

Reproductive isolation in the Elegans-Group of *Caenorhabditis*

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Received 21 January 2013; revised 20 February 2013; accepted 7 March 2013

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ABSTRACT

Reproductive isolation is the basis of the Biological Species Definition and can be a driving force of speciation. Theoretical studies have provided models of how reproductive isolation can arise within individual species. Genetic tests of these models are limited to populations in which reproductive isolation is present but not complete. Here, reproductive isolation in the Elegans-Group of the nematode genus *Caenorhabditis* is reviewed. Pre-mating barriers, assortative fertilization and post-zygotic barriers all have been observed in this clade. In some combinations of species, fertile F1 hybrids can be obtained. Therefore, the Elegans-Group of *Caenorhabditis* is poised to become an important experimental system for the study of reproductive isolation.

Keywords: *Caenorhabditis*; Pre-Mating Isolation; Assortative Fertilization; Post-Zygotic Isolation

1. INTRODUCTION

Reproductive isolation refers collectively to all genetic mechanisms that prevent or limit gene flow between populations [1]. These mechanisms are thought to result from allele-specific dysgenic interactions between two or more genes [2-5]. Reproductive isolation is the basis of the Biological Species Definition [1]. As reproductive isolation restricts gene flow, it also can facilitate genomic divergence between populations [6-9]. Categories of reproductive isolating mechanisms include pre-mating isolation, assortative fertilization and post-zygotic isolation [1,5,10,11]. Pre-mating isolation refers to mechanisms that prevent matings between individuals from different species. These mechanisms include species-specific differences in reproductive structures, behavior, ecology,

and seasonality of reproduction. Assortative fertilization refers to mechanisms that prevent fertilization after mating has occurred. These mechanisms include species-specific differences in sperm chemotaxis and sperm-ova fusion. Post-zygotic isolation refers to mechanisms that decrease the fitness of hybrids relative to that of the parental populations. These mechanisms include hybrid lethality, hybrid sterility and hybrid breakdown.

Early speciation models required the origin reproductive isolation in allopatric populations [1]. In this way, dysgenic combinations of alleles would not be subjected to negative selection before they became fixed. More recently, models of speciation with gene flow have been developed [8,9,12]. In such models, reproductive isolation does not act uniformly across the entire genome [6]. Rather, gene flow will be restricted only in regions linked to loci involved in reproductive isolation [6,9,12].

To gain a broad understanding of speciation, empirical studies in diverse taxa are required. To date, nematodes in general, and *Caenorhabditis* in particular, have contributed little to these studies [13-20]. However, in recent years, a number of new *Caenorhabditis* species have been discovered [18,21]. With these new species, four pairwise combinations that yield fertile F1 hybrids have been described [17-19]. With these new resources, *Caenorhabditis* is poised to become an important speciation genetics model system. This review provides an overview of reproductive isolation in the Elegans-Group of *Caenorhabditis*.

2. THE ELEGANS-GROUP

Caenorhabditis is a genus of microbiophagous nematodes that reproduce on rotting fruit [21,22]. *Caenorhabditis* species reproduce rapidly with generation times of three to four days at 20°C. Like other nematodes, *Caenorhabditis* species form developmentally arrested, alternative third stage, dauer larvae in conditions of environ-

mental stress [23]. Most if not all species of *Caenorhabditis* form phoretic associations with other soil invertebrates as dauers [24]. These associations are used for transport to microenvironments conducive to reproduction.

One member of *Caenorhabditis*, *C. elegans* is a model organism that has been used extensively in genetic and genomic studies [e.g. 25-27]. More recently, additional members of *Caenorhabditis* have contributed to a variety of evolutionary studies [28-30]. Mostly, other the species of *Caenorhabditis* used in these studies have been from the Elegans-Group.

The Elegans-Group is a monophyletic clade within *Caenorhabditis* (Figure 1). Live cultures of ten Elegans-Group species are available. *C. elegans* is the basal member of this group [21]. The development of this species has been characterized extensively [31,32]. All species within the Elegans-Group are morphologically similar and follow similar patterns of development [14,33]. The genomes of several Elegans-Group Species have been sequenced or are in the process of being sequenced and assembled [34-36]. Curated lists of orthologous genes can be found at wormbase.org.

