Suppression of F1 Male-Specific Lethality in *Caenorhabditis* Hybrids by *cbr-him-8*

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Suppression of F1 male-specific lethality in *Caenorhabditis* hybrids by *cbr-him-8*.

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cbr-him-8 suppresses hybrid lethality

hybrid lethality, hybrid sterility, reproductive isolation, Haldane’s rule, Darwin’s corollary

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ABSTRACT

Haldane’s Rule and Darwin’s Corollary to Haldane’s Rule are the observations that heterogametic F1 hybrids frequently are less fit than their homogametic siblings and that asymmetric results often are obtained from reciprocal hybrid crosses. In Caenorhabditis, Haldane’s Rule and Darwin’s Corollary have been observed in several hybrid crosses, including crosses of Caenorhabditis briggsae and Caenorhabditis nigoni. Fertile F1 females are obtained from reciprocal crosses. However, F1 males obtained from C. nigoni mothers are sterile and F1 males obtained from C. briggsae die during embryogenesis. We have identified cbr-him-8 as a recessive maternal-effect suppressor of F1 hybrid male-specific lethality in this combination of species. This result implicates epigenetic meiotic silencing in the suppression of F1 male-specific lethality. It also is shown that F1 males bearing a C. briggsae X chromosome are fertile. When crossed to C. briggsae hermaphrodites or F1 females derived from C. briggsae hermaphrodites, viable F2 and backcross (B2) progeny were obtained. Sibling males that possessed a C. nigoni X chromosome were sterile. Therefore, the sterility of F1 males bearing a C. nigoni X chromosome must result from dysgenic interactions between the X chromosome of C. nigoni and the autosomes of C. briggsae. The fertility of F1 males bearing a C. briggsae X chromosome provides an opportunity to identify C. nigoni loci that prevent spermatogenesis, and hence hermaphroditic reproduction, in diplo-X hybrids.

INTRODUCTION
Reproductive isolation refers collectively to all genetic mechanisms that prevent or limit gene flow between populations (Mayr, 1963; Coyne and Orr, 2004). These mechanisms can be divided into two discrete categories, prezygotic mechanisms that prevent mating or fertilization and postzygotic mechanisms that decrease the fitness hybrid progeny. Most genetic models of reproductive isolation invoke dysgenic interactions among two or more loci (Dobzhansky, 1936; Muller, 1940; 1942; Wu, 2001; Lindtke and Buerkle, 2015). Within populations, interactions among these genes are maintained. Between populations, interactions among these genes are disrupted. Genes involved in reproductive isolation “are ordinary genes that have normal functions within species” (Orr et al., 2004).

Postzygotic mechanisms of reproductive isolation include hybrid sterility and hybrid lethality. Genes involved in hybrid sterility and inviability include a 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). While reproductive isolation may evolve through non-selective mechanisms (Mayr, 1963), there is evidence that many of these and other ‘speciation genes’ are or have been under positive selection (Johnson, 2010; Ting et al., 1998; Presgraves et al., 2003; Barbash et al., 2004; Tang and Pregraves, 2009; Aripé et al., 2010; Hart et al., 2014). Therefore, speciation can result from adaptive evolution of normal cellular processes.

Two patterns frequently observed in postzygotic reproductive isolation are Haldane’s rule and Darwin’s corollary to Haldane’s rule. Haldane’s rule is the observation that when gender-specific differences are observed in hybrid fitness, it generally is the homogametic gender that is more fit (Haldane, 1922; Laurie, 1997; Coyne and Orr, 2004). Darwin’s corollary to Haldane’s rule is the observation that
reciprocal hybrid crosses often produce different results (Turelli and Moyle, 2007). These patterns are of interest because of how they inform our understanding of speciation (Coyne and Orr, 2004).

