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Mapping Hybrid Lethal Genes on the X Chromosome of C. Briggsae

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MAPPING HYBRID LETHAL GENES ON THE X CHROMOSOME OF *C. BRIGGSAE*

A thesis submitted for partial fulfillment
of the requirements for the degree of
Master of Science

By

Blaine E. Bittorf
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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY **Blaine E. Bittorf** ENTITLED ***Mapping Hybrid Lethal Genes on the X Chromosome of Caenorhabditis briggsae*** BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **Master of Science**

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Abstract

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Mapping of Hybrid Lethal Genes on the X Chromosome of *Caenorhabditis briggsae.*

In the cross of *C. nigoni* males to *C. briggsae* hermaphrodites, all F1 males arrest during embryogenesis. However in the reciprocal cross there are some viable F1 male progeny. This unidirectional male-specific lethality in the F1 hybrids has been attributed to a hybrid lethal gene in a 500 Kb region of the X chromosome of *C. briggsae*. *Cbr-him-8* is a recessive maternal suppressor of the male-specific lethal phenotype, due to the requirement of the him-8 protein for proper X chromosome pairing. Without proper pairing of any one of the chromosomes in the Caenorhabditis genome, genes present on the unpaired chromosome will be silenced due to a process known as meiotic silencing of unpaired chromosomes (MSUC). It has been proposed that MSUC-based silencing of the X-linked hybrid lethal gene is the mechanism by which the male-specific lethality is suppressed. Based on this model, a co-suppression assay was used to identify the hybrid lethal gene. Transgenic strains of *C. briggsae* were constructed via microinjection of bacterial artificial chromosomes (BACs) of small portions of the X chromosome in which the hybrid lethal gene resides. The BACs were mixed with pCFJ909, a plasmid containing a functional *cbr-unc-119* gene, this mixture was then microinjected directly into the gonad of *cbr-unc-119* mutant hermaphrodites. A proportion of the resulting progeny incorporated the injected DNA into their nucleus and formed heritable extra-chromosomal arrays. These offspring were then selected based on the rescue of the *unc-119* phenotype. Transgenic hermaphrodites were then mated to *C. nigoni* males and scored for viable F1 male progeny. Two BAC rescued the male specific hybrid lethal phenotype. Multiple other BACs failed to rescue the lethality phenotype. Focusing on a single BAC clone, using gene groupings and pCFJ909 the number of possible

genes have been narrowed to two candidate hybrid lethal genes within the BAC 08G05. As well as 5 candidate hybrid lethal genes in the non-adjacent BAC 17D03.

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Introduction

Speciation results from the inability of two populations to make viable/fertile offspring, according to the biological species concept (BSC, Mayr, 1963). This process of speciation happens slowly over many generations and in stages along a continuum (de Queiroz, 1998). In most models of speciation a dysgenic interaction of at least two loci is required (Wu 2001). The intraspecies interaction of these loci is normal, but interspecies interactions among these loci cause deleterious effects.

Two such types of speciation are, allopatric speciation and speciation with gene flow. Allopatric speciation happens passively over time due to a complete lack of mating, this type of speciation is associated with neutral genomic divergence. Neutral genomic divergence is a compilation of random mutations within populations' genomes that cause them to become different species (Wright, 1943). Speciation with gene flow is when two populations are within close quarters and able to mate, but due to variants like differences in habitat or predatory pressures the populations' genomes diverge (Nosil, 2008). The differences between species that have diverged via allopatric speciation and speciation with gene flow is that allopatric speciation will have genomic differences evenly throughout their genome and speciation with gene flow will have small areas of their genomes that have diverged more than other portions of the genome (Morjan and Rieseberg, 2004).

Regardless of the type of speciation, both result in reproductive isolation, which is any mechanism that prevents or impedes cross progeny between two populations (Mayr, 1963; Coyne and Orr, 2004). This is broken down into two subtypes of reproductive

isolation; prezygotic isolation, and postzygotic isolation. I will focus on postzygotic isolation, which is anything that reduces the fitness of the cross progeny of the two populations such as hybrid sterility, hybrid mortality, or hybrid breakdown. The incompatibility of the two genomes, with differences in as few as two loci, can cause the two populations to speciate. Hybrid incompatibility (HI) loci have been shown to code for receptor tyrosine kinase, transcription factors, nuclear pore proteins, and a histone H3 methyltransferase (Wittbrodt et al., 1989; Ting et al., 1998; Presgraves et al., 2003; Barbash et al., 2004; Tang and Pregraves, 2009; Phandis and Orr, 2009; Mihola et al., 2009). The evolution of these genes are often adaptations of normal cellular processes in the specific environment that the organism has evolved and these, canonically, are known as hybrid incompatibility (Johnson, 2010)

Often, the development of HI can impact organisms differently based on the sex chromosomes; when this occurs it is known as Haldane's rule. Haldane's rule is that the homogametic sex will be more fit than the heterogametic (Haldane, 1922; Delph and Demuth, 2016). Darwin's corollary of Haldane's rule is the observation of the effects of Haldane's rule impacting offspring differently based on the direction of reciprocal crosses (Coyne and Orr, 2004).

The most compelling theory to explain Haldane's rule is the dominance model (Wu and Davis, 1993; Turelli and Orr, 2000; Turelli, M. and L. C. Moyle, 2007). This model suggests that most deleterious hybrid genes are recessive; thus when homogametic offspring are attained they will have a functional version of the gene. By extension the heterogametic or monogametic offspring will only have the recessive, deleterious gene to transcribe, causing the unequal exhibition between sexes of these species.

