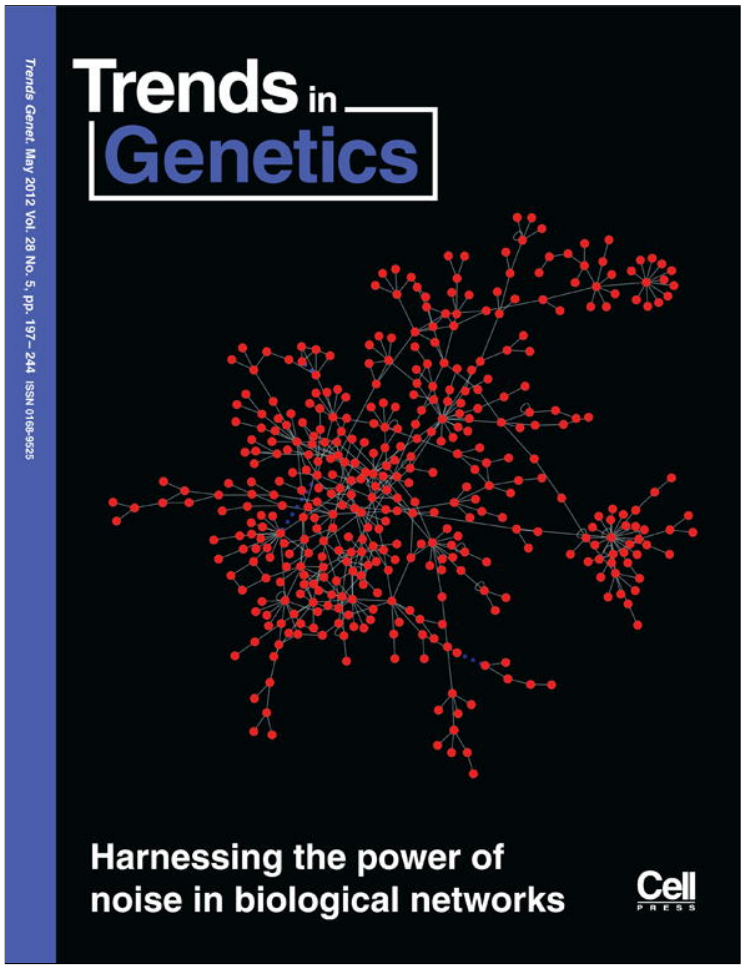

Review

# Causes and consequences of the evolution of reproductive mode in *Caenorhabditis* nematodes

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**Reproduction is directly connected to the suite of developmental and physiological mechanisms that enable it, but how it occurs also has consequences for the genetics, ecology and longer term evolutionary potential of a lineage. In the nematode *Caenorhabditis elegans*, anatomically female XX worms can self-fertilize their eggs. This ability evolved recently and in multiple *Caenorhabditis* lineages from male–female ancestors, providing a model for examining both the developmental causes and longer term consequences of a novel, convergently evolved reproductive mode. Here, we review recent work that implicates translation control in the evolution of XX spermatogenesis, with different selfing lineages possessing both reproducible and idiosyncratic features. We also discuss the consequences of selfing, which leads to a rapid loss of variation and relaxation of natural and sexual selection on mating-related traits, and may ultimately put selfing lineages at a higher risk of extinction.**

## Developmental causes and organismal consequences of reproductive mode

Since its rebirth approximately 30 years ago [1,2], a major goal of evolutionary developmental biology (EDB here, but often referred to as 'evo-devo') has been the discovery of the empirical developmental and genetic details of how anatomical novelty arises. This is difficult work, and convincing connections between the evolution of DNA sequence and morphology have only been forged during the past decade. With the first apparent victories now won, general principles are being inferred [3–5]. Much of the recent work on animal EDB has focused on somatic traits, but germ cells also evolve, and merit study for several reasons. First, changes in germ cell properties often underlie many fascinating adaptations [6]. These include abrupt shifts in reproductive mode, such as loss of a feeding larva [7] or changes in breeding systems [8]. Second, germ cell development is unusually dependent upon post-transcriptional gene regulation [9,10], in contrast to the transcriptional and coding changes that have been repeatedly implicated in the evolution of somatic traits [5]. These shifts also have large and immediate impacts on transmission genetics, effective population size, ecology and macro-evolutionary potential. As a result, research models developed for investigating the EDB of reproductive evolution can also be used to study its influence on other biological processes.

