# Clustered Organization of Reproductive Genes in the *C. elegans* Genome

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## Supplemental Experimental Procedures

### Statistical Analyses

Gamete-enriched genes were identified from a DNA microarray dataset consisting of 17,563 loci hybridized to RNA from *fem-1(hc17ts)* and *fem-3(q23gf)* mutants [S1]. A *P* value cutoff of ≤0.0005 from a two-sample t test on log10 transformed expression levels using genes with mean *fem-3/fem-1* ratios ≥2 and *fem-1/fem-3* ratios ≤0.5 identified 887 sperm-enriched genes and 523 oocyte-enriched genes, respectively. The binomial probability *(P)* of observing *k* or more sperm-enriched genes in a region given their overall frequency in the genome *(p)* and the total number of genes in the region (*n*) was computed as

$$P[x \geq k] = \Sigma \binom{n}{k} p^k (1 - p)^{n-k}.$$

Chromosome regions were defined by walking along chromosomes using nonoverlapping windows 0.5 cM long. A 2 × 2 contingency table and $\chi^2$ test were used to determine if the positions of genes in DNA microarray datasets or biological processes, which were obtained from data that is published or available at Wormbase (www.wormbase.org), are biased toward SGC-containing regions (a likelihood-ratio G test produced similar results). Tables were constructed by using the frequency of SGC-linked loci and nonlinked loci from a set of genes defined in the text and of total linked (1353 loci within 0.5 cM [Figure 2] or 3900 loci within 3.0 cM [Table 1]) and nonlinked loci in the genome, which collectively was estimated to be 20,000. Similar clustered distributions are observed with a smaller published dataset consisting of 11,917 loci [S6].

### Classifying Functional Relationships

Functionally related genes were identified by literature searches, by sequence annotations (cytoskeletal and cell cycle regulators), from published and unpublished (available at www.wormbase.edu) genetic screens, or by phenotypic analysis of mutant alleles. Relatively few processes have been characterized in sufficient detail to test whether the component genes are distributed randomly (we set a limit of n > 10 genes for analysis). Windows spanning 3.0 cM from *msp* loci were used to detect nonrandom relationships, although similar results are observed by using 2.0 and 2.5 cM. For the oocyte maturation and ovulation category, we focused on genes encoding signaling proteins rather than genes encoding proteins such as transcription factors, which may have indirect roles in regulating maturation/ovulation. For the MAPK regulation category, we included all genes implicated in regulating MAPK in the germ line. In the sperm/oocyte switch category, we considered all genes implicated in the posttranscriptional regulation of *fem-3*. Genes involved in the sperm signaling mechanism were excluded from other categories, and *msp* loci were considered as a single gene. References for these clustered loci are in Table S1. Nonclustered loci in sperm signaling and sperm/oocyte switch categories are *oma-2* (V), *sem-5* (X), *mpk-1* (III), *elf-1* (V), *mek-2* (I), *ceh-18* (X), *tra-3* (IV), *mag-1* (I), *fem-2* (III), *mog-1* (III), *mog-3* (III), *mog-4* (II), *mog-6* (II), *ipp-5* (X), *lfe-2* (I), and *let-502* (I). Smaller clusters outside of *msp*-containing regions may exist in the genome, but there are too few loci currently characterized to make definitive conclusions.

### Phenotypic Analyses

The wild-type *C. elegans* strain N2 and *C. briggsae* strain AF16 were used for phenotypic studies. *cav-1(ok270), tsp-12(ok239)*, and *inx-8(gk42)* are deletion mutants obtained from the International *C. elegans* Gene Knockout Consortium. *cav-1(ok270)* deletes 2480 bp including all exons, *tsp-12(ok239)* deletes 1379 bp including the translational start site and the first two exons, and *inx-8(gk42)* deletes 850 bp including the translational start site and first exon. These deletion mutants were backcrossed to the wild-type strain four to six times prior to phenotypic analysis. RNAi produced phenotypes identical to the mutant alleles. All other strains used are published (see Table S1); in stated cases, mutants were crossed into the *fog-2(q71)* background as previously described [S2]. Reproductive functions of genes were inferred by comparing mutant hermaphrodites to wild-type hermaphrodites or mutant *fog-2(q71)* females to *fog-2(q71)* females. Each mutant was analyzed for defects in fertility, brood size, meiotic maturation, gonadal sheath contraction, MAPK activation, and spermathecal dilation using published methods [S2, S3]. RNAi and phenotypic analysis was carried out at 20°C or 25°C as described [S2, S3]. Experiments were repeated at least twice. Quantitative data from phenotypic studies of SGC-linked loci are available in Table S1.

