Current Biology 22, 2167–2172, November 20, 2012 ©2012 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2012.09.038

Report

Simplification and Desexualization of Gene Expression in Self-Fertile Nematodes

Cristel G. Thomas,[1,2,4] Renhua Li,[3] Harold E. Smith,[3] Gavin C. Woodruff,[1] Brian Oliver,[3] and Eric S. Haag[1,2,*]
[1]Department of Biology
[2]Program in Molecular and Cell Biology
University of Maryland, College Park, MD 20742, USA
[3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Summary

Evolutionary transitions between sexual modes could be potent forces in genome evolution [1, 2]. Several *Caenorhabditis* nematode species have evolved self-fertile hermaphrodites from the obligately outcrossing females of their ancestors [3]. We explored the relationship between sexual mode and global gene expression by comparing two selfing species, *C. elegans* and *C. briggsae*, with three phylogenetically informative outcrossing relatives, *C. remanei*, *C. brenneri*, and *C. japonica*. Adult transcriptome assemblies from the selfing species are consistently and strikingly smaller than those of the outcrossing species. Against this background of overall simplification, genes conserved in multiple outcrossing species with strong sex-biased expression are even more likely to be missing from the genomes of the selfing species. In addition, the sexual regulation of remaining transcripts has diverged markedly from the ancestral pattern in both selfing lineages, though in distinct ways. Thus, both the complexity and the sexual specialization of transciptomes are rapidly altered in response to the evolution of self-fertility. These changes may result from the combination of relaxed sexual selection and a recently reported genetic mechanism favoring genome shrinkage [4] in partial selfers.

Results

Sexual selection is a core aspect of evolutionary theory. With an appropriate group of organisms, modern sequencing methods allow the role of sex in genome evolution to be addressed comprehensively. We explored the genome-wide relationship between gene expression and sexual mode by comparing RNAseq profiles of three obligately outcrossing (XO male-XX female) Caenorhabdtitis (*C. remanei*, *C. brenneri*, and *C. japonica*) with those of *C. elegans* and *C. briggsae*, which evolved self-fertile XX hermaphrodites in two separate lineages (Figure 1). These species provide replicated cases of derived self-fertility and outgroups that allowed us to infer the ancestral state. For each sex, we deeply sequenced triplicate mRNA samples pooled from the L4 and adult stages, when sexual dimorphism is obvious. Estimated average coverage of each transcribed nucleotide was 196-300X (Figure 1A, dotted lines; see also Table S1 available online). With these data, we examined both overall transcriptome complexity and patterns of sex-biased expression.

The assemblies and annotations of the genomes of outcrossing (or gonochoristic) species are less complete than that of *C. elegans*. To allow direct comparisons across the five species, we generated de novo cDNA assemblies [6–8] for each (Figure 1) and validated their accuracy with the high quality *C. elegans* genome assembly and annotations (Figure S1 and Table S2). The three outcrossing species consistently yielded many more contigs (Figure 1A) with a greater total assembly size (Figure 1B) than *C. elegans* and *C. briggsae.* We carefully excluded assembly artifacts as a source of these differences. First, the transcriptome assembly size difference held over a wide range of sequence read numbers, indicating it was not an artifact of undersampling. Second, the larger outcrossing species assemblies were also not due to the residual heterozygosity known to exist in the strains used [9], as transcriptome assembly sizes and heterozygosity estimates in the three gonochorists [9] were not correlated. Additionally, when we stripped the transcriptomes of potentially allelic contigs with high similarity to another, the relative assembly sizes were similar to those obtained with the entire sets (*C. elegans* 5.55 Mbp, *C. briggsae* 6.01 Mbp, *C. japonica* 9.40 Mbp, *C. brenneri* 9.01 Mbp, *C. remanei* 7.84 Mbp). Given the evolutionary relationships of these species (Figure 1; [8]), we conclude that the reliably detectable L4/adult transcriptomes of the selfing species have shrunk approximately one-third relative to their obligately outcrossing relatives.

Having established a link between self-fertility and transcriptome shrinkage, we next examined whether sexual specialization might have changed as well. We compared the male and female partitions of the data sets to determine sex bias in transcript levels (Figure 2). To allow direct comparisons between hermaphrodites and females, we used *C. elegans fog-2* and *C. briggsae she-1* mutants, which lack XX spermatogenesis but have normal female fertility and development [10, 11]. We examined sex bias by two methods. One used the de novo cDNA contigs as a reference onto which the reads were mapped (Figure 2A). The other used the publicly available genome sequences and associated gene predictions (Figure 2B). The estimates of bias using these two approaches were highly concordant (Figure S2 and Table S3).

