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Редакција и администрација
Институт за јужни земјоделски култури - Струмица
Гоце Делчев б.б.
2 400 Струмица, Р Македонија
тел/факс: 034 345-096

Address of the editorship
Institute of Southern Crops
Strumica
Goce Delcev b.b.
2 400 Strumica, R Macedonija
phone/fax: ++ 389 34 345-096

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COMPARATIVE GENOMICS OF GENE EXPRESSION IN THE PARASITIC AND FREE-LIVING NEMATODES *Strongyloides stercoralis* and *Caenorhabditis elegans*

Makedonka Mitreva[1, *, #], James P. McCarter[1, 2, #], John Martin[1], Mike Dante[1], Todd Wylie[1], Brandi Chiapelli[1, 2], Deana Pape[1], Sandra W. Clifton[1], Thomas B. Nutman[3], and Robert H. Waterston[1, 4]

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[1]Genome Sequencing Center, Department of Genetics, Box 8501, 4444 Forest Park Blvd., Washington University School of Medicine, St. Louis, Missouri 63108.
[2]Divergence Inc., 893 North Warson Road, St. Louis, Missouri 63141.
[3]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 4, Room 126, 9000 Rockville Pike, Bethesda, MD 20892-0425.
[4]Department of Genome Sciences, University of Washington, Box 357730, 1705 NE Pacific St, Health Sciences K-357, Seattle, Washington 98195.

КОМПАРАТИВНА ГЕНОМИКА ПОМЕЃУ ПАРАЗИТНАТА И СЛОБОДНО-ЖИВЕЕЧКАТА НЕМАТОДА *STRONGYLOIDES STERCORALIS* И *CAENORHABDITIS ELEGANS*

Краток извадок

Иако експресирањето на гени во различен период од животниот циклус на нематодите се користи за потенциално откривање на функцијата на гените, потребни се проучувања кои ке ги поврзат ваквите информации помеѓу различни видови. Во овој труд анализирани се 10,921 ESTs кои се групирани во 3,311 кластери (секој кластер кореспондира на еден ген) од прв (Л1) и трет инфективен ларвен стадиум (Л3и) на паразитната нематода *Strongyloides stercoralis.* Резултатите ги споредивме со слободно-живеечката нематода *Caenorhabditis elegans*, вид чиј Л3и-дауер стадиум кореспондира на Л3и од *S. stercoralis*. Споредувањето на *S. stercoralis* ларвен-стадиум-специфични гени со хомолози експресирани во *C. elegans* дауер или не-дауер ларвен стадиум, покажа дека *S. sterkoralis* Л1 гените почесто се совпаѓаа (беа хомологни) со *C. elegans* не-дауер гените, укажувајќи на 'конзервирање' на репертоарот на гени експресирани во тек на растот и во услови со обилна храна. На

пример, колаген транскриптите на *S. stercoralis* беа изобилни во Л1 но не и во Л3и ларвен стадиум, што е во согласност со колагените на *C. elegans*. Иако голем дел од *S. stercoralis* Л3и гени имаа хомолози во *C. elegans* дауер стадиумот, робустно 'конзервирање' на експресирањето на гени помеѓу Л3и / дауер не беше детектирано. Изненадувачки беше пронаоѓањето дека од *S. sterkoralis* гените кои имаат *C. elegans* хомолози со РКНи дезактивирање, оние со сигнификантна-Л1 експресија имаа 2 пати повеќе фенотипови одколку Л3и гени. Функционална класификација на гените е исто така прикажана во вој труд.

Клучни зборови: *Strongyloides*, *C. elegans*, компаративна геномика, `ekspressed sequence tags`, експресирани гени, RNK блокирање, колаген, паразит.

ABSTRACT
While developmental timing of gene expression is used to infer potential gene function, studies have yet to correlate this information between species. We analyzed 10,921 ESTs in 3,311 clusters from first and infective third stage larva (L1, L3i) of the parasitic nematode *Strongyloides stercoralis* and compared the results to *Caenorhabditis elegans*, a species that has an L3i-like dauer stage. Comparing *S. stercoralis* clusters with stage-specific expression to *C. elegans* homologs expressed in either dauer or non-dauer stages, matches between *S. stercoralis* L1 and *C. elegans* non-dauer expressed genes dominated, suggesting conservation in the repertoire of genes expressed during growth in nutrient-rich conditions. For example, *S. stercoralis* collagen transcripts were abundant in L1 but not L3i, a pattern consistent with *C. elegans* collagens. While a greater proportion of *S. stercoralis* L3i than L1 genes have homologs among the *C. elegans* dauer-specific transcripts, we did not uncover evidence for a robust conserved L3i / dauer 'expression signature'. Strikingly, in comparisons of *S. stercoralis* clusters to *C. elegans* homologs with RNAi knockouts, those with significant L1-specific expression were more than twice as likely as L3i-specific clusters to match genes with phenotypes. We also provide functional classifications of *S. stercoralis* clusters.

Key Words: *Strongyloides*, *C. elegans*, comparative genomics, expressed sequence tags, gene expression, RNA interference, collagen, parasite
[EST sequences are available from GenBank, EMBL, and DDJB under the accession numbers AW495499 - AW496706, AW587864 - AW588186, AW588989 - AW 589121, BE028808 - BE030358, BE223-115- BE224723,

BE579014 - BE582028, BF014868 - BF015393, BG224323 - BG227958, BI772815 - BI773227. The sequences are also available at www.nematode.net.]

INTRODUCTION

Strongyloides Pathogenesis and Biology

The human round worm *Strongyloides stercoralis* causes chronic infections of the gastrointestinal tract. In immune-competent hosts, the disease is not life threatening, but immunodeficiency can lead to dangerous disseminated infections with pulmonary hemorrhage, necrotizing colitis, and 80% mortality if untreated (Igra-Siegman et al. 1981). Strongyloidiasis is difficult to diagnose (Genta 1988) and estimates of worldwide infections range from 70 to 600 million (Chen et al. 1994). Research goals include development of vaccines (Herbert et al. 2002) and diagnostics (Siddiqui and Berk 2001). *Strongyloides* has a unique life-cycle with parasitic and free-living generations. Parasitic females in the intestine produce eggs by mitotic parthenogenesis, and L1 larvae are excreted in stool. Larvae use environmental and genetic cues to determine their developmental path, becoming free-living adults (heterogonic pathway) or L3i infective larvae (homogonic pathway) (Schad 1990; Ashton et al. 1998; Grant and Viney 2001). *S. stercoralis* free-living worms can complete one life cycle of sexual reproduction outside the host, generating progeny that must reenter parasitic development (Yamada et al. 1991). Homogonic development resembles the life-cycle of other parasitic nematodes (e.g. hookworms), whereas the heterogonic life-cycle is much like that of free-living nematodes, including *Caenorhabditis elegans*, in nutrient rich conditions. L3i, derived from either parasitic or free-living parents, are suited for long-term survival and dispersal in the environment and are the only stage capable of infection, entering the host by skin penetration before traveling to the lungs and on to the intestine. L3i of *S. stercoralis* and many parasitic nematodes are developmentally arrested, non-feeding, and resistant to extreme temperatures and desiccation. They are morphologically similar to the dauer larvae formed by free-living nematodes under unfavorable environmental conditions, a stage that has been extensive studied in *C. elegans* (Hawdon and Schad 1991; Lopez et al. 2000). *C. elegans* dauers (L3d) can arrest for months, molting to L4 when favorable conditions return, and much is known about the molecular genetic control of dauer entry and exit (Riddle and Albert 1997). In *S. stercoralis*, host factors are likely critical to the exit of L3i from arrest, but little is known about the genes involved.

Nematode Comparative Genomics

The *C. elegans* genome is complete (The C. elegans Sequencing Consortium 1998) and substantial annotation has been added by gene expression (Hill et al. 2000; Jones et al. 2001; Kim et al. 2001) and RNA interference studies (Kamath et al. 2003). Parasitic nematode genomes are being explored via expressed sequence tags (ESTs); projects on >30 species have generated nearly 300,000 parasitic nematode ESTs (McCarter et al. 2002; Parkinson et al. 2003) including collections from parasites of mammals (Tetteh et al. 1999; Daub et al. 2000; Blaxter et al. 2002) and plants (Popeijus et al. 2000; Dautova et al. 2001; McCarter et al. 2003). Comparative genomic studies that begin to look for correlation in gene expression patterns across species are an important step in understanding the degree of relevance of model species, such as *C. elegans*, to the biology of species of interest including parasites. Previous characterization of the *S. stercoralis* genome was limited to 57 ESTs (Moore et al. 1996) and studies of individual genes of interest (Siddiqui et al. 1997; Siddiqui et al. 2000; Massey et al. 2001). Strongyloididae species (*S. stercoralis*, *S. ratti*, *Parastrongyloides trichosuri*) are useful parasites for comparative studies with *C. elegans* since they can be maintained outside the host for a generation or more, depending upon the species (Viney 1999; Dorris et al. 2002). To create an inventory of *S. stercoralis* genes and to support studies of Strongyloides pathogenesis and biology, we have analyzed an estimated 2,947 genes expressed during L1 and L3i. Compared to L3i expressed genes, L1 expressed transcripts from *S. stercoralis* are more likely to have *C. elegans* homologs that are expressed and essential during growth in nutrient rich conditions.

