Sex Change by Gene Conversion in a *Caenorhabditis elegans fog-2* Mutant

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ABSTRACT

*Caenorhabditis elegans* primarily reproduces as a hermaphrodite. Independent gene conversion events in mutant obligately outcrossing populations of *C. elegans* (*fog-2* (*fl*)) spontaneously repaired the loss of function mutation in the *fog-2* locus, thereby reestablishing hermaphroditism as the primary means of reproduction for the populations.
Species within the genus *Caenorhabditis* employ one of two modes of reproduction. Nine of the eleven *Caenorhabditis* species in culture (Kiontke and Sudhaus 2006) are gonochoristic obligate female/male outcrossers. Gonochorism is thought to be the ancestral state within the genus (Schedl and Kimble 1988; Kiontke et al. 2004). The remaining two species, *C. elegans* and *C. briggsae*, have an androdioecious breeding system with populations comprising of self-fertile hermaphrodites and males at a low frequency (<0.1%) (Ward and Carrel 1979; Hodgkin and Doniach 1997). The two hermaphroditic *Caenorhabditis* species are phylogenetically separated by two gonochoristic species, suggesting that hermaphroditism (and an androdioecious breeding system) evolved convergently in *C. elegans* and *C. briggsae* (Kiontke et al. 2004). Moreover, the regulation of sperm production in hermaphrodites in these two species differs in important ways. For instance, the *fog-2* locus is specifically required for spermatogenesis in *C. elegans* hermaphrodites (Schedl and Kimble 1988; Nayak et al. 2005). The appearance of *fog-2* in the *C. elegans* genome is thought to be an evolutionarily recent event resulting from a gene duplication that may have ultimately promoted the evolution of hermaphroditism (Clifford et al. 2000; Haag 2005; Nayak et al. 2005). Furthermore, *C. elegans* also requires *fem-2*, *fem-3* and *tra-2* for spermatogenesis in hermaphrodites whereas control of sperm production in *C. briggsae* hermaphrodites occurs downstream of the *fem* genes (Hill et al. 2006).

Loss of function mutations in *fog-2* (*fog-2(If) henceforth*) in *C. elegans* result in a change from androdioecy to gonochoristic reproduction (Schedl and Kimble 1988). However, extragenic mutations that suppress, at least to some degree, the *fog-2* mutant phenotype, have been found in five different genes: *tra-2*, *fem-3*, *gld-2*, *tra-3* and *atx-2* (Barton et al. 1987; Schedl and Kimble 1988; Francis et al. 1995a, 1995b; Maine et al. 2004; Nayak et al. 2005).
These experiments have used either chemical mutagenesis or RNA interference (RNAi henceforth) to discover alleles that restore hermaphroditism in *fog-2* mutants. Here we report that spontaneous gene conversion involving the neighboring paralog with an unknown function, *ftr-1*, can restore the function of *fog-2* in experimental populations. These gene conversion events result in a fully functional hermaphrodite that replaces the original *fog-2* mutant in experimental populations and may be more frequent than point mutations in restoring the functionality of *fog-2(If)* mutants.