In both analyses, the broadest patterns of sex bias were similar in all five species, regardless of mating system (Figure 2). Contigs (Figure 2A) or gene models (Figure 2B) (collectively "transcribed units") with significantly male-biased expression were more abundant and had a wider range of expression values than those with female-biased expression. A similar pattern was found previously for *C. elegans* [12] and other animals with heterogametic males [13, 14]. However, *C. elegans* and *C. briggsae* differed from the obligate outcrossers as a group in two ways. First, the distributions of male-to-female expression ratios for highly male-biased transcribed units were less male-biased (Figure 2C). For *C. elegans*, the expression ratios remained tightly clustered as in the three gonochoristic species, albeit around a lower

[4]Present address: Department of Ecology and Evolution, University of Toronto, Toronto, ON M5S 3B2, Canada
*Correspondence: ehaag@umd.edu

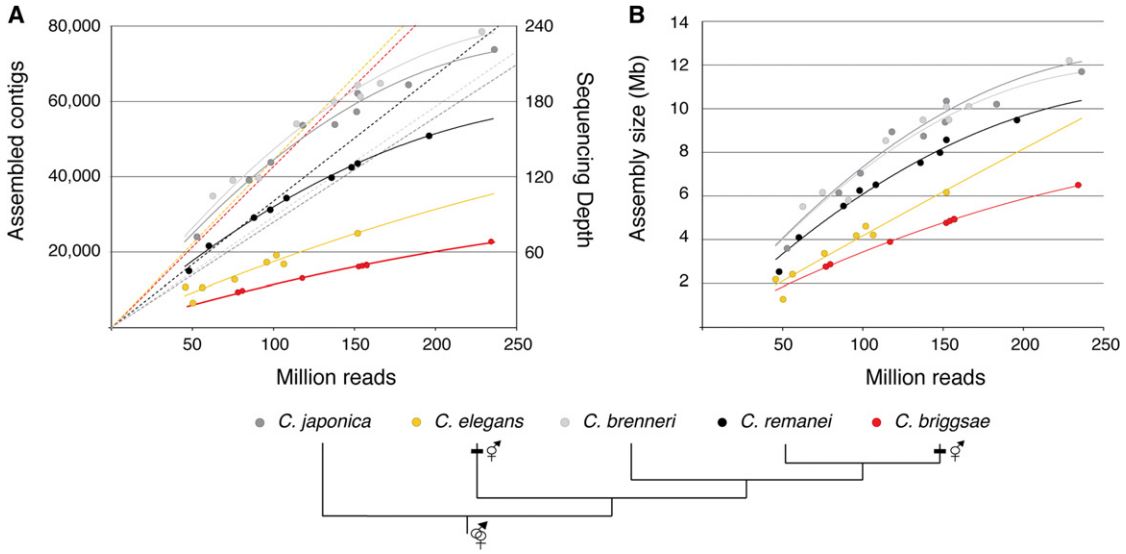

Figure 1. De Novo Transcriptome Assembly

(A and B) SOAPdenovo assemblies were generated from increasing numbers of reads in each species. The corresponding numbers of contigs assembled (A) and total cDNA assembly sizes (B) are shown for *C. japonica* (dark gray), *C. elegans* (yellow), *C. brenneri* (light gray), *C. remanei* (black), and *C. briggsae* (red), as well as the corresponding polynomial (order 2) trend lines. The sequencing depth is indicated in dotted lines for each species in (A). In the case of *C. elegans*, which has the most reliable gene annotations, 82.7% of the SOAPdenovo contigs were attributable to predicted CDS by BLASTn, irrespective of their sizes (E-value < 10^{-10}, Figure S1, see Table S2). 97.1% of the contigs whose expression is consistently detected have a significant BLASTn hit whose expression is consistently detected as well (Figure S2, Table S4). The phylogenetic relationships of the species [3, 5] are indicated as a cladogram below.

value. Comparison of sex-specific expression levels with a representative gonochorist, *C. remanei*, indicated that this shift to reduced male-biased expression was due primarily to elevated expression in *C. elegans* pseudofemales (Figure 2D). In contrast, *C. briggsae* expression ratios for highly male-biased transcribed units had a broader distribution (Figure 2C), largely explained by lower and more variable expression levels in males (Figure 2D). Thus, while the expression of the most male-biased genes evolved to be less male-specific in both selfing species, this occurred in distinct ways.

A second aspect of transcriptome-wide sex bias that distinguished the selfing species was that a lower fraction of their detected transcribed units were highly female-biased (Figures 2A and 2B, see red numbers in lower right corner of each panel). This could be because genes with highly female-biased expression were eliminated disproportionately from the *C. elegans* and *C. briggsae* genomes or because they are being created in male-female lineages at a higher rate. Consistent with the idea of ongoing gene formation, *C. remanei*-specific genes were more likely to be expressed in a highly female-biased fashion than expected (Figure 3B). This was the case for all three outcrossing species, but not for the selfing *C. elegans* and *C. briggsae*, indicating that the male-female species do evolve new genes with highly female-biased expression at a higher rate. However, the absolute number of such genes was too small to explain the 2- to 3-fold reduction of such genes observed in selfing species (Figure 2), suggesting that gene loss also occurred.

