A New Dataset of Spermatogenic vs. Oogenic Transcriptomes in the Nematode Caenorhabditis elegans

Marco A. Ortiz,* Daniel Noble,* Elena P. Sorokin,† and Judith Kimble*‡,†,‡
*Department of Biochemistry, †Graduate Program in Cellular and Molecular Biology, and ‡Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, Wisconsin 53706

ABSTRACT The nematode Caenorhabditis elegans is an important model for studies of germ cell biology, including the meiotic cell cycle, gamete specification as sperm or oocyte, and gamete development. Fundamental to those studies is a genome-level knowledge of the germline transcriptome. Here, we use RNA-Seq to identify genes expressed in isolated XX gonads, which are approximately 95% germline and 5% somatic gonadal tissue. We generate data from mutants making either sperm [fem-3(q96)] or oocytes [fog-2(q71)], both grown at 22°C. Our dataset identifies a total of 10,754 mRNAs in the polyadenylated transcriptome of XX gonads, with 2748 enriched in spermatogenic gonads, 1732 enriched in oogenic gonads, and the remaining 6274 not enriched in either. These spermatogenic, oogenic, and gender-neutral gene datasets compare well with those of previous studies, but double the number of genes identified. A comparison of the additional genes found in our study with in situ hybridization patterns in the Kohara database suggests that most are expressed in the germline. We also query our RNA-Seq data for differential exon usage and find 351 mRNAs with sex-enriched isoforms. We suggest that this new dataset will prove useful for studies focusing on C. elegans germ cell biology.

Germ cell biology is central to reproduction and fertility. The nematode Caenorhabditis elegans is a well-established model for genetic and molecular analyses of germline sex determination (Ellis and Schedl 2007; Kimble and Crittenden 2007), progression through the meiotic cell cycle (Rog and Dernburg 2013), and gametogenesis (Greenstein 2007; Kimble and Crittenden 2007), molecular analyses of germline sex determination (Ellis and Schedl 2007) and a central player in ModENCODE (Gerstein et al. 2010). Moreover, C. elegans was the first metazoan with a fully sequenced genome (C. elegans Sequencing Consortium 1998) and a central player in ModENCODE (Gerstein et al. 2010). Therefore, C. elegans is poised to serve as a model to analyze germ cell biology at a comprehensive systems level.

State-of-the-art transcriptome data lie at the foundation of virtually any modern study of biological regulation. To this end, Reinke et al. (2004) reported a pioneering analysis of spermatogenic and oogenic transcriptomes. This now classic study relied on mRNAs extracted from whole animals, custom-spotted microarrays, and a 2003 version of the C. elegans genome annotation. Other studies have also generated the following relevant transcriptomes: maternal RNAs, which can be used as a proxy for a subset of oogenic RNAs (Baugh et al. 2003); germline-specific RNAs obtained from gonads extracted from adult wild-type hermaphrodites and subjected to serial analysis of gene expression (SAGE) (Wang et al. 2009); and RNAs in isolated mature sperm (Ma et al. 2014). In addition, a study identified several hundred mRNAs whose expression depends on the sperm-specific transcription factor SPE-44 (Kulkarni et al. 2012); a proteomics study discovered proteins in isolated mature oocytes (Chik et al. 2011); and RNA immunoprecipitation studies identified RNAs associated with RNA-binding proteins in adult oogenic germlines [FBF-1 (Kersher and Kimble 2010); GLD-2 and RNP-8 (Kim et al. 2010); GLD-1 (Jungkamp et al. 2011); EFL-1 and DPL-1 (Kudron et al. 2013)]. However, the classic analysis of Reinke et al. (2004) remains the only dataset available focusing on spermatogenic vs. oogenic transcriptomes.

As background for the current work, C. elegans develops as either XX hermaphrodites or XO males; XX hermaphrodites make sperm as larvae and oocytes as adults, whereas XO males make sperm only and continuously. Because nematode XX hermaphroditism...
evolved recently, closely related species remain gonochoristic (XX females and XO males), and elimination of a single gene, *fog-2* (feminization of germline), transforms *C. elegans* into a gonochoristic strain with XX females and XO males (Schedl and Kimble 1988). A single gonadal arm in adult *C. elegans* XX hermaphrodites possesses ~1000 germ cells, with stem cells at one end and differentiating gametes at the other; each isolated gonad also possesses ~25 somatic gonadal cells. Many existent sex determination mutants affect the germline, including nonconditional and temperature-sensitive alleles of varying strengths and tissue specificities. Of particular relevance to this work are temperature-sensitive *fem-3(qf)* and homozygous *fog-2* mutants. XX *fem-3(qf)* mutants make no oocytes, but instead produce sperm continuously in a hermaphrodite/female soma (Barton et al. 1987); by contrast, *fog-2* mutants make no sperm but make oocytes continuously in an equivalent soma (Schedl and Kimble 1988).

