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X exceptionalism in *Caenorhabditis* speciation

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Abstract

Speciation genetics research in diverse organisms shows the X-chromosome to be exceptional in how it contributes to "rules" of speciation. Until recently, however, the nematode phylum has been nearly silent on this issue, despite the model organism *Caenorhabditis elegans* having touched most other topics in biology. Studies of speciation with *Caenorhabditis* accelerated with the recent discovery of species pairs showing partial interfertility. The resulting genetic analyses of reproductive isolation in nematodes demonstrate key roles for the X-chromosome in hybrid male sterility and inviability, opening up new understanding of the genetic causes of Haldane's rule, Darwin's corollary to Haldane's rule, and enabling tests of the large-X effect hypothesis. Studies to date implicate improper chromatin regulation of the X-chromosome by small RNA pathways as integral to hybrid male dysfunction. Sexual transitions in reproductive mode to self-fertilizing hermaphroditism inject distinctive molecular evolutionary features into the speciation process for some species. *Caenorhabditis* also provides unique opportunities for analysis in a system with XO sex determination that lacks a Y-chromosome, sex chromosome-dependent sperm competition differences and mechanisms of gametic isolation, exceptional accessibility to the development process and rapid experimental evolution. As genetic analysis of reproductive isolation matures with investigation of multiple pairs of *Caenorhabditis* species and new species discovery, nematodes will provide a powerful complement to more established study organisms for deciphering the genetic basis of and rules to speciation.

KEYWORDS
Caenorhabditis, reproductive isolation, sex chromosomes, speciation, worms

1 | INTRODUCTION

How important are sex chromosomes in speciation? How universal are "rules" of speciation? And what factors and processes are responsible? To gain the broadest answers to these questions requires pertinent data from the broadest diversity of organisms and sex determination systems. Species in chordate and arthropod phyla provide the most prominent systems that have revealed important insights into the evolution of sex chromosomes in reproductive isolation (Coyne & Orr, 2004). Plants with sex chromosomes provide an independent check on pattern and process (Brothers & Delph, 2010). Nematode roundworms, however, provide another independent phylum for study that is mostly neglected in research on speciation. Nematodes tend to have stable karyotypes (with $n = 6$ chromosomes) compared to other groups, and chromosomal sex determination most commonly involves an XO system as in *Caenorhabditis*, whereby males are the heterogametic sex, being diploid but hemizygous for an X-chromosome in the absence of any Y-chromosome (Triantaphyllou, Stone, Platt, & Khalil, 1983). The tractability of laboratory study with nematodes makes them increasingly appealing as systems to study general questions about speciation, especially given the genetic manipulability and rapid generation time of many species

(e.g., 2 days for many *Caenorhabditis* when reared at 25°C). This integration of nematode research, of *Caenorhabditis* nematodes in particular, with the long history of speciation studies in other organisms is invaluable for testing the generality of pattern and process in the evolution of reproductive isolation.

2 | A BRIEF HISTORY

Caenorhabditis began its life as a model in the late 1960s, featuring its elegant image in the glossy pages with all the details of its genetics, development and neurobiology (Hodgkin, 1989). Its life as a model for speciation waited a quarter century. The foundational interspecies crossing experiments by Baird and colleagues showed complete reproductive isolation between the four species of *Caenorhabditis* that were easily available in live culture at the time (Baird, Sutherlin, & Emmons, 1992). This total reproductive isolation between morphologically cryptic species stymied most experimental avenues for research on speciation. The few early studies, however, made clear the potential power to explore developmental processes in hybrid dysfunction with *Caenorhabditis* (Baird, 2002; Baird & Yen, 2000). New phylogenetic discoveries in the 2000s opened up genetic possibilities for speciation research (Kiontke et al., 2011), and the biological species concept is now applied in practice as a key criterion for species diagnosis in this group (Felix, Braendle, & Cutter, 2014). Over 50 species of *Caenorhabditis* nematodes are now available for study (Cutter, 2015; Felix et al., 2014) (K. Kiontke, personal communication), which reinvigorated these animals in speciation research starting in 2010 with the analysis of the first species pair discovered to produce viable and fertile hybrids (*Caenorhabditis briggsae* and *Caenorhabditis nigoni*) (Woodruff, Eke, Baird, Felix, & Haag, 2010). *Caenorhabditis briggsae* shares with *Caenorhabditis elegans* the derived reproductive mode of androdioecy, which they each evolved independently as a rare form of reproduction among *Caenorhabditis* (Kiontke et al., 2011). Thus, studies of speciation with *C. briggsae* also must keep in mind the accompanying evolution of selfing hermaphroditism and its many implications (see below, "Sexual mode transitions and speciation"). Active research programmes have since focused on several other *Caenorhabditis* systems with incomplete reproductive isolation as models of speciation using hybrid analysis to decipher postzygotic reproductive isolation (Baird & Stonesifer, 2012; Chang, Rodriguez, & Ross, 2015; Dey, Jeon, Wang, & Cutter, 2012; Lamelza & Ailion, 2017), as well as postmating prezygotic reproductive isolation (Ting et al., 2014). This emerging body of speciation research in *Caenorhabditis* has demonstrated that (i) Haldane's rule is upheld for both fertility and viability traits and that multiple distinct factors contribute to it, (ii) hybrid dysfunction is commonly asymmetrical in reciprocal crosses (Darwin's corollary to Haldane's rule), (iii) species contain segregating genetic variation for interspecies reproductive isolation and the magnitude of Haldane's rule and (iv) evidence is consistent with a large-X effect for hybrid male sterility.

