HIERARCHY AND FLEXIBILITY IN
Caenorhabditis elegans FORAGING

Doctoral Thesis presented by

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Thesis Director:

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Sant Joan d'Alacant, 19th April, 2021,

To whom it may concern,

*The doctoral thesis entitled "**Hierarchy and Flexibility in Caenorhabditis elegans foraging**" has been developed by myself, Saurabh Gupta. This thesis is presented in a conventional* format. It is based on theoretical/computational studies undertaken at the Neuroscience Institute of Alicante during the PhD program in neuroscience of the Miguel Hernández University.

Yours sincerely

Saurabh Gupta

Sant Joan d'Alacant, 19th April, 2021

To whom it may concern,

*The doctoral thesis entitled " **Hierarchy and Flexibility in Caenorhabditis elegans foraging**" has been developed by myself, **Saurabh Gupta** . This thesis includes the following publication(s), of which I am the first author. I declare that the   publication has not been used and will not be used in any other thesis in agreement with my thesis director Dr. Alejandro Gomez Marín:*

Poster Presentations:

1) Saurabh Gupta and Alex Gomez-Marin. The organization of posture sequences in Caenorhabditis elegans locomotion. **European Neuroscience Conference by Doctoral Students (ENCODS)**, 4-6 May 2017, Benidorm, Alicante (Spain).
2) Saurabh Gupta and Alex Gomez-Marin. The organization of posture sequences in Caenorhabditis elegans locomotion. **XVII Congreso de la Sociedad Española de Neurociencia**, 27 a 30 de septiembre de 2017, Alicante, Spain.

Yours sincerely,

Saurabh Gupta   Dr. Alejandro Gomez Marin

Sant Joan d'Alacant, 19th April, 2021

El Dr. D. Alejandro Gómez Marín, Investigador Ramón y Cajal,

Autoriza la presentación de la Tesis Doctoral titulada "**Hierarchy and Flexibility in Caenorhabditis elegans foraging**" y realizada por D. Saurabh Gupta, bajo mi inmediata dirección y supervisión como director de su Tesis Doctoral en el Instituto de Neurociencias (UMH-CSIC) y que presenta para la obtención del grado de Doctor por la Universidad Miguel Hernández.

Y para que conste, a los efectos oportunos, firmo el presente certificado.

Dr. Alejandro Gomez Marín

Sant Joan d'Alacant, 19th April, 2021

D.ª Elvira De la Peña García, Profesora Titular de Universidad y Coordinadora del programa de Doctorado en Neurociencias del Instituto de Neurociencias de Alicante, centro mixto de la Universidad Miguel Hernández (UMH) y de la Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC),

CERTIFICO:

Que la Tesis Doctoral titulada "**Hierarchy and Flexibility in Caenorhabditis elegans foraging**" has sido realizada por D. Saurabh Gupta, bajo la dirección del Dr. Alejandro Gomez Marín como director, y doy mi conformidad para que sea presentada a la Comisión de Doctorado de la Universidad Miguel Hernández.

Y para que conste, a los efectos oportunos, firmo el presente certificado.

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Contents

LIST of ABBREVIATIONS

C. elegans *Caenorhabditis elegans*

CFG Context Free Grammar

DEG/ENaC degenerin/epithelial sodium channel

HMM Hidden Markov Model

mec MEChanosensory abnormality

MR Mutual Replaceability

NDFA non deterministic push down automata

npr NeuroPeptide Receptor family

Trpc transient receptor potential cation channel subfamily C

unc UNCoordinated

ABSTRACT

Foraging is an ecologically relevant and evolutionary ancient behavior underlying some of the most important decisions made by all animals. Studying organizational principles of foraging in the nematode worm *C. elegans* presents a promising direction to uncover the molecular and neural basis of this universal behavior and could shed light on human decision making as well.

Many of the recent efforts in understanding *C. elegans* foraging behavior (consisting of roaming and dwelling states) have been dominated by markovian process dynamics, ranging from models that make the markovian assumption to identify underrepresented and over represented n-grams in worm behavioral sequences to latent variable models like hidden markov models (HMMs). The reliance of such models on linear dynamics that operate on a fixed time scale belies the apparent multiplicity of time scales and non-linearity observed in the behavior of organisms. For example, the ambiguity in meaning in the phrase 'white taxi driver' is impossible to express in a linear organizational framework, such as one consisting of transition probabilities between words (markov model) or even between categories of words (hidden markov model). However, a compositional hierarchy, where low level behavioral descriptions (like words) are grouped together into higher order chunks (like phrases) along with rules of interaction between such descriptions that define which phrases and sentences are permitted in the language, can easily capture such ambiguities.

Other efforts towards understanding worm behavior center around searching for repeatable motifs (same sub-sequences that are repeated in time) in worm behavioral sequences. While completely ignoring the question of the underlying organization of behavior, this preferential search for only repeatable motifs makes it impossible to understand the behavioral variability/flexibility that the worm is known to exhibit, even during stereotypical behavior. Thus, it is unknown whether hierarchical organization can explain *C. elegans* foraging behavior. Additionally, we lack understanding as to how the principle of hierarchy can be tied to the flexible generation of behavior.

Treating worm behavior as a sequence of changes in its body posture, we show for the first time

that worm foraging behavior (not just the stereotypical portions) is organized in accordance with a compositional hierarchy. In order to capture variability in worm behavior, we use the idea of substitution to obtain chunks containing mutually substitutable worm postures. We elucidate a grammar of worm roaming and dwelling states, outlining rules of interaction between postural chunks. We find that the stereotypical worm roaming is captured by a specific grammatical rule involving specific chunks in a particular order and that even such stereotypical behavior is characterized by variability at the lowest level of postures (degeneracy). We also delineate grammatical rules that specify how the same chunks are re-used in different ways to produce relatively less stereotypical dwelling. Borrowing ideas from computer science and linguistics, we show that the proposed foraging grammar conforms to a context free grammar (having hierarchical structure), and not to a regular grammar (based on linear organization principles characteristic of markov models). We show that the properties of the proposed grammatical rules are consistent with known experimental results about *C. elegans* foraging. Finally, using the proposed grammar, we report hitherto uncharacterized role of neuropeptide receptors *npr-3* and *npr-10* in modulating *C. elegans* foraging behavior. Although grammatical rules specify a generative model of worm behavior, the proposed grammar does not capture the switching dynamics between roaming and dwelling. Combining data driven methods like HMMs with the structure discovered in this work represents one potential avenue to address this bottleneck.

The philosopher Daniel Dennett has argued quite convincingly that the notion of computation seems to be inextricably linked to what brains do. The correspondence reported in this thesis between worm foraging and context free grammar takes that line of thought towards an interesting direction in my opinion. In the theory of computation, various automatons are defined according to what can be computed by such machines. In such a scheme, non deterministic push down automata (NDFA), that have an associated stack or memory attached to them, can recognize all context free languages. Interestingly, NDFA is more expressive than finite state machines but less capable than Turing machines. It remains to be seen if the troika of brain, behavior and computation can be combined to shed light on some of the most interesting questions in neuroscience in the future.

With the imminent possibility of recording from all the 302 neurons in freely behaving worms, this work promises to serve as a guide for behavioral neuroscientists in interpreting that neural activity. A compositional hierarchy underlying worm foraging implies that the same sequence of postures (postural chunks) can be used to generate both roaming and dwelling using different grammatical

rules. Thus, the same subset of neurons (K) might have the same combined neural activity during completely different behaviors - roaming or dwelling. The distinguishing factor amongst these behaviors should thus be sought in bigger neural circuits, that define higher order roaming or dwelling contexts and within which the smaller subset of neurons (K) is embedded. Furthermore, ideas of degeneracy, re-usability and hierarchy used in this work to understand flexible worm behavior have long been used in linguistics to understand the uniquely human competence of language. Thus, these concepts seem to be general enough to cut across different fields and could be leveraged by ethologists to understand the behavior of organisms more complex than worms.

RESUMEN

La búsqueda de alimento es un antiguo comportamiento evolutivo y ecológicamente relevante que subyace a algunas de las decisiones más importantes que toman todos los animales. Estudiar los principios organizativos de la búsqueda de alimento en el gusano nematodo *C. elegans* presenta una dirección prometedora para descubrir la base molecular y neuronal de este comportamiento universal y también podría arrojar luz sobre la toma de decisiones humanas.

Muchos de los esfuerzos recientes para comprender *C. elegans* el comportamiento de búsqueda de alimento (que consiste en estados de itinerancia y residencia) ha estado dominado por la dinámica del proceso markoviano, que van desde modelos que hacen la suposición markoviana para identificar n-gramas subrepresentados y sobrerrepresentados en secuencias de comportamiento de gusanos hasta modelos de variables latentes como modelos de markov ocultos (HMM). La dependencia de tales modelos en dinámicas lineales que operan en una escala de tiempo fija contradice la aparente multiplicidad de escalas de tiempo y la no linealidad observada en el comportamiento de los organismos. Por ejemplo, la ambigüedad en el significado de la frase "taxista blanco" es imposible de expresar en un marco organizativo lineal, como el que consta de probabilidades de transición entre palabras (modelo de Markov) o incluso entre categorías de palabras (modelo de Markov oculto). Sin embargo, una jerarquía de composición, donde las descripciones de comportamiento de bajo nivel (como palabras) se agrupan en fragmentos de orden superior (como frases) junto con reglas de interacción entre dichas descripciones que definen qué frases y oraciones están permitidas en el idioma, puede capturar fácilmente tales ambigüedades.

Otros esfuerzos para comprender el comportamiento de los gusanos se centran en la búsqueda de motivos repetibles (las mismas subsecuencias que se repiten en el tiempo) en las secuencias de comportamiento de los gusanos. Si bien ignora por completo la cuestión de la organización subyacente del comportamiento, esta búsqueda preferencial de solo motivos repetibles hace que sea imposible comprender la variabilidad / flexibilidad del comportamiento que se sabe que exhibe el gusano, in-

cluso durante el comportamiento estereotipado. Por tanto, se desconoce si la organización jerárquica puede explicar *C. elegans* comportamiento de búsqueda de alimento. Además, no comprendemos cómo el principio de jerarquía puede vincularse a la generación flexible de comportamiento.

Al tratar el comportamiento del gusano como una secuencia de cambios en su postura corporal, mostramos por primera vez que el comportamiento de búsqueda de alimento del gusano (no solo las porciones estereotipadas) está organizado de acuerdo con una jerarquía de composición. Con el fin de capturar la variabilidad en el comportamiento de los gusanos, usamos la idea de sustitución para obtener trozos que contienen posturas de gusanos mutuamente sustituibles. Aclaramos una gramática de los estados de deambulación y residencia de los gusanos, esbozando las reglas de interacción entre los fragmentos posturales. Encontramos que la itinerancia del gusano estereotipada es capturada por una regla gramatical específica que involucra fragmentos específicos en un orden particular y que incluso ese comportamiento estereotipado se caracteriza por la variabilidad en el nivel más bajo de posturas (degeneración). También delineamos reglas gramaticales que especifican cómo se reutilizan los mismos fragmentos de diferentes maneras para producir una vivienda relativamente menos estereotipada. Tomando prestadas ideas de la informática y la lingüística, mostramos que la gramática de búsqueda propuesta se ajusta a una gramática libre de contexto (que tiene una estructura jerárquica) y no a una gramática regular (basada en principios de organización lineal característicos de los modelos de Markov). Mostramos que las propiedades de las reglas gramaticales propuestas son consistentes con los resultados experimentales conocidos sobre *C. elegans* forrajeando. Finalmente, utilizando la gramática propuesta, informamos hasta ahora el papel no caracterizado de los receptores de neuropéptidos *npr-3* y *npr-10* en la modulación de *C. elegans* comportamiento de búsqueda de alimento. Aunque las reglas gramaticales especifican un modelo generativo del comportamiento de los gusanos, la gramática propuesta no captura la dinámica de cambio entre la itinerancia y la vivienda. La combinación de métodos basados en datos como HMM con la estructura descubierta en este trabajo representa una vía potencial para abordar este cuello de botella.

El filósofo Daniel Dennett ha argumentado de manera bastante convincente que la noción de computación parece estar indisolublemente ligada a lo que hacen los cerebros. La correspondencia reportada en esta tesis entre búsqueda de gusanos y gramática libre de contexto lleva esa línea de pensamiento hacia una dirección interesante en mi opinión. En la teoría de la computación, varios autómatas se definen de acuerdo con lo que pueden computar tales máquinas. En tal esquema, los

autómatas de empuje hacia abajo no deterministas (NDFA), que tienen una pila asociada o una memoria adjunta, pueden reconocer todos los lenguajes libres de contexto. Curiosamente, NDFA es más expresivo que las máquinas de estados finitos, pero menos capaz que las máquinas de Turing. Queda por ver si la troika del cerebro, el comportamiento y la computación se pueden combinar para arrojar luz sobre algunas de las cuestiones más interesantes de la neurociencia en el futuro.

Con la posibilidad inminente de registrar todas las 302 neuronas en gusanos que se comportan libremente, este trabajo promete servir de guía para que los neurocientíficos del comportamiento interpreten esa actividad neuronal. Una jerarquía de composición subyacente a la búsqueda de alimento por parte de los gusanos implica que la misma secuencia de posturas (fragmentos posturales) se puede utilizar para generar tanto la deambulación como la vivienda utilizando diferentes reglas gramaticales. Por lo tanto, el mismo subconjunto de neuronas (K) podría tener la misma actividad neuronal combinada durante comportamientos completamente diferentes: itinerancia o vivienda. Por tanto, el factor distintivo entre estos comportamientos debe buscarse en circuitos neuronales más grandes, que definen contextos de itinerancia o residencia de orden superior y dentro de los cuales está incrustado el subconjunto más pequeño de neuronas (K). Además, las ideas de degeneración, reutilización y jerarquía utilizadas en este trabajo para comprender el comportamiento flexible de los gusanos se han utilizado durante mucho tiempo en lingüística para comprender la competencia exclusivamente humana del lenguaje. Por lo tanto, estos conceptos parecen ser lo suficientemente generales como para abarcar diferentes campos y los etólogos podrían aprovecharlos para comprender el comportamiento de organismos más complejos que los gusanos.

Chapter 1: Introduction

> It's a very general principle in biology,
>
> where if we can ever use a fast loop to
>
> help a slow one, it is a good idea

Geoffrey Hinton

Biology is filled with examples of a fast process coming to aid a higher level slow process to square up with complexity. This seems to me to be one of the most important characteristics of hierarchical design where levels higher up in the hierarchy have slow scale dynamics and levels lower down have faster dynamics to tame complexity in nature.

One of the most salient manifestation of these slow and fast processes plays out in the relation between evolution and learning. In a seminal paper (Hinton and Nowlan, 1987), it was shown via computer simulations, that in situations where a large number of co-adaptations are simultaneously required, only making some of the decisions using evolution and leaving the rest of the decisions to learning can lead to significant speed up in the evolution of organisms, commonly known as the baldwin effect. Thus, in order to trump the complex process of achieving large number of co-adaptations, nature devised a hierarchical design where the slow process of evolution (higher in the hierarchy) is guided by the fast process of learning (lower in the hierarchy).

In the context of evolution of behavior in worms, it has been argued that evolution of behavior occurs by changes in signalling pathways during the life of the individual, which across generations might get ingrained in the genome (Avery, 2010). This is similar to the baldwin effect discussed above where changes across the life time of an individual facilitate changes in the genome which is a relatively slow process spanning multiple generations.

This general principle of taming complexity with hierarchy shows up in the analysis of animal behavior as well. For example: Catching a flight form the airport requires me to first leave my home, then get hold of a taxi and so on. Each of these intermediate steps are essentially faster time scale processes(as compared to my goal of reaching the airport) which in turn are defined by even faster time scale processes like my walking behavior to reach my house door or to catch a taxi.

1.1 Hierarchy and animal behavior

Humans and other animals express complex behavior in terms of extended sequence of actions, in order to achieve a variety of goals. Understanding the organizational principles underlying the

generation of spontaneous sequential behavior promises to reveal the computational primitives that can be ascribed to genetic and neural circuits underpinning such behavior. A salient characteristic of animal behavior is the variability and flexibility exhibited by the animal depending on varying contexts. The degree of freedom problem for motor systems posits that the number of neurons, muscles and joints are orders of magnitude greater than are required to make desired movements (Bernstein, 1967). This redundancy poses a significant challenge towards understanding behavior - different motor signals can generate the same behavior (degeneracy) and similar motor signals can generate completely different behaviors based on the context in which they appear (re-usability) (Tononi et al., 1999; Sporns and Edelman; Bernstein, 1967). Degeneracy and re-usability can lead to flexibility, even solely at the level of behavior. For example: a person can brush her teeth **either** by moving her head up and down **or** by keeping her head still and moving her arms up and down (*degeneracy*). Moreover, a person can move her hand up and down while brushing her teeth as well as while cleaning a window (*re-usability*). Thus any principle of behavioral organization must also account for this **either or** feature of behavior where distinct sequences of actions can be **substituted** with each other to generate flexible behavior.

Hierarchical organization has been postulated in the past as a general organizational principle of behavior (Tinbergen, 1950, 1951; Simon, 1962; Dawkins, 1976; Lashley, 1951) that can also tame the redundancy problem to generate flexible behaviors (Dawkins, 1976). As opposed to a linear chain theory of generating behavioral sequences, where the preceding action in a sequence serves as a stimulus for the next action (markovian assumption that form the basis of models like markov models and hidden markov models (HMMs)), Karl Lashley (Lashley, 1951) hypothesized that behavioral sequences are generated by a hierarchical organizational schematic, whereby low level behavioral descriptions (like words) are grouped together into higher order behavioral descriptions, i.e. chunks (like phrases), that further get grouped into still higher order "chunks of chunks" (sentences). For instance - the ambiguity in the phrase 'white taxi driver' can be easily expressed in a hierarchical schematic (Figure 1.1) but is impossible to be expressed in a linear system such as one consisting of transition probabilities between words (markov model) or even between categories of words (akin to a hidden markov model) (Dehaene et al., 2015). In the example above, 'White taxi driver' is a chunk that in turn consists of two potential sub-chunks, namely - 'taxi driver' and 'white taxi'. Also, the process of generating higher order chunks from sub-chunks follows a set of rules. For example - the sub-chunk 'taxi driver' when preceded by an adjective, 'white', gives one meaning to

the phrase 'white taxi driver' and when the sub-chunk 'white taxi' is succeeded by the noun 'driver', another meaning arises. This highlights the compositional property of hierarchical systems where higher order chunks are defined in terms of simpler constituent components according to a set of production rules. Compositionality endows flexibility to hierarchical systems by allowing the re-use of stable sub-chunks and chunks according to different rules. For example - the sub-chunk 'taxi driver' above can be re-used to generate two different sentences - 1) The id of the **taxi driver** was confiscated. and 2) The **taxi driver** hit the person on the road and fled from the scene. However, it remains unclear if the richness of animal behavior can be captured by such a compositional hierarchy and whether that organization can give mechanistic insight into ecologically relevant behaviors.
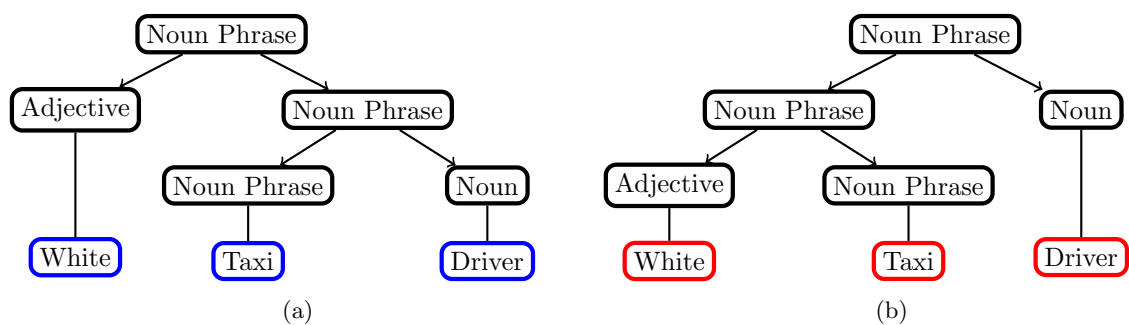

Figure 1.1: **The ambiguity in the sentence "*White taxi driver*" cannot be accounted by using flat sequential structures. Nested rules like Noun Phrase ⟶ Adjective (Noun Phrase) and Noun Phrase ⟶ (Noun Phrase) Noun, can account for the ambiguity.** (a): The rules of grammar lead to an interpretation where the taxi driver is a white person. (b): Grammatical rules lead to a different interpretation of the same phrase, in which a driver drives a white colored taxi.

Many of the ecologically important and fitness determining decisions that all animals make are related to foraging, which is one of the most critical problems faced by all animals (Mobbs et al., 2018). Modulating their foraging behavior according to internal and external conditions is critical for the survival and future reproduction of animals (Cohen et al., 2009). Studying foraging behavior in the nematode worm *C. elegans* presents a promising direction to uncover the molecular and neural basis of this universal behavior and could help decipher decision making principles in humans as well (Mobbs et al., 2018; Cohen et al., 2009). *C. elegans* foraging behavior is thought to be organized into two distinct states - the exploratory phase of roaming and the exploitative phase of dwelling (Fujiwara et al., 2002; Ben Arous et al., 2009). During roaming, the worm moves quickly across the bacterial lawn of food with low frequency of turns, whereas during dwelling, it moves rather slowly with frequent turns, thus confining itself to a very small region (Flavell et al., 2013). The same motor patterns - forward locomotion, reversals, turns, occur in roaming-specific or dwelling-

specific combinations to give roaming and dwelling states their distinct characteristics (Flavell et al., 2013). Iterative clustering of repeatable motifs in worm locomotion has been used previously (Brown et al., 2013; Gomez-Marin et al., 2016) to define phenotypes that can distinguish between worms in different environments. While still leaving open the question of organization of worm behavior, this search for only repeatable motifs renders the method incapable of capturing the flexibility that is commonly observed in *C. elegans* behavior (Chao et al., 2004; Cheung et al., 2005; Chang et al., 2006; I.). Additionally, markovian assumption has been used to identify overrepresented and underrepresented n-grams in worm behavioral sequences when the environment is changed or when its nervous system is stimulated. Aside from worms, markovian analysis is a commonly used technique to study behavior of a variety of animals like mice (Wiltschko et al., 2015). However, markovian assumption entails that there is a linear relationship between behavioral states which might not always be true as illustrated above.

Thus, it is unclear whether hierarchical organization - where primitive behavioral descriptions get grouped into chunks at a higher level of description(having relatively slower timescales) along with the rules of interaction between those chunks (compositional hierarchy), can explain *C. elegans* foraging behavior. Additionally, we lack understanding as to how the principle of compositional hierarchy can be tied to the flexible generation of *C. elegans* foraging behavior. Furthermore, the experimental tractability of *C. elegans* as a model organism holds considerable promise in elucidating molecular and neural mechanisms underlying an ecologically relevant behavior like foraging, that is critical for all animals. Indeed, receptors important for worm roaming behavior have been shown to have similarities with receptors that modulate feeding behavior in mammals (Bendena et al., 2008; Cohen et al., 2009). For example - neuropeptide receptor npr-9 has been shown to affect *C. elegans* foraging behavior (by inhibiting dwelling behavior) and is most similar to mammalian galanin receptors known to modulate feeding behavior in mammals. However, it is unclear if other neuropeptide receptors might also be involved in modulating worm foraging.

Using openly available *C. elegans* foraging data (Yemini et al., 2013; Schwarz et al., 2015), this thesis makes the following contributions. Treating worm behavior as a sequence of changes in its body posture and refraining from using any human defined labels for such postures, we show for the first time that worm foraging behavior (not just the stereotypical portions) is organized in accordance with a compositional hierarchy. With an aim to capture degeneracy and re-usability of behavioral elements, we use the idea of substitution (Maurus and Pruscha, 1973; Dawkins, 1976) to obtain

chunks containing mutually substitutable worm postures. We elucidate a context free grammar of worm roaming and dwelling states, outlining rules of interaction between such chunks. We find that the stereotypical worm roaming behavior is captured by a specific grammatical rule involving specific chunks in a particular order and that even such stereotypical behavior is characterized by variability at the lowest level of postures (degeneracy). We also delineate grammatical rules that specify how the same chunks are *re-used* in different ways to produce relatively less-stereotypical dwelling like behavior patterns.

The proposed context free grammar for worm foraging has hierarchical characteristics with higher levels in the hierarchy corresponding to slower time scale processes like roaming and dwelling and lower level process corresponding to faster time scale processes involving chunks of postures and postures themselves. We also show that the properties of the proposed grammatical rules are consistent with known experimental results about *C. elegans* foraging. Finally, using the proposed grammar for worm foraging, we report hitherto uncharacterized role of neuropeptide receptors npr-3 and npr-10 in modulating *C. elegans* foraging behavior. Such a generative grammar for worm foraging demonstrates how flexible behavior emerges from a compositional hierarchy. The importance of delineating such organization principles is manifested in their ability to elucidate novel molecular mechanisms important for regulating an ecologically relevant and evolutionarily ancient behavior like foraging.

Chapter 2:   Experiments and Data used

> Why is it that when you talk about genes
> and molecules, you are so precise and
> when you talk about behavior you are so
> vague and impressionistic
>
> *Allison J. Doupe*

The remark above made by researcher Allison J. Doupe reflects a schism between our technology to manipulate genes/neurons and the technology to measure and analyze behavior. One might also argue that without an in-depth measurement and analysis of molecular, neuronal, behavioral and environmental data, a cogent understanding of brain-behavior relationship will continue to elude us.

(Yemini et al., 2013) have tried to reduce this schism by making high quality *C. elegans* behavioral data openly available for anyone to make use of it. In this dataset, all worm types - N2, wild isolates and other mutants were picked to the centre of a patch of *Escherichia coli* OP50 on an agar plate, one at a time. The worms were allowed to habituate for 30 minutes before being tracked for a period of 15 minutes.

