Evolution and the Genotype-Phenotype map

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Title image: Ernst Haeckel, Kunstformen der Natur, Table 54

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Chapter 1

Abstract

In the course of evolution organisms adapt, their forms change, sometimes radically. This change is ultimately caused by mutations in the DNA. At the same time organisms are complex. They consist of many interacting parts. Adaptation occurs despite this complexity. How can random mutations change such a complex system without disrupting existing functions? Why is an organism not a house of cards?

In this thesis the answer is sought in the genotype-phenotype map: the relation between genotype (genetic information) and phenotype (organism form an function). The thesis question reads as follows:

*Which properties of the genotype-phenotype map cause organisms to be adaptable and robust to mutation at the same time?*

The genotype-phenotype map is studied using two abstract models of an organism. The first model, a direct mapping between genes and functions is found to be inadequate to answer the thesis question.

The second model describes a genetic regulatory network or GRN. In a GRN the genotype consists of several genes and their interactions. The phenotype is the resulting pattern of gene activation. In the GRN model the influence of the individual genes on the phenotype is characterised using information theoretic measures. Two types of influence are studied.

The first is *redundancy*, the extent to which genes influence the phenotype in the same way (i.e. convey the same information about the phenotype).

The second is *degeneracy*, the extent to which genes influence the phenotype differently in different contexts.

The results show that a genotype-phenotype map which is not degenerate does not convey adaptability to an organism. To convey adaptability and robustness at the same time, The genotype-phenotype map needs to be both degenerate and redundant.
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Chapter 2

Preface

Initially this thesis was about "evolution". Narrowing down this subject to thesis size took over two years of reading papers, books, doing experiments and losing myself in several scientific wild goose chases. I am grateful to Remko Scha for helping me to find my own subject and for supporting my enthusiasm rather than co-writing my thesis. Thanks for introducing me to numerous works which were only distantly related to the subject of my thesis but interesting nonetheless. Thank you for introducing me to D’Arcy Wentworth Thompson’s *On Growth and Form* and Goethe’s *Urpflanze*. I have yet to meet another person that can discuss Marcel Duchamp and Probability theory with equal authority.

Thank you Jaap Kaandorp for your enthusiasm and kindness. It is reassuring to know that there is someone who shares my enthusiasm for genetic regulatory networks.

Thank you Bas Heijne for dedicated math support. Thank you for telling me to stop trying to analytically optimize that function for three variables at the same time, as it is indeed impossible.

Thanks to Gideon Smeding for very critical proofreading and Jan Willem Wieland for proof reading and showing me the turtles.

This thesis only scratches the surface. It is a first serious attempt at solidifying my enthusiasm. How can mechanical laws bring forth something as complex as life? Everything is bound by the laws of thermodynamics, to fall apart and descend into a state of higher probability, how are living things the exception? Only now am I beginning to get an idea of the questions I should be asking.

I regularly think that the best improvement to this thesis would be to start over. Whenever this happens I am comforted by the words of Professor Theo Geevers, who, upon my admission that I still had not answered my thesis question remarked: "don’t worry, I’m still answering mine”. With this comforting thought in mind I abandon this attempt. Who knows I might
make another one day.
Chapter 3

Introduction

This thesis is about evolution. More specifically: how is it possible? It is known that the form of an organism changes with each generation as a result of random mutations to its genes. Yet these changes are far from random: evolution often shows slow, gradual changes to organism form. An organism’s form is to a certain extent robust to many genetic mutations.

This thesis explores the way an organism’s form can be robust and still retain flexibility.

In general, a functional, complex mechanism is hard to change. That is to say, it is hard to change its parts without disrupting its function. Such a system’s parts interact on many levels. A change in one of these parts easily disrupts an interaction. Only a well planned sequence of specific changes could possibly change such a system without disrupting its function.

Think for example of changing the speed at which a clock runs. Only by taking out specific parts and replacing others in exactly the right order will a change occur without breaking the clock.

Natural organisms are complex systems. Given their high complexity it seems unlikely that any change can be made to them without breaking them. On top of that, evolutionary changes in organisms are not planned at all. Evolution is not guided towards any end goal. There is no planning of steps to reach any goal. How can any complex system arise in this way? How can organisms adapt the many complex interactions that maintain their existence without breaking down these interactions?

Obviously this question is too big to answer in a thesis or indeed any single volume of work. This thesis approaches the question from a single perspective. It focusses on the way in which genes give rise to form. Or, in keeping with current terminology, the genotype-phenotype map.

The genotype-phenotype map describes the relation between the genetic information, or genotype, and the form or phenotype of an organism.

On the one hand it is known that the genotype mutates randomly. On the
other, it is seen that the phenotype of organisms usually develops gradually in the course of evolution.

The number of possible configurations of the parts of an organism is astronomical. The chance of producing any viable organism by randomly putting together molecules is so small that it will probably never happen in the lifetime of the universe. Gradual change allows for evolutionary adaptation and increasing complexity of already functional solutions.

The genotype-phenotype map in organisms somehow dampens the effect of mutations on the genetic level so that a single mutation will not completely disrupt the phenotype. In other words, in natural organisms the genotype-phenotype map confers robustness of the phenotype against genetic mutations.

But which features of the genotype-phenotype map cause this robustness? One could think for example of multiple genes which perform the same function. When one gene mutates, the others can take over. In this way the function is robust to mutation of that gene. However, to change the function in any way, all the genes would have to mutate in the same way. This would imply that the more robust a genotype-phenotype map is to genetic change, the more stuck it gets on its current phenotype.

The natural genotype-phenotype map: There are species which are stuck on an evolutionary route but there are always those that manage to change in the course of evolution. Are there features of the genotype-phenotype map which can help to explain this?

In other words:

Which properties of the genotype-phenotype map cause organisms to be adaptable and robust to mutation at the same time?

This thesis investigates the general principles that allow for adaptation and complexification. As such, features actually found in nature serve as successful examples of these general principles. Natural systems guide the way, but understanding natural systems in themselves is not the goal of the research.

There are many different levels of organism organisation. The highest, most integrated level is the phenotype of the entire organism. The way the organism looks and functions as a whole. This phenotype is somehow encoded in lower levels of organisation, the lowest of which is the nucleotides which make up the DNA.

In between these highest and lowest levels of organisation there are numerous intermediate levels. Levels of organisation are discussed in more detail later. What is important to note now is that the computational models used in this thesis describe only a single level of organisation. Generality is sacrificed for clarity of the results.
Chapter 4

Background

The investigations of this thesis stem from a general interest in the process of evolution. This section explores selected concepts in a narrative way. The following Section, 4.1, briefly mentions important evolutionary theory leading up to Darwin. Darwin’s theory stands out for its rejection of goal oriented or *teleological* processes in evolution. The benefits and pitfalls of teleological thinking are discussed in section 4.2.

4.1 History of evolutionary theory

From a bird’s hollow bones to a chameleon’s shooting tongue to the blowhole of dolphins to the intricacies of human anatomy, the complexity and diversity of living things has been a grateful subject of human thought for most of recorded history. Theories about the origin of the forms of nature abound.

In 550 B.C., the observation that the first human child to come into the world would not be able to survive without parental care was the basis for Anaximander’s theory of the oceanic origins of man [46].

The extraordinary adaptation of animals to their environments inspired Aristotle to postulate the *telos* or final cause that preceded the formation of every part of nature [12]. In Aristotle’s view creatures on land have lungs because they need to breathe air. Because fish do not need to breathe air they do not have lungs. This goal-oriented or *teleological* approach to describing the forms in nature dominated western thinking about the adaptedness and diversity of life for centuries [32].

During the scientific revolution, goal became less and less popular as a sufficient explanation for natural phenomena. Aristotelian biological science was not exempt from criticism. The complexity and adaptedness of organisms was no longer deemed to be explained by the necessity of such complexity and adaptation. In 1800 Lamarck was one of the first to propose
a more mechanistic view of adaptation [40]. His theory stressed the interaction between organism and environment as the driving force of adaptation.

According to Lamarck a characteristic developed during an organism’s lifetime is passed to its offspring. In a classic example the giraffe developed its long neck through centuries of stretching for high leaves.

A further decisive step away from goal-driven evolution was taken by Charles Darwin with the publication of "On the origin of species" [21] in 1859. The theory put forth in this book is the first fully mechanistic, non-teleological explanation of the origin of the complexity and adaptedness of the natural world. It does away with final cause and proposes natural selection, survival of the fittest, on a randomly mutated population as explanation for the harmonious complexity of the living world. Darwin’s evolutionary theory forms the basis of modern evolutionary theory. The theory used in this thesis is Darwinian, meaning a process is explained by describing its causes in the form of a model or mechanism.

4.2 Teleological thinking

The Copernican revolution brought about by the theory of natural evolution is effected mainly by its rejection of teleological thinking. Changes that occur in organisms are no longer considered to be directed towards any goal. Change occurs randomly and is merely maintained if it turns out to be beneficial for the reproduction of the organism.

When reasoning about the perceived extraordinary adaptation of natural systems one should keep in mind the skewed view of the products of evolution one receives. Only after an organism survived for a certain period can we study this organisms methods of survival. So naturally the only organisms which are studied are the ones which are in the classic denotation "fit" or "viable". In this way evolution shows us only its most successful face.

Biological science has in part remained a teleological science because of this. Explanations for the existence of highly adapted biological features are always stories about how natural laws caused precisely those features to be developed. For instance, fish in the ocean that were able to breathe air for short periods of time could produce more offspring than fish that could not. In this way air breathing fish proliferated and amphibians emerged. This type of evolutionary explanation works toward a specific goal and can be given for almost any biological feature found today. A certain feature has come to be because that feature is highly adapted to the environment and could persist because of its adaptedness. In this way the adaptiveness of living organisms is explained teleologically: Organisms are highly adapted to living because they need to be highly adapted to live.
Any explanation of the adaptedness of a specific exponent of natural systems is bound to be teleological in nature. While such explanations are perfectly legitimate and useful for the understanding of the world we live in, they are of limited use when theorizing about evolution. The theory of natural evolution can explain many of the adaptations witnessed in nature. The probabilistic origins of the specific features that have evolved must not be forgotten however. One needs to be careful not to conclude that because a certain feature exists, it is thus inevitable that it would come to be. The natural world we know is but one of an unknown number of possible natural worlds. It is very hard if not impossible to study the alternate worlds which might have been. A problematic feature of natural evolution in this respect is that living systems themselves influence the direction of evolution greatly.

An extreme example of life altering its own evolution is found in the so called “oxygen catastrophe” the evolution of oxyphotosynthesis (the efficient energy producing mechanism of green plants and algae) 2.7 billion years ago caused certain organisms to flood the atmosphere with oxygen. Oxygen is a highly reactive substance and it was extremely toxic to the (anaerobic) organisms living then. The evolution of photosynthesis producing oxygen subsequently caused a major extinction event\cite{20} and exiled anaerobic organisms to the fringes of the environment\textsuperscript{1}. Eventually organisms evolved that successfully incorporated oxygen in their metabolism. The descendents of these organisms stand at the basis of air breathing organisms from bacteria to humans.

Evolutionary interactions like these are commonplace in nature. A change in a single organism can profoundly affect the direction of development of many other organisms. This type of change allows for certain developments while constraining others. In oxyphotosynthesis example, oxygen made the atmosphere sufficiently energy rich to facilitate organisms with increased metabolic rates. At the same time however the development of anaerobic organisms was severely constrained.

With regard to the notion of ”direction” or ”telos” of a chain of events in evolution, an important observation can be made. The initial event (e.g. oxyphotosynthesis) was the product of chance and not inevitable. For example: today creatures can exist on land because they can breathe air with lungs that evolved from fishes floating bladders. But the existence of creatures on land is by no means a goal of evolution. It was caused by a long string of contingencies, like the existence of an oxygenated atmosphere to start with. If oxyphotosynthesis never evolved organisms would look very different indeed.

\textsuperscript{1}E.g. around the hot acid deep sea volcanic vents called black smokers.
CHAPTER 4. BACKGROUND
Chapter 5

Biological Theory

This section highlights biological topics important to this Thesis. Paragraph 5.1 introduces the current theory of evolution which is the basis of many of the assumptions made in this thesis. The second paragraph, 5.2, introduces the genotype-phenotype map as found in organisms. This information forms the basis of the computational models used in this Thesis. The most important of these computational models describes the genetic regulatory network or GRN. These networks of genes found in all organisms are described in paragraph 5.3.

5.1 The modern theory of evolution

This thesis adheres to modern evolutionary theory based on Darwin. According to Darwin evolution can be explained by two natural processes: variation and natural selection.

Variation means that an organism’s offspring differ slightly from their parents and also vary amongst themselves. Due to this variation some of this offspring survives and multiplies better, is more “fit” than their siblings.

For example an organism which looks more like its surroundings is less likely to be eaten by predators.

Organisms which produce more offspring are considered more fit. This offspring is also fitter because they inherited their parents traits. The fittest of this offspring will contribute most heavily to the next generation and so on and so on.

The reproductive advantage of the fittest organisms in each generation is known as natural selection. Natural selection on variation is shown in figure 5.2. The properties that make an organism “fit” might change along with the environment and competing species. This means that future generations are not necessarily more adapted than their ancestors. They are
just more adapted to their current environment.

The details of Darwin’s theory, many of which were unknown in his time, have since been filled in. It was not until the beginning of the twentieth century that the location of hereditary information was pinpointed in cell nuclei. Until then Darwinists had been unable to explain how traits got passed on from parent to child.

Fourty years later DNA molecules, the carriers of genetic information, were discovered. This further sharpened the understanding of hereditary mechanisms. Eventually all new discoveries were reconciled with Darwin’s theory of evolution. The so called modern synthesis of evolutionary theory was born.

In the modern evolutionary synthesis, the biomechanical processes causing genetic variation are based on the DNA molecule. Genetic information is contained in the DNA which is inside each cell in an organism. Through transcription DNA is used as a template for the creation of RNA, a molecule very similar to DNA. Through translation this RNA strand is translated into a protein. Proteins are the prime sources of cellular activity.

Proteins catalyse specific chemical reactions, act as gates on the cell membrane and are generally involved in almost every cellular process.

The form and function of a cell are ultimately encoded in its genetic information. Recent research has uncovered many different carriers of genetic information in a cell. For the purposes of this thesis it is sufficient to adhere to the traditional view of genetics. That is to locate all genetic information in the DNA. A part of DNA which encodes a single protein is called a gene. The protein which is coded for in a gene is called the gene product of that gene. DNA is subject to mutation through external influences or duplication errors.

Mutations in a reproductive cell like a sperm or egg cell are passed on to the offspring. As a result, the enzymes encoded by this mutated DNA are slightly different. Thus, the source of darwinian variation is explained in biomechanical terms.

Although basically correct, the picture of DNA coding for proteins is not as straightforward as it seems. The relation between genetic information and cell function is extremely complex. Information and structure can not be separated easily in an organism.

For example, RNA is not just a messenger for DNA but has a catalytic function as well. This means RNA can both carry information and effect chemical reactions. Also, many proteins are modified by other proteins after they are translated. This means that proteins active in a cell need not actually be encoded in the DNA in that form.

Another example of the complex interaction between genotype and phenotype is that proteins encoded by one gene can improve or inhibit the
Figure 5.1: DNA is passed on to the children of an organism. DNA determines an organism's characteristics in the following way. DNA is transcribed into RNA. RNA is translated into enzymes. Enzymes perform functions in a cell. See section 5.1. source:[16]

production of a different gene. In this way one gene regulates another. In this process the flow of information is reversed: the phenotype (proteins) influences the genotype (Genes) and not the other way around.

In summary, many levels of organisation exist which influence each other through many forward and backward connections. Information and structure cannot be separated easily in an organism.