In the nematode genus *Caenorhabditis* many species are reproductively isolated through hybrid sterility/ lethality (Baird et al., 1992; Baird and Yen, 2000; Woodruff et al. 2010; Baird and Seibert, 2013). One example of this was demonstrated by Woodruff et al (2010) in the cross of *C. briggsae* with *C. nigoni*. When *C. briggsae* males are crossed to *C. nigoni* females, both males and females are present in the F1 generation. However, in the reciprocal cross only females are present in the F1. The F1 males from these crosses differ in the derivation of the X chromosome, the source of their mitochondria and the maternal protein content in their oocytes before being fertilized. These factors are suspected to be the potential cause for the asymmetry of the Darwin's corollary of Haldane's rule (Turelli and Moyle, 2007). The male specific lethality was was shown to be expressed as a recessive maternal effect and could be suppressed using *Cbr-him-8* (Ragavapuram et al., 2016).

The structure of the crosses performed in Ragavapuram (2016) showed that the male specific lethality possibly was suppressed through meiotic silencing. Specifically they discovered that the F1 male-specific lethality is suppressed by *Cbr-him-8*. There was substantial embryonic lethality in the cross between *C. nigoni* males and *C. briggsae-him-8* mothers; however viable males were obtained from both this cross and its reciprocal. The primary defect in *Cbr-him-8* mutant hermaphrodites is the failure of the X chromosomes to pair during meiosis (Phillips et al., 2006). Unpaired chromosomes likely are transcriptionally repressed during meiosis, a phenomenon known as Meiotic Silencing of Unpaired Chromosomes (MSUC) (Kelly and Aramayo, 2007). MSUC is mediated by small RNA pathways (Weick and Miska, 2014).

The mechanism of MSUC is a trans-acting transcriptional silencer; therefore, it was possible to use a co-suppression assay to identify HI genes on the X-chromosome of *C. briggsae* (Dernburg et al., 2000). Using this assay I was able to identify two candidate HI genes that may be responsible for F1 hybrid male specific lethality. I also identified a small group of genes that may be required for the viability of F1 hybrids in these crosses.

SPECIFIC AIM To map maternal-effect F1 male-specific hybrid lethal genes on the X chromosome of *Caenorhabditis briggsae.* This aim was accomplished using a cuo-suppression assay. In this assay, selected regions of the *C. briggsae* X chromosome were transcriptionally silenced during meiosis by the presence of unpaired extrachromosomal arrays derived from BAC clones or mixtures of PCR products.

MATERIALS AND METHODS

Nematode strains and maintenance

C. nigoni EG5268 (Kiontke *et al.* 2011; Félix *et al.* 2014) was provided by Marie-Anne Félix. *C. briggsae* AF16 (Fodor *et al.* 1983) and CP99 [*cbr-unc-199(nm67)*] were obtained from the *Caenorhabditis* Genetics Center. Nematode strains were grown at 20° C on lawns of *Escherichia coli* strain DA837. Strains above are available from the *Caenorhabditis* Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

Microscopy

Crosses and routine microscopy were conducted using stereomicroscopes at magnifications of 25–50x. Micro-injections were performed using DIC optics at a magnification of 400x on a Zeiss Axiovert 35M microscope. Injections were driven by compressed air at 35 psi.

Reagents

Plasmids were obtained from Addgene (www.addgene.com). *C. briggsae* BAC clones were obtained from the Children's Hospital of Oakland Research Institute. BACs were streaked onto agar plates containing chloramphenicol. From these plates, single colonies were used to seed 50 ml liquid cultures. BAC DNA of these cultures were purified from these cultures using PSI Clone Big BAC DNA isolation kits from Princeton Separations. Selected BACs covered the region of the *C. briggsae* X chromosome from approximately 14.6 to 15.1 Mb. This region was selected based on the research done by Bi et al. (2012),

showing that this is the area of the genome that a male specific hybrid lethal gene lies. Restriction enzymes used for size confirmation of BACs and plasmids were ordered from New England Biolabs (www.NEB.com).

Co-suppression assay

Adult *Cbr-unc-119* [strain CP99] *C. briggsae* hermaphrodites were immobilized on a dehydrated 1% agar pad. Immobilized hermaphrodites were placed under 400X magnification, using a Zeiss Axiovert 35M microscope and had one arm of their distal gonad microinjected with a single BAC clone from the *C. briggsae* X-chromosome between 14.6-15.1 Mb mixed with pCFJ909 to rescue the *cbr-unc-119* phenotype. The concentration of injected DNAs were approximately 100 ng/ul, These concentrations were measured using a Nanodrop spectrometer. Injected animals were recovered and placed onto a seeded agar plate and allowed to lay eggs. The F1 population was scored based on rescue of Cbr-UNC-119 phenotype. In general, the Cbr-UNC-119 phenotype was only partially rescued (i.e. phenotypes of transgenic animals differed both for *Cbr-unc-119* and from wild-type animals). F1 transgenic animals were picked to a single *E. coli* covered agar filled petri dish (plates), and allowed to proliferate and establish separate strains. Any offspring not exhibiting the rescued phenotype were discarded throughout the establishment of strains.

Crosses

Crosses always were of three *C. nigoni* males to three *C. briggsae* transgenic females. They were conducted on freshly seeded mating plates (plates seeded with an approximately one cm spot of *E. coli*). Cross-progeny, which were identified by their wild-type motility, were scored for the presence of F1 males.

RESULTS

Preparation of BAC DNA.

