payseur 2003; Witherspoon and Robertson 2003). Direct estimates of \( \mu \) are usually within a few-fold close to inferences drawn indirectly from putatively neutral sites, although there are occasionally incongruities between direct and indirect estimates, notably a consistently twofold higher direct estimate in humans (Shendure and Akey 2015).

The question “Why so much...” takes an interesting turn in C. elegans, into “Why so little genetic variation?” Andersen et al. (2012) reported a large-scale survey of genome-wide nucleotide variation in C. elegans, and concluded that large regions of the genome must coalesce within a few hundred generations, presumably the result of one or more recent, global selective sweep. However, Thompson et al. (2015) compared the genomes of N2 and CB4856 (“Hawaii”), and reported that 2–3% of the genome appeared to harbor ancient segregating variation (“ancient” meaning an estimated average time of divergence on the order of 10⁵ generations), which they attributed to long-term balancing selection. Taken at face value, these two studies lead to the seemingly odd situation of most of the genome being genetically depauperate, but a dispersed fraction carrying ancient polymorphisms.

The question of Why so much genetic variation? applies equally to emergent phenotypic traits. If mutation and random genetic drift are the only forces at work, at mutation-drift equilibrium the standing genetic variance for the trait should equal \( V_G = 2N_eV_M \) (Lynch and Hill 1986), where \( N_e \)
is the effective population size (Box 1) and \( V_M \) the mutational variance (Appendix 1 in File S1). If an estimate of \( N_e \) is available, as it would be from segregating nucleotide variation and an independent estimate of the per-nucleotide mutation rate (because \( \theta = 4N_e\mu \)), a value of \( V_G << V_M \) implies the trait is subject to purifying selection and can be used as an approximate estimate of the strength of selection acting against mutations that affect the trait (Barton 1990). For example, Denver et al. (2005) compared \( V_M \) and \( V_G \) for hundreds of gene transcripts in four MA lines and five wild isolates of \( C. elegans \) (including N2). Small sample sizes notwithstanding, the results were impressive: of \( \sim 3700 \) genes investigated, not one had a \( V_M/V_G \) ratio greater than the neutral expectation, from which it could be concluded that transcription is under strong and ubiquitous stabilizing selection.

More recently, this approach has been used to assess pattern of variation that are expected a priori to be under different types of selection (Salomon et al. 2009; Braendle et al. 2010; Etienne et al. 2015; Farhadifar et al. 2015). The results are summarized in Figure 3 of Farhadifar et al. (2016). Three salient results emerge. First, across a broad spectrum of traits, \( V_M \) explains a large fraction of the variance in \( V_G \) (\( \sim 90\% \)), and the ratio \( V_M/V_G \) is well below the neutral expectation. That finding is entirely consistent with genetic variation in \( C. elegans \) being largely modulated by mutation and purifying selection (Rockman et al. 2010), but it also suggests that the strength of purifying selection must be quite similar among disparate traits. Second, for a large fraction of the traits, \( V_G \approx 500V_M \). These findings are also consistent with the conclusion of Andersen et al. (2012) that a large fraction of the \( C. elegans \) genome coalesces on the order of a few hundred generations, and they further suggest that random background selection across a highly linked genome predominates over trait-specific effects. However, \( V_M/V_G \) for some traits is clearly well below the trend, and the pattern is not random: life history traits and vulva development traits (Braendle et al. 2010) clearly experience stronger purifying selection than other traits.

The second class of unsupervised tests of non-neutral evolution involves the comparison of the between-population component of variance at putatively neutral marker loci (\( F_{ST} \)), with the between-population component of genetic variance of phenotypic traits (\( Q_{ST} \)) (Lande 1992; Spitz 1993). For traits experiencing significant stabilizing selection, \( Q_{ST} \) is predicted to be \( \sim F_{ST} \), whereas, for traits experiencing diversifying directional selection (i.e., local adaptation), \( Q_{ST} \) is predicted to be \( > F_{ST} \). To our knowledge, this type of test has yet to be applied in \( Caenorhabditis \). \( C. elegans \) is probably not conducive to \( Q_{ST}/F_{ST} \) comparisons due to its unusual population structure, but \( C. briggsae \), with its hierarchical population structure (Thomas et al. 2015), would seem to be an ideal candidate.