RESULTS AND DISCUSSION

As part of a larger effort to examine the genomes of parasitic nematodes, we have submitted to GenBank 5' ESTs from staged *S. stercoralis* cDNA libraries including 4,473 from L1 and 6,435 from L3i. Here we present the first large scale analysis of *S. stercoralis* genes including a comparison to gene expression patterns in *C. elegans*.

NemaGene Cluster Formation

To reduce data redundancy and determine gene representation, the 10,908 *S. stercoralis* sequences were grouped by identity into 3,479 contigs and further organized into 3,311 clusters. ESTs within a contig derive from nearly identical transcripts while contigs within a cluster may represent splice isoforms of a gene. Clusters ranged in size from a single EST (1,868 cases) to 1,097 ESTs (Figure 1). The great majority of clusters have ten or fewer ESTs. Contig building reduced the total number of nucleotides used for analysis from

4.75 to 2.73 million. The 3,311 clusters likely overestimate gene discovery, as one gene could be represented by multiple non-overlapping clusters (see L3NieAg.01, Table 1A). Such "fragmentation" was estimated at 11% using *C. elegans* as a reference genome. After discounting for fragmentation, we estimate that sequences derive from 2,947 genes for a discovery rate of 27% (2,947/10,908). Assuming 19-20,000 total genes as in *C. elegans* (The C. elegans Sequencing Consortium 1998), these clusters likely represent 15% of all *S. stercoralis* genes. Contig building successfully increased the length of assembled transcript sequences from 435±101 nucleotides for ESTs to 646±219 for multi-member contigs.

Distribution of BLAST Matches and Homologs in *C. elegans*

The Figure 2 Venn diagram combines the results of BLAST searches versus three databases for the 85% (2,826/3,311) of *S. stercoralis* clusters that had matches to proteins from other species. Strikingly, in the majority of cases where homologies were found, matches were found in all three databases – *C. elegans* proteins, other nematode sequences, and non-nematode sequences (1,785/2,826). Many gene products in this category are conserved across metazoans. The 15% of clusters with no homology may contain novel or diverged amino acid sequences specific to *S. stercoralis* or contain 3' or 5' untranslated regions (UTRs) where amino acid level homology would be lacking. A comparison of open reading frame (ORF) length in contig sequences with and without BLAST homology confirms that the mean ORF length is shorter in contigs without homology at 135 amino acids (a.a.) versus those with homology at 176 a.a. (Suppl. Figure 1), a significant difference at >99% confidence (2-tailed T-test with unequal variance) (Snedecor and Cochran 1967). The distribution of ORF length for clusters lacking homology is bimodal indicating the possible presence of two populations; the first containing novel protein coding sequences with a similar distribution of ORF sizes to that found in sequences with homology and a second of UTR sequences containing random and generally short ORFs.

As expected for a clade IVA Strongyloididae nematode with phylogenetic proximity to the clade V Rhabditida (Blaxter et al. 1998), the *C. elegans* genome provided the best source of information for interpreting *S. stercoralis* sequences. 89.5% of clusters with matches showed homology to a *C. elegans* gene product (Figure 2), a higher percentage than observed for *Meloidogyne incognita* of clade IVB (85%) (McCarter et al. 2003) or the more distant clade I nematode *Trichinella spiralis* (82%) (our unpublished observations). The most conserved sequences between *S. stercoralis* and *C. elegans* include gene products involved in cell structure, protein synthesis, and metabolism (e-values

of 1e-243 – 1e-187) (Suppl. Table 1). Representation of these clusters in L1 and L3i varied from common to rare and from shared to stage-specific. None of the most conserved gene products were nematode specific. 558 clusters (19.7% of those with matches) had homology only to nematodes, the most conserved with an e-value of 1e-106. Included among the most conserved nematode-specific clusters were homologs of previously characterized *C. elegans* structural proteins such as UNC-87 calponin (SS01345.cl) (Goetinck and Waterston 1994) and LET-805 myotactin (SS03242.cl) (Hresko et al. 1999). Cases were also identified of *S. stercoralis* sequences arising from putative ancestral nematode genes lost in the lineage leading to *C. elegans*. For example, SS01920.cl contains a prolyl oligopeptidase beta-propeller domain (IPR004106) (Rennex et al. 1991) not previously described in nematodes but present in our ESTs from *S. ratti*, *Parastrongyloides trichosuri*, and *Dirofilaria immitis*. SS00116.cl has homology to *Drosophila melanogaster* protein CG1167 (Q9VXQ8) as well as *P. trichosuri* and *D. immitis* ESTs, but lacks a *C. elegans* homolog. Several parasitic nematode species have been demonstrated to harbor prokaryotic related sequences including plant parasites that express rhizobacteria-like transcripts from their nuclear genomes (possibly as a result of horizontal gene transfer) (Scholl et al. 2003) and filarial nematodes that possess a *Wolbachia* bacterial endosymbiont (Blaxter et al. 1999). Other nematodes, such as *C. elegans*, lack prokaryotic-like sequences and endosymbionts. Surveying clusters as in McCarter et al. (2003), we found no evidence for prokaryotic-like sequences in *S. stercoralis*.

Abundant Transcripts Expressed in L1 Versus L3i

The 25 most highly represented clusters accounted for 27% of ESTs (Table 1A). Representation in a cDNA library generally correlates with abundance in the original biological sample (Audic and Claverie 1997), although artifacts can occur. Among the most abundant clusters, four have homology to known parasite antigens. Two are highly represented in L3i; IgG immunoreactive antigen (SS00012.cl) is observed in patients with chronic Strongyloidiasis (Ramachandran et al., 1997, direct submission), and L3NieAg.01, represented by 3 clusters, is a putative member of the *Ancylostoma* secreted protein (ASP) family (Hawdon et al. 1996). Among transcripts abundant in both stages are SS01569.cl with homology to genes encoding calreticulin-related antigens in *Necator americanus* (Pritchard et al. 1999) and *Onchocerca volvulus* (Rokeach et al. 1994), and SS01566.cl with weak homology to the immunodominant hypodermal SXP-1 antigen used as a filarial diagnostic tool (Dissanayake et al. 1992; Dissanayake et al. 1994; Klion et al. 2003).

Highly abundant L1-specific clusters (Table 1A, 1B) include genes encoding specific collagens (SS00069.cl, SS00001.cl, SS01498.cl, SS01492.cl, and SS01490.cl). Remarkably, thirty eight *S. stercoralis* collagen encoding clusters are detected in L1 with none found in L3i. In *C. elegans*, the cuticular collagen superfamily consists of about 100 members (Mayne and Brewton 1993) and several dozen are characterized in other nematodes (Selkirk et al. 1989; Selkirk and Blaxter 1990; Cox 1992). While sharing conserved sequences, nematode collagens are often developmentally regulated and not functionally redundant (Levy et al. 1993). In *C. elegans*, collagens are expressed in waves coinciding with the four molts (L1 to L2, etc.) (Johnstone 1994; Johnstone and Barry 1996). A survey of genes expressed in *C. elegans* dauer versus other stages found no collagen genes among 358 dauer-specific transcripts, but numerous collagens among the genes expressed during nutrient-rich conditions where molting worms were present including 6 of the 20 most abundant transcripts (Jones et al. 2001). Likewise in our survey of 5,713 ESTs from root-knot nematode *Meloidogyne incognita* L2 (infective dauer-like stage), only 3 collagen ESTs were found (0.05% of transcripts) (McCarter et al. 2003) though collagens are more common in other stages (our unpublished observations). Down regulation of collagen expression may be a general feature of the long-lived non-molting dauer/infective stage in many nematodes, a possibility that is now being explored. Abundant L3i-specific clusters (Table 1A, 1C) encode several novel proteins (SS01581.cl, SS01616.cl) as well as the first sheath protein (SHP3) encoding transcript described in *S. stercoralis* (SS01534.cl). Nematode surface proteins (SHP1-SHP5) have been studied in *Brugia malayi* and *Litomosoides sigmodontis* (Selkirk et al. 1991; Zahner et al. 1995; Conraths et al. 1997).