We directly tested gene loss by identifying genes in *C. remanei* (a surrogate for the outcrossing ancestor) for which orthologs were present in at least one other outcrossing species, but absent in *C. elegans* and *C. briggsae* (Table S5). Such genes must have been lost in at least one selfing lineage, and parallel losses will also be included in this set. *C. remanei* genes with highly sex-biased expression were significantly more likely to be missing in one or both of the selfing species than the overall set of genes with detectable expression, but this was not true for the outcrossing *C. brenneri* (Figure 3). Genes with both strong male- and strong female-biased expression were significantly overrepresented. Nevertheless, genes retaining strict orthologs in selfing species typically had similar sex biases to their counterparts in male-female species: 83.4% to 86.6% (depending on the species pair) displayed the same sign and degree of bias in their expression (Figure 4). Taken together, these results suggested that the disproportional decrease in strongly female-biased transcripts observed in *C. elegans* and *C. briggsae* is mainly due to a reduction in the number of genes of this class and not to lower origination rates or a shift in expression bias from one sex to the other.

Genes with strong sex-biased expression lost in self-fertile lineages might be expected to have functions related to sexual mode. We scrutinized *C. remanei* genes with orthologs in at least one other outcrossing species genome, absent in both selfing species, and highly sex-biased in their expression, for distinctive annotation features using Gene Ontology (GO) Consortium [15] categories (Figure S4). More than a third of those were predicted to encode integral membrane proteins. However, integral membrane protein-encoding genes as a group are significantly overrepresented in *C. remanei* genes with strong male-biased expression (Figure S3), suggesting the set of candidate "lost genes" is not unusually enriched for this class. Another significant subset of highly male-biased genes lacking homologs in selfing species was predicted to code for phosphatases (Figure S3). Phosphorylation status regulation is key for adequate sperm function in Caenorhabditis [16], consistent with a modification of the regulation of sperm function in selfing species. Among genes with highly

