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Caenorhabditis briggsae methods*

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1. The *Caenorhabditis briggsae* model system

Caenorhabditis briggsae is being developed in parallel to *C. elegans* as a model system, primarily for the study of evolution. Like *C. elegans*, *C. briggsae* is a protandrous hermaphrodite (Nigon and Dougherty, 1949) and like *C. elegans*, its genome has been sequenced (Stein et al., 2003). From this point, these two model systems diverge. The development, behavior, and physiology of *C. elegans* have been characterized through tens of thousands of genetic and molecular studies. Genetic and molecular characterizations of *C. briggsae* are relatively few. Experimental resources in *C. elegans* include a high density recombination map that is well integrated with the genome sequence. The *C. briggsae* recombination map has yet to be published and attempts to integrate it with the genome sequence are in their infancy. Despite these deficiencies, *C. briggsae* is attractive for several reasons. First as a parallel system, it can be used to test the generality of results obtained in *C. elegans*. Second, it appears that the structure of the *C. briggsae* world-wide population is qualitatively different from that of *C. elegans* and that *C. briggsae* may be more amenable to studies of gene flow, genome evolution, and speciation (Cutter et al., 2006; Baird and Hampton, unpubl. results). Finally, *C. briggsae* and *C. remanei* are sister species with *C. elegans* as an outgroup, making the *C. briggsae*–*C. remanei* species better for some aspects of comparative genomics (Kiontke et al., 2004; Cho et al., 2004; see The phylogenetic relationships of *Caenorhabditis* and other rhabditids).

2. Natural history and population biology

2.1. Natural history

Most *Caenorhabditis* species form phoretic and/or necromenic associations with other soil invertebrates (see Ecology of *Caenorhabditis* species). In phoretic associations, hosts are used for transport between microenvironments. In necromenic associations, nematodes resume development and reproduce in the bacterial bloom that occurs following the host death. A variety of snails have been identified as hosts for *C. briggsae* dauer larvae (S. Baird, unpubl. obs.). *C. briggsae* dauers also have been obtained from soil and compost (Cutter et al., 2006). As dauers can persist for several months without feeding, generation times in wild populations are not known and may vary widely between and within populations.

2.2. Biogeography and population biology

C. briggsae is a cosmopolitan species, having been obtained from collections in Asia, North America, and Europe (Figure 1A; Cutter et al., 2006). Phylogentic reconstructions of strains derived from these collections strongly support the division of *C. briggsae* into distinct tropical and temperate clades (Figure 1B; Graustein et al., 2002; Cutter et al., 2006; Baird and Hampton, unpubl. results). This clade structure should be considered when designing experiments, as tropical and temperate strains exhibit morphological and developmental differences. Consequently, different experimental results sometimes are obtained for tropical and temparate strains (Baird, 2001; 2002; Dellatre and Félix, 2001). Few collections have been made in the Southern hemisphere. However, *C. briggsae* strains have been obtained from Kenya and South Africa (E. Dolgin, personal communication).

The division of *C. briggsae* into tropical and temperate clades probably results from a recent expansion of the *C. briggsae* range into temperate latitudes. Nucleotide diversity within the temperate clade is very low and the coalescence time for this clade has been estimated to be 700 years (Cutter et al., 2006). This estimate was based on an effective population size (N_e) for the temaperate clade of 1,000 and a 60 day generation time (assuming that in wild populations, *C. briggsae* individuals spend most of their time as dauers).

