RESEARCH PAPER

Worm 1:4, 189–195; October/November/December 2012; © 2012 Landes Bioscience

Reproductive isolation in *Caenorhabditis briggsae*

Dysgenic interactions between maternal- and zygotic-effect loci result in a delayed development phenotype

Scott Everet Baird* and Rachael Stonesifer†

Department of Biological Sciences; Wright State University; Dayton, OH USA

†Current affiliation: Microbial Methods Development Branch; HFS0711; Division of Microbiology; Office of Regulatory Science; Center for Food Safety and Applied Nutrition; US Food and Drug Administration; College Park, MD USA

Keywords: reproductive isolation, maternal-effect, *Caenorhabidtis briggsae*, allopatric, speciation

Abbreviations: BDM, Bateson-Dhobzhansky-Muller; VRI, variable reproductive isolation

In sexual species, speciation occurs through the accumulation of genetic barriers to gene flow. In *Caenorhabditis briggsae*, one such barrier impedes gene flow between temperate strains and the tropical AF16 strain. Up to 20% of F2 progeny derived from crosses of AF16 to strains from the temperate clade exhibit a delayed development phenotype. This phenotype, which results from dysgenic interactions between maternal- and zygyotic-effect loci, causes a ~21% decrease in the intrinsic growth rate. The maternal-effect requires contributions from both parental genotypes. The dysgenic maternal-effect allele appears to be fixed in the temperate clade of *C. briggsae* and appears to have arisen between 700 and 15,000 years ago. The dysgenic zygotic allele appears to be present only in AF16 and also may be of recent origin.

Introduction

Reproductive isolation refers collectively to all genetic barriers that prevent or limit gene flow between populations.[1-3] In sexual species, the advent of reproductive isolation is considered to be coincident with speciation. Early theoretical studies implicated dysgenic interactions among two or more genes within an adaptive gene complex as the genetic basis to gene flow restriction.[2,4-6] While several models have been advanced that allow for single-gene speciation, the multigene Bateson-Dhobzansky-Muller (BDM) model still is considered the predominant model of speciation.[3,7]

Experimental support for the BDM Model has come from crosses between partially isolated species. These studies, mostly in Drosophila, have shown that multiple loci contribute to hybrid sterility and lethality.[4,8,9] However, the molecular identities of relatively few hybrid-incompatability genes have been determined.[10-16] Even rarer are molecular characterizations of pairs of interacting genes.[17] In part, this is because the very nature of reproductive isolation precludes exhaustive genetic analyses in most species pairs. Indeed, genetic studies of reproductive isolation are possible only in species pairs in which reproductive isolation in incomplete. This severely limits the type of studies that can be done in model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans*.

Studies of reproductive isolation between well-established species also suffer from an inability to distinguish between hybrid incompatibilities that arose coincident with and those that arose subsequent to speciation. Thus, in these studies, the genetic basis of speciation is blurred by continued divergence.[18] This problem can be addressed through genetic studies of incipient speciation. In *Drosophila pseudoobscura* subspecies, as few as five loci may account for sterility in F1 male hybrids.[9] In *C. elegans*, gene flow within a 33 kb region of chromosome I is restricted by dysgenic interactions between two loci.[14,15] These results indicate that speciation may occur through dysgenic interactions among small numbers of loci.

Generalization of these results from will require characterizations of incipient speciation in multiple taxa. One promising species for such studies is *Caenorhabditis briggsae*. *C. briggsae* is a cosmopolitan species that, like *C. elegans*, is a protandrous hermaphrodite.[19] Unlike *C. elegans*, *C. briggsae* is geographically structured with at least five distinct populations.[20,21] Moreover, some F2 progeny derived from crosses between the AF16 and HK104 strains of *C. briggsae* are subject to hybrid breakdown. These F2 hybrids exhibit a delayed-development phenotype that is associated with homozygosity of AF16 alleles on chromosome III.[22] The delayed development phenotype is sufficient to distort marker transmission ratio in recombinant inbred lines.[22,23] This paper provides a more detailed characterization of the delayed-development phenotype, provides evidence for the involvement

*Correspondence to: Scott Everet Baird; Email: scott.baird@wright.edu
Submitted: 11/21/12; Revised: 12/21/12; Accepted: 01/03/13
http://dx.doi.org/10.4161/worm.23535

Table 1. Frequencies of delayed F2 progeny

Clade	Strain	AF16	HK104
Tropical	AF16	0.003 (304)	0.205 (396)[b]
	AF16	0.009 (106)[d]	0.137 (153)[b,c]
	VT847	0.032 (284)	0.012 (260)
	PS1185	0.000 (107)	0.051 (78)
	PS1186	0.000 (62)	0.000 (123)
Temperate	HK104	0.189 (573)[a]	0.020 (202)
	HK104	0.211 (123)[a,c]	0.000 (93)[d]
	HK105	0.284 (31)	0.006 (173)
	PB800	0.234 (188)	0.007 (143)
	JU279	0.135 (170)	0.018 (55)
	JU383	0.103 (185)	0.030 (66)
	JU439	0.091 (55)	—
	JU441	0.156 (109)	—
	PB826	0.146 (48)	0.000 (58)
	PB859	0.217 (212)	0.005 (181)