Our analysis of spermatogenic and oogenic transcriptomes begins with XX animals possessing germlines of the opposite sex but housed in somas of equivalent sex, RNAs extracted from isolated gonads, RNA-Seq data based on eight biological replicates, and the most recent version of the *C. elegans* reference genome annotations available in Ensembl (Flicek et al. 2014). Other details of our experimental design differ from those used previously, as outlined in Results. Where possible, we used the Kohara *in situ* hybridization database (NEXTDB: nematode.lab.nig.ac.jp/) to validate RNAs that previously had not been identified as expressed in the germline or previously had not been annotated as spermatogenic or oogenic. We compared our data to relevant datasets mentioned above and analyzed our data for alternative splicing. We suggest that this new dataset will prove useful in combination with other datasets for studies of germline regulation and gamete differentiation.

**MATERIALS AND METHODS**

**C. elegans strains**

We used two homozygous mutant strains: *fem-3(qf6gf)* IV, which is temperature-sensitive, and *fog-2(qf1)* V. The *fem-3(qf6gf)* stocks were maintained at 15° and experimental animals grown at 22°; *fog-2* mutants were maintained at 22°. For an immunostaining control, we used wild-type Bristol strain N2, also grown at 22°.

**Dissection of gonads for immunostaining**

Synchronized young adults (0–2 hr past the L4 to adult molt) were cut just behind the pharynx in PBS-Tween (0.1% Tween20) with 0.25 mM Levamisole; dissected animals were fixed in 3% paraformaldehyde, 0.1 M K2HPO4 for 30 min and permeabilized in 100% methanol at −20° for 30 min. Samples were washed three times in PBST and blocked in PBST plus 0.5% BSA for 30 min at room temperature. Samples were incubated with primary antibodies at 4° overnight in PBST plus 0.5% BSA at the following dilution: mouse anti-SP56 (1:100) (gift from S. Strome) and rabbit anti-RME-2 (1:500) (gift from B. Grant). They were then incubated with Cye3 and FITC conjugated secondary antibodies (Jackson Immunoresearch), both at a 1:1000 dilution in PBST plus 0.5% BSA, for 1 hr at room temperature. Finally, samples were mounted on slides in VectaShield containing DAPI to visualize DNA and imaged with a Zeiss Axioimager microscope.

**Isolation of gonads for RNA extraction**

Synchronized young adults (0–2 hr past the L4 to adult molt) were first cut behind the pharynx as described above. Gonadal arms were then cut at or near the spermathecae to isolate them from the carcass. Total RNA was extracted from the gonads using TRIzol (Invitrogen) and RNeasy Mini Kit (Qiagen) following the manufacturer’s instructions.

**Replicates and sequencing**

We generated eight independent samples for *fog-2* and another eight for *fem-3*. Each sample contained approximately 30 gonadal arms and most (14/16) had a total RNA concentration of 20–34 ng/ml. The University of Wisconsin Biotechnology Center prepared libraries for each sample using the TruSeq Illumina sequencing protocol, which includes mRNA purification (poly-A selection) and fragmentation, cDNA synthesis, end repair, adapters ligation, and DNA fragment enrichment. Each library was bar-coded and sequenced in four different lanes to obtain single-end 101-bp reads using Illumina HiSeq2000. We obtained more than 36 million reads of high-quality score (>35 mean quality score) on average per sample. All sequencing data are available in the National Institutes of Health Gene Expression Omnibus database under accession number GSE57109.

**Transcript analysis**

We used TopHat2 v2.0.11 (with −g option) (Trapnell et al. 2012) to align reads to the *C. elegans* reference genome (WBcel235.75.fa) and gene annotations (WBcel235.75.gtf) in WormBase WS240 (Ensembl) (Flicek et al. 2014). For compatibility to feature-counting software, we created sorted and indexed SAM versions of the BAM files (SAMtools) (Li et al. 2009). To create a read-count dataset, we processed SAM files with python scripts described elsewhere (Anders and Huber 2010). Our cutoff was two mapped reads per gene for each of eight replicates or a minimum of 16 total reads per gene, applied to each mutant independently. Genes with ambiguous annotations or fewer reads (<16 reads/gene) were removed. To identify differentially expressed transcripts, we used R/Bioconductor package DESeq, a common method to evaluate differential expression (Anders and Huber 2010).