The primary explanation for Haldane’s rule is the dominance model (Wu and Davis, 1993; Turelli and Orr, 2000). The dominance model posits that most hybrid incompatibility genes are recessive. F1 female hybrids that are heterozygous for an X-linked hybrid incompatibility gene are viable. F1 male hybrids that are hemizygous for that gene are inviable. Support for this model in regard to hybrid lethality is especially strong (Wu and Davis, 1993). The primary explanation for Darwin’s corollary is that F1 hybrids from reciprocal crosses have different mitochondria, different maternal contributions and F1 males have different X chromosomes (Turelli and Moyle, 2007).

In the nematode genus *Caenorhabditis*, many species pairs are isolated by hybrid sterility and/or by hybrid lethality (Baird et al., 1992; Baird and Yen, 2000; Woodruff et al., 2010; Kiontke et al., 2011; Kozlowska et al., 2011; Dey et al., 2012; Baird and Seibert, 2013; Félix et al., 2014; Dey et al., 2014). Among these is the combination of *C. briggsae* and *C. nigoni* (Woodruff et al., 2010; Kozlowska et al., 2011). From crosses of *C. briggsae* males to *C. nigoni* females, fertile F1 adult females and sterile F1 adult males were obtained. Fertile adult females also are obtained from the reciprocal cross but all male hybrids die during embryogenesis. Therefore, both Haldane’s rule and Darwin’s corollary to Haldane’s rule are observed in crosses between *C. briggsae* and *C. nigoni*.

In this manuscript, *cbr-him-8* is identified as a maternal-effect suppressor of F1 male-specific lethality in crosses of *C. nigoni* males to *C. briggsae* hermaphrodites. It also is demonstrated that F1 males derived from *cbr-him-8* mutant mothers that possess a *C. briggsae* X chromosome are fertile. Finally, it is shown that fertile adult progeny can be
obtained from crosses of these C. briggsae-X bearing F1 males to C. briggsae
hermaphrodites or to F1 females derived from C. briggsae mothers.

MATERIALS AND METHODS

Nematode strains and strain 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) was obtained from the Caenorhabditis Genetics Center. The C. briggsae AF16 derivatives RE980 [cbr-him-8(v188) I] (Wei et al., 2013) and RW20120 [stIs20120 (pmyo2::GFP) X] (Yan et al., 2012) were provided by Ron Ellis and Zhongying Zhao, respectively. PB192 [cbr-him-8(v188) I; stIs20120 X] was constructed from crosses of RE980 to RW20120. PB3500 was constructed from crosses of EG5268 males to AF16 hermaphrodites. Female progeny from this cross were backcrossed to EG5268 males for ten generations. Consistent with fixation of the C. nigoni nuclear genome, PB3500 had a female reproductive mode. Fixation of the C. briggsae AF16 mitotype and of the C. nigoni X chromosome in PB3500 was confirmed by amplification of species-specific products mitochondrial and X chromosomal DNA (Figure 1). Nematode strains were grown at 20 °C on lawns of E. coli strain DA837. All strains used in this study are available from the Caenorhabditis Genetics Center.

Crosses: Crosses always were of five males mated to three females or sperm-depleted hermaphrodites and were conducted on freshly seeded mating plates (plates seeded with an approximately 1 cm spot of E. coli). Hermaphrodites were sperm-depleted by daily transfers for four to five days to fresh plates until egg laying ceased.

Microscopy: Crosses and routine microscopy were conducted using stereomicroscopes at magnifications of 25 – 50x. Pharyngeal GFP fluorescence was scored using an M2Bio fluorescence microscope (Kramer Scientific). Analyses of
gonadal morphology were conducted using DIC optics at a magnification of 400x on a Zeiss Axiovert 35M microscope.

**Reagents and Data Availability:** All strains used in this study are available from the Caenorhabditis genetics center. Supplemental data on control crosses between C. nigoni EG5268 males and C. briggsae RW20120 hermaphrodites is available at figshare.com/articles/EG5268_x_RW20120_suppl_data_xlsx/2058864.

