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# The Caenorhabditis Elegans Connectome Network

## Abstract

The *Caenorhabditis elegans* (*C. elegans*) is a type of nematode organism approximately 1 millimeter in length and diameter of 50 µm when fully grown [1]. Originally introduced for formal study in the 1970s, this small creature has been immensely helpful for biologists due to its relatively simple cellular composition and general abundance [2]. One of such key areas of study is the mapping of the neuronal connections and identifying the functions thereof. The way in which the nervous system of any organism encodes and transmits information between neurons via synapses is key to understanding physical and cognitive behaviors including decision-making, and therefore worthy of analysis and study. Treating each neuron as a node, and the connections between them via synapses as links, one can form a directed network of the entire nervous system, commonly referred to as a connectome. This analysis aims to analyze the connectome network of this nematode, extract interesting features, and compare it against generated models.

## Background

A neuron is a cell in an organism's nervous system. These cells form the basic building blocks where information can be processed. Neurons exchange information with other neurons via synapses. There are different types of synapses which can complicate the analysis. The types of synapses included in this analysis include Chemical, Electrical Asymmetric, and Electrical Symmetric. Electrical synapses can also sometimes be referred to as gap junctions. Neurons not only connect amongst themselves, but also with muscle cells. Neurons that connect with muscle tissues can be referred to as Neuromuscular and by applying an electric signal they cause the muscle to contract, thereby offering motion.

Many of the details are outside the scope of this writeup. A single neuron can have more than one connection to a single cell of all different synapse types. For example, the *C. elegans* I1L neuron has 2 different electrical synapses to neuron I2L but has 10 chemical synapses to I2L [12]! There are many different types of chemicals that can be transferred from neuron to neuron via different chemical synapses, and electric synapses communicate via action potentials that can change polarities resulting in certain ions from traveling to and from cells. This transfer can be treated as symmetric or asymmetric based on what is being analyzed, as a receiving neuron may do nothing with an increase in action potential, or it may influence cascading action potentials. For a small worm with a small number of neurons there can be a lot of information to process, and data prep needed to begin to analyze such a structure.

The hermaphrodite nematode has a lifespan of about 18-20 days. The life cycle of C. elegans is comprised of the embryonic stage, four larval stages (L1-L4) and adulthood (see Figure 1). The end of each larval stage is marked with a molt. Some neurons are only present during the pre-adult states and die off prior to adulthood, and the overall synaptic connections change as the nematode matures [11]. An example of such a change during the larval stage is the decision regarding the sex the nematode will take in adulthood. For the context of this essay all results and data will target the Adult period in the worm's life, and the Dauer state is ignored entirely (it is a special scenario stage regarding extreme scarcity of resources where the organism can survive in a semi-hibernation mode).