3 | HALDANE'S RULE

Like most organisms, *Caenorhabditis* interspecies crosses reveal Haldane's rule (Delph & Demuth, 2016; Haldane, 1922; Schilthuizen, Giesbers, & Beukeboom, 2011): heterogametic male hybrids are disproportionately inviable and sterile. This widespread empirical pattern immediately implicates the sex chromosomes as intrinsic to causing it, spawning multiple theories that aim to explain it (Delph & Demuth, 2016; Presgraves, 2008; Schilthuizen et al., 2011). The dominance theory applied to Dobzhansky-Muller incompatibilities (DMIs) could lead to Haldane's rule as a kind of epiphenomenon: loci involved in DMIs that happen to be sex-linked will inevitably expose their effects disproportionately in the heterogametic sex and not the homogametic sex when they act in a recessive manner. Other theories aim to characterize a "large-X effect" independently of dominance through the genomic density of loci that contribute to DMIs, or to their evolutionary and functional properties (Delph & Demuth, 2016; Presgraves, 2008; Schilthuizen et al., 2011). Crossing experiments require homozygous autosomal introgressions to confidently rule out dominance effects (Masly & Presgraves, 2007; Wu & Davis, 1993), and the laboratory tractability of *Caenorhabditis* makes such experiments especially feasible (Bi et al., 2015).

For Haldane's rule in *Caenorhabditis*, in particular, hybrid male sterility is strong when *Caenorhabditis remanei* females are crossed to *Caenorhabditis latens* males (but not in the reciprocal cross) due to malformation of the male gonad (Dey, Jin, Chen, & Cutter, 2014) (Figure 1). This hybrid male sterility appears to be caused by X-autosome incompatibility between these species (Bundus, Wang, & Cutter, 2017). Substantial recessive incompatibilities reveal themselves in the severe inviability in F2 hybrids of *C. remanei* and *C. latens* (F2 hybrid breakdown) (Dey et al., 2014).

Haldane's rule is even stronger between *C. briggsae* and *C. nigoni* such that hybrid males are produced in appreciable numbers in only one direction of the cross, with those hybrid males also exhibiting highly deformed gonads that render them all sterile (Kozlowska, Ahmad, Jahesh, & Cutter, 2012; Woodruff et al., 2010). Consequently, later generation hybrid analysis depends on backcrosses without the benefit of crosses between F1 individuals. The X-chromosome is implicated as the principal genomic compartment involved in male-specific hybrid sterility and inviability, with no known roles for the X in female-specific or sex-independent hybrid dysfunction (Bi et al., 2015). Although an initial report suggested that use of a laboratory mutant genetic background could recover hybrid male fertility (Ragavapuram, Hill, & Baird, 2016), this result was not replicable (Ryan & Haag, 2017). The overall magnitude of Haldane's rule does, however, depend on the combination of genetic backgrounds of the isofemale lines used in crosses from each species (Kozlowska et al., 2012). This genetic variation within species for the degree of interspecies reproductive isolation appears to be common in *Caenorhabditis* and other organisms (Cutter, 2012; Dey et al., 2014). Some hybrid embryos and sterile adults can form from crosses even between *Caenorhabditis* species with distant phylogenetic relationships; the sex of these rare sterile hybrid individuals is