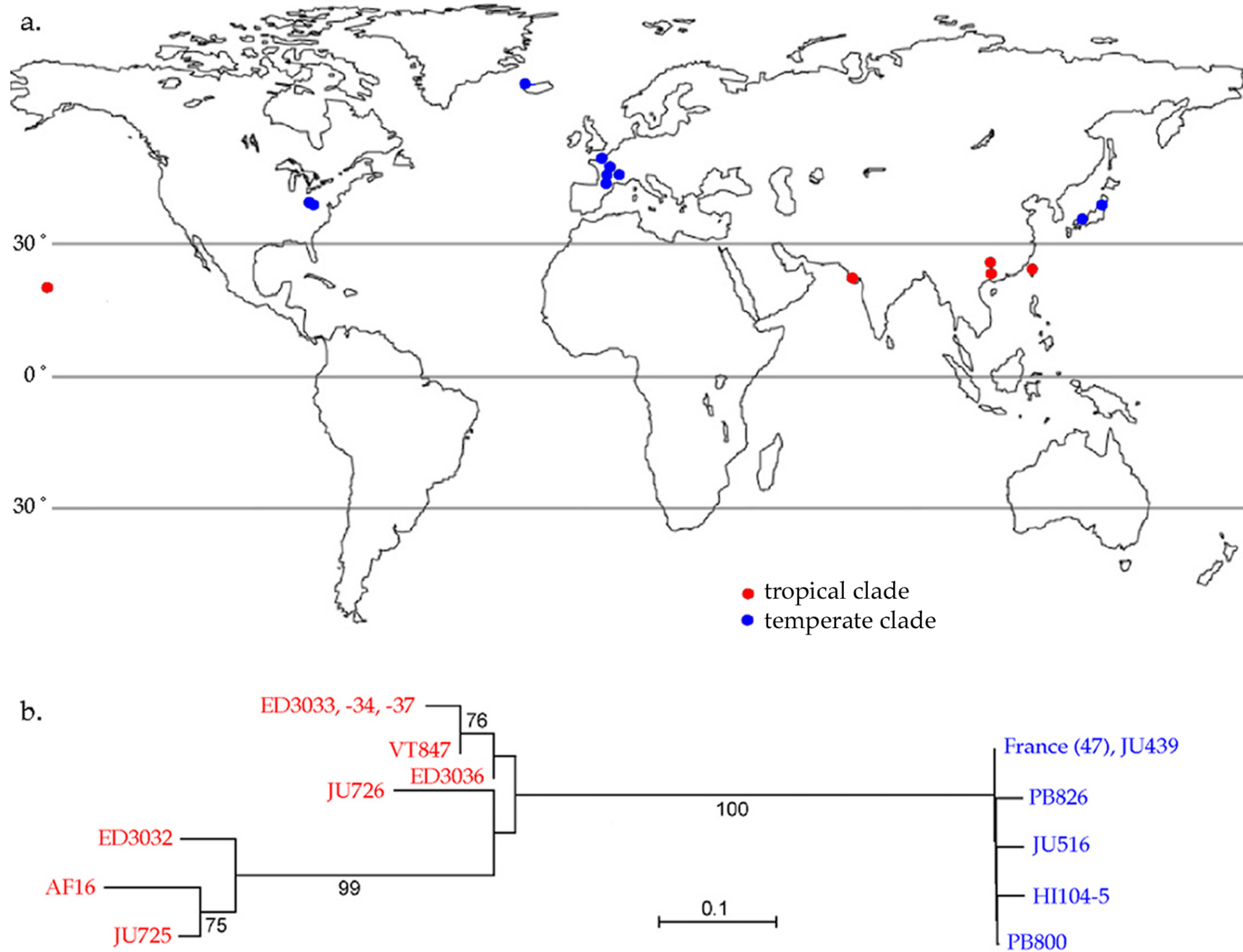

Figure 1. Biogeography of *Caenorhabditis briggsae*. a) A map of tropical and temparate collection sites from which *C. briggsae* strains have been obtained. b) An unrooted reconstruction of *C. briggsae* phylogeny based on sequence comparisons at six loci. Adapted from Cutter et al. (2006).

3. Genetics

3.1. Genetic nomenclature

Nomenclatural rules for *C. briggsae*, which currently are in flux, reflect the conflicting goals of providing unique durable gene names for mutationally identified genes while at the same time allowing for the identification of orthologs to *C. elegans* genes. The history of *C. briggsae* nomenclature rules and detailed nomenclatural suggestions can be found at the *Caenorhabditis briggsae* Research Page. One feature of this system is that *C. briggsae* gene names may change as orthology is determined. For example, the *C. briggsae mip-1* gene was identified based on mutations that cause mutant animals to twitch uncontrollably. In *C. elegans*, this phenotype results commonly from mutations in *unc-22*. Hence, *mip-1* may be the *C. briggsae* ortholog of *unc-22*. If this orthology is confirmed, *mip-1* will be renamed *Cb-unc-22*. Confusion caused by changes in *C. briggsae* gene names is expected to be minimal as gene name histories will be archived in WormBase.