2.1 *C. elegans* foraging behavior is abstracted as a sequence of 90 postures

Previous studies (Schwarz et al., 2015) have discretized this behavioral data by first finding the angles of worm midlines at 49 equally spaced points. Then, the skeleton angles in each frame are discretized by matching the current posture (denoted by the 49 angles) to the closest posture in a set of 90 postural templates that were derived from wild type N2-worms using clustering.
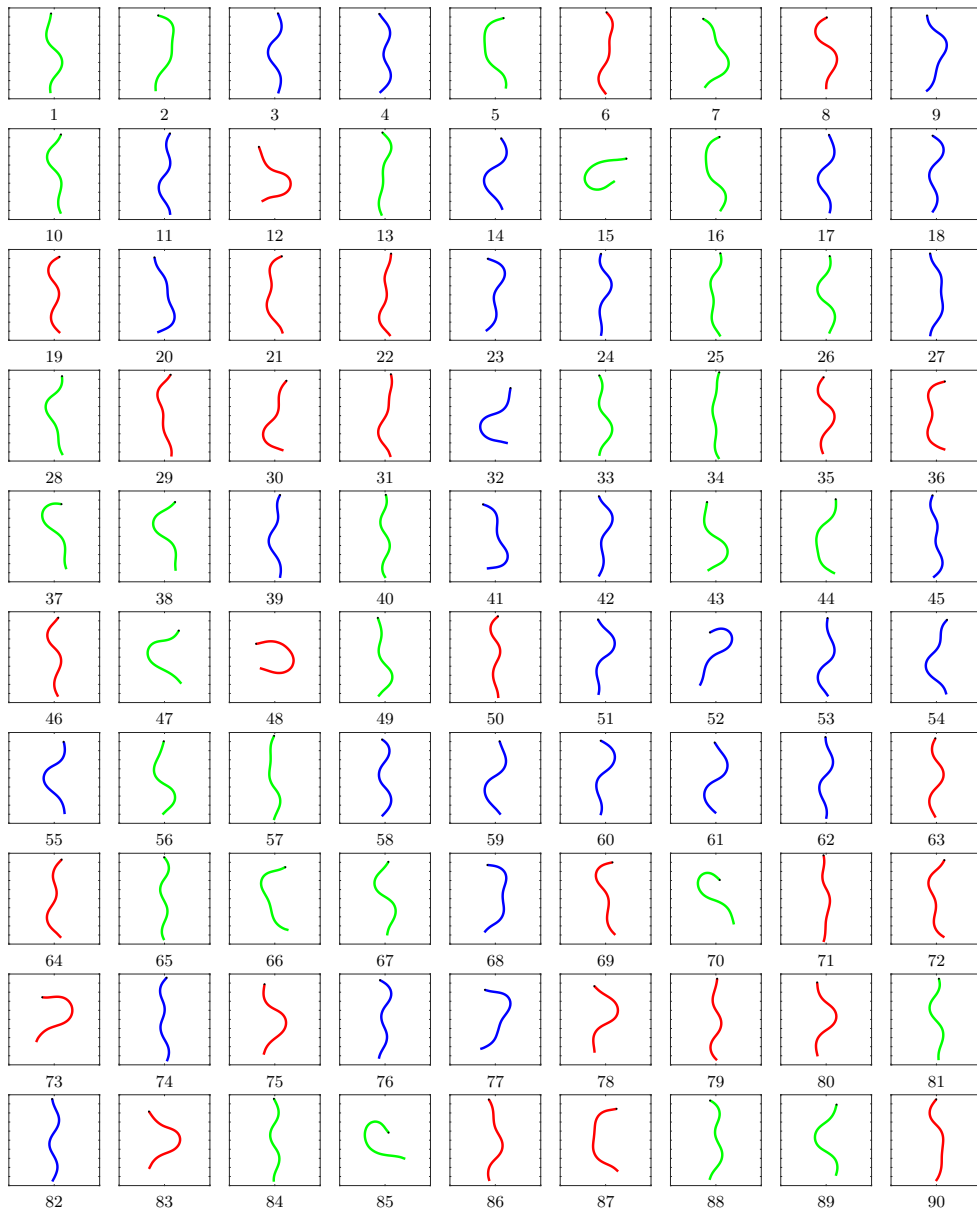
Figure 2.1: **Worm foraging behavior studied in terms of sequences of the above 90 postures.** Each worm posture (shape) is identified by a unique label ranging from 1-90. The head of the worm in each posture is indicated by a black dot.

The details of these procedures can be found in (Schwarz et al., 2015). This results in the same

template being fit to multiple consecutive frames, during which the animal might not be making huge changes to its posture. Thus, in order to disambiguate same postures repeated at different speeds, a simple non-uniform time warping procedure is used to swallow up repeats from the postural sequence. Thus the behavioral sequence 5,5,5,4,4,3,3,2 is abstracted as 5,4,3,2, but the timing information of each posture is still conserved. In this way, worm foraging behavior can now be seen as a sequence of postures with each posture having timing information, denoting the amount of time the worm stayed in that posture before moving to the next one.

This thesis makes use of this discretized worm behavior data, where the behavior of the worm is abstracted as a sequence of body postures.

Chapter 3:   Proposed Conceptual Framework to understand worm foraging

Wherein the beauty of the behavior is honored, the value of the nonconscious is confirmed, and a record is clarified.

M. Frances Stilwell

By separating and then combining different properties of movement one can then seek foci of relative variation and invariance, along a number of different dimensions and for various combinations of individually defined events.

John C. Fentress

As noted in Chapter 1, any organizational principle underlying behavior must account for the behavioral flexibility that is found in the animal kingdom. In this chapter, I introduce the ideas of substitution and mutual replaceability (MR), that can help capture variability in worm foraging behavior and can be related to the organizational principle of hierarchy.

3.1   *C. elegans* foraging behavior exhibits multiple time scales with postures used flexibly across multiple contexts

To investigate the extent of flexibility exhibited by individual postures, based on the multiple contexts in which they occur, we plotted the probability of their recurrence after a particular time interval, for all the N2 worms pooled together. Specifically, for each posture, we calculated the amount of time elapsed between all consecutive occurrences of that posture and computed the probability of recurrence for different time intervals (Figure 3.1b). We found that there are postures (D type postures - like 27,34,20) that have a considerably high probability of recurring very close together in time, indicating their enhanced role in dwelling or pause related behavioral states. This is because in pause and dwelling states, the worm remains confined to a small region in space, and hence alternates between the same set of postures in a short span of time. We also found that there are postures (E type postures - like 58,10,81,19) that have considerably higher probability of recurrence after a relatively long period of time (greater than 2 seconds). During a sustained period

of roaming, a traveling wave moves along the worm's body from its tail to the head multiple times to continuously propel it in the forward direction. The amount of time taken for the forward wave to travel along its body is usually of the order of 2-3 seconds. The similarity in time of the recurrence of forward traveling wave along the worm's body and the recurrence of E type postures illustrates that E type postures have a higher chance of being used in roaming.

More interestingly however, we can also see that D type postures also have non-negligible probability of recurring after a relatively long time interval (greater than 1.5 seconds) and the E type postures similarly have non-negligible probability of recurring after very short time intervals (less than 1 second). Thus, both the D type and E type postures participate in multiple contexts (roaming as well dwelling), and to understand the organization of behavior in *C. elegans*, we need an account of this flexibility that the worm exhibits, even at the lowest level of postures, based on context.
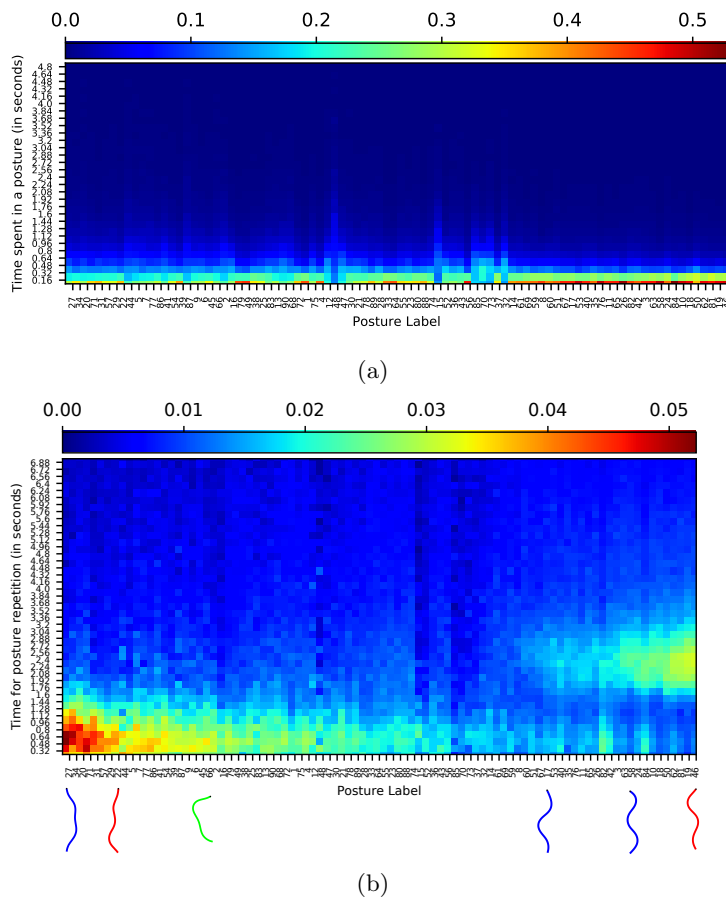
(a)

(b)

Figure 3.1: **Flexible posture usage across multiple contexts during worm foraging behavior** (a): Plot showing the probability distribution of the times spent by foraging N2 worms pooled together in each of the 90 postures. (b): Plot showing the probability of all the 90 postures to repeat after a particular time for all the N2 worms pooled together, sorted such that postures having similar time profiles for repetition are grouped together. Some postures(lets call them E) have relatively higher probability of repeating after 2-3 seconds (58,10,81) and might be related more strongly with roaming behaviors. Other postures (lets call them D) recur after very short times (27,20,25), that might be involved in pause or dwelling states. More importantly, note that D type postures also have non-negligible probabilities to recur at higher timescales (between 2-3 seconds) and similarly E type postures very frequently recur at very short time scales of less than 1 second. This exemplifies the flexibility even at the level primitive behavioral units like postures, where the same posture can be involved in *either* roaming *or* dwelling depending on the context.

3.2   *C. elegans* foraging behavior does not conform to markovian dynamics

We first sought to establish if worm foraging can be explained by a markov model. A markov model postulates that the next posture in the postural sequence is dependent on the worm's current posture. To that end, following the work in (Berman et al., 2016), we looked at behavioral transition

matrices at different time scales. Specifically,

$$[B(\tau)]_{ij} = p(pos(n + \tau) = j | pos(n) = i) \qquad (3.1)$$

where each element of the behavioral transition matrix B, denotes the probability that the worm goes to posture j from posture i after τ discrete behavioral time steps. For example, the elements in the matrix B(1) describe the probability of moving from one posture to the next, i.e. behavioral elements that are separated by just one behavioral time step. We see in Figure 3.2b that when the postures are ordered in a particular way, there is a conspicuous structure the B(1) matrix. In chapter 7, we discuss the procedure used to order the matrix in this particular manner. As we increase τ from 1 to higher values, we should expect that the structure present in the B(1) matrix to progressively degrade, because as move further in time away from the current state, the ability to predict the behavioral state decreases.

Alternatively, if the behavior of the worm were to be organized in a markovian manner, then,

$$[B_{markov}(\tau)] = [B(1)]^{\tau} \qquad (3.2)$$

The eigenvalues of B(1) (denoted by λ_i) have the property that $\lambda_1 \geq \lambda_2 \geq \lambda_3 .... \geq \lambda_n$ with the largest eigenvalue λ_1 equal to 1. The slowest time scale in a markovian system is governed by $|\lambda_2|$, resulting in a time decay of t_2 equal to $\frac{-1}{log|\lambda_2|}$. Calculating t_2 for $B_{markov}(1)$ for all the worms pooled together gives $t_2 \approx 6.4$ transitions. Hence, any memory that extends beyond 7 transitions would provide evidence for states that modulate behavior at a longer time scale. Visualizing $B(\tau)$ and $B_{markov}(\tau)$ for $\tau = 12$ ($\approx 2t_2$) in Figure 3.2c and 3.2d respectively, shows that there is some block diagonal structure that still persists in the actual behavioral data at a longer time scale as compared to a markovian system which loses all initial structure. This intuition is quantified in Figure 3.2e, where the largest eigenvalues (leaving the largest whose value is 1) of the markovian system and the actual data are plotted as a function of future time in terms of postural transitions. We can see that the rate at which the eigenvalues of the first order markovian system decay as a function of time is much greater than the actual data. These analyses demonstrate that worm foraging behavior has a longer time scale than would be predicted by a first order markov model .
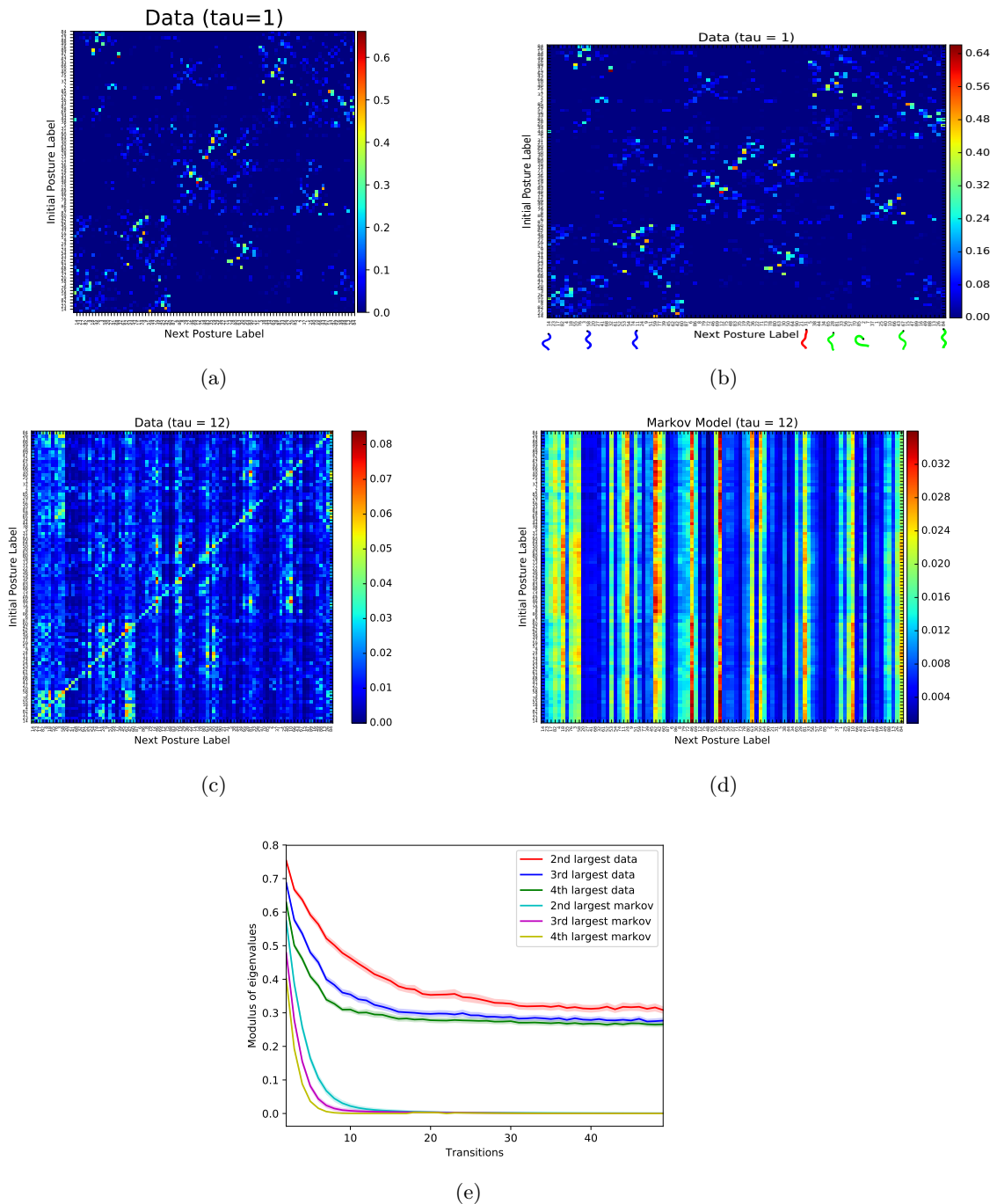
(a)

(b)

(c)

(d)

(e)

Figure 3.2: **Worm foraging behavior is not well described by a first order markov model.**
(a): Posture transition matrix denoting the probability of transition between pairs of postures for
all the foraging N2 worms pooled together. Distinct block diagonal structure can be discerned from
this matrix alone. (b): Same figure as in (a) with some representative postures shown.(c): Posture
transition matrix looking 12 steps in the future still retains some structure, indicating that the
current posture can be predictive of a posture well into the future. (d): Essentially all the structure
is lost for the first order markovian process if we look 12 states into the future. (e): Long time scales
involved in worm behavior quantified by the rate of decay of the largest k eigenvalues of posture
transition matrices characterized by both the first order markovian process as well as actual worm
behavior. The relatively slow decay in the case of actual worm behavior quantifies the intuition that
actual worm behavior is modulated at higher time scales than that given by a first order markovian
markovian process. Solid curves denote average across all the worms (N=1287) and the shaded
region denotes S.E.M.

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3.3 Degeneracy and Re-usability can be captured from behavioral sequences using substitution

Flexibility in behavior via degeneracy and re-usability coupled with hierarchical organization is nicely exemplified in the case of verbal behavior. Hierarchically organized grammatical rules between categories of words (like nouns, verbs etc.) or phrases specify the constraints according to which different sentences can be generated. Let us imagine the following grammatical rules that specify the hierarchy in Figure 3.3.

$$Rule\ 1\ =\ Sentence\ \rightarrow\ (Noun\ Phrase)\ (Verb\ Phrase)$$

$$Rule\ 2\ =\ Noun\ Phrase\ \rightarrow\ Article\ Noun$$

$$Rule\ 3\ =\ Noun\ Phrase\ \rightarrow\ Article\ Adjective\ Noun$$

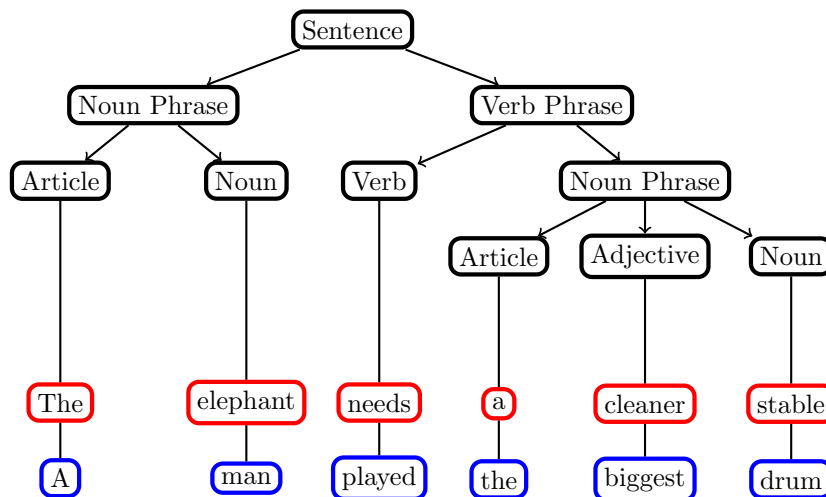$$Rule\ 4\ =\ Verb\ Phrase\ \rightarrow\ Verb\ (Noun\ Phrase)$$

Figure 3.3: **Two different ways in which substitution lends itself to flexibility by teaming with hierarchical organization.** Type 1 substitution - same low level description **Noun** gives rise to two different higher order chunks - **Noun Phrase** and **Verb Phrase** under differing contexts. Type 2 substitution - substitution at the lowest level: **elephant** being substituted by the word **man** to generate a new sentence, while the grammatical rules remain the same.

It can be seen that the same lower level chunk (description) - *Noun* is re-used to generate substitutable higher level chunks (descriptions) in the hierarchy - *Noun Phrase* and *Verb Phrase*. We define this type of substitution, that involves *re-using* the same lower level description in different context and results in the substitution between higher level chunks as type 1 substitution. Furthermore, we also see that the same grammar can generate two different sentences by substituting words belonging

to the same category like an Article or Noun (**elephant** being substituted by **man**). We define this form of substitution that achieves *degeneracy* (different words belonging to the same higher order category - Adjective) in a hierarchical system as type 2 substitution. In this way, substitution can be conceptualized to capture degeneracy and re-usability that lend flexibility to animal behavior.

3.4 Substitution uncovers modules that capture the flexibility and combinatorial choice in the generation of behavioral sequences

Given that worm foraging behavior is not explained by a first order markov model, we explored the possibility that foraging behavior might be organized hierarchically (Lashley, 1951; Schank and Abelson, 1977; Miller et al., 1960). It might thus be possible to divide the worm behavioral repertoire into meaningful modules and uncover the rules of interaction between such modules (Simon, 1962). We hypothesized that substitution dynamics coupled with hierarchical organization, implementing degeneracy and re-usability of behavioral elements at various levels in the hierarchy might help explain the flexibility that is synonymous with behavior. Taking inspiration from the twin principles of hierarchical organization and substitution dynamics in the domain of verbal behavior, we sought modules of worm postures such that they can be used in a manner that generates flexible/variable behavioral sequences. To obtain groupings of postures from behavioral sequence data that respect variability, postures are put together in a module, if they are mutually substitutable, with respect to their transitions to other postures. Specifically, two postures are put in the same module, if the
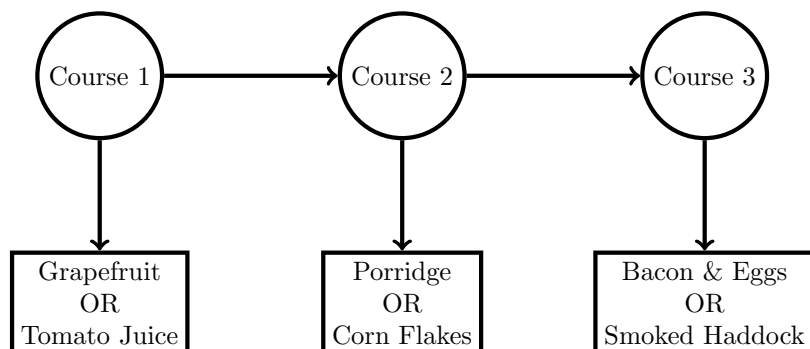

Figure 3.4: **Modules comprising substitutable items generate flexible sequences**.A meal comprising a succession of three courses (modules), each having substitutable food items. A person can choose only one item from a course leading to different sequences.

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correlation between the incoming transitions to the respective postures as well as the correlation between the outgoing transitions from them is high. The procedure is illustrated with the help of an example of a restaurant menu as shown in Figure 3.4 (Kalmus, 1969). Assuming that sequence of dishes can be taken by people respecting the constraint that only one dish can be chosen from each course, four sample dish sequences can be generated from this menu as shown below.

- Grapefruit, **_Corn Flakes_**, Smoked Haddock

- Grapefruit, **_Porridge_**, Smoked Haddock

- Tomato Juice, **_Porridge_**, Bacon & Eggs

- Tomato Juice, **_Corn Flakes_**, Bacon & Eggs

Assuming that we do not have the course content information a priori, and are only given the observed dish sequences opted by customers, then substitutability provides a way to capture the information regarding the contents of different courses. From the sequences, we observe that **_Corn Flakes_** and **_Porridge_** have similar items before and after them in a meal. Thus, **_Corn Flakes_** and **_Porridge_** can be substituted for each other and hence must be part of the same module. It must also be noted, making modules in this way based on substitution implies that elements in the same module need not occur close together in time in the observed sequence. Substitution, by capturing the combinatorial aspect of how elements are combined, leads to modules which can help generate flexible sequences like the above example. We applied the method of mutual replaceability (MR) (Maurus and Pruscha, 1973; Dawkins, 1976) designed to capture substitution principle, on the 1287 behavioral sequences of N2 worms on food, with the modules (and sub-modules) thus formed shown in Figures 3.5 and 3.6.
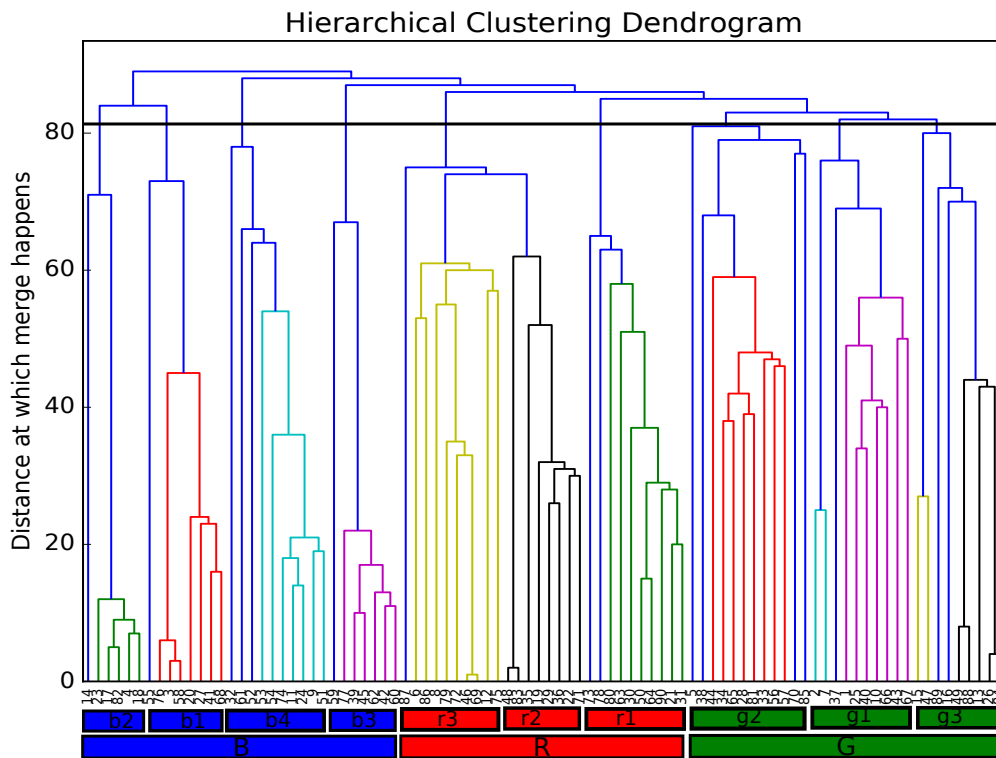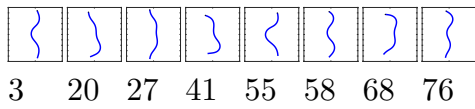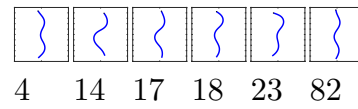
Figure 3.5: **Hierarchical dendrogram depicting the modules and sub-modules of 90 worm postures obtained by applying mutual replaceability (MR).** The tree is cut at a height of approximately 80 resulting in three big modules - B,R and G. Module B can further be divided into - b1,b2,b3 and b4 sub-modules, R into r1,r2,r3 sub-modules and G into g1,g2 and g3 sub-modules. Each module and sub-module consists of postures that are spanned by the spatial extent of the boxes corresponding to three modules and the 10 sub-modules.
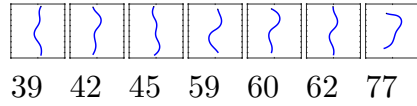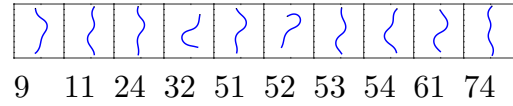
b1 (sub-module in B)

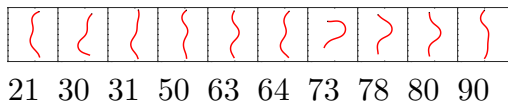3   20   27   41   55   58   68   76

b2 (sub-module in B)

4   14   17   18   23   82

b3 (sub-module in B)

39   42   45   59   60   62   77

b4 (sub-module in B)

9   11   24   32   51   52   53   54   61   74

(a)

r1 (sub-module in R)

21   30   31   50   63   64   73   78   80   90

r2 (sub-module in R)

19   22   29   35   36   48   71   83

r3 (sub-module in R)

6   8   12   46   69   72   75   79   86   87

(b)

g1 (sub-module in G)

1   10   25   37   40   43   66   67   2   7

g2 (sub-module in G)

5   28   33   34   38   44   56   57   65   81   70   85

g3 (sub-module in G)

13   16   26   49   84   88   89   15   47
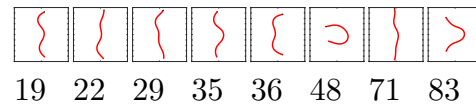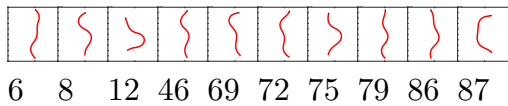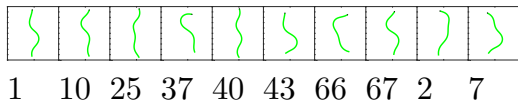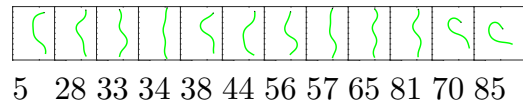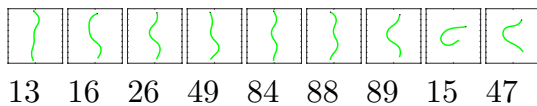
(c)

Figure 3.6: **10 sub-modules generated by the mutual replaceability criterion.** Each sub-module (sub-chunk) consists of a set of finite worm postures.

