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The evolutionary origins and consequences of self-fertility in nematodes

Ronald E. Ellis* and Shin-Yi Lin

Address: Department of Molecular Biology, Rowan University SOM, B303 Science Center, 2 Medical Center Drive, Stratford, NJ 08084, USA

* Corresponding author: Ronald E. Ellis (ellis@rowan.edu)

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Abstract

Self-fertile hermaphrodites have evolved from male/female ancestors in many nematode species, and this transition occurred on three independent occasions in the genus *Caenorhabditis*. Genetic analyses in *Caenorhabditis* show that the origin of hermaphrodites required two types of changes: alterations to the sex-determination pathway that allowed otherwise female animals to make sperm during larval development, and the production of signals from the gonad that caused these sperm to activate and fertilize oocytes. Comparisons of *C. elegans* and *C. briggsae* hermaphrodites show that the ancestral sex-determination pathway has been altered in multiple unique ways. Some of these changes must have precipitated the production of sperm in XX animals, and others were modifying mutations that increased the efficiency of hermaphroditic reproduction. Reverse genetic experiments show that XX animals acquired the ability to activate sperm by co-opting one of the two redundant pathways that normally work in males. Finally, the adoption of a hermaphroditic lifestyle had profound effects on ecological and sexual interactions and genomic organization. Thus, nematode mating systems are ideal for elucidating the origin of novel traits, and studying the influence of developmental processes on evolutionary change.

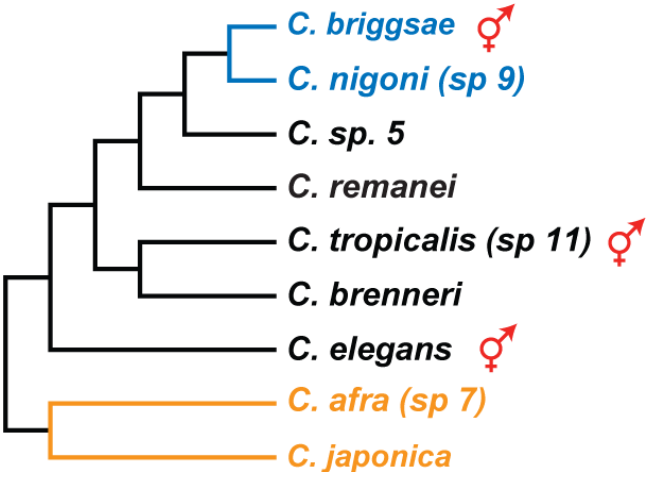
Introduction

Darwin published "On the Origin of Species" 155 years ago [1], but his theory of natural selection remained incomplete until its integration with genetics in the modern synthesis [2]. The past 30 years have seen the beginnings of a second major integration, fusing evolutionary theory with new research in development (reviewed in [3,4]). This field of evolutionary developmental biology is best known for the discovery of orthologous genes that pattern the early embryo, but it is now branching out into many other areas.

Here, we review the evolution of self-fertility in *Caenorhabditis* nematodes. The convergent evolution of hermaphrodites in this genus provides an ideal way to explore both evolutionary change and the use of alternative reproductive strategies (reviewed in [5,6]). Phylogenetic analysis implies that mating systems changed recently, which makes it easier to reconstruct many of the underlying genetic events (Figure 1). Furthermore, technical considerations make this genus ideal for study. *C. elegans* is one of the leading models for studying sex-determination, and decades of research provide the background information needed to characterize its relatives. This task is simplified by the genome sequences of *C. elegans* [7] and *C. briggsae* [8], and the partial sequences of seven related species (Figure 1). Finally, orthologous genes can be characterized by powerful reverse genetic techniques, including RNA interference and gene-editing with transcription activator-like effector nucleases (TALENs) or clustered regularly interspaced short palindromic repeats (CRISPRs) [9-13], which allow the control of mating systems to be dissected in all species.

Our discussion will focus on three major questions. First, a change in mating system requires the coordination of many genes and regulatory pathways, so we will explore

Figure 1. Hermaphrodites evolved on three independent occasions in *Caenorhabditis*



Only species with sequenced genomes are shown. Androdioecious species with males and hermaphrodites are marked with a red symbol, and the others are male/female. The species in blue are able to interbreed and produce fertile offspring, and two outgroup species are orange. Modified from Kiontke *et al.* [18] and Felix *et al.* [15].

how complex traits originate. Second, hermaphrodites are common in some phyla but rare in others, so we will consider whether the rules of development influence the evolution of self-fertility. Third, mating systems are central to sexual reproduction, so we will ask how self-fertility affects the evolutionary process.

How did androdioecy evolve in nematodes?

Most nematode species have males and females, just like other animals. However, some species display a rare mating system known as androdioecy, which uses males and self-fertile hermaphrodites. In androdioecious nematodes, the XO animals are normal males but XX animals are hermaphrodites (Figure 2). These hermaphrodites look like females, but the first germ cells to differentiate become sperm, which are stored in the spermathecae and used later for self-fertilization. Subsequent germ cells become oocytes. Because hermaphrodites are anatomically female, they cannot mate with each other, but do produce cross progeny if mated with males.