Unless otherwise indicated, data are for self-progeny, cross direction is not specified and data may be combined from reciprocal crosses. [a]F2s derived from AF16 males mated to HK104 hermaphrodites. [b]F2s derived from HK104 males mated to AF16 hermaphrodites. [c]F2 cross progeny derived from F1 males mated to F1 females. [d]Progeny of parental males mated to parental hermaphrodites.

of dysgenic heteroallelic interactions involving one or more maternal-effect genes and demonstrates that the genetic architecture responsible for this phenotype is of recent origin.

Results

Approximately 20% of F2 self-progeny obtained from F1 hermaphrodites derived from crosses between *C. briggsae* strains AF16 and HK104 exhibit a delayed development phenotype (**Table 1**). At 48 h, greater than 95% of animals scored as delayed were L2 larvae and less than 5% were L3 larvae. In contrast, their non-delayed siblings all had reached the L4 larval stage. In control crosses, nearly all AF16 and HK104 animals were L4s by 48 h (**Table 1**). Cross direction had little or no impact as similar results were obtained regardless of the P0 maternal strain (**Table 1**). Qualitatively similar results were obtained for F2 progeny derived from crosses of F1 males to F1 hermaphrodites (**Table 1**). However, only 13.7% of F2 cross progeny derived from P0 crosses of HK104 males to AF16 hermaphrodites were delayed. This cross was not repeated so it is not clear if this result was anomalously low or if it represents a consistent difference from the results obtained from the reciprocal cross and for F2 self-progeny.

Delayed F2s took approximately 15 h longer to reach reproductive maturity than their non-delayed siblings and the AF16 and HK104 parental controls (**Fig. 1**). This delay in the onset of reproduction resulted in significant decreases in intrinsic growth rates in delayed F2s (**Table 2**). The delayed-development phenotype is associated with homozygosity of AF16 alleles on chromosome III and one consequence of the decreased intrinsic growth rate in these animals was the under-representation of chromosome III-linked AF16 alleles in three independent sets of recombinant inbred lines derived from crosses of AF16 to HK104.[22,23] This distortion of allele segregation ratios on chromosome III demonstrates that selection against delayed F2s is sufficiently deleterious to have an impact on population structure.

Homozygosity of AF16 alleles on chromosome III is not sufficient to explain the delayed-development phenotype. This is evident from the reproductive schedule of the AF16 parental strain, which is not delayed (**Fig. 1**). Rather, AF16 alleles on chromosome III must be dysgenic in combination with HK104 alleles at one or more additional loci. Several attempts to identify zygotic loci with dysgenic HK104 alleles were unsuccessful. First, several loci throughout the genome were genotyped in pools of delayed F2 hybrids. None of these loci were skewed toward HK104 homozygosity (data not shown). Second, in recombinant inbred lines derived from crosses of AF16 to HK104, no chromosomal regions were observed in which HK104 alleles were under-represented.[22,23] Attempts also were made to construct fixed strains in which all progeny were delayed. These attempts failed as several generations of selection for delayed animals still resulted in the segregation of delayed and nondelayed progeny from individual hermaphrodites (see below).

The delayed-development involved a maternal-effect gene. This was determined from crosses of F1 hybrids to the AF16 parental strain. Delayed progeny were infrequent when F1 males were mated to AF16 hermaphrodites but frequent when F1 hermaphrodites were mated to AF16 males (**Table 3**), i.e., delayed progeny were obtained from heterozygous mothers that possessed HK104 alleles but not from homozygous AF16 mothers. These results also demonstrated that zygotic homozygosity of HK104 alleles was not required for the occurrence of the delayed development phenotype.

The delayed development phenotype may involve dysgenic heteroallelic interactions at the maternal-effect gene. This conclusion is based on attempts to establish a fixed strain of delayed animals. In these attempts, multiple lines were initiated, each from a single delayed F2 hermaphrodite. Delayed F2 hermaphrodites all should have been homozygous for the AF16 allele at the dysgenic zygotic locus on chromosome III.[22] These lines then were propagated, for several (> 10) generations through a single delayed hermaphrodite per generation in a attempt to fix HK104 alleles at the dysgenic maternal-effect locus. For each line, ten replicate sub-lines were established each generation. Each sub-line was scored for the presence of delayed progeny in the next generation. Delayed progeny from one sub-line then were picked to initiate the next generation. If homozygosity of HK104 alleles at a single maternal-effect locus was sufficient to cause a delayed development phenotype, then one quarter of the sub-lines in the F3 generation should have been fixed for these alleles. Delayed progeny should have been present in all F4 sub-lines from such HK104 homozygotes. However, after ten generations of selection, no strains were established in which delayed progeny were always present. One explanation for this result is that heterozygosity was required in maternal-effect genotype in order to generate delayed progeny in the next generation. In this case,