**Figure 1** Immunocytochemistry of sex-specific gamete markers in wild-type and mutants used in this work for RNA-Seq. (A–C) Extruded gonads from young adult XX worms (L4/A molt+2 hr), all raised at 22°; each image merges staining of the α-SP56 sperm-specific marker (red), α-RME-2 oocyte-specific marker (green), and DAPI DNA marker (blue).
DESeq performs normalization by applying a scaling factor to each sample; this scaling factor is the median calculated from the ratios of read counts for each gene to the geometric mean of all samples (Anders and Huber 2010; Rapaport et al. 2013). We also determined abundance as rpkm using Cufflinks v2.1.1 (with −N, −u, and −b options) (Trapnell et al. 2012) to assemble isoforms and measure isoform expression. For analysis of differential exon usage between spermatogenic and oogenic gonads, we used the DEXSeq package for R/Bioconductor (Anders et al. 2012). We obtained reads per exon from the alignment files using a script accompanying the DEXSeq package. For an exon to be scored significantly different, we set a false discovery rate of <1% and a minimum fold change requirement of two-fold. We also set a minimum expression threshold of two reads per exon. We used gene annotations in WS240 and a custom script to compare our differential exon results with annotated alternative exon usage. Custom scripts were also used to count the number of exon junction–spanning reads for the alignment files to determine whether alternative exon usage was in a coding or noncoding region and to discriminate types of alternative splicing observed. The UCSC Genome Browser cell0 (www.ucsc.genome.edu) was used to visualize alternative splicing of WIG files that were converted from BAM files using SAMtools mpileup (SAMtools) (Li et al. 2009) and a custom script.

**RESULTS AND DISCUSSION**

**Experimental design**

Many mutants affecting gamete sex must be grown at 25°C, thus reducing fertility even in wild-type animals (Hirsh et al. 1976). We avoided growth at 25°C by using fog-2(q71) mutants, whose XX germline makes oocytes and only oocytes at all standard growth temperatures (Schedl and Kimble 1988), and the strong fem-3(q96gf) allele (Barton et al. 1987), which makes sperm and only sperm when raised at 22°C even 3 days into adulthood (100%; n = 150). To ensure germline sex transformation of these two mutants, we stained for sex-specific germline marker, SP56 for spermatogenic germlines (Ward et al. 1986) and RME-2 for oogenic germlines (Grant and Hirsh 1999). For wild-type animals grown at 22°C, staining was as expected with SP56-positive sperm and RME-2-positive oocytes (Figure 1A). More importantly, fem-3(q96) sperm-only adults raised at 22°C possessed SP56 but not RME-2 (Figure 1B), and fog-2(q71) oocyte-only adults raised at 22°C had RME-2 but not SP56 (Figure 1C). Yet these two XX mutants have morphologically indistinguishable somatic tissues, including the somatic gonad (Barton et al. 1987; Schedl and Kimble 1988). We note that use of mutants was essential for this analysis and caution that a mutant transcriptome may include changes not found in wild-type and that XX sperm are only a proxy for XO sperm.

Two other features were specific to our analysis of spermatogenic vs. oogenic transcriptomes. First, we isolated gonads and discarded the main body and intestine; an isolated gonad contains approximately 1000 germ cells but many fewer somatic gonadal cells (1 DTC, 10
with lists of germline transcripts deduced by comparing
found in our work (92%; 4323/4699). We also compared our dataset
10,631 in fog-2 gonads using SAGE (Table 1). Most SAGE-identi
4699 mRNAs detected in isolated wild-type adult hermaphrodite
a total of 10,754 mRNAs comprise the polyadenylated transcriptome

Oocyte-only
fem-1(hc17) fog-2(q71

Growth temperature 25/C176
Stage Young adult L4/A molt + 2 hr
Sperm-only mutant
fem-3(q23gf) fem-3(q96gf)

Genes expressed in gonadal transcriptome
We prepared and sequenced polyadenylated RNAs from eight bio-
logical replicates of each mutant [fem-3(gf) and fog-2], with 30 iso-
lated gonads per replicate. Using TopHat (Trapnell et al. 2012),
>90% of sequence reads could be mapped uniquely to the most
recent version of the C. elegans genome (see Materials and Methods).
A gene was scored as expressed when 16 or more reads mapped to
that gene. Expression abundance was measured in normalized total
read counts per gene. Because transcript isoforms were ignored for
this first analysis, we discuss the data in terms of “genes expressed”
rather than “transcripts expressed.” For quality reference, 97% of
the reads mapped to the annotated transcriptome; of those, 1.7% map-
partly to noncoding sequences and partly to coding sequences
and 0.3% fell in ambiguous gene sequence annotations. In this way,
we identified totals of 10,733 genes expressed in fem-3(gf) gonads,
10,631 in fog-2 gonads, and 10,610 shared (Figure 2A). Together,
a total of 10,754 mRNAs comprise the polyadenylated transcriptome
of these XX gonads.