### Bioinformatics

*C. elegans* and *C. briggsae* sequences and positions were obtained from Wormbase (releases WS77 through WS91). Although *C. briggsae* supercontigs have not been linked, most are large enough (up to several megabases) to evaluate gene order relationships. To identify *C. briggsae* orthologs of *C. elegans* genes, we used the Wobble Aware Bulk Aligner (WABA) algorithm [S4] and reciprocal BlastP [S5] searches. Two genes were considered orthologous if the WABA algorithm for a given *C. elegans* gene yielded a single hit in the *C. briggsae* genome. If the algorithm yielded multiple hits, then the genes were considered homologs. If multiple *C. elegans* genes hit


Figure S1. *C. briggsae* Chromosomal Regions Contain Combinations of Reproductive Genes that Are Not Observed in the *C. elegans* Genome

Supercontig cb25.fpc2234 is syntenic to a region in *C. elegans* chromosome III containing the sex determination genes *mog-1* and *cpb-1* and seven sperm genes (black arrows). In addition to these genes, cb25.fpc2234 also harbors two *msp* loci, a gene closely related to the sex determination genes *fbf-1* and *fbf-2* and four homologs of sperm genes from the *C. elegans* SGCs (red arrows). A similar relationship is observed in *C. briggsae* supercontig cb25.fpc0071, which is syntenic to a region in *C. elegans* chromosome I containing the meiotic regulator *mek-2* and six sperm genes (black arrows). cb25.fpc0071 also contains homologs of ten sperm genes from the *C. elegans* SGCs (red arrows) and of two spermathecal valve regulators (*rme-2* and *let-502)*, and a relative of the *C. elegans* sex determination proteins *cpb-1* and *fog-1*. Genes in red occupy different relative positions in the two genomes whereas those in black are conserved.

Table S1. Position and Expression Data Table

| Gene | Name | Chr | Start (Bps) | cM | *fem-3/fem-1* | *C. briggsae* Match |
|---|---|---|---|---|---|---|
| SGC II-Linked | | | | | | |
| M03A1.1 | *vab-1* | 2 | 4572081 | −3.09 | 0.24 | cb25.fpc3052:560731..583818 |
| F26G1.7 | *msp-3* | 2 | 4785890 | | 50.49 | See Figure S2 |
| C33F10.9 | *msp-40* | 2 | 4825406 | | 51.64 | See Figure S2 |
| R05F9.8 | *msp-33* | 2 | 4900222 | | 87.17 | See Figure S2 |
| R05F9.3 | *msp-32* | 2 | 4898606 | | 15.49 | See Figure S2 |
| R05F9.13 | *msp-31* | 2 | 4916169 | | 50.6 | See Figure S2 |
| ZK546.6 | *msp-152* | 2 | 4933016 | −2.39 | 29.89 | See Figure S2 |
| F58A6.8 | *msp-45* | 2 | 5145912 | | 35.21 | See Figure S2 |
| C34F11.6 | *msp-49* | 2 | 5194350 | −1.87 | 25.9 | See Figure S2 |
| C34F11.4 | *msp-50* | 2 | 5199781 | | 61.08 | See Figure S2 |
| EEED8.5 | *mog-5* | 2 | 5399512 | | 0.61 | cb25.fpc0058:488947..493092 |
| F07F6.6 | *nmr-1* | 2 | 5440288 | −1.43 | 0.93 | cb25.fpc0058:524581..530078 |
| F56D1.4 | *clr-1* | 2 | 5466447 | −1.37 | 0.55 | cb25.fpc0058:547708..554434 |
| K05F1.7 | *msp-63* | 2 | 5797032 | −1.03 | 19.93 | See Figure S2 |
| K05F1.2 | *msp-142* | 2 | 5797894 | | 19.27 | See Figure S2 |
| ZK1248.6 | *msp-64* | 2 | 5809914 | −0.99 | 59.93 | See Figure S2 |
| F59G1.5 | *ptp-2* | 2 | 5918677 | −0.91 | 0.31 | cb25.fpc0058:1004885..1007801 |
| H12I13.A | *fbf-1* | 2 | 6078506 | | 1.63 | cb25.fpc0058:1702880..1704000 |
| F21H12.5 | *fbf-2* | 2 | 6089098 | −0.70 | 0.81 | cb25.fpc0058:1702880..1704000 |
| C15F1.F | *tra-2* | 2 | 6955552 | 0.18 | 0.29 | cb25.fpc2454:301032..315030 |
| ZK1127.1 | *nos-2* | 2 | 7065606 | 0.23 | 0.17 | NA_214:13209..14111 |
| T07F8.3 | *gld-3* | 2 | 7132575 | 0.28 | 0.53 | cb25.fpc2969:31173..34993 |
| E04F6.11 | *clh-3* | 2 | 7218345 | 0.31 | 0.53 | cb25.fpc2969:31173..34993 |
| ZK675.1 | *ptc-1* | 2 | 7893189 | 0.62 | 0.46 | cb25.fpc0058:4146090..4152274 |
| T23G7.1 | *dpl-1* | 2 | 9169866 | 1.04 | 0.18 | cb25.fpc0022:904496..908713 |
| ZK1067.1 | *let-23* | 2 | 9197428 | 1.05 | 1.54 | cb25.fpc0022:446665..482768 |
| C06C3.1 | *mel-11* | 2 | 9358445 | 1.16 | 0.72 | cb25.fpc0022:446665..482768 |
| Y53C12B.3 | *nos-3* | 2 | 9746644 | 1.68 | 0.49 | cb25.fpc0022:1414305..1417827 |
| Not cloned | *mog-2* | 2 | Not cloned | ∼−3.8 | | |
| Not cloned | *QTL* [S27] | 2 | Not cloned | ∼−0.53 | | |
| Not cloned | *QTL* [S28] | 2 | Not cloned | ∼0.16 | | |
| Not cloned | *QTL* [S27] | 2 | Not cloned | ∼1.85 | | |
| Not cloned | *mog-6* | 2 | Not cloned | ∼3.4 | | |
| SGC IV-A-Linked | | | | | | |
| C09B9.6 | *msp-55* | 4 | 5055825 | | 67.43 | See Figure S2 |
| R13H9.2 | *msp-57* | 4 | 5062772 | 1.52 | 77.54 | See Figure S2 |
| R13H9.4 | *msp-53* | 4 | 5064872 | | 75.97 | See Figure S2 |
| AC7.2 | *soc-2* | 4 | 5127596 | 1.63 | 1.18 | cb25.fpc4152:846517..851248 |
| F36H12.7 | *msp-19* | 4 | 5260782 | | 70.28 | See Figure S2 |
| ZK354.5 | *msp-51* | 4 | 5295150 | 1.77 | 98.37 | See Figure S2 |
| ZK354.4 | *msp-113* | 4 | 5298155 | | 54.62 | See Figure S2 |
| ZK354.11 | *msp-59* | 4 | 5312921 | | 78.46 | See Figure S2 |
| ZK354.1 | *msp-65* | 4 | 5316716 | | 87.73 | See Figure S2 |
| T11F8.3 | *rme-2* | 4 | 5470229 | 1.89 | 0.36 | cb25.fpc0071:604666..607888 |
| F35D6.1 | *fem-1* | 4 | 5533595 | 1.93 | 1.93 | cb25.fpc4152:459969..465744 |
| Y73B6A.A | *lin-45* | 4 | 6661424 | 3.20 | 12.87 | cb25.fpc0110:66784..70501 |
| C05B10.1 | *lip-1* | 4 | 6843152 | 3.23 | 0.32 | cb25.fpc0143:24387..25690 |
| F33D4.2 | *itr-1* | 4 | 7675362 | 3.47 | 0.92 | cb25.fpc0143:844979..861922 |
| Not cloned | *QTL* [S27] | 4 | Not cloned | ∼2.18 | | |
| Not cloned | *QTL* [S28] | 4 | Not cloned | ∼2.63 | | |
| SGC IV-B-Linked | | | | | | |
| C09G9.6 | *oma-1* | 4 | 8889272 | 3.94 | 0.31 | cb25.fpc0058:2183523..2186322 |
| C01F6.4 | *fem-3* | 4 | 9105386 | 4.07 | 0.27 | cb25.fpc4260 |
| ZK1251.6 | *msp-76* | 4 | 9689195 | | 36.98 | See Figure S2 |
| T13F2.10 | *msp-79* | 4 | 9766948 | 4.43 | 14.58 | See Figure S2 |
| T13F2.11 | *msp-78* | 4 | 9770223 | | 72.47 | See Figure S2 |
| T13F2.8 | *cav-1* | 4 | 9772709 | 4.43 | 0.25 | cb25.fpc3857:4738755..4740317 |
| K07F5.1 | *msp-81* | 4 | 9836211 | 4.45 | 43.1 | See Figure S2 |
| K07F5.2 | *msp-10* | 4 | 9837826 | | 46.77 | See Figure S2 |
| K07F5.3 | *msp-56* | 4 | 9841716 | | 103.12 | See Figure S2 |
| F32B6.6 | *msp-77* | 4 | 9894806 | | 73.68 | See Figure S2 |
| C04G2.4 | *msp-36* | 4 | 10095608 | | 91.09 | See Figure S2 |
| K08F4.8 | *msp-38* | 4 | 10145000 | | NA | See Figure S2 |