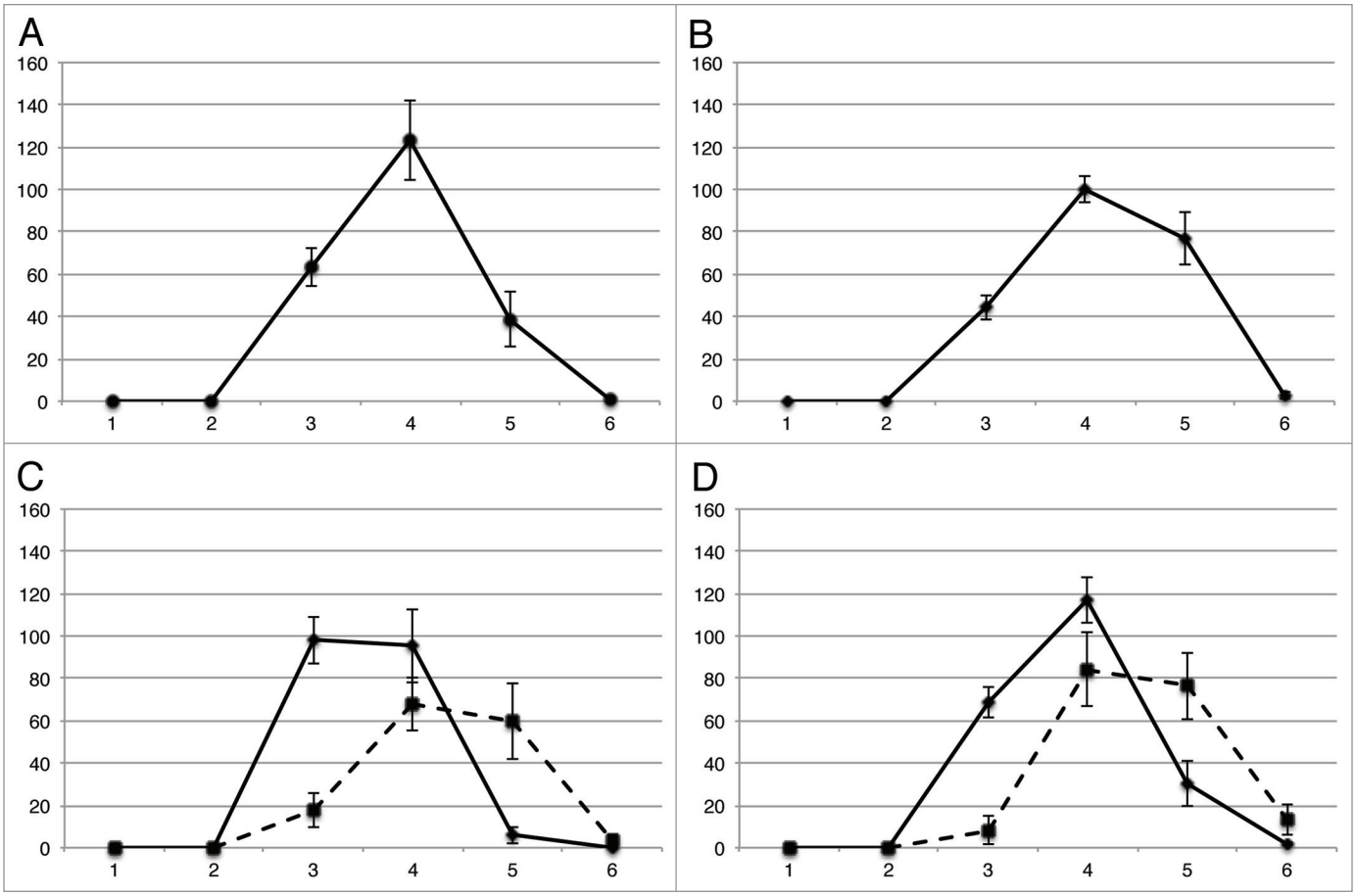

Figure 1. Reproductive schedules of F2 hybrids. Average daily counts at 20°C of progeny for (**A**) AF16 (n = 17) and (**B**) HK104 (n = 15) parental controls and for F2 hybrids derived from crosses of (**C**) AF16 males mated to HK104 hermaphrodites and (**D**) HK104 males mated to AF16 hermaphrodites. In **C** and **D**, the dotted lines represent the reproductive schedules of F2 hybrids that were scored as delayed at 48 h (**C**, n = 12; **D**, n = 11) and the solid lines represent the reproductive schedules of their nondelayed siblings (**C**, n = 27; **D**, n = 22).

heterozygous mothers would continually segregate homozygous progeny, from which no delayed progeny would be obtained. Maternal heterozygosity may have been required because of a dysgenic heteroallelic interaction or because HK104 and AF16 alleles were required at two closely linked loci.