Although comparisons of \( V_G \) and \( V_M \) have not turned up compelling evidence for balancing selection, several lines of evidence support the conjecture that balancing selection may play a role in maintaining genetic variation in natural \( C. elegans \) (Thompson et al. 2015). For example, Greene et al. (2016) recently presented evidence that balancing selection on foraging behavior, mediated by chemoreceptor genes involved in pheromone signaling, has maintained genetic variation in foraging behavior, and, further, that the genomic region involved was one identified by Thompson et al. (2015) as a putative candidate for balancing selection. Competition experiments further revealed that the fitness effects depended on environmental context, such that one allele was favored in a constant-food environment, and the other favored in a patchy-food environment. In another example, Peters et al. (2003) characterized the fitness effects of EMS-induced mutations and found that 10/19 lines had point estimates of fitness greater than the unmutated control, and that 3/19 lines were significantly overdominant; see also Manoel et al. (2007). Other examples of specific genes or gene complexes being potentially maintained by balancing selection have been found in natural \( C. elegans \) (e.g., Gloria-Soria and Azevedo 2008; Seidel et al. 2008; Ghosh et al. 2012; Ashe et al. 2013), and, during EE from standing genetic variation, balancing selection has been invoked to explain
maintenance of excess heterozygosity (Chelo and Teotónio 2013) and frequency-dependence (Chelo et al. 2013b). In addition, for outcrossing species, heterozygosity levels may have been underestimated (Barrière et al. 2009). Taken together, all these findings have important implications, because even a small number of overdominant loci (or recessive alleles at multiple loci in repulsion linkage) capable of generating balancing selection can have an outsized effect on the total inbreeding load (Dobzhansky 1955).

**Evolution of reproductive mode**

Transitions from outcrossing to selfing are believed to be common in both animals and plants (Jarne and Auld 2006; Barrett 2008), and, in particular, the case of *Caenorhabditis*, three independent transitions from dioecy to androdioecy have occurred (Könntke et al. 2004). Even though androdioecy is a relatively rare reproductive system, and *Caenorhabditis* hermaphrodites somewhat unusual in their inability to outcross with each other, EE with *Caenorhabditis* has contributed to our understanding of selection during transitions from outcrossing to selfing, as well as the maintenance of mixed breeding modes during evolution.

Provided that mutation to self-compatibility is not limiting (Hill et al. 2006; Katju et al. 2008; Baldi et al. 2009), one route to selfing involves restrictions on outcrossing between males and females (e.g., due to time required to find a mate). Hermaphrodites able to self autonomously will in this case be favored because they provide reproductive assurance and guarantee population survival (Lloyd 1976; Busch and Delph 2012). Second, selfing lineages may outcompete outcrossing lineages if the latter suffer from a “cost of males,” since males do not reproduce by themselves, but still consume ecological or developmental resources (Maynard Smith 1978; Lively and Lloyd 1990). A final route to selfing involves density-dependent selection among subpopulations (demes) for higher dispersal, which can be correlated with the ability to self and establish viable colonies without partners (Cheptou 2012).

Theologidis et al. (2014) took advantage of genetic manipulation of sex determination in *C. elegans* to show that the benefit of reproductive assurance is sufficient to explain transitions to selfing. As expected under this hypothesis, the successful invasion of hermaphrodites in male–female dioecious populations resulted in adaptation to a novel environment (high salt concentration) where outcrossing was restricted. Adaptation was not due to the loss of males, but rather to the replacement of females by hermaphrodites, as shown by the lower adaptive rates of androdioecious populations, which similarly lost males and reproduced exclusively by selfing by the end of EE. (Theologidis et al. 2014) did not test for density-dependent selection for dispersal among demes during transitions to selfing, but it is unlikely this form of selection favors selfing over outcrossing in natural *Caenorhabditis*. Not only are *C. elegans* males are more vagile than hermaphrodites (Lipton et al. 2004), they also have higher survivorship than hermaphrodites at the dauer stage (Morran et al. 2009a)—presumed to be the stage at which most dispersal occurs. The jury is nonetheless still out, and tests of the role of density-dependent selection on transitions to selfing are needed.