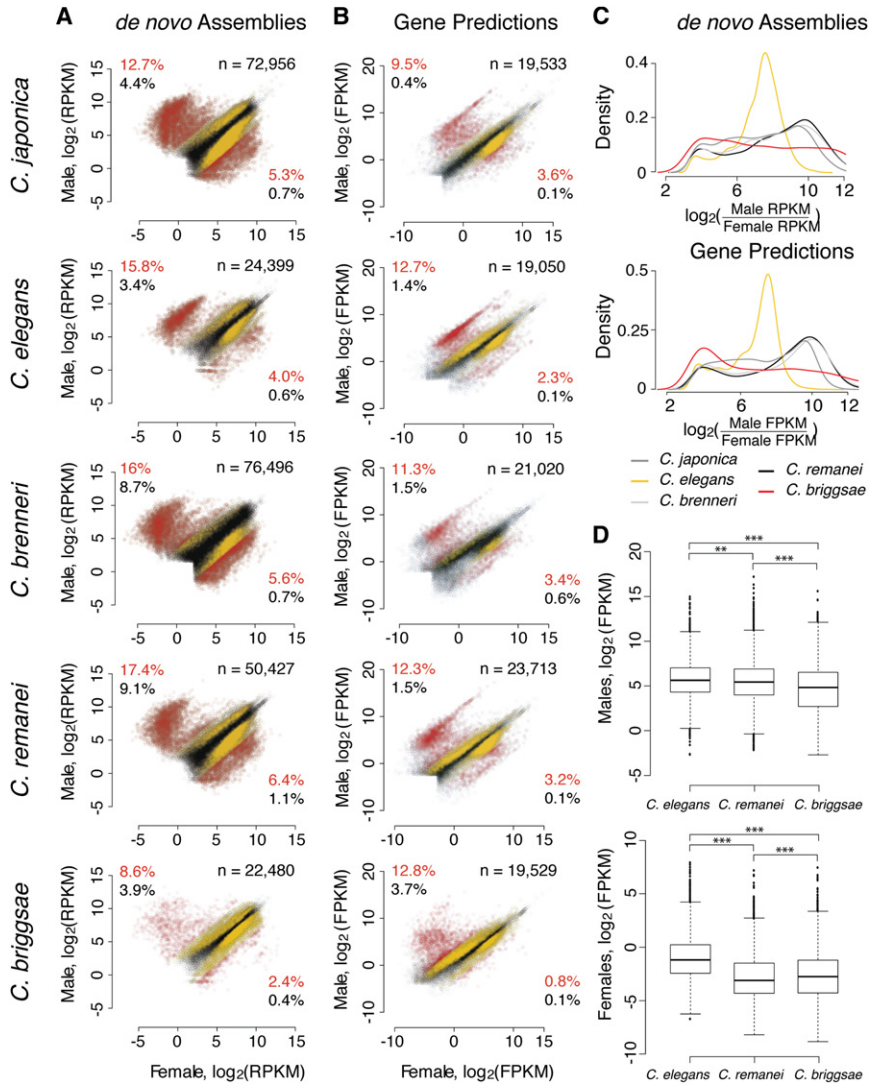

Figure 2. Sex-Biased Transcripts in Outcrossing and Selfing *Caenorhabditis*

(A) Scatter plots of the expression values for each SOAPdenovo cDNA contig in male versus female data sets for each species. Expression values are expressed as $\log_2$ of reads per kilobase per million fragments mapped (RPKM).

(B) Scatter plots of the expression values for each gene prediction in male versus female data sets for each species. Expression values are expressed as $\log_2$ of fragments per kilobase per million fragments mapped (FPKM). In both (A) and (B), differentially expressed transcripts are plotted in yellow and those displaying a difference over 10-fold between sexes in red. Red numbers in the upper left and lower right represent the fraction of total transcripts that are highly male- or female-biased, respectively. Transcripts that are consistently detected in one sex, but not detected in the other (i.e., truly sex-specific transcripts), cannot be plotted, but are indicated in the black numbers in each corner and are included in the fraction of highly sex-biased transcripts.

(C) Distribution of the male/female expression ratios for transcripts in the highly male-biased category, in the de novo assembly analysis (top), and for gene predictions (bottom).

(D) Expression values of genes with highly male-biased expression in males (top) and females (bottom) of *C. remanei* and pseudofemales of *C. elegans* and *C. briggsae* expressed as $\log_2$ FPKM. The heavy line in the boxes indicates the median. The top and bottom of the boxes indicate the upper and lower quartile, respectively. Significant differences between distributions as assessed by the Kolmogorov-Smirnov test are indicated above ($p < 0.001$; ** $\alpha < 0.01$; *** $\alpha < 0.001$).

female-biased expression, those with no orthologs in the self-fertile species were significantly enriched for transcription factors (Chi square, df = 1, $p < 0.01$), including members of the T-box, zinc finger, forkhead, and homeodomain families. These genes retain nonorthologous homologs in *C. elegans* and *C. briggsae*, suggesting they represent cases of gene family contractions.

Discussion

Caenorhabditis nematodes are ancestrally obligately outcrossing, or gonochoristic, whereas some, such as the model organism *C. elegans*, have recently evolved a mixture of males and self-fertile hermaphrodites termed androdioecy [5]. There are reasons to expect both gene gain and gene loss in the evolution of a selfing species. Hermaphrodites gain XX spermatogenesis, which requires novel genes [11, 17] and gene regulation [18]. Conversely, they tend to lose ancestral sex-specific traits related to mating [e.g., 19–21], which may allow loss of genes or modification of their expression. Selfing also leads to greatly reduced levels of natural variation and increased linkage disequilibrium [22, 23], which alter the way natural selection acts on mutations [23, 24]. These observations predict that selfing may lead to substantial changes in gene expression. The results presented here confirm that the consequences of self-fertility for global gene expression are indeed profound. In addition to becoming less complex overall, the transcriptome of self-fertile nematodes also evolves to be less sexually dimorphic in two ways.

One is that the expression of strongly male-biased genes becomes less tightly regulated with respect to sex in selfing species. In *C. elegans*, this is manifest by derepression of genes with ancestrally highly male-biased expression in XX hermaphrodites, even when the animals have been genetically altered so that they do not produce sperm. This increase in "male" gene expression in hermaphrodites transformed into spermless pseudofemales may be because phenotypically female parts of *C. elegans* hermaphrodites are cryptically masculinized. This could represent incomplete feminization by the *fog*-2 mutation, adaptive relaxation of commitment to female fate that is required for self-fertility, or a sign of relaxed selection on sexual differentiation. We favor the last possibility. Though gene expression levels in the androdioecious *C. elegans* are subject to ongoing purifying selection [25], the larger effective population sizes and frequent recombination of gonochoristic species should lead to even stronger purifying selection. In *C. briggsae* the ancestral pattern of male-biased expression appears to be disrupted in more complex ways, notably by increased variation in the range of