The allelic variants responsible for the delayed development phenotype appear to be of recent origin. This was determined from pairwise crosses between AF16, HK104 and several additional strains of *C. briggsae* and from inferences based on the phylogenetic relationships of these strains (**Fig. 2**). AF16 and HK104 are members of the tropical and temperate clades of *C. briggsae*, respectively.[20,21] AF16 and HK104 were crossed to three additional strains from the tropical clade and eight additional strains from the temperate clade. Delayed F2s were frequent whenever AF16 was crossed to any of the temperate strains, but not when AF16 was crossed to any of the tropical strains (**Table 1**).

The apparent fixation of the dysgenic maternal-effect allele in the temperate clade indicates this allele arose after the divergence of the temperate clade from the tropical clade but before divergence within the temperate clade. A coalescent times of ~8.92 × 10^5 generations has been estimated for the divergence of the tropical clade from the temperate clade.[21] A coalescent time of

Table 2. Intrinsic growth rates of *C. briggsae* parental controls and F2 hybrids[a]

Cross				
Hermaphrodite		**Male**		r_{max}
AF16	x	AF16		1.53 ± 0.03 (17)
HK104	x	HK104		1.38 ± 0.09 (15)
HK104	x	AF16	all	1.44 ± 0.06 (33)
			delayed	1.24 ± 0.07 (11)
			nondelayed	1.53 ± 0.06 (22)
AF16	x	HK104	all	1.37 ± 0.07 (39)
			delayed	1.17 ± 0.07 (12)
			nondelayed	1.51 ± 0.03 (27)

[a]Intrinsic growth rates for individual hermaphrodites were determined from reproductive schedules as described in Vassilieva and Lynch.[36] Values reported are the means ± 2 sem. Sample sizes indicated in parentheses.

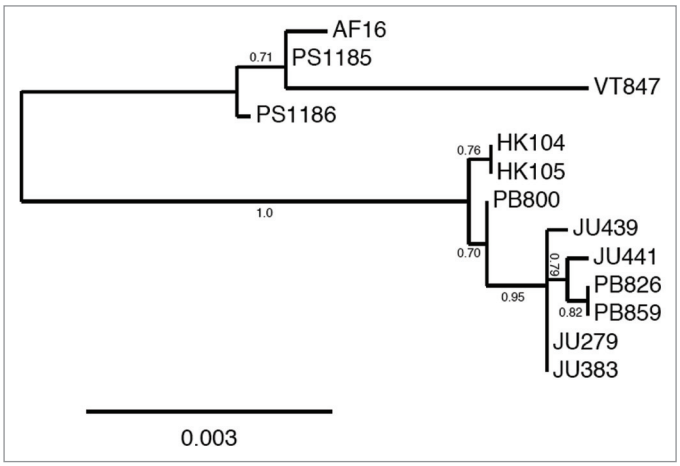

Figure 2. Phylogenetic relationships of strains used in this study. Maximum Likelihood phylogeny based on a 4,002 nucleotide set of concatenated intron sequences from *Cbr-glp-1*, *Cbr-tra-3*, *Cbr-tra-2*, *Cbr-daf-3*, *Cbr-mes-2*, *Cbr-mab-20*, *Cbr-her-1* and *Cbr-ram-5* (accession #s KC404662-KC404668, KC404670, KC404672-KC404675, KC404678-KC404685, KC404687, KC404689-KC404692, KC404695-KC404702, KC404704, KC404706-KC404709, KC404712-KC404719, KC404721, KC404723-KC404726, KC-404729-KC404736, KC404738, KC404740-KC404743, KC404746-KC404753, KC404755, KC404757-KC404760, KC404763-KC404770, KC404772, KC404774-KC404777, KC404780-KC404787, KC404789, KC404791-KC404794 and KC404797). Approximate likelihood ratio (aLRT) support values for each individual branch are indicated. The length of the VT847 branch is primarily due to variation in *Cbr-mes-2*. When *Cbr-mes-2* was excluded from the concatenated data set, the length of this branch decreased from 0.00373 to 0.00060 (data not shown).

Table 3. Segregation of the delayed development phenotype in backcrosses of F1 hybrids to parental strains

Cross[a]			Fraction	
Males		**Hermaphrodites**	**Delayed**	**N[b]**
AF16	x	HK104/AF16	0.260	127
AF16	x	AF16/HK104	0.109	128
HK104/AF16	x	AF16	0.009	111
AF16/HK104	x	AF16	0.010	105

[a]HK104/AF16 = F1 progeny from crosses of HK104 males to AF16 hermaphrodites. AF16/HK104 = F1 progeny from crosses of AF16 males to HK104 hermaphrodites. [b]n = number of progeny scored.

4.5×10^3 generations has been estimated for the divergence of strains within the temperate clade.[20,21] The dysgenic maternal-effect allele(s) likely arose some time between these upper and lower boundaries.