**RESULTS**

**F1 male-specific lethality is suppressed by cbr-him-8(v188):** Asymmetric results were observed in reciprocal crosses between the *Caenorhabditis* species *C. nigoni* and *C. briggsae* (Table 1; Figure 2 A&B). Despite considerable embryonic lethality, viable and fertile F1 hybrid females were obtained from both cross directions. From *C. nigoni* mothers, some viable but sterile F1 hybrid males were obtained. However, from *C. briggsae* mothers, all F1 hybrid males died during embryogenesis. These results were consistent with results reported by Woodruff et al. (2010) and Kozlowska et al. (2011). Woodruff et al. (2010) reported no viable males from 186 F1s scored. Kozlowska et al. (2011) reported only seven viable males from 3,705 F1s scored. Similarly, from 429 F1s scored in this study, no viable F1 males were observed (Table 1).

F1 males from reciprocal crosses in *Caenorhabditis* differ in the source of their maternally derived X chromosome, their maternally derived mitochondria and in maternal contributions to the oocyte prior to fertilization (Figure 2). These differences have been proposed as potential causes of asymmetric results in reciprocal crosses (Turelli and Moyle, 2007; Dey et al., 2014). To test for dysgenic mitonuclear interactions, *C. briggsae* males were mated to females from the PB3500 cybrid strain. This strain possessed a *C. nigoni* nuclear genome and *C. briggsae* mitochondria (Figure 1). Viable F1 males were obtained from this cross. Frequencies of F1 males obtained
from crosses of *C. briggsae* AF16 males to *C. nigoni* EG5268 and cybrid PB3500 mothers were identical (Table 1; Figure 2 C). As mitochondria are maternally inherited, males derived from PB3500 mothers would have possessed *C. briggsae* mitochondria. The viability of these F1 males is not consistent with dysgenic mitonuclear interactions as a cause of F1 male-specific lethality of F1 males derived from *C. briggsae* mothers. This result was consistent with those of Bundus et al. (2015), who found that *C. briggsae* mitochondria did not have an impact on postzygotic reproductive isolation in crosses between *C. briggsae* and *C. nigoni*.

To discriminate between maternal-zygotic and X-autosomal interactions, *C. nigoni* males were mated to sperm-depleted *C. briggsae cbr-him-8(v188)* I hermaphrodites. The *cbr-him-8(v188)* mutation results in high rates of X chromosome nondisjunction and hence in high frequencies of XO males among self-progeny of mutant hermaphrodites (Wei et al., 2013). It was thought that this cross would produce exceptional males with a paternal *C. nigoni* X chromosome (*X^Cni^*) through the fertilization of nullo-X oocytes by X-bearing sperm. Viability of these males would eliminate *C. briggsae* maternal-zygotic interactions as the cause of asymmetric F1 male-specific lethality. Viable F1 males were obtained from *C. briggsae cbr-him-8(v188)* mutant mothers (Table 1; Figure 2 D). However, only 40% of these were the expected exceptional *X^Cni^* males (Table 1). The rest of the viable F1 males possessed a maternally derived *C. briggsae* X (*X^Cbr^*) chromosome. This was determined from crosses of *C. nigoni* males to hermaphrodites from the PB192 strain of *C. briggsae*. PB192 is an AF16 derivative that was mutant for *cbr-him-8(v188)* and that also included an X-linked insertion, *stls20120*, of a *cbr-my02p::GFP* transgene. Expression from *stls20120* results in pharyngeal GFP fluorescence (Yan et al., 2012). Frequencies of F1 males obtained from crosses that included or did not include *stls20120* were identical (Table 1). As PB192 is
an AF16 derivative, the only difference between the viable X<sup>Chr</sup> F1 males derived from PB192 mothers and the inviable X<sup>Chr</sup> F1 males derived from wild-type AF16 C. briggsae mothers was the presence of cbr-him-8(v188) and stIs20120. In control crosses, stIs20120 was shown to have no affect on F1 male viability (not shown). For viable X<sup>Chr</sup> F1 males, cbr-him-8 was homozygous in the maternal genome and heterozygous in the zygotic genome. Hence, cbr-him-8(v188) was identified as a suppressor of the lethality of F1 X<sup>Chr</sup> males.