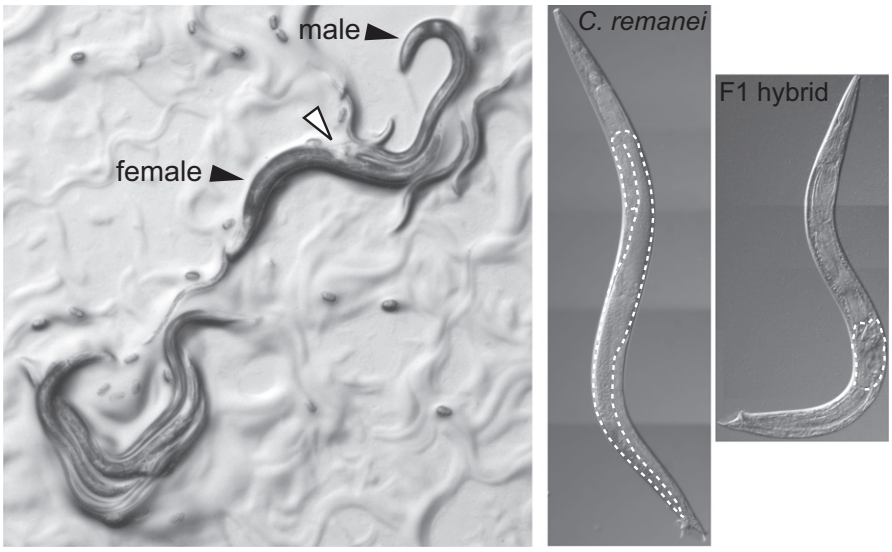

FIGURE 1 *Caenorhabditis remanei* male and female individuals mating on the surface of an agar Petri dish (left panel). The point of contact between the male tail with spicules inserted into the female vulva indicates copulation (white arrow). Small ovals on the agar surface are fertilized eggs; short and thin individuals are larval animals. In the right panels, gonad morphology for *C. remanei* and sterile F1 hybrid males (*C. remanei* female × *Caenorhabditis latens* male), outlined with white stippling, illustrates the small and defective testis of hybrid males

always female (Baird & Seibert, 2013; Baird & Yen, 2000; Baird et al., 1992), providing support for Haldane's rule at both short and long timescales of divergence (Table 1).

In addition to genetically intrinsic contributions of incompatibilities to Haldane's rule in *Caenorhabditis*, extrinsic and developmental factors also modulate the magnitude of hybrid male inviability. Specifically, hybrid male inviability and therefore Haldane's rule are disproportionately strong at low rearing temperatures for *C. briggsae-nigoni* hybrids (Bundus, Alaei, & Cutter, 2015). Moreover, not all developmental stages equally contribute to hybrid inviability. For this same species pair, given that a hybrid animal will die prematurely, the mortality that will take place will almost always manifest during embryogenesis and not later in development (Bundus et al., 2015).

Interestingly, an additional two unusual phenomena contribute to the manifestation of Haldane's rule in some hybrid crosses. First, among the rare viable hybrid offspring between *C. briggsae* and *C. remanei*, both the XX and XO sex chromosome karyotypes develop as females, thus exacerbating the magnitude of Haldane's rule (Baird, 2002). While this sexual transformation is not known from other species pairs, it suggests that developmental perturbations of chromosomal sex determination in hybrids could represent a cryptic and underappreciated contributor to skewed sex ratios among hybrid offspring. Second, in crosses between male *C. briggsae* and female *C. nigoni*, the X-bearing sperm outcompete the nullo-X sperm to result in a more strongly female-biased set of hybrid offspring than would be expected from random sperm usage in fertilization (Bundus et al., 2015). This result points to the influence of postmating prezygotic factors as modulators of the severity of Haldane's rule, with the *Caenorhabditis* mechanism especially pertinent to plant systems that experience the similar phenomenon of certation (Stehlik & Barrett, 2006).

TABLE 1 Summary of cross analysis results for the rules of speciation in *Caenorhabditis*

Species combination	Scale of divergence	Haldane's rule	Darwin's corollary	Large-X effect
>20 species combinations	Distant species	F1 inviability	Yes	Undetermined
Caenorhabditis briggsae–Caenorhabditis nigoni	Sister species	F1 male sterility and inviability	Yes	Hybrid male sterility and inviability
Caenorhabditis remanei–Caenorhabditis latens	Sister species	F1 male sterility and inviability	Yes	Undetermined
C. briggsae	Interpopulation	Not demonstrated	Yes	Absent
Caenorhabditis elegans	Interpopulation	Not demonstrated	Yes	Absent
Caenorhabditis nouraguensis	Interpopulation	Not demonstrated	Yes	Absent