Purified BAC DNA strains obtained from PSI Clone Big BAC DNA isolation kits from Princeton Separations, were digested with Bam HI and run on a 1% agarose gel to confirm BAC identities (Table 1, Figure 1). DNA was successfully purified and maintained from seven of the eight BAC clones. One BAC clone, 21F20, was difficult to maintain, and it was not possible to grow the overnight culture required for multiple DNA purifications.

Construction of transgenic strains

Initially injections were attempted on wild type *C. briggsae* (AF16) hermaphrodites with pCFJ420 and pCFJ421, plasmids that induce green florescent protein (GFP) expression; the phenotypic expression of these two plasmids failed. As did injections of pCFJ909, which contained an intact *Cbr-unc-119* gene, into *Cbr-unc-119* mutant hermaphrodites (strain CP99) the expected result being transgenic rescue of the CBR-UNC-119 mutant phenotype. However, more complex injection mixtures that included both pCFJ909 and DNA from various BAC clones were successful. From these more complex injections, transgenic strains were obtained that contained extra-chromosol arrays derived from all seven BAC clones that were injected (Table 2, Figure 2).

Cbr-unc-119 mutant animals have a phenotype that is easily distinguishable from wild-type. Mutant animals are very short and nearly completely immotile. Transgenic animals were partially rescued. They were wild-type in length and had nearly normal mobility. The utility of this partial rescue came into effect when identifying the cross progeny of transgenic L4 hermaphrodites when crossed to *C. nigoni* males (EG5268). The

Table 1 BAC Clones

BAC	BAC End Points[a] Left	Right	Size (bp)
09E01	14545731	14603560	57829
09O12	14593404	14693009	99605
17D03	14676262	14771883	95621
23C06	14764103	14853869	89766
21F20	14844877	14959558	114681
08G05	14894978	14972860	77882
23H05	14978673	15066976	88303
20O22	15036413	15145866	109453

[a] BAC left and right end positions on the *C. briggsae* X chromosome according to the cb4 genome assembly (wormbase.org)

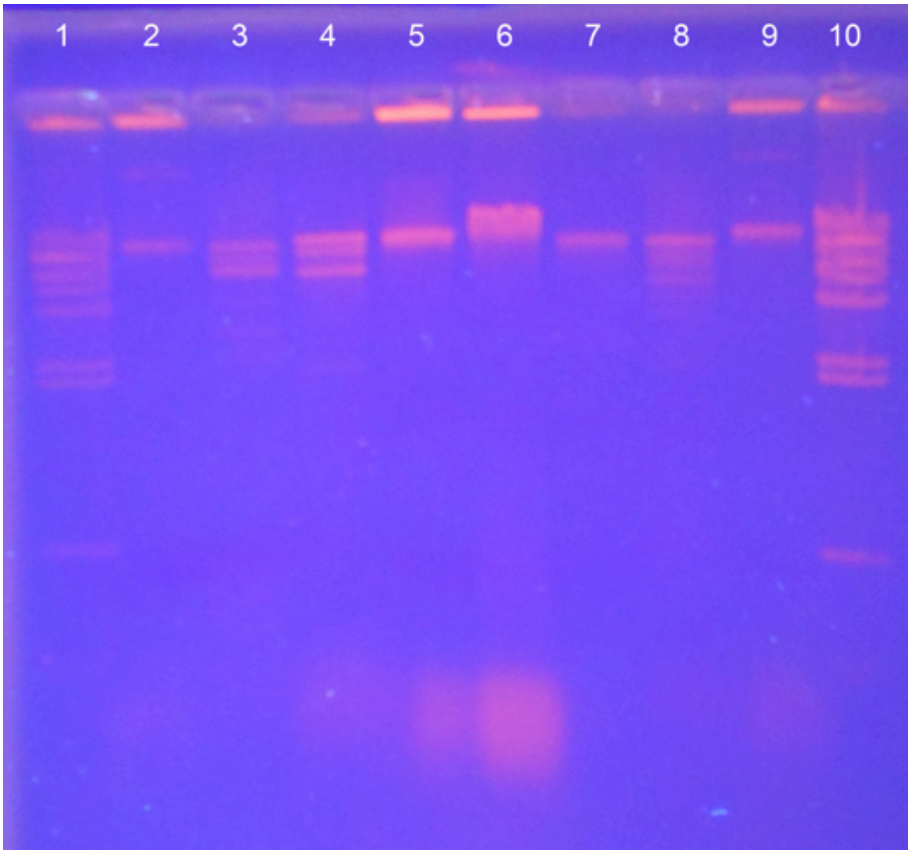

Figure 1. Bam HI digestions of *C. briggsae* BAC DNA clones. Lanes 1 and 10) Hind III-digested l DNA. Lane 2) 09E01; Lane 3) 09O12; Lane 4) 17D03; Lane 5) 23C06; Lane 6) 21F20; Lane 7) 23H05; Lane 8) 20O22; Lane 9 08G05.

Table 2: Co-Suppression Assay Results of BAC clone-derived Transgenic Strains

BAC	transgenic strains	total crosses	total progeny	males	frequency of males	average offspring per cross
09E01	3	9	78	0	0	8.67
09O12	2	6	58	0	0	9.67
17D03	8	44	367	9	0.0245[a]	8.34
23C06	4	21	278	1	0.0036[b]	13.24
21F20	-	-	-	-	-	-
08G05	2	7	46	6	0.1304[a]	6.57
23H05	4	8	50	0	0	6.25
20O22	4	7	74	0	0	10.57

[a] $p < 0.0001$

[b] $p = 0.512$

p values based on an expected male frequency of 0.00189 as reported by Kozlowska et al. 2011. Values corrected for multiple pairwise comparisons by the method of Bonferroni (1936).