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3.5 Mutual Replaceability reveals three higher order clusters with three smaller sized clusters regulating most of the transitions between the larger clusters

We then plotted the first order transition matrix between postures for the N2 and wild isolate worms as shown in Figure 3.7a and Figure 3.7b (using the modules obtained by applying mutual substitutability on N2 worms' motion on food). Specifically, we ordered the 90 postures in the

(a)   (b)

(c)   (d)
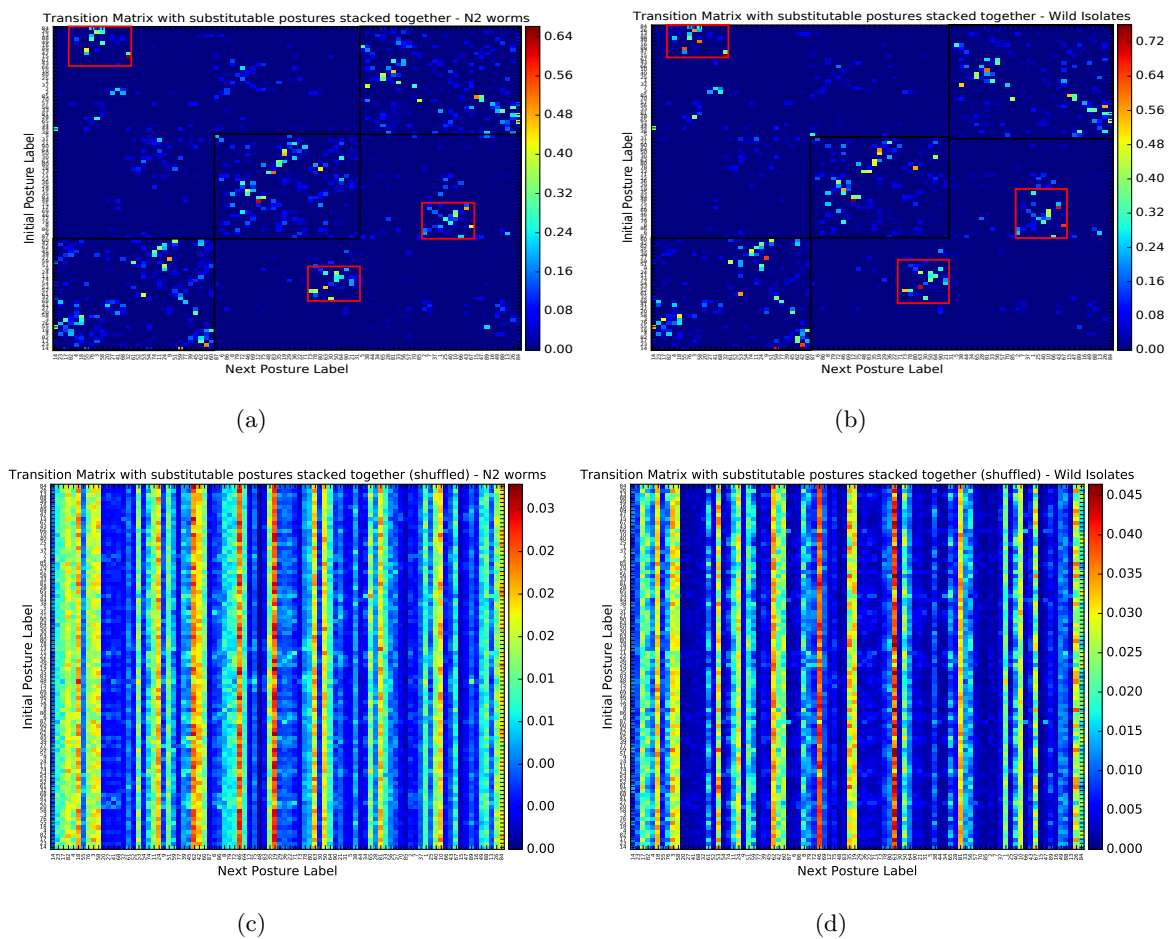
Figure 3.7: **Posture transition matrices ordered according to the modular structure given by MR reveals three big modules along the diagonal with clear cut transition structure amongst them.** (a),(b): Transition matrix between the 90 postures for all the worms pooled together for both N2 worms and wild isolate worms. The postures are ordered according to the modules discovered by mutual replaceability. Specifically, the **B,R,G** modules in the dendrogram in the previous figure are depicted by the three squares along the diagonal. (c),(d): transition matrices for N2 and wild type worms with shuffled postural sequences.

transition matrix according to the ordering given by Figure 3.5. Figures 3.7a and 3.7b reveal structure in the way in which postures are used to create worm behavioral sequences. Three big modules corresponding to B, R and G in Figure 3.5 are observed along the diagonal (marked out by black squares) of the matrices. Chunk/module B can further be divided into - b1,b2,b3 and b4 sub-chunks, R into r1,r2,r3 sub-chunks and G into g1,g2 and g3 sub-chunks, each of which consist of a set of postures as shown in Figure 3.6. The transition matrices demonstrate that there is a strong tendency for the worm to go from B to R and then to G (B $\rightarrow$ R $\rightarrow$ G), using the three smaller sized modules (marked out by red rectangles) that serve as doorways. This shows that worm foraging behavior can be decomposed into 3 higher order chunks which themselves can be decomposed into 10 sub-chunks, each of which is composed of worm postures. We next sought to determine if these decomposable chunks and sub-chunks of postures have any underlying meaning for the worm by virtue of how they interact with each other.

Chapter 4: Hierarchical organization and substitution combine to elucidate a grammar of worm foraging

We can speak of the competence (knowledge of rules, grammar, behavioural adequacy) of an individual, or we can, more generally, speak of such competences as 'rules of chess', 'English grammar' or 'efficient mating behaviour'. In this light the study of animal behaviour can be redefined as a search for the competences of animals.

Hans Kalmus

Students of animal behavior often try and distinguish between the performance and competence of an animal. Performance usually refers to the overt movements of the animal in space and time that are observed by a researcher (Kalmus, 1969). On the other hand competences are usually an inferred construct by observing the overt behavior of animals that consists of the specification of rules and strategies that can explain the overt behavioral sequences of the animal under consideration(Kalmus, 1969). The search for such competences can lead to the uncovering of general principles underlying animal behavior.

As pointed out very eloquently in (Fentress, 1991), the performance of animals does not just contain isolated movements, but those isolated movements are embedded in broader contexts. To make sense of behavior, then demands that we understand competences that explain how isolated movements are mutually dependent and what principles allow them to be strung together into context dependent actions.

In this chapter, I show how the principles of hierarchy and substitution allow us to define competences underlying worm foraging behavior.

4.1 Stereotypical yet flexible sustained forward locomotion (roaming) in wild isolates is generated by sustained application of the b1→ g3 behavioral rule

Concentrating on wild type worms (Figure 3.7b), we then investigated the meaning of the B → R → G sequence and its relation to the sub-modules (b1 through g4) for generating meaningful worm

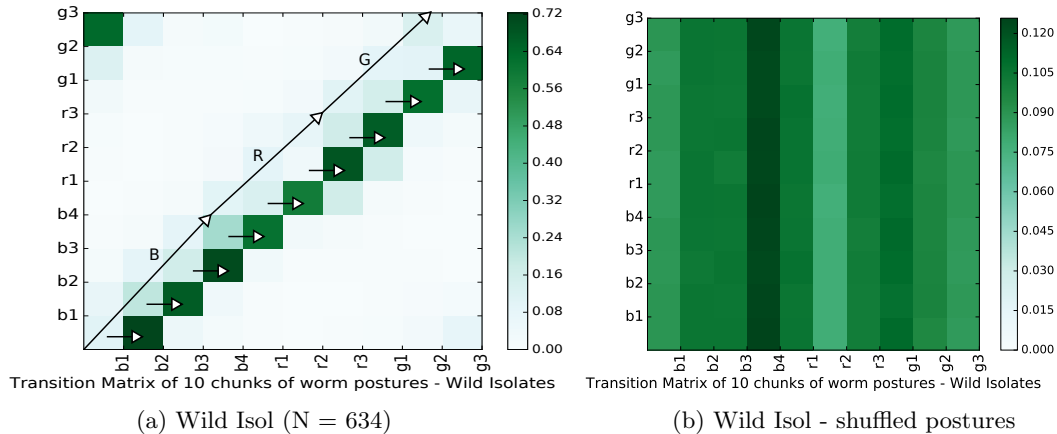behavior. As a first step, we plotted the transition matrix between the 10 sub-modules (from b1

(a) Wild Isol (N = 634)

(b) Wild Isol - shuffled postures

Figure 4.1: **Wild isolate worms move in a way such that at the higher level of sub-modules, the sequence of b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 is followed.** (a): Transition matrix between the 10 sub-modules corresponding to b1,b2,b3,b4,r1,r2,r3,g1,g2 and g3 for 634 wild isolate worms pooled together. There is very high probability of the worm to move according to the sequence b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 is followed. (b) Same as (a), but for worms whose posture sequences contain postures such that they have been shuffled as compared to the original sequences. The lack of structure in the shuffled sequences points at the non-triviality of the structure observed in (a).

through g4) as shown in Figure 4.1a. Specifically, there is a transition counted from sub-module b1 to b2 if there is a transition from a postures belonging to b1 to a posture belonging to b2. We can see from Figure 4.1a that there is a strong predictability to wild type worm foraging behavior, with the worm taking the sub-module sequence b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 for the majority of its movement time.

Since the wild type worms are known to perform roaming behavior (sustained forward locomotion with limited turns and pauses) for a significant time, we hypothesized that the B → R → G invokes the b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 sequence rule to generate roaming behavior in worms. Note that even though this is a stereotyped behavior, there is flexibility at the level of postures in the sense that whenever the worm is in a particular sub-module (say b1), it can pick any posture belonging to that particular sub-module and then move on to the next sub-module in the sequence. This flexibility results in a combinatorial explosion in the number of unique sequences that can be generated.

To test this idea of stereotypical yet flexible behavior generation, we simulated 10000 frames where each frame was represented by a worm posture. The putative forward locomotion generating sequence b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 was used to generate the sequence of 10000

frames. Whenever in a particular sub-module, the simulation randomly chose any one posture in that sub-module and moved on to the next sub-module to do the same. Note that the extremely curved postures that are definitively used for making sharp turns (2,7,70,85,15,47) were not included in these simulations. Once a sequence of 10000 postures was generated using the b1→g3 rule, we divided the sequence into 100 consecutive chunks of 100 postures (frames) each. The angles corresponding to the 48 segments of each worm posture in each of the 100 frames was then averaged across the 100 consecutive chunks to get an averaged out 100 frame chunk. The evolution of the angles corresponding to the 48 segments corresponding to each frame in the averaged out chunk of 100 frames was then visualized as shown in Figure 4.2a. The traveling wave in the forward direction (from the head to tail) in Figure 4.2a confirms the hypothesis that sustained forward locomotion in worms is generated by the sub-module sequence b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 with flexibility being rendered to this stereotypic sequence by the variable choice of postures from each sub-module in every instantiation of this behavioral rule. Thus, the red rectangles in Figure 3.7b are the postures comprising sub-modules b4, r3 and g3, enabling the chain of B→R→G to accomplish forward locomotion. If b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 encodes smooth forward locomotion in worms, then g3→g2→g1→r3→r2→r1→b4→b3→b2→b1 behavioral rule should in principle generate reversal behavior. To test this idea, we simulated 100 worms each consisting of 100 frames, using the above behavioral rule, with each frame corresponding to a particular posture. This is because of the fact that the full roaming sequence is 10 states long. Whenever in a particular sub-module, the simulation randomly chose any one posture in that sub-module and moved on to the next sub-module to do the same. Using the same procedure as was used to generate Figure 4.2a, the evolution of the angles corresponding to the 48 segments of each frame was then visualized as shown in Figure 4.2b. We can clearly see from the figure, that a wave travels from the tail to the head of the worm confirming the hypothesis that the g3→g2→g1→r3→r2→r1→b4→b3→b2→b1 grammatical rule indeed encodes reversal behavior. Note that multiple consecutive instances of this b1 → g3 rules means that at the higher level of chunks, B→R→G sequence gets instantiated multiple times like BRGBRGBRGBRG. Due to the cyclic nature of the roaming behavioral rule, we can see that BRG roaming rule is the same as R→G→B and G→B→R. During the R→G→B sequence for example - instead of starting from b1, the worm would start from r1 and follow the following sequence at the level of 10 sub-chunks to complete one roaming cycle - r1→r2→r3→g1→g2→g3→b1→b2→b3→b4.

One important point to note is that although repeated application of b1→g3 behavioral rule has

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

(i)
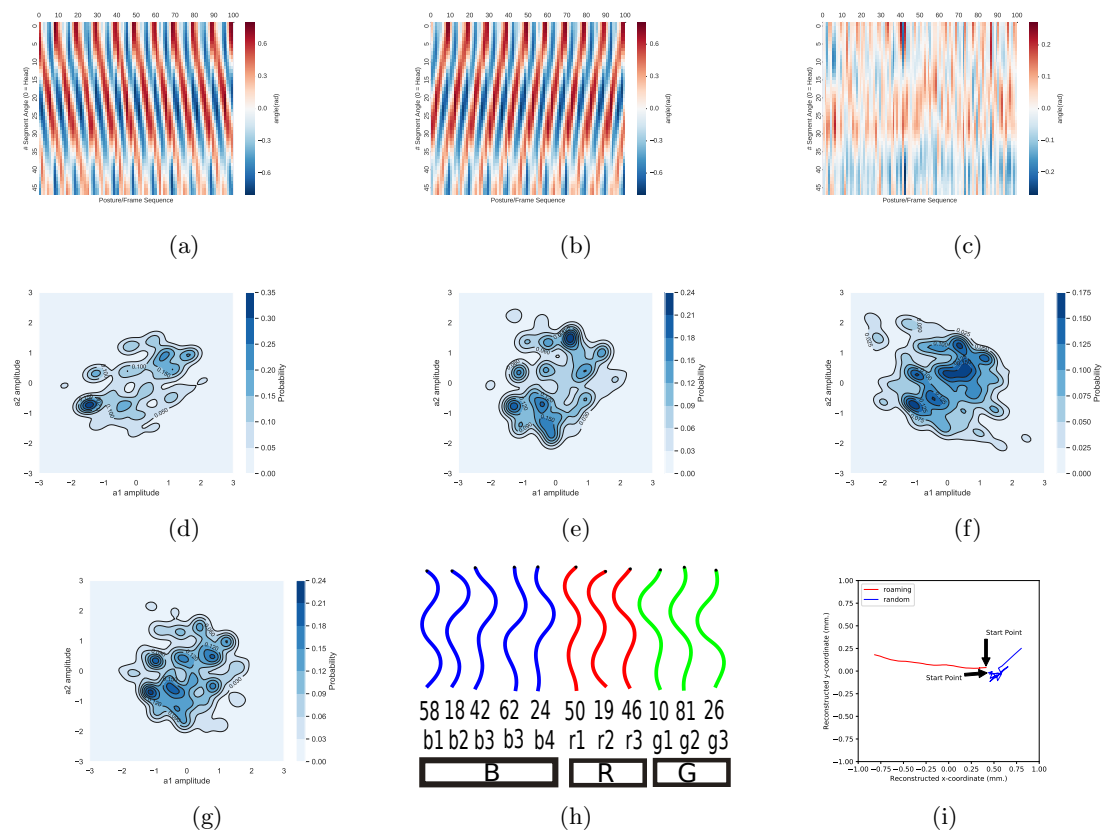
Figure 4.2: **Grammatical rule b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 invoked by the hierarchically superior rule B→R→G encodes worm roaming behavior** (a): Simulation of b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 behavioral rule shows the progression forward traveling wave along the worm body which sustains forward locomotion (roaming). Figure shows simulation of averaged worm behavior over 100 frames. (b): Simulation of the reverse behavioral rule g3→g2→g1→r3→r2→r1→b4→b3→b2→b1 generates sustained backward motion. Figure shows simulation of averaged worm behavior over 100 frames. (c): A simulation of worm locomotion where the identities of sub-modules remains the same but the sequence of b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 is broken in favor of a randomized one.Figure shows simulation of averaged worm behavior over 100 frames. (d) and (e): Probability distribution of the eigen projection of the postures involved in all the instances of the b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 sequence along the first two eigenvectors of the shape covariance matrix of freely crawling worms, for wild isolates (d) and N2 worms (e) respectively. The probability distribution in both cases is reminiscent of a ring like structure suggesting oscillatory behavior involved in forward locomotion. (f) and (g): Probability distribution of the eigen projection of the postures involved in all the instances of all the rules other than b1→g3 forward rule and g3→b1 rule, along the first two eigenvectors of the shape covariance matrix of freely crawling worms, for wild isolates (f) and N2 worms (g). Note that the probability distribution of rules other than b1→g3 is relatively more uniform than the corresponding figures in (d) and (e), inhabiting regions in the postural space that were not frequented by the b1→g3 rule. (h): Sample postural sequence that respects the b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 sequence. (i): Trajectory of centroid position during a roaming phase (red) characterized by 3 consecutive b1→g3 rules comprising approx. 30 postures from an actual N2 worm . The figure also shows worm centroid trajectory based on application of the randomized sequence rule mentioned in (c) and consisting of 30 postures.

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the capacity to generate sustained forward motion, slight variations to this rule still keep the worm in roaming state. Roaming can also consist of short reversals or pauses. Roaming mostly consists of forward motion aiding the worm in traveling to farther places, but it also involves small reorientations, consisting of short reversals, as well as pauses, so that it can change direction and then travel a long distance in that direction. For example - instead of b1→b2→b3→b4→r1→r2→r3→g1→g2→g3, b1→b2→b3→b4→r1→r2→r3→g1→g2 or b1→b2→b3→b4→r1→r2→r3→g1→g2→g3→g2→g3 also encode roaming behavior. The details of heuristics used to assign sequence of postures into roaming and non-roaming states is mentioned in chapter 7.

We then computed the eigen projections of the postures involved in all instances of b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 rule found in actual behaving wild isolate and N2 worms, along the first two eigen vectors of shape covariance matrix of freely moving N2 worms (Figures 4.2d and 4.2e). The non-uniform probability distribution in Figures 4.2e and 4.2d with low density of points in the middle is indicative of an oscillatory motion that is thought to underly forward locomotion in worms (Stephens et al., 2008; Gyenes and Brown, 2016). On the other hand, plotting the probability distribution for postures involved in sequences other than those characterized by the b1→g3 and g3→b1 rules, reveals a more uniform distribution (Figures 4.2f and 4.2g). This suggests that the postures used in sequences other than those characterized by roaming or reversal rules inhabit regions of posture space more liberally including regions where postures used in roaming type rules project to less often. In other words, the probability of those postures being used in roaming that project in the middle region of a1-a2 space (postures related to pauses and sharp turns) is low as compared to the dwelling phase, when they get used more often.

Finally, we plotted the trajectory of the worm centroid position from a particular sequence of postures in an actual behaving N2 worm, characterized by roaming behavioral rule using the model proposed in (Keaveny and Brown, 2017). As can be seen from Figure 4.2i, the trajectory of the roaming rule characterized by b1→g3, travels a far greater distance than a sequence of postures of the same length but generated by a randomized behavioral rule.

Taken together, these results show that the b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 grammatical rule encodes worm roaming behavior. Thus, *C. elegans* roaming behavior is hierarchically organized, with type 2 substitution accounting for the flexible nature of roaming behavior (Figure 4.3). We note that worm roaming behavior is hierarchically organized not because the behavioral repertoire was divided into lesser number of chunks/modules, but because rules of interaction be-

tween such modules could be meaningfully elucidated (Simon, 1962; Clarke and Crossland, 1985), establishing correspondence between worm roaming behavior and compositional hierarchy. Note that at the level of higher order chunks (B,R and G), roaming behavior is only made possible by a specific rule combining these three chunks (B→R→G) and no other rules combining them (for example - B→R→B) would generate worm roaming.
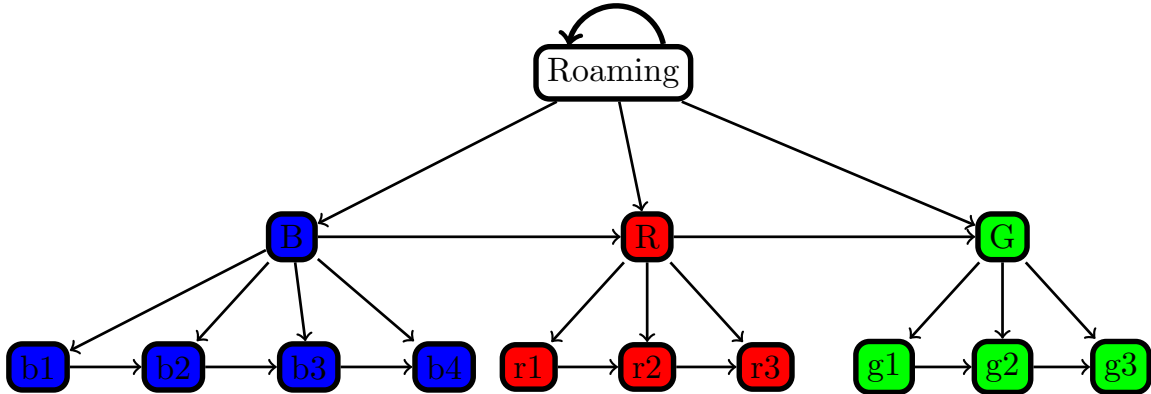
Figure 4.3: **Schematic of hierarchical organization of stereotypic yet flexible forward locomotion in worms.** Flexibility is afforded by the use of type 2 substitution, whereby when the worm is in any of the 10 sub-modules (b1 to g3), it can choose any of the multiple postures that belong to that particular sub-module. (type 2 substitution)

4.2 Body morphology cannot account for the dynamics underlying worm roaming behavior

One relatively straightforward way to make modules and sub-modules of postures involves clustering postures based on morphological similarity, i.e. - morphologically similar postures should be grouped together into one module. To investigate the difference between sub-modules based on substitution versus those that might have been generated based on body morphology similarity, we computed the silhouette scores for all the postures based on the sub-modules given by MR (Figure 4.4). The silhouette value for a posture p in a sub-module (given by MR) measures how morphologically similar p is to other postures in its own sub-module, as opposed to postures in other sub-modules. Large silhouette value indicates that the posture is tightly bound to other postures in its sub-module in terms of morphological similarity. The existence of high number of postures having negative silhouette value (60 out of the 90 postures) indicates that the sub-modules given by MR contain postures that are not morphologically similar to each other as compared to postures in other sub-

modules (Figure 4.4). Thus, sub-modules generated by MR capturing the substitutability between postures are different from what might be expected by modules generated based on the criterion of morphological similarity between postures.
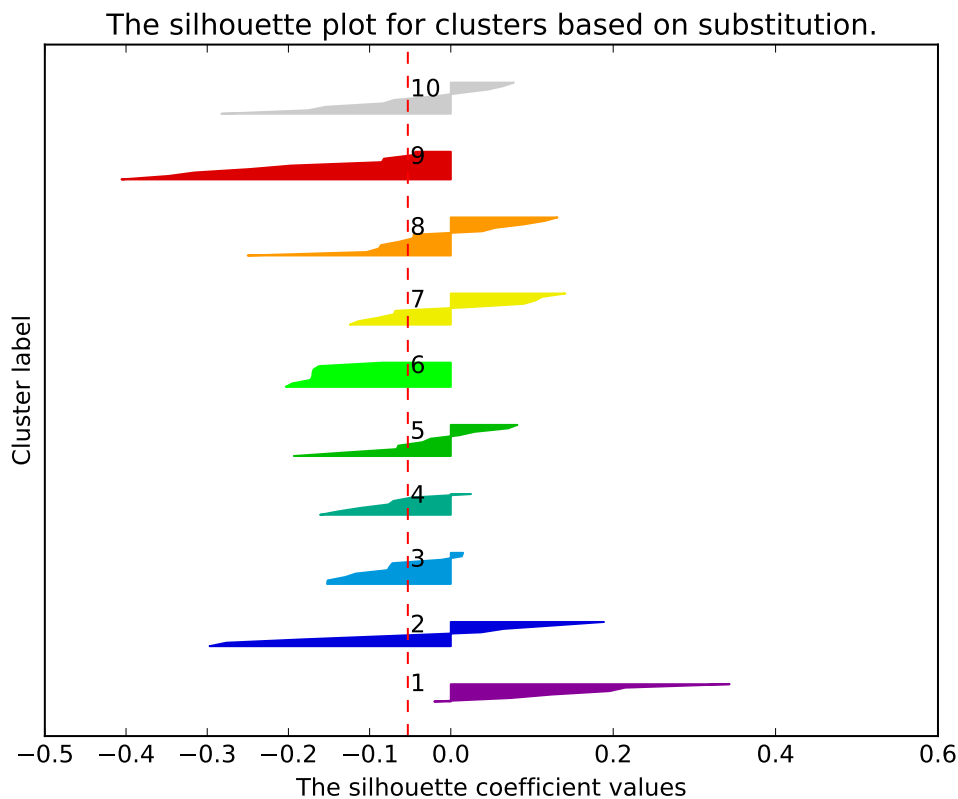
Figure 4.4: **Sub-modules of substitutable postures given by mutual replaceability (MR) are not compact in terms of morphological similarity as would be expected if substitutable postures were to be morphologically similar to each other.** Silhouette values of all postures separated according to their membership to the 10 sub-modules generated by MR. Postures in each sub-module are ordered by decreasing silhouette value. Silhouette values show low intra-module morphological similarity between postures. Negative silhouette values for most of the postures in general, indicates low morphological cohesiveness of postures with the others in the same cluster. The red vertical line shows the average silhouette value across the 10 sub-clusters.