4 | DARWIN'S COROLLARY TO HALDANE'S RULE

Uniparentally inherited factors, such as sex chromosomes, are expected to contribute to asymmetries in the degree of reproductive isolation in reciprocal crosses between species, termed Darwin's corollary to Haldane's rule (Turelli & Moyle, 2007). If only a few DMIs of large effect are responsible for reproductive isolation, however, then cross asymmetry also could result simply from stochasticity between lineages in the origin and dominance of DMI effects, irrespective of sex linkage. Striking asymmetry occurs in reciprocal crosses both for closely related species of *Caenorhabditis* (capable of forming viable and fertile hybrids; Dey et al., 2012; Dey et al., 2014; Kozlowska et al., 2012; Woodruff et al., 2010) and distantly related species (rare viable and completely sterile hybrids; Baird & Seibert, 2013; Baird & Yen, 2000) (Table 1). The conspicuous influence of the X-chromosome in interspecies hybrid dysfunction makes the X-chromosome an obvious candidate for contributing to such asymmetry, which is consistent with data for both *C. briggsae-nigoni* hybrids and *C. remanei-latens* hybrids (Bundus et al., 2015, 2017). To date, however, analysis of introgression lines between species is limited to just a single species pair and in just one direction of introgression (*C. briggsae* DNA introgressed in to *C. nigoni*) (Bi et al., 2015). The very strong reproductive isolation between these species has thwarted alternative strategies for constructing and testing reciprocal introgression lines (J.D. Bundus and A.D. Cutter, unpublished data). Incompatibilities involving the uniparental inheritance of the mitochondrial genome also could contribute to Darwin's corollary. Mito-nuclear incompatibilities do appear to play a role in incipient speciation systems for within-species hybrids (Chang et al., 2015; Lamelza & Ailion, 2017), although they were ruled out as potential contributors to Darwin's corollary in crosses between *C. briggsae* and *C. nigoni* (Ragavapuram et al., 2016).

5 | X EXCEPTIONALISM

The X-chromosome draws attention to itself as a key player for trying to understand both Haldane's rule and Darwin's corollary (Presgraves, 2008). In what ways is the X-chromosome of *Caenorhabditis* special that will be useful context for deciphering its role in the evolution of reproductive isolation? The X-chromosome is distinctive from autosomes in *Caenorhabditis* in many ways. Genetically, the recombination profile along the X-chromosome is more uniform than that found on autosomes, which have distinctive low recombination domains in the central half of each autosome and high recombination domains on the arms (Barnes, Kohara, Coulson, & Hekimi, 1995; Rockman & Kruglyak, 2009; Ross et al., 2011). In hemizygous males, there is no recombination at all on the X-chromosome, although autosomes recombine normally (in contrast to *Drosophila*). All *Caenorhabditis* chromosomes are holocentric and have no defined centromere, however, with more diffuse kinetochore attachment along the length of chromosomes (Maddox, Oegema, Desai, & Cheeseman, 2004).

The gene content on the X-chromosome also is more uniform than it is on autosomes, for which the central domains are gene-dense (*C. elegans* Sequencing Consortium 1998). Even though ~18% of *C. elegans* genes occur in polycistronic operons, only ~5% of genes on the X-chromosome are operonic (Blumenthal et al., 2002), probably because genes expressed in male and female germlines are rare on the X-chromosome and most operonic genes are expressed in the female germline (Ortiz, Noble, Sorokin, & Kimble, 2014; Reinke & Cutter, 2009; Reinke, Gil, Ward, & Kazmer, 2004; Reinke et al., 2000).

Cytogenetically, the X-chromosome is distinctive in spermatogenesis in that it compacts and becomes transcriptionally silent during meiosis: meiotic sex chromosome inactivation (MSCI) (Kelly et al., 2002). This X-specific state due to the lack of a pairing partner is reflected in repressive chromatin marking in the form of dimethylation of lysine 9 in histone 3 proteins (H3K9 methylation; and absence of H3K4 methylation that would indicate open chromatin) (Kelly et al., 2002). Histone marks dictate chromatin state in *Caenorhabditis*, which lacks DNA methylation. Disruption of proper histone regulation results in sterility in *C. elegans* mutants through several pathways, including the histone methyltransferase complex and small RNA pathways, at least in part due to improper regulation of chromatin targets on the X-chromosome (Garvin, Holdeman, & Strome, 1998; Kelly & Aramayo, 2007; She, Xu, Fedotov, Kelly, & Maine, 2009). Derepression of the sperm-derived X-chromosome does not normally occur until after several embryonic cell divisions following fertilization (Bean, Schaner, & Kelly, 2004). These observations suggest that the process of X inactivation in male meiosis would be especially sensitive to disruption in hybrids ("fragile male" hypothesis; Wu & Davis, 1993), potentially leading to hybrid male sterility. The limited available data are consistent with this idea, based on X-linked introgression lines between *C. briggsae* and *C. nigoni* that implicates chromatin misregulation via small RNA pathways (Li et al., 2016).