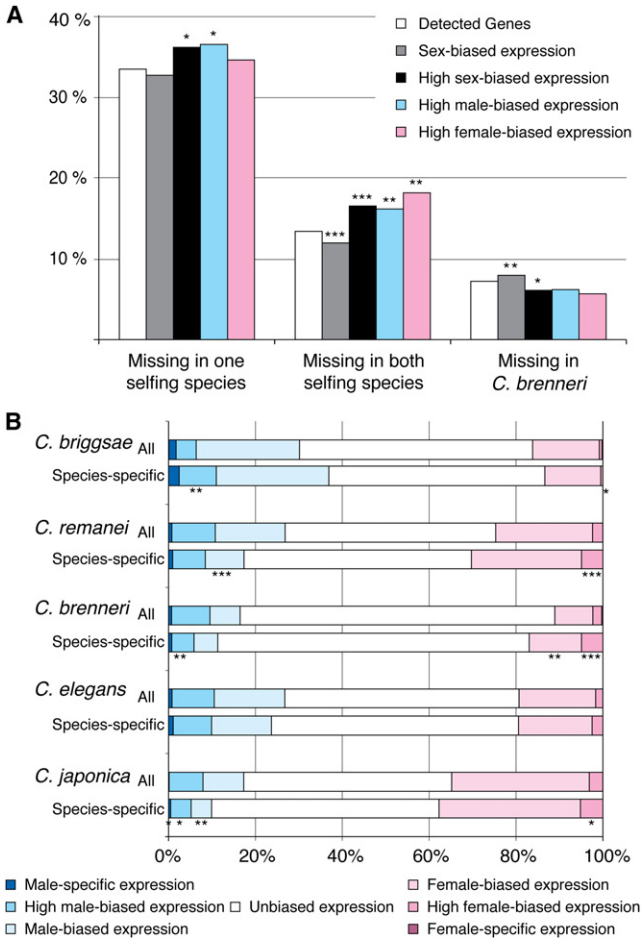

Figure 3. Relationship between Conservation and Expression Sex Bias for *C. remanei* Genes

(A) Comparison of the patterns of conservation of *C. remanei* genes whose expression is detected (white), sex-biased (gray), highly sex-biased (over 10-fold, black), highly male-biased (blue), or highly female-biased (pink). The fraction of genes with a homolog in at least one other gonochoric species but none in one or both of the selfing species are represented, as well as those missing in another outcrossing species, *C. brenneri*, for comparison. Significance of difference to detected number of genes was assessed by Chi square test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

(B) The fraction of genes male-specific (dark blue), highly male-biased (blue), male-biased (light blue), female-biased (light pink), highly female-biased (pink), female-specific (dark pink), or not biased (white) in their expression are indicated for all detectably expressed genes in the modENCODE homology data set and for genes that are species-specific. Significance of difference between all detected genes and lineage-specific genes was assessed by Chi square test as in (A). The asterisk at the right end of the *C. briggsae* bars refers to a single female-specific, species-specific gene, producing a bar too narrow to see.

male expression across the set of highly male-biased transcripts. Thus, while the outcome in terms of male-biased expression associated with androdioecy is similar, the evolutionary paths differ.

The second mode of desexualization we document in the two selfing *Caenorhabditis* species is a disproportionate loss of genes with strong female-biased expression from the genome entirely. Again, both adaptive and neutral explanations exist for why this may be. For example, genes with ancestrally female functions might interfere with XX spermatogenesis, or loss of a strictly sexually dimorphic phenotype may allow genes encoding parts of that pathway to degenerate. Functional characterization of such "lost female genes" may help explain if one, or both, is correct.

Mating systems are expected to shape genome content over generations through their effects on population genetics [1] and reproductive traits [26]. Comparison of the cruciferous plants *Arabidopsis thaliana* and *A. lyrata* provides further evidence that the evolution of self-fertility does indeed have strong effects on the genome [27]. In this case, the selfing *A. thaliana* harbors fewer endogenous protein-coding genes and fewer copies of selfish repetitive elements. It is likely that this represents simplification in the *A. thaliana* lineage, but in such a two-taxon comparison it remains formally possible that *A. lyrata* has experienced recent expansion. By employing a multispecies phylogenetic analysis, we have been able to localize transcriptome size change to the derived self-fertile species, and thus confidently polarize it as simplification in these lineages. We propose two broad mechanisms for desexualization of the selfing genome. First, weakened natural and sexual selection may allow many genes to be lost, with those related to sex being disproportionately affected. Second, sex-biased transmission of chromosome size variants in *Caenorhabditis* has recently been documented and leads to the prediction that partially self-fertile animals will quickly fix nondeleterious genomic deletions as they arise [4]. This phenomenon would promote rapid shrinkage of the genome and with it in the number of transcribed genes.