High frequencies of delayed F2s only were observed when HK104 was crossed to AF16 and not when HK104 was crossed to other tropical strains (**Table 1**). Hence, the dysgenic zygotic allele on chromosome III is not fixed in the tropical clade and likely arose after AF16 diverged from VT847, PS1185 and PS1186. Of these strains, AF16 is most closely related to PS1185. The divergence of AF16 from PS1185 is 0.0005 (**Fig. 2**). Using a mutation rate (μ) of 5.4×10^{-9} mutations/generation, a divergence time, $t = K/2\,\mu$, of ~4.6×10^4 generations can be estimated for these strains.[21,24-26]

Discussion

In sexual species, speciation can occur through the accumulation of genetic barriers to gene flow.[1,3,18] In this paper, one such barrier, which affects the time it takes *C. briggsae* F2 hybrids to reach sexual maturity, is described. At present, this dysgenic phenotype only is described as delayed development at a 48 h time point. A time course of development in delayed animals is needed for a full understanding of this developmental defect. The outcome of such studies may provide insight into the underlying genetic pathways affected in delayed F2 progeny.

The reproductive barrier that results from the delayed development phenotype is not complete. Gene flow appears to be affected only on chromosome III and even on this chromosome gene flow is limited but not precluded.[22,23] However, as speciation is thought to occur through the accumulation of multiple reproductive barriers, any dysgenic interaction that limits gene flow has the potential to contribute to a speciation event.[3,18,27]

Dysgenic interactions between at least two loci are involved in the delayed development phenotype. The first of these is a locus with a maternal effect. The involvement of maternal-effect genes in reproductive isolation is not unusual. Snail species often are isolated by chirality, which, in several taxa, is determined by maternal-effect genes.[7,28] *Drosophila simulans* alleles of the maternal-effect *mhr* gene are dysgenic in combination with *D. melanogaster* alleles of the zygotic *zhr* genes.[29,30] This interaction results in embryonic lethality in female hybrids. Maternal-effect genes also have been implicated in the control of allochronic mating differences between the corn and rice strains of the fall armyworm, *Spodoptera frugiperda*.[31] What is unusual is that the maternal effect involved in the delayed development phenotype appears to require maternal contributions from both parental genotypes. These AF16 and HK104 maternal-effect loci appear to be on the same chromosome and possibly are allelic. The HK104 allele of this locus appears to be fixed within the temperate clade of *C. briggsae* as delayed F2 progeny were observed in all crosses of AF16 to any of the temperate strains tested. A coalescent time of ~4,000 generations has been estimated for the temperate clade of *C. briggsae*, which means that this allele may have arisen as recently as 700 y ago.[20]

The second locus involved in the delayed development phenotype had a zygotic effect and was located on chromosome III.[22] The dysgenic allele of this locus did not appear to be fixed within the tropical clade as delayed F2s were frequent only when HK104 was crossed to AF16. (Alternatively, the dysgenic allele at the zygotic locus may be fixed in the tropical clade if a dysgenic tropical allele was not fixed at the maternal-effect locus.) The dysgenic allele on chromosome III also appeared to be absent from the ED3101 strain, which is a member of the Nairobi clade of *C. briggsae*.[21,32] The presence of the dysgenic zygotic-effect allele only in AF16 indicates that this allele also is of recent origin. As the tropical clade of *C. briggsae* exhibits considerable structure, it should be possible to refine estimates for the origin of this

dysgenic allele by crossing HK104 to additional tropical strains and scoring these crosses for delayed F2 progeny.[21]

The conclusion that the dysgenic alleles involved in the delayed development phenotype are of recent origin is based on the tropical and temperate clade divergence averaged across several loci located throughout the the *C. briggsae* genome (**Fig. 2**).[20,21,32] However, localized regions of the genome and hence these dysgenic alleles may differ considerably from the genome average in divergence. For example, divergence within the *peel-1/zeel-1* haplotype domain is 50-fold higher than genome-wide average divergence in *C. elegans*.[14] Therefore, a rigorous dating of the dysgenic *C. briggsae* alleles involved in the delayed development phenotype must wait until the molecular identities of these genes are known.

The skewed chromosome III segregation ratios obtained in three sets of recombinant inbred lines demonstrate that the delayed development phenotype is maladaptive.[22,23] However, the tropical and temperate populations of *C. briggsae* are geographically isolated and while migration rates between these populations are not known, they have not been sufficient to "disrupt the high differentiation and linkage disequilibrium among multilocus haplotypes."[20,21,32] In the absence of gene flow between these populations, the dysgenic alleles that result in the delayed development phenotype likely have not been exposed to negative selection. The origin of this dysgenic interaction between the allopatric tropical and temperate populations is consistent with the BDM model of speciation.[1-3,5,6]