*Caenorhabditis* nematodes are well suited for investigating the causes and consequences of reproductive mode shifts because *Caenorhabditis elegans* is an intensely studied model organism, and its androdioecious mating system (males and self-fertile hermaphrodites; see Glossary) evolved recently from gonochoristic (male and female) ancestors [11]. Androdioecy has evolved at least three times in the genus (Figure 1) and, in each case, the overt anatomical modifications required for evolution of self-fertility were confined to the germline. Therefore, *Caenorhabditis* can serve as a tractable model for how germline adaptation works and for addressing repeatability in adaptive evolution. In addition, the advanced state of *Caenorhabditis* genomics [12,13] supports investigation of the role that sexual mode plays in shaping genome content and variation. Here, we describe recent advances in both of these areas. First, we summarize recent studies that point to an important role for the control of mRNA translation in the evolution of XX spermatogenesis. Then we address other new work that highlights the major behavioral and genomic consequences of the evolution of self-fertility in *Caenorhabditis*.

## Developmental causes of self-fertility in *Caenorhabditis*

*Caenorhabditis* uses genetic sex determination; males have a single X chromosome and the XX sex is either female or hermaphroditic, depending upon the species. Hermaphrodite spermatocytes differentiate in a typically female somatic gonad and maintain their XX karyotype. Therefore, a compelling developmental biology question is

### Glossary

**Androdioecy**: a sexual system with males and hermaphrodite sexes.
**Ascarosides**: nematode-specific glycosides (modified sugars) that serve as signals for various reproductive decisions.
**Congener**: member of the same genus.
**Conspecific**: member of the same species.
**Convergent evolution**: evolution of an outwardly similar trait in multiple lineages, whose common ancestor did not possess the trait.
**Co-option**: use of a pre-existing feature (gene, organ, etc.) for a new purpose.
**Gonochory**: a sexual system with separate male and female sexes.
**Outbreeding and inbreeding depression**: loss of viability or fecundity of progeny of mating between distantly or closely related individuals, respectively.
**Selfing**: hermaphroditic reproduction through the union of gametes from the same individual.

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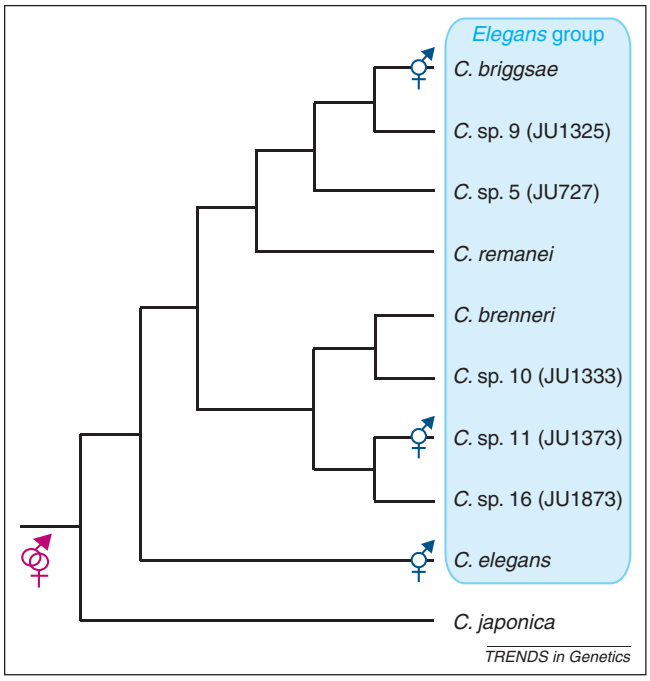


**Figure 1**. Phylogenetic relationships of the *Elegans* group of *Caenorhabditis*. The most parsimonious scenario posits that androdioecy evolved convergently in three lineages from a common gonochoristic ancestor. This is supported by the polarity of changes in sex-related traits (Table 1, main text). The outgroup species, *Caenorhabditis japonica*, belongs to the Japonica group. Modified from [11].

how limited spermatogenesis is achieved in any one hermaphrodite species. However, from the standpoint of EDB, answering this question immediately leads to several others: which aspects of the mechanisms required for limited XX spermatogenesis first evolved in hermaphroditic lineages? Which of these mechanisms are ancestral? Are those changes that are associated with self-fertility similar or different in convergent species? These questions have stimulated comparative studies of germline sex determination in *Caenorhabditis*.

