Chapter 3
Evolution of Self-Fertile Hermaphrodites

Ronald E. Ellis and Yiqing Guo

Abstract Self-fertile hermaphrodites have evolved in several independent lineages of nematodes. Surprisingly, both *C. elegans* and *C. briggsae* have recruited members of the large family of F-box genes to promote hermaphrodite development. However, *C. elegans* FOG-2 and *C. briggsae* SHE-1 have different biochemical functions, and each was created by a unique series of gene duplications. Despite these differences, they share a common target – the transmembrane receptor TRA-2, which plays a central role in the sex-determination pathway. When *tra-2* activity is knocked down in the male/female species *C. remanei*, some of the animals develop as hermaphrodites, but are unable to self-fertilize. This defect is due to the inability of their sperm to auto-activate, since knocking down a second gene that blocks sperm activation leads to self-fertility. Based on these results, we propose that hermaphroditic reproduction is a complex trait, because it requires the independent coordination of different regulatory pathways, one controlling sexual development and the other controlling sperm activation. Further analysis of the evolution of these hermaphrodites should reveal how novel traits first arise during evolution.

3.1 Animal Species with Self-Fertile Hermaphrodites Are Rare

3.1.1 The Androdioecious Lifestyle Is Adapted for Colonization

In androdioecious species, some individuals are males and others are hermaphrodites. Early models for the origin of androdioecy focused on flowering plants, where evidence suggests that ancestral populations consisted entirely of hermaphrodites

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(reviewed by Charlesworth and Charlesworth 1978). Since most hermaphroditic plants can either cross-pollinate or self-pollinate, it seemed likely that males subsequently arose by acquiring mutations that eliminated female reproductive structures and their associated costs. This idea fit in well with other analyses of the cost of sexual reproduction.

Work with animals has reopened this problem (reviewed by Pannell 2002; Weeks et al. 2006a). First, hermaphrodites from androdioecious species appear to be modified females, which lack the male reproductive structures needed to inseminate other individuals. Hence, these androdioecious species must have arisen from male/female ancestors through modification of the female sex. Second, these hermaphrodites cannot cross-fertilize, but instead only self or mate with males. As a result, a population of purely hermaphroditic animals should become completely inbred.

Given this scenario, what advantage could self-fertility confer on animals that would overcome the cost of inbreeding depression? Darwin (1876) suggested that selfing might aid in colonization, and this idea was elaborated on by Baker (1955, 1967). A beautiful example involves the European tadpole shrimp, which has male/female, male/hermaphrodite and purely hermaphroditic populations (Zierold et al. 2007). Phylogenetic studies indicate that much of the continent was repopulated by hermaphrodites following the retreat of glaciers at the end of the last ice age. By contrast, male/female populations remain more common in ancient refuges in Iberia. These data support the commonsense notion that hermaphrodites should excel at colonization, since a single individual can found a new population.

3.1.2 In Some Taxa, Androdioecy Has Evolved Repeatedly

Although the advantages of self-fertility might seem beneficial to all species that frequently colonize new environments, androdioecy is extremely rare in both plants and animals (Charlesworth and Charlesworth 1978; Pannell 2002). However, despite this general trend, certain taxa have exhibited repeated, parallel evolution of this mating system. For example, nematodes have undergone independent evolution of androdioecious mating systems on numerous occasions (Kiontke and Fitch 2005), and even in the genus *Caenorhabditis,* androdioecy has evolved in at least three different species (Fig. 3.1, Cho et al. 2004; Kiontke et al. 2004, K. Kiontke, M.-A. Félix, M. Ailion, and D. H. A. Fitch, pers. comm.). Branchiopod crustaceans have also produced many androdioecious species, like the clam shrimp *Eulimnadia texana* (Sassaman and Weeks 1993) and other members of its genus (Weeks et al. 2006b), or the tadpole shrimp *Triops cancriformis* (Zaffagnini and Trentini 1980). Phylogenetic studies imply that androdioecy also evolved independently in several of these cases (Weeks et al. 2009).

By contrast, only one self-fertilizing hermaphrodite is known among the vertebrates – the fish *Kryptolebias* (formerly *Rivulus*) *marmoratus* (Turner et al. 1992). Thus, certain taxa are predisposed to the origin of self-fertilizing hermaphrodites, whereas other taxa are not.

