

## Supplemental Data

### Genetic Flexibility in the Convergent Evolution

### of Hermaphroditism in *Caenorhabditis* Nematodes

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## Supplemental Results

### *Cb-fem-2(nm27)* Is Not Maternally Rescued or Temperature-Sensitive

In *C. elegans*, male development of XO *fem-2* homozygotes can be rescued by a single maternal wild-type allele (Hodgkin, 1986). Furthermore, feminization is completely penetrant only at elevated temperatures (25° C), even in null alleles. Crossing *Cb-fem-2(nm27)/+* hermaphrodites and males produces reduced male frequencies consistent with transformation of the 25% homozygous XO offspring (Table S1). To more rigorously test for maternal rescue, we crossed *Cb-fem-2(nm27)/+*; *dpy(nm4)/+* hermaphrodites with *Cb-fem-2(nm27)/+* III; *syIs802* X males, and scored plates that produced few or no self progeny. We observed that 20% of the non-GFP (*i.e.* XO) progeny (N=331) were hermaphrodites, indicating a single maternal copy of *Cb-fem-2* is not sufficient to rescue male development. Furthermore, sexual transformation of *Cb-fem-2(nm27)* XO animals into hermaphrodites is complete at 20°. These results show that *C. briggsae fem-2* sex determination function is neither temperature-sensitive nor maternally provided, unlike in *C. elegans*.

## Supplemental Experimental Procedures

### Primers Used in Isolation of a *Cb-fem-2* Deletion:

#### Outer PCR:

2BOL 5' AGTTTCCAGGATCTCCACTTGG 3'

2BOR 5' CGTATCGAGAAGAGATCTCG 3'

#### Inner PCR:

2BIL 5' TCATGACGTTTTCCGAGATGC 3'

2BIR 5' TCCTAAGCCTGTACTTAAGCC 3'

#### Wild-type Only Primers:

EH21 5' TGCTCCCAATACGCTGCTGGGC 3'

EH22 5' CGAGATCATCGGTCCGCCAGGG 3'

**Primers Used in Isolation of a *Cb-fem-3* Deletion:**

Outer PCR:

3A0L2 5' GTGGTGATTCTGCACATTGGACG 3'

3AOR2 5' CTGCCAAAAGCAACGATCGCGAG 3'

Inner PCR:

3AIL2 5' GAAATAGTGTGCGAAACGAGGAGG 3'

3AIR2 5' TGACTAACCCCTCTTCCAACATGGC 3'

Wild-type Only Primers:

RH05 5' AGACGTTACGAACTGATCTCCAGG 3'

RH06 5' CATCGTGATACAGTAGTCGACACG 3'

**Table S1. Sex Ratio and *Cb-fem* Genotypes**

| Mother   | Father                  | Mean Brood size <sup>1</sup> | % Males | No. Crosses       | No. Progeny |
|--|-------------------------|------------------------------|---------|-------------------|-------------|
| AF16   | self                    | 200                          | < 0.1   | 6                 | 1203        |
| AF16   | AF16                    | 288                          | 39.7    | 5                 | 1439        |
| AF16   | <i>Cb-fem-2(nm27)/+</i> | 273                          | 34.6    | 5                 | 1364        |
| <i>Cb-fem-2(nm27)</i>  | self                    | 124 <sup>2</sup>             | 0.0     | 17                | 2110        |
| <i>Cb-fem-2(nm27)</i>  | AF16                    | 209                          | 39.6    | 10                | 2093        |
| <i>Cb-fem-2(nm27)</i>  | <i>Cb-fem-2(nm27)/+</i> | 256                          | 22.8    | 8                 | 2051        |
| <i>Cb-fem-2(nm27)/+</i>                                      | self                    | 182                          | 0.0     | 5                 | 912         |
| <i>Cb-fem-2(nm27)/+</i>                                      | AF16                    | 195                          | 36.8    | 8                 | 1560        |
| <i>Cb-fem-2(nm27)/+</i>                                      | <i>Cb-fem-2(nm27)/+</i> | 302                          | 33.2    | 7                 | 2112        |
| <i>syIs802[myo-2::GFP] X/+</i>                               | self                    | 57                           | 32.5    | 7                 | 400         |
| <i>syIs802[myo-2::GFP] X/+;</i><br><i>Cb-fem-2(nm27) III</i> | self                    | 80                           | 0.0     | 4                 | 319         |
| <i>Cb-fem-3(nm63)</i>  | self                    | 234                          | 0.0     | 10                | 2341        |
| <i>syIs802[myo-2::GFP] X/+;</i><br><i>Cb-fem-3(nm63) IV</i>  | self                    | n.d. <sup>3</sup>            | 0.0     | n.d. <sup>3</sup> | 169         |
| <i>Cb-fem-2(nm27); Cb-fem-3(nm63)</i>                        | self                    | 136                          | 0.0     | 6                 | 816         |

<sup>1</sup> Scored only if hermaphrodite remained on plate for at least 48 h.

<sup>2</sup> Although this brood size is lower than wild-type, it is not seen in *Cb-fem-2(nm27); Cb-fem-3* double mutants. This could be real suppression, but the low *Cb-fem-2* brood size may be due to a quirk of the genetic background of CP36, or to a minor problem with the culture media at the time the counts were done (separated in time by over a year).

<sup>3</sup> A high degree of embryonic lethality was seen, which was somewhat alleviated by growth at 15°. This may be due to enhancement of the *syIs802*-mediated meiotic nondisjunction in the *nm63* background.