Comparative Genomics of Transcription in *S. stercoralis* L1 Versus L3i and *C. elegans* Nutrient-Rich Conditions Versus Dauer

Figure 3 displays the distribution of the 3,311 clusters in a Venn diagram based on EST library of origin. The majority of clusters had representation only in L1 or L3i with just 12% of clusters containing ESTs from both libraries. Excluding singletons, 27% of the clusters are mixed L1/L3i. The limited number of shared clusters between the two libraries is most likely a result of true differences in the abundance of gene expression between the two stages; even for transcripts with abundant representation, many clusters remain stage-specific or stage-biased (Suppl. Figure 2). Limited overlap could also result from allelic variation between the two *S. stercoralis* strains used in library production or from limited depth of sampling. Allelic variation between strains is unlikely to result in the formation of multiple clusters representing the same gene since any contigs sharing >93% nucleotide identity over ≥100 nucleotides

will share the same cluster. Nuclear polymorphisms are rarely this extensive, especially within coding regions. In *C. elegans*, comparison of the N2 and the reproductively isolated Hawaiian strain detected a polymorphism rate of 0.115% over 5.4 million bases (Wicks et al. 2001). The EST sample size is sufficient that 261 of the clusters with stage-biased expression are large enough (≥4 members for L1, ≥6 members for L3i) that bias toward one stage can be considered statistically significant by the pairwise-test ($P<0.05$) (Audic and Claverie 1997).

Based on morphology and behavior, *C. elegans* dauer larvae and infective stage L3i of many animal parasites are believed to be equivalent, and searches for homologs of *C. elegans* dauer pathway genes are underway in many parasites including *S. stercoralis* (Massey et al. 2001). As a first comprehensive comparative genomics approach to examining conservation of gene function during nematode evolution, transcripts with stage-specific or stage-biased gene expression were compared between *S. stercoralis* (L1 versus L3i) and *C. elegans* (dauer versus other stages). The aim of this comparison was to determine whether or not there is any pattern of shared expression of homologs in like-stages between species (L3i with dauer, and L1 with other stages). In *C. elegans*, gene expression in dauer versus other stages had previously been compared in our lab by serial analysis of gene expression (SAGE) (Jones et al. 2001). In that study, non-dauer stages were mixtures of all feeding larval stages (L1-L4) and adults containing embryos growing in nutrient rich conditions where L1's made up ~5% of the sample volume; for simplicity we refer to these mixed stages as "nutrient-rich-specific". Out of 11,130 detected *C. elegans* genes, 6,496 were common to both groups, 328 were identified as statistically significant dauer-specific while 489 were nutrient-rich-specific by the Fisher exact test ($P<0.05$) (Jones et al. 2001).

S. stercoralis L1 and L3i biased or specific clusters were compared to the *C. elegans* dauer-specific and nutrient-rich-specific genes at a variety of BLAST thresholds (1e-30, 1e-15, and 1e-05). In all cases, the overwhelming result was that BLAST matches were dominated by *S. stercoralis* L1 / *C. elegans* nutrient-rich matches with L1 / dauer, L3i / nutrient-rich, and L3i / dauer matches less common. This was true even though L1's made up only a fraction of the *C. elegans* mixed-stage starting material used for SAGE, perhaps because of shared expression between all feeding and growing stages or specifically between L1's and embryos. One example is given in Figure 4 using the full set of *S. stercoralis* L1 and L3i-specific clusters at the 1e-30 BLAST threshold. Based on the relative sizes of the input data-sets (1,342, 1,573, 489, 328), the null expectation is that of the 144 matches found, 39.7 would fall in the L1 /

nutrient-rich quadrant; instead, 73 such matches were observed. Assessment of the deviation of the observed from the expected values results in the highly significant chi-square (X^2) value of 39.3. A X^2 value >7.8 is required for significance at P<0.05 (Steel and Torrie 1960; Snedecor and Cochran 1967). Significant concentrations of matches in the L1 / nutrient-rich quadrant were also seen at BLAST cut-offs of 1e-15 and 1e-05.

Next, we restricted consideration to just the larger 178 L1-biased and 83 L3i-biased *S. stercoralis* clusters with statistically significant biased representation (Suppl. Figure 2) (Audic and Claverie 1997). In this case as well, the comparison to *C. elegans* showed a strong preference toward L1 / nutrient-rich matches with highly significant X^2 values at BLAST cut-offs of 1e-15 and 1e-05. At 1e-15 for instance, 36 of 53 matches were in the L1 / nutrient-rich quadrant versus an expectation of 21.6. Sample sizes were inadequate for comparison at 1e-30. In addition to the assumption that the distribution of matches would reflect the relative sizes of the starting datasets, we also considered the null hypothesis that the *S. stercoralis* L3i data-set should show a similar distribution of matches to the *C. elegans* nutrient-rich versus dauer categories as is seen with the L1 data-set. This null hypothesis was also rejected at P<0.05; the L3i-specific or L3i-biased data-sets were significantly more likely than the L1 data-sets to distribute their matches in dauer by measures of 1.3 to 3.9-fold depending on the data-sets and thresholds used.

At least two factors could account for the concentration of *S. stercoralis* / *C. elegans* BLAST matches in the L1 / nutrient-rich quadrant. First, these matches may reflect actual evolutionary conservation of expression pattern by homologs between the two species; i.e. genes excluded from L3i in *S. stercoralis* tend to be excluded from dauer in *C. elegans*. Second, genes expressed during L1 and nutrient-rich growth may be more likely to have conserved sequences than genes expressed during L3i / dauer. Evidence suggests that both these factors are involved. Addressing sequence conservation, Jones et al. (2001) noted that 15 of the 20 most abundant dauer-specific transcripts in *C. elegans* were of unknown function whereas fewer of the most abundant non-dauer specific transcripts were unknowns (8 of 20), suggesting the possibility that dauer-specific genes are more rapidly evolving or less likely to be found in other species (Jones et al. 2001). Other *C. elegans* studies have also found that genes with roles in later or specialized stages of development (i.e. dauer as opposed to embryonic or germ line), tend to be less conserved (Castillo-Davis and Hartl 2002; Kamath et al. 2003). Similarly, *S. stercoralis* L3i clusters appear to be less conserved than L1 clusters. At a BLAST threshold of 1e-05, 95% of statistically significant L1-biased clusters

have *C. elegans* homologs versus only 82% of the L3i-biased clusters. Also at 1e-05, 84% of L1-specific clusters have *C. elegans* homologs versus only 66% for L3i-specific clusters. Recalculating the expected values for the quadrants in the comparisons described above, counting only clusters with BLAST matches for input data-set sizes, still results in statistically significant X^2 values, but less severe than those seen before the adjustment. For instance, in the Figure 4 example the X^2 value changes from 39.3 to 27.8. Our current interpretation is that in part the reason for the concentration of matches in the L1 / nutrient-rich quadrant is the higher degree of sequence conservation in these stages with the remainder resulting from shared expression pattern of homologs.

While a greater proportion of *S. stercoralis* L3i-specific genes than L1-specific genes have homologs among the *C. elegans* dauer-specific transcripts, we did not uncover evidence for a robust L3i / dauer 'expression signature' conserved between the two nematodes. There are a number of challenges that may prevent use of this EST-based comparative genomics approach to identify key genes involved in these stages of presumably shared origin. First, a major limitation of this approach is sample size. By using only stage-biased or stage-specific transcripts in our comparison, we are essentially limiting analysis to 4.2% of *C. elegans* genes and 1.2% or 13.3% of *S. stercoralis* genes (assuming 19,500 genes per species). The intersection of these comparisons tends to be rather small (several dozen to several hundred matches). Second, use of stage-specificity as a criterion may be inappropriately severe since expression of a gene in other stages does not prevent it from still playing a critical role in the stage of interest (i.e. L3i / dauer). Also, we currently have no available information to make comparisons based on organ or tissue of expression; substantial changes in gene expression that occur in the context of specific cells may be lost in our whole organism analysis. Third, the speed of evolution of genes involved in L3i and dauer may make detection of homologs involved in these stages more difficult than for genes expressed in other more conserved stages. It is also possible that while structurally and functionally very similar, the *C. elegans* dauer stage and *S. stercoralis* L3i stage may have evolved to be substantially different at the molecular level or could even conceivably have arisen by convergent evolution. Overcoming these challenges would likely involve having the full *S. stercoralis* genome sequence (for ortholog mapping) as well as full genome microarray or SAGE data that could be compared to data generated by the equivalent methods in *C. elegans*.