Experimental Procedures

Nematode Strains and Culture

C. remanei strains PB4641 and EM464, *C. japonica* DF5081, *C. brenneri* PB2801, and *C. elegans* CB4108 *fog-2(q71)* were maintained at 20°C on NGM agar plates according to standard *C. elegans* methods [28], using 2.2% agar to discourage burrowing. *C. briggsae* AF16 (for males) and the AF16-derived *she-1(v47)* (for pseudofemales) were maintained at 25°C to prevent production of intersexual germ cells in the latter [11]. To avoid inbreeding depression in the obligately outcrossing strains, large populations were maintained by transferring large chunks from two independent plates per passage.

Samples and Deep-Sequencing

Worms were roughly synchronized by hypochlorite/bleach treatment, and at least 400 XX or 600 XO L4 and young adult worms per replicate were hand picked. Three biological replicates per sex per species were collected for a total of 30 samples. Worms were washed and resuspended in 50 μl of RNA-ase free water. Two hundred fifty microliters of TRI-Reagent (Molecular Research Center) were added, and the samples were frozen at −80°C. Samples were thawed and lysed using a plastic pestle. RNA was purified using phenol/chloroform extractions, isopropanol precipitations, and DNaseI treatment (New England Biolabs). Single-ended cDNA libraries were prepared according to the manufacturer's protocol (Illumina) and sequenced on either an Illumina Genome Analyzer II or HiSeq2000. Reads were trimmed to 36 nt for assembly.

Bioinformatics

For de novo transcriptome assembly, the single-end reads from all six samples for each species were used as input for SOAPdenovo v.1.05 [29], with a *k*-mer size of 23. Contigs were improved by setting a minimum length threshold of 50 bp and removing contigs at the tips of the de Bruijn graph whose lengths were below 2*k* and/or whose coverage were below 5. To quantify contig expression sex bias, reads from each replicate were mapped onto the de novo transcriptome reference using Bowtie v.0.12.7 [30] and expression level for each contig expressed in RPKM (*r*eads *p*er *k*ilobase of transcripts per *m*illion mapped reads [30]).

For predicted gene model analyses, reads were aligned to the genome reference sequences of WormBase release WS224 using TopHat [31] v.1.2.0, and the expression level was assessed using Cufflinks [32] v.1.0.3. Genome annotation files were from ENSEMBL release 9 (CJ302 for