Next, we investigated the role of body morphology in dynamics that generate worm behavior. Considering the grammatical rule for worm roaming behavior - b1→g3, we looked at the morphological similarity between successive postures during all the b1→g3 sequences in behaving N2 worms. This morphological similarity was then contrasted with the morphological similarity that would be expected if at each posture during the b1→g3 sequence, the worm transitioned to the most morphologically similar posture to the current one. As shown in Figure 4.5a, there is a large difference in the distribution of morphological similarity in the roaming case versus the most similar posture

next case (N=2033667, effect size, Cohen's d = 1.102, p<0.0001, Welch's t-test). The large effect size shows that the transitions between successive postures during roaming behavior is significantly different and cannot be captured by considering transitions between the most morphologically similar postures. Further, it can be seen that although the profile of morphological similarity structure between successive postures is the same in b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 as well as in g3→g2→g1→r3→r2→r1→b4→b3→b2→b1 behavioral rule, the behavior that these two rules generate is completely different from each other. If we assume that posture p_1 is chosen from b1 and posture p_2 is chosen from b2, then if we transition from b1→b2 or from b2→b1, the morphological similarity profile of the transition between p_1 and p_2 would remain the same. Even with the same morphological similarity structure between successive postures, the worm behavior generated by these two rules is completely different. While the former generates sustained forward locomotion (Figure 4.2a), the simulation of the latter behavioral rule generates sustained backward locomotion (Figure 4.5b). This analysis reveals the primacy of the behavioral grammar in generating behavior, where identical morphological similarity transition profiles can generate different behavior based on the grammatical rule being used by the worm.

(a)　　　　　　　　　　(b)　　　　　　　　　　(c)
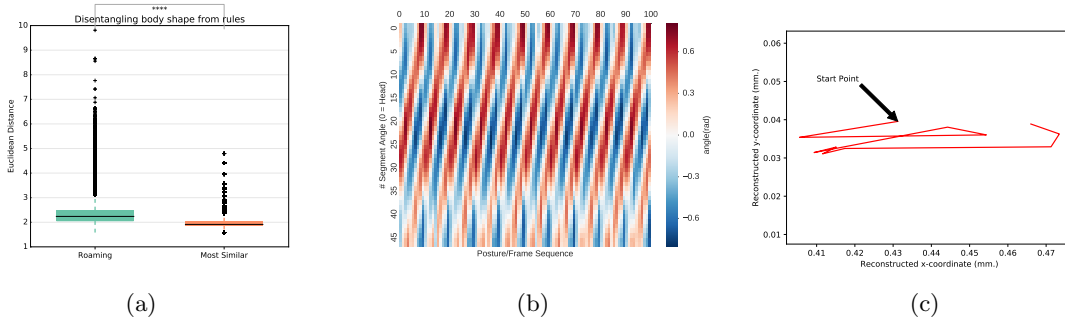
Figure 4.5: **Worm postural dynamics involved in generating roaming behavior are not explained by morphological similarity between the successive postures.** (a): The left box shows the morphological similarity between adjacent postures in all the instances of b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 behavioral rule in all N2 worms on food. For each posture belonging to the posture sequence making up the left box, the right box represents the morphological similarity distribution assuming that the most similar posture is chosen from the current posture. Box plot shows interquartile range. (b) Simulation of g3→g2→g1→r3→r2→r1→b4→b3→b2→b1 (reversal) generates a different behavior than the b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 rule (forward), even though the morphological similarity profiles are the same during the two rules. (c) A sample trajectory of worm centroid position (consisting of 20 postures) simulated from a behavioral rule that picks a posture randomly from among those postures whose morphological similarities are within the top 5 morphological similarities to the current posture. It can be seen that the simulated worm fails to maintain continuous forward locomotion that results from the roaming rule above.

4.3 Non-stereotypical dwelling behavior involves disruption of b1→g3 roaming rule by alternating between sub-modules in the b1→g3 sequence

Apart from roaming, *C. elegans* also displays dwelling behavior wherein the worm reorients itself, by generating short reversals and interrupting forward motion frequently to change its direction. Concentrating on the behavior of N2 worms in Figure 3.7a, we first note the regions in the transition matrix that were not implicated in the generation of roaming behavior in worms, marked by green rectangles in Figure 4.6b. The green rectangles in Figure 4.6b encapsulate weaker interaction strength within themselves as opposed to the red rectangles that facilitated the B→R→G behavioral rule. They correspond to interactions of the type G→R, R→B and B→G, instead of the B→R, R→G and G→B transitions represented by the red rectangles. Comparing Figure 4.6c with 4.1a, we observe

- Compared to the wild type isolates, in N2 worms, there is a stronger tendency for transitions to occur between postures of the same sub-module and between postures from the current sub-module to postures of a preceding sub-module. Any sub-sequence of the b1→g3 roaming rule must corrrespond to forward motion for a short time (e.g: b3→b4→r1). Similarly, any sub-sequence of the g3→g2→g1→r3→r2→r1 →b4→b3→b2→b1 reversal sequence (e.g.: g1→r3→r2) must correspond to shorter reversals.

- Previous work has demonstrated that worm reversals are generally associated with a decrease in their speed and form part of what is known as dwelling behavior (Flavell et al., 2013), that is not very stereotypic (Gomez-Marin et al., 2016).

- The full reversal sequence g3→g2→g1→r3→r2→r1→b4→b3→b2→b1 is not as probable as the full forward sequence b1→b2→b3→b4→r1→r2→r3→g1→g2→g3.

- Apart from the strong tendency to follow b1→g3 rule (similar as in wild isolates), N2 worms also display behavior that disrupts this b1→g3 rule more strongly than the wild isolates. This disruption is very specific in the sense that it involves an increased use of more than one posture from the current sub-module that the worm is in and/or postures belonging to the preceding sub-module in the b1→g3 sequence. It is not the case, for example, that b1 suddenly starts
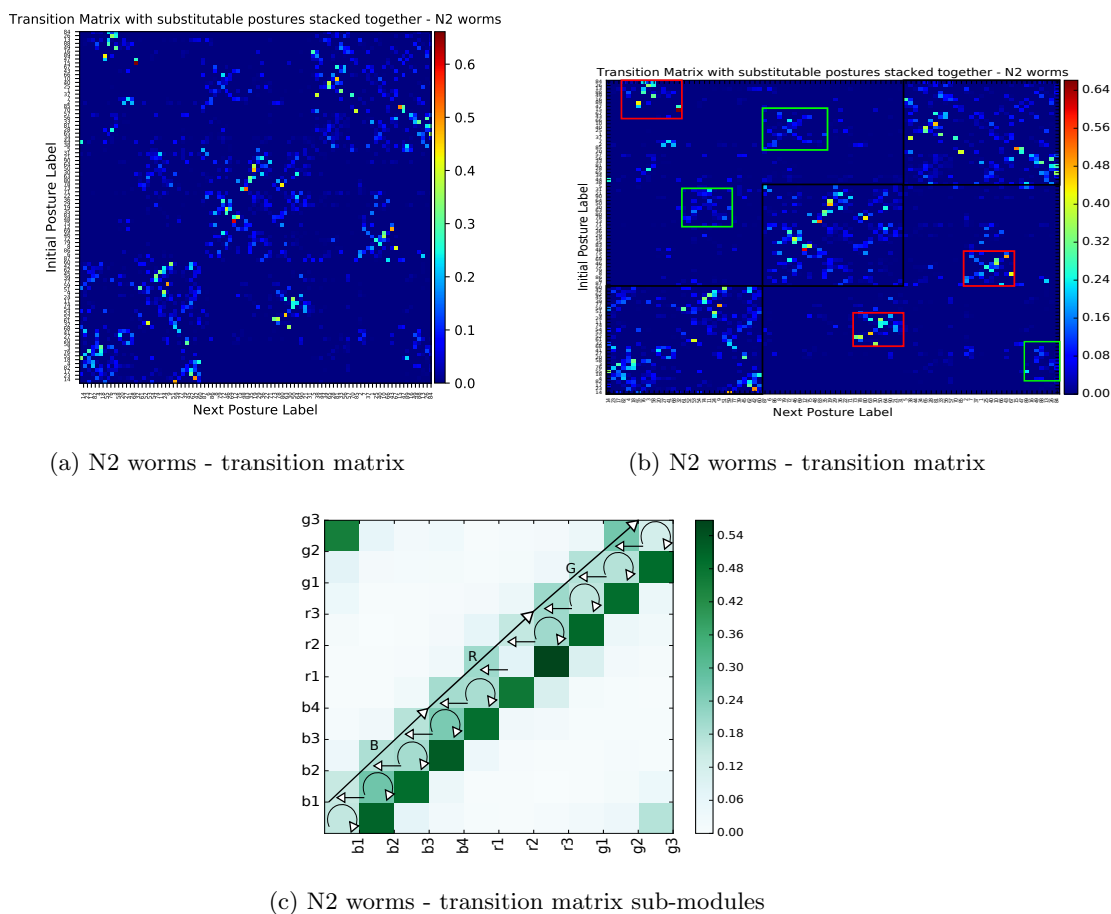
(a) N2 worms - transition matrix

(b) N2 worms - transition matrix

(c) N2 worms - transition matrix sub-modules

Figure 4.6: **As compared to wild isolates, N2 worms show an increased tendency to disrupt the b1→g3 rule by alternating between adjacent sub-modules in the b1→g3 rule sequence** (a): Transition matrix between the 90 postures for all the worms pooled together for both N2 worms . The postures are ordered according to the modules discovered by mutual replaceability. (b): Same as Figure 3.7a, with the addition of green rectangles representing interactions between the B, R and G modules that were not implicated in forward motion during analysis of wild type data. (c): Transition matrix between the 10 sub-modules for the N2 worms. The curved and the leftward arrows point to the increased interaction between postures of the same sub-module and between the current sub-module postures to preceding sub-module postures respectively as compared to wild type worms (Figure 4.1a).

making increased transitions to r3 to break the b1→g3 sequence.

- This results in the disruption of the b1→g3 sequence rule by alternating between consecutive sub-modules in the b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 rule. Note that disruption in the b1→g3 sequence, in terms of higher level modules (B, R and G) can be achieved in the following ways (Figure 4.9a) -

    - **Dwell 1**:- by alternating between sub-modules but still maintaining the B→R→G rule,

– **Dwell 2**:- achieving alternation between sub-modules by alternating at the level of higher modules by adopting rules of the form B→(R or G)→B, R→(B or G)→R and G→(B or R)→G,

– **Dwell 3**:- by alternating between sub-modules such that the smooth reversal sequence (without alternations) of g3→g2→g1→r3→r2→r1→b4→b3→b2→b1 is disrupted while still maintaining the G→R→B sequence seen in smooth reversals.

If the behavior rules corresponding to Dwell 1, Dwell 2 and Dwell 3 encode dwelling behavior in worms, then the proportion of such rules should be higher when the sequence length of the worm during the 15 minute recording is shorter. This is because during dwelling, the speed of the worm is slower, thus spending a greater amount of time per posture leading to a decrease in the number of unique postures that make up the worm's behavioral sequence. During roaming, the worm can be

(a) N2 (N = 1287 worms)

(b) Wild Isolates (N = 634 worms)
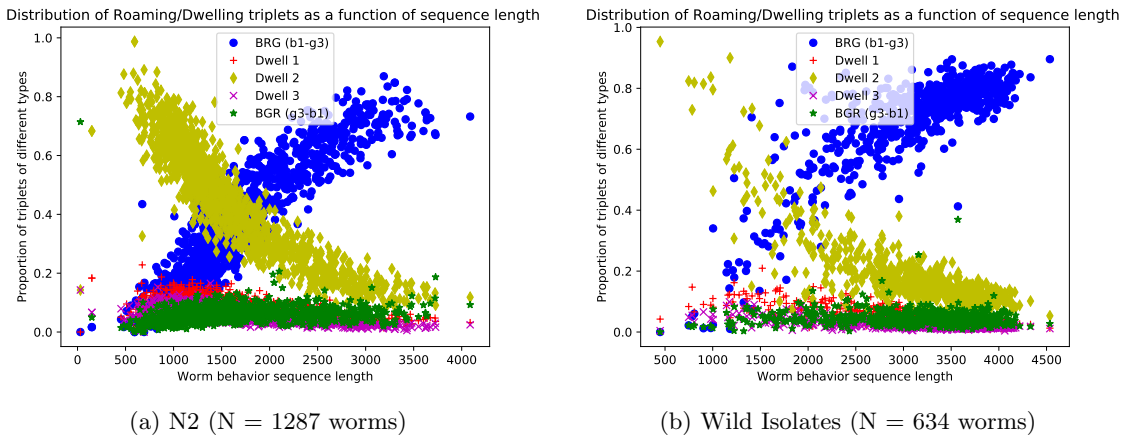
Figure 4.7: **Usage of roaming and dwelling rules vary smoothly as a function of the postural sequence length.** (a) and (b): The proportion of roaming type behavioral rule increases as a function of postural sequence length in both N2 and wild isolate worms respectively. The proportion of Dwell 1, Dwell 3 and reverse rules remain stable as function of the postural sequence length, but the Dwell 2 rules corresponding to B_B,R_R,G_G rules decrease as a function of postural sequence length.

presumed to spend relatively less amount of time in each posture due to its higher speed, thereby generating a behavioral sequence that is comparatively longer in length. We therefore plotted the proportion of purported dwelling and roaming type behavioral rules as a function of the sequence length (Figure 4.7) and found that the proportion of Dwell 2 type rules (ignoring Dwell 1 and Dwell 3 rules for the moment, as their proportion is relatively stable at a low value) decreases sharply as the sequence length increases. This decrease in the proportion is offset by a corresponding increase in the roaming type rules as the sequence length increases. Figure 4.7 lends support to the idea that

Dwell 2 type rules encode dwelling behavior. Thus, depending on the context (dwelling vs roaming), the same modules and sub-modules rearrange the way in which they interact with each other to produce different sequences.

As the worm moves faster during roaming than during dwelling (Flavell et al., 2013), we hypothesized that the speed of the worm during b1→g3 rule should be higher than during the rules hypothesized to underlie dwelling (like Dwell 1, Dwell 2 and Dwell 3). As a corollary, we reasoned that the time spent during a single instantiation of the roaming rule (b1→g3) ought to be lesser in comparison to the time spent during a single instantiation of dwelling type rules (Dwell 1, Dwell 2 and Dwell 3). Hence, we calculated the average speed and total time taken during each instantiation of all the behavioral rules (b1→g3, g3→b1, Dwell 1, Dwell 2 and Dwell 3) . We averaged the speed across all the frames belonging to a particular instantiation of a behavioral rule to get a handle on the average speed of the worm centroid during a particular postural sequence given by a particular behavioral rule. It can be seen from Figures 4.8a and 4.8b that the average speed of the worm during instantiations of the hypothesized roaming rule is higher than the average speed during all instantiations of the various rules hypothesized to underlie dwelling. It is also worth noting that the speed during instantiations of the hypothesized rule for smooth reversal (g3→g2→g1→r3→r2→r1→b4→b3→b2→b1) is also quite high. Next, as can be seen from Figures 4.8d and 4.8c, the total time spent during instantiations of roaming type rule is considerably lesser than the time spent during instantiations of rules implicated in dwelling. Specifically, the total time spent in each instantiation of all the different types of behavioral rules (Roam, Dwell 1, Dwell 2 etc.) across all the worms was computed and their distributions were then plotted. The slower speed and the higher time duration for rules of type Dwell 1 and Dwell 3 also establish their role in dwelling behavior (Figures 4.8a, 4.8b, 4.8c, 4.8d).

(a) N2 worms - Speed.

(b) Wild isolates - Speed

(c) N2 worms - timings.

(d) Wild Isolates - timings

(e) N2 worms - time inside Dwell 2 rules

(f) Wild Isol - time inside Dwell 2 rules

(g) N2 worms (N = 1287)

(h) Wild Isol (N = 634)
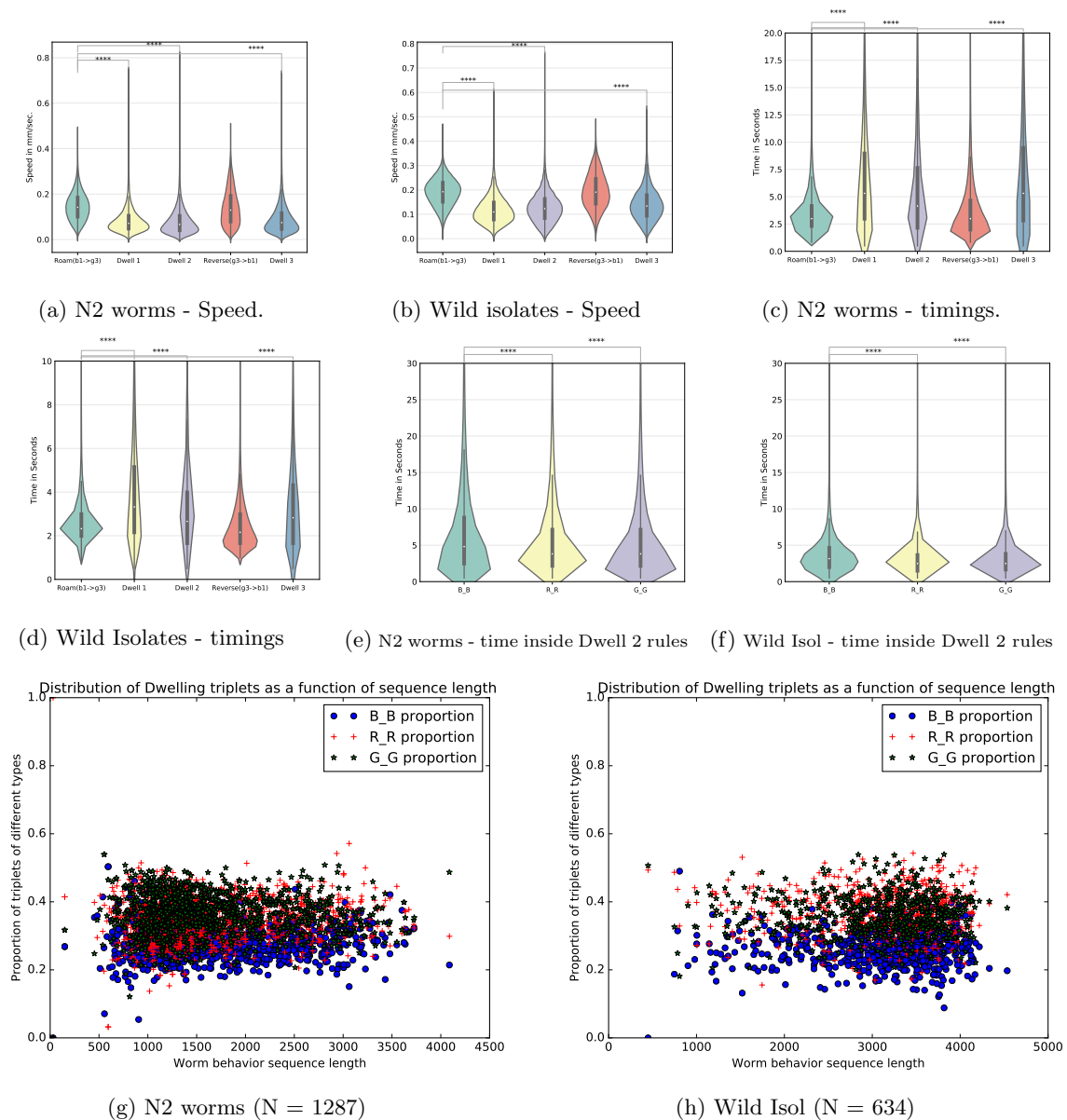
Figure 4.8: **In accordance with their functional roles for the worm, grammatical rules underlying roaming and dwelling have considerably different properties.** (a) and (b): Average speed of the worm centroid position during instantiations of the roaming type rule is considerably higher than during the behavior rules corresponding to dwelling. Violin plots showing the distribution of average speeds during each instantiation of all types of rules across all the worms. (a) d(roam,dwell1) = 0.978, d(roam,dwell2) = 1.1, d(roam,dwell3) = 0.91. N(Roam)= 267217, N(Dwell 1)= 51538, N(Dwell 2)= 240267, N(Reverse)= 39424, N(Dwell 3)= 33287. (b) d(roam,dwell1) = 1.29, d(roam,dwell2) = 1.1, d(roam,dwell3) = 0.89. N(Roam)= 424860, N(Dwell 1)= 26492, N(Dwell 2)= 96763, N(Reverse)= 27371, N(Dwell 3)= 13105. (c) and (d): To-tal time spent by the worm during all instantiations of various grammatical rules in N2 and wild isolates respectively. Violin plots show the distribution of times taken to complete each in-stantiation of all the behavioral rules across all the worms. Time spent during roaming type rules is much smaller than that spent during dwelling type rules. (c) d(roam,dwell1) = -1.05, d(roam,dwell2) = -0.51, d(roam,dwell3) = -1.19. N values same as (a). (d) d(roam,dwell1) = -1.26, d(roam,dwell2) = -0.4, d(roam,dwell3) = -0.86. N values same as (b). (e) and (f): De-constructing the time associated with dwell 2 type behavioral rules in N2 and wild isolates respec-tively. (e) N(B_B)=68986, N(R_R)=83038, N(G_G)=88243. d(B_B,R_R)=0.17, d(B_B,G_G)=0.18. (f) N(B_B)=25588, N(R_R)=36236, N(G_G)=34939. d(B_B,R_R)=0.18, d(B_B,G_G)=0.17. (g) and (h): The proportion of the three types of dwell 2 type rules is similar across varying posture sequence lengths in both N2 and wild isolates respectively. **** = p < 0.0001, Welch's t-test; effect size: d = cohen's d. Violin Plots contain box plots that show the interquartile range.

Since the proportion of rules of type Dwell 1 and Dwell 3 remains relatively stable across a variety of postural sequence length (Figure 4.7), we decided to further look into the more dynamic Dwell 2 type dwelling rules. Figures 4.8e and 4.8f show that the time taken during each of the rules of type B→(R or G)→B, R→(B or G)→R and G→(B or R)→G is consistently higher than the time taken during the rule that characterizes roaming. Also, the the timing difference between rules of type B→(R or G)→B, R→(B or G)→R and G→(B or R)→G amongst themselves is not significantly different (low effect size as shown in 4.8e and 4.8f. p values denote significance but that is likely due to the large values of N for these comparisons.) We also looked at the distribution of Dwell 2 type rules to check if there is a preference for one kind of dwelling rule as opposed to the others (Figures 4.8g and 4.8h) and found them to be nearly equally distributed.

Taken together, these observations indicate that even in worm dwelling behavior that is thought to be relatively less stereotypic than roaming behavior (Gomez-Marin et al., 2016), there is predictability owing to the hierarchical nature of behavioral organization. Specifically, if we know that the worm is in *dwelling* state, we know that the smooth b1→g3 sequence rule is broken. Furthermore, if we further know that inside the dwelling phase, the worm is in B_B state, then we know for sure that either B→R→B *or* B→G→B has to hold. Additionally, if we further know that the worm is in B→R→B we can be sure that the scaffold of sub-modules that the worm will execute is as given in Figure 4.9a. In this way, the worm dwelling behavior, not unlike the roaming behavior, is predictable yet flexible.