(*continued*)

Table S1. Continued

| Gene | Name | Chr | Start (Bps) | cM | *fem-3/fem-1* | *C. briggsae* Match |
|---|---|---|---|---|---|---|
| T14G10.6 | *tsp-12* | 4 | 10149607 | 4.53 | 0.24 | cb25.cb25.fpc0063:423198..424410 |
| K11E8.1 | *unc-43* | 4 | 10325170 | 4.57 | 1.09 | cb25.fpc0081:17847..34241 |
| C43F9.8 | *efn-2* | 4 | 10576326 | 4.68 | NA | cb25.fpc0081:132650..134651 |
| F36H1.4 | *lin-3* | 4 | 11057989 | 4.82 | 0.42 | cb25.fpc0143:1021571..1028863 |
| M04B2.1 | *mep-1* | 4 | 11524816 | | 0.5 | cb25.fpc0143:1495783..1499363 |
| ZK792.2 | *inx-8* | 4 | 11674045 | 5.17 | 1.07 | cb25.fpc0143:1371149..1373569 |
| ZK792.3 | *inx-9* | 4 | 11676556 | | 1.11 | cb25.fpc0143:1371159..1373357 |
| ZK792.6 | *let-60* | 4 | 11689218 | 5.17 | 0.65 | cb25.fpc0143:1353419..1355154 |
| F28D1.10 | *gex-3* | 4 | 12404606 | 5.85 | 0.42 | cb25.fpc0063:71602..78136 |
| C01C7.1 | *ark-1* | 4 | 12622656 | 6.41 | 0.64 | cb25.fpc3052:926992..931609 |