Most species in the Elegans-Group of *Caenorhabditis*

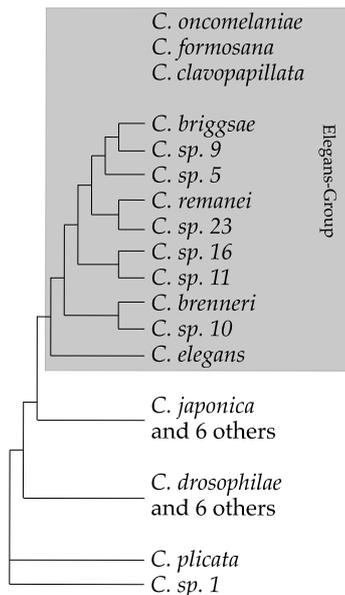


Figure 1. Phylogenetic relationships within the Elegans-Group of *Caenorhabditis* [18,21]. *C.oncomelaniae*, *C. formosana* and *C. clavopapillata* are included within the Elegans-Group based on morphological characters [37]. live cultures of these three species currently are not available, which precludes their inclusion in phylogenies, such as this one, that are based on DNA sequence comparisons.

are known only from geographically limited collection sites [21]. However, at least four, *C. elegans*, *C. briggsae*, *C. remanei* and *C. brenneri* have cosmopolitan distributions [21,37]. Of these, populations are most highly structured in *C. briggsae* [38].

Many pairwise combinations of Elegans-Group species are cross-fertile (Figure 2). In most cross-fertile combinations, F1 hybrids arrest during embryogenesis [13,14,21]. In six combinations, F1 adults are obtained (Table 1) [13,16-18]. In four of these combinations, fertile F1 adults are obtained [17,18]. These results demonstrate that the Elegans-Group of *Caenorhabditis* affords ample opportunities for genetic and genomic studies of reproductive isolation.

3. REPRODUCTIVE ISOLATION IN THE ELEGANS-GROUP

3.1. Pre-Mating Isolation

Within the Elegans-Group of *Caenorhabditis*, all pairwise combinations of species that have been tested will mate with each other [13,17,18,21]. Therefore, pre-mating mechanisms do not obviously contribute to reproductive isolation. This conclusion has two major caveats.

The first caveat is that all mating tests between *Caenorhabditis* species were conducted on agar plates with *E. coli* as a food source. In natural populations, *Caenorhabditis* species reproduce on rotting fruit while feeding on a diverse microbial community [21,22]. Pre-mating mechanisms not apparent in laboratory conditions may act in natural populations. For example, *C. elegans* and *C. briggsae* both reproduce on rotting fruit and have been found to co-occur on the same fruit [22]. These species appear

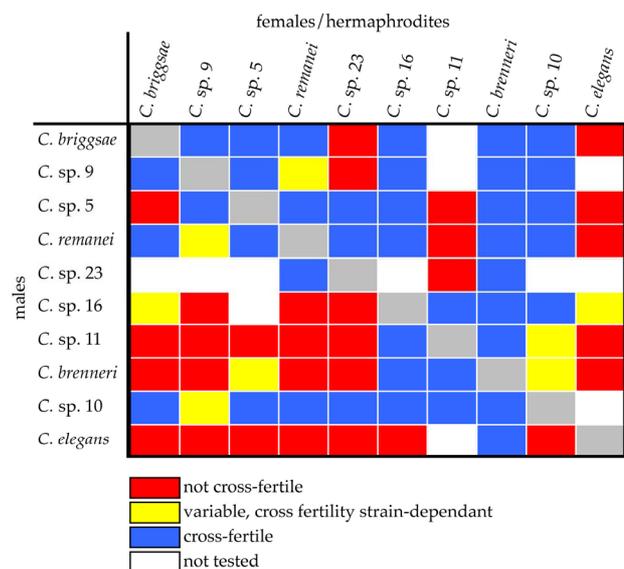


Figure 2. Cross fertility among Elegans-Group species [13-18, 21,57].

Table 1. Hybrid sterility and hybrid breakdown in the *Elegans*-Group.

males	females/herm. ^a	outcome	ref.
<i>C. briggsae</i>	<i>C. sp. 9</i>	fertile F1 females, slow developing sterile F1 males, fertile F2 females and males obtained from crosses of <i>C. sp. 9</i> males to F1 females	[17,19]
<i>C. sp. 9</i>	<i>C. briggsae</i>	fertile F1 females, no adult F1 males, fertile F2 females and males obtained from crosses of <i>C. sp. 9</i> males to F1 females	[17,19]
<i>C. briggsae</i>	<i>C. sp. 5</i>	sterile F1 females with abnormal gonads	[21]
<i>C. briggsae</i>	<i>C. remanei</i>	sterile F1 females with abnormal gonads, XO hybrids develop as males, intersexes or females, degree of sexual transformation strain-dependent	[13,16]
<i>C. remanei</i>	<i>C. sp. 23</i>	fertile F1 females and fertile F1 males, F2 hybrid breakdown (embryonic lethality)	[18]
<i>C. sp. 23</i>	<i>C. remanei</i>	fertile F1 females and fertile F1 males, F2 hybrid breakdown (embryonic lethality)	[18]