6 | MOLECULAR EVOLUTION AND THE LARGE-X EFFECT

Multiple distinct factors could lead the X-chromosome to play an outsized genomic role in generating reproductive isolation, a "large-X effect" usually framed in terms of hybrid male sterility (Coyne, 1992; Presgraves, 2008). Most simply, if the X-chromosome is especially large or contains an unusually high density of genes that influence male fertility, then this greater mutational target size alone could make incompatibilities involving the X-chromosome the predominant cause of hybrid male sterility. In *Caenorhabditis*, however, the X-chromosome is an average-sized chromosome relative to the five autosomes, with each chromosome usually comprising 15%–20% of the genome; gene density tends to be lower on the X-chromosome compared to autosomes (*C. elegans* Sequencing Consortium 1998).

Moreover, genes with highly male-biased and sperm-biased expression are exceptionally rare on the X-chromosome (Albritton et al., 2014; Ortiz et al., 2014; Reinke et al., 2000, 2004). This rarity of X-linked sperm genes, despite their fast molecular evolution in *Caenorhabditis* genomes (Artieri, Haerty, Gupta, & Singh, 2008; Cutter & Ward, 2005), would not lead us to expect their evolution to dominate the X-chromosome dynamics to support a "faster male" explanation for the creation of DMIs and a large-X effect (Wu & Davis, 1993). If the genetic network structure of the genome were such that genes with highly central "hub" positions in gene networks were enriched on the X-chromosome, then X-linked loci also might be predicted to be more likely to participate in DMIs. It remains undescribed, however, whether gene positions in genetic networks tend to biased towards different parts of the genome and available gene interaction networks in *C. elegans* are based only on the hermaphrodite sex (Lehner, Crombie, Tischler, Fortunato, & Fraser, 2006; Simonis et al., 2009). Mutational target size and "faster male" evolution driven by sexual selection thus appear unlikely to predict a large-X effect for hybrid male sterility in *Caenorhabditis*.

The "faster X" hypothesis provides another possible source of a large-X effect: if X-linked loci evolve more rapidly, then they may be more likely to instigate the formation of DMIs in hybrids (Charlesworth, Coyne, & Barton, 1987). Higher mutation rates on the X-chromosome could also provide a source of faster X-chromosome evolution. Mutation accumulation experiments do not support mutation rate heterogeneity among chromosomes (Denver et al., 2009); however, neutral divergence between species is slightly higher for X-linked loci than most other chromosomes which could be consistent with higher mutational input on the X-chromosome (Thomas et al., 2015). Divergence in protein sequence, however, is actually slightly lower for X-linked genes (Artieri et al., 2008; Cutter & Ward, 2005), and rearrangements are rarer on the X-chromosome than on autosomes when comparing the genomes of different species (Fierst et al., 2015; Ross et al., 2011; Stein et al., 2003); these kinds of changes are more likely to impact fitness and the formation of DMIs. These patterns do not strongly support the idea of the X-chromosome generally evolving faster in *Caenorhabditis*.

Yet another conceivable contributor to a large-X effect is the possibility of sex chromosome meiotic drive (Frank, 1991; Hurst & Pomiankowski, 1991). No driving X elements are known from *Caenorhabditis* through mechanisms similar to those described for *Drosophila* (McDermott & Noor, 2010; Phadnis & Orr, 2009; Tao, Hartl, & Laurie, 2001). At the postmating prezygotic level, however, greater sperm competitiveness of X-bearing sperm in *C. briggsae* mimics a drive-like process and leads to skewed sex ratios in both conspecific and heterospecific crosses (Bundus et al., 2015; LaMunyon & Ward, 1997). While this phenomenon influences the manifestation of Haldane's rule (Bundus et al., 2015), the genetic cause remains unknown and so it is unclear whether it has the potential to contribute to a large-X effect at the postzygotic level.