We compared our dataset with a previously published dataset of
4699 mRNAs detected in isolated wild-type adult hermaphrodite
gonads using SAGE (Table 1). Most SAGE-identified genes were also
found in our work (92%; 4323/4699). We also compared our dataset
with lists of germline transcripts deduced by comparing “soma plus
germline” to “soma-only” transcriptomes. Reinke et al. (2004) found
3145 germline RNAs in this manner from microarray data, whereas
Wang et al. (2009) found 1063 using SAGE. Again, most germline
RNAs previously reported were also identified in this work (84%;
2637/3145 and 95%; 1017/1063). The design differences among these
various studies (Table 1 and Table 2) were so extensive that the main
conclusion is that our data include most genes previously reported as
expressed in the gonad and doubles their number.

Gene expression enriched in spermatogenic vs. oogenic gonads: We
next identified genes whose expression was enriched in spermatogenic
vs. oogenic gonads. To this end, we used DESeq to compare the
10,733 genes expressed in fem-3(gf) spermatogenic gonads with the
10,631 in fog-2 oogenic gonads. Using thresholds of a two-fold differ-
eence in abundance and a false discovery rate of 1%, we identified 2748
genes with expression enriched in spermatogenic gonads and 1732
enriched in oogenic gonads; the remaining 6274 were not enriched in
either spermatogenic or oogenic gonads (Figure 2, Table 3, and Table
S1). We refer to these as spermatogenic, oogenic, and gender-neutral
genes, respectively. All spermatogenic, oogenic, and gender-neutral
genes are labeled as such and listed in an Excel searchable format
in Table S1, column H. Table 4 shows 12 representative genes pre-
viously determined experimentally to have gender-neutral or sex-
bias germline expression.

A comparison of our datasets (spermatogenic, oogenic, and
gender-neutral genes) with those of a similar but earlier study (Reinke
et al. 2004) reveals considerable overlap between spermatogenic and
gender-neutral genes, but less with oogenic genes (Table 3). Only 37%
of oogenic genes were found in our oogenic gene list. Where were the
missing 63%? The majority were in the gender-neutral gene list (99%).
Why were so many “oogenic” genes in the study by Reinke et al.
(2004)”gender-neutral” genes in our study? One possible explanation
is that our work queried very young adult gonads with only a few
mature oocytes, whereas Reinke et al. (2004) used older adults with
many mature oocytes. Another explanation is that our study had
greater sensitivity. Both explanations likely have some validity. We
blindly surveyed expression of all the Reinke et al. (2004) study’s
oogenic genes in the NEXTDB database and found 582 genes with
unambiguous in situ hybridization data; of these, 42% (242/582) were
enriched for germline expression in adult hermaphrodites compared
with L4s, consistent with their oogenic classification (Table S1,

Table 1 Total genes expressed in isolated gonads

| Paper            | Strain          | Stage          | Gamete       | Temperature | Method | Genome Version | Total # Genes Expressed | % Overlapa |
|------------------|-----------------|----------------|--------------|-------------|--------|---------------|-------------------------|------------|
| Wang et al. (2009)| XX wild-type    | Adult          | Sperm and oocytes | 20°C        | SAGE   | WS160 (2006)   | 4699                    | 92%        |
| This work        | XX fem-3(q23gf) and XX fog-2(q71) | Adult          | Sperm and oocytes | 22°C        | RNA-Seq | WS240 (2014)   | 10,754                   | NA         |

NA, not applicable.

a Percent of total transcripts identified by Wang et al. (2009) that were also found in this work.

Table 2 Experimental design of studies of sex-enriched expression

| Features                  | Reinke et al. (2004) | This Work   |
|---------------------------|----------------------|-------------|
| Sperm-only mutant         | fem-3(q23gf)         | fem-3(q96gf) |
| Oocyte-only               | fem-1(hc17)          | fem-2(q71)  |
| Stage                     | Young adult          | L4/A molt + 2 hr |
| Growth temperature        | 25°C                 | 22°C        |
| RNA source                | Whole animal         | Isolated gonad |
| Methods for RNA detection | Microarray           | RNA-Seq     |
| Genome version            | WS90                 | WS240       |
| Statistical threshold     | p-value = 0.01       | Adjusted p-value = 0.01 |

Table 3 Sex-enriched expression

|                   | Reinke et al. (2004) | This Work 2014 | Overlapa |
|-------------------|----------------------|----------------|----------|
| Spermatogenesis-enriched genes | 865                 | 2748            | 98%      |
| Oogenesis-enriched genes     | 1030                 | 1732            | 37%      |
| Gender-neutral        | 1250                 | 6274            | 83%      |

a Percent transcripts in Reinke et al. (2004) also found in this work.
column I), whereas 58% (243/582) were expressed at approximately the same level in both larval and adult germ lines. The percentage of the Reinke et al. (2004) study’s oogenic genes validated as enriched during oogenesis according to NEXTDB (42%) is therefore similar to the percentage overlap between oogenic genes found here and that work (37%) (Table 3). We suggest that the datasets are comparable but not identical.