Chromosomal positions and microarray data are shown for all functionally related loci referred to in the text, according to cluster. Functional data and references are shown following the table in text format. Only genes are included for which there is genetic or biochemical data related to function. See Reinke et al. [S1, S6] for microarray expression data of all genes. *fem-3/fem-1* represents mRNA enrichment ratios in mutant hermaphrodites that only make sperm (*fem-3*) to those that only make oocytes (*fem-1*) [S1]. *C. briggsae* match shows the closest relative by using the WABA alignment [S4]. Gene position is shown in contig number:basepairs.

the same *C. briggsae* gene(s), then the genes were considered paralogs (except for the *msps*, which were analyzed by using phylogenetic methods). Phylogenetic analyses of *msp* gene nucleotide sequences were performed by using parsimony and distance methods as described [S3].

We used comparative mapping to examine reproductive gene distribution in the genome of *Brugia malayi*, a nematode that diverged from *C. elegans* roughly 300–500 Mya. The *Brugia* genome has been sequenced with coverage of 5.1× based on an estimated genome size of 110 Mb. To identify *Brugia* homologs, BlastP searches [S5] were performed on 115 total protein sequences encoded by clustered *C. elegans* loci. A Blast statistical value of $10^{-6}$ was used as a minimum threshold for this comparison. In cases where multiple sequences matched with a value above $10^{-6}$, the top three were considered. Blast statistical values ranged from $10^{-1}$ to $10^{-285}$. Based on the $10^{-6}$ threshold, putative *Brugia* homologs were identified for 86 out of 115 loci. The sizes of *Brugia* contigs containing the putative homologs ranged from 2 to 200 kb. 200 kb chromosomal regions in the *C. elegans* SGCs harbor up to 24 sperm genes, indicating that these sizes are sufficient to detect conserved relationships among reproductive genes. However, this size range limited our ability to detect long distance relationships. The *Brugia* genome contained three *msp* homologs, which are all located on contig# 1131595. All three *msps* have a single intron. We performed BlastP searches with protein sequences from adjacent *C. elegans* genes located in the SGCs. Out of 68 *C. elegans* gene neighbors, only six pairs are found on the same *Brugia* contig, even when multiple sequence matches are considered. Furthermore, >90% of the genes surveyed with *Brugia* matches are located on distinct contigs, none of which contained the *Brugia msps*. These results indicate that genes in the *C. elegans* SGCs are not clustered in the *Brugia malayi* genome.

## Gene Functional Data

### *SGC II Linked*

*vab-1* encodes an Eph receptor protein-tyrosine kinase homolog. In *C. elegans*, VAB-1 is an MSP and ephrin receptor. In the absence of MSP, *vab-1* negatively regulates oocyte maturation, MAP kinase activation, and ovulation. It is also a positive regulator of basal sheath contractions [S7].

*msps* encode members of the major sperm-specific protein (MSP) family. They are sperm cytoskeletal proteins that also act as signals for oocyte maturation, MAP kinase activation, sheath contraction, and spermathecal dilation ([S3, S7]; cytoskeletal function reviewed in [S8]).

*mog-5* encodes a member of the RNA helicase, DEAH-box protein family. It is required for the hermaphrodite switch to oogenesis [S29]. MOG-5 binds the zinc finger protein MEP-1 and regulates *fem-3* posttranscriptionally [S9].

*nmr-1* encodes a putative ortholog of *H. sapiens* GRIN1 protein, a NMDA glutamate receptor subunit. *nmr-1* is a negative regulator of sperm-dependent sheath cell contractions. *nmr-1(ak4)* hermaphrodites have higher sheath contraction rates (19.0 ± 4.7 cont/min, n = 10) than WT (10.1 ± 1.4 cont/min, n = 13). Old *nmr-1(ak4)* hermaphrodites that are depleted of sperm have low contraction rates (2.3 ± 1.7 cont/min, n = 5). Also, *nmr-1(ak4)* hermaphrodites frequently lay misshapen eggs. *nmr-1* is expressed in oocytes where it functions to negatively regulate maturation, MAPK activation, and ovulation (M.A.M., unpublished data).

*clr-1* encodes a type II receptor protein-tyrosine phosphatase. CLR-1 is a possible MSP receptor or receptor regulator based on MSP-FITC staining to oocytes (M.A.M., unpublished data). *clr-1* functions with VAB-1 as a weak negative regulator of oocyte maturation and ovulation. *clr-1(RNAi), clr-1(e1745); soc-1(n1789)*, and *clr-1(n1992); egl-15(n1477)* have reduced MSP-FITC binding to oocytes when compared to WT. *vab-1(e2); clr-1(RNAi)* unmated females have a higher oocyte maturation and ovulation rates (0.39 ± 0.3 mat/hr, n = 9) than unmated females (0.09 ± 0.08 mat/hr, n = 25), *vab-1(e2)* unmated females (0.14 ± 0.11 mat/hr, n = 11), and *clr-1(RNAi)* unmated females (0.09 ± 0.17 mat/hr, n = 11).