5.2 Genotype-phenotype map

Variation is caused by mutation and natural selection acts on this variation. At first glance this is a straightforward idea. Changes in an organism are caused by mutations in the genes, and the best changes survive because the organisms carrying them reproduce more successfully.

Upon closer investigation an important step is missing from this idea. Variation acts on the genotype, while natural selection acts on the phenotype of an organism. The changes that are possible depend on the way in which the genotype gives rise to the phenotype, i.e. the genotype-phenotype map. For every change in the genetic information this map yields the resulting changes in the form of an organism. The reverse relation, that each conceivable change in form can be expressed in genetic terms, is not always true. This is because not every change in form can be achieved by changes in the genotype.

The genotype-phenotype map has attracted increasing research over the past two decades. Several important publications are mentioned in "Previous Work", section 7.
Figure 5.2: Descendants of an organism are not identical to each other but vary slightly as a result of mutation. Due to this variation some siblings will be more likely to survive and produce offspring. The surviving organisms pass on their traits to their offspring in turn. This process is called natural selection. See section 5.1
Historically several parts of the map have been researched. The color of flower petals is a classic example. The color of the petals is determined by a single gene. During the growth of a plant this gene is active and is translated into a protein with a certain color. Change the gene, change the color produced. In other words there is a straightforward one to one relationship between genotype and phenotype. This example was used by Gregor Mendel to determine the rules for genetic inheritance in plants in the nineteenth century.

The parts of the genotype-phenotype map which have been explored in detail are, almost necessarily, the simple parts. Simple relations are much easier to observe and formalise than complex ones.

This observation bias (one gene apparently controls one trait) can lead one to think that for every observable trait there is a corresponding gene. If enough research is done we could find the length-gene, the ear-size-gene, the number-of-arms gene etc.. This is not the case.

First of all the term "trait" or "feature" can never be rigorously defined. One can think of an almost infinite amount of features: length of the right toenail, curvature of the right toenail in two directions, thickness, material, speed of growth etc. etc. There are many more features than there are genes. It is impossible to assign a gene to each feature. We could try to assign certain genes to the most important or obvious features of an organism. Caution is advised however. A "feature" is a human interpretation and not objectively measurable. There is no guarantee whatsoever that the human conceptions of features coincide with the genetic basis of these features.

Is a toenail a separate entity in genetic terms? or are all toenails controlled by a single gene? Maybe there are two genes which control only the thickness of the right part of every toenail only when three other genes are not active?

This last example may be closer to the truth. Genetic research has revealed a second reason for mistrusting the one-trait one-gene hypothesis. Many factors were discovered that moderate the effect a gene has on cell function. Other genes, the environment inside the cell and the environment outside the cell (e.g. hormones) all influence gene expression.

This complex network of interactions makes it impossible to name a single source gene for all but a few traits. Or reversedly: the traits that most genes code for can not easily be conceptualised.

In summary: there are phenotypic features which are coded for by only one or a few genes but most phenotypic features can not be traced back to a single gene. The genotype-phenotype map is not that simple.

The terms genotype and phenotype have very abstract definitions. The first refers to the encoding elements of a system, such as DNA nucleotides or genes. The second refers to the form encoded by these elements. Because of

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Figure 5.3: Some of the levels of organisation in biological systems. The genotype-phenotype map can be studied at any level. For example, one can study how DNA codes for a protein, or take genes as basic units and study how gene interaction gives rise to patterns of gene expression.
this abstract definition, the term genotype-phenotype map can be applied to different levels of biological organisation. Figure 5.3 shows such different levels of organisation in biological systems. When discussing general properties in this section the term genotype-phenotype map will refer to the mapping between DNA base pairs and the structure of an entire organism. This definition of a genotype-phenotype map is the widest one can make and useful for general remarks. Many levels of organisation exist between DNA sequence and organism structure. Each of these levels complicates the total map by adding interaction steps. For detailed study it is more fruitful to define the genotype-phenotype map between adjacent levels of organisation.

For example between RNA sequence and the two dimensional folding pattern of RNA. Many studies use RNA to model the genotype-phenotype map [7][27][51][52][53] RNA has a sequence of base pairs similar to DNA. But unlike DNA, RNA can fold itself like an enzyme and it can catalyse reactions like an enzyme. The folding is based on attractions and repulsions between the base pairs it contains.

The folding of RNA is quite well understood and can be simulated on computers. In this way RNA can serve as a simple model of a genotype-phenotype map. Many interesting properties of the genotype-phenotype map exist in the RNA sequence/ RNA form map. These properties are discussed further in section 7.

The genotype-phenotype map determines the phenotypic changes that are possible given random mutations to the genotype. Consequently the structure of this map determines the evolutionary directions that are available to an organism. The genotype-phenotype map, as witnessed in natural organisms, is remarkable in several respects. First of all it confers robustness to an organism. Genetic mutations often do not have destructive phenotypic effects. This is remarkable given the complexity of even the simplest of organisms. A random change in the phenotype of such an intricate system should almost certainly break something.

Think of randomly modifying a gear in a watch. For example to double its size or to increase its number of teeth. It is very unlikely that the watch would still work afterwards. Yet an organism can take many random modifications in its genotype without breaking down.

This discrepancy suggests that a direct connection between genes and features does not exist in biological systems, that the effects of gene mutation are somehow dampened.

A second remarkable property of the natural genotype-phenotype map is that it confers phenotypic flexibility to the organisms. Through random mutation phenotypic variation is produced that natural selection can act upon. This flexibility is remarkable because with an increasing number of interacting parts, a system generally becomes harder to change without
disrupting its function.

5.3 Genetic Regulatory Networks

Genes can regulate each others’ behaviour. The gene products of one gene can improve or inhibit the production of another. Multiple genetic interactions of this kind form networks of regulation.

Genetic regulatory networks or GRN’s determine which genes are active at which time and in what order. In this thesis GRN’s are modeled and used to investigate the genotype phenotype map.

GRN’s perform an important function as they guide a multicellular organism’s growth from fertilised egg to maturity. A central publication on this subject is on the segment polarity network of the fruitfly. In 2000 von Dassow et al. published the network of genes which determine the locations of segments such as of the head, thorax and abdomen in a fruitfly [60]. This network is depicted in figure 5.4. It was also shown that the pattern of expression effected by this network is highly robust to changes in concentrations of the gene products.

Genetic regulatory networks or GRNs play an important part in evolution. Through GRNs evolution can speed up greatly by acting on a higher level of organisation.

Innovation commences slowly in the functional regions of a gene. A functional region is the part of a gene which actually encodes a protein. The timing and positioning of these proteins is based on interactions in GRNs.
Gene-gene interactions take place in so called *enhancer* and *supressor* regions. Mutation of these regions can change the interactions in a genetic regulatory network and thus the timing and positioning of proteins in a cell.

This way, the expression patterns of gene products can mutate without having to evolve new functional gene products from scratch. Mutation of GRN interaction appears to be a major source of variation among higher organisms [30].

DNA regions responsible for gene regulation are much more elaborate in higher organisms. Greater phenotypic complexity seems to be caused by evolution of gene regulation. This fact helps to explain the great conservation in genes among species despite great phenotypic differences. For instance, we share about 97\% of our DNA with mice [45] and about 50\% with bananas [44].

A human being gets drunk by consuming alcohol. When a wasp consumes alcohol it reacts in a similar way: its flight becomes uncoordinated and its behaviour is less controlled. Apparently important biological mechanisms of the central nervous system are the same in human and wasp. The body structure of human and wasp is quite different, but the basic building blocks react the same.

In conclusion, evolution in higher species seems to be mainly the result of mutations in GRN interactions.
Chapter 6

System Theory/Mechanical Theory

In this thesis two consequences of natural genotype-phenotype maps are investigated. The first is adaptability or innovation, the possibility of phenotypic change in organisms despite their complexity, explained in section 6.2. For innovation to be possible, the phenotype should not be disrupted at every genotype change. In other words, the second property, robustness is needed. Section 6.3 investigates different kinds of which are found at several levels of organisation in an organism.

the effect of genotype-phenotype map structure on robustness and innovation is explored. Which properties of the genotype-phenotype map cause robustness and innovation? Two properties are measured. One is redundancy. The other is degeneracy, explained in section 6.4 and section 6.5 respectively. A useful framework for discussing the genotype-phenotype map is explained in section 6.6.

6.1 Biological System

A biological system is collection of elements which together perform some function. This collection of elements can be specified over any level of biological organisation.

On a small scale for example, the cell compounds and proteins which together translate RNA into enzymes can be called a system. On a larger scale the term "system" can refer to an entire organism from genes to outer form.

The terms "natural system" or "living system" are used as synonyms.

An organism is regarded as a complex mechanism. It can ultimately be understood by studying its parts and their interactions. This mechanism is
not limited to the physical form but also includes the genotype-phenotype map. Thus, the phrase “biological systems display astounding adaptability” refers to the evolutionary adaptability of the phenotype and not to the adaptability of the physical biological system itself.

This thesis is not about adaptation within a single generation or organism.

6.2 Innovation

Innovation refers to the ability of a system to yield new variants of itself based on random genetic mutations.

An innovative system can produce variation based on mutation. Through this variation evolution becomes possible.

The important question here is not whether a system can produce variation. In almost any genotype-phenotype mapping mutation of the genotype will eventually cause changes in the phenotype. When genetic mutation causes a great array of random phenotypes which are never functional it can hardly be called innovation.

The question is what type of variation a system can produce based on genetic mutation. Are the variants wildly different or slightly different? Are some of the variants still able to perform all the functions that the original could?

In this thesis it is assumed that innovation which progresses gradually is preferable, as large jumps would disrupt a system’s function too easily. In order to have meaningful (gradual) innovation, a system requires a certain amount of robustness to mutation.

6.3 Robustness at different levels

Robustness means invariance in the face of disturbance. Robustness is vital for evolution. Robustness creates room for innovation. If any change to a system would cause it to stop functioning there could never be any innovation. In natural systems there is room for innovation. Robustness is ubiquitous in biological systems. Biological systems are robust in different ways. A biological system can be divided into different levels of organisation (see figure 5.3. At each of these levels robustness is observed [62, 37].

The causes for this robustness are different at each level. Because of the many guises of robustness it is important to specify what it is that is robust, and what it is robust to. In this thesis, robustness will be studied at the level of the gene network. Before explaining this level in more detail, examples of robustness on other levels are given below.
The genetic alphabet is robust. More specifically, the encoding scheme of amino acids by codons is robust to changes in the codons. A codon is a triplet of nucleotides that codes for an amino acid. The coding is not one on one, as a single amino acid is coded for by several codons. For example, the amino acid Arginine is coded for by the codons, CGU, CGA, CGG, CGC, AGA and AGG. This coding scheme can be said to degenerately code for the amino acid Arginine. This is because no two nucleotides have the same function in bringing about the coding of Arginine so there is no clear redundancy. The number of coding codons varies per amino acid. As a result, some amino acids are more susceptible to mutation than others [9].

A different example of robustness in natural systems is found in protein function, which is robust to changes in its amino acid building blocks [62, Ch.5]. Proteins make up the molecular workforce of a cell as they catalyse reactions.

The function of a protein depends mainly on its three-dimensional or tertiary structure. This tertiary structure is in turn determined by the amino acids making up the protein. Robustness in this case comes from the fact that many different amino acid combinations result in the same tertiary structure and thus the same function.

Other levels of organisation in which robustness is found include protein secondary structure which is robust to amino acid substitutions [27]. Metabolic chains’ throughput is robust to changes in the enzymes making up the chain. Gene expression is robust to changes in the regulatory regions of a gene [42] and Gene expression patterns of regulatory networks are robust against changes in network connections [60].

The function of organisms as a whole also appears to be robust to deletion of entire genes. A study in which yeast genes were systematically silenced or "knocked out" [67] showed that for more than half the genes in the genome, deletions did not influence growth rate in a food-rich or minimal environment.

In mice, gene deletion did not incur any notable phenotypic effects in up to 30% of genes. Numerous examples exist of organisms maintaining function whilst missing genes previously thought indispensable [29], [14].

Is this robustness an unavoidable consequence of the type of systems employed by organisms? Is robustness an evolved property? This question will have to be asked for each level individually and even then the answer might not be a straightforward one or the other. Any complex evolving system needs at least a minimum level of robustness because this provides space for exploration of new phenotypes [37].

To what extent robustness is an evolved property remains unclear. It is quite possible that there has been selection for robustness on the RNA level [9] [8]. The genetic code itself is still seen to evolve in several species [38].
In RBN models of gene networks it was found that evolution of robustness is possible in principle [18]. Also, robust organisms can outperform fitter and less robust organisms [66, 48] giving a foothold for natural selection.

Although robustness is achieved by different means on each level of organisation, one can discern patterns. Often a problem has many different solutions: many codons code for the same amino acid. Many amino acid sequences result in the same secondary or tertiary protein structure. Many regulatory region configurations result in the same gene expression. Many gene network interactions result in the same expression pattern.

The contrast with human engineered systems is apparent. Complex human engineered systems usually have few alternative solutions. Think of changing a gear in a watch which will almost certainly destroy the mechanism. More importantly in human engineered systems there usually is no sequence of changes which will go from one solution to another without breaking the mechanisms in intermediate steps. In biological systems the genotype can change considerably without altering the phenotype.

### 6.4 Redundancy

Redundancy is used in two related ways in this thesis. First of all redundancy is a property of the constituent elements of a system. A part is said to be redundant when there are other parts which perform the same function within the system. Think of a hospital’s power supply system which houses two power generators for example: if the first generator fails to produce electricity, the second power generator can take over its function. In this case the power generators can both be called a redundant element.

A second use of the word redundancy consists in naming a system in which there are redundant elements a redundant system. Using the word in this way the hospital’s power supply system itself can be called a redundant system.

### 6.5 Degeneracy

The robustness of many natural systems can not be explained by redundant elements alone [24]. For example, in gene networks [60] or gene network models [18, 17] robustness to changes in network parameters can not be explained by duplicate genes backing each other up [18, 64]. Robustness of natural systems which can not be explained by redundant parts is called degeneracy.

Degeneracy [24], also named distributed robustness [61], is a property of a system in which robustness is achieved without using redundant parts.
Degeneracy and redundancy are both properties of networks. Both are associated with the robustness of system function to changes in their constituent parts. A redundant system achieves robustness by means of multiple identical elements which all perform the same function (see section 6.4) while in a degenerate system robustness can not be explained by identical elements.

Examples of degeneracy abound in natural systems. Many codons code for the same amino acid. Many amino acid sequences result in the same secondary or tertiary protein structure. Many regulatory region configurations result in the same gene expression. Many gene network interactions result in the same expression pattern. The function of all these systems is robust to changes in their underlying structure.

6.6 Neutral networks

The genotype-phenotype map was defined as a mapping between genetic information and the form that this information codes for. In other words the map tells us how the form changes when the genetic information changes. In section 5.2 it was stated that a change in genetic information rarely causes a clear change in the form of an organism. In fact, studies show that a change in the genotype often does not cause a change in the phenotype at all [52, 14]. This is what the property mutational robustness designates: a mutation often leaves the phenotype intact. Mutations which do not alter the phenotype are called neutral mutations.