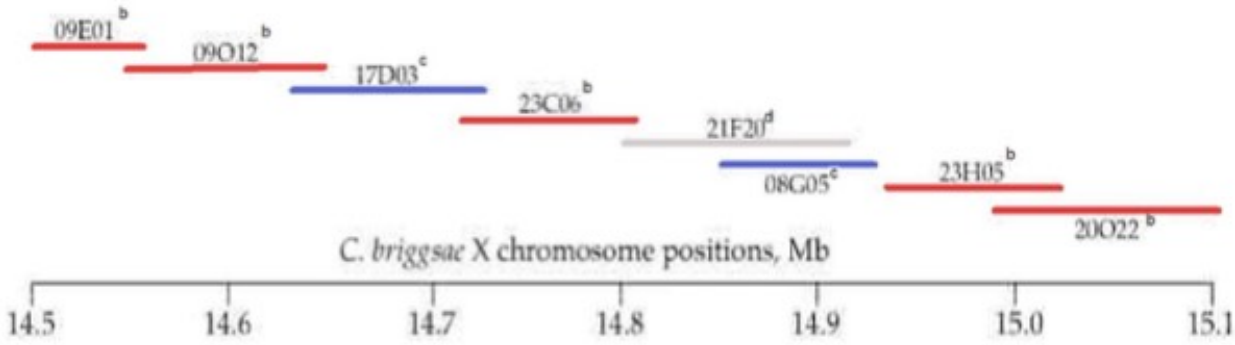

Figure 2. *C. briggsae* Bacterial Artificial Chromosome (BAC) clones. a) BAC clone sizes and positions on the X chromosome of *C. briggsae*. b) BACs indicated in red did not rescue F1 male-specific lethality. c) BAC clones indicated in blue did rescue F1 male-specific lethality. d) 21F20 was not tested for rescue of F1 male-specific lethality as it grew poorly in culture and sufficient DNA for microinjection was not obtained.

resulting hybrid progeny were completely wild-type, in size and motility. Due to this easily distinguishable phenotype of hybrid progeny transgenic hermaphrodites were not sperm depleted prior to the cross, because the self-progeny of the hermaphrodites were easily identified as either fully phenotypically mutant or only partially rescued.

From each BAC clone multiple transgenic strains were obtained (Table 2). None of these strains were entirely stable. In every generation, partially rescued and fully UNC self-progeny were observed. Because of this, transgenic strains were maintain by picking only the most motile animals from each generation to fresh plates to continue propagation of the strain. Eventually, all transgenic strains reverted back to a fully UNC phenotype and were then discarded.

Rescue of F1 male-specific lethality

Crosses of *C. nigoni* males to transgenic *C. briggsae* hermaphrodites were used to test for rescue of F1 male-specific lethality. In all crosses of *C. nigoni* males to transgenic *C. briggsae* hermaphrodites the resulting brood sizes were approximately the same as expected from the cross of these species with non-transgenic animals (Kozlowska et al. 2011). In crosses to transgenic strains derived from four of seven BAC clones, no F1 males were observed (Table 2, Figure 2). F1 males were observed in crosses to transgenic strains 17D03, 23C06 and 08G05 (Table 2, Figure 2).

23C06 singular male

In crosses to 23C06-derived strains, a single male was observed among 278 F1 hybrids (Table 2). This frequency was not significantly different from the frequency of

hybrid males obtained from wild-type crosses (Kozlowska et al. 2011; Table 2). Therefore these 23C06 BAC-derived strains did not rescue F1 male specific hybrid lethality.

Rescue by 17D03

In crosses to 17D03-derived strains the number of males were statistically significantly higher than those from wild-type crosses (Kozlowska et al. 2011; Table 2). Therefore 17D03 rescued the F1 male-specific hybrid lethality. This means that the BAC, 17D03, must contain at least one male-specific hybrid lethal gene. The BAC 17D03 contains 9 protein-coding genes. Considering that 4 of these 9 genes are also within the 2 overlapping BACs that do not rescue, only 5 protein-coding genes remain as candidates as the male-specific hybrid lethal gene (Figure 3). Due to the low frequency of males in these crosses, I did not further pursue the male-specific hybrid lethal gene in this BAC.

Rescue by 08G05

In crosses to 08G05-derived strains the frequency of hybrid males were significantly higher than those from wild-type crosses (Kozlowska et al. 2011; Table 2). Ergo the BAC-derived strains of 08G05 also rescues the F1 male-specific hybrid lethality. By extension that also means that 08G05 must contain one or more male-specific hybrid lethal genes. This BAC contains 11 protein-coding genes, the majority of which have not had their function described (Figure 4). Due to the much higher frequency of males from the 08G05-derived strains this BAC was chosen over 17D03 for the focus of further co-suppression assays.