The delayed development phenotype also is consistent with the genic and variable reproductive isolation (VRI) models of speciation.[27,33] The genic model describes four stages of speciation; (1) population differentiation without reproductive isolation; (2) further population differentiation with some maladaptive hybrid genotypes; (3) gene flow restricted between populations by reproductive isolation throughout much but not all of the genome and; (4) reproductive isolation complete with no gene flow between populations.[27] The delayed development phenotype clearly is maladaptive but restricts gene flow only on chromosome III. As such, it falls into stage ii in the genic model. VRI posits that posits that dysgenic BDM alleles will be polymorphic in partially isolated populations for significant periods of time.[33] This clearly is the case for the delayed development phenotype as the dysgenic allele of the zygotic gene was apparent only in AF16 and not in the other tropical strains that were tested. One issue relevant to VRI is how much do polymorphic reproductive incompatibilities contribute to reproductive isolation over the course of speciation. To answer this question for the delayed development phenotype, it will be necessary to (1) determine the prevalence of the dysgenic zygotic allele within the tropical clade and (2) to determine whether or not gene flow of haplotypes linked to this dysgenic allele is suppressed in the temperate clade.

It also will be of interest to determine whether or not the dysgenic alleles involved in the delayed development phenotype reside in regions of the *C. briggsae* genome that recently have been subjected to positive selection. Dysgenic alleles involved in BDM incompatibilities can become fixed through neutral mechanisms.[1,2,5,6] However, they also may become fixed due to geographically restricted positive selection.[16,33] Recent selective sweeps throughout much of the genome of *C. elegans* have been documented.[34] The occurrence of selective sweeps within *C. briggsae* populations remains to be tested.

A dysgenic interaction that partially restrict gene flow also has been identified in *C. elegans*.[14,15] This interaction, which is between two closely linked genes, *peel-1* and *zeel-1*, results in embryonic lethality.[15] As with the delayed development phenotype in *C. briggsae*, the *peel-1/zeel-1* interaction is between a zygotic-effect gene and a parental-effect gene. However, *peel-1* has a paternal- rather than a maternal-effect.[14] Also unlike the genes involved in the *C. briggsae* delayed development phenotype, *peel-1/zeel-1* haplotypes are not restricted to distinct geographic populations. Indeed, the maintenance of *peel-1/zeel-1* haplotypes appears to result from balancing selection and chromosomal regions that flank the *peel-1/zeel-1* haplotype domain recombine freely.[14] Once the loci involved in delayed development have been identified, it will be of interest to compare the evolutionary histories of these dysgenic interaction in these two species of *Caenorhabditis*.

Materials and Methods

Strains and strain maintenance. AF16 and VT847 were obtained from the *Caenorhabditis* Genetics Center, HK104 and HK105 from H. Kagawa, PS1185 and PS1186 from P. Sternberg, JU279, JU383, JU439 and JU441 from M.-A. Fèlix and PB800, PB826 and PB859 from local collections; PB800 from decaying mushrooms in Dayton OH, PB826 from a snail obtained from Hueston Woods State Park, College Corner OH and PB859 from a snail obtained from the Wright State University campus woods, Dayton OH. All strains were grown at 20°C on lawns of *E. coli* strain OP50.

Experimental crosses and intrinsic growth rate determinations. Crosses were conducted between males and sperm-depleted hermaphrodites.[35] F1 hermaphrodites were allowed to lay eggs for one hour and then removed. 48 h later, F2 progeny were counted and scored for the delayed development phenotype. Individual F2 progeny were transferred to new plates daily (beginning at 48 h) through day 6. F3 progeny were counted and removed from these plates before they reached adulthood. Intrinsic growth rates were determined from daily brood counts as described by Vassilieva and Lynch.[36]

DNA sequence analyses. Amplification primers (**Table 4**) were designed based on the AF16 genome sequence.[37] Amplified products were commercially sequenced. Sequence results were trimmed to include data only from introns. A maximum likelihood phylogeny of the *C. briggsae* strains used in this study was constructed using the "One Click" site at Phylogeny.fr (www.phylogeny.fr/version2_cgi/index.cgi) to analyze a concatenated data set of 4,002 nucleotides.[38,39] The divergence (K) of AF16 from PS1185 was determined from the length of the branch that separated these two strains. The divergence time (T) of AF16 from PS1185 was estimated as $t = K/2\mu$, using a mutation rate (μ) of 5.4×10^{-9} mutations/generation for *C. briggsae*.[24-26]