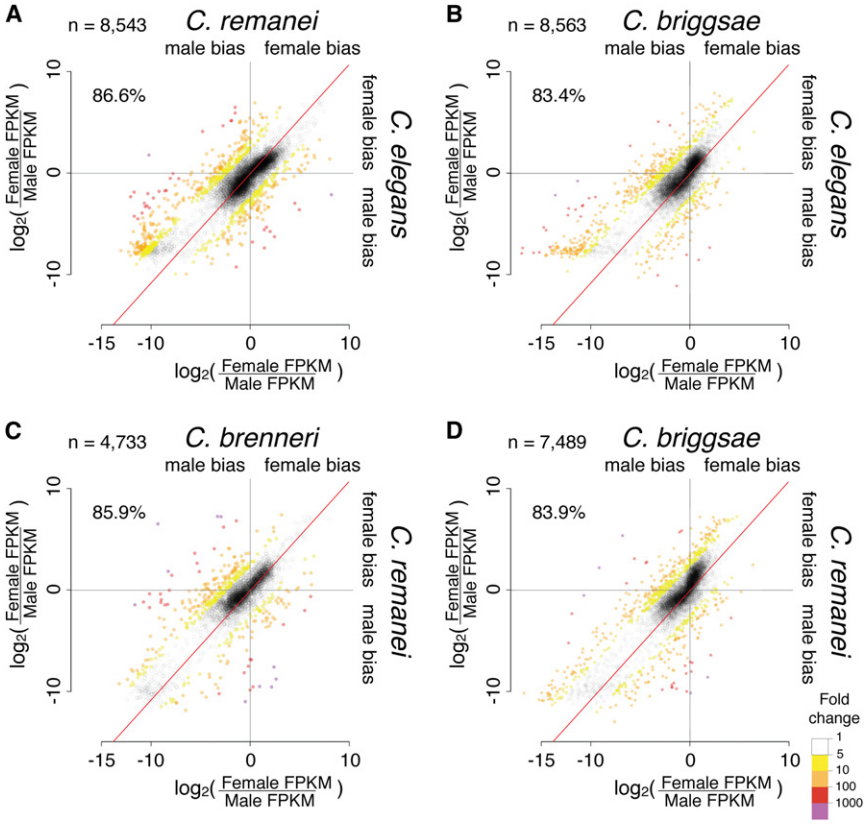

Figure 4. Conservation of Sexual Regulation of Gene Expression

(A–D) Scatter plot of the expression differential of *C. elegans* genes against that of their *C. remanei* orthologs (A), *C. elegans* genes against that of their *C. briggsae* orthologs (B), *C. remanei* genes against that of their *C. brenneri* orthologs (C), and *C. remanei* genes against that of their *C. briggsae* orthologs (D). Only genes with a 1:1 homology relationship and for which expression of both orthologs is detected in both sexes are represented. Genes for which the fold-change between expression ratios of each ortholog is between 5 and 10 are plotted in yellow, between 10 and 100 in orange, between 100 and 1000 in red, and over 1000 in purple. Orthologs whose expression ratios are less than 5-fold different are depicted in open, transparent gray circles to emphasize differences. The total number of ortholog pairs is indicated in the upper left, the percentage of these displaying the same sex bias in both species is indicated below.

C. japonica, WS220 for *C. elegans*, CB601 for *C. brenneri*, and CR2 for *C. remanei*) or release 14 (CB4 for *C. briggsae*). Expression level for each gene was expressed in FPKM (*f*ragments *p*er *k*ilobase of transcript per *m*illion mapped reads [32]). Orthologous genes were defined phylogenetically by the June 20, 2011, modENCODE ortholog data set (M. Rasmussen, M. Bansal, and Y.-C. Wu; http://compbio.mit.edu/modencode/orthologs/modencode-orths-2011-06-20/README.html).

For each species, only those transcribed units (TUs) expressed consistently across all three biological replicates were considered for further statistical analyses. On the basis of the distribution of the TUs, we applied linear regression models to identify significantly differentially expressed TUs between sexes by taking into account the effects of biological replicates and sequencing machine variation (Illumina GAII versus HiSeq). The threshold of p values was determined by permutation tests (N = 1000), under the control of false discovery rate $\leq$ 0.01. We used software packages of *R/maanova* and *R/qvalue* (http://cran.r-project.org/src/contrib/Archive/maanova/). Further analyses were performed using PERL scripts developed by CGT (available upon request).

Accession Numbers

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus, accessible through GEO Series accession number GSE41367 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41367).

Supplemental Information

Supplemental Information includes three figures and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.09.038.

Acknowledgments

The authors wish to thank Ian Korf and WormBase staff for bioinformatics assistance and the Institute for Bioscience and Biotechnology Research (IBBR) sequencing core for generating *C. briggsae* sequencing data. We thank the Caenorhabditis Genetics Center, which is supported by awards from the National Institutes of Health (NIH), and Ron Ellis for strains. This work was supported by the Intramural Research Program of the NIH, The National Institute of Diabetes and Digestive and Kidney Diseases (to B.O. and H.E.S.), and NIH grant 5R01GM079414 (to E.S.H.).

Received: February 27, 2012
Revised: July 18, 2012
Accepted: September 18, 2012
Published online: October 25, 2012

References

1. Lynch, M. (2007). The Origins of Genome Architecture (Sunderland, MA: Sinauer Associates).
2. Wright, S., and Schoen, D. (1999). Transposon dynamics and the breeding system. Genetica *107*, 139–148.
3. Kiontke, K.C., Félix, M.-A., Ailion, M., Rockman, M.V., Braendle, C., Pénigault, J.-B., and Fitch, D.H. (2011). A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. BMC Evol. Biol. *11*, 339.
4. Wang, J., Chen, P.J., Wang, G.J., and Keller, L. (2010). Chromosome size differences may affect meiosis and genome size. Science *329*, 293.
5. Kiontke, K., Gavin, N.P., Raynes, Y., Roehrig, C., Piano, F., and Fitch, D.H. (2004). Caenorhabditis phylogeny predicts convergence of hermaphroditism and extensive intron loss. Proc. Natl. Acad. Sci. USA *101*, 9003–9008.
6. Chen, S., Yang, P., Jiang, F., Wei, Y., Ma, Z., and Kang, L. (2010). De novo analysis of transcriptome dynamics in the migratory locust during the development of phase traits. PLoS ONE *5*, e15633.
7. Hao, C., Ge, G., Xiao, P., Zhang, Y., and Yang, L. (2011). The first insight into the tissue specific taxus transcriptome via Illumina second generation sequencing. PLoS ONE *6*, e21220.
8. Wong, M.M., Cannon, C.H., and Wickneswari, R. (2011). Identification of lignin genes and regulatory sequences involved in secondary cell wall formation in Acacia auriculiformis and Acacia mangium via de novo transcriptome sequencing. BMC Genomics *12*, 342.