A neutral network is a set of genotypes which all code for the same phenotype. Neutral networks can be understood in terms of phenotype space and genotype space which will both be explained below. In nature genotype space is infinite, as there is no natural limit to the number of genes or genetic elements. In simulations this infinity is a problem. Many mathematical operations are hard to perform in such an infinite space. Also the space of possibilities is so large that simulations are impossible. A common abstraction to alleviate this problem is to fix the length of a genotype to a certain length [17, 61]. In this way there exists only a finite number of genotypes and consequently a finite number of phenotypes as well. In this thesis the length of a genotype also fixed to a single value. The elements in a genotype are taken from a finite alphabet. For instance, DNA consists of a sequence of bases which can only be of the type A, C, D or G. In a model where the number of elements is fixed to \( n \) this means that a length-\( n \) genotype can be represented as a point in a finite space of \( n \) dimensions. This space, which contains all possible genotypes of length \( n \) is called the genotype space. Similarly the range of phenotypes that is encoded in is called phenotype space.
A phenotype is harder to decompose into dimensions, but for now it is sufficient to assume phenotype space contains the range of phenotypes which are possible.

Each point in genotype space maps to a certain phenotype. The distribution of phenotypes in genotype space is very important for understanding the properties of the genotype-phenotype map. For instance the property of robustness: It has been found that many mutations in natural organisms leave the phenotype intact. This signifies two things for the genotype-phenotype map. First of all, several genotypes map to the same phenotype. Secondly these genotypes are connected to each other through single mutations. These two facts together define a neutral network. A neutral network is a collection of genotypes connected by single mutations. All these genotypes result in the same phenotype. A mutation which moves the genotype on the neutral network is called a neutral mutation.

Through a neutral network the genotype of organisms can change while the phenotype stays the same. Neutral networks cause genetic robustness, because a neutral mutation will not disturb the phenotype. Each phenotype in phenotype space is associated to a neutral network. Two neutral networks can be connected to each other through mutations. An organism jumping from one neutral network to another will undergo a change in phenotype. Figure 6.1 shows several points in genotype space where points of the same color are in the same neutral net.

The view of genotypes as points in space with mutations as connections is insightful. In addition to defining neutral networks it can also be used as a general description of mutation. The set of genotypes which can be reached through a single mutation is called the mutational neighbourhood of a genotype.

Are all the neutral genetic variants which result in the same phenotype part of the same neutral network or part of small islands? These questions are better understood through the genotype space paradigm. The biological relevance of this paradigm is shown by Burch and Chao. Their analysis of the mutational neighbourhood of RNA viruses shows that this neighbourhood is important for evolutionary innovation in natural systems. The neutral nets of Random Boolean Networks (a model for GRNs, see section 10.1) have been explored by Wagner et al. The study suggests that neutral nets span genotype space. This facilitates innovation as many different genotypes can be reached without compromising the phenotype.

Robustness can be better understood in the genotype-phenotype space/neutral net paradigm. The different solutions to the same problem are all points in genotype space. Any move from one of these solutions to another does not alter the phenotype. An important question in this respect is whether such a move is probable or even possible. If the solutions are part
Figure 6.1: Several points in genotype space where points of the same color are in the same neutral net. See section 6.6. Image source: [27]
of the same neutral network, any move in this network will not alter the phenotype. Subsequently the system is robust to any mutation which keeps it on the neutral net. A population of systems will inhabit different places on a neutral net. Random mutations move the systems over the neutral net. An interesting and elegant paper by Nimwegen et al. [59] explores the relation between robustness and neutral net topology. Nimwegen et al. show that a population of systems tends to drift towards the most robust solutions. Their theory can be summed up as follows. Robust solutions are connected to many other solutions by single mutations. The probability of randomly moving to a certain solution scales with the number of paths leading to that solution. The solutions with the greatest probability of being visited are the best connected ones. These are also the most robust for the same reason: many connections to neutral neighbours means high probability of staying on the network. Nimwegen's theory has been experimentally confirmed by Bornberg Bauer and Chan [13].
Chapter 7

Previous Work

In section 6 it was asserted that the form of the genotype-phenotype map is important for the evolutionary properties of a system. The effects of a genotype-phenotype map are expressed in the jumps which are possible in genotype space and the effects of these jumps in phenotype space.

Research into the genotype-phenotype map is still in the exploratory phase and a unified perspective has yet to arise. Different explanations are not mutually exclusive. Rather they are like different pieces of a single puzzle. This section outlines previous research on the robustness and flexibility of natural systems.

7.1 Modularity

The more interactions there are in a system the harder it is to change without disrupting its function. With growing system complexity the number of interactions increases. Eventually this makes all change impossible as any change will disrupt the system’s function. For a system, one way to overcome this problem is by containing modules, i.e exhibiting modularity. A ”module” is as hard to define as a ”feature” in an organism. Both are subjective terms dependent on human perception. Efforts have been made to exclude human measurement and interpretation from the definition of modularity [6]. For the purposes of this section it suffices to regard a module as a semi-self contained unit within a system. Interactions in a single module are numerous while interactions between modules are limited. Modules can also be built up of other modules in a hierarchical fashion. In this way complex systems can be built which are still flexible because interactions are limited to mostly within modules. A change in one of the modules does not disrupt interactions in other modules. Cell organelles, hairs, eyes, wings, legs, leaves and kidneys are all modules, as are power supplies, wheels, displays, and hard
drives. Modularity is ubiquitous in natural and man made systems alike. Modules definitely exist in cells and higher levels of biological organisation. Whether modularity is also present at the genetic level is an important issue in genetics. Numerous interesting discoveries have been made. In the 80s research was started which lead to profound discoveries in genetic control[47]. The discoverers of the so called homeobox or hox genes in fruitflies were awarded the 1995 Nobel prize in medicine. Hox genes are "controller" genes which pattern development. Hox gene expression determines which type of segment develops where [30]. Researchers could for example create mutants which developed two winged segments instead of one giving a single fly four wings. The presence of hox gene product is the starting sign for a host of other genes involved in segment development. In this way a hox gene controls modules on a genetic level. Hox genes themselves are controlled by a gene network which determines the position of different segments. The genetic regulatory network active in early fruitly development has been well studied [33, 60]. It is shown in figure 5.4 in section 5.3. Hox genes have also been found other animals including humans, and in plants. They provide strong evidence of modularity on the genetic level. Support for hierarchical modular architecture on the genetic level has also been found in large scale gene expression studies [54]. Experiments in evolution on the yeast Escherichia Coli, another staple organism, showed certain genes with high interaction density or pleiotropy. The research shows a population of \( \sim 5 \times 10^7 \) bacteria adapting to a new environment within \( \sim 500 \) generations. It is observed that mutations to coarse grained genes with high pleiotropy are more common. Adaptation starts with coarse adaptations which change a lot of features. Later on smaller grained compensatory mutations restore most of the altered features to their original expression levels [57] [11]. This evolutionary pattern shows the benefits of modularity at the genetic level. Mutation of controller genes can quicken phenotypic change, while the modules which are controlled can be optimized individually. Modularity can make a species more adaptable, but how can modularity evolve?

G. P. Wagner concludes that the existence of modular architecture can best be explained by a combination of directional and stabilizing selection and that it is a product of natural selection [63]. In experiments on digital evolving organisms, Edlund et al.[25] found that at high mutation rates the genetic code tends to evolve towards greater independence and less gene interaction. Another explanation for the evolution of modularity is the DDC model [28, 43] (See figure 7.1). In this model duplicate genes both retain part of the functionality of the original. After duplication both genes are free to degenerate as long as together they perform all the functions of the original. This causes both genes retain complementary subfunctions of the original. If one of the duplicates is lost, part of the function of the original
is lost, disrupting the original function. This will cause both duplicates to be maintained by natural selection. In this way degenerative mutations facilitate maintenance of both gene copies \[43\] [28]. The original function is now split between two genes. This makes it possible for the duplicates to diverge into different genetic modules.

Modularity appears on all levels of biological organisation as well as in man made systems. Modularity, its effect on evolution or even its definition are interesting research topics. Unfortunately they go beyond the scope of this Thesis.

7.2 Gene duplication

How can an organism be robust to mutation and at the same time be flexible enough to adapt? Gene duplication might provide an insight. Gene duplication is the complete cloning of a gene resulting in two identical genes in the DNA. Duplication happens quite often due to errors in DNA repair or recombination of genes. There is even evidence of duplication of entire genomes at some points in evolutionary history. These whole genome duplication events are thought to be an important motor for evolutionary innovation [23]. Just after a duplication there are two identical genes performing the same function in a redundant fashion. Under the classical model of gene duplication [49] one of the gene duplicates will become nonfunctional due to mutation because there is no selection pressure to preserve both copies. Later genetic studies showed that this was not the case. A genome typically contains many more duplicate genes than traditionally predicted. In many cases duplicate genes remain functional but quickly diverge in function [64]. This is possibly explained by the duplication-degeneration-complementation or DDC model (section 7.1, figure 7.1).

Gene duplication can increase genetic robustness by adding redundant genes. This redundancy is not enforced by natural selection however, as duplicates quickly diverge in function [64]. Empirical evidence does support gene duplication as a major source of genotypic innovation [10].

Gene duplication could facilitate genetic robustness in a different way, by affecting the topology of gene networks. The Duplication/Diversification process in network nodes can cause these networks to acquire so called scale free architecture [56]. Scale free refers to the pattern of connectivity between nodes. Irrespective of the scale at which such a network is viewed the distribution of the number of connections per node has the same form. Few nodes with many connections and many nodes with few connections. At any scale there are a few nodes which influence are connected to many nodes. In this way a scale free network is modular on a continuous scale.
Figure 7.1: The duplication-degeneration-complementation model. After duplications both copies of a gene carry all subfunctions (black squares) of the original. Due to redundant functions there is no selection pressure to preserve both copies of a subfunction. As a result subfunctions can degenerate (white squares) and become nonfunctional in either the one or the other duplicate. Degeneration eventually causes both duplicates to have complementary functions. This model helps to explain the evolution of modularity and the retention of gene duplicates. Image source: MacCarthy and Bergman BMC Evolutionary Biology 2007 7:213.
At any level there are a few "controller" nodes which control many others.

### 7.3 Shape space covering

The genotype-phenotype map found in natural systems has many unexpected features. The existence of neutral networks (section 6.6) for example. Genetic changes on the neutral net do not cause phenotypic changes. But what happens when a mutation moves the genotype off a neutral net? Some studies assume that a move off a neutral net is always fatal [59, 66]. If this was the case in reality no phenotypic change would ever be possible. This is not so. In evolution new phenotypes are discovered. For a natural system there exist many lethal mutations, but paths to other phenotypes do exist. Neutral nets belonging to different phenotypes are connected in genotype space. But how are neutral nets distributed in genotype space? Are they densely connected or sparsely? Is the genetic distance between two phenotypes large or small? Can certain phenotypes only be reached through a specific sequence of phenotypes or are there many routes? Given the enormous number of possible phenotypes it seems unlikely that any two viable phenotypes should be close in genotype space. It seems improbable enough to find any genotype which codes for a viable phenotype. The chance to find two or more close together in genotype space seems even smaller.

It turns out however that first impressions are misleading. Several studies show that natural genotype-phenotype maps are shape space covering. This means that almost any shape or phenotype can be reached in only a few steps in genotype space. In other words, shapes or phenotypes are scattered though genotype space and not concentrated in a certain area. In RNA it was shown that any two RNA phenotypes could be connected through only a few mutations in their genotype, their nucleotide sequence[53, 52, 27]. Analysis of RBNs revealed an interesting property of their genotype-phenotype map. Any two neutral networks of viable phenotypes are neighbours in genotype space at some point.[17].

A similar result was shown empirically by Schultes and Bartel using RNA molecules [51]. They showed that a mutational path exists between two RNA sequences catalysing two different reactions. RNA sequences can transform into each other in 40 mutation steps without losing functionality at any step along the way. This means that the neutral nets of the catalysts are only 40 mutations apart in genotype space. Also the intermediate genotypes all code for a viable phenotype. Research on the yeast *Escherichia coli* showed that different populations of the same strain of bacteria travel different genotypic routes towards the same (optimal) end goal [57, 11].

These results are surprising. Of all possible phenotypes few are viable.
In systems as complex as organisms there are many ways in which functionality can break down. Each viable phenotype has a neutral network associated with it. This neutral network consists of one or more genotypes. The intuition is that as there are so many phenotypes which are non-viable, the viable phenotypes are scattered through phenotype space. Subsequently one would think that the neutral networks associated with these phenotypes are also scattered through genotype space. The surprising result is that although phenotypes are scattered through phenotype space, their corresponding neutral networks are often very close to each other at some point in genotype space. This does not mean that any mutation or jump in genotype space results in a viable phenotype. Many mutations can be lethal. It just shows that for any two phenotypes there exists a short sequence of mutational steps which connects the two. With regard to the thesis question this is interesting. Neutral nets confer robustness as certain mutations do not change the phenotype, while at the same time neutral nets keep the door open for innovation by allowing for transitions between viable phenotypes.

The surprise at these results illustrates the uselessness of our spatial intuitions when reasoning about multidimensional spaces. Keep in mind genotype space is not 2 or 3 dimensional as the neutral net depicted in image 6.1 (section 6.6) would have us believe. Rather, there are as many dimensions as there are genotype elements. The genotype space of amino acids coding for a single protein can easily be 600-dimensional. On the level of an entire organism the number of dimensions runs in the millions. In these spaces our spatial intuitions are of no use. Common notions such as adjacency and neighbourhood have a different meaning.

It appears that many functional structures in natural systems can be connected by a short sequence of genetic steps. Whether this is an evolved property remains unclear. Perhaps the evolutionary observational bias confounds us yet again. Had the different structures not been close together in genotype space they would not have been found by evolution and would not be observed. Therefore we only observe structures which are close together in genotype space.
Chapter 8

Methods

This section describes the approach taken in answering the thesis question. First, the way this question is translated into a model is discussed. Secondly, the terms degeneracy, redundancy, robustness and innovation are defined.

8.1 Approach

To focus the investigations adaptation and robustness are studied only as far as they can be explained by the structure of the genotype-phenotype map. This approach excludes theories on species-level evolutionary dynamics such as co-evolution and island theory. Explanations are sought only on the level of a single biological system (see section 6.1).

The technical approach consists in defining an abstract model of a biological system and studying the genotype-phenotype map in this model. Two models are used in this thesis. Both models focus on different aspects of the genotype-phenotype map. Both models are introduced in the next section.

8.2 Choosing the model

A model to answer the thesis question needs to have the following properties:

- **model aspects of a biological system** The thesis question stems from the observation that biological systems possess evolutionary properties such as robustness and innovation which are lacking in man-made systems. To study these properties in a model, the model needs to describe a biological system. When conclusions are drawn about certain features, the model needs to coincide with biological systems on these features.
• **degeneracy and redundancy can be distinguished** Degeneracy and redundancy are both properties of the genotype-phenotype map. These properties are studied for their connection to innovation and robustness. It should be possible to define measures of degeneracy and redundancy for instances of the model. The minimal requirement of these measures is that it is possible to describe an instance as more or less degenerate or redundant than another instance.

• **the neutral net of systems generated by the model is mappable** This entails that given an instance of the model and mutational dynamics, the number of neutral neighbours can be ascertained in theory (computational limitations notwithstanding). This model property is important for connecting the thesis research to neutral net theory (see section 6.6).

In this thesis two models are considered. The *explicit mapping* model and the *Random Boolean Network* or *RBN* model.

The explicit mapping model (section 9) defines the genotype-phenotype map in the most basic way possible. Genes, functions and the relations between them are modeled explicitly as nodes with edges signifying relations. This model is used to investigate degeneracy. Degeneracy is ubiquitous in natural systems and might be important for properties such as robustness and innovation [24] (see section 6.5). The conjecture investigated with model 1 is that the prevalence of degeneracy in natural systems is the result of the connectedness of degenerate systems on the neutral net. This conjecture is based on neutral net theory by Nimwegen et al.[59]. It is found that model 1 is not discriminant enough to investigate this claim. This problem is discussed in sections 9.4 to 9.6.