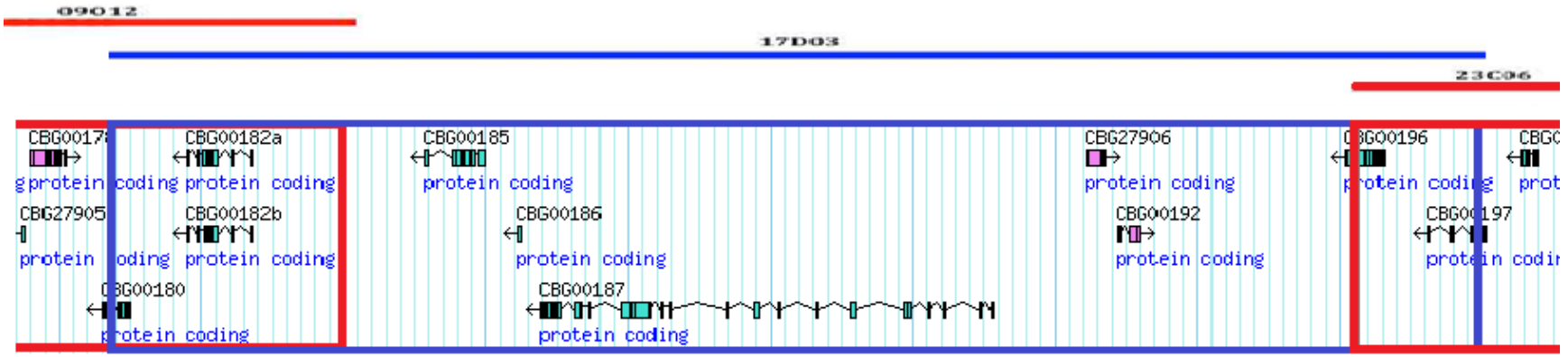

Figure 3. Candidate hybrid lethal genes in 17D03. All genes within the blue box lie within the BAC 17D03. Genes within the red boxes lie within adjacent BACs, 09O12 and 23C06. As 09O12 and 23C06 did not rescue F1 male-specific lethality, only genes in 17D03 that are not also present in the adjacent BAC were considered to be candidate hybrid lethal genes.

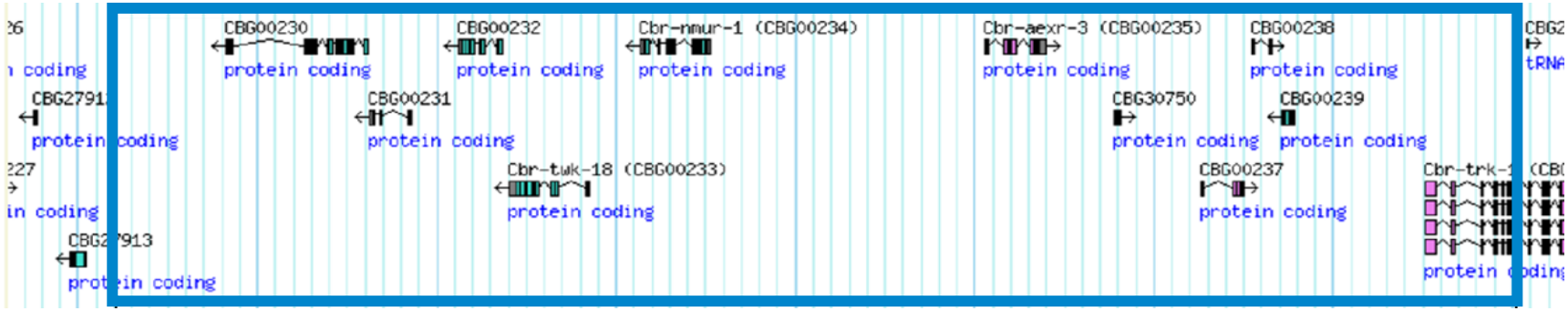

Figure 4. Candidate hybrid lethal genes in 08G05. All or part of eleven predicted protein-coding genes were contained within 08G05. Initially, these all were considered candidate hybrid lethal genes. Subsequently, most of these candidates were eliminated from consideration by co-suppression assays using PCR products.

Hybrid lethal genes in 08G05

To identify the male-specific hybrid lethal gene within 08G05, I performed another co-suppression assay. For the co-suppression of the genes within the BAC 08G05 each gene had a pair of primers designed to capture 1000- 2000 bp of flanking 5' DNA relative to the start of the gene. The flanking DNA was captured to ensure regulatory regions are included in these PCR products, as required to invoke endogenous gene silencing (Adamo et al. 2012). Primers were also designed to capture at least the first exon of the gene (Table 3). Two of the 11 predicted genes within the BAC 08G05, CBG00230 and CBG00231, are part of a single operon. Because these genes are derived from a single primary transcript only the first gene in the operon needed to be targeted to suppress the expression of both. For that reason only CBG00231 was targeted in the co-suppression assay. The primers were tested and confirmed for amplification by electrophoresis on agarose (Figure 5).

The 10 genes were co-suppressed using the micro-injection technique previously described for the BACS, but the genes were broken down into 4 sub-groupings (Table 4). The first half of the genes injected gave no males. The second half of genes injected also resulted in only females. When the genes from the even numbered genes were injected they also had only females. When the odd numbered genes were injected they produced 2 male hybrids. With a frequency of 0.333 males and when comparing these statistics to the expected number of males to be present in wild-type *C. briggsae* crossed to *C. nigoni*, this number of males is significantly different with a p-value much lower than any crosses performed previously (Table 4). Based on the results of these transgenic hybrid crosses

Table 3. Primers for the amplification of genes within 08G05.[a]