Self-fertile hermaphrodites have arisen independently many times during evolution [14]. Even within a subgroup of the genus *Caenorhabditis*, hermaphroditic reproduction evolved in three different species — *C. elegans*, *C. tropicalis* (formerly *C. sp. 11* [15]) and *C. briggsae* (Figure 1) [16-18]. Comparative studies, particularly between *C. elegans* and *C. briggsae*, have elucidated the genetic control of self-fertility.

Caenorhabditis nematodes share a core set of sex-determination genes

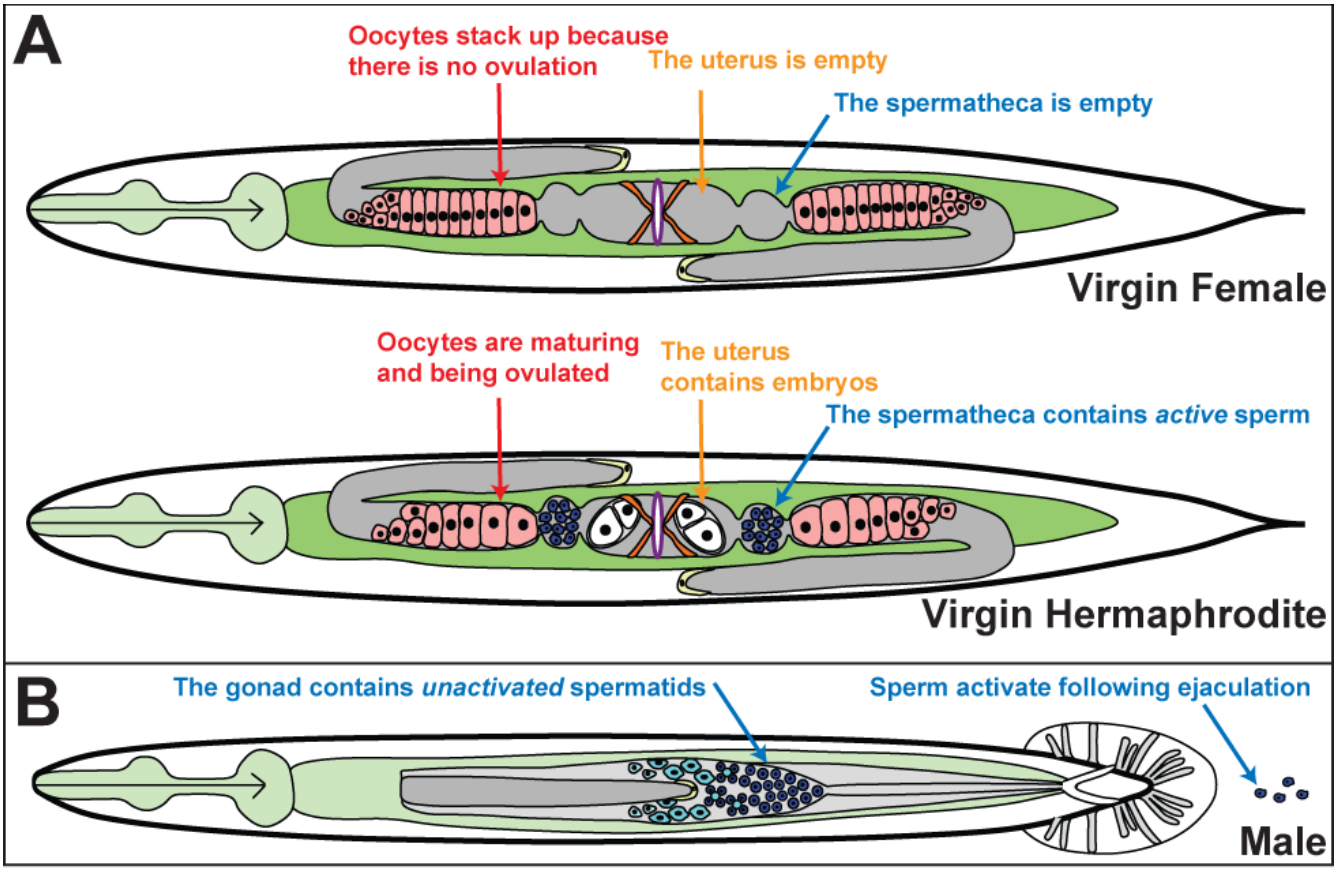
Decades of research with *C. elegans* have defined a signal transduction pathway that regulates sexual development in both the somatic tissues and the germ line (Figure 3; reviewed in [19,20]). In signaling cells, the ratio of X chromosomes to autosomes controls *xol-1*, a gene that specifies male development. Next, XOL-1 acts through the syndecan (SDC) proteins to control the production of a hormone, HER-1 (human epidermal growth factor receptor-1), that causes cells throughout the body to adopt male fates. The ultimate target of this pathway is TRA-1, a transcription factor related to the Gli proteins [21].

In males, HER-1 binds to and inactivates its receptor, TRA-2 (Figure 3A). This interaction allows the FEM proteins and the ubiquitin ligase CUL-2 to mark TRA-1 for degradation [22] (shown as TRA-1^{ubi} in Figure 3). However, some full-length TRA-1 remains [23] and is likely to work with the Tip60 histone acetyltransferase (HAT) complex to promote the expression of genes needed for spermatogenesis [24]. These genes include *fog-1* and *fog-3*, which have TRA-1 binding sites in their promoters [25,26] and are required for germ cells to become sperm rather than oocytes [27,28]. FOG-1 and FOG-3 are likely to work by regulating the translation of messenger RNAs (mRNAs) [26,29,30].