Alleles, strains, polymorphisms, rearrangements, transgenes, and other variants will be given unique identifiers following *C. elegans* nomenclatural conventions (Horvitz et al., 1979; CGC). These identifiers will include a lab strain or allele designation followed by a unique strain or allele number for that lab. For example, *bd101* is a *C. briggsae* mutation that is present in several strains including PB101, PB107 and PB108. These names will be unique and durable.

Finally, it should be noted that a different convention is used by WormBase to name predicted *C. briggsae* genes. In this convention, each gene prediction gets a CBG (*C. briggsae* gene) prefix followed by a unique five digit identifier, e.g., CBG00123. Gene predictions in WormBase are continually refined as algorithms are improved. Therefore, predicted CBG gene names also may change over time.

3.2. Single nucleotide polymorphisms

The genome sequence of *C. briggsae* is that of the tropical strain, AF16 (Stein et al., 2003). To facilitate genetic studies, 13,000 randomly selected clones derived from a temperate strain, HK104, also have been sequenced. From these sequences over 26,000 single nucleotide polymorphisms (SNPs) have been identified giving a SNP density of greater than 0.2% (Hillier, Miller, Chinwalla, Fulton, Kolboldt, and Waterston, personal communication).

3.3. Recombination maps

Two recombination maps have been constructed for *C. briggsae*. One is a SNP-based map that was constructed based on the segregation of SNP alleles in recombinant inbred lines (RIL) (Hillier, Miller, Chinwalla, Fulton, Kolboldt, and Waterston, personal communication). These RIL were derived from a cross between *C. briggsae* strains AF16 and HK104 (Baird et al., 2005). Each RIL was initiated with a single L4 F2 hermaphrodite and inbred through a single hermaphrodite per generation to F11. The current draft SNP map (v3.3) includes segregation data for 290 SNPs in 93 RIL. RIL genotypes for each SNP were determined using a fluorescent polarization-terminal dye incorporation (FP-TDI) assay (Hsu et al., 2001). This assay does not require any post-reaction product purification and has been adapted for the high-throughput genotyping of *C. briggsae* RIL. The other is a mutation-based map that was constructed based on the segregation of visible phenotypes in F2 progeny (B. Gupta, P. Sternberg, and D. Baillie, personal communication). Both maps identified six linkage groups. Integration of the SNP- and mutation-based maps is in progress (R. Miller and B. Gupta, personal communication). Nearly all *C. briggsae* induced mutations were derived from strain AF16. Therefore, map integration will be accomplished by mapping mutant alleles relative to HK104 SNPs. This will entail the genotyping of individual mutant F2s for multiple SNPs. To facilitate this, template DNA from F2 single-worm lysates will be amplified by whole genome amplification (Dean et al., 2001). Preliminary experiments have demonstrated the whole genome amplification provides template sufficient for several thousand genotyping reactions (S. Baird, unpubl results; B. Gupta and R. Miller, personal communication).

3.4. Bulk segregant analysis

In *C. briggsae*, as in other organisms, most SNPs do not affect restriction endonuclease recognition sites. Therefore it is difficult and/or expensive to distinguish between AF16 and HK104 SNP alleles with using commonly available lab equipment. One way around this, at least for linkage determination, is bulk segregant analysis. In *C. briggsae* a bulk segregant assay is initiated by crossing an AF16-derived mutant strain to HK104. Pools of 10–25 mutant F2 progeny then are collected and lysed following a scaled-up (25 µl) single-worm protocol. Primers for these reactions are designed to amplify short (200–500 bp) products that contain a polymorphic nucleotide at least 50 nucleotides from either end. Amplification products are sequenced and polymorphisms are detected as ambiguities in the sequence traces. For unlinked loci, both alleles, i.e. an ambiguity, will be apparent in the sequence trace. For linked loci, only the AF16 allele will be present. In control reactions, a strong allelic bias was observed from lysates made from a 9:1 ratio of HK104 to AF16 hermaphrodites (Figure 2). In F2 progeny, a 9:1 ratio would be expected for loci located 10 mu from the selected mutation. Thus, two-fold redundant coverage of the *C. briggsae* genome can be achieved with as few as four SNPs per chromosome. This approach has been used successfully to identify loci involved in fitness loss in AF16::HK104 F2 hybrids (Hampton and Baird, unpubl. results).