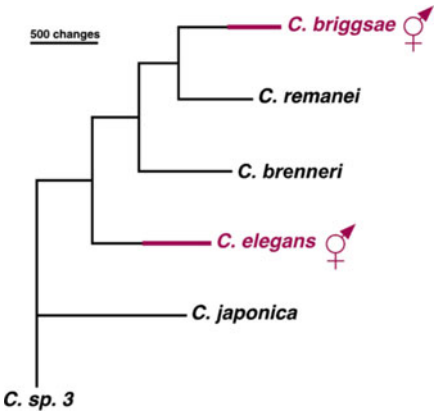

Fig. 3.1 Hermaphrodites evolved independently in *C. elegans* and *C. briggsae*. Maximum Likelihood tree prepared by Cho et al. (2004). Androdiocious lineages are maroon. All branches are significantly positive ($P < 0.01$)

The parallel evolution of androdioecy makes it an ideal model for the origin of complex traits, since theories about how change occurs can be tested by comparative analyses of related species. This task is facilitated in nematodes by the recent origin of selfing in several *Caenorhabditis* lineages. Finally, the lack of androdioecy in other taxa makes it an excellent model for probing the role that developmental biases play in evolutionary change.

3.2 *C. elegans* Is a Model for Self-Fertile Hermaphrodites

The nematode *C. elegans* was originally selected as a model for studying development and neurobiology (Brenner 1974). One of the key advantages Sydney Brenner considered was the fact that these animals are androdioecious (Nigon 1949), since the ability of hermaphrodites to self-fertilize dramatically simplifies mutant screens. Eventually, *C. elegans* also became a model for the genetic control of sexual identity (reviewed by Goodwin and Ellis 2002), and for the population dynamics of hermaphrodites (reviewed by Barriere and Felix 2005).

3.2.1 The Hermaphrodite Soma Is Essentially Female in Structure

In *C. elegans,* about a third of all cells are sexually dimorphic (Fig. 3.2, reviewed by Zarkower 2006). For example, some hypodermal cells produce the vulva in hermaphrodites, but do not divide in males (Fig. 3.2, purple structure). Many muscles are also sexually dimorphic, such as the hermaphrodite sex muscles, which control the opening of the vulva (Fig. 3.2, orange cells). Moreover, some neurons are specific to one sex or the other; in particular, the HSN neurons innervate the sex

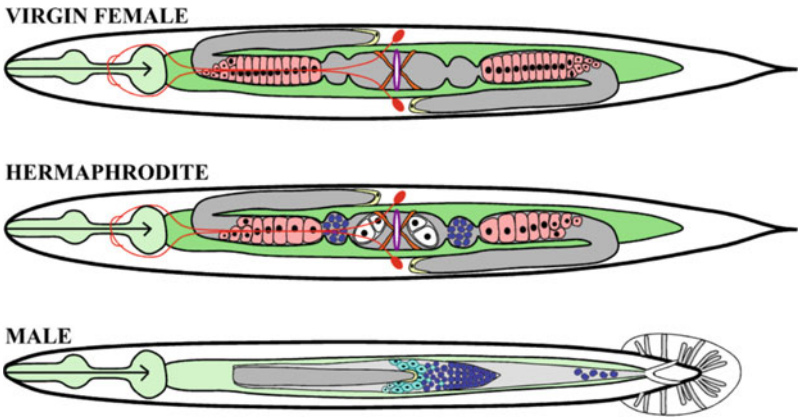

Fig. 3.2 Nematode hermaphrodites are modified females. Ventral views of each adult sex, with anterior to the left. The digestive system is colored *light green*, except for the female and hermaphrodite intestines, which are *dark green* to denote the production of yolk. The HSN neurons are *red*, the sex muscles *orange*, and the vulva *purple*. In each sex, the somatic gonad is *grey*, with oocytes colored *pink* and male germ cells *light blue* for spermatocytes and *dark blue* for sperm. For clarity, the mitotic and early meiotic germ cells are not included in the diagram, since they appear similar in each sex

muscles and control egg laying in hermaphrodites, but die in males (Fig. 3.2, red cells). As one might expect, the gonads of the two sexes also differ dramatically; the hermaphrodite ovotestes is a bilobed structure devoted to nurturing oocytes, whereas the male testes has a single lobe that connects to the cloaca and is specialized for nurturing spermatocytes (Fig. 3.2, gray structures). Even the intestine differs between the sexes. In hermaphrodites, it produces yolk (Fig. 3.2, dark green) but in males it does not (Fig. 3.2, light green).