Of the many *C. elegans* mutations with germline-specific sex-determining phenotypes (Box 1), only those that affect hermaphrodites are of particular interest here. The characterization of these mutants revealed a major role for translational regulation in germline sex determination. Whereas loss-of-function mutations in *tra-2* (*transformer-2*) and *fem-3* (*feminized-3*) cause body-wide masculinization and feminization, respectively, the dominant, gain-of-function *tra-2(gf)* and *fem-3(gf)* alleles cause germline-specific sex reversals that are the opposite. The dominant alleles alter small regulatory elements in the 3′ untranslated region (UTR) of each gene, the direct repeat element (DRE) [14] and point mutation element (PME) [15], respectively. The loss-of-function phenotypes of the RNA-binding proteins (RBPs) that recognize these elements (GLD-1, germline defective 1, for *tra-2* [16], FBF, *fem-3*-binding factor, for *fem-3* [17]) are consistent with their repressive function. The F-box protein FOG-2 (feminization of germline 2) has been indirectly implicated in mRNA regulation, as it complexes specifically with GLD-1 [18]. These factors define a set of elements that are necessary for limited hermaphrodite spermatogenesis in *C. elegans* and, thus, are prime subjects for comparative studies.

**Box 1. An overview of germline sex determination in *Caenorhabditis elegans***

The study of *C. elegans* sex determination helped pioneer developmental genetics [98]. Decades of work has shown that a twofold difference in X dosage is first converted to a binary signal by *xol-1* (XO-lethal-1) [99] and, through a series of interactions, this is converted into differential abundance of the zinc finger transcription factor, TRA-1 [100,101]. In males, TRA-1 levels are kept low by the combined action of FEM-1 (*feminized 1*), FEM-2 and FEM-3, which form a complex that targets TRA-1 for ubiquitination and degradation by the proteasome [29]. TRA-1 abundance is sufficient to explain essentially all sexually dimorphic somatic traits, but in the germline things become more complicated. First, male *tra-1* mutants cannot sustain spermatogenesis and frequently produce oocytes [102,103]. In addition, *tra-1; fem* double mutants have an unexpected feminized (Fem) phenotype in the germline (but not the soma) [103,104]. These results are not fully understood, but are consistent with *tra-1* having both positive and negative effects on spermatogenesis, and with it being only one of a larger set of germline sex determiners.

Germline-specific sex determination mutations have also been discovered [34], generally falling into 'no sperm' (*feminization of germline*, or *fog*) and 'too many sperm' (*masculinization of germline*, or *mog*) categories. Loss-of-function mutations in *fog-1* and *fog-3* eliminate both male and hermaphrodite sperm [105–107], whereas *fog-2* mutations only feminize hermaphrodites [108]. By contrast, the *mog*-class loss-of-function mutations produce an excess of hermaphrodite sperm [109,110]. Loss of *gld-1* and *fbf-1* and/or *fbf-2* activity also ablates or overproduces XX sperm, respectively, but with pleiotropic defects in hermaphrodites and no effect on male sex determination [25,111]. Through clever genetic screens, gain-of-function mutations affecting the 3′ UTRs of *tra-2* and *fem-3* with germline-specific effects were also found [14,15,41,112]. Eventually, it was discovered that the loss-of-function *gld-1* and *fbf-1* and/or *fbf-2* phenotypes stemmed from an inability to bind RNA motifs defined by the *tra-2* and *fem-3* gain-of-function alleles [16,113]. FOG-1 [106,107] and several of the MOG proteins [114–116] are also implicated in mRNA regulation.

The global sex-determination pathway of *C. elegans* is conserved across at least the *Elegans* group of *Caenorhabditis*, as are sperm-promoting factors acting downstream of *tra-1* [19]. However, an exception is *fog-2*, a recently duplicated gene specific to *C. elegans* [20]. FOG-2 is unique among its closest *C. elegans* paralogs in affecting sex determination, and also in possessing a GLD-1-binding domain in its divergent C terminus [20]. Presumably, FOG-2 is involved in the post-transcriptional regulation of *tra-2* mRNA. Thus, *fog-2* is a new gene with a new function, and it is a strong candidate for a key step in the gain of XX spermatogenesis in the *C. elegans* lineage. Interestingly, the *Caenorhabditis briggsae*-specific F-box gene *she-1* is also necessary for hermaphrodite spermatogenesis at elevated temperatures [21]. However, in contrast to FOG-2, SHE-1 does not interact with *C. briggsae* GLD-1. Thus, although *fog-2* and *she-1* are both implicated in hermaphrodite spermatogenesis, they probably exert their roles through different mechanisms. Characterization of these mechanisms is an important area for future research.