**Suppression of hybrid by cbr-him-8(v188) is a maternal effect:** In C. elegans, mutations in him-8 exhibit two distinct and separable phenotypes. Homozygosity of him-8 results in high rates X chromosome nondisjunction (Hodgkin et al., 1979). This is caused by defects in X chromosome pairing during meiosis (Phillips et al., 2005). In somatic cells, him-8 mutations are dominant suppressors of missense mutations in transcription factor binding domains (Nelms and Hanna-Rose, 2006; Sun et al., 2007). If C. briggsae cbr-him-8(v188) exhibits both of these phenotypes, then suppression of F1 male-specific lethality could be the result of maternal homozygosity or zygotic heterozygosity.

To distinguish between maternal and zygotic modes of suppression, C. nigoni males were crossed with cbr-him-8/+ C. briggsae heterozygotes. The X chromosome nondisjunction phenotype of cbr-him-8(v188) is recessive. If suppression results from X chromosome pairing defects during meiosis, then few if any F1 males would be expected from cbr-him-8 heterozygous mothers. Conversely, half of F1 male progeny from heterozygous mothers would inherit the mutant allele of cbr-him-8. These males would be genetically identical to F1 males derived from cbr-him-8 homozygotes. If suppression results from somatic suppression of transcription factor binding defects, then the abundance of viable F1 X<sup>Chr</sup> males derived from heterozygous mothers would
be expected to be half of that observed from *cbr-him-8* homozygotes. From crosses of *C. nigoni* males to *C. briggsae cbr-him-8/+* hermaphrodites, a single F1 male was observed among 354 viable F1 progeny scored (Table 2; Figure 2 E). This result excludes zygotic suppression but is consistent with maternal pairing defects as the cause of suppression of $X^{Cbr}$ F1 male-specific lethality.

**F1 $X^{Cbr}$ males are fertile:** F1 $X^{Cbr}$ males derived from crosses of *C. nigoni* males to *C. briggsae cbr-him-8* mutant hermaphrodites had well-developed gonads and were fertile (Figure 3; Table 3). When F1 $X^{Cbr}$ males were crossed to *C. nigoni* females, fertilized embryos were observed. All of these embryos arrested prior to hatching.

When F1 $X^{Cbr}$ males were mated to *C. briggsae* hermaphrodites, viable F2 adult progeny were obtained. When F1 $X^{Cbr}$ males were crossed to F1 females, the result varied depending upon the source of F1 females. When crossed to F1 females derived from *C. nigoni* mothers (F1Cni females), only arrested embryos were observed. When crossed to F1 females derived from *C. briggsae* mothers (F1Cbr females), viable F2 adults were obtained approximately a third of the time. Further crosses will be required to determine if these differences are significant.

Adult male, female and hermaphrodite progeny were obtained from crosses of F1 $X^{Cbr}$ males to *C. briggsae* hermaphrodites and F1Cbr females (Table 3). However, the frequencies of these different progeny types were not consistent with expectations. Among cross progeny, haplo-X males were expected at a frequency of 0.50. From crosses to *C. briggsae* hermaphrodites, observed frequency of males, 0.20, was significantly lower than this expectation ($p << 0.0001$). From crosses to F1Cbr females, F2 males were sometimes, but not always, abundant. From both crosses, nearly all diplo-X progeny were self-fertile. Self-sterile (female) and self-fertile (hermaphrodite) diplo-X progeny both were expected from these crosses. However, Woodruff et al. (2010)
demonstrated that self-sterility (female reproductive mode) was dominant and they observed very low frequencies (< 3%) of self-fertility among progeny of 2nd or 3rd generation hybrid males crossed to *C. briggsae* hermaphrodites. The high rates of self-fertility, ≥ 0.98, observed among diplo-X progeny was not consistent with this observation.

