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Abstract

When *C. briggsae* females are mated to *C. nigoni* males fertile F1 females are obtained. However, all of the F1 males arrest during embryogenesis. In the reciprocal cross of *C. nigoni* females mated to *C. briggsae* males, fertile F1 females and some sterile F1 male adult hybrids are obtained. The goal of this study was to determine the terminal phenotypes of the arrested embryos in these crosses. From these terminal phenotypes, tissue-specific defects in the development of hybrid embryos were inferred. Hybrid crosses were set and allowed to mated overnight. The following day gravid females were dissected to release hybrid embryos. These embryos were allowed to incubate overnight so that any viable nematodes would hatch. The arrested embryos were scored for terminal phenotypes by microscopic observation at a magnification of 1,000x. From *C. briggsae* mothers, 19 of 40 hybrid embryos failed to hatch. Of the 19 arrested embryos, 18 of these failed to gastrulate and 1 arrested at the comma stage. Failure to gastrulate is associated with defects in intestinal cell development while the arrested comma stage is associated with defects in actin cytoskeleton development. From C. *nigoni* mothers, 6 of 10 hybrid embryos failed to hatch. Of the 10 arrested embryos, 6 of these failed to gastrulate. This failure to gastrulate is once again associated with defects in intestinal cell development.

Introduction

Reproductive isolation is the mechanism by which the flow of genes is restricted or prevented from one population to another (Baird & Yen, 2000). The process can be observed in many species with effects ranging from hybrid lethality to hybrid sterility. The species of *Caenorhabditis* are one such organism which reflects the effects of hybrid lethality observed as a product of dysgenic interactions. Specifically, this lethality can be observed notably in crosses between the species of *C. briggsae* and *C. nigoni*. The *C. briggsae* species consists of both hermaphrodites and males whereas the *C. nigoni* species consists of males and females. In crosses between *C. briggsae* males and *C. nigoni* females some fertile females and sterile males are obtained while others arrest during embryogenesis. The reciprocal cross yields some fertile females but all of the males die during embryogenesis (Fig. 1) (Woodruff *et al*. 2010).

Despite the arrest observed in the stages of embryogenesis, the process is conserved among the wild type of all the species of *Caenorhabditis*. It is only when the mixing of genomes as in hybrids that the associated lethality and terminal phenotypes are observed. The terminal phenotypes or physical death traits of the embryos reveal important tissue-specific defects and indicate possible genes involved in the stages of arrest for embryogenesis. For instance, arresting at gastrulation would indicate a defect with intestinal formation, arresting at compaction would confer a defect with the development of the actin cytoskeleton, and arresting at elongation would suggest a problem with the musculature of the worm.

Similarly, observations of the cell lineage of the organism reveals important information such as the appropriate manner of cell division and development in contrast to the differences observed in defective hybrids. Ultimately, the aim of this experiment is to characterize the stages

of arrest observed in crosses between the species of *C. briggsae* and *C. nigoni* while also gaining a better understanding of the process by which reproductive isolation occurs in these species.

C. nigoni ♀ x *C. briggsae* ♂	*C. briggsae* ♀ x *C. nigoni*
↓	↓
F1 females	F1 females
59.4% viable, fertile adults	60.0% viable, fertile adults
40.6% embryonic arrest	40.0% embryonic arrest
F1 males	F1 males
30.9% viable, sterile adults	100% embryonic arrest
69.1% embryonic arrest	

Figure 1. Results from reciprocal crosses of *Caenorhabditis briggsae* and *Caenorhabditis nigoni*. Asymmetric results were obtained from these crosses. In both crosses, frequencies of F1 female lethality are the same. The females that do survive are fertile. However, frequencies of F1 male lethality differ between these crosses. From *C. nigoni* mothers, some sterile adult F1 males are obtained. From *C. briggsae* mothers, all F1 males die during embryogenesis.