### *Functional evolution of RNA-binding proteins and translational control*

As noted above, *C. elegans* hermaphrodite germline sex-determination requires the translational control of *tra-2* and *fem-3* expression. Early comparative studies suggested that the 3′ UTR elements required for these controls

were conserved, even in species that were gonochoristic [22–24]. More recent work has focused on the evolution of the *trans*-acting RBPs that recognize these elements. As a result, an evolutionary picture of the translational control networks that regulate germline sex determination is emerging, one that suggests that both quantitative and qualitative changes matter for producing novel phenotypes.

In addition to promoting XX spermatogenesis, *C. elegans gld-1* is also necessary for germline tumor suppression, oocyte maturation and meiotic progression [25,26]. It has recently been shown that *gld-1* also plays these latter roles in females of gonochoristic species (e.g. *Caenorhabditis japonica*, *Caenorhabditis brenneri* and *Caenorhabditis remanei*), but that it has no discernible impact on sex determination [27]. This suggests that the ancestral role of *gld-1* was to regulate oogenesis, and that it was co-opted into sex determination recently. Remarkably, *C. briggsae gld-1* has also acquired a role in hermaphrodite sexual patterning, but as a limiter of sperm production [20,27]. Thus, *gld-1* has been co-opted into hermaphrodite sex determination at least twice, which suggests some reproducibility in evolution. However, it functions in opposite manners in two species, which indicates an important role for chance or the influence of initial conditions in incipient hermaphroditic lineages.

How can *Ce-gld-1* and *Cbr-gld-1* have opposite effects on germline sex? Cross-species rescue experiments rule out the possibility that *gld-1* itself is the locus of functional evolution [27]. One clue is that Ce-GLD-1 associates strongly with *tra-2* mRNA *in vivo*, as expected, but Cbr-GLD-1 does not [27]. Furthermore, the *Cbr-tra-2* transcript lacks DREs [27] and has fewer GLD-1-binding elements than does *Ce-tra-2* [14,28]. This suggests that *gld-1* has divergent functions, at least in part, because of lineage-specific evolution of target mRNA 3′ UTRs. The robust association of GLD-1 with *tra-2* mRNA in *C. elegans* may synergize with the ability of its cofactor, FOG-2, to recruit an E3 ubiquitin ligase complex [29] (Figure 2). This has been proposed to target translation-stimulating factors associated with the 3′ end of the mRNA for degradation [30].

In *C. elegans*, the factors regulating *fem-3* translation are the PUF family RBPs, FBF-1 and FBF-2 [17]. These RBPs also indirectly promote female fate by serving as negative regulators of GLD-1 expression. *C. briggsae* lacks strict *fbf* orthologs, but members of the related PUF-2 subfamily (*Cbr-puf-1.2* and *Cbr-puf-2*) have nevertheless been implicated in *C. briggsae* hermaphrodite germline sex determination. Pairwise RNAi knockdown of either *Cbr-puf-1.2* and *Cbr-puf-8* [27] or *Cbr-puf-1.2* and *Cbr-puf-2* [31] both feminize the hermaphrodite germline. This is in complete opposition to the roles of *fbf-1*, *fbf-2* and *Ce-puf-8*, which are redundantly necessary for hermaphrodite oogenesis in *C. elegans* [17,32]. The unexpected sperm-promoting roles of Cbr-PUF-2 and Cbr-PUF-1.2 can be explained by their ability to negatively regulate the *Cbr-gld-1* transcript, which (as noted above) promotes female fate in *C. briggsae* [31]. Translation of *gld-1* appears to be regulated by PUF proteins in all *Caenorhabditis* species [31], probably indicative of an ancestral role in the regulation of germ cell proliferation (rather than sex). Thus, the