**F1 X\textsuperscript{Cni} males are sterile regardless of cross direction:** F1 X\textsuperscript{Cni} males derived from *C. nigoni* mothers have gonad defects and are sterile (Woodruff et al., 2010). In general, these F1 males were defective in gonadal outgrowth (Table 4). Gonad outgrowth in *C. nigoni* and *C. briggsae* is nearly identical to gonad outgrowth in *C. elegans*. Gonad outgrowth in *C. elegans* is regulated by the migration of the linker cell (Kimble and Hirsh, 1979; Kato and Sternberg, 2009). The linker cell initially migrates anteriorly along the ventral body wall until the L2 larval molt. It then migrates to the dorsal body wall where it turns and migrates posteriorly during the L3 and L4 larval stages. The result of these migrations is a thin tubular gonad with an anterior reflex (180° bend) near the posterior bulb of the pharynx. In some F1 X\textsuperscript{Cni} males, there is an apparent complete failure in gonad outgrowth. These males possess gonads that differ little from the gonad primordium present in L1 larvae at hatching. In other F1 X\textsuperscript{Cni} males, there is an apparent failure in the dorsal turn of the linker cell at the L2 molt. These males possess swollen, ovoid gonads that lack an anterior reflex (Table 4).

F1 X\textsuperscript{Cni} males obtained from *C. briggsae cbr-him-8(v188)* mutant mothers had the same gonadal outgrowth defects as those observed in F1 X\textsuperscript{Cni} males derived from *C. nigoni* mothers (Figure 3; Table 4). The only genetic difference between these males and their F1 X\textsuperscript{Cbr} male siblings, which had well-developed functional gonads, was the X chromosome. Based on these results, the gonadal outgrowth defects observed in F1
X_Cni^1^ males must result from the presence of a hybrid sterile gene on the X chromosome of C. nigoni.

DISCUSSION

In crosses between C. nigoni males and C. briggsae hermaphrodites, almost all F1 male hybrids die during embryogenesis. This F1 hybrid male-specific lethality was suppressed by the cbr-him-8(v188) mutation. This result was unexpected. There is evidence that F1 male-specific lethality results from dysgenic interactions between a C. briggsae X-linked locus and C. nigoni autosomal loci (Bi et al., 2015). cbr-him-8 does not correspond to this X-linked gene as it is located on chromosome I (www.wormbase.org). Rather, cbr-him-8 must be acting as a suppressor of this hybrid lethality gene.

In C. elegans, mutations in him-8 are pleiotropic. The HIM-8 protein binds to the pairing centers of the X chromosomes and is required for the meiotic pairing of X chromosomes (Phillips et al., 2005). Consequences of disrupted meiotic pairing include X-specific nondisjunction and an expansion of recombination distances on the X chromosome (Hodgkin et al., 1979; Broverman and Meneely, 1994). The X-specific nondisjunction phenotype of cbr-him-8(v188) and the conservation of HIM-8 proteins in these species indicate that the role of HIM-8 in X chromosome pairing is conserved in C. briggsae (Phillips and Dernberg, 2006; Wei et al., 2013). C. elegans him-8 mutations also act as dominant suppressors of missense mutations in the DNA-binding domains of transcription factors (Nelms and Hanna-Rose; 2006; Sun et al., 2007). Conservation of this phenotype in C. briggsae has not been tested.

The suppression of F1 male-specific lethality by cbr-him-8 likely results from defects in X chromosome meiotic pairing during oogenesis in C. briggsae. This was evident from crosses of C. nigoni males to C. briggsae cbr-him-8/+ hermaphrodites. The
X-nondisjunction phenotype, and hence the pairing defects, of cbr-him-8 are recessive.

However, half the F1 hybrids derived from cbr-him-8/+ heterozygous mothers would also have been heterozygous for cbr-him-8. Thus, the absence of viable F1 male progeny from cbr-him-8/+ mothers demonstrates that zygotic heterozygosity of cbr-him-8(v188) is not sufficient to suppress male-specific lethality in F1 hybrids.