*ptp-2* encodes a Src homology-2 (SH2) domain-containing protein-tyrosine phosphatase. *ptp-2* function is required for the sperm-dependent increase in the oocyte maturation and ovulation rate, and MAP kinase activation. *ptp-2(op194) unc-4(e120)* unmated females have low oocyte maturation and ovulation rates (0.05 ± 0.08 mat/hr, n = 7) that do not increase when sperm are present (0.12 ± 0.19 mat/hr, n = 7). Also, *ptp-2(op194) unc-4(e120)/+* heterozygous unmated females have an impaired ability to inhibit oocyte maturation and ovulation (0.75 ± 0.66 mat/hr, n = 12). *ptp-2(op194) unc-4(e120); him-5(e1490)* hermaphrodites do not have activated MAP kinase in the most proximal oocytes (n = 40 gonads).

*fbf-1* and *fbf-2* encode RNA-binding proteins. They are redundantly required for the hermaphrodite switch to oogenesis and are thought to act as posttranscriptional regulators of *fem-3* through a direct interaction with the fem-3 3′UTR [S10].

*tra-2* encodes a transmembrane protein. *tra-2* promotes female development in hermaphrodites. TRA-2 and FEM-3 interact directly to control somatic sex determination. In the germline, *tra-2* is posttranscriptionally regulated to allow spermatogenesis (reviewed in [S11]).

*nos-2* encodes a protein that functions in development of the germ lineage and has similarity to *D. melanogaster nanos*. It functions in the sperm/oocyte switch in hermaphrodites, although it may not physically interact with FBF [S12].

*gld-3* encodes a Bicaudal-C homolog. GLD-3 promotes the sperm fate, a sex determination effect opposite to that of FBF [S13].

*clh-3* encodes a chloride channel protein and putative ortholog of H. sapiens CLCN1 gene product (chloride channel 1). CLH-3 is activated during oocyte meiotic maturation and may function in part to modulate ovulatory contractions of gonadal sheath cells [S14].

*ptc-1* encodes a putative ortholog of *D. melanogaster* membrane protein PTC (patched). PTC-1 is expressed on the surface of oocytes in the proximal gonad. *ptc-1(ok122) unc-4(e120)* unmated females