The sub-module sequence (like **b1..b4r1..r3r2r1b4b3..b1** generated by **B→R→B** sequence) provides a scaffold with sub-sequences that could get arbitrarily long while still maintaining the structure imposed by the rules. This capacity of memory is a property of hierarchical systems where the time spent in a sub-chunk inside a bigger chunk can extend to arbitrary time scales as highlighted by the posture sequence in Figure 4.10a. It shows a dwelling sequence of type B→R→B like in Figure 4.9b with almost the same set of postures making up the two sequences. But the posture sequence in Figure 4.10a is considerably longer than the other one. Hierarchical organization that treats B→R→B as one single unit permits sequences where the posture sequence generated by the first B chunk inside the B→R→B unit can be arbitrarily long, still having memory to generate posture sequences from the R and B chunks after finishing the posture sequence from the first B chunk to make up the B→R→B unit. On the other hand, linear models can only look so far back in time and usually find it difficult to handle long term dependencies in sequential data.

(a)

(b)

(c)
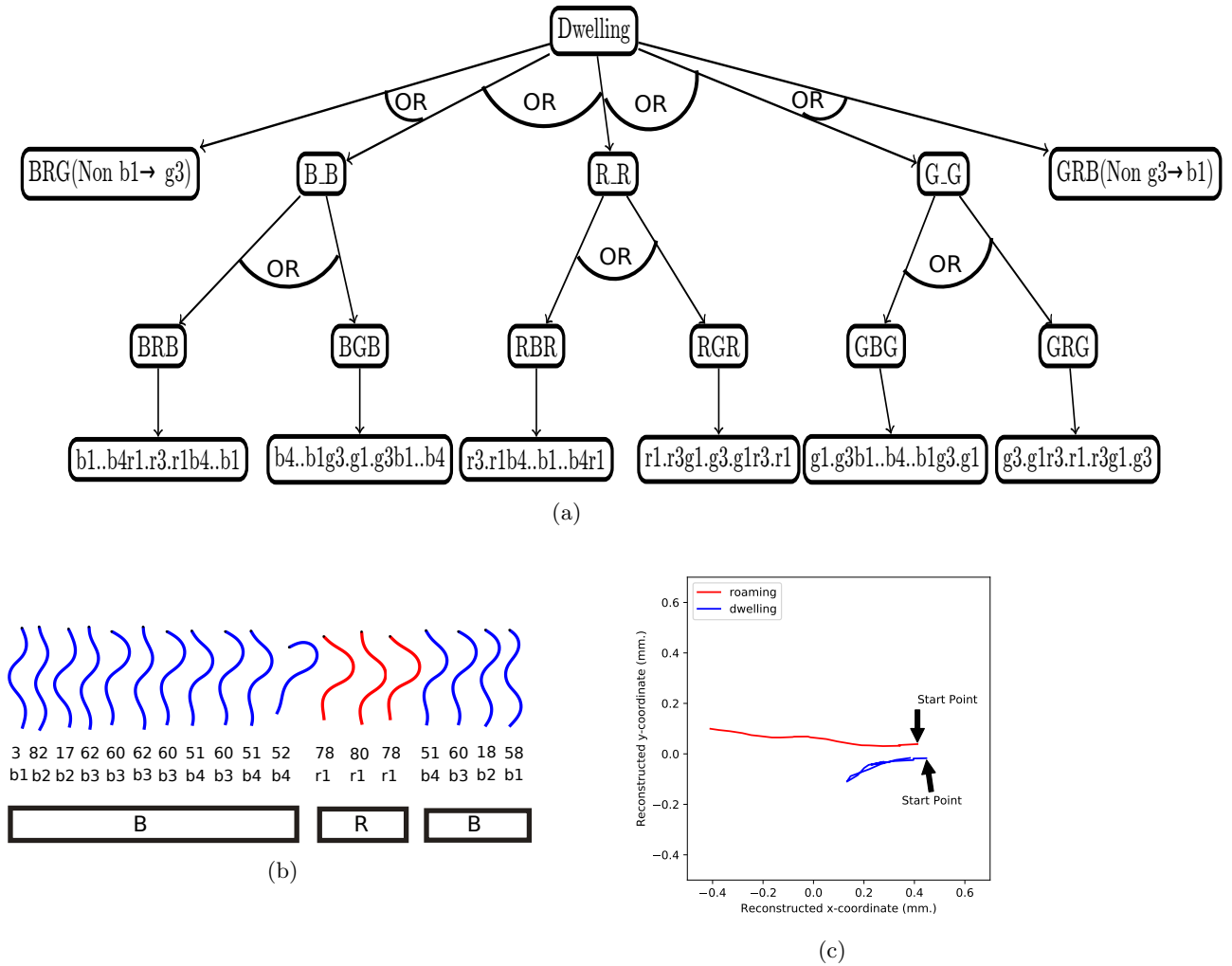
Figure 4.9: *C. elegans* **dwelling behavior abstracted as a set of grammatical rules.** (a): Schematic of *C. elegans* dwelling behavior with three higher order dwelling rules. **Dwell 1** = {BRG(Non b1→ g3)}, **Dwell 2** = {B_B,R_R,G_G} and **Dwell 3** = {BGR(Non g3→b1)}. The multiple rules for realizing dwelling lend it a relatively less stereotypic character than roaming behavior. There is type 2 substitution at the level of postures as well as at the level of sub-modules, where the different sub-modules can be involved in the generation of same dwelling behavior. (b): A sample dwelling sequence (rule of type BRB) taken from an actual foraging N2 worm. (c):Reconstructed trajectory of worm centroid based on the posture sequence in (b). The roaming trajectory in red denotes the roaming sequence (2 consecutive b1→g3 rules) involving roughly the same of postures as the dwelling sequence (around 20). The blue trajectory includes forward locomotion frequently interspersed with backward locomotion, that is characteristic of dwelling behavior

To quantify the predictability afforded by dividing the 90 postures into 10 sub-modules, we calculated the H_0, H_1 and H_2 entropy values for the two conditions (90 postures versus 10 sub-modules). H_0 denotes the uncertainty in predicting the next event (posture/sub-module), if all the events are equi-probable, H_1 denotes the reduced uncertainty afforded by the knowledge of individual event probabilities and H_2 denotes the reduced uncertainty afforded the knowledge of
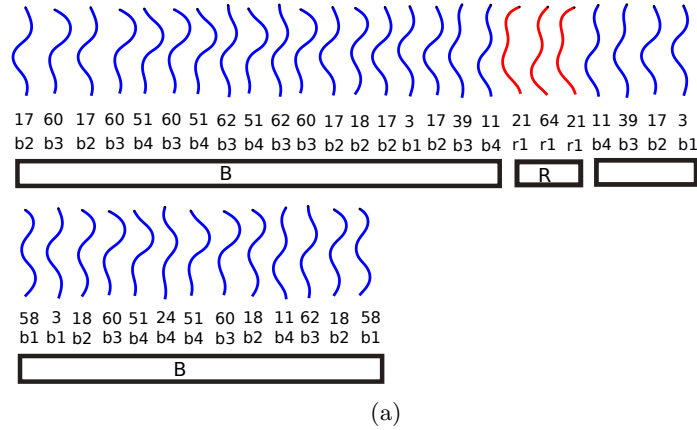
(a)
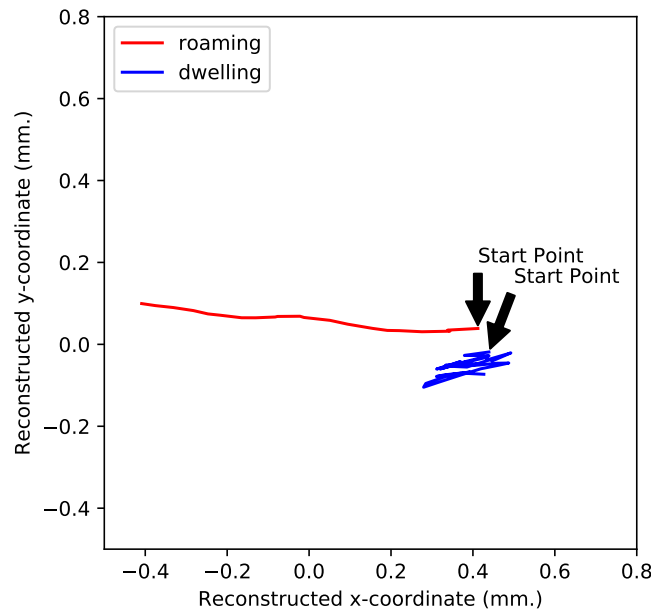
(b)

Figure 4.10: ***C. elegans* dwelling behavior can be arbitrarily long while still conforming to the grammatical constraints for dwelling.** (a): A sample dwelling sequence (rule of type BRB) taken from an actual foraging N2 worm. (b):Reconstructed trajectory of worm centroid for the posture sequence in (a), showing a relatively longer period of dwelling as compared to the trajectory shown in Figure 4.9c described by a similar type of grammatical rule. The corresponding roaming trajectory in red corresponds to application of 2 consecutive b1→g3 rules consisting of around 20 postures.

first order transition probabilities between the individual postures. The values of H_0^{10}, H_1^{10} and H_2^{10} (for the 10 sub-modules) were 3.32, 3.31 and 2.02 bits respectively, as compared to the H_0^{90}, H_1^{90} and H_2^{90} values of 6.49, 6.19 and 3.122 respectively for the 90 postures. The reduction in entropy values for the 10 sub-module scenario indicates that we can achieve greater predictability by dividing up the

90 posture into 10 sub-modules. Note that this analysis might appear a bit non-informative because the entropy is bound to decrease once you decrease the number of objects under consideration. To control for this, we calculated the unigram normalized perplexity (PPLu) scores (Roh et al., 2020) for the two vocabulary settings(one for 90 postures and one for the 10 sub-module scenario) taking into account the vocabulary sizes(see Methods chapter for details). The log (PPLu) value for bigram probabilities, calculated for each worm and the values then averaged over the total number of worms in the 90 posture scenario is -1.21 as compared to -3.03 in the 10 sub-module scenario indicating that there is a greater reduction in uncertainty (hence higher predictability) in the 10 sub-module scenario even after accounting for the lesser number of symbols in the 10 sub-module scenario. Therefore, the way in which we decompose the 90 postures into sub-modules, and then specify the dynamics characterizing interaction between them leads to functionally relevant worm behavior, thus indicating the significance of both the sub-modules and the dynamics operating on top them.

Thus, worm roaming and dwelling behavior can be described in terms of interaction rules between the 10 sub-modules, the interactions between which are in turn dictated by the interaction rules between three higher order chunks - B,R and G, leading to predictability and flexibility simultaneously and pointing to a hierarchical organization of behavior. Note that type 1 substitution comes into the picture when we view the whole worm foraging behavior from a level of description that comprise both roaming and dwelling behavior. At that level of description, there is type 1 substitution because the same sub-module, let's say b1 can be involved in roaming or dwelling (using roaming or dwelling specific rules) based on the context. At a higher level of abstraction, the chunk B can either be used during roaming with the invocation of the B→R→G sequence rule while the same B chunk can be used during dwelling through the B→G→B and other sequence rules. This is the essence of hierarchical organization where the transition probabilities between chunks does not remain constant, instead depends on the higher order unit which subsumes the chunks and sub-chunks. Similar argument holds for the 10 sub-chunks - b1 to g3 and finally for the 90 postures, with the same posture being used during roaming and dwelling phase with different probabilities. These roaming and dwelling specific interaction rules between chunks and sub-chunks give rise to a grammar of worm foraging.

Chapter 5: Computational Principles underlying *C. elegans* foraging

> In order to be a perfect and beautiful computing machine, it is not requisite to know what arithmetic is.

Daniel C. Dennett

In my opinion, one of the most important scientific achievements of 20th century science is Turing's idea of a universal computer : 'It is possible to invent a single machine which can be used to compute any computable sequence.' Like Darwin, he showed that one could get to higher order cognitive functions by recursively building up on mindless processes that have no smidgen of understanding in them (Dennett, 2017). I think that 'competence without comprehension' (Dennett, 2017) afforded by the idea of computation presents a promising direction in understanding the complexity of behavior exhibited by organisms - from worms to humans.

In this chapter, I show how worm foraging can be approximated by a context free grammar (CFG) that has particular import in the theory of computation. Specifically, CFGs can be recognized by non-deterministic push down automata that are more expressive than a finite state machine but less powerful than turing machines. Only future will tell if insights from the theory of computation can help us shed more light on the brain behavior nexus in worms and other animals.

5.1 A context free grammar for worm foraging behavior

The results obtained in the previous chapters point to the existence of grammatical rules governing *C. elegans* foraging behavior, where a grammar specifies rules for transforming higher order chunks into a sequence of postures. For example: roaming behavior can be transformed into B$\longrightarrow$R$\longrightarrow$G and so on to generate posture sequences that lead to roaming behavior.

One way to specify transformation rules is as shown in Equations 5.1 and 5.2, where each symbol on the left of the $\longrightarrow$ generates (is substituted) by the symbols on the right hand side of the $\longrightarrow$. Symbols that never occur to the left of any grammatical rule, in other words, those symbols that cannot generate any new symbols are known as terminal symbols. All the other symbols are known as non-terminal symbols. For example, the toy grammar in Equations 5.1 and 5.2 can produce strings of type $a^n b^n$, where $n = 1,2,3.......$

$$A \longrightarrow aAb \tag{5.1}$$

$$A \longrightarrow \epsilon \tag{5.2}$$

where A is a non-terminal symbol and a,b and ϵ (which denoted empty or null symbol) are the terminal symbols. A grammar generates multiple strings of terminal symbols, each of which is part of the language generated by the grammar. Thus, the strings, ab, aabb, aaabbb, are all part of the language generated by the grammar given by Equations 5.1 and 5.2.

Keeping these preliminaries in mind and combining them with the knowledge of roaming and dwelling gleaned from the previous chapters, we can formulate the following grammatical rules for *C. elegans* foraging. Each of the 90 postures serves as a terminal symbol and all the other symbols are the non-terminal symbols.

$$Roam \longrightarrow B_{NRG} \mid R_{NGB} \mid G_{NBR} \tag{5.3}$$

$$B_{NRG} \longrightarrow b_{1_u} R_{NG_p} \tag{5.4}$$

$$b_{1_u} \longrightarrow P_{b_1} b_{2_u} \tag{5.5}$$

$$b_{2_u} \longrightarrow P_{b_2} b_{3_u} \tag{5.6}$$

$$b_{3_u} \longrightarrow P_{b_3} b_{4_u} \tag{5.7}$$

$$b_{4_u} \longrightarrow P_{b_4} \tag{5.8}$$

$$R_{NG_p} \longrightarrow r_{1_u} g_{1_u} \tag{5.9}$$

$$r_{1_u} \longrightarrow P_{r_1} r_{2_u} \tag{5.10}$$

$$r_{2_u} \longrightarrow P_{r_2} r_{3_u} \tag{5.11}$$

$$r_{3_u} \longrightarrow P_{r_3} \tag{5.12}$$

$$g_{1_u} \longrightarrow P_{g_1} g_{2_u} \tag{5.13}$$

$$g_{2_u} \longrightarrow P_{g_2} g_{3_u} \tag{5.14}$$

$$g_{3_u} \longrightarrow P_{g_3} \mid P_{g_3} B_{NRG} \mid G_{(NBG)_2} \mid G_{(NRG)_1} \tag{5.15}$$

$$Dwell \longrightarrow B_{(NRB)_1} \mid B_{(NRB)_2} \mid B_{(NGB)_1} \mid B_{(NGB)_2} \mid R_{(NGR)_1} \mid R_{(NGR)_2} \tag{5.16}$$

$$Dwell \longrightarrow R_{(NBR)_1} \mid R_{(NBR)_2} \mid G_{(NBG)_1} \mid G_{(NBG)_2} \mid G_{(NRG)_1} \mid G_{(NRG)_2} \tag{5.17}$$

$$B_{(NRB)_1} \longrightarrow b_{1_a} R_{NB_1} \tag{5.18}$$

$$B_{(NRB)_2} \longrightarrow b_{4_a} R_{NB_1} \tag{5.19}$$

$$b_{1_a} \longrightarrow P_{b_1} b_{1_a} \mid P_{b_1} b_{2_a} \tag{5.20}$$

$$b_{2_a} \longrightarrow P_{b_2} b_{1_a} \mid P_{b_2} b_{3_a} \mid P_{b_2} b_{2_a} \tag{5.21}$$

$$b_{3_a} \longrightarrow P_{b_3} b_{2_a} \mid P_{b_3} b_{4_a} \mid P_{b_3} b_{3_a} \tag{5.22}$$

$$b_{4_a} \longrightarrow P_{b_4} b_{3_a} \mid P_{b_4} \mid P_{b_4} b_{4_a} \tag{5.23}$$

$$R_{NB_1} \longrightarrow r_{1_a} b_{4_h} \tag{5.24}$$

$$r_{1_a} \longrightarrow P_{r_1} r_{1_a} \mid P_{r_1} r_{2_a} \mid P_{r_1} \tag{5.25}$$

$$r_{2_a} \longrightarrow P_{r_2} r_{1_a} \mid P_{r_2} r_{3_a} \mid P_{r_2} r_{2_a} \tag{5.26}$$

$$r_{3_a} \longrightarrow P_{r_3} r_{2_a} \mid P_{r_3} r_{3_a} \tag{5.27}$$

$$b_{1_h} \longrightarrow P_{b_1} b_{1_h} \mid P_{b_1} b_{2_h} \mid B_{(NRB)_1} \mid B_{(NGB)_2} \mid B_{(NRG)} \mid P_{b_1} \tag{5.28}$$

$$b_{2_h} \longrightarrow P_{b_2} b_{1_h} \mid P_{b_2} b_{3_h} \mid P_{b_2} b_{2_h} \mid P_{b_2} \tag{5.29}$$

$$b_{3_h} \longrightarrow P_{b_3} b_{2_h} \mid P_{b_3} b_{4_h} \mid P_{b_3} b_{3_h} \mid P_{b_3} \tag{5.30}$$

$$b_{4_h} \longrightarrow P_{b_4} b_{3_h} \mid P_{b_4} b_{4_h} \mid B_{(NGB)_1} \mid B_{(NRB)_2} \mid P_{b_4} \tag{5.31}$$

$$B_{(NGB)_1} \longrightarrow b_{4_c} G_{NB_2} \tag{5.32}$$

$$B_{(NGB)_2} \longrightarrow b_{1_c} G_{NB_2} \tag{5.33}$$

$$b_{1_c} \longrightarrow P_{b_1} b_{1_c} \mid P_{b_1} b_{2_c} \mid P_{b_1} \tag{5.34}$$

$$b_{2_c} \longrightarrow P_{b_2} b_{1_c} \mid P_{b_2} b_{3_c} \mid P_{b_2} b_{2_c} \tag{5.35}$$

$$b_{3_c} \longrightarrow P_{b_3} b_{2_c} \mid P_{b_3} b_{4_c} \mid P_{b_3} b_{3_c} \tag{5.36}$$

$$b_{4_c} \longrightarrow P_{b_4} b_{3_c} \mid P_{b_4} b_{4_c} \tag{5.37}$$

$$G_{NB_2} \longrightarrow g_{3_c} b_{1_i} \tag{5.38}$$

$$g_{1_c} \longrightarrow P_{g_1} g_{1_c} \mid P_{g_1} g_{2_c} \tag{5.39}$$

$$g_{2_c} \longrightarrow P_{g_2} g_{1_c} \mid P_{g_2} g_{3_c} \mid P_{g_2} g_{2_c} \tag{5.40}$$

$$g_{3_c} \longrightarrow P_{g3} g_{2_c} \mid P_{g3} g_{3_c} \mid P_{g3} \tag{5.41}$$

$$b_{1_i} \longrightarrow P_{b_1} b_{1_i} \mid P_{b_1} b_{2_i} \mid B_{(NRB)_1} \mid B_{(NGB)_2} \mid B_{(NRG)} \mid P_{b_1} \tag{5.42}$$

$$b_{2_i} \longrightarrow P_{b_2} b_{1_i} \mid P_{b_2} b_{3_i} \mid P_{b_2} b_{2_i} \mid P_{b_2} \tag{5.43}$$

$$b_{3_i} \longrightarrow P_{b_3} b_{2_i} \mid P_{b_3} b_{4_i} \mid P_{b_3} b_{3_i} \mid P_{b_3} \tag{5.44}$$

$$b_{4_i} \longrightarrow P_{b_4} b_{3_i} \mid P_{b_4} b_{4_i} \mid B_{(NRB)_2} \mid B_{(NGB)_1} \mid P_{b_4} \tag{5.45}$$

$$P_{b_i} \longrightarrow \{Postures\ in\ subcluster\ b_i\} \mid \epsilon \ ; i = \{1, 2, 3, 4\} \tag{5.46}$$

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$$P_{r_i} \longrightarrow \{Postures\ in\ subcluster\ r_i\} \mid \epsilon\ ; i = \{1, 2, 3\} \tag{5.47}$$

$$P_{g_i} \longrightarrow \{Postures\ in\ subcluster\ g_i\} \mid \epsilon\ ; i = \{1, 2, 3\} \tag{5.48}$$

where $\epsilon = \{\}$, an empty symbol. Figure 5.1 shows how the grammatical rules corresponding to Equations 5.3 to 5.15 are instantiated to generate a posture sequence that implements worm roaming behavior (posture sequence 58,18,42,24,50,19,46,10,81,26). The symbol '|' stands for the logical OR operator denoting choice in the expansion of non-terminal symbols in a grammatical rule. For example - Equation 5.21 denotes that b_{2_a} can either be substituted by P_{b_2} followed by b_{1_a} or P_{b_2} followed by b_{3_a} or P_{b_2} followed by b_{2_a}. Figure 5.1 shows the instantiation of a roaming postural sequence through the B_{NRG} (B→R→G) grammatical rule, but similar grammatical rules can be made for R_{NGB} (R→G→B) and G_{NBR} (G→B→R), all of which are known to generate roaming behavior. Equation 5.15 also depicts that after completing one bout of forward locomotion through the b1→g3 rule, the worm can switch to dwelling by entering the G→R→G rule (either $G_{(NRG)_1}$ or $G_{(NRG)_1}$). Note that Equation 5.16 denotes that one way to achieve dwelling behavior is through the behavioral unit B→R→B ($B_{(NRB)_1}$ and $B_{(NRB)_2}$). Once inside the BRB unit, the grammatical rules that follow lay the groundwork for generating dwelling behavior through B→R→B unit, i.e - the rules corresponding to Equations 5.18 to 5.48. The grammatical rules corresponding to other units like R→G→R, R→B→R, G→B→G and G→R→G can be generated in a similar manner. The assignment of individual postures to sub-clusters is done according to sub-cluster identities defined in Figure 3.6. For example

$$P_{b_1} \longrightarrow \{3, 20, 27, 41, 55, 58, 68, 76\} \tag{5.49}$$

which means that whenever the pre-terminal symbol P_{b_1} is encountered, it is replaced by any of the postures (terminal symbols in our case) contained in the set of postures given by {3,20,27,41,55,58,68,76}. The grammatical rules described above constitute a context free grammar (Chomsky, 1965), whose underlying structure is hierarchical. This is because in a context free grammar, a non terminal symbol on the left side can transform into (or generate) more than one non terminal symbols (for example in the rules 5.4 and 5.18 in the above grammar). And each non-terminal on the right hand side gets transformed into sequence of terminal symbols, rather than only a single terminal symbol.
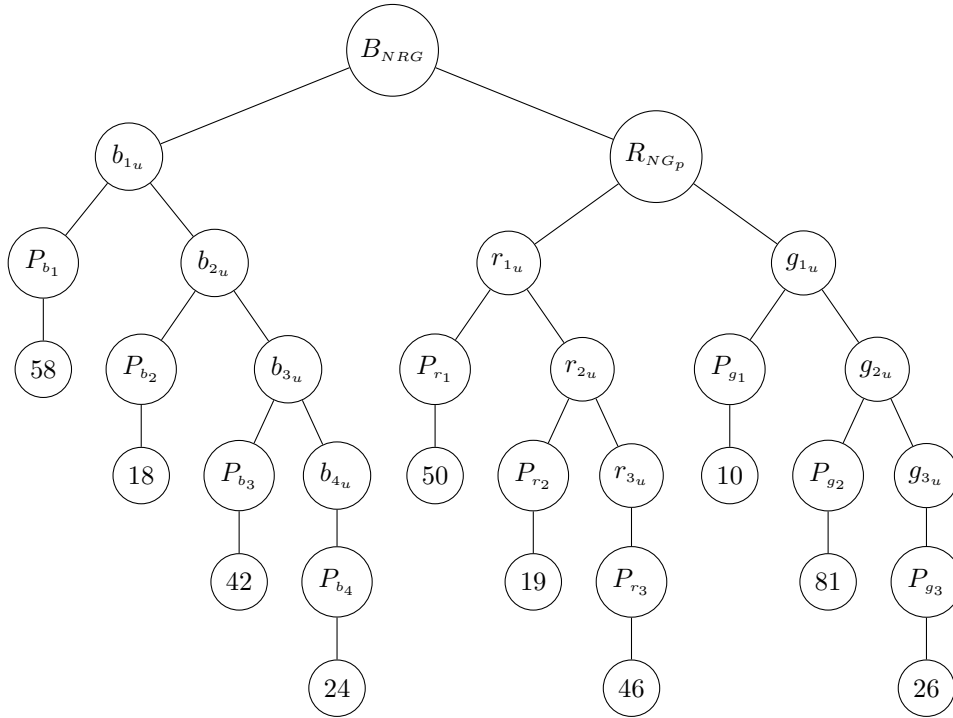
Figure 5.1: **Roaming behavior can be generated by the application of specific grammatical rules.** Rules corresponding to Equations 5.3 to 5.15 can generate the posture sequence 58,18,42,24,50,19,46,10,81,26, that generates worm roaming behavior as shown in Figure 4.2h. B_{NRG}, b_{1_u}, b_{1_u}, b_{1_u}, b_{1_u},b_{2_u},b_{3_u},b_{4_u},$r_{1_u}r_{2_u}$, r_{3_u}, g_{1_u},g_{2_u}, g_{3_u} serve as non-terminal symbols while each of the postures in the sequence 58,18,42,24,50,19,46,10,81,26 serve as terminal symbols in the grammar. The grammar provides a way to transform non-terminal symbols into sequence of terminal symbols according to set of production (grammatical) rules.