We also compared our data with recent studies of sex-biased gene expression and evolution (Thomas et al. 2012; Albritton et al. 2014). In agreement with those works, we found that the average fold change in expression of spermatogenic genes was greater than that of oogenic genes (Figure 3A). We also found that oogenic genes were significantly enriched compared with total gonadal genes on chromosome X (28% oogenic vs. 22% gonadal on X) but underenriched on autosomes (72% vs. 78% on autosomes); conversely, spermatogenic genes were significantly underenriched on chromosome X (7% spermatogenic vs. 12% gonadal on X) but enriched on autosomes (93% vs. 88% on autosomes) (Figure 3B, see legend for p-values), confirming previous results (Reinke et al. 2000, 2004). Finally, those previous studies found more spermatogenic genes than oogenic genes, in agreement with our results (Figure 2, Figure 3A, and Table S1). Therefore, recent studies using RNA-Seq from whole worms are consistent with this analysis of gonadal sex-biased gene expression.

We hypothesized that genes involved in common germline processes, such as meiosis, would be enriched in the gender-neutral expression category. To test this idea, we searched for genes involved in meiosis according to Gene Ontology classification and RNAi experiments reported in WormBase. Our gender-neutral list included 82% (231/281) of those genes with meiosis-related functions (Table S2). Thus, meiotic cell cycle genes are enriched in our gender-neutral dataset compared with sex-enriched datasets.

Gonadal genes identified specifically in this study: Using the NEXTDB database, we explored the tissue expression of gonad-expressed mRNAs identified in this work but not in previous studies of isolated gonads (Wang et al. 2009) or germline expression (Reinke et al. 2004). NEXTDB archives in situ hybridization results for approximately half the annotated C. elegans genes and includes unambiguous staining patterns for 1522 of the 2567 genes found specifically in this study. Of these 1522 staining patterns, 92% of spermatogenic (178), 90% of oogenic (294), and 74% of gender-neutral (743) genes were expressed in the germline tissue according to NEXTDB (Table S1, column I). Moreover, a sampling of genes was scored for gender expression. Most genes classified as sperm-enriched were visibly more abundant in L4 spermatogenic than adult oogenic germ lines (69%; n = 103); most classified as oocyte-enriched were visibly more abundant in adult than L4 germ lines (75%; n = 216); and most classified as gender-neutral were found at both stages with no obvious visible difference in abundance (78%; n = 363). Therefore, our dataset provides a new source for genes expressed in the germline.

**Other comparisons:** We also compared our spermatogenic, oogenic, and gender-neutral datasets with several others (Figure 4). First, we compared them to lists of target genes or target mRNAs of crucial gamete regulators. The spe-44 transcription factor drives spermatogenesis with many predicted target genes (Kulkarni et al. 2012). Most spe-44 predicted targets were found in our list of spermatogenic genes (475/668) but not in our list of oogenic genes (55/668) (Figure 4A). GLD-2/RNP-8 and EFL-1/DPL-1 heterodimers drive the process of

---

**Table 4 Representative genes with known sex bias expression**

| Gene Name | Sex-Biased Expression | Original Reference for Sex Bias | fog-2 Expression Value* | fem-3 Expression Value* | log2 fog-2/fem-3 Expression Value* | Adjusted p-value (FDR)* |
|-----------|-----------------------|---------------------------------|-------------------------|-------------------------|------------------------------------|-------------------------|
| cpb-1     | Spermatogenic         | Luitjens et al. (2000)          | 16.0E+02                | 55.0E+02                | 1.77                               | 5.68E−12                |
| fog-1     | Spermatogenic         | Luitjens et al. (2000)          | 6.26E+02                | 38.8E+02                | 2.63                               | 1.08E−27                |
| fog-3     | Spermatogenic         | Chen et al. (2000)              | 0.195E+02               | 56.9E+02                | 8.18                               | 1.7E−18                 |
| spe-44    | Spermatogenic         | Kulkarni et al. (2012)          | 5.32E+02                | 47.8E+02                | 3.16                               | 1.13E−48                |
| ima-1     | Oogenic               | Detwiler et al. (2001)          | 90.9E+02                | 40.5E+02                | −1.16                              | 8.84E−08                |
| pie-1     | Oogenic               | Tenenhaus et al. (1998)         | 34.6E+02                | 4.84E+02                | −2.83                              | 5.78E−41                |
| rme-2     | Oogenic               | Grant and Hirsh (1999)          | 184.8E+02               | 33.5E+02                | −2.46                              | 1.22E−38                |
| tra-2     | Oogenic               | Okkema and Kimble (1991)        | 21.5E+02                | 0.574E+02               | −1.90                              | 5.78E−20                |
| him-3     | Gender-neutral        | Zetka et al. (1999)             | 15.8E+02                | 28.9E+02                | 0.88                               | 5.76E−06                |
| ima-1     | Gender-neutral        | Geles and Adam (2001)           | 44.7E+02                | 26.2E+02                | −0.76                              | 2.01E−04                |
| ima-3     | Gender-neutral        | Geles and Adam (2001)           | 80.4E+02                | 75.8E+02                | −0.08                              | 5.26E−24                |
| spo-11    | Gender-neutral        | Dernburg et al. (1998)          | 18.7E+02                | 15.4E+02                | −0.63                              | 4.93E−03                |