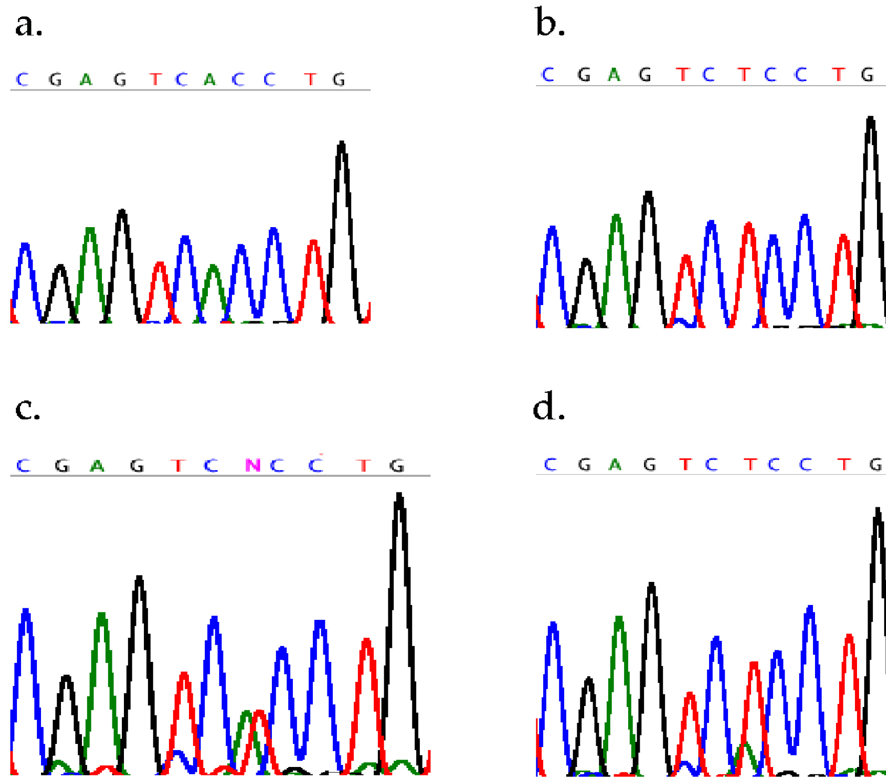

Figure 2. Bulk segregant analysis of *C. briggsae* SNPs. Sequence traces from C. *briggsae* strains a) AF16 and b) HK104, showing an A/T polymorphisms. Sequence traces from lysates made from c) a mixture of five AF16 and five HK104 hermaphrodites and d) one AF16 hermaphrodite and nine HK104 hermaphrodites.

4. Reverse genetic methods

4.1. Identification of *C. elegans–C. briggsae* orthologs

C. elegans and *C. briggsae* share a similar number of protein-coding genes (just under 20,000), with an estimate of 12,200 orthologous genes. Another 6,500 genes in *C. briggsae* have one or more *C. elegans* homolog, leaving about 800 genes as *C. briggsae*-specific (Stein et al., 2003). *C. elegans–C. briggsae* orthologs are annotated in Wormbase (http://www.wormbase.org/) according to the criteria of Stein et al. (2003). Each ortholog was identified either as the reciprocal top BLASTP match when queried against the genome of the other species, or was identified as a homolog (but not the reciprocal best match) with conserved synteny (gene order) with respect to other genes on the chromosome.