In contrast linear systems based on the markovian assumption, for example - hidden markov models (HMMs) approximate a regular grammar (Chomsky, 1965), where each non-terminal symbol on the left hand side of a rule does not get transformed into more than one non-terminal symbol on the right hand side. For example, if we were to consider a grammar for worm foraging that consisted only of the following grammatical rules, then we would have a regular grammar. Note that we have only specified the grammatical rules for B cluster and not shown the rules for the R and G cluster that can be made in a similar manner as below.

$$b_1 \longrightarrow P_{b_1}b_1 \mid P_{b_1}b_2 \tag{5.50}$$

$$b_2 \longrightarrow P_{b_2}b_1 \mid P_{b_2}b_3 \mid P_{b_2}b_2 \tag{5.51}$$
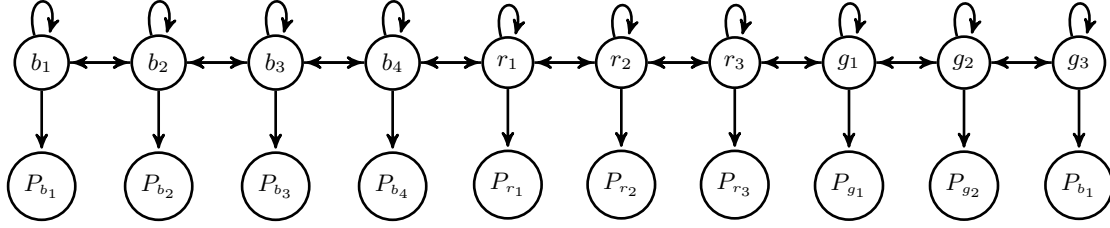
Figure 5.2: **HMMs approximate regular grammars by defining a linear relationship between hidden variables that account for actual observations like worm postures.** The transition rules between the hidden variables (sub-chunks b_i, r_i and g_i) remain the same, whether the worm is roaming or dwelling, which is not what is seen in worm behavior. When roaming, the transition relationship between hidden variables is different from the one used by the worm during dwelling, as exemplified by the grammatical rules defined above.

$$b_3 \longrightarrow P_{b_3} b_2 \mid P_{b_3} b_4 \mid P_{b_3} b_3 \tag{5.52}$$

$$b_4 \longrightarrow P_{b_4} b_3 \mid P_{b_4} \mid P_{b_4} b_4 \tag{5.53}$$

$$P_{b_i} \longrightarrow \{Postures\ in\ subcluster\ b_i\} \mid \epsilon\ ; i = \{1, 2, 3, 4\} \tag{5.54}$$

Note that $P_{b_1}, P_{b_2}, P_{b_3}$ and P_{b_4} are not actually considered as non-terminal symbols, but rather pre-terminal symbols as they only generate terminal symbols (Equation 5.46) with no associated non-terminal symbols and are only ever used in the same way. An HMM can be considered as a stochastic version of a regular grammar (Equations 5.50 to 5.54) as shown in Figure 5.2, where the non-terminal symbols b_1, b_2, b_3 and b_4 correspond to hidden states and each hidden state generates a posture with some associated probabilities and transitions to another hidden state. For example, rule given by Equation 5.51 says that the worm in hidden state b_2 generates any posture belonging to P_{b_2} (according to some probability distribution) and then the worm transitions to the hidden state b_1 or b_3 or stays in the same hidden state, based on certain probabilities. Note that in this conception, there is no memory in the system, that is, there would be no difference in the transition probabilities between b_1, b_2 etc. depending on whether the worm is roaming or dwelling. However, we know that when the worm is roaming the probability of the worm transitioning from b_2 to b_1 is very close to 0 and the probability of transitioning from b_2 to b_3 is close to 1. And the worm

follows different transition probabilities between the the 10 sub-clusters when in dwelling phase. This is the problem with regular grammars and by extension HMMs, where they fail to account for structure that is hierarchical and only capture linear relationships. On the other hand, a context free grammar gives a better account of worm behavior because the identity of b_1 and hence the transition rules that it participates in, is intimately tied to the higher order chunk that it belongs to. For example if b_1 is instantiated as b_{1_a} (Equation 5.20), we know that the worm is dwelling according to $B_{(NRB)_1}$ (B→R→B unit) rule (Equation 5.18) and the transitions between the sub-clusters is governed according to Equations 5.18 to 5.31. Whereas if b_1 is instantiated as b_{1_u} (Equation 5.5), we know that the worm is in roaming state - B_{NRG} (B→R→G unit) (Equation 5.4) and hence the transitions are different and are governed by rules given by Equations 5.4 to 5.15. This gives a hierarchical conception of behavior where the transition between lower level elements (whether sub-clusters or individual postures) is not fixed (like in markov models), but rather where the identity of the higher order chunk that the organism is in, governs the transition patterns (rules) between the chunks below (Fentress and Stilwell, 1973). Thus, a generative grammar provides a scaffolding for worm behavior, indicating sequences that can and cannot be generated by the worm.

Chapter 6: Bridging the gap between worm behavioral grammar and genes as well as environment

> Reductionism is a dirty word, and a kind of 'holistier than thou' selfrighteousness has become fashionable.

Richard Dawkins

I want to begin this chapter by extolling the virtues of reductionism as I see them and how I think we cannot escape reductionism in our quest to understand brain, behavior and everything in between, below and above. I might very well be wrong in my understanding, but in science, one often is.

Contrasting good reductionism from greedy reductionism as in (Dennett, 1996), I want to briefly point out the virtues of the former. As a case study, let us take a look at neural networks that are a rage right now. Neural networks have once again emerged from wilderness and many neuroscientists are trying to use them to better understand brain function. Now, this research might well turn out to be a dead end but at the moment, it does offer opportunities that presumably many smart neuroscientists do not want to miss out on. Looking back at the history of these computational devices, I think reductionism has had a rather venerable role in their existence. For every McCulloch and Pitts, there was a Santiago Ramón y Cajal and many other neuroscientists who gave McCulloch and Pitts ideas to model their computational devices on. For every Kunihiko Fukushima and Yann LeCun, there were neuroscientists like Hubel and Wiesel who gave Fukushima and Yann LeCun the idea of using receptive fields in their models. The advancements in models came directly through the work done using reductionist philosophy prevalent in biology.

I think that when dealing with complex problems like the brain behavior nexus, we generally do not know the privileged level to solve the problem apriori (there might not be any privileged level in the first place), and attacking the problem from various levels with an aim towards unifying knowledge gained from these various levels is our best bet. In the process, the knowledge gained from either a reductionist philosophy or a more abstract computational philosophy might be able to inform each other in a constructive manner, like the examples given above.

Motivated by a desire to unify knowledge across various levels of inquiry, in this chapter, I relate the computational principles of worm behavior that I discovered in the previous chapters, with what is known about the genetic basis of worm behavior.

6.1 Worm foraging grammar reveals hitherto uncharacterized role of *npr-3* and *npr-10* genes in affecting foraging behavior

We next asked if the discovered worm foraging grammar can give insights into the molecular mechanisms underlying foraging. Neuropeptides and their receptors have been shown previously to affect foraging behavior. *npr-1(ad609)* mutants show increased roaming behavior in the presence of food (Reddy et al., 2011; Gloria-Soria and Azevedo, 2008; De Bono and Bargmann, 1998; Cheung et al., 2004), while *npr-9(tm1652)* mutants show impaired roaming behavior on food (Bendena et al., 2008). We first sought to confirm if our proposed worm grammar for roaming and dwelling can replicate these findings. As shown in Figure 6.1a, we found that the total time spent by *npr-1* mutants in roaming type grammatical rules is significantly higher than wild type N2 worms. Furthermore, the amount of time spent by *npr-9* mutants in roaming type rule is significantly lower than N2 worms (Figure 6.1b). Note that the time spent in roaming type rules is the time spent in all rules of type b1⟶g3 in a worm whereas the time spent in dwelling type rules is computed as the combined time spent in Dwell 1, Dwell 2 and Dwell 3 type rules. For each worm, total amount of time spent in all roaming and dwelling type rules is used to calculate the proportion of total time spent roaming and dwelling.

Building on these findings, we next sought to find if the worm grammar can help implicate hitherto uncharacterized genes that affect worm foraging behavior. We found that *npr-3(tm1583)* mutants show significantly increased propensity to roam (Figure 6.1c) whereas *npr-10(tm1568)* mutants show an opposite tendency to dwell (Figure 6.1d), as compared to N2 worms. It should be noted that not all neuropeptides and their receptors show significant differences in their foraging patterns as compared to N2 worms. For example, we found no significant difference in roaming and dwelling between *npr-13* mutants and N2 worms (Figure 6.1e).

Finally, we also looked at more than 300 worm strains and isolated those strains that differ significantly (p value < 0.001, Welch's t-test accompanied with a large effect size, cohen's d > 0.8) from N2 worms in the time spent in roaming and dwelling states as determined by the grammatical rules (Figure 6.1f). Yellow values (exact values equal to cohen's d statistic) indicate the behavior type in which the time spent by the corresponding mutant strain (on the y axis) is considerably

higher as compared to N2 worms and vice versa for dark blue values. We can see that apart from the mutant strains belonging to the neuropeptide class elucidated above, *egl-30* mutants as well as mutants belonging to the mec class (*mec-14*, *mec-10*, *mec-12* and *mec-7*) show strongly different foraging patterns than N2 worms. Mutation in *egl-30(ep271)* (known to be involved in chemosensory behavior and locomotion) leads to worms spending considerably more time in roaming than N2 worms whereas mutation in *mec-14(u55)* (known to be involved in mechanosensory behavior) results in worms spending considerably more time in dwelling than N2 worms.

Prior work (Flavell et al., 2013) has also implicated *mod-1* gene in regulating roaming behavior in worms, however we did not find any significant difference in the foraging patterns between *mod-1* mutants and N2 worms, according to our proposed grammatical rules. We think that this could be because of the difference in experimental conditions, for example - the data we use involves a habituation period of 30 minutes before the actual worm tracking begins. This habituation period is absent in the study above. We must also note that the previous study (Bendena et al., 2008) that found that *npr-9* mutants have impaired roaming behavior, a result that we also find using our behavioral grammar, does involve a habituation period of 30-60 minutes, thus making their experimental conditions more similar to the experimental conditions of the behavioral data that we use.

These findings demonstrate that organization principles encapsulated by the proposed grammatical rules capture essential aspects of worm foraging in a way that can end up distinguishing between worm strains based on behavior. Moreover, the importance of delineating such organization principles is highlighted by their ability to elucidate novel molecular mechanisms important for regulating worm foraging behavior.

(a) *npr-1* timing comparison with N2 (N(*npr-1*) = 12, N(N2) = 44)

(b) *npr-9* timing comparison with N2 (N(*npr-9*) = 28, N(N2) = 44)

(c) *npr-3* timing comparison with N2 (N(*npr-3*) = 20, N(N2) = 61)

(d) *npr-10* timing comparison with N2 (N(*npr-10*) = 21, N(N2) = 44)

(e) *npr-13* timing comparison with N2 (N(*npr-13*) = 29, N(N2) = 61)

(f)
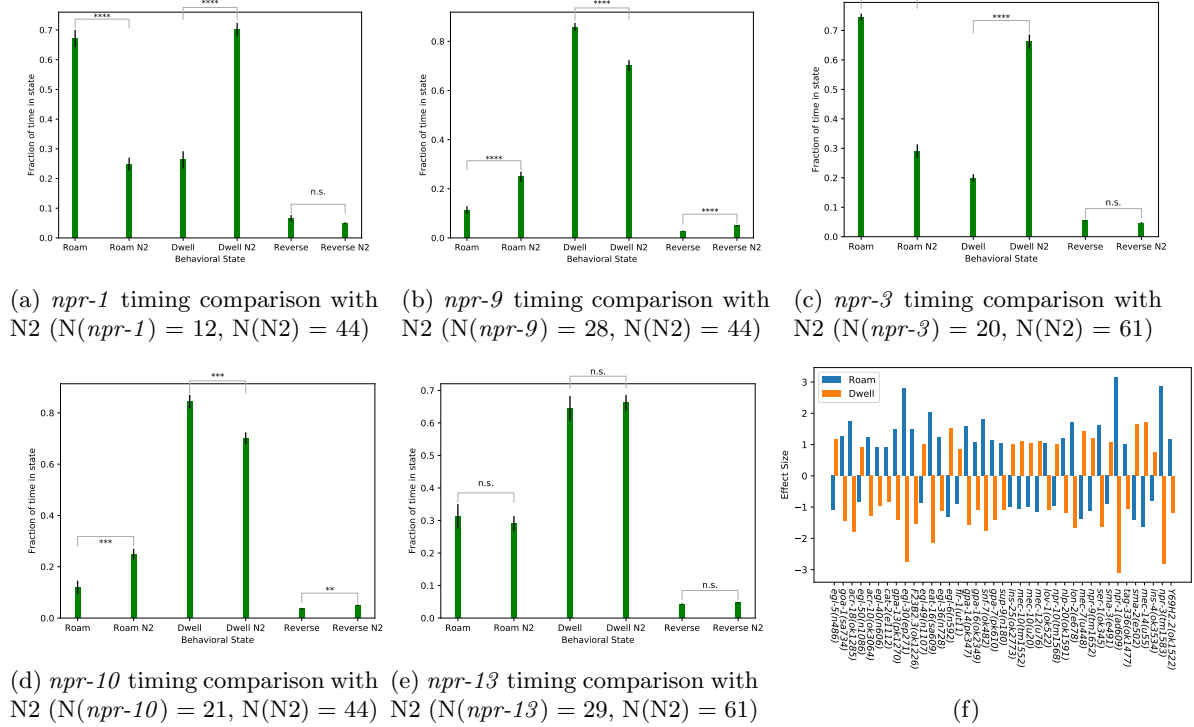
Figure 6.1: **Time spent in the grammatical rules underlying roaming and dwelling implicates novel genes underlying *C. elegans* foraging behavior**. (a): Fraction of total time spent in roaming and dwelling type rules in *npr-1* mutants. d(roam(*npr-1*),roam(N2)) = 3.14 ; d(dwell(*npr-1*),dwell(N2)) = -3.08. (b):Fraction of total time spent in roaming and dwelling type rules in *npr-9* mutants. d(roam(*npr-9*),roam(N2)) = -1.1 ; d(dwell(*npr-9*),dwell(N2)) = 1.2. (c): *npr-3* mutants spend significantly higher time roaming as compared to N2 worms. d(roam(*npr-3*),roam(N2)) = 2.84 ; d(dwell(*npr-3*),dwell(N2)) = -2.79. (d): *npr-10* mutants spend significantly lower time roaming as compared to N2 worms. d(roam(*npr-9*),roam(N2)) = -0.95 ; d(dwell(*npr-9*),dwell(N2)) = 1.00. (e): *npr-13* mutants don't differ in the time they spend in roaming and dwelling states as compared to N2 worms. (f): Mutant strains that differ from N2 worms significantly (p value (Welch's t-test) < 0.001 and |cohen's d| > 0.8) in the time spent roaming and dwelling as given by the rules of worm foraging grammar. Colors in each cell are used to indicate the actual effect size value (cohen's d) for the comparison between the time spent in either roaming or dwelling states for each of the mutant strains and N2 worms. Yellow values indicate the behavior type in which the time spent by the mutant strain is considerably higher as compared to N2 worms and vice versa for dark blue values. (a) to (e): All results shown as mean ± s.e.m. across all the worms per strain. N values denote the number of worms considered for each mutant as well as N2 worms. **** = p < 0.0001, *** = p < 0.001, n.s. = not significant, Welch's t-test; effect size: d = cohen's d

6.2 Relation between proposed grammatical rules and change in environment

As shown in Figure 6.2a, we found that the amount of time spent by N2 worms (denoted as N2-) in roaming type rules is considerably higher than the time spent in dwelling type rules, in off food conditions. Also shown in the same Figure is the complete opposite behavior shown by N2 worms on food (denoted by N2+), where the amount of time spent dwelling is considerably higher than the time spent roaming.

A complementary way to visualize the difference between N2 worms' behavior on food vs off food is shown in Figure 6.2b. For all the different lengths of posture sequences corresponding to worm behavior off food, the proportion of the roaming type rules (b1⟶g3) always exceeds that of the dwelling type rules (except for one worm whose posture sequence length is very low, approximately 100). Note that this is in sharp contrast to Figure 4.7a depicting the behavior of N2 worms on food, where the proportion of roaming type rules remains in the range of 0.2 even when the length of the posture sequence exceeds 1000. This contrast exhibits how change in environment leads to a complete change in behavior and highlights how the proposed worm foraging grammar can account for such effects of environment on worm behavior.

Further, the average speed during roaming type rules across all worms, in off food conditions, is significantly greater than the speed during dwelling type rules (Figure 6.2c) but the total time spent in roaming type rules does not differ from the time spent in Dwell 1 and Dwell 3 type rules (Figure 6.2d). This suggests that during the dispersal (roaming) period, the worm reorients itself for a very short time to change its direction (hence the low speed and relatively low time spent in dwelling type rules), to again continue with its roaming behavior.
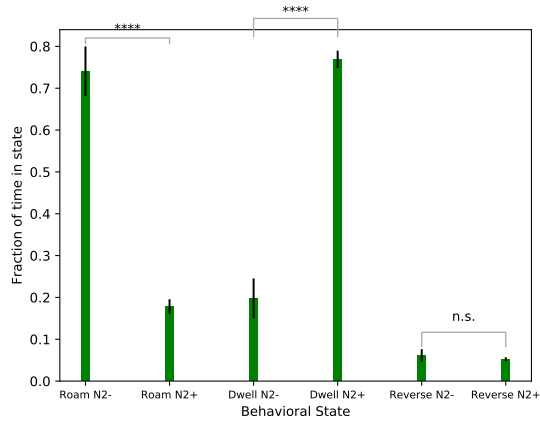
(a) off vs on food timing comparison N(N2 -) = 16, N(N2 +) = 30
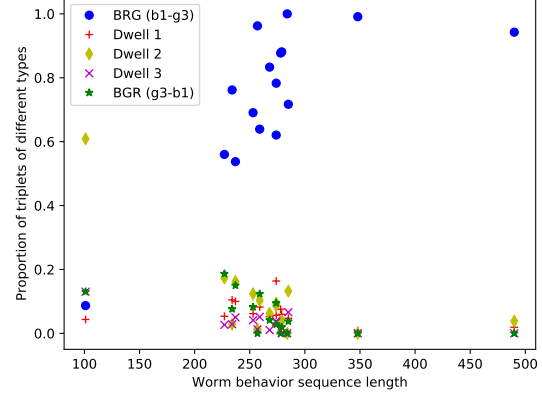
(b) N2 off food (N = 16 worms)
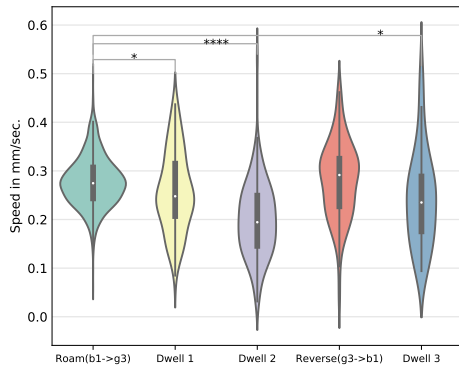
(c) N2 worms off food - Speed
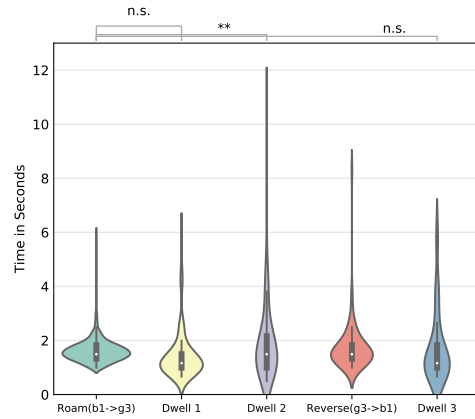
(d) N2 worms off food - timings.

Figure 6.2: **Relation between grammatical rules underlying worm foraging and environmental changes.** (a): Fraction of total time spent in roaming and dwelling type rules by N2 worms in off food (denoted by -) vs on food (denoted by +) environments. d(roam(N2 -),roam(N2 +)) = 3.56 ; d(dwell(N2 -),dwell(N2 +)) = -3.94. Results shown as mean $\pm$ s.e.m. across all the worms per strain. (b) The proportion of roaming type behavioral rule increases as a function of postural sequence length in both N2 worms off food, as in the on-food condition. (c) Violin plots showing the distribution of average speeds across all the instantiations of all types of rules across all the worms. d(roam,dwell1) = 0.36, d(roam,dwell2) = 1.44, d(roam,dwell3) = 0.64. N(Roam)= 1217, N(Dwell 1)= 92, N(Dwell 2)= 120, N(Reverse)= 81, N(Dwell 3)= 39. (d) Violin plots showing the distribution of times taken to complete individual instantiations of all the behavioral rules across all the worms. d(roam,dwell2) = -0.71. N values same as in (c). **** = $p < 0.0001$, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, Welch's t-test; effect size: d = cohen's d. Violin Plots contain box plots that show the interquartile range.

6.3 The relational properties between the various behavioral rules remain qualitatively invariant across different mutant strains on food

We then looked at the properties of roaming and dwelling rules derived from N2 and wild isolates, in the various mutant strains. Following the division of various mutant strains into classes as described in (Brown et al., 2013), we found that the roaming and dwelling rules have qualitatively similar properties in all the mutant strains. Specifically, the relationship between the usage frequency of various kinds of rules with the behavioral sequence length follows the same pattern as in the wild isolates and N2 worms. Proportion of roaming type rules increases as a function of postural sequence length and vice versa for dwelling type rules (Dwell 2) for all the mutant strain classes like DEG/ENaC, unc, Trpc etc. (Figure 6.3).

(a) Acetylcholine Receptor(268 worms)   (b) Deg/Enac Channel(581 worms)   (c) Egl(969 worms)

(d) G-protein Related(741 worms)   (e) Monoamine Related(740 worms)   (f) Neuropeptide(881 worms)

(g) Trpc(373 worms)   (h) Unc(1412 worms)
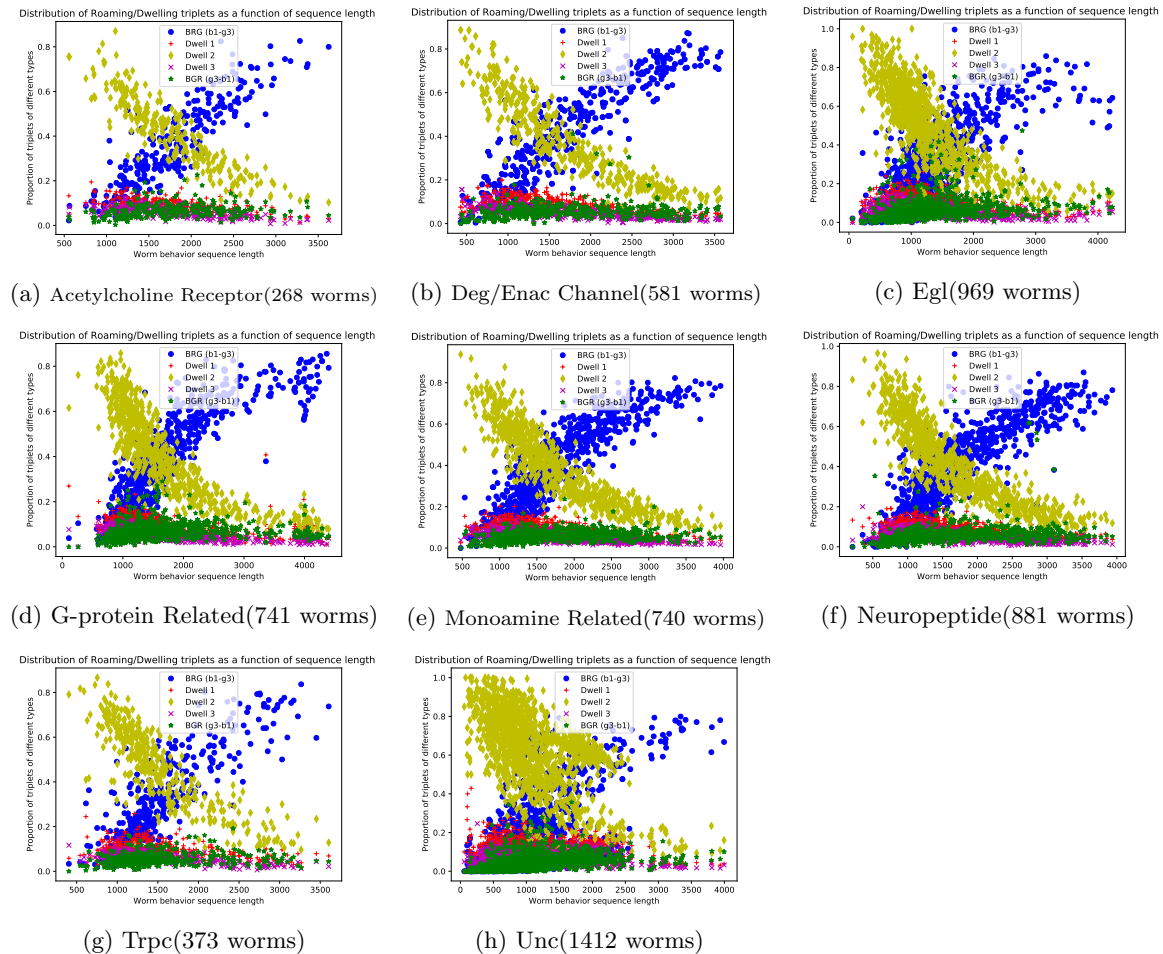
Figure 6.3: **Similar usage frequency characterize the roaming and dwelling rules in different mutant strain classes as in the N2 and wild isolate worms.**

As with N2 and wild isolate worms, we next found that the speed of the worm centroid for all the mutant strain classes during (Figure 6.4) the roaming type rules is significantly greater than that during dwelling type rules. These results suggest that the meaning of the rules (roaming specific vs dwelling specific) remains unchanged across mutant strain classes, with roaming specific rules having properties different from dwelling specific rules in precisely the manner as would be expected from the functional properties of roaming and dwelling worm behavior.