Homologs of *C. elegans* Genes Involved in Dauer Determination and Biology

As an additional approach to uncover *S. stercoralis* genes involved in L3i / dauer biology, clusters were examined for homologs of 37 genes involved in dauer entry or maintenance in *C. elegans* (Jones et al. 2001). Twenty five such *C. elegans* genes identified 36 *S. stercoralis* homologs, including 12 with high identity matches that may indicate orthology (Table 2). Included among the list are eight homologs of *daf* (dauer formation defective) genes (Georgi et al. 1990; Estevez et al. 1993; Larsen et al. 1995; Lin et al. 1997), as well as glutathione peroxidase genes (Vanfleteren 1993) and superoxide dismutase. Five *S. stercoralis* homologs showed a bias toward expression in L3i versus L1 including homologs of the *daf-12* nuclear hormone receptor (SS01351.cl), F26E4.12 glutathione peroxidase (SS01468.cl), F38E11.2 heat shock protein (SS01374.cl), F22F1.1 histone H1 (SS01412.cl), and most strikingly a homolog of T26C11.2 (SS00028.cl) with 136 L3i ESTs and zero L1 ESTs. The availability of these sequences will aid in a more thorough study comparing *C. elegans* dauer and *S. stercoralis* L3i.

Comparison to *C. elegans* Genes with RNAi Phenotypes

RNA interference (RNAi), whereby the introduction of a sequence-specific double stranded RNA leads to degradation of corresponding mRNAs (Fire et al. 1998), has allowed the surveying of thousands of *C. elegans* genes for knockout phenotypes (Fraser et al. 2000; Gonczy et al. 2000; Maeda et al. 2001; Kamath et al. 2003). Such information is potentially transferable to understanding which genes play crucial roles in other nematodes including parasites. RNAi has been demonstrated in three parasitic nematodes (Hussein et al. 2002; Urwin et al. 2002), but is not yet adaptable to rapid screening. A list of 4,786 *C. elegans* genes assayed by RNAi as of June 2002 was compared to the list of the 2,528 *S. stercoralis* clusters with *C. elegans* homologs. RNAi experimental information was available for the most closely related homolog in 1,059 cases and a phenotype was apparent in 401 cases (38%) (Suppl. Tables 2 and 3). In contrast, RNAi surveys of all predicted genes in *C. elegans* resulted in phenotypes in just 10-14% of cases (Kamath et al. 2003) and 27% for genes with evidence of expression (Maeda et al. 2001). Additionally, *C. elegans* genes with expressed *S. stercoralis* homologs were more likely to have severe RNAi phenotypes such as embryonic lethality and sterility (Figure 5). Previously, a correlation between severity of phenotype in *C. elegans* and sequence conservation across phyla had been shown by selecting genes with homologs in *Saccharomyces*, *Drosophila*, and human (Fraser et al. 2000; Gonczy et al. 2000). Here we show the same trend following detection of homology with an expressed gene in another nematode species.

To determine whether genes expressed at various stages and levels in *S. stercoralis* differ in the likelihood that their *C. elegans* homologs have RNAi phenotypes, we compared phenotypes observed for the best scoring homologs of L1 and L3i expressed clusters. *C. elegans* homologs of *S. stercoralis* clusters with significant L1-biased (178) or L3i-biased (83) expression, show a significant difference with 69% (62/90) of L1 homologs having phenotypes versus only 30% for L3i (10/33) (X^2 test, $P<0.05$) (Snedecor and Cochran 1967). Nearly half of the L1 homologs with phenotypes are ribosomal proteins (20) or structural proteins such as actin and myosin (9), categories not found among the L3i-biased genes. Clusters with lower levels of expression did not show a statistically significant difference between L1 and L3i; using the full sets of *S. stercoralis* L1-specific (1,342) and L3i-specific (1,573) clusters resulted in *C. elegans* homologs with phenotypes for 42% of L1 (230/551) versus 37% of L3i clusters (209/563). Previous data has shown that evidence of expression in *C. elegans* or other nematodes enriches for genes with RNAi phenotypes (Fraser et al. 2000; Maeda et al. 2001; McCarter et al. 2003). The comparison here between L1 and L3i demonstrates that the particular stage and level of expression in another nematode is also an important predictor of phenotype, with *C. elegans* genes having *S. stercoralis* homologs highly expressed in L1 being nearly six times as likely to have a phenotype as the average *C. elegans* gene surveyed by RNAi. High level expression in L3i does not have quite as dramatic an effect of enriching for genes with phenotypes (2.5-fold versus 6-fold increase) for perhaps two reasons. First, high throughput RNAi screens in *C. elegans* have observed nematodes during growth in nutrient rich conditions when dauer larvae are not present. Screening for RNAi phenotypes in worms induced to enter dauer may detect phenotypes not seen in standard screens. It is also possible that the repertoire of genes expressed in dauer / L3i are truly less likely to result in phenotypes following knock-out as genes active in L1.

Functional Classification Based on Gene Ontology and Kegg Assignments

To categorize transcripts by function, we utilized the Gene Ontology (GO) classification www.geneontology.org. Interproscan was used to match *S. stercoralis* clusters to Interpro protein domains which themselves are already mapped into the GO hierarchy. Of 3,311 clusters, 1,298 (39%) align to InterPro domains and 870 (26%) map to GO. Among the more highly expressed stage-biased clusters, 49% of L1-biased clusters map to the GO hierarchy versus only 36% for L3i. GO representation for *S. stercoralis* clusters is shown by biological process, cellular component, and molecular function (Figure 6, Suppl. Table 4). GO representation among 4 nematode

species is shown in Suppl. Table 5 (Quackenbush et al. 2001). The greater percentage of extracellular mappings in *S. stercoralis* is largely attributable to 17 contigs with homology to the VAP venom allergen family (Blaxter 2000; Ding et al. 2001). As an alternative categorization method, clusters were assigned to metabolic pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database www.genome.ad.jp/kegg. Enzyme commission numbers were assigned to 484 of 3,311 clusters (15%) which allowed mapping of 285 (9%) to KEGG (Table 3). Metabolic pathways well represented by the *S. stercoralis* clusters include glycolysis/gluconeogenesis, and biochemical networks involving pyruvate, butanoate, purines, pyrimidines, glutamate, tyrosine, and glycerolipids. Four enzymes involved in nucleotide sugar metabolism were expressed in L1 whereas none were expressed in L3i. These include SS00993.cl, encoding a putative UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9), and SS0508.cl and SS01928.cl encoding dTDP-glucose 4,6-dehydratases (EC4.2.1.46). The *C. elegans* homolog of this enzyme, F53B1.4, is strongly expressed in most life-cycle stages but absent from dauer larvae (Hill et al. 2000; Jones et al. 2001). Genes encoding nucleotide sugar biosynthesis enzymes in *C. elegans* are expressed in many tissues, including gut, neurons and the vulva. Mutations in some of these genes affect vulval invagination, oocyte development, and embryogenesis (Herman and Horvitz 1997; Herman et al. 1999). The observed down-regulation of nucleotide sugar metabolism in L3i and dauer is consistent with the lack of new cell division and DNA replication in these developmentally arrested stages. Complete lists of GO and KEGG mappings for *S. stercoralis* are available at www.nematode.net.

CONCLUSIONS

Increasing information on stage of gene expression now makes possible comparisons of gene expression patterns between related species. In one of the first such studies, we examined expression of homologous genes in *S. stercoralis* and *C. elegans*, observing conservation of genes expressed during growth in nutrient rich conditions and exclusion of collagen expression from the dauer equivalent stage in both species. Information on additional species and stages will help to refine our view of how patterns of gene expression have changed during nematode evolution. However, based on this analysis we anticipate that detecting robust stage-specific 'expression signatures' conserved between distant nematodes will be quite challenging. Microarray experiments, which can better detect levels of gene expression than ESTs will aid in these comparisons. As recently as February 2000, only 57 ESTs from Strongyloididae nematodes had been deposited in dbEST. As of March 2003, our submissions have brought that number to 30,115, including ESTs from the

closely related species *S. stercoralis* (11,392), *S. ratti* (10,760), and *Parastrongyloides trichosuri* (7,963) (Dorris et al. 2002). Stages represented include L1, L2, L3, and adult, and derive from both the homogonic and heterogonic life cycles. Unlike most parasites, the heterogonic cycle of Strongyloididae species (Viney 1999) allows maintenance of cultures outside the host mammal in lab conditions identical to those used for *C. elegans*. Strongyloididae species are therefore more amenable to attempts at transferring techniques developed in *C. elegans* to parasitic nematodes including transformation (Lok and Massey 2002) and RNAi, as well as mutagenesis and gene mapping. The number of generations which a Strongyloididae species can be maintained in culture away from its host varies greatly from only one for *S. stercoralis* to upwards of fifty for *P. trichosuri* (W. Grant, pers. comm. and our unpublished observations). Such technical advantages for study, as well as the medical importance of *S. stercoralis*, make Strongyloididae species good candidates for the eventual generation of a draft genome sequence.