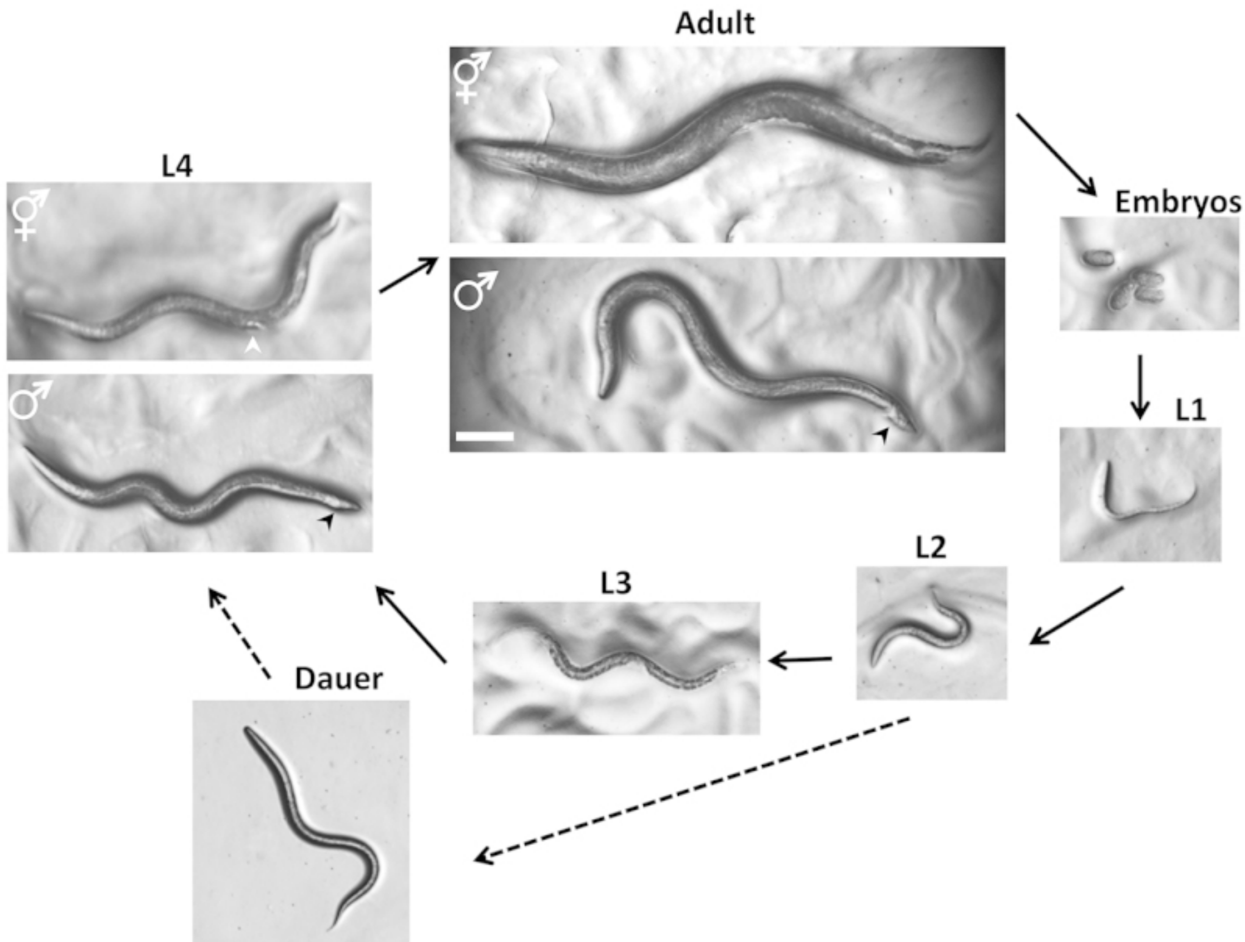


Figure 1 - *C. elegans* lifespans and sex identifications [10]

## Sexual Dimorphism Context

One noteworthy feature of the *C. elegans* is that this species does not have the traditional male and female variations, and instead consists of male and hermaphrodite sexes [1, 2, 3]. The well-studied hermaphrodite possesses 302 neurons, and the male has more than 380 (disputed amongst sources regarding the exact number) [6, 8]. The hermaphrodite (which composes more than 99% of the species population in the wild) can impregnate itself and birth what is essentially a clone of itself, while the male is only able to procreate with other hermaphrodites [8]. Therefore, reproduction between the male and hermaphrodite are the only means of increasing genetic diversity amongst a community, theoretically protecting from disease or other survival antagonists. The reproductive components reside in the tail-end of the nematode and the male has a more complex nervous system in this portion than the hermaphrodite. A 2012 study examining just this tail portion shows that there are 81 male-specific neurons and 89 shared neurons with the hermaphrodite [4]. But this is disputed by a 2021 article stating there were only 56 male-specific neurons [8]. Due to the conflicting reports regarding the male sex, and its low incidence in this species, the analysis that follows will only target the hermaphrodite sex to avoid confusion and to limit scope.

## Data Source

A useful source for anything related to the *C. elegans data* is the Worm Atlas research resource [9]. This resource has compiled various papers, databases, and more into a single repository with free and clear information. The first data source used for the midterm portion of this project is found in the Neuronal Wiring section of this resource, specifically a reference to a 2007 Doctoral Dissertation Thesis by Beth Chen that includes neuron connectivity data for the hermaphrodite sex as an excel file [13]. When first accessed it may appear that this is all the data one might need. But things do not add up.

The adult *C. Eleg*ans hermaphrodite nervous system contains 302 neurons and is divided into the pharyngeal nervous systems containing 20 neurons and the somatic nervous system contains 282 neurons. The original data source referenced by Chen [13] only contains the somatic region - as the pharyngeal section had been outlined many years prior by Albertson and Thomson [5]. Reading more into the data reveals why it was split up this way. Practically speaking, there are only two neurons that link the pharyngeal nervous system to the somatic nervous system - and it is largely a one-way street! The larger somatic system can be thought of as the worm's brain, while the pharyngeal system is something akin to a mouth, used to take in nutrients. No directed chemical path exists from the pharyngeal neurons to the somatic system - only vice versa. And there are few electrical synaptic connections amongst these two systems as well [11].