In hermaphrodites, TRA-1 is cleaved to produce a repressor (TRA-1^{100} in Figure 3) [23], which turns off male genes like *mab-3*, *egl-1*, *fog-3* and numerous other targets (Figure 3B) [25,31-33]. These repressive interactions appear to be the predominant method by which *tra-1* controls somatic sex. The fact that the full-length isoform appears to activate sperm genes, whereas the cleaved form represses them, makes TRA-1 bipotential, a trait shared with many other Gli proteins [24].

Orthologs of these genes have been shown to function in the sex-determination pathway in both hermaphroditic and male/female species of *Caenorhabditis*. Analysis of mutants in the hermaphroditic species *C. briggsae* confirms that sexual development is controlled by *tra-1* [34,35], *tra-2* [35,36], *tra-3* [35], *trr-1* [24], *fem-2* [37], *fem-3* [37] and *fog-3* [25]. Furthermore, RNA interference shows that *tra-2* [38], *fem-3* [39] and *fog-3* [40] regulate sexual development in the male/female species
C. remanei. Thus, it appears that core genes of the sex-determination pathway have been conserved throughout *Caenorhabditis* (reviewed in [5]). This conservation could extend farther, since an ortholog of *tra-1* controls sexual development in the distant relative *Pristionchus pacificus* [41].

Figure 2. Self-fertile hermaphrodites are modified females that make and use sperm



A. Comparison of virgin female and hermaphrodite nematodes. Ventral up, anterior to the left. Oocytes are pink and sperm are blue. In the soma, the gonad is gray, the pharynx is light green, the intestine is dark green, the sex muscles are orange, the distal tip cells are yellow and the vulva purple.
B. Male nematode. Primary spermatocytes are light blue hexagons, residual bodies are light blue circles, and spermatids are dark blue circles.

Hermaphrodites evolved through independent changes in the sex-determination pathway

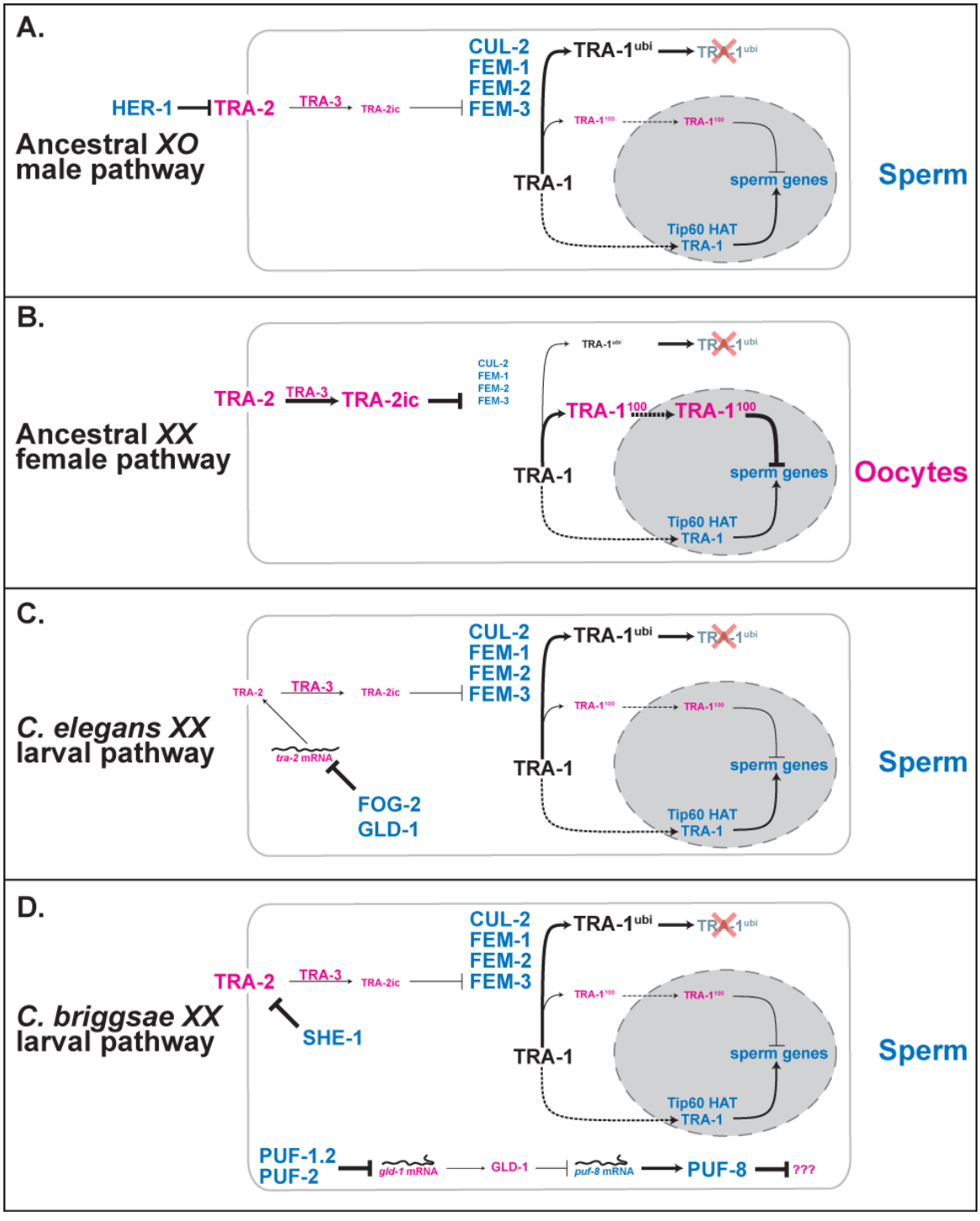
Because hermaphroditism arose independently in *C. elegans* and *C. briggsae*, comparing their sex-determination pathways can reveal what types of genetic changes led to self-fertility. To date, all known mutations that allow XX larvae to produce sperm fall into one of three separate categories: the generation of novel genes by duplication, the recruitment of known germline genes to the sex-determination pathway, and the modification of core genes within the pathway (compare Figure 3C/3D with 3A/3B).