MATERIAL AND METHODS

Libraries and EST Generation

The L1 cDNA library was created from $2x10^3$ *S. stercoralis* larvae recovered from jirds (*Meriones unquiculatus*) infected with a strain maintained in dogs. The L3i cDNA library used $4x10^5$ larvae from a strain passed repeatedly in *Patas* monkeys (Harper et al. 1984). The genetic or environmental propensity of the strains to favor heterogonic versus homogonic development is not known. Unidirectional libraries were constructed in Uni-ZAP XR (Stratagene, Cedar Creek, TX, USA) (McCarrey and Williams 1994). The L1 library had an unamplified titer of $1x10^5$ plaque-forming units per ml (pfu/ml), an average insert size of 675 bp, and ~15% non-recombinants. The L3i library had an unamplified titer of $1.5x10^6$ pfu/ml, an average insert size of 957 bp, and ~3% non-recombinants. For sequencing, the phagemid was excised and replicated in XL-1 Blue MRF' cells. Sequencing and EST processing were performed as described (Hillier et al. 1996; Marra et al. 1999; McCarter et al. 2000; McCarter et al. 2003). Prior to dbEST submission, sequences were processed to assess quality, trim vector, remove contaminants and cloning artifacts, and identify BLAST similarities (Hillier et al. 1996). www.nematode.net provides information on trace files and clone ordering. From 14,950 attempts, 11,335 sequences (76%) passed filtering and were submitted to dbEST (www.ncbi.nlm.nih.gov/dbEST). The average submitted read length was 435±101 nucleotides (457 for L1, 420 for L3i). The 10,921 ESTs analyzed here include 4,473 L1 and 6,435 L3i submissions. An additional 414 ESTs submitted later are not included. Reads were failed for poor trace quality (~

19.8% of all reads); missing insert (~ 3.7%); *E. coli* contamination (~ 0.4%); and small insert size (~ 0.1%).

Clustering and Sequence Analysis

Clustering was performed as described (McCarter et al. 2003) using Phred, Phrap, Consed, and BLAST programs (Ewing et al. 1998; Ewing and Green 2000). The completed assembly, NemaGene *Strongyloides stercoralis* v 2.0, is available at www.nematode.net. Fragmentation, defined as the representation of one gene by multiple non-overlapping clusters, was estimated by examining *S. stercoralis* clusters with homology to *C. elegans*. Best-scoring BLASTX matches against Wormpep found matches to 2,348 non-overlapping regions of 2,090 *C. elegans* proteins, for a fragmentation index of 11%. 216 proteins were represented in 2 non-overlapping regions, 16 proteins in 3 regions, and 2 proteins in 4 regions. Overlapping matches by multiple clusters to the same region of a *C. elegans* gene was not considered fragmentation since these clusters had already been directly compared to one another by BLASTN. WU-BLAST sequence comparisons were performed as described (Altschul et al. 1990; Gish 1996-2002; McCarter et al. 2003) using contig sequences as queries versus multiple databases including the SWIR v.21 (5/19/2000) protein database, Wormpep v.54 *C. elegans* protein database (Wellcome Trust Sanger Institute, unpublished), and internal databases constructed using intersections of Genebank data, such as nematode sequences excluding *C. elegans* and *S. stercoralis*. This allows examination of sequences in specific phylogenetic distributions (Wheeler et al. 2001). Homologies were reported for e-value scores of 1e -05 and better. TRANSLATE was used to translate contigs for ORF analysis (S. Eddy, unpublished).

Comparison of *S. stercoralis* and *C. elegans* Stage-specific Transcripts

To examine shared gene expression patterns between nematodes, all *S. stercoralis* 1,342 L1-specific and 1,573 L3i-specific clusters were compared by BLAST to 489 non-dauer-specific and 328 dauer-specific *C. elegans* genes (Jones et al. 2001). Additionally, 178 L1-biased clusters and 83 L3i-biased clusters from *S. stercoralis* with statistically significant stage-biased expression based on sample size were selected using a pairwise test (Audic and Claverie 1997). Null hypotheses about the distribution of matches between datasets were tested using the Chi-square statistic with one or three degrees of freedom as appropriate (Steel and Torrie 1960). Comparisons used BLASTX to match cluster nucleotide sequences to *C. elegans* gene translations in Wormpep v.54 at 1e-05, 1e-15, and 1e-30. SAGE tag sequences were used only as a means of identifying *C. elegans* genes (Jones et al. 2001), and were not used in sequence comparisons to *S. stercoralis*.

Functional Assignments

Clusters were assigned putative functional categorization as described (McCarter et al. 2003) using InterProScan v.3.1 (ftp://ftp.ebi.ac.uk/pub/software/unix/iprscan), InterPro domains (11/08/02) (InterProScan ; Apweiler et al. 2001; Zdobnov and Apweiler 2001), InterPro to GO mappings, and Gene Ontology categorization (go_200211_assocdb.sql) (The Gene Ontology Consortium 2000). Mappings are stored in a MySQL database and displayed using AmiGo (11/25/02) (www.godatabase.org/cgi-bin/go.cgi). Clusters were assigned by enzyme commission number to metabolic pathways using the KEGG database (Kanehisa and Goto 2000; Bono et al. 1998; IUBMB 1992). To identify cases where *S. stercoralis* homologs in *C. elegans* have been surveyed for knockout phenotype using RNAi, Wormpep BLAST matches were cross-referenced to a list of all 7,212 available *C. elegans* RNAi experiments (6,107 genes) (5/5/2002) (www.wormbase.org). For each *S. stercoralis* cluster, only the highest scoring *C. elegans* match was considered.

SUPPLEMENTAL DATA FILES

The following files are available online: **Table 1** – Most conserved nematode genes between *S. stercoralis* and *C. elegans*. **Table 2** - Complete list of *C. elegans* RNAi phenotypes for genes with *S. stercoralis* homologs. **Table 3** - Classification of *C. elegans* RNAi phenotypes for genes with *S. stercoralis* homologs. **Table 4 –** *S. stercoralis* Gene ontology mappings – A. biological process, B. cellular component, C. molecular function. **Table 5** - Comparison of gene ontology mappings among nematode species. **Figure 1** - Distribution of contigs by size of longest open reading frame. **Figure 2** - Histogram for *S. stercoralis* Nemagene v2.0 clustering showing the distribution of clusters by EST origin in L1 or L3i.

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FIGURE LEGENDS

Figure 1

Histogram for *S. stercoralis* Nemagene v2.0 clustering showing the distribution of ESTs by cluster size. For example, there were 3 clusters of size 26 containing a sum of 78 ESTs. Cluster size (X-axis) is shown to scale for 1 to 50 members with the size of larger clusters indicated. 17% of all clusters are singletons and 53% of clusters contain 10 or fewer members. Distribution of contigs sizes is not shown.

Figure 2

Venn diagram showing distribution of *S. stercoralis* cluster BLAST matches by database. Databases used were: for *C. elegans*, Wormpep v.54 and mitochondrial protein sequences; for other nematodes, all GenBank nucleotide data for nematodes except *C. elegans* and *S. stercoralis*; and for non-nematodes, SWIR v.21 with all nematode sequences removed.

Figure 3

Venn diagram showing distribution of *S. stercoralis* clusters based on library of origin of each cluster's EST members. The majority of clusters are either L1-specific or L3i-specific.

Figure 4

Data-sets of *S. stercoralis* L1-specific and L3i-specific clusters (lower left) were used to search sets of *C. elegans* nutrient-rich-specific and dauer-specific clusters (top right) at 1e-30 resulting in 144 BLAST matches distributed in four quadrants (lower right). Each quadrant shows the number of observed matches and expected matches, based upon the null hypothesis that the distribution of matches would reflect the relative sizes of the input data-sets. Matches were found to be concentrated in the L1 / nutrient-rich quadrant (gray triangle). Although individual *S. stercoralis* clusters may lack adequate sample size to be considered significantly biased in their expression alone, the full set of L1 and L3i-specific clusters including singletons can be used in this comparison since the collection as a whole is significantly biased. Restriction of the L1 and L3i sets to clusters with statistically significant L1 and L3i-biased expression shows the same type of distribution with a concentration in the L1 / nutrient-rich quadrant.

Figure 5

A comparison of phenotype distribution between all RNAi-surveyed *C. elegans* genes (left) versus only those genes with homology to *S. stercoralis* (right). Large columns display percent with and without phenotype. Small columns display percent breakdown of various phenotypes observed. *C. elegans* genes with *S. stercoralis* homologs are significantly more likely to have phenotypes by RNAi.

Figure 6

Percentage representation of gene ontology (GO) mappings for *S. stercoralis* clusters by (A) biological process, (B) cellular component, and (C) molecular function. See Suppl. Table 4 for details. Note that individual GO categories can have multiple mappings. For instance, GO:0015662: P-type ATPase (cluster-SS01525, Interpro domain IPR004014), is a nucleic acid binding protein, a hydrolase enzyme, and a transporter.