^a*C. briggsae* is a hermaphroditic species. Hermaphrodites are anatomically female but make and store sperm during the last (L4) larval stage. Hermaphrodites are self-fertile but can not fertilize other hermaphrodites. gene flow can occur in these species through crosses of males to hermaphrodites.

to be temporally isolated with *C. briggsae* being found primarily at warmer temperatures (e.g. during summer and early fall and *C. elegans* being found primarily at cooler temperatures (e.g. during late fall) [22]. *Caenorhabditis* species also may be isolated based on host preferences. Most if not all *Caenorhabditis* species form phoretic associations with soil invertebrates [24]. These associations are as dauer larvae and are used for transport to suitable reproductive environments. Species-specific host preferences may result in differences in reproductive environments utilized. This does not appear to be true of *C. briggsae*, *C. elegans* and *C. remanei*. All of these species form phoretic associations with a variety of soil invertebrates including terrestrial isopods, snails and beetle larvae [22,39,40]. Host preferences of other members of the *Elegans*-Group remain to be characterized.

The second caveat is that no quantitative mate choice experiments have been conducted for any combination of *Caenorhabditis* species. In *Drosophila*, many species will mate with each other but quantitative differences between con- and inter-specific matings in these species can be detected in single- and multiple-choice tests [41, 42]. Similar differences may be present in *Caenorhabditis*. If present, these differences are likely to be mediated by the male-specific ray sensilla. *Caenorhabditis* males exhibit an exploratory (wandering) behavior that is suppressed in the presence females or hermaphrodites [43]. The suppression of this behavior requires contact with females or hermaphrodites and is mediated by the neurons of the ray sensilla and the male-specific EF interneurons [44]. Male exploratory behavior and its suppression by contact with conspecific females or hermaphrodites has been described in *C. elegans*, *C. briggsae*, and *C. remanei* [43]. Contact with congeneric fe-

males and hermaphrodites also suppresses male-exploratory as males are retained on plates during interspecific crosses [13,21]. The efficacy of inter- vs con-specific in suppressing male-exploratory behavior has not been determined.

3.2. Assortative Fertilization

Assortative fertilization results from species-specific interactions between sperm and ova. In sea urchins, chemotaxis of sperm toward ova, activation of the acrosome reaction and fusion of the sperm and egg all are species-specific [45-47]. In species with internal fertilization, responses of the reproductive tract to interspecific sperm also may limit or prevent fertilization after mating has occurred. In some combinations of *Drosophila* species, the insemination reaction may serve this purpose [48-50].

Fertilization in *Caenorhabditis* is internal. During mating amoeboid sperm are injected into the uterus and then crawl to the spermathecae where fertilization occurs (**Figure 3**) [15,51,52]. In *C. elegans*, migration of sperm to the spermathecae is in response to prostaglandins signals that are secreted from oocytes [51,52]. Also in *C. elegans*, several genes have been identified that are required for fertilization [53]. Two of these, *spe-9* and *spe-38*, are expressed on the plasma membranes of sperm pseudopods and may encode sperm ligands [54,55]. Another two, *egg-1* and *egg-2* are expressed on the surface of oocytes and may encode receptors for sperm ligands (although not necessarily for SPE-9 and/or SPE-38) [56].

Defects in sperm chemotaxis have been observed in some, but not in all, cross-infertile combinations of species in the *Elegans*-Group [15,56]. Moreover, a limited



Figure 3. Sperm chemotaxis in *Caenorhabditis*. (A) An adult *C. remanei* female. the position of the vulva is indicated by the arrow. The positions of the spermathecae are indicated by the arrowheads; (B) Fluorescently labeled *C. remanei* sperm localized to the spermathecae in a *C. remanei* female; (C) Fluorescently labeled *C. remanei* sperm randomly distributed throughout the uterus in a *C. sp. 9* female. Scales bars, panel A = 100 μm , panels B and C = 50 μm .

amount of cross fertilization can occur even when defects in sperm chemotaxis are apparent [57]. Therefore, species-specific chemotaxis does contribute to assortative fertilization in the Elegans-Group, but other reproductive barriers, such as species-specific receptor-ligand interactions involved in sperm fusion must act as well.