First, new genes have been created by duplication and recruited to the pathway. For example, *C. elegans* hermaphrodites require *fog-2* to produce sperm during larval development [42]. The FOG-2 protein works with GLD-1 to block the translation of *tra-2* mRNAs in the XX germline [43,44] that allows the expression of male genes needed for spermatogenesis. This system is unique to *C. elegans*, since *fog-2* was created by a recent duplication event [44] and has no ortholog in *C. briggsae* [45].

A different gene, *she-1*, is required for *C. briggsae* hermaphrodites to make sperm [46]. Although SHE-1 also regulates the activity of *tra-2*, it does not associate with GLD-1 and its molecular function remains unknown. As with *fog-2*, the *she-1* gene was produced by a recent duplication event. Surprisingly, both genes are distant members of the large F-box family [47]. Perhaps the adaptive radiation of this family provided opportunities for novel functions to arise.

Second, existing germline genes have been independently recruited to the sex-determination pathway. In *C. elegans*, the *fbf* genes encode conserved RNA-binding proteins that block spermatogenesis by preventing the translation of *fem-3* mRNAs in the germ line [48].

Figure 3. Modifications to the sex-determination pathway allow XX larvae to make sperm



Proteins promoting spermatogenesis are blue, and those promoting oogenesis are pink. Positive interactions are shown as solid lines with arrowheads, negative ones as lines with bars, and nuclear import by dashed lines. Line thickness and font size represent the strength of each interaction. The TRA-2 receptor can be cleaved by the calpain protease TRA-3 to form the intracellular form TRA-2_{ic} [111,112]. The target of the *Caenorhabditis briggsae puf-1.2, puf-2, gld-1* and *puf-8* pathway is not yet known, but it is likely that PUF-8 represses a gene needed for oogenesis [49,52]. Likewise, the secondary role that the three *fem* genes play downstream of *tra-1* in *C. elegans* [56] and possibly in *C. briggsae* [113] is not shown because their targets remain unclear. Finally, additional genetic interactions are needed for adult hermaphrodites to switch back to oogenesis, which are reviewed elsewhere [20]. For other details, see the text.

By contrast, two other PUF proteins promote spermatogenesis in *C. briggsae* hermaphrodites by blocking the translation of *gld-1* mRNAs [49]. Thus, different members of the PUF family of proteins, which normally function in the germ line, were independently recruited to the sex-determination pathway in order to control self-fertility.

The *gld-1* gene also belongs to this class. GLD-1 plays numerous roles in the XX germ line [50-52]. In *C. elegans*, it also promotes hermaphrodite spermatogenesis by working with FOG-2 to block the translation of *tra-2* mRNAs, and its physical interaction with this target is stronger than in other nematodes [43,44]. By contrast, *C. briggsae* GLD-1 blocks hermaphrodite spermatogenesis [45,52], in part by regulating *puf-8* [52]. The ultimate target of this pathway is not yet known, but it is likely that PUF-8 represses a gene needed for oogenesis in *C. briggsae* [49,52]. Thus, these species recruited GLD-1 for opposing roles in sex determination.

Also, the nucleasome remodeling factor complex regulates gene expression by moving histones [53] and controls germ cell proliferation in many animals, including *C. elegans* [54]. In *C. briggsae*, it was recruited for a unique role — the control of spermatogenesis [55] . It appears to carry out this function by allowing TRA-1 access to the *fog-1* and *fog-3* promoters.

Third, the core pathway itself has been modified, changing the relative importance of different factors. We know that the FEM proteins and the Tip60 HAT complex are core members of the pathway, since they both influence germ cell fates in sensitive genetic backgrounds, and double mutants in either species cause synthetic feminization [24]. However, the three FEM proteins are required for spermatogenesis in *C. elegans* hermaphrodites [56] but dispensable in *C. briggsae* ones [37]. By contrast, the Tip60 HAT complex is required for spermatogenesis in *C. briggsae* but plays only a minor role in *C. elegans* [24].