Examination of females from related species of *Caenorhabditis* reveals that they are almost identical to *C. elegans* hermaphrodites, with one exception – the hermaphrodites produce sperm when they are young, but females do not.

3.2.2 *The Hermaphrodite Germ Line Produces Sperm as well as Oocytes*

In hermaphroditic nematodes, the first germ cells begin to differentiate during the fourth and final larval stage, and become spermatocytes. Later, the animals switch to oogenesis after molting into adults. Since this switch is irrevocable, each hermaphrodite must produce all of the self sperm it will need during larval development. Furthermore, the timing of the switch is of critical importance. Mutants that undergo extended spermatogenesis cannot compete with the wild type, despite the fact that they produce more self-progeny, because the delay in beginning oogenesis is too costly (Hodgkin and Barnes 1991).

3.2.3 *Genes That Regulate Sex-Determination Influence Hermaphrodite Development*

The genetic control of sex determination in *C. elegans* is now understood in great detail (Fig. 3.3, reviewed by Goodwin and Ellis 2002; Zarkower 2006). Briefly, it involves three steps.

First, a group of genes responds to the difference in *X* chromosome dose to regulate XOL-1. In males, XOL-1 is active and represses the SDC genes, thus blocking dosage compensation and allowing the expression of the secreted protein HER-1. In hermaphrodites, XOL-1 is inactive, which allows dosage compensation to proceed and blocks the production of HER-1. Second, sexual identity is coordinated by HER-1. In males, HER-1 diffuses throughout the body and inactivates the TRA-2 receptor. In hermaphrodites, the absence of HER-1 allows TRA-2 to direct female development in each cell. Third, a signal transduction cascade controls the activity of the master transcription factor TRA-1 in each cell. In males, the FEM proteins promote the ubiquitinylation and degradation of TRA-1 (Starostina et al. 2007), whereas in hermaphrodites, TRA-2 downregulates the FEM proteins, so that

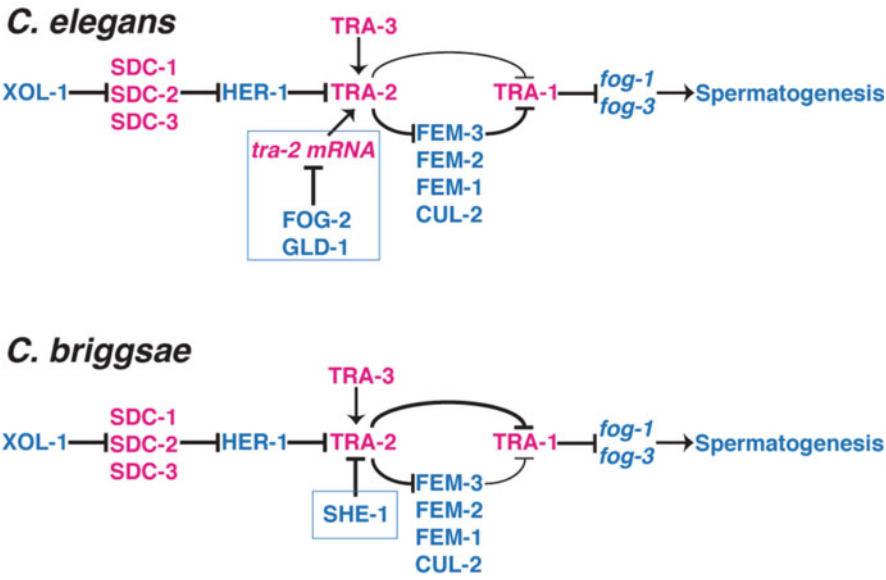

Fig. 3.3 Nematode sex determination pathways. Genes promoting spermatogenesis are shown in *blue*, and those promoting oogenesis in *pink*. Positive interactions are indicated with an *arrow*, negative interactions with a *barred line*, and relatively weak interactions with a *thin line*. Regulatory circuits that specifically promote hermaphrodite spermatogenesis are *boxed*. For simplicity, some genes that regulate sexual development in germ cells are not pictured (reviewed by Ellis 2008)

TRA-1 can be processed into a repressor that blocks male cell fates (Schvarzstein and Spence 2006).