A final category of explanation relates to chromatin regulation of the sex chromosomes in the male germline (Jablonka & Lamb, 1991; Lifschytz & Lindsley, 1972). As described above ("X exceptionalism" section), molecular studies in *C. elegans* demonstrate an important role for MSCI in male fertility (Garvin et al., 1998; Kelly & Aramayo, 2007; She et al., 2009). What do the interspecies hybrid data show?

Nearly the entire X-chromosome is associated with hybrid incompatibilities between *C. briggsae* and *C. nigoni*, although most autosomal regions are similarly linked to incompatibilities for this strongly reproductively isolated species pair (Bi et al., 2015). However, these experiments with homozygous introgressions of *C. briggsae* DNA inserted into a *C. nigoni* genomic background demonstrated that the X-chromosome is associated with all of the male-specific hybrid dysfunction in terms of fertility and viability (Bi et al., 2015). Spermatogenesis, in particular, is perturbed by these X-linked introgressions and leads to incompatibility mediated by misregulation of small RNAs (Li et al., 2016). The biogenesis of this particular class of small RNA, known as 22G RNAs, involves post-transcriptional processing of coding sequences with subsequent targeting and modulation of chromatin in conjunction with the CSR-1 and other argonaute proteins (Claycomb, 2012). By contrast, the X-chromosome was not found to be associated with hybrid inviability of females (Bi et al., 2015). Thus, despite the rarity of male-biased genes on the X, the X-chromosome is still disproportionately associated with hybrid male incompatibility phenotypes; and despite the enrichment of female-biased genes on the X, the X-chromosome is unusually depauperate in loci that contribute to hybrid inviability of females. These experiments showing a prevailing contribution of the X-chromosome to hybrid male defects between *C. briggsae* and *C. nigoni* used homozygous introgression lines and so can rule out dominance effects (Bi et al., 2015). The especially rapid loss of genes with male-biased expression in the self-fertilizing *C. briggsae* lineage also plausibly contributes to hybrid male dysfunction, especially for those loci linked to the *C. briggsae* X-chromosome (see "Sexual mode transitions and speciation" below; Fierst et al., 2015; Thomas et al., 2012; Yin et al., 2017). Hybrid male sterility between *C. remanei* and *C. latens* also appears to be caused by X-autosome incompatibility, whereas hybrid inviability involves combinations of loci across the genome (Bundus et al., 2017). The combined evidence to date supports the idea of a "large-X effect" for hybrid male dysfunction in *Caenorhabditis*.

7 | SEXUAL MODE TRANSITIONS AND SPECIATION

Populations of the sister species *C. nigoni* and *C. briggsae* do not just comprise distinct species that are partially interfertile, but also represent distinct reproductive modes. *Caenorhabditis nigoni* has the familiar male–female (dioecious) mode of reproduction that is ancestral in the genus and found in >90% of *Caenorhabditis* species (Cutter, 2015; Kiontke et al., 2011). By contrast, *C. briggsae* has a derived reproductive mode with populations comprised primarily of self-fertilizing hermaphrodites and rare males that enable outcrossing (androdioecy). The most famous species in this genus, *C. elegans*, also independently evolved the derived state of selfing

hermaphroditism like *C. briggsae* and a third species named *Caenorhabditis tropicalis*; but this reproductive mode is unusual among *Caenorhabditis* overall. The genomic consequences of these evolutionary transitions to selfing hermaphroditism are profound (Fierst et al., 2015; Thomas et al., 2012; Yin et al., 2017). In particular, the genome shrinks by 20%–40% in selfers, with genes that have male-biased expression getting lost disproportionately from the genome. This rapid and drastic evolution of genome architecture and gene loss ought to facilitate especially rapid accumulation of incompatibilities, and indeed most of the *C. briggsae* genome appears complicit in hybrid inviability or sterility (Bi et al., 2015). This rapid genome shrinkage, thought to be a molecular evolutionary by-product of adaptation to a highly selfing lifestyle (Fierst et al., 2015; Yin et al., 2017), provides a means for the evolution of selfing to enforce its intrinsic process of "assortative mating" and, in turn, drive speciation (Coyne & Orr, 2004; Lenormand, 2012). Hermaphrodites have evolved mating avoidance behaviours as part of the suite of traits contributing to the "selfing syndrome" in these species (Garcia, LeBoeuf, & Koo, 2007), which also could act as premating isolating barriers. The X-chromosome has the most species-specific DNA of any chromosome, in the case of *C. briggsae* compared to *C. nigoni* (Yin et al., 2017). Given the male bias in reproductive isolation traits associated with the *C. briggsae* X-chromosome (Bi et al., 2015), this divergence in chromosome content could tie in to the large-X effect for hybrid male sterility between these species in addition to disruption of MSCI.