(a) Acetylcholine Receptor(268 worms)

(b) Deg/Enac Channel(581 worms)

(c) Egl(969 worms)

(d) G-protein Related(741 worms)

(e) Monoamine Related(740 worms)

(f) Neuropeptide(881 worms)

(g) Trpc(373 worms)

(h) Unc(1412 worms)
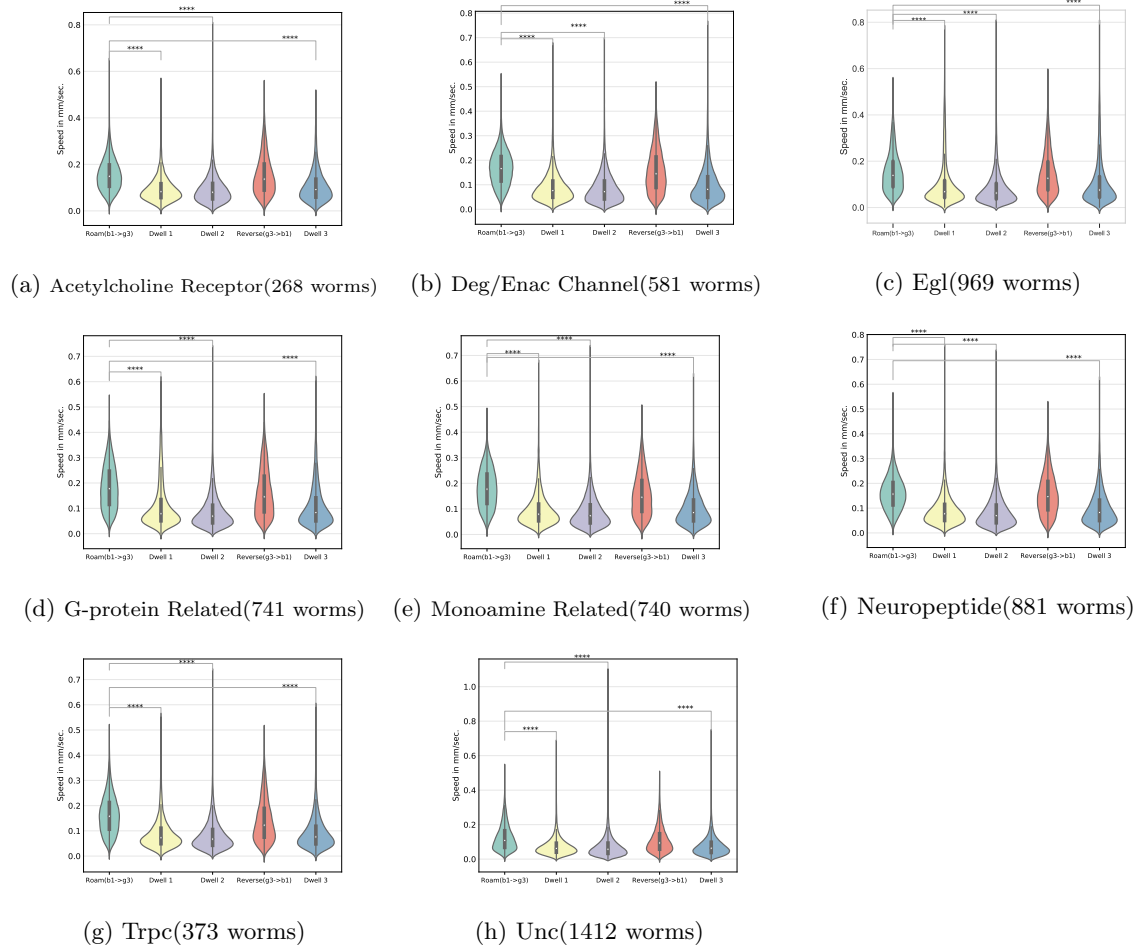
Figure 6.4: **The speed associated with the roaming type rules is much greater than the speed associated with dwelling type rules as expected, for all the mutant strain classes.** Average speed of the worm centroid position during instantiations of the roaming type rule is considerably higher than during the behavior rules corresponding to dwelling. Violin plots showing the distribution of average speeds during each instantiation of all types of rules across all the worms per mutant strain class. (a) d(roam,dwell1) = 0.94, d(roam,dwell2) = 1.02, d(roam,dwell3) = 0.72 . N(Roam)= 62178, N(Dwell 1)= 12898, N(Dwell 2)= 53980, N(Reverse)= 10122, N(Dwell 3)= 8242. (b) d(roam,dwell1) = 1.13, d(roam,dwell2) = 1.22, d(roam,dwell3) = 0.97. N(Roam)= 154215, N(Dwell 1)= 22534, N(Dwell 2)= 94293, N(Reverse)= 17699, N(Dwell 3)= 12847. (c) d(roam,dwell1) = 0.58, d(roam,dwell2) = 0.939, d(roam,dwell3) = 0.529 . N(Roam)= 145805, N(Dwell 1)= 40371, N(Dwell 2)= 181788, N(Reverse)= 30975, N(Dwell 3)= 24929. (d) d(roam,dwell1) = 0.85, d(roam,dwell2) = 1.25, d(roam,dwell3) = 0.88. N(Roam)= 177213, N(Dwell 1)= 33080, N(Dwell 2)= 132831, N(Reverse)= 28067, N(Dwell 3)= 19713. (e) d(roam,dwell1) = 1.15, d(roam,dwell2) = 1.34, d(roam,dwell3) = 1.05. N(Roam)= 194924, N(Dwell 1)= 32882, N(Dwell 2)= 139543, N(Reverse)= 25179, N(Dwell 3)= 20196. (f) d(roam,dwell1) = 1.08, d(roam,dwell2) = 1.2, d(roam,dwell3) = 0.92. N(Roam)= 216351, N(Dwell 1)= 36003, N(Dwell 2)= 157452, N(Reverse)= 29545, N(Dwell 3)= 21268. (g) d(roam,dwell1) = 0.995, d(roam,dwell2) = 1.23, d(roam,dwell3) = 0.96. N(Roam)= 70560, N(Dwell 1)= 17428, N(Dwell 2)= 73622, N(Reverse)= 10557, N(Dwell 3)= 9873. (h) d(roam,dwell1) = 0.76, d(roam,dwell2) = 0.8, d(roam,dwell3) = 0.69. N(Roam)= 109010, N(Dwell 1)= 58221, N(Dwell 2)= 295363, N(Reverse)= 27357, N(Dwell 3)= 33939. **** = p < 0.0001, Welch's t-test; effect size: d = cohen's d. Violin Plots contain box plots that show the interquartile range.

Further, the total time spent in roaming type rules across all worms of a particular class is significantly lesser than that spent in the dwelling type rules (Figure 6.5).

(a) Acetylcholine Receptor(268 worms) (b) Deg/Enac Channel(581 worms) (c) Egl(969 worms)

(d) G-protein Related(741 worms) (e) Monoamine Related(740 worms) (f) Neuropeptide(881 worms)

(g) Trpc(373 worms) (h) Unc(1412 worms)
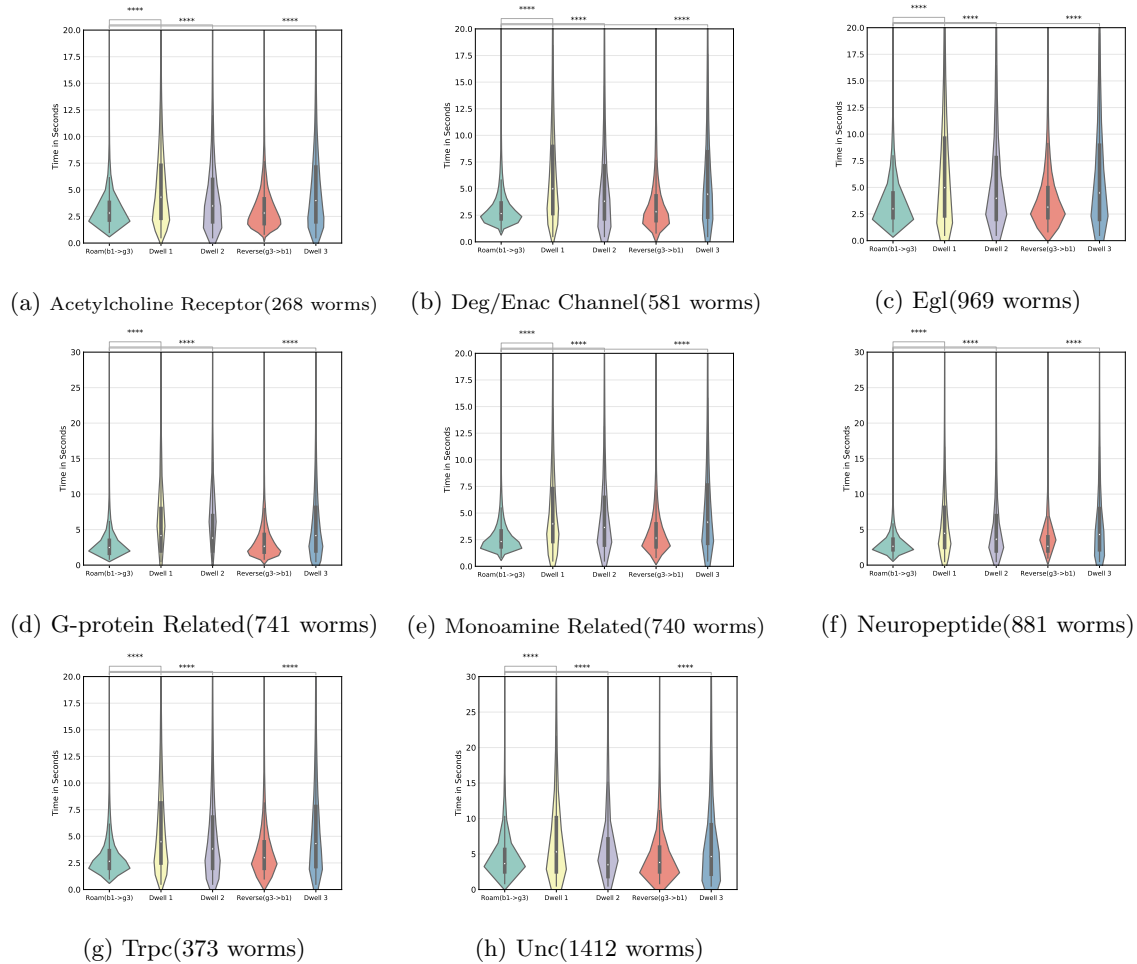
Figure 6.5: **Time spent during roaming type rules is lesser than the time spent during the dwelling type rules in all the mutant strain classes.** Total time spent by the worm during all instantiations of various grammatical rules. Violin plots show the distribution of times taken to complete each instantiation of all the behavioral rules across all the worms per mutant train class. Time spent during roaming type rules is much smaller than that spent during dwelling type rules. (a) d(roam,dwell1) = -0.89, d(roam,dwell2) = -0.43, d(roam,dwell3) = -0.86. (b) d(roam,dwell1) = -1.21, d(roam,dwell2) = -0.56, d(roam,dwell3) = -1.21. (c) d(roam,dwell1) = -0.74, d(roam,dwell2) = -0.39, d(roam,dwell3) = -0.78. (d) d(roam,dwell1) = -0.81, d(roam,dwell2) = -0.568, d(roam,dwell3) = -1.14. (e) d(roam,dwell1) = -1.09, d(roam,dwell2) = -0.6, d(roam,dwell3) = -1.26. (f) d(roam,dwell1) = -1.05, d(roam,dwell2) = -0.528, d(roam,dwell3) = -1.09. (g) d(roam,dwell1) = -0.97, d(roam,dwell2) = -0.54, d(roam,dwell3) = -1.02. (h) d(roam,dwell1) = -0.42, d(roam,dwell2) = -0.18, d(roam,dwell3) = -0.46. **** = $p < 0.0001$, Welch's t-test; effect size: d = cohen's d. Violin Plots contain box plots that show the interquartile range.

Chapter 7: Methods

7.1 Mutual Replaceability (MR)

Considering all the 1287 N2 worms on food, each of whose foraging behavior is described by a sequence of 90 postures, a first order 90×90 behavioral transition matrix B was created for all the worms pooled together. Each entry B_{ij} in the transition matrix denotes the number of times the worm made a transition from posture i to posture j across all the 1287 worms taken together.

Mutual Replaceability (MR) as described in (Maurus and Pruscha, 1973; Dawkins, 1976) was applied to first order markov transition matrix to obtain chunks of mutually replaceable worm postures. To capture the various permutations in which postures can combine to produce sequences, MR seeks to cluster those behavior patterns together that do not necessarily occur close by in time, but whose transition relationships with members of other clusters are similar. Thus, these behavior patterns are mutually substitutable in those parts of the transition matrix, B, that do not involve their interactions with behavior patterns in their own cluster (Dawkins, 1976). For instance, two adjectives might usually not occur together in time in a sentence, but certain words in adjective cluster are mutually substitutable because they can be substituted in their interactions with other words in the noun cluster.

Starting with B, MR calculates index of mutual replaceability for each pair (i and j) of postures. Let the row in B, denoting the transition structure from posture i to all other postures be denoted as r_i and the row corresponding to posture j as r_j. Analogously, let the column in B corresponding to the transition structure from each of the 89 different postures to posture i be denoted as c_i and the column corresponding to posture j as c_j. For postures i and j, the Pearson correlation coefficient R_{ij} between r_i and r_j (excluding mutual interactions) and C_{ij}, the Pearson correlation coefficient between c_i and c_j (excluding mutual interactions between i and j) is first computed. The index of mutual replaceability, M_{ij} for i and j is finally computed as the mean of R_{ij} and C_{ij}. The pair of postures, s and t, that has the maximum associated M_{st} value is then put in the same cluster and the transition matrix B is collapsed so as not to make any distinction between postures s and t by adding their corresponding entries. The same procedure is carried out on the reduced matrix, so that the next clustering might involve two different postures or one posture with an already made cluster in a previous iteration of the procedure. The procedure comes to a halt when only two entries remain in the ever reducing matrix. This process of iteratively forming clusters, gives rise to a dendrogram in which the portions that get merged higher up in the dendrogram are less substitutable than the

pairs that get merged at a lower height in the tree. We cut the tree thus formed at a height of around 81 in Figure 3.5, giving us 10 visibly distinct clusters (b1,b2,b3,b4,r1,r2,r3,g1,g2,g3).

The silhouette value of each posture p in a cluster is computed according to the following equation

$$S_p = \frac{b_p - a_p}{max(a_p, b_p)} \tag{7.1}$$

where a_p denotes the average morphological distance between the pth posture and all the other postures belonging to the same cluster as posture p, b_p denotes the minimum average morphological distance between posture p and all the other postures belonging to a different cluster than posture p, minimized over all the clusters. Euclidean distance is used to compute the morphological distance between two postures p and q as

$$d(p,q) = \sqrt{\sum_{i=1}^{48}(p_i - q_i)^2} \tag{7.2}$$

7.2 Shuffling postures

In the control experiments with shuffled postures, each worm postural sequence is shuffled such that individual posture occurrence frequencies are maintained. For example: an original sequence 1,2,3,1,3,4 might be shuffled as 3,1,2,1,4,3 to form a new shuffled sequence.

7.3 Obtaining transition matrix between chunks obtained by MR

After applying MR on the postural sequence data of all the N2 worms combined, each of the 90 postures are assigned to one of the 10 clusters (b1,b2,b3,b4,r1,r2,r3,g1,g2,g3). Given a one-to-one mapping of each posture to one cluster, the postural sequences of the all the worms can be abstracted in terms of transitions between the 10 clusters. A transition from posture i to posture j is counted as a transition between C_i and C_j, where C_i and C_j are the clusters containing postures i and j respectively. In this way, a transition matrix between the 90 postures for the all the worms taken together can be transformed into a transition matrix between 10 clusters.

7.4 Analyzing transitions between postures and clusters using entropy

Assuming that all the events are equiprobable, the uncertainty in predicting the next event (either posture or cluster) can be calculated as

$$H_0 = log_2(n) \quad bits \tag{7.3}$$

where n is the number of values that can be taken by the variable under consideration.

The reduced uncertainty that is afforded by the knowledge of individual event (posture or cluster) probabilities apriori is given by

$$H_1 = -\sum p_i log_2 p_i \quad bits \tag{7.4}$$

where p_i is the probability of state i.

Furthermore, the reduced uncertainty resulting from the additional knowledge of first order transition probabilities between events (postures or clusters) is given by

$$H_2 = -\sum_i p_i \sum_j p_{ij} log_2 p_{ij} \quad bits \tag{7.5}$$

where p_i is the probability of i and p_{ij} is the probability of going to j given the current state is i. The unigram normalized perplexity score is calculated as

$$PPLu = \left(\prod_{t=1}^{T} \frac{P(w_t|w_{t-1})}{P(w_t)}\right)^{\frac{-1}{T}} \tag{7.6}$$

where T is the length of the sequence.

7.5 Mapping 10 clusters to three higher order clusters

Mapping the 10 clusters from the dendrogram to the squares along the diagonal in Figure (refer to the figure corresponding to decomposability matrix), we observe that b1,b2,b3 and b4 make up one square of postures (henceforth referred to as B), r1,r2 and r3 another (henceforth referred to as R) and g1,g2,g3 make up the third (henceforth referred to as G).

7.6 *Generating* worm roaming behavior

Worm roaming behavior was *generated* by simulating the precise sequence of chunks (clusters) - b1→ b2 → b3→b4→r1→r2→r3→g1→g2→g3 repeatedly. Inside a particular chunk in one iteration of the above sequence, a posture in that chunk was randomly chosen and the control passed onto the next chunk in the sequence, repeating the process.

7.7 Properties of sequence of chunks during worm foraging

A one-to-one mapping between the 90 postures and clusters B, R and G enables the encoding of worm postural sequences into sequences of B,R and G. A particular worm postural sequence could be abstracted as a sequence of frames characterized by the cluster identities like B,B,G,G,G,R,R,R,B,B,R, R,R,G,G. From such a cluster sequence, we can ascertain various properties of worm behavior based on the properties of triplets like B→R→G and B→R→B etc. Cluster triplets were considered according to a sliding window that advanced one cluster at a time in the behavioral sequence. For example, in the behavioral cluster sequence above, the triplets that formed part of the analysis were B→G→R, G→R→B, R→B→R, B→R→G. We treat the triplets B→R→G, R→G→B and G→B→R as being equivalent and count all of them as the B→R→G triplet. Similarly, triplets of type B→G→R, G→R→B and R→B→G are counted as the same reversing B→G→R triplet. The data used in this work (Yemini et al., 2013; Schwarz et al., 2015) sampled worm videos at 6 frames per second that is used to calculate the time spent in each frame (1/6 seconds) in a sequence of clusters as shown above. Time in seconds spent in each frame is added to compute the time spent in each of the identified triplets. This frame based addition of time takes into account the repeats (same posture identified in consecutive frames) in the behavioral postural sequence. For instance, time computations on the postural sequence 1,1,1,2,3,4,4,4,5 takes into account the time spent in each of the frames (which is equal to 1/6 seconds) even if consecutive frames have the same posture in them. Thus for the sequence - 1,1,1,2,3,4,4,4,5, the total time taken by the worm to complete this sequence is 9*(1/6) seconds. The assignment of sub-cluster labels to a postural sequence is done after ignoring the repeats. For the sequence - 1,1,1,2,3,4,4,4,5, repeats are first ignored such that relevant postural sequence becomes 1,2,3,4,5, for which the sub-cluster sequence becomes g1, g1, b1,b2, g2. At a higher level, the sequence becomes an instantiation of the G→B→G rule. Thus,

the time spent in this particular instantiation of the G→B→G rule is 9*(1/6) seconds. Also, if we imagine a postural sequence 1,2,3,4,5,4,3,2,1 (after repeats have been ignored), the sequences generated at higher levels of abstraction become g1, g1, b1,b2, g2,b2,b1,g1,g1 and G,G,B,B,G,B,B,G,G. According to the sliding window protocol used in all the analysis in this work, the cluster triplets whose various properties are computed are - G→B→G and B→G→B. In this way, posture sequences are converted into sub-cluster and cluster sequences and the time spent in each instantiation of a behavioral rule is computed.

The disruption of the b1→b2→b3→b4→r1→r2→r3→g1→g2→g3 is achieved by alternating between consecutive sub-modules. For example, a sequence such as b1→b2→b1→b2→b3→b4→r1→r2→r1 →r2→r3→g1→g2→g3 involves two alternations (b2→b1 and r2→r1) as opposed to the smooth b1→g3 sequence. A sequence of triplets (in terms of B,R and G) when investigated at the corresponding level of 10 sub-clusters is deemed non-smooth if the number of alternations in the sequence are greater than 2. If the worm is in the regime of B→R→G rule, but satisfies either of the following conditions:

- The underlying sub-cluster sequence is non-smooth and at least one posture is repeated more than two times, OR

- The underlying sub-cluster sequence is non-smooth

then that particular sub-sequence of postures is assigned to the Dwell 1 rule. If on the other hand, the worm is in the regime of B→R→G rule, and satisfies the following condition:

- The underlying sub-cluster sequence is smooth and the number of unique sub-clusters in the sequence is greater than 5

then that particular sub-sequence is classified to belong to the b1→g3 rule (roaming rule), even though it might have less than 10 sub-clusters. We take a heuristic of greater than 5 smooth sub-clusters in a sequence, as that means that the worm moves a relatively long distance as compared to a situation in which the sub-cluster sequence is smooth but the the number of unique sub-clusters are only 2 wherein the worm actually does not move much farther from its previous position. If the worm is in the regime of rules of type B→R→B, B→G→B, R→G→R, R→B→R, G→B→G and G→R→G, then that particular behavioral sub-sequence is encoded as Dwell 2 type rule. Finally, if the worm is in the regime of rules of type B→G→R, then a smooth B→G→R sequence involves no alternation in the sub-cluster sequence g3→g2→g1→r3→r2→r1→b4→b3→b2→b1. In this case

a non-smooth sub-cluster sequence would involve at least 2 sub-cluster transition in the opposite direction, e.g. - a transition of type b2→b3 or r3→g1. If for such a sequence of B→G→R rules, the following conditions hold

- The underlying sub-cluster sequence is non-smooth OR

- At least one posture is repeated more than once

then that particular sub-sequence is classified as Dwell 3. Otherwise, the B→G→R sub-sequence is classified as g3→b1 or reversal rule.

To compute the proportion of all the different rules for each worm postural sequence, the number of occurrences of a particular type of grammatical rule in a particular worm behavioral sequence is computed and divided by the total number of occurrences of all the five types of grammatical rules observed in that sequence. Such a proportion for each rule was calculated for each worm and plotted as a function of the number of postures in the behavioral sequence of that worm. Number of postures are counted in the postural sequences where repeats (same posture identified in consecutive frames) have been removed. The number of postures in the postural sequence 1,2,2,2,3,4,4 is 4 and not 7, based on the sequence 1,2,3,4 that is generated by ignoring the repeats. Once the postural sequence of a worm's behavior is encoded into a sequence of different grammatical rules as defined above, further properties associated with the grammatical rules like the time spent in each type of rule and the speed of the worm during each kind of rule is computed. To compute the average speed of worm centroid, first the speed across each of the individual frames making up a postural sequence corresponding to a particular instantiation of a behavioral rule is calculated, using the model discussed in (Keaveny and Brown, 2017). Then the speed across all the frames is averaged to get a handle on the average speed of the worm centroid during a particular instantiation of a behavioral rule. In this way, a distribution of speeds during all instantiations of rules of a particular type is obtained. The model proposed in (Keaveny and Brown, 2017) is also used to obtain trajectory of worm centroid position from a sequence of postures.

In the analysis comparing the time spent in roaming and dwelling type rules in mutant strains and N2 worms, for each mutant strain worm, only those N2 worms were used as controls that were imaged within 1 week (before or after) from the time the particular mutant strain worm was imaged. This is due to month-month variability in the behavior of N2 worms (Yemini et al., 2013).

Chapter 8: Discussion

> For a large class of cases–though not for all–in which we employ the word "meaning" it can be defined thus: the meaning of a word is its use in the language.
>
> *Ludwig Wittgenstein*

It is enticing to look at animal behavior through the lens of "meaning" (Clarke and Crossland, 1985) - where behavior is not just looking at the kinematics or trajectories traced by the animal under study but also about enriching these lower level descriptions with intuitions about the behavioral "meaning" that operates on top of these physical descriptions. In language, Ferdinand de Saussure made suggestions to analyze its structure and meaning in terms of two kinds of relations between the words that make up the language (Harre, 1976).

- Syntagmatic Relations: Rules that define how individual words should be strewn together to generate higher order structures like phrase and sentences.

- Paradigmatic Relations: Rules that define how different words can be associated with each other based on form or meaning by playing the same role in a sentence.

In this work, we used the ideas about syntagmatic and paradigmatic relations to analyze the structure of foraging behavior in the nematode worm *C. elegans* and uncovered rules that define how worm postures and higher order chunks of worm postures are combined to generate worm foraging. Grammatical rules specify how chunks of postures can be re-used in different contexts to produce different behaviors - roaming or dwelling. Such a generative grammar of worm foraging demonstrates how flexible behavior is generated by a compositional hierarchy.

Hierarchical organization has long been thought as a general principle of behavior (Tinbergen, 1950, 1951; Dawkins, 1976) but clear demonstrations of its existence during spontaneous behavior are conspicuous by their absence (Brown and de Bivort, 2018). A further missing piece in understanding behavior is how hierarchical organization aids in generating flexible animal behavior. Treating worm behavior as a sequence of body postural changes, we first demonstrate that worm foraging behavior (not just the stereotypical portions) is hierarchically organized. We use mutual replaceability (Dawkins, 1976; Maurus and Pruscha, 1973) to obtain chunks containing mutually substitutable worm postures (paradigmatic relations between postures), just like in the context of verbal behavior,

the *adjective* chunk contains mutually substitutable words like "black" and "white". The resulting chunks are then used to demonstrate how the worm might be able to generate flexible behavior. Specifically, we elucidate a grammar of worm foraging outlining rules of interaction between such chunks containing substitutable postures (syntagmatic relations). We find that the stereotypical worm roaming behavior (sustained forward motion, with few turns and pauses) is captured by a specific grammatical rule involving specific chunks in a particular order and that even such stereotypical behavior is characterized by variability at the lowest level of postures. A single rule specifying interactions between specific chunks can generate flexible roaming behavior where there is flexibility in the choice of postures used by the worm from each chunk. Stereotypy in behavior patterns has been used effectively in the past to demonstrate hierarchical organization underlying various stereotypic behaviors in Drosophila (Berman et al., 2016). While stereotypy is indeed a central concept in behavior, relatively less stereotypic behavior patterns can constitute upto half of the behavior in some animals (Berman, 2018). In this work we fill this gap in the context of non-stereotypical *C. elegans* dwelling behavior by delineating grammatical rules that specify how the same chunks (as the ones used in stereotypical worm roaming behavior) are used in different combinations to produce non-stereotypical dwelling (involving termination of sustained forward motion) like behavior patterns. Such a generative grammar for worm foraging demonstrates how flexible behavior emerges from a hierarchically organized behavioral scaffolding. We also show that the properties of our proposed worm foraging grammar are consistent with known experimental results about the behavior of various mutant strains as well as the behavior of wild isolates that encounter changes in their environment. Finally, we report hitherto uncharacterized role of neuropeptide receptors *npr-3* and *npr-10* in modulating *C. elegans* foraging behavior.