Because outcrossing in androdioecious *Caenorhabditis* is necessarily linked to the presence of males, transitions to selfing will be more difficult if male fitness components evolve prior to females being replaced by hermaphrodites—see Theologidis et al. (2014) for such an example. Similarly, because outcrossing allows for the continued generation of adaptive genotypes not easily accessible through selfing, any factor that reduces effective recombination may reduce the likelihood of the transition to selfing. The latter hypothesis was recently supported by the *C. elegans* EE study of Slowinski et al. (2016). In this example, in which populations evolved under ever-changing environmental conditions and fluctuating selection (in the form of a coevolving pathogen), hermaphrodites were unable to invade dioecious populations. In contrast, hermaphrodites readily invaded and reached high frequencies in populations evolved under constant environmental and selective conditions, either because they provided reproductive assurance, or because populations no longer paid a cost of producing males.

Once the transition from outcrossing to selfing has been achieved, classical theory suggests that the degree of standing inbreeding depression generated by deleterious recessive alleles will determine whether selfing persists or the population reverts to outcrossing (e.g., Lande and Schemske 1985). Given sufficient time, selfing populations are expected to suffer less inbreeding depression than outcrossing populations since deleterious recessive alleles will be more effectively purged by selection. EE results with *C. elegans* are remarkably consistent with this hypothesis. Although inbred *C. elegans* populations with experimentally elevated mutation rates (e.g., DNA-repair deficiencies or mutagenesis) do not maintain males (Cutter 2005; Manoel et al. 2007), whenever there is opportunity for the build-up of inbreeding depression because of high mutation rates, males are clearly favored and outcrossing can be maintained at higher rates than in unmutagenized controls (Morran et al. 2009b). And, as expected from theory, comparative evidence between selfing and outcrossing *Caenorhabditis* species indicates that male–female populations, such as those of *C. remanei*, maintain higher deleterious loads than predominantly selfing populations, such as those of *C. elegans* (Dolgin et al. 2007). The EE results from Chelo et al. (2013a) similarly suggest that partially selfing populations can maintain lower deleterious recessive loads than obligately outcrossing populations.

Not surprisingly, whenever there is standing genetic variation for fitness components related to outcrossing, like male reproductive success (LaMunyon and Ward 1998; Teotónio et al. 2006; Wegewitz et al. 2008; Murray et al. 2011), adaptation to novel environments is correlated with the evolution of higher outcrossing rates (Morran et al. 2009a, 2011; Teotónio et al. 2012; Slowinski et al. 2016). Adaptation is faster in obligate outcrossing populations than in facultative
selfing populations, which in turn evolve faster than obligate selfing populations (Morran et al. 2009b). These observations are consistent with (i) balancing selection on overdominant loci favoring outcrossing (since more heterozygotes are produced by outcrossing than selfing); and/or (ii) outcrossing increasing effective recombination, and therefore generating more adaptive genotypes than selfing. Both of these scenarios have received experimental support. In the experiments of Teotónio et al. (2012), more heterozygosity was maintained than that expected by genetic drift and associative overdominance of linked deleterious partially recessive alleles (Chelo and Teotónio 2013), suggesting that outcrossing was favored because of balancing selection on overdominant loci. Further, consistent with multi-locus theory (Navarro and Barton 2002). Chelo and Teotónio (2013) inferred the presence of (negative) epistatic selection from the observed diminishing returns of fitness with increasing heterozygosity. Intriguingly, this form of epistasis can in part explain the evolution of recombination modifiers under partial selfing (Roze and Lenormand 2005), although no EE study has yet been undertaken to provide direct support for such an idea.

Regarding the second scenario, Morran et al. (2011) showed that increased outcrossing rates were only maintained during evolution in a fluctuating novel environment [as in (Slowinski et al. 2016), in the form of a coevolving pathogen], but not during evolution in a novel but constant environment in which case outcrossing rates increased initially and then decreased soon thereafter. Although rapid fitness recovery from mutationally degraded backgrounds can be achieved by selfing alone (Estes et al. 2004; Morran et al. 2010), these results strongly suggest that outcrossing increases effective recombination and allows the generation of more adaptive genotypes than does selfing.