The random boolean network model (section 10) models a biological system in a more complex fashion. In particular it is found to be a good model for genetic regulatory networks (see section 5.3). This model is used to investigate whether the genotype-phenotype-map properties of degeneracy (section 6.5) and redundancy (section 6.4) can be used to predict the evolutionary properties of adaptability and robustness. This is done by investigating correlations. What is the correlation of network degeneracy with the robustness to mutation of that network? Is degeneracy a good predictor of a network’s mutational robustness? Are redundancy and degeneracy complementary or mutually exclusive? It is suggested that degeneracy is an important factor in maintaining functional adaptability [24]. Is a network with higher values of degeneracy likely to be adaptable (i.e. discover new phenotypes upon mutation)?
Chapter 9

Model 1: explicit mapping

The first model used to investigate the thesis question is a computationally and conceptually straightforward model of gene/function interaction within an organism. In this model the genotype-phenotype map is represented in the most basic terms. A genotype is a collection of "gene" nodes and a phenotype is a collection of "function" nodes. The map between them consists of connections from genes to functions. This is depicted in Figure 9.1. Each gene in the sequence influences certain functions within a cell. A gene can mutate and as a result influence different functions. This influence can either disrupt or aid a function, making its state "active" or "inactive". For a complete description of the explicit mapping model see section 9.1.

The explicit mapping model was pursued for its simplicity and the direct way in which it models the genotype-phenotype map. However, after rectifying a flawed assumption (section 9.4) the following conclusion is reached: The explicit mapping model can not be used to answer questions regarding degeneracy of coding. This is because degenerate and non-degenerate mapping schemes have identical effects on neutral net connectivity. In the explicit mapping model neutral net connectivity (and consequently robustness to mutation) is a function only of the number of connections per gene and is unaffected by the specific relationship between any specific gene and any specific function. As degeneracy is a property of this specific relationship the explicit mapping model is not suited for studying the relationship between degeneracy and robustness to mutation. Section 9.4 explores this shortcoming in more detail.

9.1 Introduction

What is the cause of the many examples of degeneracy witnessed in natural systems? In this section an answer is sought in selection for mutational
robustness in neutral [59],[66] or semi-neutral [48] evolution. can the concept of selection for robustness help to explain the ubiquity of degenerate mapping in organisms? This direction of investigation is prompted by the following observation.

Given evolution on a neutral net (see section 6.6, a population of sequences will achieve a stable distribution among the possible sequences on the net. This distribution is not uniform: sequences which are highly connected to other sequences are more frequent than sparsely connected sequences[59]. Highly connected sequences are intrinsically more robust to mutation because their one-distance mutants are likely to still be functional. This robustness can be a decisive factor in evolution [66].

The effect described above is only dependent upon the connectivity of the neutral graph on which a population of sequences evolves. In the paper by Nimwegen et al.[59], sequences consist of non specified symbols. It is suggested that the symbols could stand for nucleotides but this is not a specific requirement. Nimwegen’s conclusions are valid for any type of symbol as long as the sequences created by the symbols are connected and the symbols can mutate into each other.

Based on standard evolutionary theory one would expect to find a more "efficient” instantiation relation; as long as at least one element instantiates the function there is no direct fitness bonus for any other element to instantiate the same function. One would expect to find that one of the elements which perform the same function would quickly become non-functional [49]. This behaviour is not observed in actual biological systems however[24]. The explicit encoding model will investigate the degeneracy in functional relations using the neutral network results from Nimwegen et al.[59].

9.2 Model

The explicit mapping model is an extension to the ”NK” Adaptive Landscape model described by Lee Altenberg ([5],p.26). It was originally proposed by S.A. Kaufman in 1989. The ”NK” model describes the interaction between genes and fitness on a very abstract level. This model was chosen for its simplicity and direct control over the interactions in the genotype/phenotype (gene/function) map. Lee Altenberg ([5],p.26) describes the model as follows (see figure 9.1):

1. The genome consists of $n$ binary-valued genes, that exert control over $f$ phenotypic functions, each of which contributes a component to the total fitness.

2. Each gene controls a subset of the $f$ fitness components, and in turn
each fitness component is controlled by a subset of the $n$ genes. This genotype-phenotype map can be represented by a matrix,

$$M = [m_{i,j}], i = 1...n, j = 1...f,$$  \hspace{1cm} (9.1)

of indices $m_{i,j} \in \{0,1\}$, where $m_{i,j} = 1$ indicates that gene $i$ affects fitness component $j$;

3. The columns of $M$, called the polygeny vectors, $g_j = [m_{i,j}], i = 1...n$, give the genes controlling each fitness component $j$;

4. The rows of $M$, called the pleiotropy vectors, $p_i = [m_{i,j}], j = 1...f$, give the fitness components controlled by each gene $i$;

5. If any of the genes controlling a given fitness component mutates, the new value of the fitness component will be uncorrelated with the old. Each fitness component $\phi(i)$ is a uniform pseudo-random function of the genotype, $x \in \{0,1\}^n$:

$$\phi(x) = \Phi(x \circ g_i, i, g_i) \sim \text{uniform}_{[0,1]},$$  \hspace{1cm} (9.2)

where $\Phi : \{0,1\}^n \times \{1,\ldots,n\} \times \{0,1\}^n \mapsto [0,1], \circ$ is the Schur product ($x \circ g_j = [x,m_{ij}], i = 1...n$).

6. If a fitness component is affected by no genes, it is assumed to be zero:

$$\Phi(x \circ g_i, i, g_i) = 0 \text{ for all } x, \text{if } g_i = [0...0];$$

7. The total fitness is the normalised sum of the fitness components:

$$w(x) = \frac{1}{f} \sum_{i=1}^{f} \phi_i(x).$$  \hspace{1cm} (9.3)

### 9.3 Model modification

The NK model defined in the previous section has been used to illustrate the fitness effects of adding new genes to the gene collection\5. In this thesis I will use it to study neutral evolution and its effects on degeneracy of encoding. To this end I will alter and extend the model in several ways:

1. In the NK model the value $\phi_j$ of each fitness component/function $j$ is a (pseudo-)random function of the polygeny vector $g_j$. $\phi_j$ is distributed
CHAPTER 9. MODEL 1: EXPLICIT MAPPING

Figure 9.1: The NK model as a map between functions and genes. Each gene $i = 1...n$ can affect several functions $j = 1...f$ and each function can be affected by multiple genes. The mapping can be represented by a matrix (see section 9.2)

uniformly on $[0, 1]$. This means that any change to $g_j$ has a .5 chance of turning $j$ on or off.

The assumption made in the the NK model that any gene sequence will support each function with probability .5 entails that a gene will on average support half of the functions and vice versa a function will on average be supported by half of the genes. Although the classic view of one gene per function has long been abandoned, the .5 support probability probably overestimates the pleitropy present in biological systems. It will be attempted to find a more biologically plausible value.

In this thesis I choose to use a different function for $\phi_j$. I am modeling the elements which support a certain function. My interest in the difference between duplicate elements which support a function and structurally different elements. As a result I which to have a clear model of several elements supporting (implementing) a function. I which to model the superfluous nature of subfunctions more naturally.

In this relation $\phi_j = 1$ when there is any gene in polygeny vector $g_j$ supporting $j$. The new relation can be written as

$$\phi_j = \begin{cases} 1 & \text{if } \sum g_j > 0 \\ 0 & \text{if } \sum g_j = 0 \end{cases} \quad (9.4)$$

2. I am modelling neutral evolution. In accordance with previous work
9.4 MODEL WITHOUT STACKING GENE SUPPORT

[59], all viable gene sequences will have the same fitness. All non viable sequences will have fitness zero. The \( f \) fitness components defined in section 9.2 are recast as essential functions. This means that any gene sequence \( x \in \{0,1\}^n \) which does not attain \( \phi_{ij} = 1 \) on all fitness components/functions is not viable.

Rather than a variable fitness \( w(x) \), the only sequences considered in the model are those for which

\[
\frac{1}{f} \sum_{i=1}^{f} \phi_i(x) = 1.
\]  

(9.5)

3. In the original NK model the \( n \) genes that make up a sequence of genes are unrelated. A mutation to any of the genes causes random effects in the \( f \) functions. There exists evidence that gene mutation moves the phenotype between several distinct "frequent types" which have different and often non-deleterious effects on the phenotype (cite relevant RNA/higher level).

In my model the \( n \) genes that make up a sequence are chosen from a collection \( C \) of \( t \) gene types. Similar to a pleiotropy vector \( P_i \) defined in section 9.2, a gene type \( k \) defines which of the \( f \) functions are affected by a gene of type \( k \). Collection \( C \) can be viewed as a \( f \times t \) matrix in which each column represents a function and each row represents a gene type. Each of the \( k \) gene types can mutate into each other according to graph \( H \). By default, \( H \) is assumed to be fully connected, meaning any gene type can mutate into any other gene type. See figure 9.2 for further reference.

4. Sequences on the neutral net consist of a fixed number of genes, \( m \). In figure 9.3, a neutral network of three-gene sequences is shown. The alphabet of genes used in these sequences is 1, 2. In this example, gene 1 instantiates the only function (\( a \)) and gene 2 does not. In this way the sequence 1 1 1 codes for function \( a \) three times while sequence 1 2 2 only codes for the function once and further contains only non-functional DNA. In figure 9.3 the genes 1 and 2 can mutate into each other in only one simple way: 1 can become 2 and vice versa.

9.4 Model without stacking gene support

This section discusses the consequences of changing the way a "mutation" event is modelled in a system.
Figure 9.2: Example gene type collection and mutation graph. For a sequence to be viable, all functions (here a, b, c) need to be instantiated. Gene type matrix element $C_k, l$ is white when gene type $k$ instantiated function $l$. For example gene type 1 instantiates functions $a$ and $c$ but not $b$. In this example the sequence $[1,2]$ would be viable while $[1,1]$ would not, because function $b$ is not instantiated by $[1,1]$. 
There are only 2 gene types (1, 2) and only one function (a). Gene type 1 instantiates function a while gene type 2 does not. This means that as long as gene type 1 is represented in the gene sequence, the sequence will be viable. Note that sequence [2,2,2] is not part of the network as it is not viable.
CHAPTER 9. MODEL 1: EXPLICIT MAPPING

Modification 1 to the explicit model (section 9.3) states that a function will be implemented as long as there is at least one gene supporting it. This means genes are thought to "stack" support for a function. This assumption implicitly states that a mutation can not have direct deleterious effects. When a mutation causes a gene to interact with a known function, this gene will always help to support this function. This contradicts empirical findings [14],[67],[29].

The assumption originally made by Altenberg et al. regarding mutation effects of genes is this: upon mutation of a gene, all functions that are influenced by this gene will be disturbed with probability \( p_d \). Reverting to the original assumption has consequences for the model. The effect of gene mutation on functions is defined as a probabilistic event. There are no explicit gene types a gene can mutate into, there is just abstract "mutation". It is not possible to differentiate between a system's genotype and its 1-mutant neighbour genotypes. Consequently it is not possible to explicitly map out the neutral net on which viable systems can move. The lack of an explicit neutral makes it impossible to count the number of neutral connections a system has on a neutral net. However, comparisons between systems can still be made as it is possible to define the ratio with which mutations cause a system to leave the neutral net.

Consider a system \( M \) containing a single gene \( i \) which is associated with one function \( f \). A mutation in \( i \) will cause function \( f \) to be disturbed with probability \( p \)

\[
P_{M \text{fail}} = p \tag{9.6}
\]

A gene can be associated with several functions. The vector of functions associated with gene \( i \) is designated \( P_i \). Upon mutation of \( i \), each function in \( P_i \) will be disturbed with probability \( p \). This will cause at least one of the functions \( P_i \) to be disturbed with probability

\[
P_{M \text{fail}} = 1 - (1 - p)^{c_i} \tag{9.7}
\]

in which \( c_i = |P_i| \) is the number of functions connected to gene \( i \). For a system \( M \) of \( N \) genes and \( f \) functions as defined in section 9.2, the probability that the mutation of a randomly chosen single gene disturbs a function and thus moves the system off the neutral network is given by:

\[
P_{M \text{fail}} = \frac{1}{N} \sum_{i=1}^{N} 1 - (1 - p)^{c_i} \tag{9.8}
\]
9.5 Properties of the neutral net

Equation 9.8 defines the probability that a system $M$ will move off the neutral network given mutation of a randomly chosen gene in $M$. This probability can be interpreted as a measure of robustness of the system to mutation. Low $P_{M}^{fail}$ indicates a robust system, High $P_{M}^{fail}$ indicates a fragile system.

With equation 9.8 the relation between genes and function connections and the robustness of a system can be investigated. Before going into degenerate or redundant encodings let’s first look at the distribution of connections between genes and functions. Which is the more robust, a system in which all genes share the available connections or a system in which some genes are better connected than others? To answer this question the two most extreme cases will be investigated: System $M_{d}$ which distributes the total number of connections $C$ equally among each of it’s $N$ genes, and system $M_{c}$ which concentrates all $C$ connections on a single gene and leaves the other genes without any connections. The random mutation failure probability for system $M_{d}$ is given by

\[
P_{M_{d}}^{fail} = \frac{1}{N} \sum_{i=1}^{N} 1 - (1 - p)^{\frac{C}{N}} \]

(9.9)

\[
= 1 - (1 - p)^{\frac{C}{N}}
\]

(9.10)

The random mutation failure probability for $M_{c}$ is given by

\[
P_{M_{c}}^{fail} = \frac{1}{N} (1 - (1 - p)^{C} + \sum_{i=2}^{N} 1 - (1 - p)^{0})
\]

(9.11)

\[
= \frac{1}{N} (1 - (1 - p)^{C} + \sum_{i=2}^{N} 0)
\]

(9.12)

\[
= \frac{1}{N} 1 - (1 - p)^{C}
\]

(9.13)

$P_{M}^{fail}$, the probability that a system will move off the neutral net attains a minimum at $C = 0$ for both $M_{d}$ and $M_{c}$. This would mean that the most robust system is one where there are no connections between genes and functions. For equal $N$ and equal $p$ ($0 \leq p < 1$)

\[
\lim_{C \to \infty} P_{M_{d}}^{fail} = \frac{1}{N}
\]

(9.14)

\[
\lim_{C \to \infty} P_{M_{c}}^{fail} = 1
\]

(9.15)
Which means that for large numbers of connections the system with the lowest probability of breaking down and thus the largest robustness is the concentrated system $M_c$. In fact $P_{M_c}^{\text{fail}} \leq P_{M_d}^{\text{fail}}$ for all values of $N, C, p$.

9.6 Conclusion

A system using mutation dynamics as defined in section 9.2 but defining a mutation event with the probability of disturbing each connected function was investigated. Greater concentration of connections on one gene was found to correspond to a larger proportion of neutral neighbours. Systems maintaining this greater concentration will be more robust to mutation and thus poses greater fecundity [66] [48]. The effects of degenerate and redundant connection schemes can not be investigated in these types of models as the two are indistinguishable. This can be inferred from the fact that the probability $P_{M_i}^{\text{fail}}$ is computed using only the number $C_i$ of connections for gene $i$ and not the specific functions that $i$ is connected to. Because degeneracy and redundancy both describe the way genes are connected to specific functions they can not be distinguished in this model.
Chapter 10

Model 2: Random Boolean Networks

Because of the limitations of the explicit mapping model a different model has to be used to investigate the phenotype genotype map. The explicit mapping model was found to be too simple to differentiate between degeneracy and redundancy. A more complex model is needed. The boolean network model, a network model in which each node determines its activation based on the other nodes in the network (see section 10.1) is chosen for the following reasons:

- **model aspects of a biological system** RBNs are considered to model important aspects of Genetic Regulatory Networks and are frequently used as such [17][18] [4].