During an experimental evolution study comprising 74 *fog-2(If)* lines derived from the same ancestral pair, we identified two independent instances in which *fog-2* mutants (normally obligate outcrossers) had reverted spontaneously to hermaphroditism. Revertant 1 appeared in an experimental phase that involved repeated population bottlenecks of two individuals per generation in conjunction with knockdown of the mismatch repair gene *msh-2* by a standard RNAi feeding protocol (Kamath et al. 2000). Revertant 2 appeared during the second phase of the experiment involving population expansion in the absence of *msh-2* RNAi. In each instance, a putative case of reversion to hermaphroditism was detected by observing extremely biased sex ratios in the offspring generation, namely the near complete absence of males (male-female crosses yield 50:50 offspring sex ratios whereas males are rare or absent in selfing hermaphroditic populations of *C. elegans*). Reversion to functional hermaphroditism was confirmed by the production of self progeny by individually plating L4 larvae. To determine the genetic basis of reversion to hermaphroditism, the *fog-2* gene was PCR-amplified and sequenced in (i) the wild-type *C. elegans* laboratory strain, N2, (ii) the *fog-2* mutant strain, and (iii) the two experimental *fog-2* mutant strains that reverted to hermaphroditism. The two sex reversal events each resulted from a gene conversion whereby a short segment of a paralogous gene *ftr-1*
recombined with the *fog-2(II)* mutant allele, replacing the premature stop codon with a tryptophan codon (Figure 2). Both gene conversion events are relatively short, replacing at minimum 56 and 32 nucleotides of *fog-2* sequence with *ftr-1* sequence, respectively (maximum possible lengths of the gene conversion tracts are 145 and 121 bp respectively). The length of these gene conversion tracts are well within the average range of converted lengths found between paralogs in the *C. elegans* genome (Semple and Wolfe 1999) although considerably shorter than the >200 bp conversion tracts detected in an assay of DNA double strand break repair employing an extrachromosomal DNA template (Plasterk and Groenen 1992).

A comparison of *fog-2* and *ftr-1* found signatures of past gene conversion in their evolution. Although the overall sequence divergence between *fog-2* and *ftr-1* over their homologous coding regions is 16%, there are a few large segments that are completely identical between the two genes. Using Geneconv, a software that employs statistical tests to detect gene conversion, we found three statistically significant regions (*p-values* = 0.0000, 0.0021 and 0.0415) ranging from 39 to 75 nucleotides in length that are identical between *fog-2* and *ftr-1* (Sawyer 1999). However, the directionality of these past gene conversion events is unknown, with the possibility that either *ftr-1* or *fog-2* sequence tracts have served as the donor sequence.

Our sample size is clearly too small to draw any definitive conclusions about the relative rates of point mutations and gene conversion in the *C. elegans* genome. One of the gene conversion events occurred during *msh-2* knockdown by RNAi, which might be expected to increase the rates of gene conversion. Conversely, it is also expected to increase the nucleotide substitution rate and hence the rate at which *fog-2* reverts to wild-type by point mutation. However, the fact that we found gene conversion events and no direct reversion to wild-type by point mutation suggests that gene conversion is at least as common in the *C. elegans* genome as
point mutations, if not more frequent. The genomic proximity of the \textit{ftr-1} and \textit{fog-2} loci (FIGURE 1) may also facilitate a high frequency of gene conversion between them. Indeed, studies of gene conversion events in both \textit{C. elegans} (Semple and Wolfe 1999) and yeast (Drouin 2002) have found a negative correlation between the frequency of gene conversion events and the distance between gene pairs (unlinked versus linked genes). Finally, the chromosomal location of \textit{fog-2} and \textit{ftr-1} may further enhance the rate of gene conversion. Both genes reside close to the right end of Chromosome V. Chromosomal arms in \textit{C. elegans} are known to have higher recombination rates relative to the center (Barnes et al. 1995; Hillier et al. 2007) and crossing over increases the probability of gene conversion (Jeffreys and May 2004).

These gene conversion events during experimental evolution in the laboratory raise the question whether similar events (\textit{i.e.} gene conversion between \textit{fog-2} and members of the \textit{ftr} family) are important in nature. Most \textit{Caenorhabditis} species are obligate outcrossers and it is tempting to speculate that in some environments where outcrossing is favored, a loss of function mutation in \textit{fog-2} could be advantageous in \textit{C. elegans}. A high rate of gene conversion would make such loss of function mutations more reversible than by point mutations alone. However, this is unlikely to have been important in the recent evolutionary history of \textit{C. elegans}. Despite the fact that male sperm readily overwhelms hermaphroditic sperm in the event of a male-hermaphrodite mating, \textit{fog-2} mutants are at a severe disadvantage in mixed populations of the two (Chasnov and Chow 2002; Stewart and Phillips 2002), even under experimental conditions imposing a high mutational load when outcrossing may be more beneficial (Cutter 2005; Manoel et al. 2007). Moreover, mating behavior in \textit{C. elegans} appears to have degenerated relative to other obligate outcrossers in the genus, such as \textit{C. remanei} and \textit{Caenorhabditis} spp. 4 (Rene Garcia et al. 2007). Nonetheless, gene conversion between \textit{ftr-1}
and *fog-2* has the potential to shape genetic variation at these loci in natural populations, thereby modifying the number of sperm produced by hermaphrodites with important implications for the degree of inbreeding versus outcrossing in nature.