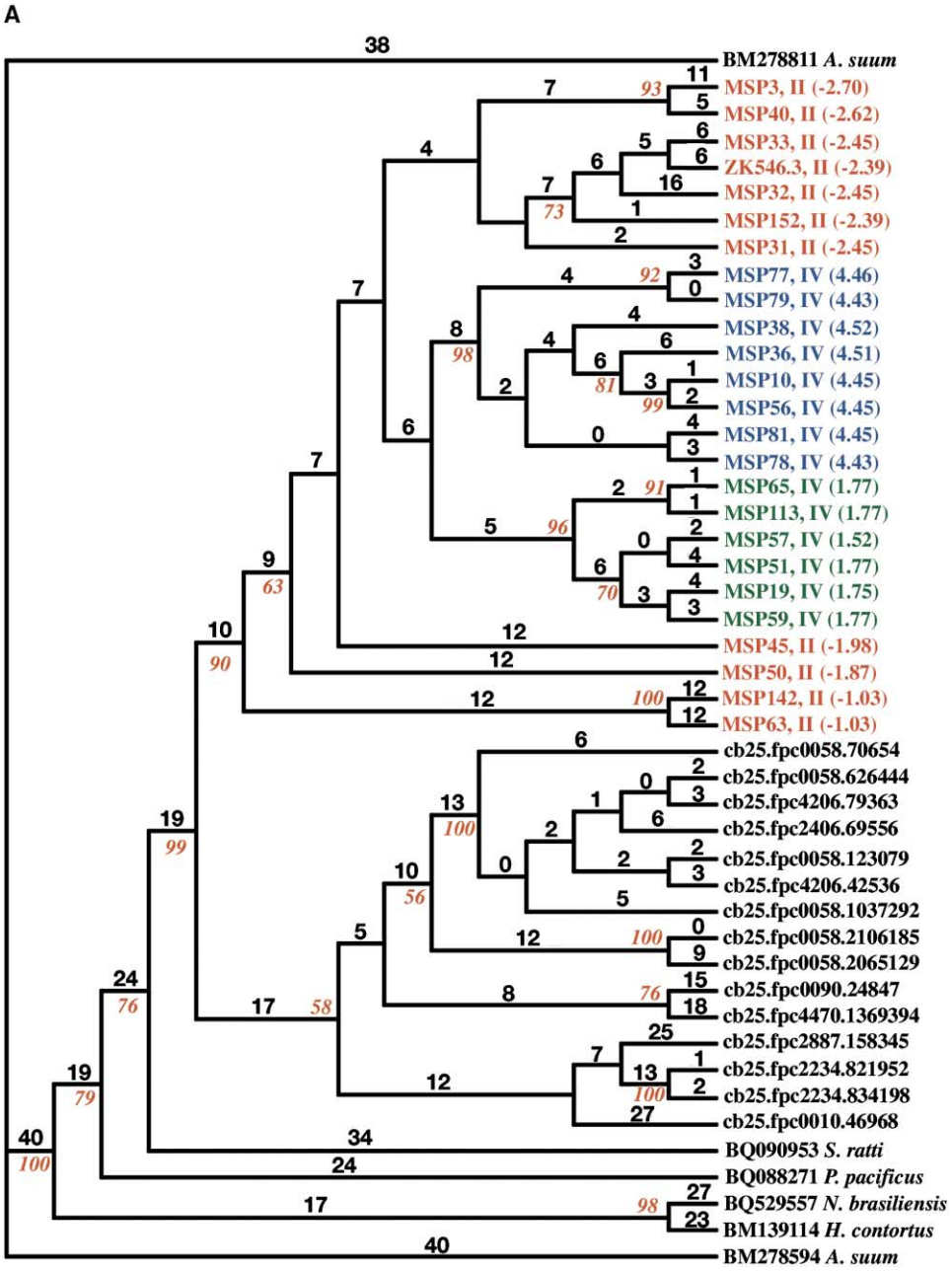


Figure S2. *Continued on next page.*

have an impaired ability to inhibit oocyte maturation and ovulation (0.56 ± 0.26 mat/hr, n = 8). Also, oocytes do not accumulate in the proximal gonads of unmated *ptc-1(ok122) unc-4(e120)* females, which contrasts with the gonads of unmated females. Spermathecal valve dilation appears delayed in *ptc-1(ok122) unc-4(e120)* hermaphrodites resulting in the uncoupling of maturation and ovulation (and infertility).

*dpl-1* encodes a putative transcription factor that has strong similarity to *D. melanogaster* E2F-related transcription factor DP. *dpl-1* mutants have more oocytes containing active MAP kinase in the gonad compared to wild-type [S15].

*let-23* encodes a protein-tyrosine kinase receptor of the EGF-receptor family that functions downstream of the EGF ligand *lin-3*. The oocyte signals spermathecal valve dilation in response to MSP. Dilation is mediated through the EGF-like ligand LIN-3 and the receptor tyrosine kinase LET-23 [S21].

*mel-11* encodes a myosin phosphatase regulatory subunit. MEL-11 is expressed in the spermatheca of the adult somatic gonad and is required for proper spermathecal valve dilation [S16].

*nos-3* encodes a protein with weak similarity to *D. melanogaster* nanos over the zinc finger domain. NOS-3 interacts directly with FBF to control fem-3 mRNA and the sperm/oocyte switch in hermaphrodites [S12].

*mog-2* is not cloned. *mog-2* mutant hermaphrodites have a masculinized germ line that does not switch to oogenesis.

***SGC IV-A-linked***

*msps* encode members of the major sperm-specific protein (MSP) family. They are sperm cytoskeletal proteins that also act as signals