Upon finding Cook et. al's data one might begin to celebrate, having found a unified data set that contains both sexes and directed synapse counts across the whole worm. And yet there is more research to be done. Cook's data includes more than just neurons, and includes neuromuscular junction cells, and other cell types that can receive synaptic signals from neurons, but they themselves are not necessarily considered neurons. The adult hermaphrodite only has around 900 cells total, and with roughly 300 being neurons, sometimes lines begin to blur.

One of such discrepancy involved the CANL and CANR neurons. After troubleshooting for many hours and pouring over obscure research, one can be assured that the CANL and CANR neurons are indeed considered neurons if one is to reach the desired 302 total number! These neurons only receive signals and do not send anything out. They are associated with the excretory system in the worm. Only after stumbling across a random 2019 PHD post on Twitter [14] might one have any indication that "most people increasingly don't consider them neurons." It is not clear why exactly that is.

Finally, there remains the issue with the male sex of this species. The male sex is less than one percent of the natural population of this species, and is only widely studied in a controlled lab environment. Nevertheless, due to its much higher neuron count and increased complexity, there are efforts underway to compare and contrast stimuli response, behavior, and more amongst the sexes [8]. While the male sex is included in much of this project and analysis, it is ultimately treated as lesser "noisier" data in that it was never determined which cells if any were being included extraneously as neurons. The male sex allegedly has 385 neurons total, but without analyzing individual neurons it is hard to tell for sure which ones should or should not make the cut.

**Extract, Transform, and Load**

Finally, agreeing to use Cook's data [11] one can take the nicely formatted .xlsx connectome files (see Table 1) and transform them into comma separated value files for input into a Python program. Python is used for this project due to its integration with NetworkX and being the advised methodology for the entire class. This data has the pre-synaptic neuron as the row vector, and the post-synaptic neuron as the column vector. This implies a directed graph, and each cell is assigned a weight or is null. The weight is the number of synapses that correspond to the type of synapse being analyzed. Note that even though there may be X number of chemical synapses from neuron A to neuron B, the synapses may not be for the same chemical necessarily - but this detail is not captured in the data.

| | I1L | I1R | I2L | I2R | I3 | I4 | I5 | I6 | M1 | M2L | M2R | M3L | M3R | M4 | M5 | MCL | MCR | MI | NSML | NSMR | pm1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I1L | | | 10 | | 3 | | 2 | 1 | | 3 | | 8 | 2 | | | 2 | 2 | 2 | 3 | | 2 |
| I1R | | | | 6 | 1 | | 2 | | 1 | 1 | 3 | 1 | 6 | | | 3 | 4 | 1 | 1 | 2 | 2 |
| I2L | 2 | | | 3 | | 13 | 2 | 2 | 1 | | | | 3 | 4 | | | | | 14 | 24 | |
| I2R | | 1 | 3 | | | 20 | 2 | 3 | 3 | | 2 | | 2 | 3 | | 1 | | | 34 | 7 | 1 |
| I3 | 1 | 1 | | | | | 2 | 1 | 2 | 2 | | 2 | 2 | 2 | | 1 | 2 | 1 | | 2 | |
| I4 | | | | 7 | | | 2 | | | 5 | | 6 | 22 | | | | | 1 | 8 | 15 | |
| I5 | 2 | | 3 | 1 | 1 | 7 | | | 3 | 4 | | 4 | 2 | 19 | 2 | | | 4 | 1 | 5 | |
| I6 | | 1 | 1 | 4 | 12 | | | | | | | 10 | | 15 | | | | | 24 | 23 | |
| M1 | | 1 | 3 | 2 | 8 | 1 | 2 | | | | | | | 1 | 1 | | | 6 | 2 | | |
| M2L | | | | | 2 | 8 | 2 | | | | | 3 | 1 | | | | | | | | |
| M2R | | | | | | 1 | 3 | | | | | 2 | 1 | | | | | | | 1 | |
| M3L | | | | | | | | | | | | | | | | | | | 1 | | |
| M3R | | | | | | | | | | | | | | 2 | | | | | | | |
| M4 | | | | | | 5 | 1 | | | 1 | | 1 | | 15 | | 1 | | | | | |
| M5 | | | | | | | 1 | | 1 | | | | | | | | | | | | |
| MCL | | | | | | | | | | | | | | | | | | | | | |
| MCR | | | | | | | | | | | | | | | | | | | | | |
| MI | 1 | | | | 2 | 1 | | | 7 | 3 | 2 | 2 | 2 | | | 3 | | | 1 | 2 | |
| NSML | | | | | | 1 | | 1 | | | | 21 | 1 | 13 | | | | | | 7 | |
| NSMR | | | 1 | 1 | | 3 | | 4 | | | | | 24 | | | | | | | | |