Table 4. Amplification primers

Gene	Forward	Reverse
Cbr-mab-20	TGC TCT TCG GTT GGA ATG CGA C	CGC TTT TTT GGT TTG ATG GTG GG
Cbr-tra-2	GCA ACT ACA CCG TCA GAA TGG AC	TAT GGC GAG CCC ACT CTT TG
Cbr-tra-3	CAT CTT TTT GTG GAA GGA GCA TCG C	TGA TAC ACC TCT CTC TTT GCC CGC
Cbr-mes-2	CAG GCG AAA AAT GCT CTG ACA AGT G	AAT CCG TTC CGT TGA TGG AGG C
Cbr-glp-1	TAT GGG ACA GAA GTG CGA GAA GGC	CGA AAC CAC ATC CAA CGA AGC
Cbr-her-1	CGT CAG TCA TTG ATT GGT CGG	CAT CTA CTC GGA GAG ACA GTT GCG
Cbr-daf-3	TGA ATG TAG TCC CTT TGT TGG TGG	TAC CCT TCT TCG GAA CTC GTC G
Cbr-ram-5	TGC GGA AAG TAG CAC AAC ACC	TCG TCA AAT CTC CAG TCT GCG

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank A.D. Cutter, E. King, S. Seibert, A.D. Aldreiwish and our reviewers for comments on this manuscript. Some strains were provided by the *Caenorhabditis* Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The research presented in this manuscript was supported in part by NIH R15 GM065847 to S.E.B.

References

1. Mayr E. (1963) Animal Species and Evolution, Belknap Press, Cambridge MA.
2. Dobzhansky T. (1970) Genetics of the Evolutionary Process. Columbia University Press, NY.
3. Coyne JA, Orr HA. (2004) Speciation. Sinauer, Sunderland MA.
4. Dobzhansky T. Studies on hybrid sterility II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics 1936; 21:113-35; PMID:17246786.
5. Muller HJ. Isolating mechanisms, evolution, and temperature. Biol Symp 1942; 6:71-125.
6. Bateson W (1909). Heredity and variation in modern light, pp 85-101 in Darwin and Modern Science, ed. Seward AC, Cambridge University Press. Cambridge MA.
7. Orr HA. Is single-gene speciation possible? Evolution 1991; 45:764-9; http://dx.doi.org/10.2307/2409927.
8. Pontecorvo G. Viability interactions between chromosomes of *Drosophila melanogaster* and *Drosophila simulans.* J Genet 1943; 45:51-66; http://dx.doi.org/10.1007/BF02982774.
9. Orr HA, Irving S. Complex epistasis and the genetic basis of hybrid sterility in the *Drosophila pseudoobscura* Bogota-USA hybridization. Genetics 2001; 158:1089-100; PMID:11454758.
10. Wittbrodt J, Adam D, Malitschek B, Mäueler W, Raulf F, Telling A, et al. Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus.* Nature 1989; 341:415-21; PMID:2797166; http://dx.doi.org/10.1038/341415a0.
11. Ting CT, Tsaur SC, Wu ML, Wu CI. A rapidly evolving homeobox at the site of a hybrid sterility gene. Science 1998; 282:1501-4; PMID:9822383; http://dx.doi.org/10.1126/science.282.5393.1501.
12. Barbash DA, Siino DF, Tarone AM, Roote J. A rapidly evolving MYB-related protein causes species isolation in *Drosophila.* Proc Natl Acad Sci U S A 2003; 100:5302-7; PMID:12695567; http://dx.doi.org/10.1073/pnas.0836927100.
13. Presgraves DC, Balagopalan L, Abmayr SM, Orr HA. Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila.* Nature 2003; 423:715-9; PMID:12802326; http://dx.doi.org/10.1038/nature01679.
14. Seidel HS, Rockman MV, Kruglyak L. Widespread genetic incompatibility in *C. elegans* maintained by balancing selection. Science 2008; 319:589-94; PMID:18187622; http://dx.doi.org/10.1126/science.1151107.
15. Seidel HS, Ailion M, Li J, van Oudenaarden A, Rockman MV, Kruglyak L. A novel sperm-delivered toxin causes late-stage embryo lethality and transmission ratio distortion in *C. elegans.* PLoS Biol 2011; 9:e1001115; PMID:21814493; http://dx.doi.org/10.1371/journal.pbio.1001115.
16. Presgraves DC. The molecular evolutionary basis of species formation. Nat Rev Genet 2010; 11:175-80; PMID:20051985; http://dx.doi.org/10.1038/nrg2718.
17. Kamei N, Glabe CG. The species-specific egg receptor for sea urchin sperm adhesion is EBR1,a novel ADAMTS protein. Genes Dev 2003; 17:2502-7; PMID:14561772; http://dx.doi.org/10.1101/gad.1133003.
18. Orr HA, Turelli M. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. Evolution 2001; 55:1085-94; PMID:11475044.
19. Haag ES. The evolution of nematode sex determination: *C. elegans* as a reference point for comparative biology. WormBook 2005; http://dx.doi.org/10.1895/wormbook.1.120.1.
20. Cutter AD, Félix MA, Barrière A, Charlesworth D. Patterns of nucleotide polymorphism distinguish temperate and tropical wild isolates of Caenorhabditis briggsae. Genetics 2006; 173:2021-31; PMID:16783011; http://dx.doi.org/10.1534/genetics.106.058651.
21. Cutter AD, Yan W, Tsvetkov N, Sunil S, Félix MA. Molecular population genetics and phenotypic sensitivity to ethanol for a globally diverse sample of the nematode *Caenorhabditis briggsae.* Mol Ecol 2010; 19:798-809; PMID:20088888; http://dx.doi.org/10.1111/j.1365-294X.2009.04491.x.
22. Ross JA, Koboldt DC, Staisch JE, Chamberlin HM, Gupta BP, Miller RD, et al. *Caenorhabditis briggsae* recombinant inbred line genotypes reveal inter-strain incompatibilities and inter-species divergence of recombination rate. PLoS Genet 2011; 7:e1002174; PMID:21779179; http://dx.doi.org/10.1371/journal.pgen.1002174.
23. Hillier LW, Miller RD, Baird SE, Chinwalla A, Fulton LA, Koboldt DC, et al. Comparison of *C. elegans* and *C. briggsae* genome sequences reveals extensive conservation of chromosome organization and synteny. PLoS Biol 2007; 5:e167; PMID:17608563; http://dx.doi.org/10.1371/journal.pbio.0050167.
24. Cutter AD. Divergence times in *Caenorhabditis* and *Drosophila* inferred from direct estimates of the neutral mutation rate. Mol Biol Evol 2008; 25:778-86; PMID:18234705; http://dx.doi.org/10.1093/molbev/msn024.
25. Baer CF, Shaw F, Steding C, Baumgartner M, Hawkins A, Houppert A, et al. Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. Proc Natl Acad Sci U S A 2005; 102:5785-90; PMID:15809433; http://dx.doi.org/10.1073/pnas.0406056102.
26. Denver DR, Dolan PC, Wilhelm LJ, Sung W, Lucas-Lledó JI, Howe DK, et al. A genome-wide view of *Caenorhabditis elegans* base-substitution mutation processes. Proc Natl Acad Sci U S A 2009; 106:16310-4; PMID:19805298; http://dx.doi.org/10.1073/pnas.0904895106.
27. Wu CI. The genic view of the process of speciation. J Evol Biol 2001; 14:851-65; http://dx.doi.org/10.1046/j.1420-9101.2001.00335.x.
28. Gittenberger E. Sympatric speciation in snails; a largely neglected model. Evolution 1988; 42:826-8; http://dx.doi.org/10.2307/2408875.
29. Sawamura K, Taira T, Watanabe TK. Hybrid lethal systems in the Drosophila melanogaster species complex. I. The maternal hybrid rescue (mhr) gene of Drosophila simulans. Genetics 1993; 133:299-305; PMID:8436276.
30. Sawamura K, Yamamoto MT, Watanabe TK. Hybrid lethal systems in the Drosophila melanogaster species complex. II. The Zygotic hybrid rescue (Zhr) gene of D. melanogaster. Genetics 1993; 133:307-13; PMID:8436277.
31. Schöfl G, Heckel DG, Groot AT. Time-shifted reproductive behaviours among fall armyworm (Noctuidae: *Spodoptera frugiperda*) host strains: evidence for differing modes of inheritance. J Evol Biol 2009; 22:1447-59; PMID:19467132; http://dx.doi.org/10.1111/j.1420-9101.2009.01759.x.