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**FIGURE LEGENDS**

**FIGURE 1.** — Schematic depicting the regions of homology between paralogs *fog-2* and *ftr-1*. Shaded narrow rectangles denote exons, horizontal lines represent introns and duplicated flanking regions where applicable. The duplicated region is highlighted in gray. Duplicated segments as determined by shared sequence homology between the two paralogs are also depicted by the correspondence of regions with identical color and pattern. The figure is drawn to scale. *ftr-1* comprises four exons encoding 314 amino acids. The exon-intron structure of *fog-2*, comprising five exons (encoding for 327 aa) exhibits both similarities and dissimilarities relative to *ftr-1*. Homology between *fog-2* and *ftr-1* commences approximately 170 bp upstream of the start codon, encompassing the first three exons and introns and terminating at nucleotide position 91 of the terminal exon (total length 186 bp). The last 95 bp of the terminal exon of *ftr-1* as well as its 3′ downstream region bear no homology to the corresponding C-terminal region of *fog-2*. The K_s value between *ftr-1* and *fog-2* over the region of homology comprising the duplication span (1248 bp) is 0.22 with the NEI-GOJOBORI method (NEI and GOJOBORI 1986) and 0.26 if corrected for multiple hits under the JUKES-CANTOR model (JUKES and CANTOR 1969). Regards *fog-2*, the latter 66 bp of exon 4 (total 157 bp), and intron 4 (45 bp) and exon 5 (23 bp) in their entirety comprise unique sequence bearing no obvious homology to *ftr-1*. In order to determine an alternative genomic source for this nonhomologous sequence tract in the C-terminal end of *fog-2*, this 134 bp of unique ORF (comprising both exonic and intronic regions) in isolation as well as in conjunction with 500 bp of the *fog-2* 3′ downstream region was queried against the *C. elegans* genome sequence in Wormbase using a BlastN search. In addition, we queried a 37 aa long sequence coded by the unique exonic regions of *fog-2* against Wormbase.
using a tBlastN search. All three queries failed to yield any alternate hits suggesting that this stretch of sequence unique to fog-2 may have been assimilated into its reading frame via recruitment of novel neighborhood sequence from its new genomic location. Both paralogs, fir-1 and fog-2, loci reside on Chromosome V as tandem genes with positive strand orientation and are separated by a 763 bp stretch of unique sequence. The genomic proximity of fog-2 and fir-1 suggests unequal exchange or slippage as the mechanism of duplication and conforms to the general pattern of genomic location observed for evolutionarily young gene duplicates in C. elegans (Katju and Lynch 2003, 2006).

**Figure 2.** — Nucleotide sequence alignments representing two independent gene conversion events at the fog-2 locus by fir-1 resulting in a switch from obligate outcrossing to hermaphroditism in two fog-2(lf) mutant lines. In-frame nucleotide positions 200-499 of exon 3 (total length 640 bp) are displayed. The small, clear box displays the nonsense mutation G→A in the fog-2(lf)q71 allele resulting in a nonfunctional gene relative to the wild-type. The larger boxed area in gray represents the minimum gene conversion tracts by the upstream fir-1 locus in sex-revertants 1 and 2. Indels are indicated by dashed lines and dots represent identical nucleotides to the fog-2 wild-type sequence.
**FIGURE 1**

![Genetic Diagram](image)
Figure 2

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1

Fog-2 wild-type
Fog-2(Ifq71
Revertant 2
Revertant 1
Ftr-1