| | pm2D | pm2VL | pm3D | pm3VL | pm3VR | pm4D | pm4VL | pm4VR | pm5D | pm5VL | pm5VR | pm6D | pm6VL | pm6VR | pm7D | pm7VR | mc2DL | mc2DR | mc2V | mc3V | e3D | e3VL | e3VR | g1p | g1AL | g1AR | g2L | g2R | bm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I1L | | | | 7 | | 1 | 2 | | | | | | | | | | | | | | | | | | | | | | |
| I1R | | | 2 | | 5 | 1 | | 2 | 2 | | 1 | | | | | | | | | | | | | | | | | | |
| I2L | | | | 1 | | | 2 | | 1 | 2 | 1 | | | | | | | | | | | 1 | | | | | | | |
| I2R | | | | | 1 | 2 | 1 | | | 1 | 4 | | | | | | | | | | | | 1 | | | | | | |
| I3 | | | | | | 2 | | | 4 | | | | | | | | | | | | | | | | | | | | |
| I4 | | | | | | 1 | | | | 11 | 2 | | | | | | | | | | | | | | | | | | |
| I5 | | | | | | | | | | 4 | 14 | | 1 | | | | | | | | | | | | 9 | 6 | | | |
| I6 | | | | | | | | | 13 | | | | | | | | | | | | | | | 2 | | | | | 2 |
| M1 | 1 | 1 | | 1 | | 1 | | | 3 | | | | | | | | | | | | 3 | 1 | | 5 | | | | | |
| M2L | | | | | | 3 | 9 | | 2 | 21 | | | | | | | | | | | | | | | 8 | | | | |
| M2R | | | | | | 3 | | 3 | | | 12 | | | | | | | | | | | | | | | 3 | | | |
| M3L | | | | | | 17 | 6 | | 4 | 2 | | | | | | | | | | | | | | | | | | | |
| M3R | | | | | | 6 | | 1 | | | | | | | | | | | | | | | | | | 3 | | | 1 |
| M4 | | | | | | | | 1 | 69 | 50 | 72 | | | | | | | | | | | | | 46 | 43 | 49 | 3 | | |
| M5 | | | | | | | | | | 1 | 3 | 2 | 2 | 2 | 3 | 2 | | | | 4 | | | | | 3 | 1 | 2 | 1 | |
| MCL | | | | | | | | | | | | | | | | | | 7 | 13 | | | | | | | | | | |
| MCR | | | | | | | | | | | | | | | | | 6 | 4 | 11 | | | | | | | | | | |
| MI | | | | | | 2 | 1 | 7 | 5 | | | | | | | | | | | | | | | | | | | | |
| NSML | | | | | | | 2 | | 38 | 63 | 8 | | | | | | | | | | | | | | | | | | 88 |
| NSMR | | | | | | 3 | | | 9 | 1 | 53 | | | | | | | | | | | | | | | | | | 72 |

Table 1 - Adult Hermaphrodite Pharyngeal Chemical Connectome [11]

Writing a python script to take the CSV as input, parsing out "extra" cells that are deemed not to be neurons, loading into a NetworkX object, writing to a .gml file, importing into Gephi, and forcing the atlas view reveals a first picture of the worm brain. Seemingly quite simple but an interesting decision-making system.