- **degeneracy and redundancy can be measured and distinguished** measures of degeneracy and redundancy exist [24] but need to be adapted for use in RBNs. The adaptations required are worked out in section 10.4.

- **the neutral net of systems generated by the model is mappable** This is the case for RBNs. The number of genotypes scales exponentially with the number of nodes so exhaustive mapping is limited to small networks. The neutral net of large RBNs is investigated for connectedness by [17][18] and connectedness is measured indirectly by some authors [19][31]

10.1 Random Boolean networks: Theory

Random boolean networks or RBNs are discrete dynamical models used to model gene regulatory networks. An RBN consists of a number of nodes
CHAPTER 10. MODEL 2: RANDOM BOOLEAN NETWORKS

with connections between them. A node state is boolean. It is either 1 or 0. The state of each node can change through time. Time is measured in discrete intervals. In each time step the state of each node is updated based on the activations of the nodes it is connected to. Each node is associated with a boolean function over the states of the connected neighbours (see figure 10.1). An RBN is basically a network of nodes which continuously exhibits new patterns of activation based on the previous pattern. It is insightful to think of all possible RBN states as existing in a state space. At each time step an RBN moves from one state to another. In this way an RBN traces out a path through this space. The number of states in RBN state space is finite. As each node can only take on two states an n node RBN can take on $2^n$ different states.

The term "random" in random boolean networks is a historical peculiarity. Boolean networks were first studied by Stuart Kauffman in the late 60s [35]. In these studies the general behaviour of randomly generated boolean networks was studied. Accordingly Kauffman spoke of the study of random boolean networks or RBNs. Afterwards any study building on this early work has referred to their object of study as RBNs, whether they were generated randomly or not [17, 18, 31, 4]. In this thesis this convention is maintained. Any specific instance of a boolean net als referred to as RBN even though there is nothing random about the net itself.

An RBN can be seen as an abstract model of a genetic regulatory network. Similar models have been used for studying amongst others early development of drosophila [33], the evolution of genetic robustness [61] and innovation [17, 4]. Recall that genetic regulatory networks consist of genes which influence each others’ activation in various ways (section 5.3). In RBNs genes are represented by nodes. The continuous activation of real genes is abstracted to two node states. The influence among genes is represented in an RBN by the connections that exist between nodes. Each node in the network updates its state based on a boolean function over all its neighbours. To keep the focus on general network properties the boolean functions are generated randomly. The functions are generated randomly but once generated they are fixed and a node behaves in a deterministic way. For each possible combination of neighbour activations of a node the resulting activation is recorded in a table. Figure 10.1 shows a small RBN with the update rules for a single node given as an example.

RBNs are abstract models but capture important dynamics of gene networks [35, 36, 68]. For example robustness to noise and cyclic behaviour [50] and degeneracy and evolvability [26]. Simple boolean networks can generate a very large array of spatial patterns [55]. They have also been used to study the role of gene duplication in evolution[31, 4]. An important feature of the RBN model is its ability to model different phases or modes of activity of
Figure 10.1: A Random boolean network consisting of three nodes. To the right the boolean update rules for node B are shown. When A and C are both 0, node B will be 0 in the next timestep. Otherwise node B will be 1. Note that nodes can have self-connections. This means that the current state of the node itself is also used in the boolean function determining the next state.
a genetic network. A "mode of activity" manifests itself as a *cycle* in the state space of an RBN. Each biological cell contains the same set of genes. Mediated by external factors different modes of activity are displayed. RBN cycles have been compared to different cell types in biological cells[34, 36]. They have also been used to model the cell cycle of organisms, for example the yeast *Schizosaccharomyces Pombe* [22].

The genotype of an RBN is given by the connections between its nodes and the boolean functions which govern the nodes. The connections between RBN nodes are implicitly contained in the structure of the boolean functions. If a neighbour is represented in a node’s boolean function then it follows that there exist a connection between the two. This means that information on the connections between nodes is superfluous when the boolean functions are known. The connections between nodes can be regarded as a tool for visualising the structure of the boolean functions. As such the *genotype* of an RBN is the set of boolean functions for each node. The *phenotype* of an RBN is in this thesis identified with the cycle an RBN ends up in. In this way the genotype-phenotype map modeled by RBNs exists at a very specific level of organisation. The genotype-phenotype map of genetic regulatory networks can be studied. In biological terms the genotype is given by the influences genes have on each other. The phenotype is the phase in which a regulatory network ends up.

The state of an RBN is the activation pattern of its nodes. For example in a network with nodes [A,B,C] a state could be [1,1,0], meaning nodes A and B are in state 1 and C is in state 0. A cycle is a collection of states which infinitely transition into each other. The existence of cycles in state space is unavoidable in an RBN. Each RBN state is fully determined by the previous state. There are a finite number of states. This means that at some point a previously visited state will be encountered. When this happens the RBN will trace out the exact same route through state space until it comes to this state again and so forth in an infinite loop. For example, if state [1,1,0] leads to state [0,1,1] and [0,1,1] leads to [1,1,0] then an RBN which arrives at any of these two states will infinitely fluctuate between them.

An RBN will not leave a cycle once it has entered it. Any path through state space will eventually lead into a cycle. Therefore a cycle is also called an *attractor cycle*. There can be many states leading up to an attractor cycle even if the cycle itself consists of only a few states. A visual representation of the way RBN states transition towards an attractor cycle can be found in figure 10.2. There can even be cycles of length 1. This happens when a state transitions to itself. These single state cycles are easier to use in calculations than multi state cycles. They have been used in research exploring large state spaces [18, 17]. Length 1 attractors are rare though and present only a small portion of possible attractors. Any initial state of an RBN will
eventually lead into an attractor. The state space can thus be divided into attractors and states leading up to these attractors. The latter are called basins [39].

RBNs are used in the investigations of this thesis. Therefore a concise definition of RBN related terminology is given.

- **RBN** A Random Boolean Network is a deterministic dynamic system. It consists of nodes. Connections can exists between nodes. At discrete time steps the state of all nodes is updated simultaneously. Each node is updated according to a deterministic boolean function over its neighbours.

- **RBN state** The RBN state is a binary sequence representing the state of all nodes in the RBN.

- **RBN state space** The space of all possible RBN states. For $n$ nodes there are $2^n$ states. An RBN traces out a route through state space.

- **RBN state transition** The transition of one state to the next based on the node update rules of an RBN. Each RBN has a unique set of transitions which link all states in statespace. A graphical representation of state transitions can be found in figure 10.2.

- **Attractor cycle** Abbreviated attractor. A sequence of states which transition into each other. The last state transitions to the first. This means the RBN loops through each state in the attractor cycle indefinitely. any path through statespace will lead into a cycle eventually. The length of a cycle is defined as the number of states contained in it. A cycle can be length 1 in case of a state transitioning into itself.

- **Attractor state** A state which is part of an attractor cycle.

- **Basin of attraction** Abbreviated basin. All states which will cause the RBN to eventually transition to a specific attractor. One can speak of the basin of attraction of a certain attractor. A large basin means that a large number of states will lead the RBN to an attractor. A basin does not include the attractor itself.

- **Basin state** A state which is part of the basin of attraction for a certain attractor. An attractor state is not a basin state.

The number of connections per node in an RBN has a strong influence on the way the network behaves [35]. When a network has few connections it will quickly settle on a single state and remain there. This is called the ordered phase. When a network has many connections the path traced
through state space becomes erratic and attractors tend to be very large. This type is called the chaotic phase. The most interesting behaviour arises at the threshold between these two phases, called the “edge of chaos” [41]. An RBN at the edge of chaos usually contains multiple attractors which are quite short, with large basins leading to them. The configuration of natural gene networks is said to exist at the edge of chaos [36]. An RBN is at the edge of chaos when on average there are 2 connections per node. This is the connectivity which is used in the experiments of this thesis.

10.2 Boolean network and neutral network: disambiguation

Random boolean networks and neutral networks are both used extensively in this thesis. It is rather unfortunate that both concepts incorporate the word “network”. While both are networks, they exist at very different levels of organisation. In this thesis the neutral net of random boolean networks is studied.

A random boolean network models a single physical system. Its genotype can be mutated and as a result its phenotype can change. On the other
10.3. MEASURES OF DEGENERACY AND REDUNDANCY

Figure 10.3: A neutral net exists on a different level from a random boolean network (RBN). An RBN models a single system while a neutral network models the mutational connections between multiple systems. In this way each node in a neutral network can be an RBN.

hand a neutral network exists in the space of all possible genotypes. It defines the mutations which are possible to a genotype without changing the phenotype. A neutral network can thus describe the neutral mutations that can be made to an RBN. A neutral network is comprised of multiple points in genotype space and each of these points can be an RBN. Figure 10.3 shows the distinction between a neutral network and a random boolean network.

The same naming problem caused Wagner et al. to rename neutral net to ”metagraph” as it describes a graph whose nodes are networks [19, 17]. In this thesis the term neutral net is kept as it conforms to existing research.

10.3 Measures of degeneracy and redundancy

In order to investigate and properly separate degeneracy and redundancy, quantitative measures are required. Tononi et al.[58] provide measures of degeneracy and redundancy based on information theory which are suited to this purpose. These quantitative measures form the basis of the measures used here.

The measures proposed by Tononi et al. [58] pertain to networks which differ from RBNs in a number of ways. However, the differences manifest
CHAPTER 10. MODEL 2: RANDOM BOOLEAN NETWORKS

Figure 10.4: The type of network used by Tononi et al. to calculate degeneracy and redundancy over. $X_j^k$ represents the $j$th size-$k$ subset of the input nodes $X$ and $O$ represents a separate output layer. Nodes in this network simply propagate activation and do not generate activation themselves.

themselves mainly in the specifics of calculation (section 10.7). Therefore we can use measures which are to a large degree similar to the measures proposed by Tononi et al. In this section the latter will be introduced. In subsequent sections 10.5 and 10.6 the adaptations needed for application to RBNs are discussed.

The measures of degeneracy and redundancy are defined by Tononi et al. using the information theoretical concept of mutual information. Specifically, the main constituent of both measures is the mutual information between the input $X$ and the output $O$ of a network:

$$MI(O; X) = H(O) - H(O|X)$$

(10.1)

In which $H(O)$ is the entropy of the output and $H(O|X)$ is the conditional entropy of the output given that the input is known. Entropy is a measure of uncertainty over the outcome of a random variable. If a variable has high entropy the outcome of that variable is hard to predict. Entropy of a variable $X$ is defined as

$$H(X) = -\sum_{i=1}^{n} p(x_i) \log_2 p(x_i)$$

(10.2)

in which $p(x_i) = p(X = x_i)$ is the chance of the variable $X$ having value $x_i$. The entropy is maximized when all $p(x_i)$ are equal. Think for example of a coin toss: when heads or tails are equally probable ($p("heads") = p("tails") = .5$) uncertainty and entropy are maximized. When the coin is
unfair \( p("heads") = .7, p("tails") = .3 \) there is less uncertainty because the result of a toss is usually "heads". Consequently the entropy of the unfair coin toss is lower than the entropy of the fair one.

Entropy is used in describing mutual information. The mutual information between \( O \) and \( X \) is the amount of uncertainty about \( O \) which is taken away by knowing \( X \) (entropy after subtracting \( H(O|X) \)). If the value of \( X \) fully determines the value of \( O \) then knowing \( X \) will reduce the uncertainty about \( O \) to zero and mutual information will be maximized. On the other hand, if \( X \) and \( O \) are independent then the uncertainty about \( O \) will be the same whether \( X \) is known or not. In this case the mutual information will be zero.

The measures given by Tononi et al. apply to the system shown in figure 10.4: consider a system \( X \) of \( N \) nodes. Connections exist between the \( N \) nodes forming a network structure. A node spreads its activation to other nodes based on these connections. Certain nodes in the system are connected to a collection of output nodes \( O \). The output nodes are not part of \( X \). A subset of all nodes \( X \) is designated by \( X^k_j \). In this designation superscript \( k \) denotes the size of the subset. For example, in figure 10.4 the size of the subset (grey area) is 2. Usually there are many different size \( k \) subsets of \( X \). The subscript \( j \) denotes which size \( k \) subset is meant. The particular order of all numbering all size \( k \) subsets is not important as all formulas used in this thesis either average or sum over all \( j \). Given the system defined above, Tononi et al. define the degeneracy \( D_N(X, O) \) of input \( X \) with respect to output \( O \) as

$$D_N(X, O) = \sum_{k=1}^{N} \left[ < MI^P(X^k_j; O) > - (k/N) MI^P(X; O) \right]$$ \hspace{1cm} (10.3)

In which the angled brackets \(< >\) denote an averaging over all \( j \):

$$< MI^P(X^k_j; O) >= \frac{1}{j_{max}} \sum_{j=1}^{j_{max}} MI(X^k_j; O)$$ \hspace{1cm} (10.4)

Redundancy \( R(X, O) \) is defined by summing the information content of every single node \( (X^1_j) \) in the network and subtracting the information content of the entire system as a whole:

$$R(X, O) = \sum_{j=1}^{N} < MI^P(X^1_j; O) > - (MI^P(X; O))$$ \hspace{1cm} (10.5)

In these equations \( MI^P(X^k_j; 0) \) designates the mutual information between the \( j^{th} \) size \( k \) subset of \( X \) and the output. The Term \( MI^P() \) is used
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by Tononi et al. to designate *perturbation*, referring to the method by which $MI()$ is calculated from their networks. This method is not applicable to the RBN case. The calculation of $MI()$ in RBNs is discussed in section 10.7. The differences in calculation do not however affect the intuitive notion of mutual information. This means that $MI^P()$ can be read as $MI()$ as long as no explicit calculation of $MI$ is involved.

Using equations 10.3, 10.5 and 10.4, degeneracy can be intuitively described as the amount by which the information on the output contained in size $k$ subsets of the input ($MI^P(X^k_j; O)$) is larger than a linear expectation ($(k/N)MI^P(X; O)$) of this information. Similarly redundancy can be described as the amount by which the sum of information given by each single node ($MI^P(X^1_j; O)$) surpasses $MI^P(X; O)$, the total information in the system.

The system $X$ considered by Tononi et al. (see image 10.4) consists of nodes which are dead: they simply propagate incoming activation to all connected neighbours based on the connection weight. There is no internal state or activation of nodes. RBN nodes have a different node type in which a node is an active entity: a boolean function over the inputs of a node determines the output in the next time step. This difference has consequences for the way in which degeneracy and redundancy are calculated in RBNs. These consequences are discussed in the next section.

10.4 Measures of degeneracy and redundancy applied to RBNs

The rules for signal propagation and the definition of input and output in RBNs differ from the models used by Tononi et al. This section explores whether degeneracy and redundancy can be adapted to apply to the RBN model.

An RBN does not contain separate input and output layers such as the Tonini model described in section 9. Rather the input and output in an RBN are distinguished by time. RBN input consists of the initial state of its nodes. Based on this state an RBN deterministically transitions to a next state in each time step. After a number of steps a stable state is reached. This is the output of an RBN. RBN input and output are defined more specifically in sections 10.5 and 10.6.

There have been to my knowledge no previous attempts to define measures of degeneracy and redundancy for an RBN as a whole. The basis for the measures used here is defined in equation 10.3 and 10.5 in section 10.3.
10.5 Defining RBN Input

The purpose of computing $MI(O; X)$ is to measure the information about $O$ which is contained in the input nodes $X$. In other words, to measure the influence the state of $X$ has on the state of $O$. To measure this influence systematically, all states of $X$ need to be sampled uniformly.
The input state of an RBN can be characterized by the state of each of its $N$ binary nodes at timestep 1 (see section 10.1). For example, an instantiation $x$ of the input state variable $X$ for a 4 node RBN could be $x = 0001$ or $x = 0010$ etcetera. A uniform sampling of all input states entails that each of these $2^N$ states be equally likely (or equiprobable). When $N$ is small all possible states of $X$ can be enumerated. For large $N$ however, uniform sampling is necessary to keep calculations tractable. In summary, the input $X$ of an RBN is defined as a variable which produces each RBN input state with equal probability.

