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Note

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(lf)*] 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 11 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, *Caenorhabditis elegans* and *C. briggsae*, have an androdioecious breeding system with populations composed 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(lf)*] 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,b; Maine *et al.* 2004; Nayak *et al.* 2005). These experiments have used either chemical mutagenesis or RNA interference (RNAi) 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(lf)* mutants.

During an experimental evolution study comprising 74 *fog-2(lf)* 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

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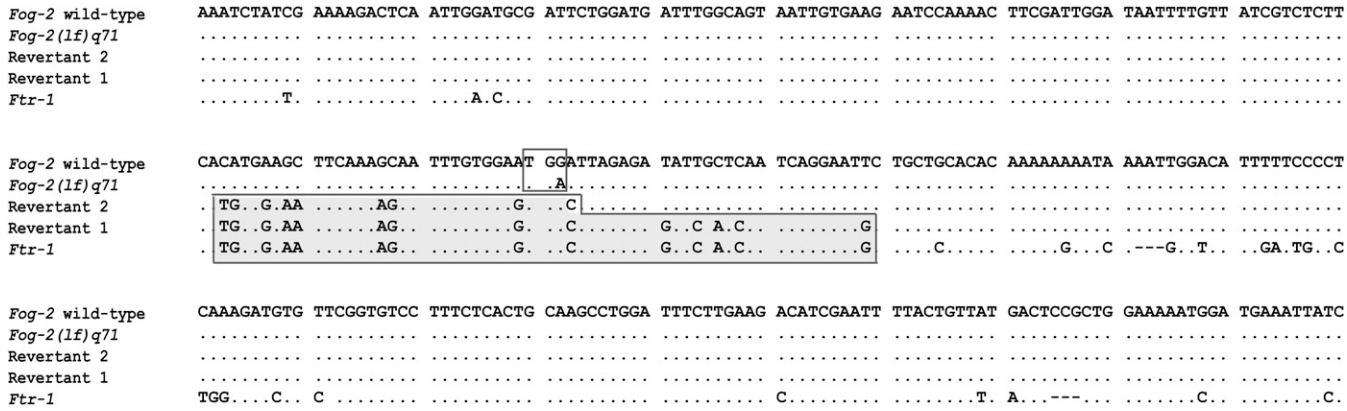

FIGURE 1.—Nucleotide sequence alignments representing two independent gene conversion events at the *fog-2* locus by *ftr-1* resulting in a switch from obligate outcrossing to hermaphroditism in two *fog-2(lf)* mutant lines. In-frame nucleotide positions 200–499 of exon 3 (total length 640 bp) are displayed. 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 shaded boxed area represents the minimum gene conversion tracts by the upstream *ftr-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.

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. Each of the two sex reversal events resulted from a gene conversion whereby a short segment of a paralogous gene *ftr-1* recombined with the *fog-2(lf)* mutant allele, replacing the premature stop codon with a tryptophan codon (Figure 1). 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%, a few large segments 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 *ftr-1* and *fog-2* loci (Figure 2) may also facilitate a high frequency of gene conversion between them. Indeed, studies of gene conversion events in both *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 *vs.* linked genes). Finally, the chromosomal location of *fog-2* and *ftr-1* may further enhance the rate of gene conversion. Both genes reside close to the right end of chromosome V. Chromosomal arms in *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 (*i.e.*, gene conversion between *fog-2* and members of the *ftr* family) are important in nature. Most

FIGURE 2.—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 shaded. 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 ~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). Regarding *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*. 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. The loci of both paralogs, *ftr-1* and *fog-2*, 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 *ftr-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).

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 *fog-2* could be advantageous in *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 *C. elegans*. Despite the fact that male sperm readily overwhelms hermaphroditic sperm in the event of a male-hermaphrodite mating, *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 *C. elegans* appears to have degenerated relative to other obligate outcrossers in the genus, such as *C. remanei* and Caenorhabditis spp. 4 (RENE GARCIA *et al.* 2007). Nonetheless, gene conversion between *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 *vs.* outcrossing in nature.

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LITERATURE CITED

BARNES, T. M., Y. KOHARA, A. COULSON and S. HEKIMI, 1995 Meiotic recombination, noncoding DNA and genomic organization in *Caenorhabditis elegans*. Genetics **141:** 159–179.

BARTON, M. K., T. SCHEDL and J. KIMBLE, 1987 Gain-of-function mutations of *fem-3*, a sex determination gene in *Caenorhabditis elegans*. Genetics **115:** 107–119.

CHASNOV, J. R., and K. L. CHOW, 2002 Why are there males in the hermaphroditic species *Caenorhabditis elegans*? Genetics **160:** 983–994.

CLIFFORD, R., M. LEE, S. NAYAK, M. OHMACHI, F. GIORGINI *et al.*, 2000 FOG-2, a novel F- box-containing protein, associates with the GLD-1 RNA-binding protein and directs male sex determination in the *C. elegans* hermaphrodite germline. Development **127:** 5265–5276.