Gene	Primer[b]	Sequence[c]	Length, bp
cbg00231	231L	ccaagacggtaccgaagaaa	20
	231R	agccgaagcagctgtagaag	20
cbg00232	232L	tgatatcatgtcccgcttca	20
	232R	cgaaatgcacaaattcaacg	20
cbg00233	233L	atggggatgagagattggtg	20
	233R	caaagaatggccccattaga	20
cbg00234	234L	gtgcagctccgaaaatgact	20
	234R	ggggaaactccccaactatt	20
cbg00235	235L	cccaaaacttctcacggtgt	20
	235R	tttggctcattcacacatgg	20
cbg30750	30750L	agccctgctagcaatttcac	20
	30750R	ccgaaacttgattggaggaa	20
cbg30927	30927L	gaggaagtggggtacattgg	20
	30927R	agacccacaaactggtgctt	20
cbg00238	238L	tccggaaatttcaaaggcta	20
	238R	tttgagtgccgagattcctc	20
cbg00239	239L	tcctgagctctgcgattctt	20
	239R	ttttcccacgacgtaagacc	20
cbg00240	240L	acgaagccgaaagctgtcta	20
	240R	tgatccttcaaatccacacg	20

[a] Primers were designed using the Primer3 design tool (molbiol-tools.ca/PCR.htm). *C. briggsae* sequence data was obtained from wormbase.org (cb4 genome assembly).

[b] Primers denoted with "L" are upstream of 5' end of genes according to the cb4 genome assembly, those denoted with "R" are downstream of the AUG codon.

[c] All primers were designed to pair with the CB4 genome (wormbase.org)

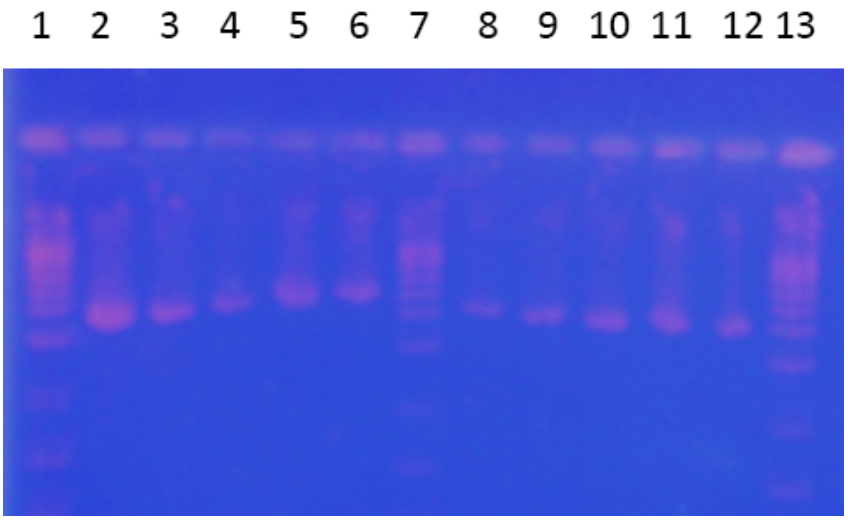

Figure 5. Amplification products of candidate hybrid lethal genes from 08G05. Lanes 1, 7 and 13) Midranger marker DNA. Lane 2) CBG00231; Lane 3) CBG00232; Lane 4) CBG00233; Lane 5) CBG00234; Lane 6) CBG00235; Lane 8) CBG30750; Lane 9) CBG30927; Lane 10) CBG00238; Lane 11) CBG00239; Lane 12) CBG00240.

Table 4. F1 Male Rescue and Hybrid Brood Sizes from 08G05 gene-derived strains.

[a] Injection Mixtures	Transgenic Strains	Total crosses	Total progeny	Males	Frequency of males	Progeny per cross
First half [b]	2	2	47	0	0	23.50
Second half [c]	4	6	5	0	0	0.83
Even Genes [d]	3	4	4	0	0	1.00
Odd Genes [e]	2	5	6	2	0.33	1.20

[a]Gene grouping were selected to narrow possible hybrid lethal genes while also keeping sufficient complexity in the injection mixture.

[b]First half: CBG00231, CBG00232,CBG00233, CBG00234, CBG00235.

[c]Second half: CBG30750, CBG30927, CBG00238, CBG00239, CBG00240.

[d]Even Genes: CBG00232, CBG00234, CBG30750, CBG00238,CBG00240.

[e]Odd Genes: CBG00231,CBG00233,CBG00235, CBG30927, CBG00239

there are only two possible genes, CBG30297 and CBG00239, which can be the male-specific hybrid lethal gene (Figure 6).

CBG00239 and CBG30927

CBG00239 and CBG30927 are the remaining possible hybrid lethal genes in 08G05 (Figure 7). For both of these genes their DNA sequence in not very informative. For CBG00239 there are no known homologs in *C. elegans* or in any other species. Searches of interpro using inferred amino acid sequenced failed to identify any known protein domains. For CBG30927 there are four orthologs in *C. elegans* all of which are predicted genes that have no described function. When searching the amino acid sequence using tBLASTn, the hits include a C-type lectin, which can be involved in many aspects of homeostatic capabilities in *Caenorhabditis.* However, the region of similarity in this sequence does not contain a lectin fold at all.