The suppression of F1 male-specific lethality by cbr-him-8 may result from meiotic silencing of the C. briggsae X chromosomes during oogenesis or from epigenetic suppression of X-linked gene expression during embryogenesis. In C. elegans, unpaired chromosomes are dimethylated on lysine 9 of histone H3 (H3K9me2) during meiosis (Bean et al., 2004; Bessler et al., 2010). H3K9me2 is a highly conserved epigenetic mark that is associated with transcriptional repression and meiotic silencing (Turner, 2007; Kelly and Aramayo, 2007; Kota and Feil, 2010; Maine, 2010; Mozzetta et al., 2015). Acquisition of H3K9me2 on unpaired X chromosomes in C. elegans her-1 XO hermaphrodites is associated with meiotic repression of transcription of X-linked genes (Bean et al., 2004). However, the repressive epigenetic imprint acquired by the X chromosome during spermatogenesis also can persist through the 14-cell stage of embryogenesis (Kelly et al., 2002; Bean et al., 2004). It should be possible to test for suppression by meiotic silencing by generating a mutation in cbr-met-2. In C. elegans, met-2 is required for dimethylation of H3K9 (Bessler et al., 2010). If suppression of F1 male-specific lethality results from H3K9me2 of X chromosomes in cbr-him-8 mutant hermaphrodites, then XCbr F1 males derived from cbr-him-8; cbr-met-2 doubly mutant hermaphrodites should die during embryogenesis.

Our results also demonstrated that the sterility of XCni F1 males was caused by dysgenic interactions between the X chromosome of C. nigoni and the autosomes of C. briggsae. From C. briggsae cbr-him-8 mothers, both XCni and XCbr F1 males were obtained.
The $X^{Cbr}$ F1 males had well-developed gonads and were fertile whereas their $X^{Cni}$ siblings had defects in gonad development and were sterile. The $X^{Cbr}$ and $X^{Cni}$ males obtained from these crosses shared the same maternal and mitochondrial genotypes. They differed only in the identity of their X chromosomes. Moreover, unpaired X chromosomes in male spermatogenesis (i.e. $X^{Cni}$) were expected to share similar epigenetic modifications as unpaired X chromosomes in hermaphrodite oogenesis in $cbr$-$him$-8 mutant mothers (Bean et al., 2004). Thus, the cryptic asymmetry observed in F1 male fertility likely results from divergence of one or more loci on the $C. briggsae$ and $C. nigoni$ X chromosomes.

Finally, the fertility of F1 $X^{Cbr}$ males provides an opportunity to define the genetic requirements for hermaphroditic reproduction in $C. briggsae$. Woodruff et al. (2010) demonstrated that the hermaphroditic mode of reproduction was recessive to the female mode in diplo-X hybrids. We found females to be rare among diplo-X backcross progeny of $X^{Cbr}$ males mated to $C. briggsae$ hermaphrodites. Genotyping of these rare backcross females should allow for the identification of $C. nigoni$ loci that suppress spermatogenesis in female hybrids.

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Table 1. Frequency of F1 males derived from *C. briggsae* mothers.

| cross                                                      | ♀♀ | ♂♂ | ♀♂ | ♂♂ fraction X_{Cbr} (N) |
|------------------------------------------------------------|-----|-----|-----|------------------------|
| *C. briggsae* AF16 ♀♂ x *C. nigoni* EG5268 ♀♀<sup>a</sup>   | 293 | 32  | 0.098<sup>e</sup> |
| *C. nigoni* EG5268 ♀♂ x *C. briggsae* AF16 ♀♀<sup>b</sup>   | 429 | 0   | 0.000 |
| *C. briggsae* AF16 ♀♂ x PB3500 cybrid ♀♀<sup>c</sup>       | 383 | 39  | 0.092<sup>e</sup> |
| *C. nigoni* EG5268 ♀♂ x *C. briggsae* RE980 ♀♀<sup>d</sup>  | 330 | 68  | 0.171 |
| *C. nigoni* EG5268 ♀♂ x *C. briggsae* PB192 ♀♀<sup>d</sup>  | 634 | 142 | 0.183 |
| *C. nigoni* EG5268 ♀♂ x *C. briggsae cbr-him-8*(v188)* ♀♀<sup>d,f</sup> | 964 | 210 | 0.179 |