B

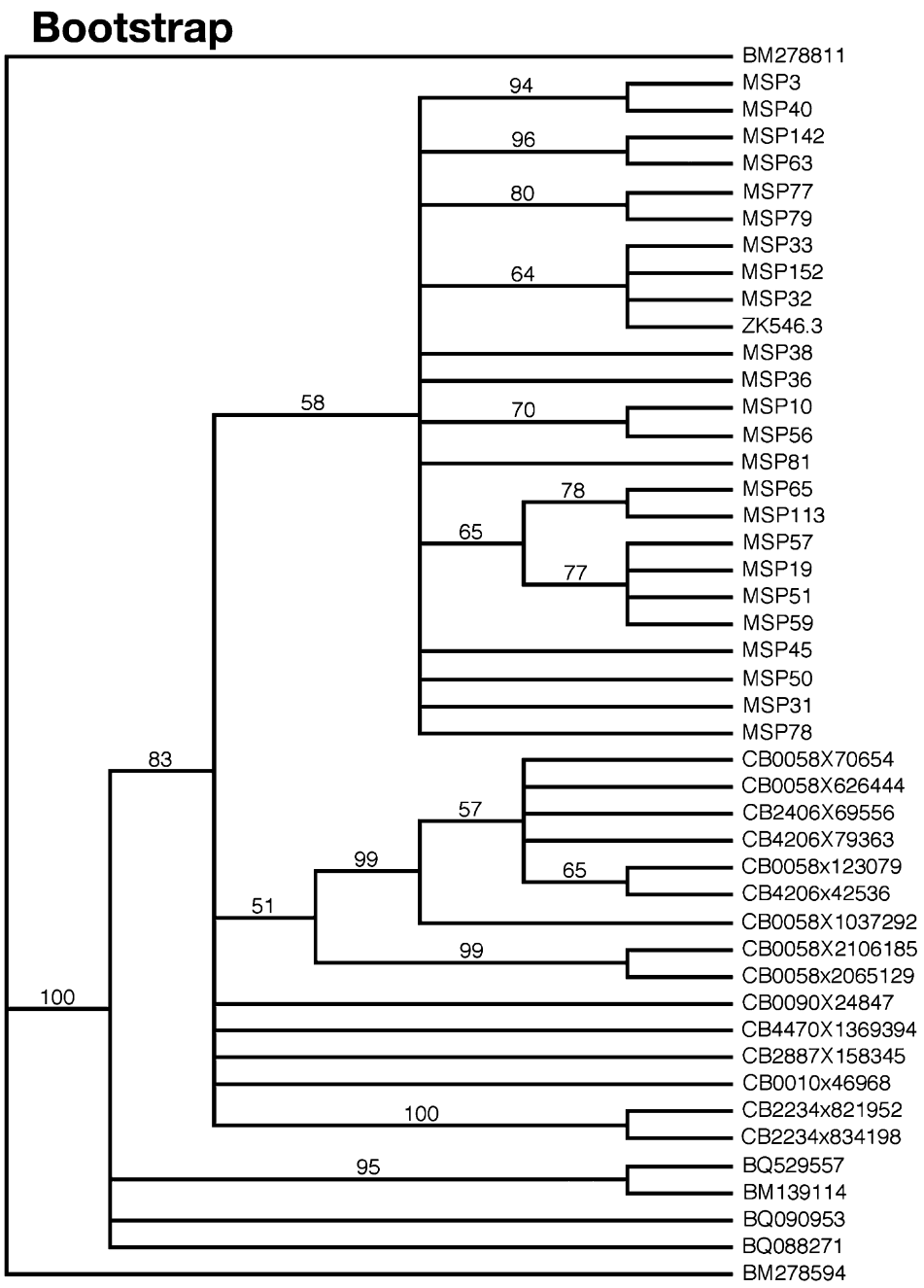


Figure 2. Neighbor-Joining and Maximum Parsimony Phylogenetic Analyses of *msp* Loci

For the neighbor-joining analysis (A), black numbers indicate branch distances and red numbers indicate bootstrap proportions. For the maximum parsimony analysis (B), bootstrap statistical support values are shown. These results support the hypothesis that *msp* loci have duplicated independently in *C. elegans* and *C. briggsae* genomes. Sequences are from www.wormbase.edu and GenBank (accession numbers are listed for *A. suum, S. ratti, P. pacificus, N. brasiliensis*, and *H. contortus*). *C. elegans msps* are listed according to gene name and chromosome position (cM). *C. briggsae msps* are listed according to their position (supercontig#. nucstartsite). Only *msp-3* and *ZK546.3*, which is a pseudogene, share the same nearest gene neighbors in *C. elegans* and *C. briggsae*.

for oocyte maturation, MAP kinase activation, sheath contraction, and spermathecal dilation ([S2, S3]; cytoskeletal function reviewed in [S8]).

*soc-2* encodes a leucine rich repeat protein. *soc-2(n1774)* hermaphrodites have fewer MAP kinase positive oocytes in the proximal gonad (2.07 $\pm$ 1.19, n = 41 gonads) compared to WT (3.21 $\pm$ 1.68, n = 38 gonads). No other sperm signaling phenotypes were detected.

*rme-2* encodes a transmembrane receptor of the LDLR family. RME-2 is expressed at the oocyte cell surface and mediates the

uptake of yolk [S17]. After oocyte maturation, spermathecal valve dilation is frequently delayed or irregular in *rme-2(b1008)* hermaphrodites. This results in the generation of large numbers of unfertilized oocytes and fertilized, misshapen eggs.

*fem-1* encodes a protein with ankyrin repeats. It is one of three sex determination fem genes required for spermatogenesis and somatic male development. *fem* gene activity must be downregulated to allow oogenesis in hermaphrodites (reviewed in [S11]).

*lin-45* encodes a raf protein serine/threonine kinase. *lin-45* functions in a pathway with the ras homolog *let-60*, the MAP kinase kinase *mek-2*, and the map kinase *mpk-1* to control the progression of oocytes through meiosis [S18, S19].

*lip-1* encodes a MAP kinase phosphatase homolog. *lip-1* regulates MAP kinase activation in the hermaphrodite germline [S20].

*itr-1* encodes a putative inositol (1,4,5) trisphosphate receptor. It functions downstream of *lin-3* and *let-23* to control dilation of the spermatheca [S21]. ITR-1 is expressed in the germ line, sheath cells, and spermatheca [S21, S22]. *itr-1* functions in sheath cells to promote contraction and in the germ line to inhibit oocyte maturation, ovulation, and MAPK activation (M.A.M., unpublished data).

***SGC IV-B-linked***

*oma-1* encodes a protein containing a putative zinc finger domain. *oma-1* and *oma-2* are redundantly required for oocyte maturation and ovulation in hermaphrodites. Both are expressed in oocytes. MAP kinase activation is not sustained in oocytes from *oma-1;oma-2* mutant hermaphrodites [S23].

*fem-1* encodes a novel protein. *fem-3* promotes spermatogenesis in the germ line and male development in somatic tissue. *fem-3* is regulated posttranscriptionally in the germ line to allow the onset of oogenesis. *fem-3* regulation is mediated by the *mog* genes, *fbfs, nos-3*, *gld-3*, and *mep-1* (reviewed in [S11]).

*msps* encode members of the major sperm-specific protein (MSP) family. They are sperm cytoskeletal proteins that also act as signals for oocyte maturation, MAP kinase activation, sheath contraction, and spermathecal dilation ([S2, S3]; cytoskeletal function reviewed in [S8]).