* This work.
oogenesis (Kim et al. 2009; Kudron et al. 2013). Many GLD-2/RNP-8 (178/317) and EFL-1/DPL-1 (130/309) predicted targets were on our list of oogenic genes, and the majority in a combined list of oogenic plus gender-neutral genes [GLD-2/RNP-8 (313/317); EFL-1/DPL-1 (290/309)], whereas overlap with spermatogenic genes was low [GLD-2/RNP-8 (4/317); EFL-1/DPL-1 (19/309)] (Figure 4, B and C).

Second, we compared our datasets with transcriptomes obtained from isolated mature sperm (Ma et al. 2014) or zygotes (Baugh et al. 2003). Both contained many of our gender-neutral expression gene set. However, the mature sperm transcriptome contained almost half of our spermatogenic gene dataset (1216/2748) and the zygote transcriptome contained 64% of our oogenic dataset (1117/1732), which represent 45% and 23% of the sperm and zygote transcriptomes (Figure 4, D and E). These comparisons therefore provide additional validation for our spermatogenic, oogenic, and gender-neutral datasets.

Datasets for differential exon usage in spermatogenic vs. oogenic gonads: To further characterize spermatogenic and oogenic transcriptomes, we analyzed the original Seq data to look for transcript level differences. Out of 99,984 total exon–exon junctions identified by TopHat in both transcriptomes, 86,452 (86.5%) represented canonical junctions (consecutive exons) and 13,532 (13.5%) represented non-canonical junctions (nonconsecutive exons) (Table S3). Transcripts were assembled in each gonad transcriptome using Cufflinks. We detected 25,461 expressed transcripts in spermatogenic gonads and 24,333 expressed transcripts in oogenic gonads, including isoforms in both cases (Table S4).

To identify isoforms enriched in spermatogenic and oogenic gonads, we analyzed differential exon usage between the gonadal transcriptomes. We identified 577 differentially expressed exons (DEXSeq; FDR < 1%) affecting 351 genes (Table S5). Of the 351 genes, 326 genes (93%) have annotated gene models on WormBase that correspond to our exon usage analysis. We also found that 253 genes (73%) were affected in protein-coding exons. As an example, we visualized mapped reads to the fog-1 gene locus with the UCSC Genome Browser. In agreement with the exon usage analysis, we found both the long and short fog-1 isoforms with enrichment of the “long” isoform in spermatogenic gonads and enrichment of the “short” isoform in oogenic gonads (Figure 5A). This finding mirrors fog-1 isoforms found previously using Northern blots (Luitjens et al. 2000). We also identified, as another example, sex-specific enrichment for the first
exon of the mRNA encoding Argonaute protein CSR-1 (Figure 5B), which is intriguing given the role of CSR-1 in spermatogenesis (Conine et al. 2013). Among the 351 genes with sex-enriched exons, 50% occurred in spermatogenic, 30% occurred in oogenic, and 17% occurred in gender-neutral genes (Table S5). To further characterize these 351 mRNAs, we performed Gene Ontology (GO) analysis. The following biological processes were over-represented compared with all gonad-expressed genes: cell cycle (P < 0.005); glucose metabolism (P < 0.007); and post-transcriptional gene regulation (P < 0.04). Molecular functions relating to ATP binding were also enriched (P < 0.003).

We next located differentially expressed exons within their transcripts: 19.2% (111/577) were the first exon; 17.7% (102/577) were the last exon; and 63.1% (364/577) were in the middle (Figure 5C). These middle exons reveal exon-skipping events predicted to alter the proteomes of spermatogenic vs. oogenic gonads. Although splicing factors have dramatic effects on gamete specification (Puoti and Kimble 1999, 2000; Kerins et al. 2010), the affected RNAs are not yet known. This dataset of differently expressed exons in spermatogenic and oogenic gonads may be a useful resource for finding events relevant to germline development or more broadly for studies of alternative 5’ and 3’ end formation as well as alternative splicing.

CONCLUSIONS
This work provides new datasets for spermatogenic, oogenic, and gender-neutral genes expressed in the C. elegans gonad. The major advantages over earlier datasets are doubling the number of genes found expressed in the gonad, with most likely expressed in germline tissue, and identification of sex-enriched mRNA isoforms.

ACKNOWLEDGMENTS
Antibodies were generous gifts from Barth Grant (RME-2) and Susan Strome (SP56). E.P.S. was supported by the Biotechnology Training Program (NIH T32 GM08349). This work was supported in part by NIH grant GM069454 (to J.K.). J.K. is an investigator at the Howard Hughes Medical Institute.