Over time, selfing can lead to the evolution of epistatic gene complexes (by local adaptation or genetic drift) whose break-up by outcrossing will lead to outbreeding depression. Comparison of crosses between wild isolates in several Caenorhabditis species, followed by inbreeding experiments have indicated that the break-up of coevolved gene complexes could explain why selfing predominates over outcrossing in nature (Dolgin et al. 2007; Seidel et al. 2008; Gaertner et al. 2012; Chelo et al. 2013a; Gimond et al. 2013). Vexingly, the conundrum that outcrossing in C. elegans appears to be quite rare in nature but is not particularly difficult to maintain in the laboratory remains unresolved (Chasnov and Chow 2002; Stewart and Phillips 2002; Anderson et al. 2010). A complex balance between different forms of selection operating at different levels may explain the maintenance of partial selfing under a variety of conditions, some of which have been explored with EE, including: the evolution of sexual conflict between males and hermaphrodites (Chasnov 2013; Carvalho et al. 2014; Palopoli et al. 2015), the allocation of resources toward self-spermatogenesis or oogenesis in hermaphrodites (Anderson et al. 2010; Murray and Cutter 2011; Poulet et al. 2016), rapid adaptation to specialized environments (Morran et al. 2009a; Masri et al. 2013; Slowinski et al. 2016), and/or unresolved inbreeding depression (Chelo et al. 2013a). The topic of evolution of breeding modes will surely keep Caenorhabditis EE researchers occupied for many years to come.

**Evolution in variable environments**

The difference in trait values displayed by a single genotype across multiple environments is known as phenotypic plasticity, long thought to be a key element in structuring both the response to selection in variable environments, and in the evolution of developmental and physiological systems (Via et al. 1995; Ghalmabor et al. 2007). Upon encountering a new environment, population survival will depend on plastic genotypes closely matching the “optimum” phenotype (Price et al. 2003; Chevin et al. 2010). But, because the optimum phenotype can be matched by existing genotypes, the strength of directional selection is weakened and adaptation possibly hampered. Even if plasticity obviates the need for short-term adaptation, there may still be opportunity for selection of genotypes that improve performance in the novel environment, particularly if there is a cost associated with maintaining the developmental and physiological programs underlying phenotypes that are only rarely expressed. Initial plasticity in this case will facilitate long-term adaptation by allowing time for the “genetic assimilation” of the optimum phenotype in the novel environment by novel mutational or segregation/recombination input. The study of Sikkink et al. (2014b) in C. remanei has provided convincing evidence for such genetic assimilation, in line with classic work in Drosophila (Waddington 1953). Sikkink and colleagues adapted large, polymorphic populations to environments generating high levels of temperature and oxidative stress and observed rapid and fairly independent responses to selection alongside diminished plasticity when evolved populations were returned to their ancestral environments. The role that stochastic trait variation may play in genetic assimilation and in the evolution of trait “robustness” is currently untested (Felix and Barkoulas 2015). However, several C. elegans studies have found significant heritability for stochastic traits that could be under selection (Braendle and Felix 2008; Braendle et al. 2010; Duveau and Felix 2012).

A particularly fascinating form of plasticity results from the transmission of environmental effects experienced by a parent to the phenotype expressed by the offspring, i.e., transgenerational plasticity. When developing individuals do not have the possibility to assess the environment they will face at the time of reproduction, phenotypic plasticity has little opportunity to be selected. Instead, and as long as there is an environmental correlation between generations, it is expected that mothers will provision, or cue, their offspring accordingly. Maternal effects could thus be especially important for adaptation to fluctuating environments. Dey et al. (2016) explicitly evaluated this idea by exposing C. elegans populations to either regularly or irregularly fluctuating normoxia–anoxia larval hatching environments. They observed
the evolution of anticipatory maternal effects such that hermaphrodites were able to shift glycogen provisioning to developing embryos to achieve levels appropriate for the environment experienced by their broods in the next generation. Contrary to theoretical predictions (Proulx and Tei\'tonio 2017), however, populations experiencing irregularly fluctuating environments failed to evolve an anticipatory response in the form of maternal “bet-hedging,” in which mothers randomize offspring phenotypes. Instead, evolution of longer-term (>2 generations) transgenerational effects may have been selected and promoted adaptation to fluctuating environments. Whether such long-term transgenerational effects are necessarily adaptive is still controversial (Wolf et al. 1998; Badyaev and Uller 2009; Uller et al. 2013; Burgess and Marshall 2014). Within C. elegans, it is increasingly apparent, however, that heritable nongenetic carry-over effects can persist for long periods—up to 30 generations or more (Katz et al. 2009; Burton et al. 2011; Luteijn et al. 2012; Shirayama et al. 2012; Ashe et al. 2013; Rankin 2015).