EG5268 = *C. nigoni* wild-isolate  
AF16 = *C. briggsae* wild-isolate  
PB3500 = EG5268 nuclear genome and AF16 mitochondria.  
RE980 = *C. briggsae cbr-him-8*(v188) I  
PB192 = *C. briggsae cbr-him-8*(v188) I; stIs20120 [pmyo2::GFP] X  
RE980 and PB192 both are AF16 derivatives  
<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup> These crosses are diagrammed in Figure 2 panels A, B, C and D, respectively.  
<sup>e</sup> ♂♂ frequencies not significantly different, p = 0.677 chi squared test, expected frequency = 0.098.  
<sup>f</sup> sum of results from crosses using RE980 and PB192 ♀♀  
<sup>g</sup> pharyngeal expression of GFP observed in 79 of 131 F1 males scored
Table 2. Tests of zygotic and maternal suppression hypotheses.

| observed                                      | females | males | p value   |
|------------------------------------------------|---------|-------|-----------|
| $C.\ nigoni \times C.\ briggsae\ cbr$-$him$-$8+$ | 353     | 1     |           |
| expected                                      |         |       |           |
| zygotic suppression                          | 331.3   | 22.7  | $2.567 \times 10^{-6}$ |
| maternal suppression                         | 353.3   | 0.7   | 0.685     |

*a* $C.\ nigoni\ EG5268\ \varnothing\ \times\ C.\ briggsae\ cbr$-$him$-$8$(v188)$/+ I; stIs20120 [p-myo2::GFP] X, or $C.\ nigoni\ EG5268\ \varnothing\ \times\ C.\ briggsae\ cbr$-$him$-$8$(v188)$/+ I; stIs20120 [p-myo2::GFP]/+ X

*b* An expected male frequency of 6.4% was based on the expected 50% transmission rate of $cbr$-$him$-$8$(v188)$ from maternal heterozygotes and on the 12.8% frequency of viable adult X$Cbr$ males from $cbr$-$him$-$8$(v188)$ homozygous mothers.

*c* An expected male frequency of 0.19% was based on the frequency of viable males obtained from crosses of $C.\ nigoni\$ males to wild-type $C.\ briggsae$ hermaphrodites (Kozlowska et al., 2011).

*d* p values from chi squared tests using the expected male frequencies for the zygotic and maternal suppression hypotheses described above.

*e* This cross is diagrammed in Figure 2, panel E.
| cross | result | self-fertile F2 female fraction N<sup>c</sup> | F2 male fraction (N) |
|-------|--------|---------------------------------------------|----------------------|
| F1 X<sup>Cbr</sup>♂ x C. nigoni ♀ | dead embryos (5) no progeny (3) | | |
| F1 X<sup>Cbr</sup>♂ x C. briggsae ♀ | viable adults (16) 0.98 (48) 0.20 (869) | | |
| F1 X<sup>Cbr</sup>♂ x F1<sup>Cni</sup>♀ | dead embryos (2) | | |
| F1 X<sup>Cbr</sup>♂ x F1<sup>Cbr</sup>♀<sup>d</sup> | dead embryos (6) viable adults (3) nd ~ 0.50<sup>e</sup> viable adults (1) 1.00 (30) 0.005 (208) no progeny (1) |

<sup>a</sup> F1 X<sup>Cbr</sup>♂ = GFP<sup>+</sup> males derived from PB192 mothers, F1 ♀<sup>Cni</sup> = F1 females derived from C. nigoni mothers. F1 ♀<sup>Cbr</sup> = F1 females derived from C. briggsae mothers.

<sup>b</sup> Number of crosses for each given result indicated in parentheses.

<sup>c</sup> Fraction of anatomically female (i.e. XX) F2s that laid eggs. Number scored indicated in parentheses.

<sup>d</sup> Includes results of full sib crosses as well as results of F1 X<sup>Cbr</sup> males from PB192 mothers crossed to F1 females from AF16 mothers.