32. Dolgin ES, Félix MA, Cutter AD. Hakuna Nematoda: genetic and phenotypic diversity in African isolates of *Caenorhabditis elegans* and *C. briggsae*. Heredity (Edinb) 2008; 100:304-15; PMID:18073782; http://dx.doi.org/10.1038/sj.hdy.6801079.
33. Cutter AD. The polymorphic prelude to Bateson-Dobzhansky-Muller incompatibilities. Trends Ecol Evol 2012; 27:209-18; PMID:22154508; http://dx.doi.org/10.1016/j.tree.2011.11.004.
34. Andersen EC, Gerke JP, Shapiro JA, Crissman JR, Ghosh R, Bloom JS, et al. Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. Nat Genet 2012; 44:285-90; PMID:22286215; http://dx.doi.org/10.1038/ng.1050.
35. Baird SE, Sutherlin ME, Emmons SW. Reproductive isolation in Rhabditidae (Nematoda: Secernentea); mechanisms that isolate six species of three genera. Evolution 1992; 46:585-94; http://dx.doi.org/10.2307/2409629.
36. Vassilieva LL, Lynch M. The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. Genetics 1999; 151:119-29; PMID:9872953.
37. Stein LD, Bao Z, Blasiar D, Blumenthal T, Brent MR, Chen N, et al. The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. PLoS Biol 2003; 1:E45; PMID:14624247; http://dx.doi.org/10.1371/journal.pbio.0000045.
38. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 2008; 36(Web Server issue):W465-9; PMID:18424797; http://dx.doi.org/10.1093/nar/gkn180.
39. Dereeper A, Audic S, Claverie JM, Blanc G. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol Biol 2010; 10:8; PMID:20067610; http://dx.doi.org/10.1186/1471-2148-10-8.