These sex-determination genes act throughout the body, so additional regulatory interactions are needed in the germ line, to allow hermaphrodites to make sperm. These germline regulators control the expression of *fog-3* during larval development, which must occur in *XX* hermaphrodites, but is not observed in *XX* females (Chen et al. 2001). FOG-3 itself cooperates with FOG-1 to promote spermatogenesis (Barton and Kimble 1990; Ellis and Kimble 1995).

3.2.4 *The F-Box Protein FOG-2 Specifies Hermaphrodite Development in* C. elegans

The *fog-2* gene plays a critical role in hermaphrodite development, since the mutants form male/female strains (Schedl and Kimble 1988). FOG-2 interacts with the translational regulator GLD-1 (Clifford et al. 2000), which in turn binds *tra-2* messenger RNAs (Fig. 3.3, Jan et al. 1999). Because FOG-2 and GLD-1 repress the translation of *tra-2,* they lower the overall activity of the gene enough to allow spermatogenesis to proceed during larval development.

Several questions about these genes remain unanswered. First, since GLD-1 is a STAR protein that normally represses its RNA targets (Jones and Schedl 1995), why does it require FOG-2 as a cofactor? Along the same lines, since FOG-2 is an F-box protein that can interact with SKR-1, why doesn't it cause the degradation of GLD-1? Finally, is either GLD-1 or FOG-2 activity modulated in adult hermaphrodites to allow the beginning of oogenesis?

3.3 In *C. briggsae*, a Novel F-Box Protein Specifies Hermaphrodite Development

Although *fog-2* plays a central role in hermaphrodite development in *C. elegans,* it has no ortholog in *C. briggsae* (Nayak et al. 2005). To learn how *C. briggsae XX* animals become hermaphrodites, we screened for mutations that create male/female strains (Guo et al. 2009). All of the mutations we identified were recessive and failed to complement each other. Since they mapped to a new location, we named the gene *she-1,* for *s*permless *he*rmaphrodites.

By constructing double mutants with alleles of other genes that control sex determination, we showed that *she-1* acts upstream of *tra-2* to repress its activity, much as *fog-2* does in *C. elegans* (Fig. 3.3, Guo et al. 2009). However, *she-1* seemed unlikely to work directly with *gld-1,* because *gld-1* promotes oogenesis in *C. briggsae* (Nayak et al. 2005), whereas it promotes spermatogenesis in *C. elegans*.

3.3.1 *SHE-1 Is a Novel F-Box Protein*

Thus, to learn how *she-1* controls sexual development, we used SNP mapping to clone the gene (Guo et al. 2009). After narrowing the region that could contain *she-1* to about 100 kb, we used RNA interference to test candidate genes, and confirmed our identification by sequencing DNA from mutant strains, all of which turned out to have lesions in *she-1*. This work showed that the sequenced genome (Stein et al. 2003) and existing SNP database (Hillier et al. 2007) could be used to identify *C. briggsae* genes that had been known only through mutations.

We found that *she-1* encodes a novel F-box protein. Genetic tests had indicated that SHE-1 was unlikely to interact with GLD-1, and yeast two-hybrid data confirmed this prediction. Thus, SHE-1 and FOG-2 have distinct biochemical activities. Two results suggest that SHE-1 functions like most other F-box proteins. First, the *v35* missense mutation alters a conserved residue in the F-box, which implies that this domain is essential for function. Second, yeast two-hybrid assays show that SHE-1 can bind to SKR-1, a component of E3 ubiquitin-ligase complexes. Moreover, this interaction is abolished by the *v35* missense mutation. Thus, SHE-1 is likely to regulate development by controlling the ubiquitinylation and degradation of a target protein. So far, the direct target remains unknown, although it must regulate TRA-2 activity.