In the combination of *C. briggsae* and *C. sp. 9*, interactions between sperm and the reproductive tract also limit cross fertility [17]. Reciprocal matings between these species are cross-fertile. However, *C. briggsae* hermaphrodites become sterile two days after mating with *C. sp. 9* males despite the continued presence of sperm within their *spermathecae*. The cause of this mating-induced sterility is not known.

3.3. Post-Zygotic Isolation

Most cross-fertile combinations of Elegans-Group species are reproductively isolated by hybrid lethality [13, 21]. Most lethality occurs during embryogenesis [13,21]. Terminal phenotypes of hybrid embryos in four combinations of Elegans-Group species have been characterized [14]. The embryonic cell lineage of *C. elegans* is known and is nearly invariant [14]. Embryonic cell lineages of other members of the Elegans-Group are similar, if not identical to that of *C. elegans* [14]. Because of this, terminal phenotypes can be used to identify which developmental processes are defective in hybrid embryos. Most hybrid embryos either failed to gastrulate or failed to elongate from an ovate ball of cells to a tube-shaped worm.

Transcriptionally repressed *C. elegans* embryos arrest at the onset of gastrulation indicating that zygotic tran-

scription is first required at this stage [59]. As gastrulation is the earliest arrest stage observed in Elegans-Group hybrids, dysgenic interactions in these embryos likely involve at least one zygotically expressed gene. However, in this context it should be noted that in *C. elegans*, the paternally delivered PEEL-1 protein causes lethality well after the completion of gastrulation [60]. Embryonic elongation occurs during the later half of embryogenesis. During elongation, the embryo changes from an ovoid ball of cells into a tube-shaped worm. Many hybrid embryos either failed to initiate elongation or arrested at the two-fold stage. The initial stages of elongation are driven by circumferential actin microfilaments in the epidermis [61]. Continued elongation beyond the two-fold stage requires proper development of the body wall muscles that underlie and are connected to the basement membrane and epidermis [62,63]. Elegans-Group hybrids that arrest at the onset of elongation likely have defects in epidermal development and those that arrest at the two-fold stage likely have defects in the development or function of the body wall musculature. PEEL-1-induced lethality in *C. elegans* intraspecific hybrids occurs at the two-fold stage and results from defects in muscular and epidermal tissues [60].

In a few combinations of Elegans-Group species, F1 hybrids arrest during larval development. The arrest stages of these hybrid larvae have not been well characterized. Characterizations of terminal phenotypes in these larvae may provide insights into the development processes that are disrupted by dysgenic interactions.

In six pairwise combinations of Elegans-Group species, adult F1s are obtained (Table 1) [16-18,21]. Sterile female F1 hybrids were obtained from crosses of *C. briggsae* males to *C. remanei* females and from crosses of *C. briggsae* males to *C. sp. 5* females [16,21]. Gonad development in F1 females derived from both of these crosses was abnormal.

Fertile F1 female hybrids were obtained reciprocal crosses of *C. briggsae* to *C. sp. 9* [17,19]. Fertile female and male F2 hybrids were obtained when F1 females were mated to *C. sp. 9* males. The frequencies of viable and fertile F2 males were consistent with the segregation of dysgenic *C. briggsae* alleles at a small number of autosomal loci. When *C. briggsae* males were mated to F1s, F2 hybrids arrested during embryogenesis. Sterile F1 adult males were obtained from crosses of *C. briggsae* males to *C. sp. 9* [17,19]. These males were small and sickly and took twice as long to reach adulthood as did F1 females. F1 adult males were absent or present at much lower frequencies from crosses of *C. sp. 9* males to *C. briggsae* hermaphrodites [17,19]. This difference likely resulted from differences in X-chromosomes, mitochondrial cytotyping and/or maternal inheritance in reciprocal F1 males [19].

From reciprocal crosses of *C. remanei* to *C. sp.* 23 fertile F1 females and males were obtained [18]. Most F2 progeny from these crosses died during embryogenesis. Results from crosses of F1 progeny back to parental species have not been reported. However, this combination of Elegans-Group species presents an opportunity to characterize the genetic architecture of hybrid breakdown.

3.4. Haldane's Rule in the Elegans-Group

In many crosses between closely related species a gender bias is observed in the fitnesses of F1 hybrids [5,64,65]. In almost all instances, it is the heterogametic gender that is less fit. This association between sexual karyotype and hybrid fitness is referred to as Haldane's Rule. Haldane's Rule is of interest because it is thought to result from a common genetic mechanism of speciation in diverse taxa [5].