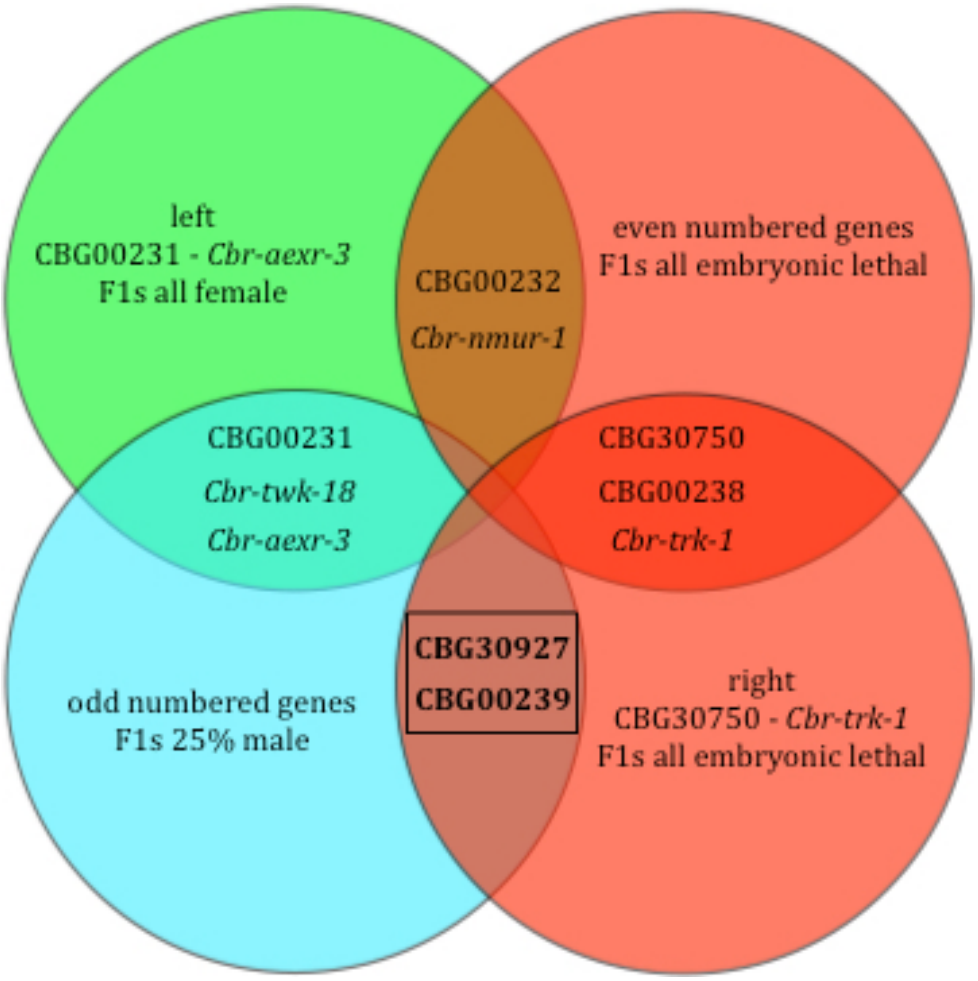

Figure 6: Injected gene subgroups used are visually broken into groups and the most likely two hybrid lethal genes are shown to be *cbg30927* and *cbg00239*, based on the phenotypic results of each injection group.

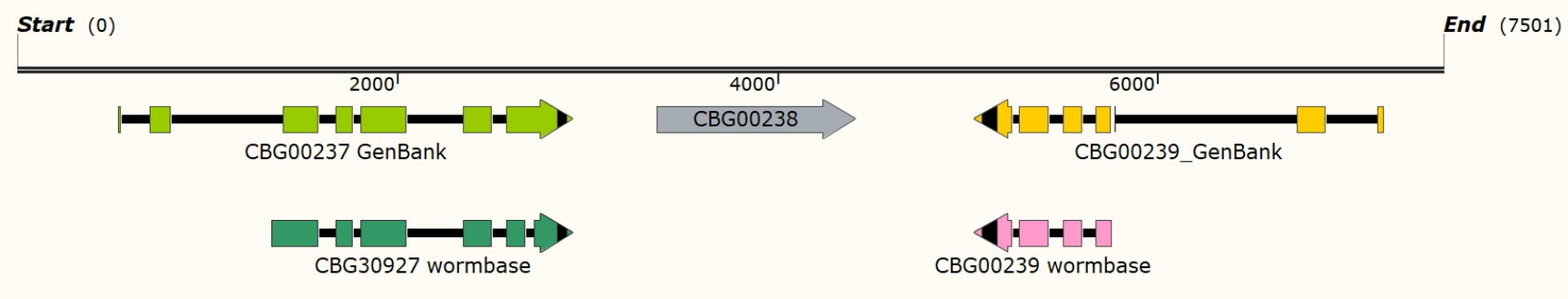

Figure 7. Structures of the CBG30927 and CBG00239 candidate hybrid lethal genes. Shown are both models derived from GenBank cDNA records (CBG00237 = XM_002645268.1, CBG00239 = , XM_002645270.1) and wormbase.org algorithmic predictions from the *C. birggsae* cb4 genome assembly. CBG00238, while included in this diagram has been eliminated as a candidate hybrid lethal gene and does not have the intron-exon struction shown.

DISCUSSION

Meiotic Silencing of Unpaired DNA

The suppression of the male-specific hybrid lethality by meiotic silencing of unpaired DNA is consistent with the model of suppression proposed for *Cbr-him-8* mutants hypothesized in Ragavapuram et al. (2016). In the non-disjunction model suggested in Ragavapuram et al. (2016) there are two possible genotypes of males in the *Cbr-him-8* animals. Some oocytes produced by *Cbr-him-8* mutant hermaphrodites will contain no X chromosome, this nullo-X oocyte can lead to F1 males that receive their X chromosome paternally (X^{Cni}) which would not be subject to the male-specific hybrid lethal gene on the *C. briggsae* X chromosome since these animals do not possess a *C. briggsae* X chromosome. These animals end up being completely sterile, due to a malformed gonad. Another way that *cbr-him-8* hermaphrodites crossed to *C. nigoni* males could result in hybrid males would be for the hybrid males to receive their X chromosome maternally (X^{cbr}). To get hybrid X^{cbr} males the male-specific hybrid lethal gene would have to be suppressed. The resulting X^{cbr} males are fertile when backcrossed to *C. briggsae* as well as when they are crossed to F1 females. This difference in hybrid cross fertility and gonad formation could only occur if the X-chromosome came from different species.