A subset $X_j^k$ of $X$ is defined similarly to $X$ with the exception that $X_j^k$ only samples the states of the $k$ nodes in subset $X_j^k$.

A convenient property of uniform sampling of $X$ and $X_j^k$ is that it can be analytically shown that $H(X) = N$ and $H(X_j^k) = k$ (see appendix, section B). This property makes it unnecessary to compute entropy $H$ using computationally costly statistics.

### 10.6 Defining RBN output

Assigning any single value to RBN output is hampered by the cyclic behaviour of the output. The problem is that when presented with a single input state $x_i$ with probability 1 ($H(x_i) = 0$), an RBN will often output a set of states $[o_1, o_2, ..., o_n]$ ending in a cycle (entropy $H([o_1, o_2, ..., o_n]) > 0$). This is unwanted behaviour when one wants to investigate mutual information with the input nodes. For example: an RBN is fed a single input state and subsequently the output states are collected into an output set. A node which is active in half of the states in the output set will have maximum entropy. The input state which caused this entropy is a single state. The problem here is that the entropy effect of varying the input is supposed to be investigated i.e. measuring the entropy in the output based on entropy of the input of an RBN. The entropy of a single input state is 0. Subsequently, the entropy of the output based on this input should also be 0. The output can not be defined as a list because this would make the entropy effects of varying the input indistinguishable from the inherent entropy or noise caused by RBN cycles.

A second problem in defining entropy over the entire set of output states is weight assignment between the states leading up to a cycle and the cycle itself (basin states and attractor states respectively, see section 10.1). Both these types of states contribute to the entropy of the RBN output. The entropy contribution of the cycle states becomes more important as more cycles are included in the calculations. In the limit the basin states’ relative contribution approaches 0 and cycle states are the only cause of entropy.
10.6. DEFINING RBN OUTPUT

Figure 10.6: The input and output of an RBN. Input is defined as a single state, output is defined as a single symbol which represents a set of states leading to the same attractor, including the attractor states themselves. In other words, a single output consists of an attractor (cycle) plus the basin states for this attractor.

This causes the basin states which are not part of the attractor cycle to become virtually invisible which will cause the method to ignore part of the statespace. To overcome these problems, cycles will either have to be disallowed as output or defined as a single invariable entity. Different characterizations of the output thus need to be investigated. In doing so it is important to keep in mind the aspects of biological networks which RBNs are thought to model. A key feature of RBNs is the existence of attractors in the state space (see section 10.1 on RBNs). An important hypothesis proposed by Kauffman [35, 36] is that RBN attractors correspond to cell types and states in genetic networks (see also section 10.1). It is desirable to incorporate the concept of attractors into the definition of RBN output. The natural solution is to stay close to the biological relevance of RBNs and define output as a single value. This single value will then represent a set of states which is relevant to the attractor landscape of the RBN in question. This set is constructed in the following way: The state space of every RBN contains one or more attractors. Each input state eventually maps into one of these attractors. This means the state space of an RBN can be partitioned into disjoint sets based on the attractor they lead to. By regarding each of these sets as a single output category a suitable RBN output definition is constructed. This definition circumvents the aforementioned problems with output cycles because the output is now a single value. The definition also describes RBN output on a biologically relevant level and makes it possible to calculate mutual information with RBN input meaningfully. The RBN input and output definitions are represented graphically in figure 10.6.
Figure 10.7: The state space of an RBN can be partitioned into disjoint sets based on the attractor each state leads to. Depicted are all possible states of (the nodes of a) three node RBN. Arrows indicate transitions in the RBN. In this example any of the input states [001, 010, 011, 100] lead to the same attractor cycle (001, 010). This means that if the RBN receives any of these input states, the output is denoted $\mathcal{A}$. See section 10.4. Note: state transitions in this figure are not actually derived from the RBN displayed but merely serve as illustrations.

10.7 Computing mutual information in RBNs

Input $X$ and output $O$ of and RBN have been defined in sections 10.5 and 10.6 respectively. But how to compute $MI^P(X;O)$, the central building block of the formulae for degeneracy and redundancy? In this section the computation of $MI^P(X^k_j;O)$ in an RBN is explored.

In the original measures proposed by Tononi et al. [58] the term $MI^P(X^k_j;O)$ is used. The superscript $P$ is added in the original measures to designate perturbation. Perturbation is only a means to achieve a goal which is to measure the mutual information $MI(X;O)$ between input and output. In an RBN mutual information can be computed more directly by defining $MI(X^k_j;O)$ as the information gained about $O$ when the value of $X^k_j$ is known. In information theoretical terms this is expressed as

$$MI(O;X^k_j) = H(O) - H(O|X^k_j)$$  \hspace{1cm} (10.6)$$

which is to say that the mutual information between $O$ and $X^k_j$ is equal to the change in entropy of $O$ which results from knowing the value of $X^k_j$. 
One of the basic formulae of information theory states that

\[ H(Y|X) = \sum_{x \in QX} P(x)H(Y|X = x) \]  

(10.7)

where \( QX \) is the set of all possible values of variable \( X \). Substituting equation 10.7 in equation 10.6, mutual information can be expressed as

\[ MI(O; X^k_j) = H(O) - \sum_{x \in QX^k_j} P(x)H(O|X^k_j = x) \]  

(10.8)

in which \( x \in QX^k_j \) denotes all possible states the nodes in \( X^k_j \) can be in. For example, in the system depicted in image 10.5, the possible states of subset \( X^2_1 = [A, B] \) are \([00, 01, 10, 11]\). As noted in section 10.5 all possible states of the input are defined to be equiprobable. For a size-\( k \) input there are \( 2^k \) input states. This being the case equation 10.8 can be written as

\[ MI(O; X^k_j) = H(O) - \sum_{i=1}^{2^k} \frac{1}{2^k} H(O|X^k_j = x_i) \]  

(10.9)

In which the summation in the second term describes an average over all states \( x_i \) of a single subset.

The conditional entropy \( H(O|X^k_j = x) \) is computed by collecting the RBN output values of each \( x_i \in X^k_j \) and computing the entropy of these outputs. Perturbation is not used in determining mutual information in an RBN. As a result the superscript \( P \) is dropped. In further formulae the term \( MI(X^P_j; O) \) is substituted by \( MI(X_j; O) \). Now that the computation of \( MI(O; X^k_j) \) for an RBN is defined, equations 10.3 and 10.5 for degeneracy and redundancy respectively can be computed.

### 10.8 Computing RBN degeneracy and redundancy

Degeneracy is defined as the the degeneracy of the node activation of an RBN with respect to the attractor the RBN moves to. In other words degeneracy is the tendency of configurations of RBN input nodes to influence the attractor an RBN moves to. Redundancy defined in similar terms as the tendency of individual RBN input nodes to influence the attractor an RBN moves to. To calculate the degeneracy and redundancy of a particular \( N \) node RBN the following steps are taken

- For each of the \( 2^N \) input states of the RBN, determine the successor state. This allows identification of attractors in the state space and basin states leading into these attractors.
PARTITION THE STATE SPACE INTO DISJOINT SETS, \(A, B, \ldots\) WHICH ALL LEAD TO OR ARE PART OF THE SAME ATTRACTOR. THE OUTPUT \(O\) OF AN RBN IS DEFINED TO BE ONE OF THESE SETS (SEE FIGURE 10.7).

FOR DEGENERACY AND REDUNDANCY, CALCULATE

\[
D_N(X, O) = \sum_{k=1}^{N} \left[ < MI(X_k^j; O) > - (k/N)MI(X; O) \right]
\]

\[
R(X, O) = \sum_{j=1}^{N} < MI(X^1_j; O) > - (MI(X; O))
\]

RESPECTIVELY. MUTUAL INFORMATION IS COMPUTED USING EQUATION 10.9 FROM SECTION 10.7:

\[
MI(O; X^k_j) = H(O) - \sum_{i=1}^{2^k} \frac{1}{2^k} H(O|X^k_j = x_i)
\]

NOTICE THE USE OF \(MI\) INSTEAD OF \(MI^p\) IN THESE EQUATIONS. THIS IS BECAUSE THERE IS NO PERTURBATION INVOLVED IN COMPUTING \(MI\) IN AN RBN (SEE SECTION 10.7).

10.9 MEASURES OF ROBUSTNESS IN RBNs

ROBUSTNESS, OR OUTPUT INVARiance IN THE FACE OF PERTURBATION OF THE INPUT IS UBiqUITOUS IN ALL LEVELS OF ORGANISM ORGANISATION. THE MECHANISMS WHICH ENFORCE THIS INVARiance ARE OFTEN VERY DIFFERENT HOWEVER[18]. ON THE LEVEL OF THE GENETIC CODE IT IS REDUNDANCY OF CODONS CODING FOR AMINO ACIDS. ON THE LEVEL OF PROTEIN TERTIARY STRUCTURE FOLDING DYNAMICS ARE UNAFFECTED BY CHANGES IN MANY AMINO ACIDS BUT STRONGLY AFFECTED BY CERTAIN COMBINATIONS OF MUTATIONS. ON THE LEVEL OF METABOLIC PATHWAYS THE WAY MUTATIONS CHANGE ENZYME ACTIVITY IS VERY IMPORTANT FOR ROBUSTNESS. IN THIS THESIS ROBUSTNESS IS DISCUSSED AT THE LEVEL OF GENETIC REGULATORY NETWORKS I.E. THE LEVEL OF GENES EXCITING AND INHIBITING EACH OTHERS ACTIVITY.

THE STRONG EVERY DAY CONNOTATION OF THE WORD ROBUSTNESS TENDS TO OBEscURE THE NECESSITY OF EXACT DEFINITION OF THE TERM. CURRENTLY ALMOST EVERY RESEARCH PAPER WHICH DEALS WITH NETWORK ROBUSTNESS USES A DIFFERENT MEASURE. THIS IS ONLY PARTLY DUE TO DIFFERENCES IN THE MODELS WHICH ARE USED. THIS SECTION DESCRIBES SEVERAL EXISTING DEFINITIONS OF NETWORK ROBUSTNESS. SUBSEQUENTLY THE MOST APPROPRIATE MEASURE IS CHOSEN AND ITS APPLICATION TO THE MODELS IN THE THESIS DISCUSSED.
To become technically useful the broad term "robustness of a biological system" will be defined in more concise terms. First of all the biological level at which robustness is regarded is of prime importance.

Robustness at the GRN level is either robustness to perturbation of connections or perturbation of initial state. These two types of perturbation correspond to gene mutation and environmental noise respectively. In this thesis only gene mutation is discussed. Several authors define robustness at the level of gene regulation differently:

- Wagner et al.[18] investigate the structure of the neutral net of boolean networks which attains a single given end state $S_\infty$. In particular the robustness of the networks to mutation and noise is assessed. The robustness to mutation (changes in connections between nodes) in an RBN is measured as the number of 1-mutation neighbours of the RBN which still attain $S_\infty$. Robustness to noise (changes in input state) is measured with three different metrics. Firstly the probability that a change in one node’s activation will change $S_\infty$. Secondly the fraction of input node activations that need to change to make the probability of preserving $S_\infty$ fall below $\frac{1}{2}$. Thirdly the chance that clamping the value of a single gene disturbs $S_\infty$. The measure used by Cilliberti et al. is computationally efficient. This is because only a single state $S_\infty$ is allowed as output. An RBN either attains this stable output state or it does not, which can easily be checked. This restriction allows for easy exploration of the neutral net of RBNs achieving $S_\infty$. With regard to the aims of this thesis the problem of this measure is that it is centered around a single output $S_\infty$. This measure of robustness excludes any RBN which produces cyclic output. However, RBNs very often produce such cyclic output (see section 10.1). As this thesis investigates all possible inputs and their effect on RBN output, RBN output cycles can not be excluded. The measure could be adapted to include cycles. With reasonable ease it can be ascertained whether a mutated RBN attains the same attractor cycle as the original. However, attractor cycles often change slightly as a result of RBN mutation. It is not desirable to discard only slightly differing attractor cycles, as these slight changes might be of key importance for another interesting biological property innovation. In order to use cycles in the Cilliberti measure, attractor cycle similarity would have to be defined. This would undo the conceptual and computational straightforwardness of the measure.

- Gershenson et al.[31] define robustness to mutations by measuring the similarity in state transitions between the original and a mutated network. For a size $n$ RBN $A^t_1$ and a 1-bit mutated RBN $M^t_1$ the distance
in their state transitions is expressed as
\[ dS = \frac{1}{2^n} \sum_{i} 2^n H(A_t^{i+1}, M_t^i) \] (10.12)
in which \( H(x, y) \) denotes the normalized Hamming distance between \( x \) and \( y \). The greater the distance between \( A \) and \( M \) the less robust \( A \) is.

This measure assumes that normalised Hamming distance (i.e. the fraction of nodes with divergent activation) corresponds to distance in RBN state space. This assumption imposes a \( N \)-dimensional topology on the state space based on the regular ordering of all states, and is unaffected by the connections of a specific RBN. However, state space topology is highly dependent upon RBN structure. For instance, consider a very simple RBN in which only two nodes \( A \) and \( B \) exist. \( A \) and \( B \) excite each other, meaning that if at time \( t \) node \( A \) is active than node \( B \) will be active at \( t + 1 \). The collection of states of the network is

\[ [00, 01, 10, 11] \]

and the attractors are

1: 00 → 00
2: 11 → 11
3: 01 → 10 → 01

Attractor 3 contains two states which have maximal hamming distance(2). One would like these two states to be more similar because they are highly related given the structure of this RBN. Hamming distance can not do this as it is insensitive to RBN structure. A state space topology based on attractor and basin states seems a better choice to capture the inherent structure of RBN state space.

- Aldana et al. [4] define robustness to mutation in RBNs using the concept of attractor cycle retention. First all attractor states are identified for an RBN. Robustness to mutation of RBN \( R \) is then characterized by \( P(q) \), the probability that a fraction \( q \) of these attractor states are retained after changing one of the connections in \( R \). \( P(q) \) can be graphed to view probabilities of different retention percentages. Or, by taking \( q = 1 \) the probability of retention of all attractor states is obtained.
This measure of robustness incorporates the concept of attractor cycles in a meaningful way. An added advantage is that $P(q)$ can be approximated by sampling the effects of random mutations to an RBN. This means that the state space does not have to be exhaustively searched.

For an attractor to be "retained" it does not have to reoccur identically in the behaviour of the mutated network. Retention may involve expansion in which new states are incorporated into existing attractors. The definition of attractor retention is wide enough to include new attractor states as long as the original attractor states are all retained.

The symbolic partition of the space of attractor transformations is somewhat arbitrary. For example, if the addition of one or more states to an attractor is called "retention", why not call the subtraction of one or more states retention as well? Regardless of this the partitioning acknowledges the importance of attractors in RBN functioning. This is the measure for robustness that will be used in this thesis. To further focus the investigation, only $q = 1$ will be used.

10.10 Measures of innovation in RBNs

Innovation is, like robustness, based on Aldana et al. [4]. Innovation is defined as the proportion of RBNs which after mutation retain all attractor states and add at least one new state. This measure works well with attractors in state space and its value can be approximated by sampling.
Chapter 11

Experiment

This section describes the implementation of the two models and the tools used for data visualisation.