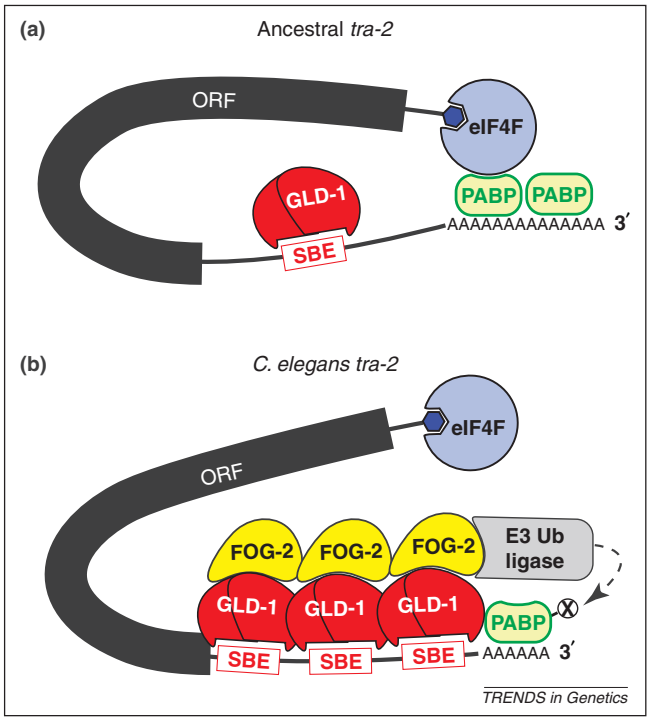


**Figure 2**. Evolution of the *tra-2* translational control module in *Caenorhabditis elegans* hermaphrodite sex determination. Orthologs of *tra-2* are found across *Caenorhabditis*, and some level of GLD-1 binding to *tra-2* mRNA may be ancestral [22,24]. This starting condition is depicted in **(a)**, in which GLD-1 is bound, but does not interfere with the interaction between poly-A-binding protein (PABP) and eIF4F, the protein complex that binds the 7-methylguanosine cap (hexagon). As a result, translation of *tra-2* occurs at an appreciable level in germ cells. In *C. elegans* **(b)**, the tandemly duplicated direct repeat elements (DREs) [14] encode three STAR-binding elements (SBEs) [28]. The DREs are unique to *Ce-tra-2* and may stabilize the GLD-1–*tra-2* mRNA complex *in vivo* [27]. Synergizing with this is a *C. elegans*-specific F-box-containing cofactor, FOG-2 [18,20], which probably recruits an E3 ubiquitin ligase. Although the targets of FOG-2-mediated ubiquitination remain unknown, one plausible scenario is that they are translation-promoting factors, such as PABP. As a result, poly-A-tails are shortened and translation is strongly inhibited. Thus, loss of FOG-2, GLD-1 or the SBEs leads to major TRA-2 overexpression and germline feminization. Abbreviations: eIF4F, eukaryotic translation initiation factor 4F; FOG-2, *feminization of germline 2*; GLD-1, germline development defective-1; ORF, open reading frame; *tra-2*, *transformer 2*; PABP, poly-A binding protein; STAR, signal transduction activator of RNA metabolism.

FBF and PUF-2 sub-families were apparently 'co-opted by association' in each hermaphrodite owing to their long-standing linkage with *gld-1*.

### *Possible genetic complexity of self-fertility in Caenorhabditis*

The above studies have deepened our understanding of how hermaphrodite development evolved in the *C. elegans* and *C. briggsae* lineages, and they have even guided a successful effort to engineer selfing in *C. remanei* females [33]. However, they have not defined the historical genetic variants distinguishing females from hermaphrodites with certainty. More generally, the comparative candidate-gene approach is problematic when the trait is not completely understood in the model system [34] and when taxa are substantially diverged [35]. Because simple genetic changes can produce dramatic sexual transformation, and because self-fertility has evolved repeatedly (Figure 1), it may be expected that only a few changes are necessary for hermaphroditic self-fertility to evolve. However, it is also possible that hermaphroditism evolves through multiple

changes of moderate to minor effect. Quantitative variation in self brood size [36–39] and cryptic variation in somatic sex determination [40] exist in *C. elegans*. Furthermore, *C. elegans* sex-determination mutations that disrupt wild-type self-fertile hermaphroditism on their own can produce fertile animals in combination [41]. Thus, the use of natural variants is a desirable alternative in determining the causes of self-fertility.