One explanation for these results is that the sperm/oocyte decision might be controlled by a balance between activating and repressing activities of TRA-1 (Figure 3). In hermaphrodites, upstream regulators of the sex-determination pathway, like those described here, could alter this balance, so that larvae make sperm and adults make oocytes. Several lines of evidence support this idea. First, the analysis of mutations in the *C. elegans fog-3* promoter suggests that TRA-1 both promotes and represses the expression of *fog-3* [25]. Second, adult hermaphrodites normally accumulate far more of the TRA-1^{100} isoform than adult males [22,23]. However, *C. elegans cul-2* or *fem* mutations create similar proportions of TRA-1^{100} in XO animals [22], leading to oogenesis [57]. Third, some mutations in accessory genes appear to favor activation or repression by TRA-1. For example, the Tip60 HAT complex requires TRA-1 to promote the expression of *fog-3* [24]. By contrast, two WDR-5 proteins are needed for TRA-1^{100} to repress *fog-3*, and appear to work by controlling its import to the nucleus [58]. Finally, the relative importance of the FEM proteins and the Tip60 HAT complex in *C. elegans* and *C. briggsae* differ dramatically [24,37], which shows that different types of changes in the regulation of TRA-1 could lead to self-fertility.

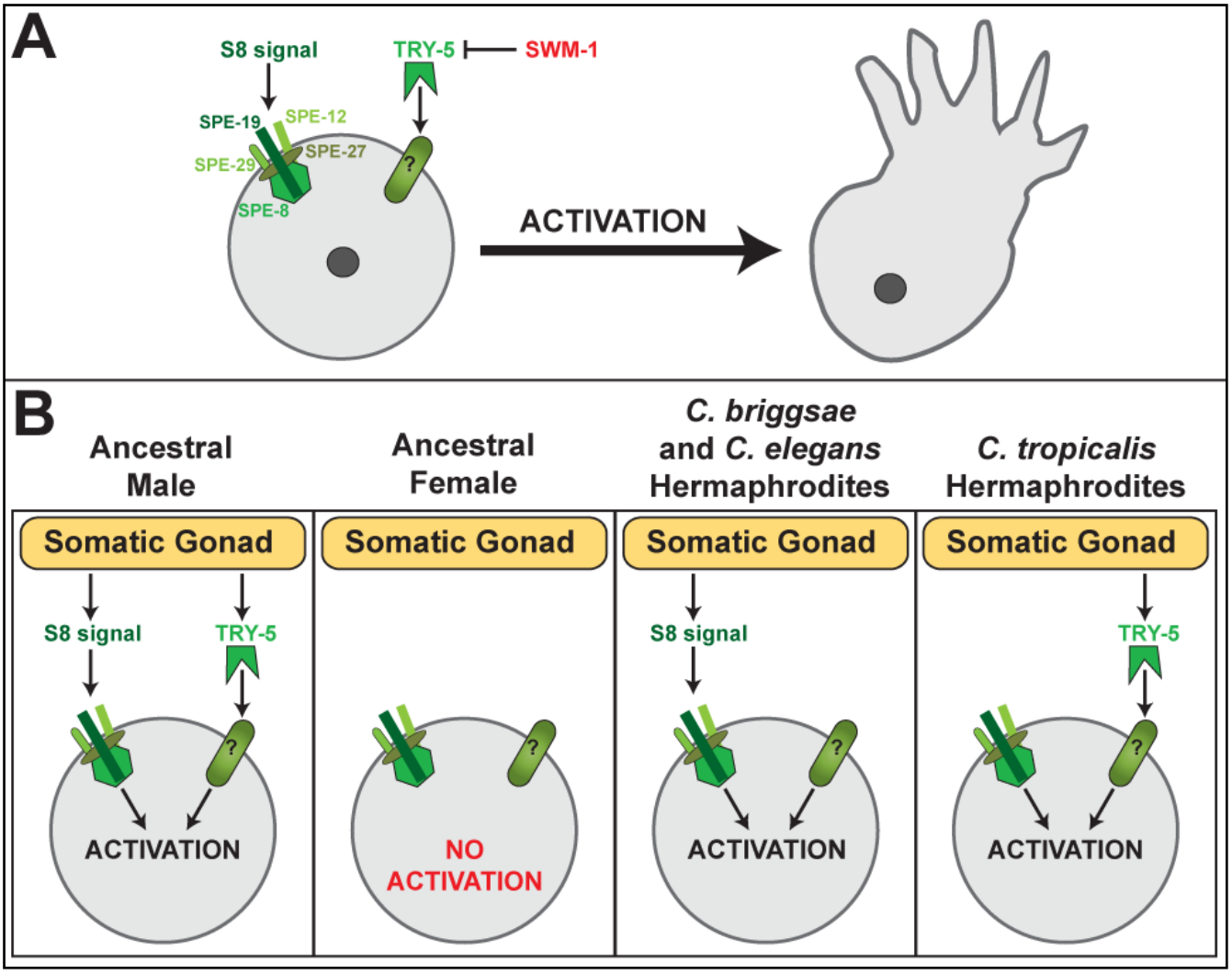
The large number of alterations found in the sex-determination pathway (compare Figures 3C and 3D) suggests that self-fertility has been refined by selection for additional modifiers. These mutations might influence the precise amount and timing of spermatogenesis, since the analysis of weak *tra-3* mutants implies that the number of sperm made by each hermaphrodite is under intense selection [59]. Thus, mutations that affect when the germ line switches to oogenesis could maximize the number of self progeny, while minimizing the delay in oogenesis.