3.3.2 *In* she-1 *Null Mutants, the Environment Determines Sexual Development*

Some of the *she-1* alleles we identified are molecular null alleles; in particular, *v49* is an early stop mutation, and *vDf2* deletes most of the gene. Like other *she-1* mutations, these alleles are temperature sensitive – at 25° all *XX* animals develop as females, but at 15° about half of them become hermaphrodites. Thus, prior to the origin of *she-1,* the development of *XX* animals in *C. briggsae* might have responded to environmental conditions, so that they became females in some circumstances and hermaphrodites in others. If so, this ability might have helped them navigate the period of inbreeding depression that should occur during the transition from a male/female species to a purely androdioecious one.

3.3.3 *The* she-1 *Gene Was Created by a Recent Gene Duplication Event*

One of the most common paradigms for evolutionary change involves the alteration of the *cis* regulatory sequences that control the expression of critical genes (Weatherbee et al. 1999; Prud'homme et al. 2006; Williams et al. 2008; Chan et al. 2010).

Such changes can create new regions of expression, while at the same time preserving all of the ancestral functions of the gene. Although gene duplications are known to play a significant role in evolution, the most striking examples of recent duplications involve structural genes (e.g., Zhang et al. 2002).

However, our studies show that hermaphrodite development in *C. briggsae* depends on *she-1*, a gene created by a recent duplication event (Fig. 3.4). Furthermore, hermaphrodite development in *C. elegans* also relies on a gene created by a

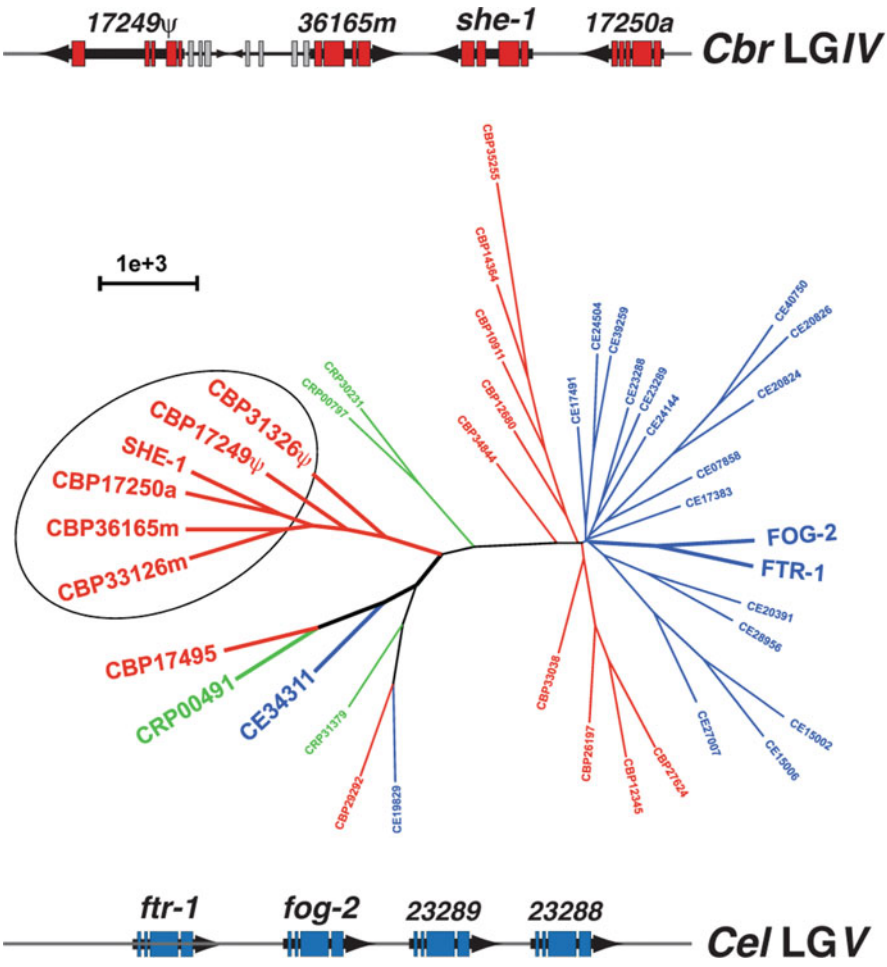

Fig. 3.4 Independent gene duplications regulate hermaphrodite development. Maximum likelihood tree of select F-box genes (Guo et al. 2009), flanked by diagrams of the *she-1* and *fog-2* genomic regions (above and below, respectively). In the phylogeny, *C. briggsae* proteins are shown in *red*, *C. elegans* proteins in *blue*, and *C. remanei* proteins in *green*. In the diagrams, the chromosomes are *gray*, *C. briggsae* F-box genes are *red*, *C. elegans* F-box genes are *blue*, and non-F-box genes are *gray*. *Arrows* connect exons and point toward the 3′ end of each gene