Caenorhabditis species are diploid with five pairs of autosomes [66]. Females and hermaphrodites are diplo-X and males are haplo-X [30]. Therefore, hermaphrodites and females are homogametic, producing only X-bearing gametes, and males are heterogametic, producing both haplo-X and nullo-X sperm. Haldane's rule is observed in *Caenorhabditis*. In four combinations of species a gender bias is observed in the viabilities and/or fertilities of F1 hybrids [13,16,17,19,21]. In all cases, F1 hybrid males are less fit than F1 hybrid females.

From crosses of *C. briggsae* males to *C. remanei* females, sterile F1 adult hybrids were obtained [13]. The genders of adult hybrids obtained in this combination varied depending upon the strains of *C. briggsae* and *C. remanei* used [16]. In most combinations of strains, haplo-X hybrids that should have developed as males were partially or completely transformed into females. Variation in the degree of sexual transformation of XO hybrids was linked to allelic variation in the *C. briggsae* *Cbr-tra-2* sex determination gene [67]. To our knowledge, this is the only instance in which Haldane's Rule results from sexual transformation [65].

From cross of *C. briggsae* males to *C. sp.* 5 females, sterile F1 females with gonadal abnormalities were obtained [21]. F1 males were not observed. It was not determined whether the absence of F1 males was due to male-specific hybrid lethality or sexual transformation of XO hybrids.

From reciprocal crosses of *C. briggsae* to *C. sp.* 9, fertile F1 female hybrids were obtained [17,19]. F1 males were obtained from crosses of *C. briggsae* males to *C. sp.* 9 females. As noted above, these males were sickly, sterile and took twice as long to reach adulthood than did their sibling females. F1 adult males were absent or extremely rare from crosses of *C. sp.* 9 males to *C. briggsae*

hermaphrodites [17,19]. These results indicate that the viability of F1 hybrid males was impacted by the source of the X chromosome, the mitochondrial cytotype and/or the maternal genotype [19].

3.5. Genic Isolation

Nascent reproductive isolation is not expected to restrict gene flow throughout the entire genome [6]. Rather, restrictions to gene flow are expected only in regions linked to loci involved in dysgenic interactions. In the Elegans-Group of *Caenorhabditis*, two examples of localized restriction of gene flow are known [20,68].

In *C. elegans*, gene flow is restricted between "Bristol-compatible" and "Hawaii-compatible" strains in a 33 kb region of chromosome I [68]. In Bristol-compatible strains, this region contains *peel-1* and *zeel-1*. These genes are absent from Hawaii-compatible strains. *peel-1* is a paternal-effect gene that encodes a sperm-delivered toxin that causes lethality during embryonic elongation [60]. *zeel-1* is a zygotic-effect gene that suppresses PEEL-1-induced lethality. Lethality is observed in F2 hybrids that lack *zeel-1*.

In *C. briggsae*, gene flow is restricted on chromosome III in crosses between temperate strains and the AF16 tropical strain [20,69]. Approximately 20% of the F2 progeny in these crosses exhibit a delayed development phenotype (DDP) in which affected animals take four days to reach sexual maturity in contrast to their non-delayed siblings the reach sexual maturity in three days. This delay in maturation causes a 25% decrease in the intrinsic growth rate. The DDP results from a dysgenic interaction between recessive AF16 alleles at a zygotic acting gene on chromosome III and temperate alleles at an unmapped maternal-effect gene [20]. Because of the decreased intrinsic growth rate in delayed animals AF16 alleles on chromosome III were under-represented in recombinant inbred lines constructed from crosses of AF16 to the HK104 temperate strain [69,70].

4. CONCLUSION

C. elegans has long been an important model system for developmental genetics. With the maturation of this system, increasing attention has been given to other species within *Caenorhabditis*. This has resulted the recent discovery of multiple new species and in a rapid increase in evolutionary studies in this genus. Within the Elegans-Group of *Caenorhabditis*, many cross-fertile combinations of species have been identified. These include four combinations that yield fertile F1 hybrids. Also, intraspecific reproductive isolation has been described *C. elegans* and in *C. briggsae*. With these resources, genetic and genomic studies of reproductive isolation and speciation are possible in the Elegans-Group *Caenorhabditis*.

5. ACKNOWLEDGEMENTS

We thank E. E. King for helpful comments on this manuscript.

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