Based on the above data, it was expected that the males and females would arrest at different stages of embryogenesis given the disparity between the arrest profiles for each sex (Fig. 1).

Materials and Methods

Crosses consisted of mating five males to three females or sperm depleted hermaphrodites on new seeded mating plates. These plates were seeded with approximately 1 cm drop of *Escherichia coli* strain DA837. In order to sperm deplete, hermaphrodites were placed on new plates each day for 4-5 until laid embryos no longer appeared on the plate (Ragavapuram *et*

al. 2016, Baird & Yen 2000). The nematodes were allowed to mate overnight. The next day resultant gravid females were dissected in an egg salt buffer on a cover slip to release the hybrid embryos. The cover slip was put inside a humid chamber and allowed to incubate overnight at 23°C room temperature. The following day the cover slip was put on a 2% agarose pad and observed under microscopy at 1000X magnification with oil immersion. The observed embryos were then scored based on the arrested stage of embryogenesis or the hatching into a viable L1 larvae.

Results

Embryogenesis in *Caenorhabditis* is well conserved (Fig. 2). During the first half of embryogenesis, an invariant pattern of cell divisions is observed. First described in *C. elegans*, we observed this same of embryonic cell divisions in *C. briggsae* and *C. nigoni*. During the second half of embryogenesis, the embryo elongates into a tube-shaped worm. Microscopic observations (400x, DIC optics) of embryogenesis *C. nigoni* and *C. briggsae* were made from fertilization through gastrulation. No differences in cell division patterns or cellular migrations were noted between these species and *C. elegans* (Sulston *et al*., 1983) or *C. brenneri* (Baird & Yen, 2000).