Besides variable abiotic environments, interactions between host organisms and their pathogens and/or parasites are expected to generate strong fluctuating selection. Of special interest is the extent to which host–pathogen relationships are genotype-specific, and the ways in which evolution with coevolving pathogens (or hosts) differs from evolution in the presence of nonevolving pathogens (or hosts) (Box 3 and Figure 6). Two studies exemplify advances in our understanding of host–pathogen coevolution with C. elegans EE. In one, Schulte and colleagues allowed 20 replicate populations of C. elegans to coevolve with the bacterial pathogen Bacillus thuringiensis (Bt) for 48 generations (Schulte et al. 2010). As controls, 10 replicates of the Bt pathogen, and 20 replicate populations of C. elegans were allowed to evolve in parallel in the absence of the other. As expected, the coevolved pathogen evolved increased virulence and the coevolved host evolved increased resistance when compared to the non-coevolved controls. Also as expected, the coevolved traits came with a fitness cost: several life-history traits (presumably correlated with relative fitness) decreased in the coevolved host and pathogen populations compared to the non-coevolved controls.

Genetic diversity was quantified for three toxin genes in the pathogen, and nine unlinked microsatellite loci in the host (Schulte et al. 2010). Results for the pathogen were clear: coevolution led to an increased rate of evolution, reduced diversity within populations, and greater diversity between populations (see also Masri et al. 2015). Results for the host were less clear-cut, with different loci exhibiting different patterns of evolution, although the overall rate of evolution was greater in the coevolved host than in the control. Coevolution led to an apparently increased rate of recombination in the pathogen but not in the host.

In one of the most influential coevolution experiments to date, Morran and colleagues allowed experimental populations of C. elegans with varying degrees of outcrossing to coevolve with the bacterial pathogen Serratia marcescens (Morran et al. 2011). As briefly discussed above, the frequency of outcrossing increased in populations in which the pathogen was allowed to coevolve with the host, whereas populations of obligate selfers went extinct. Conversely, males were lost in populations in which the evolving C. elegans were exposed to non-coevolving pathogens. Similar results were observed in the pathogen, wherein coevolving populations evolved significantly greater infectivity than did populations evolving in the presence of a non-coevolving host. The results of these experiments are in concordance with predictions of the Red Queen hypothesis for the evolution of outcrossing (Jaenike 1978). However, the potential remains that Hill-Robertson effects resulting from fluctuating directional selection imposed by the pathogen rather than fluctuating epistatic selection resulting from negative frequency-dependent selection—the signature of the Red Queen (Barton 1995)—led to the advantage of outcrossing. More generally, it is unclear the extent to which sexual selection reverses, or reinforces, natural selection during coevolution, as different sexes can have different resistance and tolerance to pathogens (Masri et al. 2013).

How the composition of microbial communities can generate variable and fluctuating selection is, at present, unknown, and much work is needed in order to characterize these communities and their potential effects on Caenorhabditis. Are they constant during individual lifetime? Do they have high turnover rates when populations are challenged with novel environments? Two recent studies, by Dirksen et al. (2016) and Samuel et al. (2016), provided the first systematic sampling of the bacterial microbiota associated with field-collected C. elegans. Samuel and colleagues cultured C. elegans on >500 different bacterial isolates; there were consistent effects of bacterial taxon on nematode demography, and on multiple indicators of physiological stress. Dirksen et al. (2016) sampled the microbiomes associated with C. elegans and the congeneric C. briggsae and C. remanei, and reported consistent differences between the microbiotas of C. remanei and those of C. elegans and C. briggsae. Further, they found that populations of C. elegans initiated with a cocktail of 14 bacterial taxa consistently retained only a subset of taxa, but that the specific subset retained were specific to both (host) genotype and developmental stage.

**Future directions and conclusions**

While Caenorhabditis EE has begun to touch on many aspects of population and quantitative genetics, its potential use in addressing questions related to evolution in structured populations remains largely untapped. An exception is the study of Gloria-Soria and Azevedo (2008) that showed that mutations at the npr-1 locus could lead to differences in dispersal rates and the maintenance of polymorphism driven by behavioral characteristics. Another exception is the recent study of Greene et al. (2016), showing that mutations at the srx-43 locus could lead to density-dependent selection. But few studies have used the systematic manipulation of migration
Box 3 Host–pathogen coevolution experiments