Recent work on interspecies *Caenorhabditis* hybrids with divergent reproductive modes has shown the utility and limitations of this approach. The F1 hybrid progeny of *C. briggsae* and *C.* sp. 9 (a recently discovered gonochoristic species) are fertile females [42]. That self-fertility is recessive indicates that no *C. briggsae* gene is sufficient for self-fertility at a single dose, and that that the *C.* sp. 9 XX germline may be highly canalized in the female state. Therefore, an important aspect of the evolution of self-fertility may have been a weakening of the commitment to female germline sexual identity [43]. Certain informative backcrosses in the *C. briggsae–C.* sp.9 system are inviable, but those that can be performed are unable to regenerate self-fertility [42]. This result is consistent with a requirement for the homozygosity of *C. briggsae* alleles of multiple unlinked genes. Therefore, hybridization eliminates self-fertility, suggesting that incipient hermaphrodites achieved the requisite fixation of multiple genetic variants promoting XX spermatogenesis via isolation and inbreeding. A quantitative genetic approach for understanding the evolution of self-fertility may be a fruitful one for future studies.

## Consequences of the evolution of selfing in *Caenorhabditis*

Self-fertility may not only provide a way to invade a new ecological niche or survive drastic times, but it also has major consequences for genome content and sexual traits. Research that collectively highlights these consequences is summarized below.

### *Population genetics*

Selfing leads to progressive homozygosis, which in turn unmasks deleterious recessive mutations and often results in a decline in fitness called inbreeding depression [44]. To survive, an incipient selfing population must rid itself of residual deleterious mutation through a process called purging. Because the fitness effects of alleles often depend upon epistatic effects of alleles at other loci, this process is expected to eventually produce unique homozygous genotypes. Consistent with this prediction, selfing *Caenorhabditis* nematodes do not suffer from general inbreeding depression [45,46], but instead suffer from outbreeding depression [47]. This is in stark contrast to their gonochoristic congeners, which display severe inbreeding depression [47,48].

The succession of inbreeding depression and purging of deleterious mutations predict that self-fertilizing species should retain less genetic diversity than should their dioecious counterparts. Indeed, higher selfing rates lead to a decrease in the effective population size for neutral alleles of autosomal genes [49]. The neutral theory of evolution predicts that outcrossing species will display at least twice as much genetic diversity as will selfing species [50], and other factors are likely to reduce genetic diversity in selfing species further [51–53]. For example, severe population bottlenecks are facilitated by the fact that any single individual can found a new population. Moreover, progressive homozygosis leads to extensive linkage disequilibrium across the genome of selfing species. The impact of natural selection is then limited because both deleterious and advantageous loci are effectively linked to one another.

The above dynamics might lead to reduced selection against weakly deleterious mutations and thereby accelerate rates of evolution in sequences located in non-recombining parts of the genome. By contrast, selection for a new beneficial mutation would be expected to sweep a large swath of the genome along with it, lowering population-level variation. Consistent with the latter, dramatically less nucleotide diversity is observed in the genomes of selfing *Caenorhabditis* compared with gonochoristic *Caenorhabditis* [54–56] and is lowest in the low-recombination central domain of the *C. elegans* chromosomes [57]. Similar findings have been described in plants (e.g. [58,59]).

In addition to lower genetic diversity, the smaller effective population sizes of selfing should lead to weaker selection against various forms of selfish elements, which would in turn inflate genome size [60]. This is consistent with observations of the abundance of transposable elements (TE) in low-recombining regions of the *Drosophila melanogaster* genome [61] and a higher copy number of certain TEs in *Arabidopsis thaliana* [62] and *C. elegans* [63] compared with their obligately outcrossing congeners. However, more recent studies contradict some of the above predictions. In *Arabidopsis*, the genome size of the selfing *A. thaliana* is smaller than that of outcrossing relatives [64–66]. Furthermore, in the obligately outcrossing *Arabidopsis lyrata*, TEs are more active and younger than in the selfing *A. thaliana* [67]. In *Caenorhabditis*, the genome sequences of *C. elegans* and *C. briggsae* also harbor fewer repeats than those of three gonochoristic species (*Caenorhabditis* Repeat Libraries, Release R1.0, 2008; http://genome.sfu.ca/projects/worm_genomes/REPEATS/RELEASE1.0).

Ten *Caenorhabditis* species have or will soon have their genomes completely sequenced, annotated and available [13,68]. Preliminary assemblies of the gonochoristic *C. japonica, C. remanei* and *C. brenneri* genomes suggests that they have larger genome sizes than do *C. elegans* and *C. briggsae*, even when the substantial amounts of heterozygosity retained in the sequenced strains [69] is considered. Although genome sizes and the emergence of selfing could be unrelated, the intriguing possibility of repeated genome shrinkage in selfing lineages remains. If this is borne out by more direct measures, it may be driven by an interaction between partial selfing and the preferential segregation of deletion-bearing autosomes with the X chromosome in male meiosis [70].