LITERATURE CITED
Albritton, S. E., A. L. Kranz, P. Rao, M. Kramer, C. Dieterich et al., 2014 Sex-biased gene expression and evolution of the X chromosome in nematodes. Genetics 197: 865–883.
Anders, S., and W. Huber, 2010 Differential expression analysis for sequence count data. Genome Biol. 11: R106.
Anders, S., A. Reyes, and W. Huber, 2012 Detecting differential usage of exons from RNA-seq data. Genome Res. 22: 2008–2017.
Barton, M. K., T. B. Schedl, and J. Kimble, 1987 Gain-of-function mutations of fem-3, a sex-determination gene in Caenorhabditis elegans. Genetics 115: 107–119.
Baugh, L. R., A. A. Hill, D. K. Slonim, E. L. Brown, and C. P. Hunter, 2003 Composition and dynamics of the Caenorhabditis elegans early embryonic transcriptome. Development 130: 889–900.
C. elegans Sequencing Consortium, 1998 Genome sequence of the nematode C. elegans: a platform for investigating biology. Science 282: 2012–2018.
Chen, P.-J., A. Singal, J. Kimble, and R. E. Ellis, 2000 A novel member of the Tob family of proteins controls sexual fate in Caenorhabditis elegans germ cells. Dev. Biol. 217: 77–90.
Chik, J. K., D. C. Schriemer, S. J. Childs, and J. D. McGhee, 2011 Proteome of the Caenorhabditis elegans oocyte. J. Proteome Res. 10: 2300–2305.
Conine, C. C., J. J. Moresco, W. Gu, M. Shirayama, D. Conte, Jr. et al., 2013 Aragonautes promote male fertility and provide a paternal memory of germline gene expression in C. elegans. Cell 155: 1532–1544.
Dernburg, A. F., K. McDonald, G. Moulder, R. Barstead, M. Dresser et al., 1998 Meiotic recombination in C. elegans initiates by a conserved mechanism and is dispensable for homologous chromosome synopsis. Cell 94: 387–398.
Detwiler, M. R., M. Reuben, X. Li, E. Rogers, and R. Lin, 2001 Two zinc finger proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in C. elegans. Dev. Cell 1: 187–199.
Ellis, R., and T. Schedl, 2007 Sex determination in the germ line. WormBook Mar 5: 1–13. 10.1895/wormbook.1.82.2.
Flicek, P., M. R. Amode, D. Barrett, K. Beal, K. Billis et al., 2014 Ensembl 2014. Nucleic Acids Res. 42: D749–D755.
Geles, K. G., and S. A. Adam, 2001 Germline and developmental roles of the nuclear transport factor importin α3 in C. elegans. Development 128: 1817–1830.
Gerstein, M. B., Z. J. Lu, E. L. Van Nostrand, C. Cheng, B. I. Arshinoff et al., 2010 Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project. Science 330: 1775–1787.
Grant, B., and D. Hirsh, 1999 Receptor-mediated endocytosis in the Caenorhabditis elegans oocyte. Mol. Biol. Cell 10: 4311–4326.
Greenstein, D., 2005 Control of oocyte meiotic maturation and fertilization. WormBook Dec 28 1–12. 10.1895/wormbook.1.53.1.
Hirsh, D., D. Oppenheim, and M. Klass, 1976 Development of the reproductive system of Caenorhabditis elegans. Dev. Biol. 49: 200–219.
Jaramillo-Lambert, A., M. Ellesfon, A. M. Villeneuve, and J. Engebrecht, 2007 Differential timing of S phases, X chromosome replication, and meiotic prophase in the C. elegans germ line. Dev. Biol. 308: 206–221.
Jungkamp, A. C., M. Stoeckius, D. Mecenas, D. Grun, G. Mastrobuoni et al., 2011 In vivo and transcriptome-wide identification of RNA binding protein target sites. Mol. Cell 44: 828–840.
Kerins, J. A., M. Hanazawa, M. Dorsett, and T. Schedl, 2010 PRP-17 and the pre-mRNA splicing pathway are preferentially required for the proliferation vs. meiotic development decision and germline sex determination in Caenorhabditis elegans. Dev. Dyn. 239: 1555–1572.
Kersner, A. M., and J. Kimble, 2010 Genome-wide analysis of mRNA targets for Caenorhabditis elegans FBF, a conserved stem cell regulator. Proc. Natl. Acad. Sci. USA 107: 3936–3941.
Kim, K. W., K. Nykamp, N. Suh, J. L. Bachorik, L. Wang et al., 2009 Antagonism between GLD-2 binding partners controls gamete sex. Dev. Cell 16: 723–733.
Kim, K. W., T. L. Wilson, and J. Kimble, 2010 GLD-2/RNP-8 cytoplasmic poly(A) polymerase is a broad-spectrum regulator of the oogenesis program. Proc. Natl. Acad. Sci. USA 107: 17445–17450.
Kimble, J., and S. L. Crittenden, 2007 Controls of germline stem cells, entry into meiosis, and the sperm/oocyte decision in Caenorhabditis elegans. Annu. Rev. Cell Dev. Biol. 23: 405–433.
Kudron, M., W. Niu, Z. Lu, G. Wang, M. Gerstein et al., 2013 Tissue-specific direct targets of Caenorhabditis elegans Rb/E2F dictate distinct somatic and germline programs. Genome Biol. 14: R5.
Kulkarni, M., D. C. Shakes, K. Guevel, and H. E. Smith, 2012 SPE-44 implements sperm cell fate. PLoS Genet. 8: e1002678.
L’Hernault, S. W., 2006 Spermatogenesis. WormBook Feb 20 1–14. 10.1895/wormbook.1.85.1.
Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan et al., 2009 The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078–2079.
Luitjens, C., M. Gallegos, B. Kraemer, J. Kimble, and M. Wicken, 2000 CPEB proteins control two key steps in spermatogenesis in C. elegans. Genes Dev. 14: 2596–2609.
Ma, X., Y. Zhu, C. Li, P. Xue, Y. Zhao et al., 2014 Characterisation of Caenorhabditis elegans sperm transcriptome and proteome. BMC Genomics 15: 168.
Morgan, D. E., S. L. Crittenden, and J. Kimble, 2010 The C. elegans adult male germline: Stem cells and sexual dimorphism. Dev. Biol. 346: 204–214.
Okkema, P. G., and J. Kimble, 1991 Molecular analysis of tra-2, a sex determining gene in Caenorhabditis elegans. EMBO J. 10: 171–176.
Puoti, A., and J. Kimble, 1999 The Caenorhabditis elegans sex determination gene mug-1 encodes a member of the DEAH-box protein family. Mol. Cell. Biol. 19: 2189–2197.