Although ortholog predictions by Stein et al. are fairly robust, false positives can result if best matches are not orthologous due to gene loss or divergence, or gene amplification events. Reciprocally, false negatives can result in the cases of rapid gene evolution or from errors in genome annotation. Consequently, researchers interested in a specific gene or set of genes will want to perform additional analysis to confirm orthology. Commonly, this can be achieved by phylogenetic reconstruction using all sequences of homologs available from different *Caenorhabditis* species, as well as an outgroup species. Homologs can be identified using BLAST to identify conserved domains as well as other genes (or translated gene products) that contain the domains. The relationship among the genes/proteins can be determined using a neighbor joining program with confidence assessed by bootstrapping methods (e.g., PHYLIP). True orthologs should exhibit fewer sequence differences and thus join, or cluster, with each other (e.g., Jovelin and Phillips, 2005; Nayak et al., 2005; Thomas, 2006).

4.2. Transgenesis and heterologous gene expression

Introduction of transgenes into *C. briggsae* allows for mutant rescue experiments, reporter transgene assays, and heterologous gene expression, both for ectopic expression studies, and inter-specific functional assays (e.g., Streit et al., 1999; Wang and Chamberlin, 2002; Wang and Chamberlin, 2004). High copy number

extrachromosomal transgenes can be introduced into *C. briggsae* by microinjection of DNA into the hermaphrodite germline (see Transformation and microinjection) following protocols developed for *C. elegans* (Mello et al., 1991). Transformation markers from *C. elegans* that are effective in *C. briggsae* include *ce-rol-6(d)*, *ce-myo-2::gfp*, *ce-myo-3::gfp*, *ce-hsp-16-48::gfp*, and *ce-sur-5::gfp* (Streit et al., 1999; Wang and Chamberlin, 2002) M.-A. Felix, personal communication). Extrachromosomal arrays can be integrated (see Transformation and microinjection) into the chromosome using 3000–4000 rad γ-irradiation and following *C. elegans* protocols (Inoue et al., 2002).

C. briggsae exhibits differences from *C. elegans* with respect to the efficacy of transgene formation, and they exhibit greater transgene silencing and mosaicism. In general, similar DNA mixtures and injection conditions result in 3-10 fold fewer stable transgenes in *C. briggsae* than in *C. elegans*, with reduced efficiency at both F1 and F2 generations (M.-A. Felix, personal communication). Although some of these differences may reflect the use of heterologous [usually *C. elegans* (Li et al., 2006)] DNA in transformation experiments, these results suggest that producing transgenes in *C. briggsae* will benefit from further optimization of protocols.

4.3. RNA-mediated interference

As in *C. elegans*, double stranded RNA-mediated gene interference (RNAi; see Reverse genetics) can provide a relatively easy method to assess the function of *C. briggsae* genes *in vivo* (e.g., Haag et al., 2002; Rudel and Kimble, 2001; Stothard et al., 2002; Streit et al., 1999). For this method, introduction of double-stranded RNA corresponding to a gene of interest results in the specific degradation of the corresponding mRNA, allowing phenotypic analysis of animals depleted for the gene. However, in contrast to *C. elegans*, an effective RNAi response is generally obtained in *C. briggsae* only when double stranded RNA is introduced directly into animals, such as by microinjection. *C. briggsae* is resistant to RNAi introduced by soaking or bacterial feeding methods (M. K. Montgomery, personal communication; C. Hunter, personal communication). This resistance reflects a difference between *C. briggsae* and *C. elegans* animals in availability of RNAi uptake and spreading mechanisms that allow RNAi transport into the animal and across epithelial boundaries. Consequently, RNAi can frequently be less effective in *C. briggsae* than *C. elegans*, and negative results or differences in RNAi phenotypes between the species must be interpreted with caution. Recent work has shown that the *sid-2* gene required for environmental uptake of RNA by *C. elegans* is altered and not functional in *C. briggsae*. Consequently, introduction of *ce-sid-2* as part of a transgene enhances RNAi uptake in *C. briggsae*, and confers sensitivity to RNAi by feeding onto this species (W. M. Winston, M. Sutherlin, A. J. Wright, E. Feinberg, and C. Hunter, unpublished results). We anticipate that these transgenic animals or other genetically modified or mutant *C. briggsae* strains will allow for enhanced RNAi response and a greater range of methods to introduce RNAi.