8 | INCIPIENT SPECIATION

The three species *C. elegans*, *C. briggsae* and *C. tropicalis* are the only species in the genus known to have evolved selfing hermaphroditism, which arose independently in the ancestry of each of them (Kiontke et al., 2011). The genetic differentiation among lineages within these species gets magnified by very strong linkage disequilibrium across the genome (Barrière & Félix, 2005; Cutter, 2006; Cutter, Félix, Barriere, & Charlesworth, 2006; Gimond et al., 2013; Haber et al., 2005; Thomas et al., 2015) and may foster the evolution of genetic incompatibilities among conspecific lineages. This might even occur despite the small absolute divergence among interfertile populations relative to the huge molecular divergence observed between "good" species of *Caenorhabditis* (Cutter, 2008; Dey et al., 2012; Thomas et al., 2015). Such within-species incompatibility is indeed observed, often termed outbreeding depression (Dolgin, Charlesworth, Baird, & Cutter, 2007; Gimond et al., 2013). However, it is useful also to consider negative epistatic interactions in the context of the evolution of reproductive incompatibilities and incipient speciation (Snoek et al., 2014). Because genetically effective outcrossing in nature appears to be extremely rare (Thomas et al., 2015), "faster male" theories motivated by sexual selection are unlikely to apply to reproductive isolation that evolves within selfing lineages (Wu & Davis, 1993).

Caenorhabditis briggsae is the species most investigated as a model for incipient speciation, given the divergence among phylogeographic groups and hybrid dysfunction in some intergroup crosses (Cutter et al., 2006; Dolgin, Félix, & Cutter, 2008; Ross et al., 2011; Thomas et al., 2015). Crosses between some strain combinations yield outbreeding depression or a developmental-delay phenotype indicative of reduced hybrid fitness (Baird & Stonesifer, 2012; Dolgin et al., 2008; Ross et al., 2011). Recombinant inbred lines produced from intercrossing strains from two such phylogeographic groups led to segregation distortion across much of three of the autosomes, but not the X-chromosome; the distortion often was stronger in one mitochondrial background than the other (Ross et al., 2011). Cyto-nuclear incompatibility also appears to be present in crosses of some *C. briggsae* genetic backgrounds (Chang et al., 2015), whereas molecular divergence between phylogeographic groups is stronger for the X-chromosome than for autosomes (Thomas et al., 2015). Curiously, crosses between distinct *C. briggsae* genetic backgrounds provide support for paternal leakage of the mitochondria (Ross, Howe, Coleman-Hulbert, Denver, & Estes, 2016). Outbreeding depression also is found in crosses among genetic backgrounds of *C. tropicalis* (Gimond et al., 2013), suggesting that this species might also be amenable as a model for incipient speciation. However, it remains unknown what role, if any, that the X-chromosome or mitochondrial genome might play for either sex in reduced fitness of hybrid crosses within *C. tropicalis*.

In addition to these cases of cyto-nuclear incompatibility, fully autosomal intraspecies incompatibility systems have been characterized in both *C. elegans* and *C. briggsae*, involving neither the X-chromosome nor the mitochondrial genome (Baird & Stonesifer, 2012; Seidel, Rockman, & Kruglyak, 2008; Seidel et al., 2011). The *peel-1/zeel-1* gene interaction linked to *C. elegans* autosomal chromosome I acts as a toxin–antidote system that segregates as a polymorphism in nature, in which the paternal-effect PEEL-1 sperm-derived toxin gets eliminated by zygotically expressed ZEEL-1 only if the embryo has the appropriate allele of *zeel-1*; consequently, ~25% of the offspring from F1 crosses are inviable (Seidel et al., 2008, 2011). Also in *C. elegans*, a number of synthetic interactions between loci lead to reduced fitness from egg-laying defects, primarily involving autosomal loci, as identified from crosses involving introgression lines and recombinant inbred lines (Snoek et al., 2014). Interestingly, genomic regions of exceptionally high divergence are especially rare on the X-chromosome between *C. elegans* strains that otherwise have overall strong genomic differentiation (Thompson et al., 2015). These intraspecies incompatibilities of isogenic strains for these selfing species contrast with the apparent lack of reduced fitness in crosses between individuals from different continents for hyperdiverse outcrossing species like *Caenorhabditis brenneri*, despite enormous allelic divergence (Dey, Chan, Thomas, & Cutter, 2013).