*cav-1* encodes a member of the caveolin protein family. CAV-1 is expressed in the hermaphrodite germline [S24]. *cav-1(ok270)* hermaphrodites have an impaired ability to inhibit oocyte maturation and ovulation after the depletion of sperm (137.4 $\pm$ 66.4 unfertilized oocytes laid per hermaphrodite [n = 9] versus 19.4 $\pm$ 11.0 (n = 13) for WT). *cav-1(ok270)* hermaphrodites also have elevated levels of MAP kinase activation in oocytes of the proximal gonad (n = 45). The oocyte maturation and ovulation rate in *cav-1(ok270)* hermaphrodites is higher than in WT (3.5 mat/hr [n = 6] for cav-1(ok270) vs 2.5 mat/hr for WT) 48 hr after the L4 stage. The oocyte maturation and ovulation rate in unmated *cav-1(ok270)* females is similar to unmated females.

*tsp-12* encodes a protein with strong similarity to human tetraspanins. *tsp-12(ok239)* hermaphrodites have an impaired ability to inhibit oocyte maturation and ovulation after the depletion of sperm (82.3 $\pm$ 22.2 unfertilized oocytes laid per hermaphrodite [n = 7] versus 19.4 $\pm$ 11.0 [n = 13] for WT). Also, *tsp-12 (RNAi)* unmated females have a higher oocyte maturation and ovulation rate (0.50 $\pm$ 0.13 mat/hr, n = 14) than unmated females (0.09 $\pm$ 0.08 mat/hr, n = 25). More oocytes of the proximal gonad have activated MAP kinase in *tsp-12(ok239)* hermaphrodites than WT (n = 30), a phenotype similar to *vab-1(dx31)* hermaphrodites [S3]. *tsp-12(ok239)* hermaphrodites have higher broods (343.3 $\pm$ 11.0, n = 7) than WT (268.8 $\pm$ 13.3, n = 13) at 25°C. Spermatogenesis in *tsp-12(ok239); fog-2(q71)* mutant males appears normal by DIC microscopy and mating tests.

*unc-43* encodes a calcium/calmodulin-dependent serine/threonine kinase type II (CaMKII). *unc-43* functions as a negative regulator of sperm-dependent sheath contractions. *unc-43(sa200)* hermaphrodites have higher basal sheath contraction rates (17.2 $\pm$ 3.5 cont/min, n = 9) than WT (10.1 $\pm$ 1.4 cont/min, n = 13). Old *unc-43(sa200)* hermaphrodites that have run out of sperm have low rates (2.0 $\pm$ 2.0 cont/min, n = 6). *unc-43* functions in oocytes to promote maturation, MAPK activation, and ovulation (M.A.M., unpublished data).

*efn-2* encodes an ephrin/Eph receptor ligand. It is required for the inhibition of oocyte maturation in the absence of sperm. *efn-2* mutants generate fewer sperm than wild-type [S3].

*lin-3* encodes a putative ligand for the LET-23 receptor. The oocyte signals spermathecal valve dilation in response to MSP. Dilation is mediated by the EGF-like ligand LIN-3, which likely functions in the oocyte, and the receptor tyrosine kinase LET-23, which likely functions in the spermatheca [S21].

*mep-1* encodes a zinc finger protein. MEP-1 acts with the MOG DEAH box proteins to control the sperm/oocyte switch via the *fem-3* 3′ untranslated region [S9].

*inx-8* and *inx-9* encode members of the OPUS/Innexin (gap junction) protein family. INX-8 and INX-9 are expressed in the somatic sheath cells covering the oocytes of the proximal gonad arm [S25]. *inx-8(gk42)* hermaphrodites have a higher basal sheath cell contraction rate (14.1 $\pm$ 3.3 cont/min, n = 13) than WT (10.1 $\pm$ 1.4 cont/min, n = 13). The oocyte maturation and ovulation rate in unmated *inx-8(gk42)* females is similar to unmated females. *inx-9(RNAi)* unmated females have a higher oocyte maturation and ovulation rate (0.89 $\pm$ 0.57 mat/hr, n = 14) than unmated females (0.09 $\pm$ 0.08 mat/hr, n = 25).

*let-60* encodes a GTP-binding protein of the ras superfamily. *let-60* functions in a pathway with the raf homolog *lin-45*, the MAP kinase kinase *mek-2*, and the map kinase *mpk-1* to control the progression of oocytes through meiosis [S18, S19].

*gex-3* encodes a putative ortholog of the *Drosophila* HEM protein, a transmembrane domain containing protein. *gex-3* is required for the normal sperm-dependent increase in the basal sheath contraction rate. *gex-3(zu196)* hermaphrodites have lower basal sheath contractile activity (3.4 $\pm$ 1.4 cont/min, n = 8) compared to WT (10.1 $\pm$ 1.4 cont/min, n = 13). Also, unmated *gex-3(RNAi)* females have a higher oocyte maturation and ovulation rate (0.88 $\pm$ 0.4 mat/hr, n = 10) than unmated females (0.09 $\pm$ 0.08 mat/hr, n = 25).

*ark-1* encodes a putative protein-tyrosine kinase with similarity to human p21cdc42 protein kinase and to *D. melanogaster* PR2 protein. ARK-1 inhibits LET-23 mediated spermathecal dilation [S26].

## Supplemental References


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