9. Barrière, A., Yang, S.P., Pekarek, E., Thomas, C.G., Haag, E.S., and Ruvinsky, I. (2009). Detecting heterozygosity in shotgun genome assemblies: Lessons from obligately outcrossing nematodes. Genome Res. *19*, 470–480.
10. Schedl, T., and Kimble, J. (1988). *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics *119*, 43–61.
11. Guo, Y., Lang, S., and Ellis, R.E. (2009). Independent recruitment of F box genes to regulate hermaphrodite development during nematode evolution. Curr. Biol. *19*, 1853–1860.
12. Jiang, M., Ryu, J., Kiraly, M., Duke, K., Reinke, V., and Kim, S.K. (2001). Genome-wide analysis of developmental and sex-regulated gene expression profiles in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *98*, 218–223.
13. Small, C.M., Carney, G.E., Mo, Q., Vannucci, M., and Jones, A.G. (2009). A microarray analysis of sex- and gonad-biased gene expression in the zebrafish: evidence for masculinization of the transcriptome. BMC Genomics *10*, 579.
14. Zhang, Y., Sturgill, D., Parisi, M., Kumar, S., and Oliver, B. (2007). Constraint and turnover in sex-biased gene expression in the genus Drosophila. Nature *450*, 233–237.
15. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al.; The Gene Ontology Consortium. (2000). Gene ontology: tool for the unification of biology. Nat. Genet. *25*, 25–29.
16. Nishimura, H., and L'Hernault, S.W. (2010). Spermatogenesis-defective (spe) mutants of the nematode Caenorhabditis elegans provide clues to solve the puzzle of male germline functions during reproduction. Dev. Dyn. *239*, 1502–1514.
17. Nayak, S., Goree, J., and Schedl, T. (2005). *fog-2* and the evolution of self-fertile hermaphroditism in Caenorhabditis. PLoS Biol. *3*, e6.
18. Beadell, A.V., Liu, Q., Johnson, D.M., and Haag, E.S. (2011). Independent recruitments of a translational regulator in the evolution of self-fertile nematodes. Proc. Natl. Acad. Sci. USA *108*, 19672–19677.
19. Garcia, L.R., LeBoeuf, B., and Koo, P. (2007). Diversity in mating behavior of hermaphroditic and male-female *Caenorhabditis* nematodes. Genetics *175*, 1761–1771.
20. Palopoli, M.F., Rockman, M.V., TinMaung, A., Ramsay, C., Curwen, S., Aduna, A., Laurita, J., and Kruglyak, L. (2008). Molecular basis of the copulatory plug polymorphism in *Caenorhabditis elegans*. Nature *454*, 1019–1022.
21. Chasnov, J.R., So, W.K., Chan, C.M., and Chow, K.L. (2007). The species, sex, and stage specificity of a *Caenorhabditis* sex pheromone. Proc. Natl. Acad. Sci. USA *104*, 6730–6735.
22. Cutter, A.D., Dey, A., and Murray, R.L. (2009). Evolution of the *Caenorhabditis elegans* genome. Mol. Biol. Evol. *26*, 1199–1234.
23. Rockman, M.V., Skrovanek, S.S., and Kruglyak, L. (2010). Selection at linked sites shapes heritable phenotypic variation in *C. elegans*. Science *330*, 372–376.
24. Cutter, A.D., and Payseur, B.A. (2003). Selection at linked sites in the partial selfer Caenorhabditis elegans. Mol. Biol. Evol. *20*, 665–673.
25. Denver, D.R., Morris, K., Streelman, J.T., Kim, S.K., Lynch, M., and Thomas, W.K. (2005). The transcriptional consequences of mutation and natural selection in Caenorhabditis elegans. Nat. Genet. *37*, 544–548.
26. Fobis-Loisy, I., Miege, C., and Gaude, T. (2004). Molecular evolution of the s locus controlling mating in the brassicaceae. Plant Biol. (Stuttg.) *6*, 109–118.
27. Hu, T.T., Pattyn, P., Bakker, E.G., Cao, J., Cheng, J.-F., Clark, R.M., Fahlgren, N., Fawcett, J.A., Grimwood, J., Gundlach, H., et al. (2011). The *Arabidopsis lyrata* genome sequence and the basis of rapid genome size change. Nat. Genet. *43*, 476–481.
28. Wood, W.B., ed. (1988). The Nematode Caenorhabditis elegans. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).
29. Li, R., Zhu, H., Ruan, J., Qian, W., Fang, X., Shi, Z., Li, Y., Li, S., Shan, G., Kristiansen, K., et al. (2010). De novo assembly of human genomes with massively parallel short read sequencing. Genome Res. *20*, 265–272.
30. Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. *10*, R25.
31. Trapnell, C., Pachter, L., and Salzberg, S.L. (2009). TopHat: discovering splice junctions with RNA-Seq. Bioinformatics *25*, 1105–1111.
32. Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., Salzberg, S.L., Wold, B.J., and Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. *28*, 511–515.