### *Role of males and outcrossing*

Although neither *C. elegans* nor *C. briggsae* hermaphrodites need to mate with males to reproduce, males exist in wild populations. They are produced spontaneously from meiotic X chromosome non-disjunction or through fertilization of XX individuals by males, the latter allowing sex

ratios of up to 50%. In laboratory cultures of the *C. elegans* reference strain, N2, the male frequency is close to that of the X chromosome non-disjunction rate [45,71]. Nevertheless, outcrossing can be detected in wild populations of *C. elegans* and *C. briggsae* [72–76] and, in some isolates of *C. elegans*, males persist at a higher rate than in N2 and vary widely in their abilities to mate [37,71,76]. At the same time, a natural population of *C. elegans* carrying a mutation in *mab-23* (*male abnormal 23*), which renders the males unable to mate, has been reported [37,77], illustrating the fact that males are not needed, at least for the short-term survival of the population.

Why males are still retained in androdioecious species is a subject of open debate [45,78–80]. In laboratory environments, *C. elegans* hermaphrodites can invade gonochoristic populations generation after generation, even when starting with a population composed of nearly 50% males [45,71,79] (although this may not be true for *C. briggsae* [21]). Although this suggests that androdioecious males are often useless and perhaps on their way out of existence, another theory is emerging. When environmental conditions are not optimal (i.e. scarce food resources, overpopulation and unfavorable temperatures), *Caenorhabditis* nematodes have the ability to go into a resistant larval phase called the dauer. Males survive dauer arrest better than do hermaphrodites, and outcrossing rates increase after starvation [81]. In addition, in populations subjected to environmental stresses or an increased mutation load, the progeny resulting from crosses between males and hermaphrodites have a higher fitness than do those resulting from selfing [82]. Thus, outcrossing allows both avoidance of inbreeding depression and more rapid adaptation to new environments for *C. elegans*, as is the case for most species. This also squares well with theoretical calculations that suggest *C. elegans* would go extinct in a few thousand years were it to lose all ability to outcross [83].

### *Degradation of mating behavior: the selfing syndrome*

Mating in *C. elegans* relies on sex-specific anatomy and on chemosensory and neuronal signals. Briefly, upon contact between the rays and ventral sensilla of the male tail with the hermaphrodite cuticle, the male presses his tail against the body of the hermaphrodite and starts scanning the hermaphrodite to find the vulva. When the male tail eventually makes contact with the vulva, the male stops moving and inserts his spicules in the vulval slit. The male then ejaculates and deposits a gelatinous copulatory plug. Larger, male-derived sperm is stored in the hermaphrodite spermathecae along with the sperm of the hermaphrodite, where it has a competitive advantage because of its larger size [84,85]. In the absence of a mate, males wander away from the food source, presumably in search of mates [86].

Because mating is not required for reproduction in androdioecious species, mating-related traits should be under weaker stabilizing and sexual selection. Indeed, in *C. elegans* and *C. briggsae*, both sexes have lost their ability to behave as reliable mating partners compared with gonochoristic *Caenorhabditis* species (Table 1) [45,79,87,88]. Most laboratory crosses between males and hermaphrodites in *C. elegans* or *C. briggsae* use mobility-impaired hermaphrodites or a large excess of males to make up for the low frequency of successful matings. Furthermore, males from androdioecious species do not discriminate between the sexes, and tend to initiate mating behaviors upon contact with any cuticle, including their own [89].

**Table 1. Comparison of mating-related traits in androdioecious and gonochoristic *Caenorhabditis***

| Trait | Gonochoristic | Androdioecious | Refs |
|---|---|---|---|
| Mate discrimination | Excellent | Poor | [89] |
| Pheromone potency | High | Low | [87,90] |
| Female immobilization | Yes | No | [89] |
| Mating length | Approx. 40 min | 2 min | [45] |
| Mating efficiency | 100% | 5% | [45] |
| Copulatory plug | Always | Polymorphic | [37,117] |

Several steps in the mating process are degraded in androdioecious species. Females from dioecious species attract conspecific males as well as *C. briggsae* and *C. elegans* males, whereas hermaphrodites do not elicit such a reaction from males from any species [45,87,89]. *C. elegans* hermaphrodites do produce a mixture of characteristic glycosides, the ascarosides, that attract both conspecific males and males from other species to varying lesser extent [90]. Females from *C. remanei* and *C. brenneri* secrete a sex pheromone to attract males, which seems to be either not produced by hermaphrodites or is less efficient [87]. It is unclear whether the attractants secreted by the gonochoristic females are chemically similar to those secreted by *C. elegans* hermaphrodites. In addition, both males and females in dioecious species wander in the absence of potential mates, whereas in selfing species, hermaphrodites do not search for mates [91].