When Ryan and Haag (2017) tried to replicate these experiments they did not obtain the same results. To justify their inability to get X^{cbr}, males they stated that the males retrieved from the Ragavapuram et al. (2016) crosses, X^{cbr}, had to be a result of self-fertilization of the hermaphrodites. However, This is not consistent with the observed differences in the fertility profiles of *C. briggsae* males and males identified as F1 hybrids by

Ragavapuram et al. (2016). My results using co-suppression assays (Dernburg et a 2000; Adamo et al. 2012) showing that the mechanism of meiotic silencing of unpaired DNA does suppress male-specific lethality and is not consistent with the model of Ryan and Haag (2017).

Suppression of F1 male-specific lethality by co-suppression

I was able to suppress the male-specific lethality with two of the seven BAC clones injected in co-suppression assays(Dernburg et al. 2000; Adamo et al. 2012). The results of hybrid males from these crosses are consistent with the meiotic silencing model purposed by Ragavapuram et al. (2016). Based on the result of these two separate non-overlapping BACs producing hybrid males, I can deduce that there are at least two maternal-effect male-specific hybrid lethal genes; with a minimum of one hybrid lethal gene within each of these regions covered by the BACs on the X-chromosome. BAC: 17D03, yielded males with a frequency of 2.5%, and it has 5 candidate hybrid lethal genes. BAC: 08G05, yielded males with a frequency nearly the exact same as that of *cbr-him-8* at 13%, has 2 candidate hybrid lethal genes remaining. Synergistic and/ or additive interaction between the hybrid lethal genes in 17D03 and 08G05 are not expected, as lack of pairing of the entire X chromosome in *Cbr-him-8* mothers resulted in an identical male frequency to that obtained from 08G05-derived strains. This could be tested by co-injection of 17D03 and 08G05.

Function of remaining candidate male-specific hybrid lethal genes

Within the two BACs that rescued there are a total of 16 candidate hybrid lethal genes. Of these 16 candidate genes only 8 have a known function. After the completion of the co-suppression assay that was performed on smaller sub-groups of genes within

08G05, the total number of candidate hybrid lethal genes is now narrowed to 7 possible genes with only 3 of those having known functions. With BAC: 17D03 containing all three genes with known functions: (CBG00182) G-protein coupled receptor signaling, (*cbr-ajm-1*) component of apical cell junctions, and (CBG00192) TBP associated factor 11. Since BAC: 17D03 contains all of the genes with known functions, I can state that at least one of the candidate hybrid lethal genes does not have a described function. Furthermore based on the fact that 2 genes that have already been eliminated as candidates in BAC: 08G05 function in G-protein coupled receptor signaling, it is unlikely that the remaining gene that also functions in G-protein coupled receptor signaling has any effect on male-specific hybrid lethality. Based on this information at least one of the hybrid lethal genes is going to be of unknown phenotypic impact.

BAC: 08G05

Neither of the candidate genes in BAC: 08G05, *cbg30927* or *cbg00239,* have functional or phenotypic characterization. *Cbg30927* does have orthologues in *C. elegans* and this gene codes for a C-type lectin, however when comparing the transcripts of the two proteins the lectin fold, of the protein coded for by *cbg30927,* is missing therefore would not likely have the same function. In the gene *cbg00239* there are no orthologues and no information about the possible function or similar genes when a BLAST search was conducted.

Limitations of co-suppression

No single gene could be identified using co-suppression due to the dwindling complexity of the injection mixtures. As the injection mixtures became less and less

complex the animals retained the rescued phenotype for shorter and shorter periods of time. When injecting the BACs and the *cbr-unc-119* rescue plasmid, the resulting animals would retain the rescued phenotype for upwards of a month. By comparison, when injecting the subgroups of genes (Table 2) with the *cbr-unc-119* rescue plasmid, the resulting rescued animals would only retain the rescued phenotype for three to five generations before the injected animals could no longer be discerned from *cbr-unc-119* animals. Coupled with the extremely fast loss of the rescued phenotype, the rescued animals had few self-progeny and, when crossed, had even fewer hybrid progeny.

In summation: the resulting strains from these relatively simple injection mixtures had an extremely short phenotypic exhibition of the rescue and most also had very few cross progeny to be scored. This resulted in the decision of using a complete gene knockout to be done by a proceeding graduate student to test these final two genes. The disproportionate results of the number of offspring from the crosses of the gene subgroupings does also suggest the possibility of a hybrid vital gene residing in 08G05.

Based on how the crosses were structured *cbg30750, cbg00238, or cbr-trk-1* are all possible hybrid viable genes. *Cbg30750* has no orthologues and when a BLAST search was performed no similar genes or possible function were described. *Cbg00238* has a unique nucleotide sequence to *C. briggsae.* However, the resulting protein has regions that are highly conserved throughout the *Caenorhabditis* genus, though none of the proteins that hit in the blast search had a particularly low E value. Lastly c*br-trk-1* is a highly conserved protein coding gene throughout eukaryotes and is a protein tyrosine kinase, which is a key element in protein phosphorylation. The possibility of a single one of these genes or

combination of genes being necessary for hybrid viability is an area which has been opened for further experimentation based on this research.

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