<sup>e</sup> F2 males abundant but not counted. It is not clear why males were abundant in some crosses but not in others.
Table 4. Gonadal phenotypes of F1 $X^{Cni}$ males.

| cross | no outgrowth$^a$ | defective outgrowth$^b$ | N |
|-------|------------------|------------------------|---|
| $C. briggsae$ AF16 ♂♂ x $C. nigoni$ EG5268 ♀♀ | 9 | 10 | 19 |
| $C. briggsae$ PB192 ♂♂ x $C. nigoni$ EG5268 ♀♀ | 6 | 15 | 21$^c$ |
| $C. nigoni$ EG5268 ♂♂ x $C. briggsae$ PB192 ♀♀ | 5 | 2 | 7$^c$ |

$^a$ Small ventral ovoidal masses of gonadal tissue, or degenerate vacuoles, located at midbody.

$^b$ Larger masses of gonadal tissue extending anteriorly toward the pharynx but lacking the anterior reflex. Differentiated and/or tumorous cells often observed.

$^c$ Distributions of gonadal phenotypes in $X^{Cni}$ males derived from PB192 ♂♂ x EG5268 ♀♀ and EG5268 ♂♂ x PB192 ♀♀ do not differ significantly from the distribution of phenotypes derived from AF16 ♂♂ x EG5268 ♀♀, p values 0.084 and 0.20, respectively.
Figure 1. Confirmation of PB3500 cybrid genotypes. A) Mitochondrial amplification products. Primers: cbr-nad-5 - AGCCAAACTCTAACCACCT and cbr-nad-3 - TTCTTGGGGATTTTAGTTTCTGA. A 506 bp amplification product was expected from *C. briggsae* AF16 mitochondria. No product expected from *C. nigoni* EG5268 mitochondria. B) Amplification products from the X-linked cbr-vab-3 and cni-vab-3 orthologs. Amplification products of 334 and 297 bp were expected from *C. briggsae* AF16 and *C. nigoni* EG5268, respectively. Primers: exon 4 - TGCACCTCGGCTACTGTAA and exon 6 - TGTACAACGGGCTCAGTCAG.

Figure 2. Chromosome and mitochondrial segregation and maternal contributions in *C. briggsae x C. nigoni* hybrid crosses. In all panels, *C. briggsae* and *C. nigoni* genotypes are indicated in red and blue, respectively. In F1 hybrids, maternal chromosomes are shown above paternal chromosomes. In panels D and E, the v188 mutant allele of cbr-him-8 is indicated by a closed circle on chromosome I. In panel E, an open circle on chromosome I indicates that half of F1 hybrids were expected to be heterozygous for cbr-him-8(v188). Diagrammed are crosses between A) *C. nigoni* females and *C. briggsae* males, B) sperm-depleted *C. briggsae* hermaphrodites and *C. nigoni* males, C) PB3500 cybrid females and *C. briggsae* males, D) sperm-depleted *C. briggsae* cbr-him-8 mutant hermaphrodites and *C. nigoni* males and E) sperm-depleted *C. briggsae* cbr-him-8/+ heterozygous hermaphrodites and *C. nigoni* males.

Figure 3. Gonad morphology in F1 male hybrids. A) *C. nigoni* EG5268, B) F1 X<sup>Cni</sup> and C) F1 X<sup>Cbr</sup> males. Contrast of gonads enhanced in all panels. Boxes correspond to regions enlarged in insets. In panels A and C, the distal arm is outlined with a dashed line in the large insets to emphasize the tubular structure of the gonad. This tubular structure is absent in the F1 X<sup>Cni</sup> male shown in panel C. Anterior reflex (ar), distal tip (dt), sperm (sp) and tumorous cells (tu) indicated in insets. The C. F1 X<sup>Cni</sup> male was an
‘exceptional’ GFP− male obtained from crosses on C. nigoni EG5268 males to C. briggsae PB192 [cbr-him-8(v188) I; stIs20120 (pmyo2::GFP) X] hermaphrodites. The F1 Xcbr male was a GFP+ male obtained from the same cross.