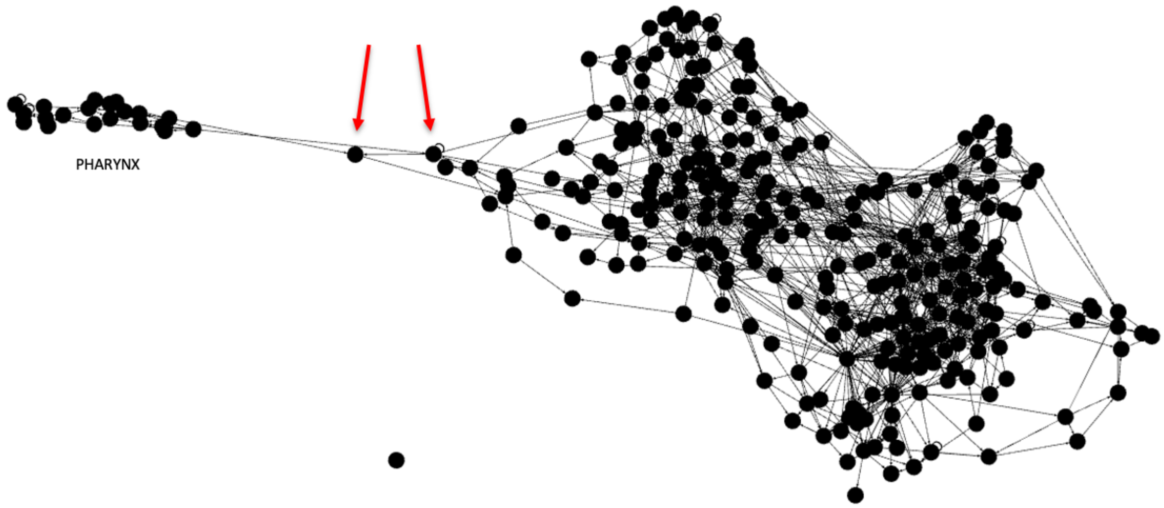


Figure 2 - Adult Hermaphrodite Asymmetric Electrical Connectome [11] - Note the RIPL and RIPR nodes have arrows accentuating their betweenness criticality.

**Comparing Against Generated Graphs**

Once the data is loaded, one can compare it against generated graphs. For this project the Erdos-Renyi, WattsStrogatz, Gilbert, and Barabasi-Albert graphs were used. Each graph was generated 10 times (a configurable parameter) and the results were averaged. The python codes run in the following general flow (Figure 3).

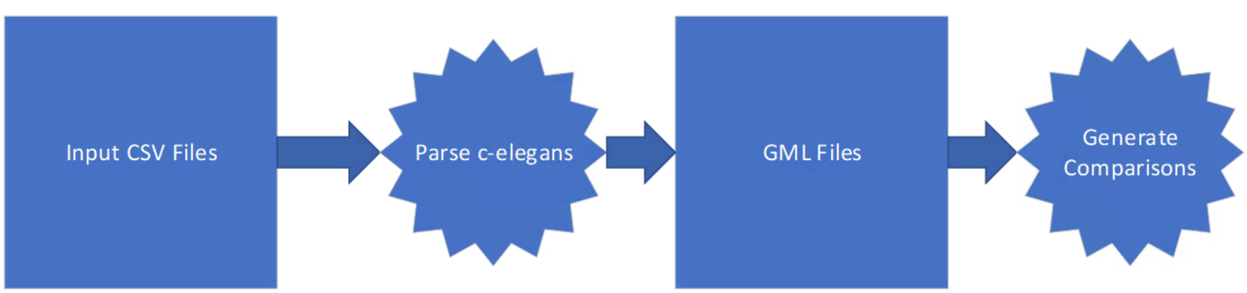


Figure 3 - General flow of python code

Each run of the "generate-comparisons.py" code calculates the "deltas" of the selected model vs. the averaged generated model. In this case, a smaller delta value would be indicative that the generated model was a closer fit for that particular metric. See the readme in the included zip project for more information.

**Conclusions and Next Steps**

The degree distributions do indeed appear to show some type of scale-free approach regardless of sex or synapse type.

It would be prudent to spend more time studying the male sex of this species. Even with so few specimens occurring naturally, the diversity of its connections might help better understand the hermaphrodite as well. It may also be interesting in seeing how the two sexes' datasets might combine for a "mutant" worm. If the hermaphrodite has 302 neurons with unique connections, and the male has 385 neurons with unique connections, what if they were all connected by either sexes link weights?

More research is needed into the symmetric vs. asymmetric electrical synapses. Being well versed in biology would help with this analysis.

More debugging on clustering is needed. This project spent many hours on clustering trying to get it to work with only limited success. Please see the slides for an example dendrogram that was created for one synapse type. It may be that the "noisy" data is contributing to the failure of some of these types.

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