Once males from gonochoristic species have found a female partner, they are able to immobilize it to facilitate spicule insertion and subsequent copulation. It has been suggested that males produce a 'soporific factor' that allows them to immobilize the female and facilitates spicule insertion by widening the vulval opening [89]. However, hermaphrodites seem to be unresponsive to this signal, and most of the matings fail because the hermaphrodite moves away from its partner before copulation occurs. Interestingly, *C. briggsae* males induce the same inactive behavior in females from both gonochoristic species tested, whereas *C. elegans* males do not [89]. Similarly, a strain of *C. briggsae* otherwise able to mate seems to lack a sex drive and displays neither mating nor self-plugging behaviors [89]. In addition, hermaphrodites have been observed to eject male sperm if their reproductive tracts already contain their own, particularly if they are young [92]. This behavior is currently unexplained, and stands at odds with the well-documented fertilization advantage experienced by male sperm in a mixed brood [85,93]. However, both may be true, especially if ejaculate ejection can be overcome by repeated mating.

The above studies indicate that androdioecious species have lost (or are in the process of losing) characteristics that would otherwise make them efficient cross-fertilizers. The genes underlying these traits and/or their regulation may have evolved in response to relaxation of selection [88] and some are still responsive to selection [94]. However,

given that androdioecious species have clear mating defects, some historically important genes associated with mating success may be more readily identified in a gonochoristic species, such as *C. remanei*, work that is currently underway (C.G. Thomas and E.S. Haag, unpublished).

### *Macroevolutionary consequences*

The complex molecular biology and repeated evolution of self-fertility in *Caenorhabditis* suggests that it is an adaptation, perhaps driven by selection for reproductive assurance at low density. Selfers can also enjoy a large boost in intrinsic growth rate once males, who cannot produce eggs, are largely eliminated from the population. The power of this can be seen in laboratory cultures of the obligately outcrossing *C. elegans fog-2* mutant, which have been repeatedly observed to revert to selfing by rare gene conversion events that repair the mutation [95] and restore self-fertility.

However, despite the above advantages, across the nematode family Rhabditidae multi-taxon clades of selfing species are rarely seen [96] and, within *Caenorhabditis*, all three known cases are clearly recently evolved from gonochorism [11,53]. This suggests that conversion to selfing has powerful short-term benefits to species that can evolve XX spermatogenesis and overcome the resulting inbreeding depression, but in the long run extinction generally occurs before speciation. A similar macroevolutionary cost of selfing has been observed in plants of the nightshade family [97] and may occur in many taxa that colonize disturbed or ephemeral habitats. The above results suggest the paradoxical result that selfing nematodes need to maintain the ability to outcross to withstand stress and ongoing mutation, yet at the same time, standing variation falls to very low levels and mating-related traits are gradually degraded. The eventual endpoint may be selfing species that are so poor at mating they cannot respond to an environmental shift and succumb. From this macroevolutionary perspective, the impressive achievement of hermaphrodite development appears to be a Faustian bargain.

## Concluding remarks

In this review, we have shown how *Caenorhabditis* nematodes represent a useful experimental system for the integrative study of shifts in sexual mode. Early comparative studies on the EDB causes of selfing focused on the genetic control of sex determination, and important insights were gleaned from this work. A promising new area will be to examine the consequences of selfing, both for the genome overall and for reproductive traits. As the natural history and ecological niche of the genus is being refined [11], it is also possible to start to address another outstanding question: what ultimately explains the phylogenetic distribution of selfing? An adaptationist hypothesis would be that it is a facile transition driven by ecological shifts to a particular lifestyle. However, it is also possible that gonochoristic lineages have a more or less constant probability of spinning off selfing derivatives, which derive short-term gains but then go extinct. In any case, we can look forward to many interesting connections being made.

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