Hermaphrodites also required changes in a sperm activation pathway

Based on these results, it seemed possible that a single mutation that altered the sexual fates of germ cells would be sufficient to make self-fertile hermaphrodites. This mutation would have to allow XX animals to make sperm as well as oocytes, perhaps by decreasing *tra-2* activity. To test this hypothesis, RNA interference was used to target *tra-2* in *C. remanei* females [60]. Some of the *Cre-tra-2(RNAi)* XX animals did indeed produce sperm and oocytes in a female body. Surprisingly, their spermatids failed to activate and fertilize oocytes, so they did not make progeny. Thus, alteration of the sex-determination pathway is not sufficient for self-fertility.

How is sperm activation controlled? *C. elegans* males use two redundant pathways, one dependent on SPE-8 and the other dependent on TRY-5, whereas hermaphrodites use only the SPE-8 pathway (Figure 4, reviewed in [61]). The five genes of the *spe-8* group encode sperm proteins [62-66] that appear to respond to labile zinc [67]. By contrast, TRY-5 is a protease that activates sperm by cleaving unknown targets [68]. Prior to ejaculation, TRY-5 activity is kept in check by the inhibitor SWM-1 (Figure 4A) [69]. Because *C. elegans* hermaphrodites normally use the *spe-8* pathway to activate sperm, mutants in these genes are not self-fertile. However, their spermatids can be activated by exposure to male seminal fluid in a process

Figure 4. Hermaphrodites have co-opted one of two redundant sperm activation pathways



A. Studies of *Caenorhabditis elegans* and *C. briggsae* males show that two redundant pathways control sperm activation. One pathway uses the SPE-8 group of sperm proteins to respond to an unknown signal (denoted "S8 signal"). The other pathway uses an unknown receptor (denoted "?") to respond to the TRY-5 protease. Males defective for both pathways are sterile.

B. *C. briggsae* and *C. elegans* hermaphrodites rely on the SPE-8 pathway to activate sperm, whereas *C. tropicalis* hermaphrodites rely on the TRY-5 pathway. For details, see the text (Wei et al., *unpublished data*).

called transactivation [70]. This is done experimentally by mating sterile *spe-8* hermaphrodites with males, resulting in the activation of some hermaphrodite sperm and the production of self-progeny. Transactivation implies that TRY-5 targets remain functional in hermaphrodite sperm, even if they are not normally used.

To see if the spermatids in *C. remanei tra-2(RNAi)* XX animals could be activated and fertilize oocytes, they were crossed with sterile males. This resulted in the production of self-progeny, so the XX sperm functioned normally after transactivation by male seminal fluid. Likewise, simultaneous knockdown of both *Cre-tra-2* and *Cre-swm-1* also produced self-fertile hermaphrodites [60]. Thus, two coordinated changes are sufficient to produce self-fertility: one in the sex-determination pathway that allows XX animals to make sperm and another that causes these sperm to activate and fertilize oocytes [60].

All of the sperm activation genes from both pathways are conserved throughout *Caenorhabditis* (Wei et al., unpublished data). Since both pathways operate in *C. briggsae* males, they must have controlled sperm activation in the male/female ancestor. However, *C. elegans* and *C. briggsae*

hermaphrodites use the SPE-8 pathway to control sperm activation, but *C. tropicalis* hermaphrodites use TRY-5 (Figure 4B) (Wei et al., unpublished data). Thus, newly evolving hermaphrodites appear to have co-opted either one system or the other, probably by expressing the appropriate signal in the somatic gonad.

What are the consequences of androdioecy in nematodes?

Once self-fertility has been acquired, it has far-reaching consequences as organisms adapt to and refine the hermaphroditic lifestyle. Work in *Caenorhabditis* and other androdioecious species has begun to shed light on how changes in mating systems can affect the ecology, fitness, and genomic organization of these hermaphroditic species (reviewed in [6]).

Hermaphrodites facilitate the colonization of new habitats

Although we are beginning to learn about the natural ecology of *Caenorhabditis* [18], we do not know what selective pressures favored the origin of self-fertility. However, the idea that hermaphrodites are better suited for colonization [71] is supported by studies of the European tadpole shrimp [72]. This species has male/female, male/hermaphrodite and hermaphrodite populations. As glaciers retreated north following the end of the last ice age, new habitats were preferentially filled by hermaphrodites. This advantage in colonization could be due to the ability of single animals to open up new territories without needing to find mates. Ecological studies with *Pristionchus* nematodes, which also include male/female and male/hermaphrodite species, should help test this hypothesis (reviewed in [73]).

Hermaphroditism alters the genetic structure of populations

Population structure plays a critical role in evolution, since it determines what allelic combinations will be available for selection. In large sexual populations, new mutations will most likely be present within the population in heterozygous form, so the production of favorable combinations within the same individual would be rare. However, newly evolving hermaphrodites can escape these sexual dynamics by selfing, which should make it easier to produce homozygotes with new allele combinations. This feature of self-fertile populations might accelerate the evolution of androdioecy, and also influence how alleles controlling other traits propagate within the population.