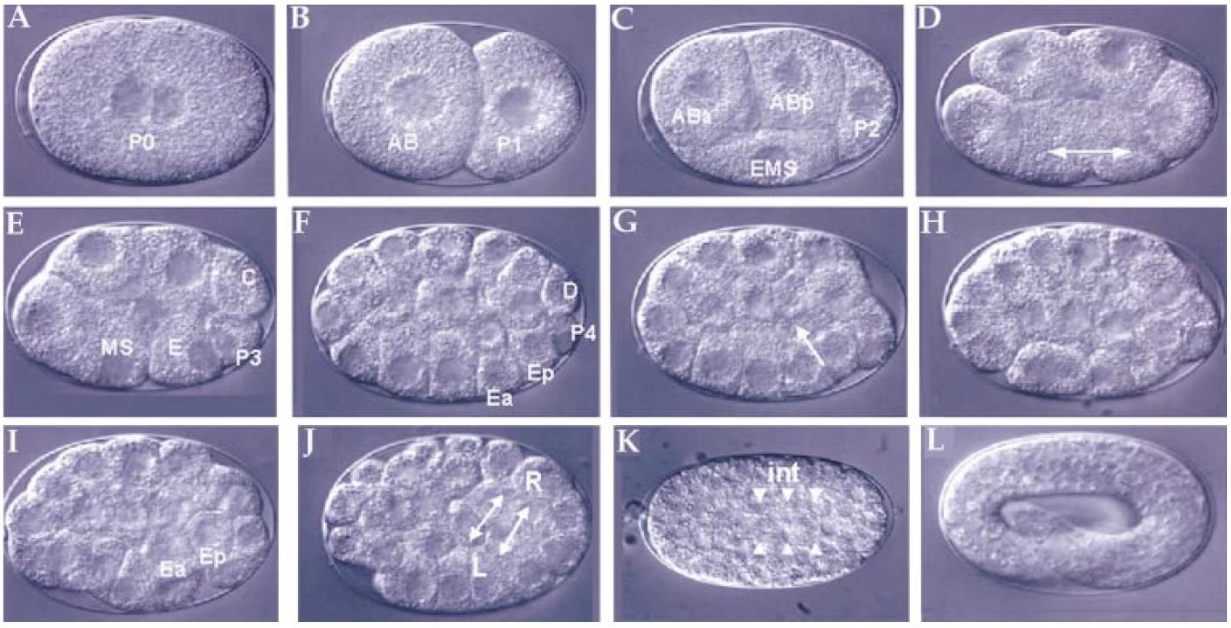

Figure 2. Embryogenesis in *Caenorhabditis*. Early cell divisions in *Caenorhabditis* embryos generate a 28-cell embryo that contains the intestinal precursor cells, Ea and Ep (panel F). These cells migrate internally (panel G), which initiates gastrulation. Immediately before elongation, the entire embryo goes through a brief period of compaction (panel K). The embryo then elongates into a tube-shaped worm (panel L). An intermediate stage during elongation is the two-fold stage (not shown). In prior studies, terminal phenotypes have included; defects in gastrulation (intestinal cell defects), defects in elongation (epidermal cell defects) and arrest at two-fold (muscle cell defects) (Yen & Baird, 2000).

The cross between *C. briggsae* females and *C. nigoni* males resulted in approximately half of the embryos arresting during embryogenesis. Out of a cumulative count of 40 embryos, 18 of the embryos failed to gastrulate whereas 1 embryo arrested at the comma stage of embryonic compaction. In contrast, the remaining 21 embryos hatched into viable L1 larvae (Table 1). The cross between C. nigoni females and C. briggsae males also resulted in

approximately half of the embryos arresting during embryogenesis. Out of a total count of 10 embryos, 6 of the embryos failed to gastrulate. In contrast, the remaining 4 embryos hatched into viable L1 larvae (Table 2). The phenotypic frequencies of the scored embryos show an dominance of failure to gastrulate despite the singular occurrence of an embryo at elongation (Fig. 3).

Table 1. The number of hybrid embryos observed that either arrested at the gastrulation or elongation stage of embryogenesis or hatched into viable L1 larvae out of a total of 40 embryos are recorded below.

C. briggsae ♀ x *C. nigoni* ♂	
Stage of Development	Embryos Scored
Gastrulation	18
Compaction	0
Elongation	1
L1	21

Table 2. The number of hybrid embryos that either failed to gastrulate or hatched into viable L1 larvae out of a total of 10 embryos are recorded below.