Signature features of coevolution are that the fitness effects of alleles in one (host or pathogen) species may depend on genotype frequencies in the other species, and that the effects of alleles in coevolved host populations confer high(er) fitness in the presence of the coevolved pathogen genotype. A straightforward prediction is that some salient property(s) of coevolved hosts and pathogens will differ from hosts evolved in the presence of non-coevolving pathogens, and vice versa, if coevolution has played an important role in the evolution of either partner. To unambiguously demonstrate such a difference, hosts must be evolved in the presence of coevolving pathogens, and in the presence of non-coevolving pathogens, and, similarly, pathogens must be evolved in the presence of coevolving hosts and with non-coevolving hosts (Figure 6), and the relevant properties compared among these groups and with the nonevolved ancestors. A comprehensive experiment would have five treatment groups: (1) Pathogens evolving to the laboratory environment in the absence of hosts (this would not be possible with an obligate parasite such as a virus); (2) pathogens evolving with nonevolving hosts (this can be easily done by serially passaging pathogens onto populations of naïve hosts); (3) hosts evolving in the absence of pathogens (as in 1); (4) hosts evolving with nonevolving pathogens (as in 2); and (5) coevolving hosts and pathogens. Nonevolved cryopreserved ancestral pathogens, and hosts, would constitute the baseline control for such an experiment.

Caenorhabditis provide a uniquely powerful model system with which to experimentally investigate coevolution because they are so readily cryopreserved. C. elegans can host a wide spectrum of bacteria, fungi, protozoa, and viruses, as well as combinations thereof (e.g., Dirksen et al. 2016). Moreover, unlike dipterans, which fly, and plants, which attract pollinators and herbivores that fly, nematodes carrying pathogens can be safely contained with a minimum of effort and expense. Those factors have made C. elegans a popular model system for characterizing the genetic basis of pathogen resistance and avoidance. Many studies have focused on specific mutants on the N2 background (e.g., Mahajan-Miklos et al. 1999; Aballay and Ausubel 2001; Garsin et al. 2003; Troemel et al. 2006), but a number of studies have employed standard quantitative genetic line-cross analysis between N2 and the Hawaiian strain CB4856 to dissect the genetic basis of pathogen-related traits (Reddy et al. 2009; Andersen et al. 2014; Glater et al. 2014; Nakad et al. 2016). The latter have revealed significant genetic variation for a variety of pathogen-related traits (e.g., olfactory behavior, oxygen avoidance, longevity, and innate immunity), often revealing genes that explain a significant fraction of the genetic variation. Most investigations of host–pathogen interactions involving C. elegans have understandably focused on host traits, but there is also a significant body of work focusing on pathogen or parasite traits (e.g., Tan et al. 1999; Sifri et al. 2003; Huber et al. 2004; White et al. 2016).

Theoretical characterizations of the genetics of host–pathogen relationships have focused on interactions between small numbers of host genes and pathogen genes (Frank 1994; Parker 1994; Agrawal and Lively 2002), so empirical investigations of the genetics of host susceptibility to infection have understandably focused on identifying genes of large effect. However, the prevalence of variation among host genotypes in pathogen susceptibility suggests that pathogen susceptibility may behave more like a classical polygenic trait than a Mendelian trait. Of note, Etienne et al. (2015) quantified the mutational input of genetic variation for susceptibility to the bacterial pathogen Pseudomonas aeruginosa in C. elegans and found that, indeed, mutational variance for susceptibility to P. aeruginosa accumulated at a rate similar to typical quantitative traits.

Rates to examine, say, the influence of local adaptation on rates of genomic change, or the evolution of dispersal traits (Friedenberg 2003). Indeed, a stack of Petri dishes, each containing its own population, would seem to be the perfect representation of S. wrights original idealization of a collection of demes (Wright 1969).

Adding population structure to existing approaches would provide a nice complement of spatial variation in selection to previous studies that have largely concentrated on temporal variation in selection. As more environmental variables are added, investigation of transgenerational inheritance will also become increasingly important in Caenorhabditis EE studies, since experience of previous generations can clearly influence a wide variety of physiological and life history responses (Miska and Ferguson-Smith 2016). Caution is warranted to ensure that any work building upon our knowledge of transgenerational carry-over effects be conducted in a rigorous population genetic framework, as there is already a set of theory that deals with the genetics and selection for intergenerational interactions (e.g., Lande and Price 1989; Lande and Kirkpatrick 1990; Slatkin 2009; Furrow and Feldman 2014; Proulx and Teotónio 2017). Part of the surprise from studies conducted to date, however, is that some of these effects appear to be extremely persistent, and, therefore, have the potential to obscure effects that would ordinarily be attributed to genetic changes.