8.1 Limitations of current work and its promise in playing a complementary role in building more powerful generative models of behavior

One limitation of our work in its current form is that it is not fully generative in its design. Our grammar does not capture richer statistical characteristics of behavior that can specify when a simulated worm should shift from roaming to dwelling. Also, our grammar does not have adequate

mechanisms built-in to specify which of the multiple rules that form part of Dwell 2 type dwelling rules should be used at a particular instant to generate dwelling behavior. It seems that these aspects are statistical in nature and such structure could in principle be captured by hidden markov models (HMMs) (Wiltschko et al., 2015) or recurrent neural network models (Li et al., 2017). Although immensely powerful in their ability to capture statistical regularities, HMMs do have the limitation of being linear and memoryless in theory and hence can fail to capture more long term relationship in behavioral sequences (Collado-Vides et al., 1996). Although one can in principle learn hierarchical HMMs, but the optimization underlying such learning process is liable to getting stuck in local optima, hence there are high chances of learning the wrong structure (Collado-Vides et al., 1996). One avenue for future research would be to identify structure beforehand (like the grammar identified in this work) and then feed this structure to learning algorithms like HMMs or RNNs, such that both the inherent structure and the statistical regularities within such structure can be leveraged simultaneously to build powerful generative models of behavior.

8.2 Posture sequences versus a more continuous representation of worm behavior

One limitation of this work is that instead of using a more natural continuous representation of worm behavior, we make use of discretized version of an originally continuous representation. This can result in significant loss of information and might cast doubts on the validity of findings reported in this thesis. In what follows, I try to discuss how the posture based representation used here presents a complementary perspective in the understanding of worm behavior.

The central question being asked in this work is about the nature in which worm behavior is organized. One relatively straightforward way in which questions about the organization of anything can be tackled is by analyzing how units combine with each other, for which a discrete representation of the units being analyzed can be more amenable. To be sure, this is not to say that questions about organization cannot be answered in a continuous data regime. To that end, I contend that the perspective and findings reported here present only a complementary perspective of worm behavior.

Any perspective that claims to be complementary, has to first establish whether facts that are known to be true in the literature can be accounted for in the complementary perspective. Only then, can it proceed to reveal something new about the phenomena being studied.

To establish that the grammatical perspective based on postural sequences can account for a myriad facts known about worm behavior, I analyzed mutant worm data and found that the mutants reported in prior work, to have defective roaming or dwelling behavior, also have the same defective patterns when analyzed through the lens of my grammatical framework based on postural sequences. Further, the proposed grammatical rules correctly predict worm behavior patterns in off food environment as well. Finally, as a comparison of the posture based representation with a more continuous and dynamic vision of behavior as exemplified by the eigen worm projections (Figures 4.2e and 4.2d), I found the projections from discrete postures during roaming and dwelling closely mirrored the projections of a more continuous behavioral conception as reported in (Stephens et al., 2008; Gyenes and Brown, 2016). This recapitulation of already known results about worm behavior from prior literature, confirms that the grammatical framework and its associated use of discrete postures offer a reasonable framework in which to find something new about worm behavior.

After confirming the validity of the postural representation in reproducing known aspects of worm behavior, I have shown for the first time that worm behavior is organized in a hierarchical fashion rather than in a linear organizational framework. Finally,the 90 postures used in this study capture upwards of 80% of the variance in the shapes taken by the worm over its entire behavior. Hence these 90 postures can be seen as capturing a significant chunk of shapes taken by the worm.

8.3 Clustering and its role in uncovering hierarchical organization

To demonstrate that *C. elegans* foraging behavior is hierarchically organized, we perform clustering based on substitutability criteria to obtain chunks of postures. However, it has been argued in (Berman et al., 2016; Brown and de Bivort, 2018), that the fact that one can cluster behavior into chunks does not necessarily imply that the underlying organization is actually hierarchical. This apparent limitation in our approach is mitigated by complementing the knowledge of behavioral chunks with a behavioral grammar that specifies how the chunks obtained by clustering might be used to actually *generate* behavior sequences by the worm. This conception of hierarchical organization is in line with Herbert Simon's notion of hierarchy (Simon, 1962), in which he argued that hierarchy does not only mean obtaining partitions of data but it also involves elucidation of the rules of interaction between the obtained partitions.

8.4 Discrete representation as loss of essential behavioral information

We make use of a discrete representation of *C. elegans* behavior where the worm's foraging behavior is abstracted as a sequence of any of 90 pre-defined template postures. The data comes from previous studies (Yemini et al., 2013; Schwarz et al., 2015), in which the 90 template postures were found to capture more than 80% variance in the whole repertoire of postures actually taken by the worm. Although capturing a sizable variance, the 90 template postures still leave out some shapes that the worm takes during foraging. To check whether our findings are qualitatively immune to such changes in the number of template postures used to capture the totality of worm locomotion, we changed the number of postures from low (45) to high (150). We found that qualitatively similar patterns characterize the transition matrix between the sub-modules as shown in Figure 8.1.

(a) 45 postures

(b) 150 postures

(c) 45 postures
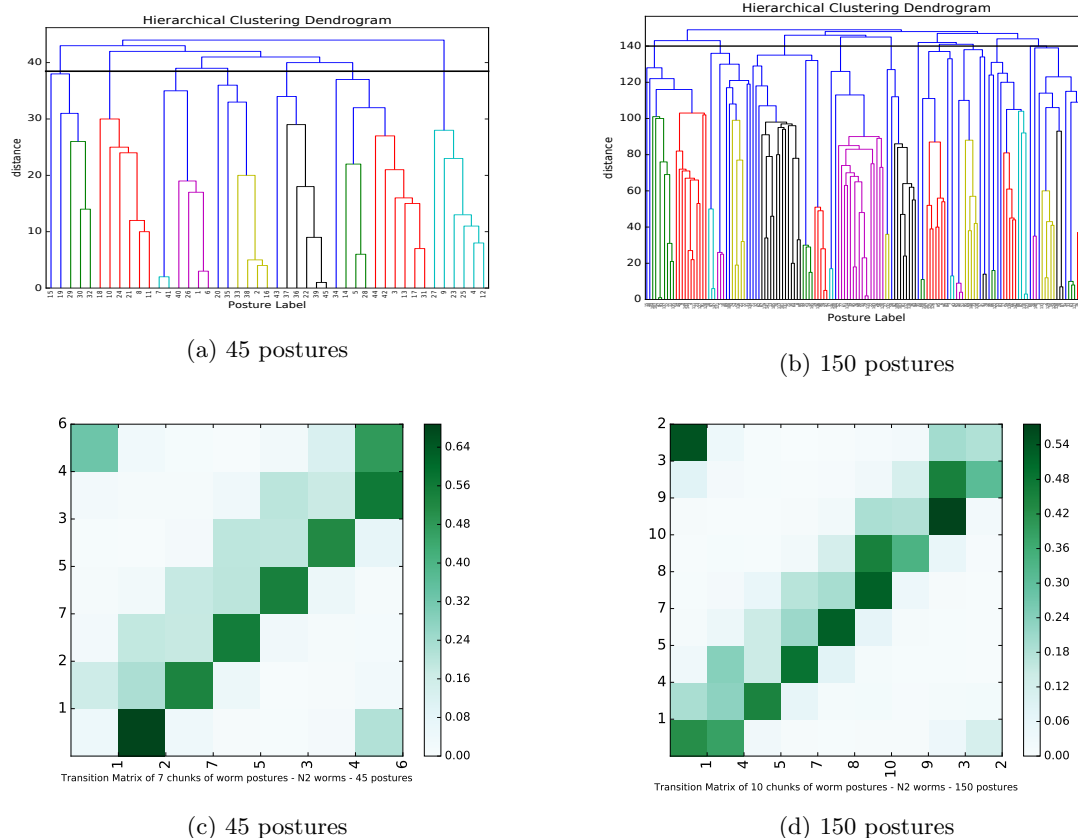
(d) 150 postures

Figure 8.1: **Decreasing or increasing the number of template postures used in the analysis of worm foraging behavior reveals qualitatively the same patterns as are seen with 90 postures.** (a): Dendrogram depicting the sub-module structure in N2 worms with 45 postures, based on mutual replaceability (MR). (b): Dendrogram depicting the sub-module structure in N2 worms with 150 postures. (c): MR applied on the all the N2 worms pooled together with only 45 postures describing all the shapes taken by the worm during foraging. (d) MR applied on the all the N2 worms pooled together with 150 postures describing all the shapes taken by the worm during foraging.

8.5 Hierarchical organization and substitution combine to elucidate a grammar of worm foraging

We have attempted to marry the idea of flexible behavior generation with hierarchical organization to elucidate a behavioral grammar for worm foraging behavior. The generative model afforded by the behavioral grammar proposed here, takes into account different timescales at which animal behavior manifests itself. This is in contrast to some other generative models of behavior which operate at a single time scale (Wiltschko et al., 2015). Earlier attempts at obtaining a behavioral grammar have been characterized by human defined action labels, which severely limits the reproducibility of the

analysis due to the biases inherent in human classification. Here, we try to reduce that limitation by working with a representation which is comparatively more unbiased and use it to spell out a behavioral grammar for worm foraging. Such an approach based on clustering of substitutable behavioral descriptions can be used across organisms in a relatively robust manner. The approach outlined in this work can result in fairly precise dynamical understanding of human behavior and be used to characterize subtle changes in human behavioral organization, resulting from many diseased states.

Recent studies have shown that stereotyped animal behaviors are hierarchically organized. Whilst stereotypy indeed is a general principle of behavior (Berman et al., 2014), a lot of animal behavior consists of engaging in non-stereotyped behaviors (Berman, 2018). We have demonstrated that all the portions of *C. elegans* foraging behavior: the more stereotyped roaming and less stereotyped dwelling behavior, are hierarchically organized. This demonstration of behavioral hierarchy in worms lends credence to a principle whose demonstration in behaving animals has been rare in the past (Brown and de Bivort, 2018).

8.6 Hierarchy, predictability and flexibility in *C. elegans* foraging behavior

It is an often expressed lament that in spite of extensive knowledge about the anatomy and connectivity patterns in the nervous system of the worm, we still have not found ways to predict its behavior at a sufficient level of detail. When studying animal behavior, that is modulated by a variety of factors including the environment and the animal's internal state and exhibits inherent flexibility, one is forced to ask about the level of abstraction at which we want to predict behavior. It becomes even more pertinent in scenarios where we study unrestrained behavior for a relatively long time rather than a behavior systematically paired with a stimulus. In such a system that can change in a number of ways, the nature of predictions about behavior has to take a different form from, lets say, the stimulus-response behavioral paradigms (Hofstadter, 1996; Hayek, 1964). Hierarchical organization helps in taming complexity by giving predictability at a higher level of abstraction than the lower most level of representation (postures in our case) (Fentress and Stilwell, 1973). It follows that, in a hierarchically organized behavior, the predictability about the identity of lower levels of representation increases if we also know the chunk in which the animal is currently in. For example,

in worm foraging behavior, if we know that the worm is in roaming state, we can deduce that it is in the B→R→G regime. If we further know it is roaming and is currently in B chunk, then we can deduce that it is in either of b1,b2, b3 or b4 chunks with the particular ordering of b1→b2→b3→b4. Finally, if we know that the roaming worm is in b1 chunk then our prediction ability improves further, because we know that there are only a few postures from which one can be chosen, reducing the search space from 90 original postures. One way in which flexibility is afforded in this system is that there is relative freedom in the identity of posture that is used from each chunk as chunks are created by looking for substitutable postures in the first place (type 2 substitution). Another way in which flexibility comes in the system is through the re-usability of the same structures in generating different behaviors like roaming and dwelling (type 1 substitution).

8.7 Neural dynamics subservient to hierarchically generated flexible behavioral dynamics

The concepts of degeneracy and re-usability that we have leveraged to tie hierarchical organization with flexible behavior have been shown to exist at the interface between nervous system and behavior in *C. elegans*. For example - octanol avoidance behavior is driven primarily by ASH nociceptive neurons in well-fed worms. But after an hour of starvation, the same behavior is mediated by ASH, AWB and ADL nociceptive neurons (degeneracy) (Chao et al., 2004). Moreover, differential activation of npr-1 neuropeptide receptor allows ASH neurons to generate different behaviors by facilitating two different neuromodulatory states - with aerotaxis behavior occurring irrespective of modulation and aggregation behavior occurring only when npr-1 activity is low (re - usability of the same neural circuitry to generate different behaviors) (Cheung et al., 2005; Chang et al., 2006; I.). One possibility for future work would be to determine if the degeneracy (type 2 substitution) and re-usability (type 1 substitution) in the organization of behavior outlined in this work can help elucidate degenerate and re-usable neural circuit mechanisms related to flexible behavior.

Studies investigating the relationship of neural activity with behavior in *C. elegans* have found that the whole brain dynamics lie on a low-dimensional manifold. Trajectories of neural activity through this manifold can then be mapped to different behavioral states in the worm (Kato et al., 2015). It has been reported that reasonably differentiated trajectories though this manifold can correspond to the same behavioral state like reversals. Stereotyped behaviors like roaming that are

generated by a single grammatical rule with flexibility in the choice of postures, might be expected to trace trajectories relatively close to each other. On the other hand, less stereotyped dwelling behavior is characterized by multiple grammatical rules. Different grammatical rules (corresponding to Dwell 1, Dwell 2 and Dwell 3) should be expected to correspond to substantially differentiated trajectories through the neural manifold.

Finally, our finding that npr-3 and npr-10 play a role in modulating worm foraging can be used to further investigate the neural dynamics underlying roaming and dwelling. Since we have a fair understanding of neural circuits underlying worm roaming and dwelling (Gray et al., 2005), investigations geared towards understanding how neuropeptides like npr-3 and npr-10 influence the known neural circuitry associated with roaming and dwelling would help us in getting a more complete understanding of how the concerted action of genes and neural circuits leads to behavior.

8.8 Role of syntax in understanding evolution of behavioral flexibility

One way in which higher animals are usually distinguished from relatively simple ones, is by noting the higher levels of behavioral flexibility in complex as compared to simpler animals in the evolutionary tree. The ideas of hierarchical organization and a grammar (outlining ways of combining elements) have fertilized inquiries both in linguistics as well as ethology (Peters, 1981; Lashley, 1951; Chomsky, 1965; Kalmus, 1969; Fentress and Stilwell, 1973).

Grammar (either in the context of motor behavior or linguistics) involves defining ways in which elements may be permuted/combined at different levels of hierarchical organization to generate different sequences flexibly. Due to such multi-level permutations, even a small increase in number of elements and rules of combination can lead to disproportionate increase in the number of novel behavioral repertoires. Suggestions have been made in the past about how behavioral flexibility in higher animals can be seen as a series of evolutionary steps where the fixed action patterns of simpler organisms are first isolated into component primitive units that are then recombined in a variety of ways (using rules of grammar) in descendant and more complex animals (Peters, 1981). Consequently, a similarity in the rules of combination of behavior units (grammar) between animals, irrespective of the identity of the behavioral units might indicate evolutionary relationship, driving researchers to not only look for homologues of behavioral elements but also homologues of rules for

combining behavioral elements (Peters, 1981).

Chapter 9: Conclusions

Foraging is an ecologically relevant and evolutionary ancient behavior underlying some of the most important decisions made by all animals. Foraging in the nematode worm *C. elegans* is thought to be organized into two distinct states - the exploratory phase of roaming and the exploitative phase of dwelling. During roaming, the worm moves quickly across the bacterial lawn of food with low frequency of turns, whereas during dwelling, it moves rather slowly with frequent turns, thus confining itself to a very small region. This thesis was aimed at understanding the organizational principles underlying foraging behavior in the nematode worm *C. elegans*. It is currently unknown whether hierarchical organization can explain *C. elegans* foraging behavior. Given also that flexibility is an integral part of animal behavior, this thesis also aimed towards understanding how the principle of hierarchy can be tied to the flexible generation of behavior by the worm.

- We first observed that the principle of substitution can relate hierarchical organization with flexible behavior generation using *C. elegans* foraging behavior as a use case. Substitution in a behaving system that leads to flexible behavior could be realized via:

  - Degeneracy : Different motor signals can generate the same behavior
  - Re-usability : Similar motor signals can generate completely different behaviors based on the context in which they occur.

- Treating worm behavior as a sequence of changes in its body posture and refraining from using any human defined labels for such postures, we show that worm foraging behavior (not just the stereotypical portions) is hierarchically organized. We use mutual replaceability to obtain chunks containing mutually substitutable worm postures, just like the chunk named adjective contains mutually substitutable words like "black" and "white".

- Furthermore, we elucidate a grammar of worm roaming and dwelling states, outlining rules of interaction between such chunks containing substitutable postures. We find that the stereotypical worm roaming behavior is captured by a specific grammatical rule involving specific chunks in a particular order and that even such stereotypical behavior is characterized by variability at the lowest level of postures. Variability in worm roaming behavior comes about through substitution where postures in a chunk can be substituted with each other without changing the nature of the higher order behavior – roaming (degeneracy).

- We also delineate grammatical rules that specify how the same chunks are used in multiple combinations to produce relatively less-stereotypical dwelling like behavior patterns. Thus

substitution, in terms of re-use of the same chunks in different ways can generate two different worm behaviors - roaming and dwelling (re-usability).

- Body morphology cannot account for the dynamics underlying worm roaming behavior.

- Such a generative grammar for worm foraging demonstrates how flexible behavior emerges from a hierarchically organized behavioral scaffolding.

- We then tied the grammar of worm foraging with ideas that are commonplace in computer science and linguistics. Specifically, we proposed that the worm foraging grammar could conform to a context free grammar (CFG in computer science and linguistics parlance) that has hierarchical structure built into it. And indeed, we defined a CFG that can capture the worm foraging behavior. It was also shown that a less expressive grammar - a regular grammar, cannot capture worm foraging behavior.

- The organization principles captured by the proposed grammar can elucidate novel molecular mechanisms important for regulating worm foraging behavior. After validating the robustness of the proposed grammar by replicating previous findings that npr-1 and npr-9 mutants show increased and impaired roaming behavior respectively. We then showed that the proposed worm foraging grammar reveals hitherto uncharacterized role of npr-3 and npr-10 genes in affecting foraging behavior.

- The proposed grammatical rules correctly predict worm behavior patterns in off food environment as well. We found that the proposed grammatical rules can recapitulate variations in worm behavior that are caused by changes in its environment. Specifically, the amount of time spent by N2 worms in roaming type grammatical rules is considerably higher than the time spent in dwelling type rules, in off food conditions, as reported in prior literature.

Chapter 10:   Conclusiones

La búsqueda de alimento es un antiguo comportamiento evolutivo y ecológicamente relevante que subyace a algunos de los decisiones más importantes que toman todos los animales. Buscando alimento en el gusano nematodo *C. elegans* se cree que está organizado en dos estados distintos: la fase exploratoria de itinerancia y la fase de explotación de vivienda. Durante la deambulación, el gusano se mueve rápidamente a través del césped bacteriano de alimentos con baja frecuencia de vueltas, mientras que durante la morada se mueve bastante lento con giros frecuentes, confinándose así a una región muy pequeña. Esta tesis tuvo como objetivo comprender los principios organizacionales comportamiento de alimentación subyacente en el gusano nematodo *C. elegans*. Actualmente se desconoce si La organización jerárquica puede explicar *C. elegans* comportamiento de búsqueda de alimento. Dado también que la flexibilidad es una parte integral del comportamiento animal, esta tesis también tuvo como objetivo comprender cómo el principio de jerarquía puede vincularse a la generación flexible de comportamiento por parte del gusano.

- Primero observamos que el principio de sustitución puede relacionar la organización jerárquica con la generación de comportamiento flexible usando el comportamiento de búsqueda de alimento de *C. elegans* como un caso de uso. La sustitución en un sistema de comportamiento que conduce a un comportamiento flexible podría realizarse a través de:

  - Degeneración: diferentes señales motoras pueden generar el mismo comportamiento
  - Reutilización: las señales motoras similares pueden generar comportamientos completamente diferentes según el contexto en el que ocurren.

- Tratando el comportamiento del gusano como una secuencia de cambios en su postura corporal y absteniéndose de usar etiquetas definidas por humanos para tales posturas, mostramos que el comportamiento de búsqueda de alimento del gusano (no solo las porciones estereotipadas) está organizado jerárquicamente. Usamos la reemplazabilidad mutua para obtener fragmentos que contienen mutuamente posturas de gusano sustituibles, al igual que el fragmento llamado adjetivo contiene palabras mutuamente sustituibles como "negro" y "blanco".

- Además, aclaramos una gramática de los estados de deambulación y residencia de los gusanos, esbozando las reglas de interacción entre esos trozos que contienen posturas sustituibles. Encontramos que el comportamiento estereotipado de vagabundeo del gusano es capturado por una regla gramatical específica que involucra fragmentos específicos en un orden particular y que incluso ese comportamiento estereotipado se caracteriza por la variabilidad en el nivel más

bajo de posturas. La variabilidad en el comportamiento de vagabundeo de los gusanos se produce mediante la sustitución, en la que las posturas de un fragmento pueden sustituirse entre sí sin cambiar la naturaleza del comportamiento de orden superior: vagabundeo (degeneración).

- También delineamos reglas gramaticales que especifican cómo se usan los mismos fragmentos en múltiples combinaciones para producir patrones de comportamiento de vivienda relativamente menos estereotipados. Por lo tanto, la sustitución, en términos de reutilización de los mismos fragmentos de diferentes maneras, puede generar dos comportamientos de gusanos diferentes: itinerancia y vivienda (reutilización).

- La morfología corporal no puede explicar la dinámica subyacente al comportamiento de itinerancia de los gusanos.

- Esta gramática generativa para la búsqueda de alimento de los gusanos demuestra cómo el comportamiento flexible surge de un andamiaje conductual organizado jerárquicamente.

- Luego vinculamos la gramática de la búsqueda de alimento de gusanos con ideas que son comunes en informática y lingüística. Específicamente, propusimos que la gramática de búsqueda de gusanos ajustarse a una gramática libre de contexto (CFG en el lenguaje informático y lingüístico) que tiene estructura jerárquica incorporada en él. Y de hecho, definimos un CFG que puede capturar la comportamiento de búsqueda de alimento del gusano. También se demostró que una gramática menos expresiva, una gramática regular, no puede capturar el comportamiento de búsqueda de alimento del gusano.

- Los principios de organización capturados por la gramática propuesta pueden dilucidar la novela mecanismos moleculares importantes para regular el comportamiento de búsqueda de alimento de los gusanos. Después de validar el robustez de la gramática propuesta al replicar hallazgos previos que npr-1 y npr-9 los mutantes muestran un comportamiento de itinerancia aumentado y deteriorado, respectivamente. Luego mostramos que el La gramática de búsqueda de gusanos propuesta revela un papel hasta ahora no caracterizado de npr-3 y npr-10 genes que afectan el comportamiento de búsqueda de alimento.

- Las reglas gramaticales propuestas predicen correctamente los patrones de comportamiento de los gusanos en off entorno alimentario también. Descubrimos que las reglas gramaticales propuestas pueden recapitular variaciones en el comportamiento del gusano causadas por cambios

en su entorno. Específicamente, la cantidad de tiempo que pasan los gusanos N2 en reglas gramaticales de tipo itinerante es considerablemente más alto que el tiempo empleado en las reglas del tipo de vivienda, en condiciones de no comer, como se informó en la literatura anterior.

Chapter 11: Poster presented at ENCODS 2017

The organization of posture sequences in

Caenorhabditis elegans locomotion

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Humans and other animals express behavior in sequences — when coated with dust, a fly first cleans its head, followed by its abdomen and then wings. Current methods allow for high-resolution behavioral measurement, and can generate big datasets of actions. But, having cut our cloth, we must sew it. How is behavior organized? Ethology's main tool is the ethogram, a catalogue of behaviors linked by arrows depicting the probability that one follows another. Yet, it seems unlikely that this is really how nervous systems control actions. Half a century ago, Simon mapped complexity to hierarchy, as organization in interconnected nested modules. Later, Dawkins proposed hierarchical organization as a general principle for serial behavior. Starting at the top of the hierarchy, animals make global decisions, progressively making narrower sub-decisions, until each act is instantiated. Ascending from the bottom, behavioral units (actions) get grouped into "chunks" and further into "chunks of chunks". As opposed to the traditional ethogram, knowledge of the chunk in which the previous action occurs, increases predictability of the next action. Guided by this idea, we are studying locomotion in *Caenorhabditis elegans*. Using a publicly-available high-resolution database of worm behavior, we have grouped postures via mutual replaceability (body postures grouped based on similar transition probabilities, yet, not necessarily co-occurring). We have found that postures can be arranged in three distinct chunks, in turn decomposable into sub-chunks, whose precise temporal ordering maps to worm behavioral states (forward locomotion, reversals, turns). These descriptive rules can be used in a generative grammar to produce postural dynamics. From this mechanistic viewpoint, worms would control locomotion by executing hierarchical procedure calls. Mapping such postural dynamics to biomechanical models of body-substrate interactions, we plan to predict locomotion trajectories, bridging the gap from neural activity to muscular contractions, to body dynamics, and finally to foraging strategies.

Chapter 12: Poster presented at SENC 2017

THE ORGANIZATION OF POSTURE SEQUENCES IN *CAENORHABDITIS ELEGANS* LOCOMOTION

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Topic:

- Systems Neuroscience

- Cognitive and Behavioral Neuroscience

- Theoretical and Computational Neuroscience

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