C. nigoni ♀ x *C. brigssae*♂	
Stage of Development	Embryos Scored
Gastrulation	6
Compaction	0
Elongation	0
L1	4

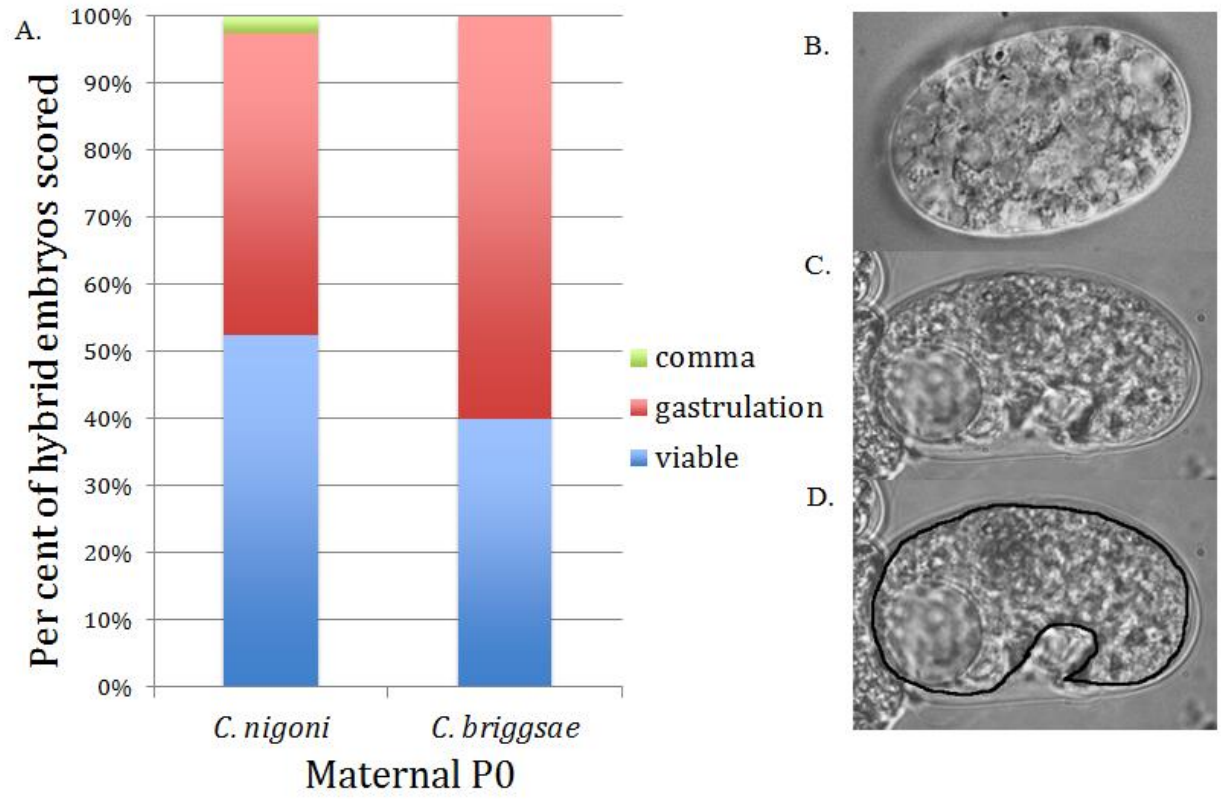

Figure 3. Terminal Phenotypes of scored hybrid embryos. A) Phenotypic frequencies of F1 hybrid embryos. B) An embryo arrested at gastrulation. C) An embryo arrested at the 'comma stage' of embryogenesis. D) An outline highlighting the 'comma' structure of the arrested embryo.

Discussion

The majority of the arrested embryos observed in the cross of *C. briggsae* females to *C. nigoni* males revealed that there is a tissue-specific defect with the gastrulation process. Given that the hybrid embryos arrested predominantly at gastrulation, there is an indication that a problem is occurring with the intestinal cells of the nematode to properly migrate and/or localize to the appropriate area for the development of the intestines. Therefore, it is possible that genes involved with the formation of the gut may be experiencing dysgenic interactions resulting in the

arrested embryos being observed. One notable gene that is important in the gastrulation event is *gad-1* and may be a part of the problem resulting in the hybrid lethality of embryos arresting at this stage.

It is important to note that there was a single embryo that arrested at the comma stage which is inclusive of embryonic compaction. An embryo arresting at this stage would suggest a defect in the cytoskeletal development and thus genes pertaining to the formation of this structure could be subject to further experimentation in order to draw more conclusive results. Based on previous arrest rates and proportions, it is expected that 100% of the males would be arrested in a population of progeny resulting from the above cross (Woodruff *et al.* 2010). Taking the law of assortment into consideration and applying this concept to the embryos scored, one would be able to suggest that any males present in the population of scored embryos would have arrested predominantly at gastrulation with possibly a singular occurrence at compaction. In the reciprocal cross, the sample size is too small to draw conclusions from, however, it is interesting to note the similarity distribution of phenotypic frequencies between the two crosses.

Originally, this difference in survivability would be primarily linked to the X-chromosome inherited as the males only receive one sex chromosome from the mother whereas the females receive one from both parental organisms; this idea was further bolstered by a comparison made between the rates of arrest seen in the reciprocal cross as sterile adult males are obtained (Woodruff *et al.* 2010). However, it was expected that different stages of embryonic arrest would be observed in males and females. Counter to expectations, virtually all observed male and female embryos failed to gastrulate. This lack of distinction in terminal phenotypes observed in the embryos suggests another pathway causing this failure to gastrulate.