recent duplication (Fig. 3.4, Clifford et al. 2000). Surprisingly, these genes were both recruited from the F-box family, even though they appear to have distinct biochemical functions. Why should this one family have produced evolutionary novelties in two independent lineages? In nematodes, the F-box genes form one of the largest and most rapidly diversifying families (Thomas 2006). If a large number of F-box genes are constantly being created by duplication and altered by mutation and drift, these high numbers should increase the odds that some family members will adopt novel functions.

In addition, the structure and function of regulatory pathways might also influence the types of change that occur during evolution. Many of the models now being studied involve changes in spatial patterning (Weatherbee et al. 1999; Prud'homme et al. 2006; Williams et al. 2008; Chan et al. 2010). In one respect, the recruitment of tissue-specific regulatory genes like *fog-2* or *she-1* is analogous to the tissue-specific changes in enhancers seen in other systems. However, hermaphrodite development requires more than altering the sex-determination pathway in the germ line but not the soma; it also involves precise temporal regulation.

To allow for self-fertilization, the hermaphrodite germ line first produces male cells, and later makes female cells. Thus, the relative activity of genes in the sex-determination pathway must be able to flip around the time the animals molt into adults. Perhaps the recruitment of genes like *she-1* and *fog-2* allowed this flip by lowering the activity of *tra-2* without eliminating it. Thus, investigating additional evolutionary changes that involve developmental timing or heterochronic phenotypes might broaden our understanding of how regulatory pathways evolve.

3.3.4 *The* tra-2 *Gene Might Be a Hot Spot for Changes to the Sex-Determination Pathway*

In fruit flies, almost all of the changes that have altered trichome patterns during evolution affect the transcription factor Shavenbaby (McGregor et al. 2007). This gene probably plays a privileged role because it acts at a nexus in the pathway that controls trichome development (reviewed by Stern 2007; Stern and Orgogozo 2009). Upstream genes that regulate the expression of *shavenbaby* are highly pleiotropic, so mutations that affect them are likely to be deleterious. Downstream mutations affect the structure of trichomes themselves, and might damage them. However, mutations in the *shavenbaby* promoter affect where the gene is expressed, and thus where the developmental subprogram that produces trichomes is active. These favorable conditions appear to make it a hotspot for evolutionary change.

Since independent genes that regulate hermaphrodite development impinge on the sex-determination pathway at *tra-2* (Fig. 3.3), it also appears to be a hotspot. However, TRA-2 is not a transcription factor, so the reason it is favored must be different. We note that the genes that act upstream of TRA-2 influence tissues throughout the body through the secreted protein HER-1, whereas TRA-2 is a

cell-autonomous receptor. Hence, only *tra-2* or downstream genes are likely to be altered to create the hermaphrodite germ line. Perhaps *tra-2* is the most likely target because it controls two separate branches of the sex-determination pathway (Fig. 3.3).

3.4 The Origin of Self-Fertility Requires Two Separate Adaptations

To test our model that small decreases in *tra-2* activity can create self-fertile hermaphrodites, we studied the male/female species *C. remanei* (Baldi et al. 2009).

3.4.1 Lowering the Activity of tra-2 *Allows Sperm Production in* XX *Females*

Although the complete inactivation of the sex-determination gene *tra*-2 transforms *XX* animals into imperfect males (Hodgkin and Brenner 1977), we used a low dose for RNA interference that only partially knocked down its activity. In *C. remanei,* this treatment produced a range of weaker phenotypes among the *XX* progeny. Most importantly, we found individuals with a normal female soma that produced sperm while young, and oocytes when older (Baldi et al. 2009). Although these animals strongly resembled *C. elegans* hermaphrodites, they were not self-fertile (Fig. 3.5). Thus, we refer to them as pseudohermaphrodites.