In many cases, simply observing trait changes over time is sufficient for addressing the question at hand. Nevertheless, one of the strong appeals of using such well-developed model systems as Caenorhabditis for EE is the potential to identify
the underlying genetic changes responsible for an evolutionary response—a goal quite apart from that of traditional mutagenesis studies. In this age of genomics, many candidate polymorphisms with potentially minor or rare contributions to a phenotype would be expected to emerge when millions of such polymorphisms are examined simultaneously. This can make it quite difficult to distinguish natural selection on individual loci from background levels of genetic drift across the whole genome. One of the primary advantages of *C. elegans* and its relatives over other metazoan models for EE is that it is reasonable to use very large population sizes, and, thus, to capture (and characterize) more such variants. Current studies frequently utilize thousands of individuals within a single population. Future experiments performed using liquid culture of *Caenorhabditis* or novel rearing approaches should allow populations to be maintained at sizes in the millions or more, making EE more typical of natural populations. While still perhaps not at yeast or bacteria population sizes, moving in this direction—especially in sexual species—has the potential to qualitatively change the way that experiments are conducted.

Once putative genetic changes have been identified, application of new genetic transformation methods will allow functional hypotheses to be more readily tested, especially outside of *C. elegans*. Perhaps more interestingly, the ability to make identical allelic substitutions in different genetic backgrounds opens up a new world of potential experiments. Chief among these will be the ability to more precisely test for epistatic effects between loci within different genetic backgrounds. One can readily imagine using CRISPR to manipulate the genetic backgrounds of different base populations that serve as the basis of later evolutionary experiments (Dickinson and Goldstein 2016). How dependent are evolutionary outcomes on initial genetic background? How are compensatory changes structured across different classes of mutations? What is the role of structural variation in determining the response to selection? These are a few of the kinds of questions that are now tractable with precision genomic editing.

Clearly, there are major gaps to be filled by future work aimed at identifying and characterizing the genetic and phenotypic bases of adaptation and by “translational” studies to connect such results to evolution in nature. Although functional genetic information for *Caenorhabditis* is still largely lacking (Petersen et al. 2015), some genetic factors mediating *C. elegans*’ tolerance to various forms of environmental challenges likely to be encountered by natural populations, such as pathogen exposure, or osmotic, thermal, and oxygen stress, have started to be dissected (e.g., Lithgow et al. 1995; Lamitina *et al.* 2004; Frazier and Roth 2009; Reddy *et al.* 2009; LaRue and Padilla 2011; Andersen *et al.* 2014). Application of EE techniques wherein populations are exposed to such conditions would appear to hold great promise for uncovering the patterns, population genetic requirements, and genetic bases of adaptive responses (e.g., Sikkink *et al.* 2014b, 2015; Dey *et al.* 2016).

Finally, the fundamental problem of evolution is the problem of the phenotype. One advantage of *Caenorhabditis* is that these nematodes are relatively easy to manipulate via a variety of methods, allowing high-throughput, high-precision phenotyping to be applied (Husson *et al.* 2013; Andersen *et al.* 2015). The potential of these approaches, e.g., microfluidics (McCormick *et al.* 2011; Yan *et al.* 2014; Gupta and Rezai 2016), has not really begun to be utilized for EE. Similarly, the physical transparency of *Caenorhabditis* allows their cellular and developmental structure and function to be examined with exquisite detail in an EE context (Braendle *et al.* 2010; Farhadifar *et al.* 2015; Phillips and Bowerman 2015). This opens the possibility creating a new and rigorous evolutionary cell and developmental biology, with *Caenorhabditis* as a central player (Braendle *et al.* 2011; Phillips and Bowerman 2015). The role that EE might play in such an effort is currently undefined.

*Caenorhabditis* species have come of age as models for EE. EE with these nematodes have provided significant insights into the origin and evolution of reproductive modes, adaptation to changing environments and into mutation rates, their genomic context and fitness effects. Use of *Caenorhabditis* allows unprecedented control over the properties of standing variation, population sizes, transgenerational effects, and degree of sexuality for a metazoan. Coupled with our extensive understanding of their genetics, and their cellular and developmental biology, future EE studies with *Caenorhabditis* promise to unravel many of the outstanding problems of evolutionary biology.

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