Maternal transcripts are sufficient for gastrulation in *C. elegans* (Edgar et al., 1994). In contrast, the zygotic transcripts are not needed to successfully facilitate gastrulation. These results indicate a connection to the process known as maternal-to-zygotic transition. This concept describes the process by which an embryo stops utilizing maternal transcripts and instead switches over to its own zygotic transcripts to facilitate its developmental processes. While zygotic transcript activity has been detected at early stages of embryonic development, the transition period occurs later past gastrulation (Robertson & Lin, 2015).

Given that virtually all embryos failed to gastrulate, disruption of gastrulation must result from a dysgenic maternal-zygotic interaction. The zygotic transcripts could thus be suppressing the activity of the maternal transcripts causing the embryos gastrulation failure. This is consistent with the maternal-effect suppression of F1 male-specific lethality by a mutation in *Cbr-him-8* (Ragavapuram *et al*., 2016).

Based on current data, the next step in experimentation would be to inhibit zygotic transcripts in the hybrid embryos. By doing this, one could determine whether these transcripts are indeed inhibiting the maternal transcripts necessary for gastrulation to occur as embryos failing to gastrulate would no longer be observed. Additionally, more embryos from *C. briggsae* mothers could be scored to see if different terminal phenotypes are observed. The singular occurrence of an embryo arresting at the comma stage while currently being an outlier could potentially be observed at a greater frequency.

This could then possibly support the original expectation of males and females displaying different terminal phenotypes based on previously established arrest profiles. Another experiment to be considered would be obtaining more scored embryos from the reciprocal cross given that the total sample size was so small. This made it difficult to obtain accurate

conclusions from the available data. Finally, confirmation of the results that have been obtained from hybrid embryos of *C. briggsae* mothers could be done by polymerase chain reaction analysis. Specifically, the sex of the nematode would be determined from a known X-linked gene, *vab-3*. Hybrid males obtain one X-chromosome from the parental generation while hybrid females obtain two X-chromosomes. Additionally, the size of these gene products are known for both species (Ragavapuram *et al*. 2016). Therefore, origin of the X-chromosome (inherited from *C. briggsae* or *C. nigoni*) could be determined in addition to the sex of the embryo.

Ultimately, all but one of the scored embryos that arrested failed to gastrulate. The singular event of an embryo at the comma stage might warrant further experimentation as outlined above. The results obtained give future experiments that might reveal the underlying mechanism and/or pathway that results in the hybrid lethality observed in crosses between the species of *C. briggsae* and *C. nigoni*. In conclusion, this experiment was successful in characterizing the terminal phenotypes scored in resultant hybrid embryos and provided useful information relating to the causes of these hybrid lethal embryos.

Literature Cited

Baird, S., & Yen, W. (2000). Reproductive isolation in *Caenorhabditis*: terminal phenotypes of hybrid embryos. *Evol & Dev* 2: 1 – 9.

Edgar, L., Wolf, N., & Wood, W. (1994). Early transcription in *Caenorhabditis elegans* embryos. *Development* 120: 443 – 451.

Ragavapuram, V., Hill, E., & Baird, S. (2016). Suppression of F1 male-specific lethality in *Caenorhabditis* hybrids by *Cbr-him-8*. *Genes Genomes Genetics* 6: 623 – 629.

Robertson, S., & Lin, R. (2015). The Maternal-to-Zygotic Transition in C. elegans. *Current Topics in Developmental Biology the Maternal-to-Zygotic Transition*, 1-42.

Sulston, J., Schierenberg, E., White, J., & Thomson, J. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol 100: 65 – 119.

Woodruff, G., Eke, O., Baird, S., Félix, M., & Haag, E. (2010). Insights into species divergence and the evolution of hermaphroditism from fertile interspecies hybrids of *Caenorhabditis* nematodes. *Genetics* 186: 997 – 1012.