Volume 4 September 2014 | RNA-Seq of Germline Sex | 1771
Puoti, A., and J. Kimble, 2000  The hermaphrodite sperm/oocyte switch requires the *Caenorhabditis elegans* homologs of PRP2 and PRP22. Proc. Natl. Acad. Sci. USA 97: 3276–3281.

Rapaport, F., R. Khanin, Y. Liang, M. Pirun, A. Krek et al., 2013  Comprehensive evaluation of differential gene expression analysis methods for RNA-seq data. Genome Biol. 14: R95.

Reinke, V., I. S. Gil, S. Ward, and K. Kazmer, 2004  Genome-wide germline-enriched and sex-biased expression profiles in *Caenorhabditis elegans*. Development 131: 311–323.

Reinke, V., H. E. Smith, J. Nance, J. Wang, C. Van Doren et al., 2000  A global profile of germline gene expression in *C. elegans*. Mol. Cell 6: 605–616.

Rog, O., and A. F. Dernburg, 2013  Chromosome pairing and synopsis during *Caenorhabditis elegans* meiosis. Curr. Opin. Cell Biol. 25: 349–356.

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.

Tenenhaus, C., C. Schubert, and G. Seydoux, 1998  Genetic requirements for PIE-1 localization and inhibition of gene expression in the embryonic germ lineage of *Caenorhabditis elegans*. Dev. Biol. 200: 212–224.

Thomas, C. G., R. Li, H. E. Smith, G. C. Woodruff, B. Oliver et al., 2012  Simplification and desexualization of gene expression in self-fertile nematodes. Curr. Biol. 22: 2167–2172.

Trapnell, C., A. Roberts, L. Goff, G. Pertea, D. Kim et al., 2012  Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 7: 562–578.

Trapnell, C., B. A. Williams, G. Pertea, A. Mortazavi, G. Kwan et al., 2010  Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28: 511–515.

Wang, X., Y. Zhao, K. Wong, P. Ehlers, Y. Kohara et al., 2009  Identification of genes expressed in the hermaphrodite germ line of *C. elegans* using SAGE. BMC Genomics 10: 213.

Ward, S., T. M. Roberts, S. Strome, F. M. Pavalko, and E. Hogan, 1986  Monoclonal antibodies that recognize a polypeptide antigenic determinant shared by multiple *Caenorhabditis elegans* sperm-specific proteins. J. Cell Biol. 102: 1778–1786.

Wickham, H., 2009  *ggplot2: elegant graphics for data analysis*. Springer, New York.

Zetka, M. C., I. Kawasaki, S. Strome, and F. Müller, 1999  Synapsis and chiasma formation in *Caenorhabditis elegans* require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. Genes Dev. 13: 2258–2270.

*Communicating editor: M. Walhout*