3.4.2 Altering tra-2 *Activity Is Not Sufficient to Activate Sperm in* XX *Animals*

Closer analysis of the pseudohermaphrodites showed that their sperm neither activated nor moved into the spermatheca, but were lost following the first ovulation (Fig. 3.5). In *C. elegans,* hermaphrodite sperm must be activated to fertilize oocytes and avoid being lost when pushed into the uterus during ovulation (reviewed by L'Hernault 2006). Thus, we dissected individual pseudohermaphrodites, and found that their sperm were indeed inactive, but could be activated by treatment with pronase. Taken together, these results implied that pseudohermaphrodites were not self-fertile because their spermatids could not self activate.

In *C. elegans,* male seminal fluid can activate sperm (reviewed by L'Hernault 2006). Thus, we tested our hypothesis by crossing pseudohermaphrodites with sterile males or *C. elegans* males. In both crosses, male seminal fluid activated sperm in the pseudohermaphrodites, leading to fertilization and the production of self progeny. These results imply that altering the sex-determination pathway is necessary to create hermaphrodites, but not sufficient for self-fertility.

3.4.3 *The* swm-1 *Genes Plays a Conserved Role in the Activation of* Caenorhabditis *Sperm*

In *C. elegans,* the *swm-1* gene prevents the premature activation of sperm in males (Stanfield and Villeneuve 2006). It encodes a protease inhibitor with two TIL domains, which suggests that the ability of proteases to activate sperm in vitro reflects normal regulation in vivo. In addition, genetic tests indicated that *swm-1* also plays a weak role in hermaphrodites. Thus, we analyzed the function of *swm-1* in the male/female species *C. remanei*.

Each of the nematode species in Fig. 3.1 has a single *swm-1* gene with a highly conserved sequence (Baldi et al. 2009). In addition, the two hermaphroditic species have independent and highly divergent duplications of *swm-1* that have no known function. We found that using RNA interference to knock down *swm-1* in *C. remanei* promotes the activation of male sperm, which implies that its function has been conserved in nematodes.

Moreover, when both *tra-2* and *swm-1* were knocked down in *C. remanei,* some of the *XX* animals developed as self-fertile hermaphrodites (Fig. 3.5, Baldi et al. 2009). Thus, *tra-2(RNAi) XX* pseudohermaphrodites are sterile because their sperm

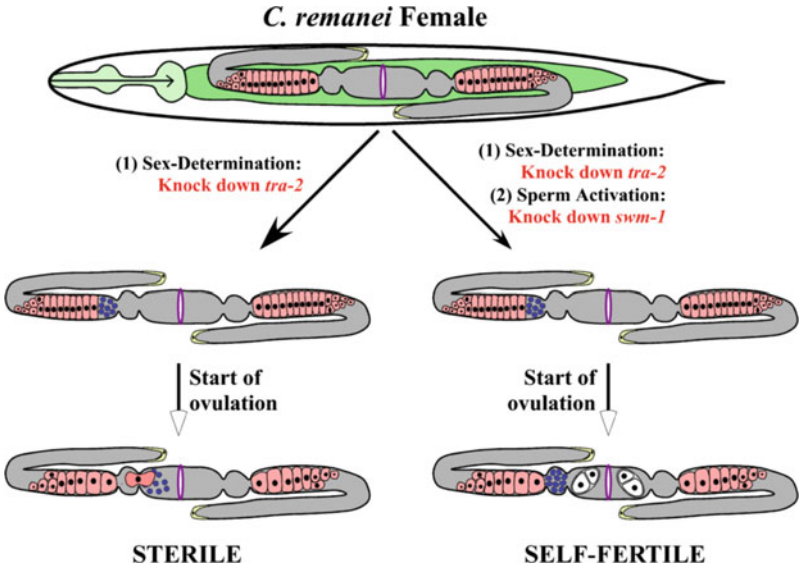

Fig. 3.5 Two independent changes are required to produce hermaphrodites. Summary of experiments that use RNA interference to alter the development of *C. remanei* females. The diagrams use the same conventions as Fig. 3.2, but focus on the germ line, since it plays a central role in self-fertilization. If only *tra-2* is knocked down, the animals produce inactive sperm, which are pushed into the uterus during ovulation and lost (*left*). If *swm-1* is also knocked down, the sperm activate; they can fertilize oocytes, and crawl back into the spermatheca to avoid being lost (*right*)

are unable to activate. Moreover, the *swm-1* gene is expressed in these *XX* animals and is capable of blocking sperm activation.