11.1 Model 1: simulation

Model 1 regards the behaviour a population of organisms for which a mutation is either fatal or neutral. In other words the population is trapped on a single neutral net. Given enough cycles of death and birth a population will achieve a stable distribution over the genotypes in the neutral net. This distribution is skewed: genotypes with a higher proportion of links to other genotypes on the neutral net are overrepresented \[59\]. These overrepresented links are also more robust to mutation as a high proportion of mutations are neutral mutations. The intuition investigated in model 1 is that a system made up of degenerate elements is better connected in the neutral network and therefore more robust. This increased robustness can be selected for by natural selection, providing a clue to the ubiquity of degenerate coding in natural systems. In other words, degeneracy causes robustness better then redundancy, therefore it gets selected by natural selection, therefore it exists at all levels of biological organisation.

In order to investigate the possible link between degeneracy, neutral net topology and robustness, a model was built in matlab. The implementation features the following:

- Model an organism as a genotype and phenotype as described in section 9. In other words, as a row of genes and a row of functions with explicit connections in between.
- Map the neutral net the organisms move on by finding all configurations of genes which still support all functions.
• Compute the steady state distribution of a population of organisms which is allowed to procreate freely on the neutral net. This distribution can be found by computing the principal eigenvector of the connection matrix of the neutral net.

The connections between genes and functions in a model affect the steady state distribution of organisms among genotypes. Well connected genotypes will be more prevalent in the population.

The basic assumptions in this model turned out to be unsound. This makes the majority of the results worthless. Figure 11.1 shows some interesting preliminary results. The figure shows how the structure of the neutral net influences the stable state distribution. A genotype which is well connected to other viable genotypes, which is central to the neutral net is home to a larger proportion of the population. Peripheral genotypes are clearly less common in the population. When genotypes which were previously peripheral are connected, as shown in the top two circles of figure 11.1, the distribution evens out again.

11.2 Model 2: experiment setup

In order to study the evolutionary properties of RBNs the following experiment is set up.

1. An RBN is generated consisting of six nodes (see section 11.3 below for implementation). Connections between nodes are in such a way as to have a mean number of 2 connections per node (See section 10.1, "edge of chaos").

2. The state space of the RBN is mapped exhaustively, finding all basin an attractor states. Degeneracy and Redundancy in the RBN are computed as described in section 10.

3. Mutation is applied. A mutation flips a bit in one of the boolean tables governing RBN node behaviour. For example, if node A=1 and node B=0 caused node C=1, after mutation A=1,B=0 causes C=0. This type of mutation is small enough to be applied to 6-node RBNs but powerful enough to cause noticeable changes in RBN behaviour.

4. After mutation, the state space is mapped again and compared.

5. Step 3 and 4 are repeated 150 times for each RBN. Comparison of the attractors before and after mutation yields the values of Robustness and Innovation. Robustness is defined as the proportion of mutations
11.2. MODEL 2: EXPERIMENT SETUP

Figure 11.1: Several neutral networks with steady state distributions. Each node represents a genotype on the neutral net. Each edge represents a mutational step between them. Next to each number is the proportion of the population which resides on the node after many life cycles.
which retains all original attractor states. Innovation is defined as the proportion of mutations which retain all original attractor states and introduce at least one new state. See section 10.9.

6. The values of degeneracy, redundancy, robustness and innovation are computed for 10,000 RBNs.

7. Degeneracy and redundancy are based on the uncertainty regarding the attractor an RBN will move to. When there is only a single attractor this uncertainty is zero and degeneracy and redundancy are indistinguishable. Therefore RBNs which contain only a single attractor are discarded. In six node RBNs this applies to about 50%, making the final dataset about 5000 RBNs.

11.3 Model 2: simulation

The implementation of model 2 is based on the random boolean network simulator created by Carlos Gershenson [1]. Figure 11.2 shows a screenshot. The simulator is called RBNLab and has been used in several of Gershenson’s papers. For example in studying the addition of duplicate nodes in RBNs [31]. The author was kind enough to give full access to the code. This allows for extensions to be added.

In the extended version of RBNLab the properties described in model 2 can be computed. The computations are all based on the attractor landscape of an RBN. In order to perform these computation this landscape is first mapped using the ”basins” button. This finds all attractor cycles or basins of an RBN and then labels each RBN state by the attractor that it leads to. For more information on the RBN attractor landscape see section 10.1. An important extension added to RBNLab is the ability to output data in a format readable by Matlab. The ability to run batch experiments has been added. The results can then be processed and visualised further in Matlab. Figure 11.3 shows a screenshot of the extended version of RBNLab.

11.4 Model 2: visualisation

The batch experiments run in RBNLab produce data in a Matlab readable format. The output takes the form of a Matlab structure. This structure contains global information about the batch experiment, like the parameters used and the time taken. The structure also contains for each RBN that was tested its computed properties. It contains the degeneracy, redundancy, number of nodes etcetera for each RBN that was tested. This data can be
Figure 11.2: The JAVA-based program RBNlab by Carlos Gershenson. The implementation of Model 2 is based on this program. On the left the successive states of the RBN are printed. Each state is also represented as a column in the bottom window. At each time step the new state is appended on the right side of this window. States are repeating as the RBN has entered an attractor cycle. This can be seen from the regular pattern in the bottom window. On the right side the topology of the network is shown.
Figure 11.3: The extended version of RBNlab. In this version the basins in an RBN can be determined. Based on these basins the properties described in model 2 can be computed. The bottom bar can be used to run a batch experiment and output the results in a matlab readable format.
visualised in Matlab. Several visualisation styles are used to explore the data:

**global experiment information** Output from the experiment includes global information on the data. For example the time an experiment finished, the number RBNs which were investigated and RBN parameters. In figure 11.4 these parameters are seen right of the scatter plot. Information specific to the visualisation is depicted above the figure (see figure 11.4). For a scatterplot this information consists of several elements. Firstly the data depicted in the figure and the number of points which are shown. "Scatter" shows the amount of noise added to the data points. Scatter shows the maximum random deviation in each direction. Noise is added to identify multiple identical data points. "Filter" shows the filter which has been applied to the data (if any). In the case of figure 11.4 the filter nBasins > 1 only shows the data of RBNs which have more then 1 basin. This is a standard filter as RBNs which can not switch between at least 2 basins automatically have a degeneracy and redundancy of zero. In figure 11.4 the effect of the filter can be seen as there are only 2823 points displayed while the data set contains 5000.

**scatter plot** Represent each instance as a point in 2 or 3 dimensional space. Each dimension represents a measured property. The measurement value determines the position of the point in this dimension. An example scatter plot is depicted in figure 11.4. An additional property can be visualised by using colour. The value range of a property is mapped to a color range. The color of a point then represents the value of the property. See figure 11.5.

**plot means** A scatter plot allows close inspection of single data points. Discerning global patterns can be hard however. The means plot provides a very coarse view of the relation between two variables. The means plot divides the data into regularly spaced bins along one dimension. In example figure 11.6 this is the dimension "degeneracy" (named "degTotal"). Subsequently the mean score for another dimension, in figure 11.6 "innovation" is shown for each bin. The leftmost blue bar in figure 11.6 shows that the points with a degeneracy value up to $-2.90$ have a mean innovation value of around 0.015.

**surface** The surface plot combines the preciseness of the scatter plot with the means plot ability to see global patterns. The surface plot can be seen as a fine scale two dimensional histogram. Unlike in a histogram however, the surface height does not display the number of points in
CHAPTER 11. EXPERIMENT

Figure 11.4: A scatter plot. To the right information about the data set is displayed. The figure title shows information about the visualisation.

Figure 11.5: A colored scatter plot. An additional property can be visualised by using colour. The value range of a property is mapped to a color range. The color of a point then represents the value of the property.
Figure 11.6: A means plot. The means plot provides a very coarse view of the relation between two variables. It shows the mean value of one variable for regularly spaced bins of another variable.
Figure 11.7: A surface plot. The surface plot can be seen as a fine scale two dimensional histogram. Unlike a histogram however height does not display the number of points in a bin but rather the average value of the points in a bin. An example surface plot can be seen in figure 11.7. Usually color is linked to the height of the map for increased clarity.

The surface plot shows the average value of all the points in a bin. The number of points this average is based on cannot be discerned however. Without this information a single freak reading can severely alter the shape of the curve and lead to wrong conclusions. To prevent this the translucency of the surface has been coupled to the number of points on that part of the surface. In this way averages which are based on many points are solid and averages based on few points are translucent. An example is shown in figure 11.8.
11.4. MODEL 2: VISUALISATION

Figure 11.8: A surface plot with alpha map. The translucency of the surface has been coupled to the number of points on that part of the surface. In this way averages which are based on many points are solid and averages based on few points are translucent. This visualisation allows a clearer understanding of the data underlying the surface.
Chapter 12

Results

This section discusses the output from the experiments defined in section 11.2.

12.1 Value ranges

Here the data is discussed as a whole, without looking at specific patterns. See figure 12.1.

Robustness As can be seen from figure 12.1, Robustness ranges from 0 to 1. This is in accordance with the measures proposed by Aldana et al. [4] which is used in this thesis (see section 10.9). Robustness is defined as the proportion of mutations which does not cause a change in an RBN’s attractor cycles. A robustness value of 0 means that every mutation disrupts the RBN’s attractor cycles. A value of 1 means that attractor cycles remain as they are, no matter what single mutation is applied to the RBN. Figure 12.1 shows that robustness values are quite varied, ranging from 0 to 1 with a preference for average values.

Innovation Innovation is also defined based on Aldana et al. and is defined similarly to robustness. As described in section 10.10, innovation denotes the proportion of RBNs which, after mutation, keep all original attractor cycles while at the same time adding states to these cycles. This is a rather strict definition as no attractor state may be lost. As it denotes a proportion, innovation ranges from 0 to 1. A value of 0 means that no mutation causes the described effect, while a value of 1 means that every mutation causes the described effect. Figure 12.1 shows that innovation ranges from 0 to about .2 with very few outliers at .3.
Figure 12.1: The value ranges for the evolutionary properties innovation and robustness are shown on the left. The ranges for the genotype-phenotype properties degeneracy and redundancy are shown on the right.

Degeneracy Figure 12.1 shows that degeneracy ranges from 0 to -3. The causes of this value range are discussed below. In section 10.8 degeneracy is described as follows:

$$D_N(X;O) = \sum_{k=1}^{N} [< MI(X^k;O) > -(k/N)MI(X;O)]$$

This equation compares the average information content of all size $k$ input subsets with a naive expectation. The term $(k/N)MI(X;O)$ represents this naive expectation. The term describes a proportion of the information present in the entire RBN input. Why is this latter term always larger than the first, causing a negative value range? The term $MI(X;O)$ depends only on the entropy of the RBN output $HO$ (see section 10.7. Entropy in an RBN is defined in section 10.6 as the uncertainty about the attractor cycle the RBN will move to given randomness of part of its input state. For instance if the state of two of the four nodes of an RBN is unknown an RBN might move to one or the other attractor. The more equal the chances of arriving at either attractor, the higher the uncertainty about the outcome. When the RBN input is completely unknown, all attractors could be reached, resulting in high uncertainty. However, when part of the RBN input is known, certain attractors might not be reached. This means that
the uncertainty over the outcome is lowered. This loss of attractors in entropy calculation occurs in the term $MI(X^k_j; O)$ but does not occur in the term $(k/N)MI(X; O)$. This helps to explain why degeneracy takes on negative values. However, the highest degeneracy attainable is 0. The lower the degeneracy value, the less a systems parts perform the same function in different ways.

The value of degeneracy is not bounded by -1. Its computation involves the entropy of a multi variate system, the RBN. Take for example the entropy of the RBN input $H(X)$. In the RBN input $X$ each input node is a Bernoulli variable. The maximal entropy for a single Bernoulli variable is 1 (see Appendix B). The maximal entropy for $n$ Bernoulli variables is $n$. For the RBNs used in this Thesis $n = 6$. This means that the principle lower bound for degeneracy is -6. In the experiments however, this value has not been lower than -3. In conclusion, the basic entropy computations for RBNs are not bounded by 1. Therefore the value of degeneracy isn’t either.

**Redundancy** Figure 12.1 shows that redundancy, like degeneracy, ranges from 0 to -3. In section 10.8 redundancy is defines as

$$R(X, O) = \sum_{j=1}^{N} < MI(X^1_j; O) > - (MI(X; O))$$

The value range of redundancy is explained in the same way as for degeneracy. The term $(MI(X; O))$ always encompasses all available attractors while the term $MI(X^1_j; O)$ does not. This causes the values to be negative. The RBN input $X$ is multivariate, making the maximal entropy over it to be greater then 1. This causes degeneracy values outside the range of $[-1, 0]$

### 12.2 Data coverage

For the final result plots a dataset of 10000 RBNs was used (see section 11.2). For each of these RBN’s the relevant properties were computed. Figures 12.2 and 12.3 show the spread of datapoints for robustness and innovation. There is a clear central cluster of datapoints. The mean values of degeneracy are represented as color. The peripheral values are less certain as there are few datapoints making up the average. However several identical batches were run and showed a similar pattern for degeneracy and redundancy. Therefore the results are deemed representative, even for peripheral values.
Figure 12.2: A surface visualisation with superimposed the data points which were used to form the surface. Each point represents a 6 node RBN. Color of the surface denotes the degeneracy value.
Figure 12.3: A two dimensional histogram showing the number of datapoints recorded for each combination of innovation and robustness.
12.3 Redundancy

Redundancy is the tendency of a system to have identical system parts perform the same function (see section 6.4). In Equation 10.11, section 10.8, redundancy was defined for an RBN.

\[ R(X, O) = \sum_{j=1}^{N} < MI(X_j^1; O) > - (MI(X; O)) \]

Mutual information is computed using equation 10.9 from section 10.7:

\[ MI(O; X_j^k) = H(O) - \sum_{i=1}^{2^k} \frac{1}{2^k} H(O|X_j^k = x_i) \]

First the average effect of redundancy in an RBN is investigated. Figure 12.4 shows that for increasing redundancy the average robustness also increases. Interestingly the same is true for average innovation, as figure 12.5 shows.
12.3. REDUNDANCY

Figure 12.5: The way redundancy corresponds with mean values of innovation.
CHAPTER 12. RESULTS

Figure 12.6: The way redundancy corresponds with robustness and innovation.

A more detailed view of redundancy is given in figure 12.6. From this figure it can be confirmed that high redundancy corresponds to both high robustness and high innovation in an RBN. However the correlation with robustness is more clear-cut. A redundant RBN can have low innovation (red is present on the line innovation = 0.05). A redundant RBN always has high robustness (there is no red below the line robustness = 0.5).

12.4 Degeneracy

Degeneracy is the tendency of a system to have structurally diverse parts which still influence the system in the same way (see section 6.5. In Equation 10.10, section 10.8, degeneracy was defined in for an RBN as follows:

$$D_N(X, O) = \sum_{k=1}^{N} \left[<MI(X^k_j; O)> - (k/N)MI(X; O)\right]$$

Mutual information is computed using equation 10.9 from section 10.7:
12.4. DEGENERACY

\[
MI(O; X^k_j) = H(O) - \sum_{i=1}^{2^k} \frac{1}{2^k} H(O | X^k_j = x_i)
\]

First the average effect of degeneracy in an RBN is investigated. Figure 12.7 shows that for increasing degeneracy the average innovation value also increases. Figure 12.8 shows that degeneracy does not correspond strongly to the average robustness of an RBN.