Suppl. Figure 1 (supplemental data on line)

Distribution of contigs by size of longest open reading frame. Solid line = contigs with any database homology by BLASTX (1,445). Dotted line = contigs without database homology (353).

Suppl. Figure 2 (supplemental data on line)

Histogram for *S. stercoralis* Nemagene v2.0 showing the distribution of clusters by number of ESTs in L1 and L3i. Clusters with a statistically significant bias in expression are bounded by a triangle, blue for L1-biased and red for L3i-biased. The X and Y axis are each linear from 0 to ~35. The 14 largest clusters are not shown to scale; see Table 1A for actual number of L1 and L3i ESTs in the largest clusters.

TABLES

Table 1A. The most abundantly represented transcripts in the *S. stercoralis* cDNA libraries

	Cluster	ESTs per cluster	ESTs from library L1	ESTs from library L3i	non-redundant GenBank Best identity descriptor	non-redundant GenBank Accession SW / TR	non-redundant GenBank E - value	*C. elegans* Gene Wormpep
1	SS00012.cl	1097	8	1089	*S. stercoralis* IGG immunoreactive antigen	O44394	6e - 91	F54B11.2*
2	SS00013.cl	234	4	230	*Crithidia fasciculata* MURF-2 protein	Q34096	5e - 06	-
3	SS00010.cl	159	1	158	*Podocoryne carnea* POD-EPPT, pod-EPPT protein	Q25677	2e - 31	T05A10.1*
4	SS00028.cl	136	0	136	*Zea mays* HRGP, hydroxyproline-rich glycoprotein	Q41814	3e - 18	Y22D7AR1*
5	SS01581.cl	131	0	131	novel	-	-	-
6	SS01580.cl	129	6	123	*S. stercoralis* L3NIEAG.01	Q9UA16	3e - 40	C07A4.3*
	SS00064.cl	56	0	56	*S. stercoralis* L2NIEAG.01	Q9UA16	3e - 141	C07A4.3*
	SS01574.cl	48	2	46	*S. stercoralis* L3NIEAG.01	Q9UA16	1e - 40	C07A4.3*
7	SS01578.cl	109	101	8	novel	-	-	-
8	SS01394.cl	96	19	77	*Anisakis simplex* troponin-like protein	Q9U3U5	7e - 99	F54C1.7*
9	SS01577.cl	70	0	70	*C. elegans* HCH-1, metalloprotease	Q21059	2e - 53	F40E10.1
	SS01564.cl	38	0	38	*C. elegans* HCH-1, metalloprotease	Q21059	2e - 52	F40E10.1
10	SS01515.cl	49	47	2	*C. elegans* ADP/ATP carrier protein	O45865	8e - 178	T27E9.1
11	SS00004.cl	49	48	1	*C. elegans* 60S acidic ribosomal protein	Q93572	2e - 146	F25H2.10
12	SS01616.cl	43	0	43	novel	-	-	-
13	SS01569.cl	42	26	16	*Necator americanus* calreticulin precursor	O76961	1e - 185	Y38A10A.5*
14	SS00165.cl	42	36	6	*Onchocerca volvulus* disulfide isomerase precursor	Q25598	4e - 209	C07A12.4*
15	SS01567.cl	40	37	3	*C. elegans* 40S ribosomal protein S8	P48156	3e - 88	F42C5.8
16	SS01566.cl	39	22	17	*Loa loa* SXP-1, immunodominant hypodermal antigen	Q9GU97	1e - 05	-
17	SS01565.cl	39	2	37	*C. elegans* trehalase precursor	Q22195	3e - 94	T05A12.2
19	SS01563.cl	38	38	0	*C. elegans* ACT-4, actin 4	P10986	1e - 241	M03F4.2
19	SS01562.cl	37	19	18	*C. elegans* EF-1-ALPHA, elongation factor 1-alpha	P53013	4e - 186	F31E3.5
20	SS01355.cl	35	19	16	*C. elegans* FTT-2, 14-3-3 like protein	Q20665	9e - 131	F52D10.3A
21	SS00503.cl	35	33	2	*C. elegans* COX1, cytochrome C oxidase polypeptide I	P24893	5e - 108	-
22	SS00069.cl	34	34	0	*C. elegans* COL-34, cuticular collagen	Q20087	6e - 154	F36A4.10
23	SS01559.cl	33	27	6	*C. elegans* RPL-4, ribosomal protein L1	O02056	4e - 141	B0041.4
24	SS00810.cl	32	25	7	*C. elegans* RPS-9, ribosomal protein S9	Q20228	6e - 97	F40F8.10
25	SS01557.cl	31	0	31	*C. elegans* EGL-3, prohormone convertase 2	Q10575	5e - 120	C51E3.7A

Table 1B. Abundantly represented L1-specific transcripts#

	Cluster	ESTs per cluster	non-redundant GenBank Best identity descriptor	Accession SW / TR**	E - value	*C. elegans* Gene Wormpep
1	SS00001.cl	26	*C. elegans* COL-6, cuticle collagen 6	P18831	5e - 134	ZK1290.3
2	SS01536.cl	22	*C. elegans* arginine kinase	Q10454	1e - 145	F46H5.3
3	SS01533.cl	21	*Brugia pahangi* HSP 90, heat shock protein 90	O61998	6e - 234	C47E8.5*
4	SS01520.cl	18	*C. elegans* H3, histone 3	Q10453	8e - 81	F45E1.6
5	SS00723.cl	16	*C. elegans* 40S ribosomal protein S16	Q22054	2e - 58	T01C3.6
6	SS01504.cl	15	*C. elegans* UNC-54, myosin heavy chain	O02244	2e - 220	F11C3.3
7	SS00026.cl	15	*Monodelphis domestica* ribosomal protein S4 Y isoform	O62739	7e - 90	Y43B11AR.4*
8	SS01498.cl	14	*C. elegans* COL-10, cuticle collagen 10	Q17460	1e - 124	B0222.8
9	SS01492.cl	14	*C. elegans* cuticle collagen	Q20778	8e - 29	F54D8.1
10	SS01490.cl	14	*C. elegans* COL-1, cuticle collagen 1	Q19763	1e - 134	F23H12.4

Note: The two largest L1-specific clusters appear in Table 1A.

Table 1C. Abundantly represented L3i-specific transcripts##

	Cluster	ESTs per cluster	non-redundant GenBank Best identity descriptor	Accession SW / TR**	E - value	*C. elegans* Gene Wormpep
1	SS01556.cl	31	*Drosophila melanogaster* CG16995 prtoein	Q9VQA7	2e - 13	-
2	SS01540.cl	23	*C. elegans* protein id: CAB02955.1	O62173	8e - 54	F15D3.6
3	SS01145.cl	22	*Dictyostelium discoideum* SRF related protein	O76853	8e - 14	T05A10.1*
4	SS00011.cl	22	*C. elegans* lipase	O16380	3e - 32	K12B6.3
5	SS01534.cl	21	*Litomosoides sigmodontis* SHP3, microfilarial sheath protein	Q25402	4e - 25	F55B11.3*
6	SS01532.cl	21	*C. elegans* MAP1A/1B, microtubule associated protein	Q09490	2e - 65	C32D5.9
7	SS01528.cl	20	*C. elegans* tyrosine aminotransferase	Q93703	7e - 105	F42D1.2
8	SS01517.cl	17	*C. elegans* HSP70, heat shock protein 70	Q22758	4e - 62	T24H7.2
9	SS01508.cl	16	*C. elegans* protein id: AAB65951.1	O16458	2e - 94	F41E6.9
10	SS01505.cl	15	C. elegans protein id: CAB03200.1	P90845	4e - 33	F25B3.6

Note: The seven largest L3i-specific clusters appear in Table 1A.
* *C. elegans* homolog present but with a lower probability match than the best GenBank descriptor.
** SW/TR is Swiss-prot and TrEMBL Proteinknowledgebase (http://us.expasy.org/sprot/).