CUTTER, A. D., 2005 Mutation and the experimental evolution of outcrossing in *Caenorhabditis elegans*. J. Evol. Biol. **18:** 27–34.

DROUIN, G., 2002 Characterization of the gene conversions between the multigene family members of the yeast genome. J. Mol. Evol. **55:** 14–23.

FRANCIS, R., M. K. BARTON, J. KIMBLE and T. SCHEDL, 1995a *gld-1*, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. Genetics **139:** 579–606.

FRANCIS, R., E. MAINE and T. SCHEDL, 1995b Analysis of the multiple roles of *gld-1* in germline development: interactions with the sex determination cascade and the *glp-1* signaling pathway. Genetics **139:** 607–630.

HAAG, E. S., 2005 The evolution of nematode sex determination: *C. elegans* as a reference point for comparative biology, in *Wormbook*, edited by THE *C. ELEGANS* RESEARCH COMMUNITY. http://www.wormbook.org.

HILL, R. C., C. E. DE CARVALHO, J. SALOGIANNIS, B. SCHLAGER, D. PILGRIM *et al.*, 2006 Genetic flexibility in the convergent evolution of hermaphroditism in *Caenorhabditis* nematodes. Dev. Cell **10:** 531–538.

HILLIER, L. W., R. D. MILLER, S. E. BAIRD, A. CHINWALLA, L. A. FULTON *et al.*, 2007 Comparison of *C. elegans* and *C. briggsae* genome sequences reveals extensive conservation of chromosome organization and synteny. PLoS Biol. **5:** 1603–1616.

HODGKIN, J., and T. DONIACH, 1997 Natural variation and copulatory plug formation in *Caenorhabditis elegans*. Genetics **146:** 149–164.

Jeffreys, A. C., and C. A. May, 2004 Intense and highly localized gene conversion activity in human meiotic crossover hot spots. Nat. Genet. **36:** 151–156.

Jukes, T. H., and C. R. Cantor, 1969 Evolution of protein molecules, pp. 21–123 in *Mammalian Protein Metabolism*, edited by H. N. Munro. Academic Press, New York.

Kamath, R. S., M. Martinez-Campos, P. Zipperlein, A. G. Frazer and J. Ahringer, 2001 Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegan*s. Genome Biol. **2**:research0002.

Katju, V., and M. Lynch, 2003 The structure and early evolution of recently arisen gene duplicates in the *Caenorhabditis elegans* genome. Genetics **165:** 1793–1803.

Katju, V., and M. Lynch, 2006 On the formation of novel genes by duplication in the *Caenorhabditis elegans* genome. Mol. Biol. Evol. **23:** 1056–1067.

Kiontke, K., N. P. Gavin, Y. Raynes, C. Roehrig, F. Piano *et al.*, 2004 *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. Proc. Natl. Acad. Sci. USA **101:** 9003–9008.

Kiontke, K., and W. Sudhaus, 2006 Ecology of *Caenorhabditis* species, in *Wormbook*, edited by The *C. elegans* Research Community. http://www.wormbook.org.

Maine, E., D. Hansen, D. Springer and V. E. Vought, 2004 *Caenorhabditis elegans atx-2* promotes germline proliferation and oocyte fate. Genetics **168:** 817–830.

Manoel, D., S. Carvalho, P. C. Phillips and H. Teotónio, 2007 Selection against males in *Caenorhabditis elegans* under two mutational treatments. Proc. R. Soc. Lond. Ser. B **274:** 417–424.

Nayak, S., J. Goree and T. Schedl, 2005 *fog-2* and the evolution of self-fertile hermaphroditism in *Caenorhabditis*. PLoS Biol. **3:** 57–71.

Nei, M., and T. Gojobori, 1986 Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. **3:** 418–426.

Plasterk, R. H. A., and J. T. M. Groenen, 1992 Targeted alterations of the *Caenorhabditis elegans* genome by transgene instructed DNA double strand break repair following Tc1 excision. EMBO J. **11:** 287–290.

Rene Garcia, L., B. LeBoeuf and P. Koo, 2007 Diversity in mating behavior of hermaphroditic and male-female Caenorhabditis nematodes. Genetics **175:** 1761–1771.

Sawyer, S. A, 1999 GENECONV: a computer package for the statistical detection of gene conversion. Washington University, St. Louis. http://www.math.wustl.edu/~sawyer.

Schedl, T., and J. Kimble, 1988 *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics **119:** 43–61.

Semple, C., and K. H. Wolfe, 1999 Gene duplication and gene conversion in the *Caenorhabditis elegans* genome. J. Mol. Evol. **48:** 555–564.

Stewart, A. D., and P. C. Phillips, 2002 Selection and maintenance of androdioecy in *Caenorhabditis elegans*. Genetics **160:** 975–982.

Ward, S., and J. S. Carrel, 1979 Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. Dev. Biol. **73:** 304–321.

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