4.4. Targeted deletion screens

Although functional studies using RNAi are efficient, the gold standard in genetic analysis is chromosomal mutations. Strategies developed to isolate deletion alleles (see Reverse genetics) in *C. elegans* genes have been applied effectively to *C. briggsae* (Edgley et al., 2002), although the frequency of allele recovery may be somewhat lower than in *C. elegans*. Hill et al. (2006) report a single deletion allele each of *Cb-fem-2* and *Cb-fem-3* recovered from a library of 1.1 million EMS mutagenized gametes.

4.5. *Mos* mutagenesis

The Drosophila transposon *Mos1* has been shown to mobilize and cause heritable mutations in *C. elegans*, providing a method for rapid tagging and recovery of mutant genes (Williams et al., 2005). Following a modified protocol (below), M.-A. Felix and her group have shown that *Mos1* mutagenesis can be applied to *C. briggsae* as well. Although the SNP-based polymorphism map (v3.3) combined with genomic sequence will provide the tools for positional cloning of genes in *C. briggsae*, transposon tagging may allow a more efficient strategy for gene cloning in some cases.

In *C. elegans*, *Mos1* mutagenesis is achieved using two transgenes: an extrachromosomal array that expresses the transposase under control of a heat-shock promoter, and a second transgene that includes the transposon (Bessereau et al., 2001). Expression of the transposase is induced in parental (P0) hermaphrodites, and then offspring are screened in the F1 or F2 for mutant phenotypes. Following outcross of the mutant allele, the tagged gene can be isolated using PCR. A detailed protocol for *Mos1* mutagenesis is available (Bessereau et al., 2006).

In *C. briggsae*, *Mos1* mutagenesis can be achieved following the *C. elegans* protocol, substituting transgenes *mfEx18* (transposase) and *mfEx24* (transposon) (M.A. Felix, personal communication). Heat-shock conditions are

also modified to allow for the greater heat-tolerance of *C. briggsae* animals. Mos1 germline insertions and mutant alleles have been obtained using the following heat-shock treatment of P0 young adults:

1. 37° C for one hour, 20° C for one hour, 37° for one hour, and 15° overnight.

2. 37° C for 2.5 hours, 20° C for 35 minutes, 37° for one hour, and 25° overnight.

5. Online resources for *C. briggsae* genetics and genomics

As the development of *C. briggsae* as a model system is in its infancy, information on its genetics and genomics is changing rapidly. The most valuable resources for staying up to date are a set of *C. briggsae* web sites that are the repository of considerable published and unpublished data.

5.1. The *Caenorhabditis briggsae* Research Page

wormlab.caltech.edu/briggsae

This is the best site for *C. briggsae* experimental genetics. Of primary importance are archival lists of *C. briggsae* mutations, linkage data, and recombination maps based on visible mutations. This site also has links to a variety of other resources including a list of *C. briggsae* labs.

5.2. The *C. briggsae* SNP map

snp.wustl.edu/snp-research/c-briggsae

In addition to the SNP map, this site is a download site for *C. briggsae* sequence data, including strain-specific nucleotide polymorphisms.

5.3. WormBase

www.wormbase.org

C. briggsae genomics data have been incorporated into WormBase. Features include *C. briggsae* gene preditions, the identification of *C. briggsae*-*C. elegans* orthologs and syntenic alignments of *C. briggsae* and *C. elegans*.

5.4. The WashU *C. briggsae* Genome server

http://genome.wustl.edu/genome.cgi?GENOME=Caenorhabditis%20briggsae

The *C. briggsae* genome server offers sequence data downloads and a link to *C. briggsae*-specific BLAST server.

5.5. The *Caenorhabditis* Genetics Center

biosci.umn.edu/CGC/CGChomepage.htm

The CGC is a repository of *C. briggsae* wild isolates and mutant strains.

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