3.4.4 Two Independent Pathways Must Be Altered to Produce Self-Fertile Hermaphrodites

These results imply that two independent pathways were altered during evolution to create self-fertile hermaphrodites. One set of changes affected the sex-determination pathway, and led to the production of sperm during larval development in otherwise female animals. The *fog-2* gene plays a critical role in this process in *C. elegans,* and the *she-1* gene does so in *C. briggsae*. Additional genes that have not yet been identified might assist them.

The second set of changes allowed the sperm produced by *XX* animals to activate and fertilize oocytes. Although these changes could have been caused by mutations in *swm-1,* the process of sperm activation involves a complex signal transduction pathway (reviewed by L'Hernault 2006), and many other candidates exist. This topic remains a wide-open area for research.

3.5 A Model for the Origin of Self-Fertility

Because the origin of hermaphrodites required the coordination of changes in at least two independent pathways, self-fertility is a complex trait. The analysis of intraspecies hybrids between *C. briggsae* and *C. sp. 9* supports our conclusion that multiple genes were involved in the origin of hermaphroditism (Woodruff et al. 2010). Thus, it provides a model for how other complex traits originated. Two types of explanations seem possible.

In the first, the initial genetic change was neutral, and set the stage for mutations affecting the second trait to sweep through the population. For example, mutations that caused the expression of proteases in the female spermatheca might have conferred the ability to active sperm. In a male/female population, these mutations would probably be selectively neutral. They might have accumulated to low frequencies, or become fixed in small, isolated populations. If so, mutations that altered the sex-determination pathway could then have immediately led to self-fertility.

In the second, these changes proceeded through a selectively favorable intermediate. For example, if nematodes of different species copulate as frequently in the wild as they do in the laboratory, it is possible that "pseudohermaphrodites" would have been able to reproduce using male seminal fluid to activate their own sperm. If so, then an initial change that altered the sex-determination pathway might have been advantageous on its own, even before the acquisition of a second mutation that allowed incipient hermaphrodites to activate self-sperm on their own.

In either scenario, the origin of self-fertility would push nematodes into a niche that should favor additional changes. For example, the population would need to eliminate recessive lethal mutations to minimize inbreeding depression (Dolgin et al. 2007), the number of hermaphrodite sperm would have to be optimized (Hodgkin and Barnes 1991), and the transition from spermatogenesis to oogenesis would need to be sharpened to prevent the production of sexually ambiguous cells. In addition, genomic databases imply that a large-scale reduction in genome size occurs in androdioecious species, and comparative studies show the size of spermatocytes declines in both androdioecious sexes (LaMunyon and Ward 1999). Finally, the structure of the sex-determination pathway itself might drift in hermaphrodites, as shown by the differing importance of the *fem* genes in *C. elegans* and *C. briggsae* (Hill et al. 2006).

To date, we know very little about which characteristics set the stage for the independent, parallel evolution of hermaphrodites in many species of nematodes and branchiopod crustaceans. One factor that might be important in nematodes is their *XO* mating system. Because of it, *XX* females contain all of the genetic information needed to make male tissues. By contrast, many animal species use an *XY* mating system, so the *XX* females lack crucial genes on the *Y* chromosome that might be needed for spermatogenesis. We know less about the sex-determination system in branchiopod crustaceans, but it seems likely that in the ancestral state males were homozygous *ZZ* and females were heterozygous *ZW* (Weeks et al. 2010), which is consistent with our hypothesis. Other traits, such as a gonad that would facilitate the ability of sperm in newly evolving hermaphrodites to find and fertilize oocytes, might be critical as well.

Three factors should make this decade a golden age for evolutionary studies in nematodes. We can now analyze the origin of a complex trait, and recreate it in the laboratory. Moreover, a large suite of changes occurred in response to the origin of hermaphrodites, and can be probed with genetic and genomic tools. Finally, these changes happened recently, in several parallel lineages.

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