In contrast to these selfing species, a recent study used the obligatorily outcrossing *Caenorhabditis nouraguensis* as a model for incipient speciation (Lamelza & Ailion, 2017). They demonstrated cyto-nuclear incompatibility segregating within *C. nouraguensis*, but no elevated hybrid male sterility or inviability, nor results consistent

with a contribution of the X-chromosome to hybrid inviability (Lamelza & Ailion, 2017). To date, these studies of incipient speciation seem to point to a less prominent role of the X-chromosome in hybrid dysfunction than for fully distinct species.

9 | SPECIATION IN THE LABORATORY AND SPECIATION IN THE WILD

Caenorhabditis has proven itself as supremely suited to experimental evolution approaches for testing evolutionary hypotheses (Gray & Cutter, 2014), but there is little application of experimental evolution to questions in speciation so far. One exception is divergent experimental adaptation regimes applied to *C. remanei* that led to the evolution of premating barriers but not postzygotic barriers (Castillo, Burger, Lively, & Delph, 2015). However, the genomic context of this evolutionary response remains unknown. The sex determination system of *C. elegans* can be manipulated genetically to change the XO chromosomal system to create neo-sex chromosomes to mimic XY, ZW or environmental sex determination systems (Chandler, Chadderdon, Phillips, Dworkin, & Janzen, 2012; Hodgkin, 2002). Similar approaches should be feasible in other species (Haag & Liu, 2013). Experimental tests of hypotheses about the role of sex chromosomes in the evolution of reproductive isolation may be attainable in *Caenorhabditis* by combining experimental evolution with divergent adaptation regimens and genetic manipulations of chromosomal sex determination.

Laboratory experiments to decipher the genetic underpinnings of reproductive isolation are the strength of *Caenorhabditis*. Field studies in nature, however, present a challenge for this group, given that they are microscopic and transparent and live in microbe-rich rotting environments (Cutter, 2015; Frézal & Félix, 2015). Despite widespread geographic distributions for many species of *Caenorhabditis*, no examples of clinal trait variation or hybrid zones are known. Tropical regions are most speciose and so offer the greatest opportunity for sympatry of closely related species (Cutter, 2015; Felix et al., 2013; Ferrari et al., 2017; Kiontke et al., 2011). Given that many species are known from just a single sampling location, often on islands (Cutter, 2015; Kiontke et al., 2011), it remains to be determined how crucial is geographic isolation in allopatry and to what extent sex chromosome evolution plays into the evolution of reproductive isolation upon secondary contact. For example, it remains unknown whether interspecies hybrids ever form in nature. Under laboratory conditions, most *Caenorhabditis* species intermate promiscuously and virgin females produce pheromones that attract males of other species (Chasnov, So, Chan, & Chow, 2007), although some instances of premating isolation are apparent and mate recognition does occur at intergeneric phylogenetic scales (Baird et al., 1992; Ting et al., 2014). Even with a lack of premating barriers, sperm-induced harm may mediate interspecies reproductive isolation as a postmating prezygotic barrier (Ting et al., 2014), such that postzygotic genetic barriers involving the X-chromosome might not even manifest in the wild. As new *Caenorhabditis* species get discovered and clever field studies emerge, new insights into the natural context of species origination and maintenance should reveal themselves.

10 | CONCLUSION

Applications of *Caenorhabditis* nematodes to questions in speciation genetics have a short history, but nonetheless they have expanded our phylogenetic and mechanistic view of sex chromosomes in the evolution of reproductive isolation. Species of *Caenorhabditis* follow Haldane's rule for hybrid fertility and viability traits, as well as Darwin's corollary to Haldane's rule. In the species pair that has been examined in most detail (*C. briggsae* × *C. nigoni*), the X-chromosome is central to hybrid male sterility and inviability, with small RNA pathways and chromatin misregulation on the X-chromosome of hybrid males implicated in defects in sperm development. Evolutionary transitions in sexual mode, genetic variation within species for the extent of interspecies reproductive isolation, gametic isolation barriers involving sperm-induced harm or fertilization biased towards X-bearing sperm and cyto-nuclear incompatibilities in incipient speciation models all provide biologically intriguing character to the speciation process in these animals. The manipulative experimental tractability, accessibility of developmental and cell biological processes, and potential for experimental evolution all point to continued discoveries with *Caenorhabditis* nematodes about the role of sex chromosomes in speciation.

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