Table 2. *C. elegans* Genes with Potential Roles in Dauer having *S. stercoralis* Homologs

S. stercoralis Homolog	*C. elegans* Gene	Description	E-value	Possible Ortholog?	Other Homologs* Gene	Description	E-value	*S. stercoralis* ESTs L1	L3i
SS00947.cl	C05D2.1	daf-4 TGF-B receptor	2e -22	**yes**	see below			0	3
"	F29C4.1	daf-1 kinase	1e -07	no	see above			"	"
SS01351.cl	F11A1.3	daf-12, nuclear hormone receptor	3e -58	**yes**	F33D4.1	nhr-8, nuclear hormone receptor	8e -08	0	7
SS03246.cl	R13H8.1	daf-16 forkhead domain	5e -46	**yes**	R13H8.2		7e -29	1	0
SS02087.cl	"	"	2e -13	no	none			1	0
SS00592.cl	"	"	1e -13	no	F26D12.1	fkh-7, forkhead domain	1e -33	0	2
SS03322.cl	"	"	3e -07	no	none			1	0
SS02393.cl	B0334.8	daf-23 phosphoinositide 3-kinase	3e -10	no	F35H12.4	phosphatidylinositol 4-kinase	1e -57	0	1
SS01344.cl	C15F1.b	sod-1 superoxide dismutase	8e -64	**yes**	see below			2	5
"	F55H2.1	sod-4 superoxide dismutase	1e -36	no	see above			"	"
"	ZK430.3	superoxide dismutase	2e -57	no	see above			"	"
SS01223.cl	C08A9.1	sod-3 superoxide dismutase	8e -72	**yes**	F10D11.1	sod-2 superoxide dismutase	3e -74	4	1
SS00590.cl	C11E4.1	glutathione peroxidase	2e -90	**yes**	see below		5e -82	0	2
"	C11E4.2	glutathione peroxidase	5e -82	**yes**	see above		2e -90	"	"
SS01468.cl	F26E4.12	glutathione peroxidase	2e -60	**yes**	R05H10.5	glutathione peroxidase	2e -60	2	10
"	R03G5.5	glutathione peroxidase	2e -54	no	"	"	"	"	"
SS00462.cl	T24H7.1	prohibitin	3e -41	no	Y37E3.9	prohibitin	3e -80	2	0
SS01126.cl	R11A8.4	sir-2 silancing information regulator	1e -17	no	none			1	3
SS02217.cl	H42K12.1	pdk-1 protein kinase	7e -09	no	none			0	1
SS02646.cl	T28B8.2	ins-1 insulin like	3e -12	no	none			0	1
SS01374.cl	F38E11.2	hsp-12, alpha-B-crystallin	3e -9	no	C14B9.1	hsp-12, alpha-B-crystallin	3e -13	0	8
SS03005.cl	R02C2.3	G-protein receptor	2e -21	no	T14D7.2		4e -100	1	0
SS00028.cl	T26C11.2	novel	1e -10	no	Y22D7AR.1	tyrosine phosphatase	4e -16	0	136
SS03404.cl	F36D3.9	cysteine protease	2e -08	no	Y65B4A.2	cysteine proteinase	5e -45	1	0
SS01412.cl	F22F1.1	histone H1	3e -35	**yes**	F59A7.4	histone H1	3e -34	0	9
SS00818.cl	"	"	3e -40	**yes**	M163.3	his-24 histone	2e -42	3	0
SS01412.cl	C30G7.1	histone H1	1e -18	no	see above			0	9
SS00818.cl	"	"	1e -24	no	see above			3	0
SS00190.cl	C12D8.10	akt-1 kinase	5e -25	no	B0545.1B	tpa-1 protein kinase	2e -87	0	2
SS03259.cl	"	"	3e -23	no	T01H8.1B	spk-1 protein kinase C	1e -44	1	0
SS03125.cl	"	"	6e -27	no	ZK303.2B	kin-1 protein kinase	1e -34	0	1
SS02139.cl	F56C9.1	protein phosphatase 1	1e -73	**yes**	F23F11.6	serine/threonine phosphatase	6e -73	1	0
SS00017.cl	"	"	2e -51	**yes**	F23F11.6	"	1e -55	1	3
SS00691.cl	"	"	6e -37	no	F38H4.3	"	1e -68	2	0
SS00509.cl	"	"	4e -46	no	C05A2.1	"	5e -54	0	2
SS02292.cl	"	"	5e -45	no	Y75B8A.30	"	3e -61	0	1

***** The highest matching additional *C. elegans* homolog of the *S. stercoralis* cluster.

The following *C. elegans* genes with potential roles in dauer did not have *S. stercoralis* homologs among the clusters:

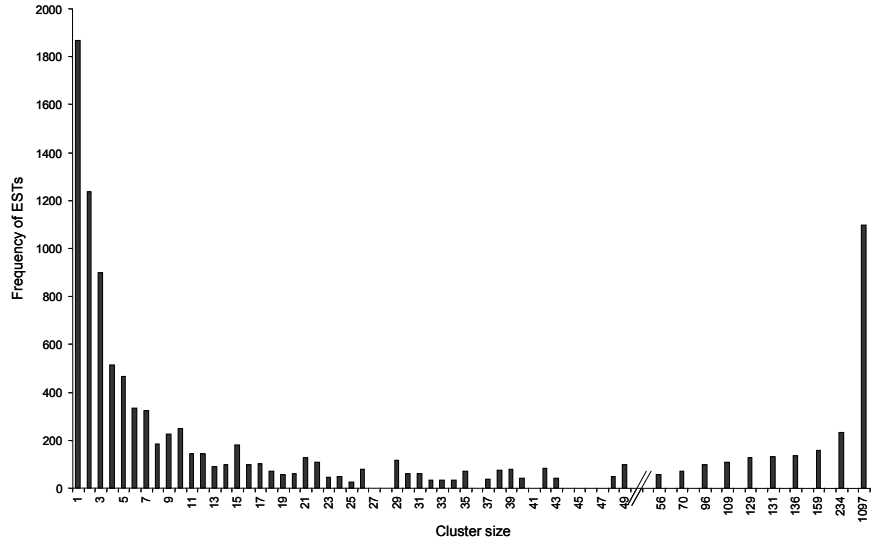
Y55D5A.b, *daf-2*	ZC395.6, *gro-1*	ZC395.2, *clk-1*
F25E2.5, *daf-3*	R10H10.2, *spe-26*	Y54G11A.6, *ctl-1* catalase
B0412.2, *daf-7*	C08H9.5, *cold-1*	Y54G11A.5, peroxisomal catalase

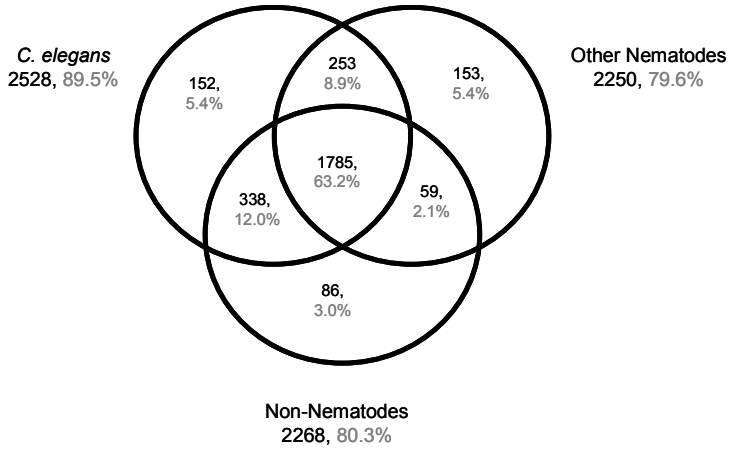
Selected *C. elegans* genes include those involved in signalling pathways for dauer entry and exit, genes known to play a role in dauer, and genes of interest with high levels of dauer expression in comparison to other stages (Jones et al., 2001).