A more detailed view of degeneracy is given in figure 12.9. This figure confirms the correspondence of degeneracy and innovation. It can also be seen that the values of degeneracy and redundancy are not clearly related. However, a high innovation score signifies a minimum value for robustness. This can be inferred from the lower right triangle of the figure in which no datapoints exist. On average a degenerate RBN has high innovation but can have any robustness value above a certain minimum. This minimum robustness increases with increasing innovation.
Figure 12.8: The way degeneracy corresponds with mean values of robustness.
Figure 12.9: The way degeneracy corresponds with robustness and innovation.
12.5 Relative effects

The effects redundancy and degeneracy have been viewed independently in the previous sections. In this section they are compared.

RBN robustness is shown as color in figure 12.10. In this figure it can be seen that robustness scales mainly with redundancy. A highly robust rbn can have low degeneracy: at degeneracy = -2.5 there is plenty of red to be seen. However a highly robust rbn can not have low redundancy: the color gradient moves to blue with decreasing values of redundancy.

RBN innovation is shown as color in figure 12.11. In this figure it can be seen that innovation scales mainly with degeneracy. A highly innovative rbn can have low redundancy: at redundancy = -2.5 there is still red at high degeneracy values. A highly innovative RBN can not have low degeneracy: the color gradient moves to blue with decreasing values of degeneracy.
12.5. RELATIVE EFFECTS

Figure 12.11: The relative effect of redundancy and degeneracy on innovation.
Chapter 13

Discussion

In this section the research findings are put into broader perspective.

13.1 Model 1: explicit mapping

Work on the explicit mapping model was quite extensive. Much energy was expended on investigating and documenting several of its aspects. It was in a late stage of investigation that a flaw in its assumptions was found. The discovery and correction of this flawed assumption had two effects. Firstly it made the largest part of the results obtained unusable. The results pertained to a model which could not claim to model a biological system at any level. Secondly the corrected version of the model was shown to be inadequate in addressing the thesis question (see section 9.5). Specifically, degeneracy and redundancy had indistinguishable effects. Model 1 was found to be too simple to allow further study of thesis question.

13.2 Model 2: Random Boolean Networks

The results in section 12 show that degeneracy correlates with innovation and that redundancy correlates with robustness. These results are not trivial. Redundancy and degeneracy are properties of the genotype-phenotype map of an RBN. Innovation and robustness are properties of the evolutionary dynamics of an RBN. In other words they give information about the reaction of an RBN to mutations. The results show a connection between properties of the genotype-phenotype map and evolutionary dynamics. The results show only a connection: the one can not be seen as causing the other. Even so, for random boolean networks it can be concluded that a robust networks needs to have a high redundancy value, and that an innovative network needs to have a high degeneracy value. The almost diametric
opposition of the color gradients in figures 12.6 and 12.9 and in figures 12.10 and 12.11 show that degeneracy and redundancy measure different aspects of the genotype-phenotype map of RBNs.

The measures of degeneracy redundancy and used in this thesis are based on measures by Tononi et al. [58]. One of the conclusions presented in that paper is that "to be degenerate, a system must have a certain amount of functional redundancy". This conclusion was based on experimentation with a different type of networks (see section 10.5). This conclusion can be confirmed for the RBN case. From figures 12.10 and 12.11 it can be seen that high values of degeneracy can not exist when redundancy is low($< -2$). Conversely, high values of redundancy cannot exist when degeneracy is very low. Degeneracy and redundancy in RBNs are not independent. The higher the value of the one, the more constrained to high values the other. This effect is especially true for degeneracy. When redundancy is very high, near 0, the variation in degeneracy tends to 0. This can be inferred from the converging top left portion of figure 12.10.

It is also interesting to note that in an RBN, robustness and innovation are not mutually exclusive. The relation between robustness and innovation can be read from the shape of figure 12.9. Higher robustness allows for greater innovation, as the surface is broader at higher robustness values. By using the measures of degeneracy and redundancy this relation can be dissected: Higher robustness allows for greater innovation only if the RBN has a high degeneracy value. This can been seen from figures 12.10 and 12.11. The dark red upper left part of figure 12.10 represents very robust RBNs. The same part in figure 12.11 shows that these highly robust RBNs are only robust with high degeneracy values.
Chapter 14

Conclusion

The thesis question reads as follows:

Which properties of the genotype-phenotype map cause organisms to be adaptable and robust to mutation at the same time?

This question was investigated for a specific natural system: the genetic regulatory network. The GRN was modeled as a random boolean network and appropriate measures of robustness and adaptability/innovation were defined. Degeneracy and redundancy, two properties of the genotype-phenotype map were also defined. It was found that the genotype-phenotype map of an RBN needs to be both redundant and degenerate to be robust and adaptable at the same time. Robustness correlates strongly with redundancy and adaptability correlates with degeneracy.
Chapter 15

Future work

The aim of this thesis is broad. The basic question asked is about the relationship between the genotype-phenotype map and evolutionary dynamics. This question is general enough to support an entire field of research. This field already exists and goes by the unfortunate name evo-devo, for evolutionary developmental biology. In this thesis a minute part of this question was investigated. Even asking the thesis question specifically for random boolean networks can keep a researcher occupied for a long time. Many questions remain. Some building directly upon this research and some investigating the question in a different way.

What are the topology and state space structure of robust and innovative RBNs?
This research has shown that RBNs which possess degeneracy and redundancy are at the same time robust and innovative. But what do these RBNs look like? Are their nodes connected in certain ways? Do they have a specific topology? Does their topology perhaps show modularity, as was found for different network models [58]. And what is the structure of their transitions in statespace compared to other RBNs? Do they arrive at an attractor quickly or do they take a long time? Do they possess many or few attractors? Can a recipe be given for creating an RBN which is robust and innovative?

Degeneracy and redundancy in other models Define degeneracy and redundancy for genotype-phenotype models other than RBNs. In this way the generality of the findings of this thesis can be determined. Is the co-occurrence of degeneracy with innovation a general trend or specific to RBNs?

Calculate degeneracy and redundancy for known GRNs RBNs can be used to model genetic regulatory networks at an abstract level. This means that an RBN can be modeled after a natural GRN, for instance
the segment polarity network of the fruitfly. It would be interesting to see what value of degeneracy and redundancy is found in RBNs modeled after natural systems. Are these values constant over different systems? This would be interesting as this research shows that the values are quite varied for random RBNs. Constant values in natural systems would perhaps indicate important properties of the natural genotype-phenotype map.

**How simple can the genotype-phenotype map get?** Natural systems have the property of being robust and innovative at the same time. This makes the system evolvable by allowing for change without being fragile. In this thesis it was shown that a direct mapping between "genes" and "functions" was not powerful enough to show this property. The property was found to exist in RBNs. However RBNs have a quite complex relation between genotype and phenotype. In this thesis an RBN phenotype is not a single state but rather a cycle of states. Also this phenotype is only reached after a certain path through genotype space. Which aspect of RBN function causes robustness to coexist with innovation? Can the RBN model be abstracted further? Perhaps a model is possible which does not require computation of all node states at several time steps to arrive at a cycle? Or perhaps the RBN model is the simplest possible without losing important properties. Only further research will tell.

**RBN state space memory and modularity** Modularity is unavoidable in building complex viable systems. Organisms appear to be modular in many respects. A property of RBNs which has been noted by other authors [17] implements a type of modularity.

All states of an RBN point towards a next state, like railroad switches. Inevitably there will be states which point to each other in a circle. These circles are called attractors. The state space can be divided into attractors and basin states leading towards an attractor. The basin states form paths of states leading towards an attractor (see figure10.1).

Mutation of the connections or boolean rules in an RBN changes the paths through statespace, although it does not affect the number of possible states. Only part of the states are redirected though. Dependent upon the type of mutation a certain portion of the paths through statespace is retained. The robustness of RBN attractors to mutations is in part due to the many paths that lead towards the same attractor. When a state in a path is redirected there is a high chance that it will point towards a different path towards the same attractor.
Consider a static initial state of an RBN which leads towards attractor A. Upon mutation of the RBN several things can happen. An initial state might still lead towards attractor A but through a different path, attractor A might have changed slightly, or the path might lead to a different attractor B.

Seen form a static initial state, a mutation might cause a switch of the RBN from attractor A to attractor B. However, many of the paths leading to attractor A are still intact in state space. Because of this further mutations have a high probability of restoring the path from the initial state to attractor A. This behaviour is called the memory of an RBN. This memory implements a type of modularity: the attractors can be seen as modules. These modules can be switched by mutations and are "remembered" in the structure of state space. The memory effect has been noted before [17] but has not been connected to modularity. Further investigation can yield important insights into the way modularity arises in natural evolution.

Mutations might cause a sudden switch from one attractor to another. This would look like a large jump in phenotype. However the genotype might have been expressed and optimized earlier in evolution and remained implicitly coded in structure of the statespace. In this way state space structure might help to explain phenotypic jumps.

**Degeneracy analysis of evolutionary algorithms**

The brittleness of simulated evolution is notorious in AI. As the evolved solutions become more complex changes become harder and harder. Soon any mutation disrupts the solutions found and change stops. Complex solutions might be possible but reaching this solution would require a large number of specific simultaneous mutations. In light of this thesis the traditional approach of directly changing the phenotype is naive. Well chosen mutational parameters can alleviate the problems only for so long. Eventually the lack of adaptability will stop any complexification. It would be interesting to try to compute degeneracy and redundancy for such basic evolutionary algorithms. Can the lack of adaptability be explained by a lack of degeneracy?

Another recurring theme in evolutionary algorithm research is that of "might help to find new solutions". Any feature which makes the phenotype insensitive to genotype mutations 'might help to find new solutions” because there is a certain amount of genetic freedom. This thesis has shown that such talk can be clarified by distinguishing between degeneracy and redundancy as causes of robustness. If a feature only increases robustness by adding redundancy the feature might im-
pede the finding of new solutions. An analysis of the structure of the neutral nets in genotype-phenotype space might help as well in this case. Does the feature allow connections to many different neural nets, increasing innovation? Or does the feature just increase the current neutral net, making the current solution more robust but also less able to change? Any "might help" conclusions are vacuous without such further analysis.
Appendix A

Mutations and probability

assumptions:

- minimal organism is needs 124 proteins of 400 amino acids [3]. Using the modern genetic code this comes to $124 \times 400 \times 3 = 1011600$ or about a million nucleotides to code this information

- currently there are an estimated $4 - 6 \times 10^{30}$ unicellular organisms on the planet [65]

- unicellular organisms double at rates between 1 day and 10 minutes

- the time frame between the advent of prokaryotes and eukaryotes is 2 billion or $2 \times 10^9$ years

The following are some crude calculations to determine whether beneficial mutations in organisms are possible/probable through random mutations given different assumptions for the evolutionary process and the structure of the genotype-phenotype map. The question that is investigated is whether it is necessary to postulate a great organising principle that makes organisms evolvable.

case 1: Asexual reproduction of a minimal organism population. Assume that only a single combination of mutations at specific locations in the genome can produce a fitter mutant. A second assumption is that a fitter organism needs only to appear once to spread through the population. The third assumption is that the entire sea contains the same species of prokaryotes. These assumptions are allowed in my opinion because the calculation is only intended to investigate whether beneficial mutations are possible at all. How long would it take for different length mutations to be achieved by a population with high probability?

assumptions:
minimal organism contains about ($\approx 10^6$ nucleotides).

number of prokaryote organisms produced in the world seas per year $\approx 9 \times 10^{29}$ [65](table 7(marine heterotrophs)). This number is taken as the number of mutation events taking place per year.

high probability = .95

A minimal organism contains about ($\approx 10^6$ nucleotides). This means that the chance of a distance-1 specific mutation $P_1 \approx 10^{-6}$. Given that the number of nucleotides is much larger than the number of mutations, $P_n$, the chance of an n-distance specific mutation can be approximated by:

$$P_n \approx P_1^n \cdot n!$$ (A.1)

with $P_1, (0 < P_1 < 1)$ The number mutation events (E) needed for .95 probability of a single specific mutation occurring is given by:

$$E = \frac{\log(1 - .95)}{\log(1 - p)}$$ (A.2)

Combination with equation A.1 gives

$$E_n = \frac{\log(1 - .95)}{\log(1 - P_1^n \cdot n!)}$$ (A.3)

Where $E_n$ = the number of years needed and $p_1$ = the probability of the event = $1/10^6$ in the case of a minimal organism.

Conclusion: given equation A.3, the $P_1^n$ term dominates with increasing n by becoming very small very quickly. This means that assuming only a single mutation is profitable, the probability of that mutation occurring tend to zero, even for the number of cells on earth and evolutionary timescales.
Appendix B

Bernoulli variables

The input to an RBN consists of one or more boolean nodes. To each of these nodes an activation is assigned which is either 1 or 0. The set of these nodes needs to be modeled as a random variable. Each node is a variable that can only have one of two possible outcomes. This type of system can be described by a so-called Bernoulli process. A well known example of a Bernoulli process is the repeated coin toss: a single coin is tossed and the outcome of this toss is either heads or tails. Formally the outcome of a single coin toss \( y \) can be defined as \( y = h \) for heads or \( y = t \) for tails and is called a Bernoulli trial. Multiple consecutive tosses are named a Bernoulli process and the random variable describing the outcome of this process would be named \( Y \) in this example. Certain properties of \( Y \) can be computed. For example If the coin is fair, the proportion \( y = h \) would approach 0.5 given enough tosses. In this case the entropy or uncertainty about the outcome of the process will be maximized as neither heads or tails is more likely to occur than the other. To be precise, given a fair coin the probability of either outcome is equal \( P(y = h) = P(y = t) = 0.5 \) and the entropy is maximized \( H(Y) = 0.5 \times \log(0.5) + 0.5 \times \log(0.5) = 1 \). This maximized entropy at equiprobable outcomes is not only true for a single tossed coin but also for multiple concurrently tossed coins or Bernoulli trials. The entropy of a process composed of \( n \) Bernoulli trials is maximized when in each individual trial the possible outcomes are equally probable. In this case the combined entropy of the \( n \) trials turns out to be

\[
H(X_1, X_2, ..., X_n) = n \tag{B.1}
\]

with \( X_i \) a 0.5 Bernoulli variable. By modeling each input node as a Bernoulli process the entropy of the set of input nodes can thus be determined analytically. Given that a single node can be modelled by a single Bernoulli process, a set of input nodes \( X^k_j \) can be modelled by a process consisting of
$k$ Bernoulli processes. Each Bernoulli process is defined to be equiprobable and thus result in node state 1 or 0 with equal probability. By extension each state of the set of input nodes is also equally likely to occur and all possible input states are sampled uniformly.
Appendix C

Before and after the war

A mathematical example of the way in which a soldier survives many battles purely by chance. This example shows a misconception which is also easy to make when thinking about evolution.

Think of a veteran soldier who took part in many lethal battles and always managed to come out unscathed many of his comrades were killed. It is easy to assume, as people often do, that this veteran is special. He seems to be guarded from above. The deception here is that there were countless soldiers at the beginning of the war, all of whom could have survived to become a veteran. It is only after one of them survives that we turn our attention to his case. We can reconstruct his actions in all the battles he took part in and use him as an example of a great soldier. It is not unlikely that he will be an able or even a great soldier. Being an expert marksman and having tactical insight will certainly increase his chances of survival. But the fact remains even if none of the soldiers are special, and the survival of each soldier in a battle was merely a product of chance, it is very probable that one will become a veteran. After a veteran is found it seems very unlikely that this specific man would survive each time purely by chance. However, before the war it is almost certain that a soldier will survive to become a veteran. Figure C.1 shows that for 10,000 soldiers and a .5 chance to survive each battle, there is a very high probability that someone will survive 40 battles. When the story of this survivor is viewed in retrospect it will seem impossible that this survival is due to chance.
Figure C.1: Approximation of probability distribution. 10,000 agents have a .5 chance of survival each round. The number of rounds until no agent is left is counted. This is repeated 10,000 times and plotted in this histogram.)
Bibliography