However, selfing does come at a cost. Self-fertile populations can show inbreeding depression, a decrease in fitness caused by progeny that are homozygous for harmful mutations. There is a huge reservoir of genetic diversity in male/female *Caenorhabditis* species [74], so when the ancestor of *C. elegans* began to self-fertilize, it would have faced a crisis until lethal mutations were purged from the gene pool. Many incipient hermaphrodite populations might have died out during this stage. However, once inbreeding depression was overcome, hermaphrodites should have been well adapted to their environments. Indeed, modern isolates of *C. elegans* actually show *outbreeding* depression for some traits, presumably because beneficial allele combinations have become fixed in the population [75].

Despite the impact of selfing, the existence of males in androdioecious species suggests that some out-crossing still occurs. This conclusion is bolstered by the direct observation of heterozygosity among *C. elegans* isolated from the wild [76,77]. Indeed, some out-crossing might be necessary for the long-term survival of the species. The existence of populations with both out-crossing and selfing animals has made *Caenorhabditis* ideal for exploring the role of sexual reproduction, the importance of Muller's ratchet and other mechanisms for eliminating deleterious mutations [78,79].

Androdioecious males show a decline in male fitness

In male/female species, each sex is under selective pressure to find and mate with the other. By contrast, hermaphrodites do not need males to reproduce. Indeed, wild *C. elegans* populations are highly skewed towards XX animals [76,77]. Consequently, many traits involved in mating have degraded from their state in the male/female ancestor. For example, *C. elegans* hermaphrodites no longer secrete a pheromone to attract males [80], and do not remain immobile during copulation [81]. Furthermore, males from numerous wild isolates of *C. elegans* have lost the ability to produce a mating plug [82]. Finally, sperm from androdioecious males are less aggressive than those from gonochoristic males, and interactions between sperm and the XX gonad have changed significantly [83]. All of these changes make *C. elegans* males less effective than their counterparts from gonochoristic species [80,81]. Genetic studies suggest that hermaphroditic nematodes evolved recently [84], so it is not clear if this trend will eventually result in the elimination of males altogether.

Androdioecious species show decreased sperm size

The evolution of hermaphroditism also involved two different reductions in sperm size. First, all hermaphrodites make smaller sperm than males of the same species [85,86]. This difference is probably due to a developmental bias, since sperm made by XX females following genetic manipulation are also smaller than male sperm [86]. Genetic analyses in *C. elegans* suggest that this bias

could be due to the unsuitability of the hermaphrodite gonad and germ line for the development of large sperm.

Second, males from androdioecious species make much smaller sperm than males from gonochoristic ones [85,86]. Since larger sperm are more likely to fertilize oocytes in controlled experiments, intense sperm competition in male/female species probably favors large sperm [87,88]. Perhaps the smaller sperm in androdioecious males is another example of decreasing male effectiveness in populations that are largely composed of hermaphrodites.

Androdioecious species show a decrease in genome size

The genomes of *C. elegans* and *C. briggsae*, as well as their sets of all transcribed genes, are dramatically smaller than those of their male/female counterparts [89]. Not unexpectedly, genes with sexually dimorphic patterns of expression are most likely to have disappeared from the hermaphroditic species. However, these changes are not driven solely by selection. Crosses with *C. elegans* show a transmission distortion, in which shorter chromosomes are preferentially segregated to hermaphrodite progeny [90]. Thus, deletions might accumulate in selfing lineages.

A model for the origin of self-fertile hermaphrodites

Based on these studies, we propose that the evolution of self-fertile hermaphrodites in *Caenorhabditis* required three stages. In the first one, a small number of genetic changes combined to make self-fertile animals. The simplest possibility is that this process started with a neutral mutation causing XX animals to produce a sperm activation signal, and was completed by a mutation that caused them to make sperm as well as oocytes [60]. This sequence would avoid a stage in which XX animals wasted resources by making sperm they could not use. Even so, these incipient hermaphrodites probably had small broods and severe reproductive problems.

During the second stage, harmful recessive mutations were purged, to avoid the consequences of inbreeding. At the same time, selection would have favored modifying mutations that increased the precision and effectiveness of hermaphroditic reproduction. The impact of selfing probably explains the rapid establishment of these mutations in the population. Newly evolving androdioecious species might have had three sexes during this stage. One *Rhabditis* species currently makes males, females and hermaphrodites [91]. Furthermore, *C. briggsae she-1* mutants are inherently temperature-sensitive, so perhaps this species once made both females and hermaphrodites too, depending on environmental conditions [46]. Eventually, females were eliminated, both because hermaphrodites are better at colonization, and because opportunities to mate with males declined as the population came to rely more on selfing.

The third stage would have been the longest, and may still be going on. In it, all aspects of the animal's behavior and genome slowly adapted to the hermaphroditic lifestyle. These changes have led to a dramatically smaller genome and lower male effectiveness in the androdioecious species of *Caenorhabditis*.

Given this reconstruction of events, how does the analysis of nematode mating systems fit into the broader context of evolutionary and developmental studies? Two topics are of critical importance: the role of co-option in producing new traits, and the importance of developmental biases.

Co-option plays a central role in the origin of novel traits

Some of the most detailed studies of evolutionary change involve the reduction or loss of traits. Examples include the reduction of pigmentation in beach mice [92] and pelvic reduction in stickleback fish [93]. Androdioecious mating systems are common in plants, and are thought to have arisen by a similar mechanism — the transformation of some hermaphrodites into males through the loss of female reproductive structures (reviewed in [94]).