Table 3. Kegg biochemical pathway mappings for *S. stercoralis* clusters

KEGG CATEGORIES REPRESENTED*	Clusters	Clusters per library			Enzymes
		L1	L3i	L1/L3i	
1.1 Glycolysis / Gluconeogenesis	21	14	6	1	13
1.2 Citrate cycle (TCA cycle)	19	13	2	4	8
1.3 Pentose phosphate cycle	14	10	3	1	9
1.4 Pentose and glucuronate interconversions	2	2	0	0	2
1.5 Fructose and mannose metabolism	15	10	5	0	9
1.6 Galactose metabolism	7	5	2	0	5
1.7 Ascorbate and aldarate metabolism	3	1	2	0	2
1.8 Pyruvate metabolism	24	12	7	5	12
1.9 Glyoxylate and dicarboxylate metabolism	10	7	1	2	5
1.10 Propanoate metabolism	14	7	7	0	8
1.11 Butanoate metabolism	15	7	8	0	11
2.1 Oxidative phosphorylation	22	10	5	7	4
2.5 Methane metabolism	1	1	0	0	1
3.1 Fatty acid biosynthesis (path 1)	8	6	1	1	2
3.2 Fatty acid biosynthesis (path 2)	5	2	2	1	2
3.3 Fatty acid metabolism	18	7	7	4	6
3.4 Synthesis and degradation of ketone bodies	1	0	1	0	1
3.5 Sterol biosynthesis	3	2	1	0	2
3.6 Bile acid biosynthesis	9	5	3	1	6
3.8 Androgen and estrogen metabolism	7	3	3	1	4
4.1 Purine metabolism	20	4	12	4	12
4.2 Pyrimidine metabolism	19	4	12	3	11
4.3 Nucleotide sugars metabolism	5	5	0	0	4
5.1 Glutamate metabolism	17	7	7	3	10
5.2 Alanine and aspartate metabolism	17	8	7	2	8
5.3 Glycine, serine and threonine metabolism	14	7	6	1	9
5.4 Methionine metabolism	3	3	0	0	3
5.5 Cysteine metabolism	5	3	2	0	4
5.6 Valine, leucine and isoleucine degradation	11	4	6	1	6
5.7 Valine, leucine and isoleucine biosynthesis	5	4	1	0	4
5.8 Lysine biosynthesis	1	0	1	0	1
5.9 Lysine degradation	14	4	10	0	7
5.10 Arginine and proline metabolism	16	9	7	0	8
5.11 Histidine metabolism	7	2	5	0	3
5.12 Tyrosine metabolism	15	6	8	1	10
5.13 Phenylalanine metabolism	11	6	3	2	8
5.14 Tryptophan metabolism	18	6	10	2	7
5.15 Phenylalanine/tyrosine/tryptophan biosynthesis	9	4	5	0	6
5.16 Urea cycle and metabolism of amino groups	4	3	0	1	2
6.1 beta-Alanine metabolism	11	7	4	0	6
6.2. Taurine and hypotaurine metabolism	4	2	2	0	3
6.3 Aminophosphonate metabolism	5	2	2	1	2
6.4 Selenoamino acid metabolism	4	4	0	0	4
6.5. Gyanoamino acid metabolism	2	1	1	0	2
6.6 D-Glutamine and D-glutamate metabolism	3	2	0	1	2
6.7 D-Arginine and D-ornithine metabolism	1	0	1	0	1
6.9 Glutathione metabolism	6	2	2	2	4
7.1 Starch and sucrose metabolism	15	10	3	2	9
7.2 Glycoprotein biosynthesis	5	3	1	1	4
7.4 Aminosugars metabolism	4	2	2	0	3
8.1 Glycerolipid metabolism	19	8	9	2	14
8.2 Inositol phosphate metabolism	4	1	2	1	4
8.4. Phospholipid degradation	2	1	1	0	2
8.5 Sphingoglycolipid metabolism	4	1	2	1	3
8.8 Prostaglandin and leukotriene metabolism	2	1	1	0	2
9.2. Riboflavin metabolism	1	0	1	0	1
9.3 Vitamin B6 metabolism	1	1	0	0	1
9.4 Nicotinate and nicotinamide metabolism	2	0	2	0	2
9.5 Pantothenate and CoA biosynthesis	5	2	3	0	4
9.8 One carbon pool by folate	1	1	0	0	1
9.11 Ubiquinone biosynthesis	25	7	15	3	3

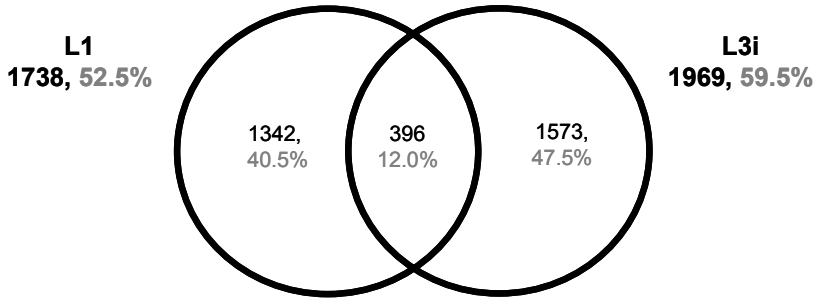
555 total and 312 unique mappings; 61 metabolic pathways are represented out of 83 possible.

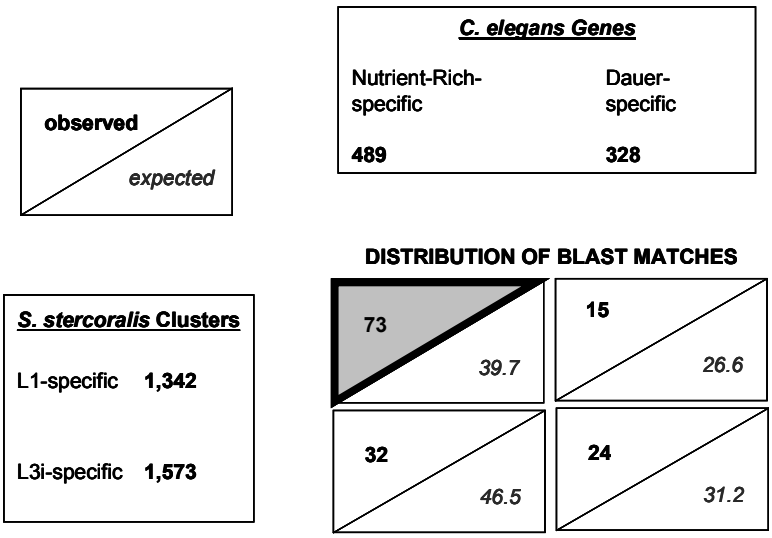




Mitreva_Fig1

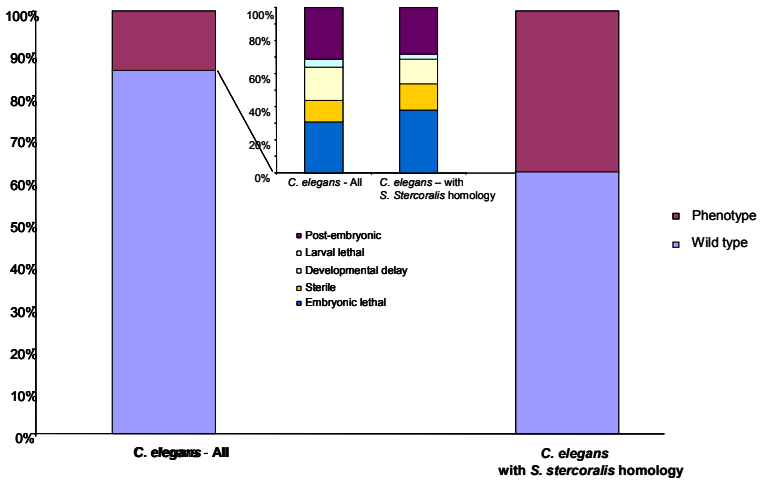
Mitreva_Fig2



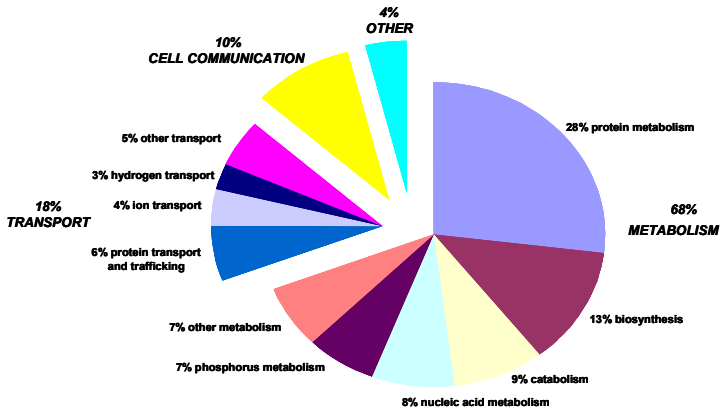


Mitreva_Fig3

Mitreva_Fig4



Mitreva_Fig5



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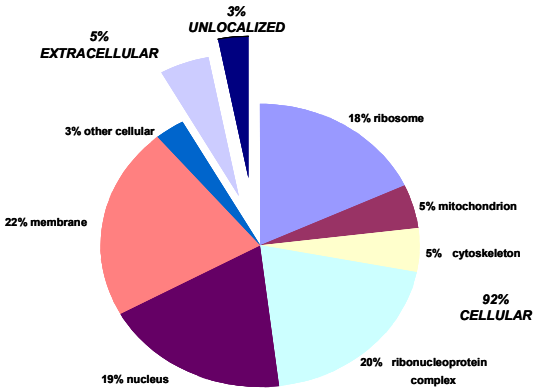
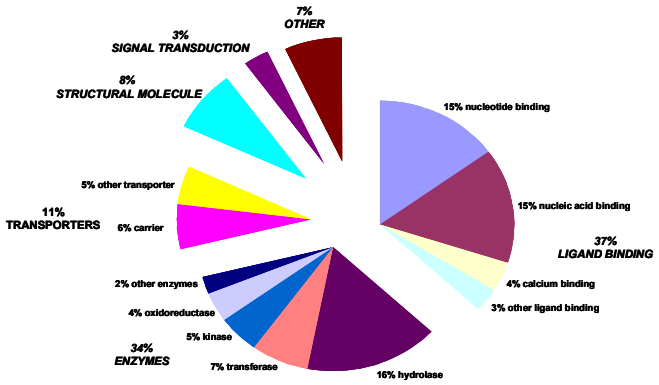


Mitreva_Fig6B



Mitreva_Fig6C