By contrast, self-fertility in nematodes was caused by the gain of new functions in XX animals. Hermaphrodites acquired these traits by using genetic programs previously restricted to males. They co-opted the spermatogenesis program through changes in the sex-determination pathway in germ cells, and appear to have co-opted one of the sperm activation pathways by producing a male signal in the somatic gonad. Darwin first proposed that existing traits could be co-opted for new roles, based on his observation that our lungs had evolved from the swim bladder [1]. The origin of self-fertility provides molecular examples of how co-option occurs.

Furthermore, self-fertile hermaphrodites provide a model for the origin of complex traits, since their inception requires changes in two signal transduction systems (the sex-determination pathway and the sperm activation pathways), involving at least two tissues (the germ line and somatic gonad). Without co-option, this type of coordinated change would have been impossible.

Developmental biases favor androdioecy in nematodes

Darwin suspected that "laws of growth" helped shape the pattern of evolutionary change [1]. These effects are now known as developmental biases or constraints (reviewed

in [95]). For example, a developmental bias caused nematode sperm to have sexually dimorphic sizes [86]. Although the general significance of this process is still being debated, it is striking that self-fertile hermaphrodites evolved independently in many species of nematodes [16-18] and branchiopod crustaceans [96-98] but never in insects or mammals (reviewed in [99]). Three factors suggest that this broad pattern of evolutionary change is caused by developmental biases.

First, it should be difficult for XX hermaphrodites to evolve in species that use a Y chromosome to specify male development because the Y usually contains sperm genes. Nematodes use an XX/XO system, so this is not a problem. Studies from the branchiopod crustacean *Eulimnadia texana* support this model, since it uses a ZW/ZZ system to specify sex, which also ensures that newly evolving hermaphrodites would not lack sperm genes [100]. By contrast, fruit flies and mammals have Y chromosomes that contain sperm genes (e.g. [101,102]), so it should not be surprising that neither group has produced self-fertile hermaphrodites. Instead, we note that the only androdioecious vertebrates are fish of the genus *Kryptolebias* [103]. In this group, males can be induced by environmental perturbations, so they also lack an XX/XY system [104]. Hence, we propose that a genetic constraint prevents the evolution of self-fertile hermaphrodites in many taxa but does not affect nematodes.

Second, the structure of the sex-determination pathway might facilitate the origin of self-fertility. All hermaphrodites in *Caenorhabditis* make sperm as larvae and oocytes as adults; no other arrangement has been observed. Furthermore, mutations that eliminate *tra-1* cause younger animals to make sperm and older ones to make oocytes [35,105,106]. These patterns suggest that each androdioecious species has found a way to exploit a predisposition towards male fates in the larval germ line. Mutations that slightly altered the delicate balance between the activating and repressing activities of TRA-1 might have led to the production of sperm and oocytes in the same animal. Hence, the structure of the sex-determination pathway could favor the evolution of hermaphroditism in nematodes.

Finally, the presence of two redundant pathways to activate male sperm might also favor the evolution of self-fertility. Experiments using *C. remanei* suggest that changes in both the sex-determination and sperm activation pathways are necessary for the evolution of self-fertility [60]. Furthermore, either the SPE-8 or TRY-5 pathway can be co-opted for use in newly evolving hermaphrodites (Wei *et al.*, unpublished data). Thus, the existence of these redundant pathways might increase the number of strategies that can produce hermaphrodites without compromising male fertility.

Parallel evolution is a major topic of research [107], and parallel changes in mating systems are common. For example, asexual mating systems have arisen several times in the fungal genus *Neurospora* [108]. Here, we suggest that shared developmental constraints — the XX/XO sex-determination system, the structure of the sex-determination pathway, and redundancy in the sperm activation pathways — could explain why the parallel evolution of hermaphroditism is common in nematodes.

A bright future

Studies of the origin of self-fertile hermaphroditism in *Caenorhabditis* have contributed significantly to our understanding of the evolution of novel, complex traits. In addition, new work is beginning to reveal the effects of androdioecy on the ecology, sexual selection, and genomes of these hermaphroditic species. Recent developments suggest that the most exciting results are yet to come. First, gene-editing techniques that use TALENs [10-12] or CRISPRs [11,13] now allow rapid and unrivaled precision in evolutionary comparisons among nematode species [55] (Wei *et al.*, unpublished data). Second, the ability to study hybrids between the male/female species *C. nigoni* (formerly *C. sp. 9* [15]) and the male/hermaphrodite species *C. briggsae* should allow sophisticated tests of evolutionary models [109]. And third, experimental evolution with nematodes is now feasible [88,110], so laboratory studies can explore how selection pressures drive sexual reproduction and the choice of specific mating systems. These technical advances will allow us to compare *Caenorhabditis* species with more sophistication, reconstruct ancestral and intermediate stages in the path towards self-fertility, and test population genetic theories in the laboratory.

Abbreviations

CRISPRs, clustered regularly interspaced short palindromic repeats; HAT, histone acetyltransferase; HER-1, human epidermal growth factor receptor-1; mRNA, messenger RNA; TALENs, transcription activator-like effector nucleases.

Disclosures

The authors declare that they have no disclosures.

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