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Dynamical systems and lineage decision making: a systematic approach for the evaluation of a phenomenological mathematical model

Master thesis

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Abstract

In this thesis, we analyze and evaluate a phenomenological model for cell differentiation based on Hill-function type interaction kinetics. This is an extension of a model formulated by Dr. Sui Huang that has been proposed by Dr. Stefan Semrau to explain the observations of retinoic-acid driven mouse embryonic stem cells differentiation. Thereby, our main goal in this thesis is to evaluate the proposition that the model suffices as a conceptual mechanism of the performed experiments. Towards this end, we investigate how Frege's theory of judgment can be used along with Kant's theory of judgment to provide a systematic evaluation of phenomenological mathematical models.

Acknowledgements

First of all, I would like to thank my thesis advisor Dr. Sander Hille for his inspirational lectures during the course of my master training at Leiden University, from whom I have learned many of the special techniques, which I know, so as to analyze mathematical models in a biological context. Dr. Sander Hille could in a very short time point me out how I ought to look at a problem. Besides that he could also tell in which way we should be describing it before reducing it to the equations. These are higher order thinking skills that I will keep pursuing as a mathematical biologist. Likewise, I would like to thank Dr. Stefan Semrau for having given me a project with which I believe that I have developed necessary skills to keep doing research in this field. Dr. Stefan Semrau was always available to discuss some related questions by coming up with suitable ideas which were extremely necessary for my understanding of the project and, subsequently, for our accomplishment. His thinking fluidity was a motor in the process of sharpening the analysis. Lastly, I would like to thank my family for having believed in me since my childhood until now.

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List of Relevant Symbols

$\bigvee$	there exists
$\bigwedge$	for all
$\bot$	a contradiction
$\mathcal{A}$	the set of relevant aspects on model $\mathcal{M}$ for the judging agent
$\mathcal{L}^*$	a formal language for the model $\mathcal{M}$
$\mathcal{M}$	a phenomenological mathematical model
$\mathcal{SC}^{\mathcal{M}}$	the scenario space with respect to the model $\mathcal{M}$ and to the relevant aspects $\mathcal{A}$
Π	the knowledge-transformation mapping
$\sim$	symbolizing similarity between a scenario and an observation
$\top$	a tautology
$\vdash A$	a judgment as a logical notion, with A representing the judgeable content
$\vdash$	the assertoric force or/and the symbol of a syntactic proof on the basis of natural deduction
$\vdash^{\Psi} A$	a judgment as an empirical notion, with A representing the judgeable content
$\vdash^{\Psi}$	the act of judging based on empirical evidence
$\vee$	or
$\wedge$	and
$\Xi[\mathcal{O}]$	the set of all relevant properties of an observation $\mathcal{O}$ described and stipulated by the modelling agent
$\Xi[sc^{\mathcal{M}}_{\lambda}]$	the aspects in $\mathcal{A}$ that are true on scenario $sc^{\mathcal{M}}_{\lambda}$
$A^{\mathcal{O}}$	a formalized assertion in $\Xi[\mathcal{O}]$, that is, a property of the observation $\mathcal{O}$
$sc^{\mathcal{M}}_{\lambda}$	a scenario generated by an admissible parameter setting λ

Preface

Over one year ago, I started attending the lectures of the subject " Logic and the First-person" given by Dr. Maria van der Schaar at Leiden University. But, why did I delve into the philosophy of logic to accomplish my master project? In fact, I did so because I was trying to evaluate a phenomenological mathematical model when I got deeply confused about what I had exactly been doing up to that moment. After being faced with some inconsistencies in my own thinking, I started questioning the mentality to which I had been subject as regards phenomenological mathematical models. Despite having gotten profoundly frustrated, I was feeling very motivated to go through that process and learn as much as possible therefrom.

But, what was the motor of my thinking until that moment ? In fact, the driving force of my actions towards an evaluation procedure was the aphorism attributed to Dr. George Box : "Essentially, all models are wrong, but some are useful.". However, if the concept of *model* fundamentally means an approximated representation of an ontological counterpart and if such a representation is inherently simplified and idealized, then the respective aphorism offers no elucidation to how we ought to be performing an evaluation procedure. In fact, knowing which observations are found in the model is not logically equivalent to knowing which ones are not therein. Furthermore, being able to tell which ones are not produced by the model, might either unravel properties that contradict the ontological counterpart, which, in turn, would strongly suggest that I should rule out the model, or might provide myself with a suitable strategy to modify or extend it.

On the other hand, such a task seemed not to be doable given that it purportedly entails a continuous search within the parameter space. Hence, I knew that if I intended to come up with an evaluation procedure enabling that, then I should ensure that it could be done algorithmically, that is, by constructing a method with which I could reduce it from a continuous to a discrete search in a systematic way.

Next, after this primary process through which I acknowledged that "my thinking activity" had been engulfed in a domain wherein *psychologism* was governing it, that is, in which I allowed that inherited beliefs (ideas regarded as "mental laws") formed the basis of my reasoning as to phenomenological mathematical models, I was then entirely convinced that I was in need of a suitable philosophical framework to approach such an envisaged evaluation procedure.

Nonetheless, what does it have to do with *Frege's theory of judgement*? In fact, a necessary condition for one to evaluate a phenomenological mathematical model is that one makes judgements about that. So, I must make assertions on the model that can be proven true or false. The later elucidation reveals that the *first person perspective* cannot be neglected in our investigation. And what is the role of *logic* in the latter process? In fact, *logic* gives the rules of inference with which I can prove mathematical assertions on the model. The latter essentially stipulates the style in which I have tried to write the thesis. Indeed, the writing is performed in the domain of analytical philosophy wherein argumentative clarity and precision—by means of formal logic—are central points. The idea was to annihilate any bias in the reasoning to the extent that I could function optimally and let logic speak by itself. Consistently, I have often used logical symbols instead of known mathematical jargon when constructing the mathematical assertions given that I feel much comfortable with the former than with the later. Presumptively, the adopted writing style partly explains why the thesis has become rather extensive with respect to known standards for a master thesis.

Now, why does *Frege's theory of judgement* suit the purpose? In fact, drawing upon the approach of Dr. Maria van der Schaar, the notion of judgement in Frege's theory has a logical account—an acknowledgement of the truth of an assertion. Thereby, if we regard a phenomenological mathematical model as a mere mathematical object and if the truth of mathematical assertions on it must be objective, then Frege's notion of judgement is a suitable concept to understand the essence of the judgements made by the judging agent, which, in turn, is interpreted as being a transcendental ego. On the other hand, a phenomenological mathematical model is the description of a phenomenon taking place in the world so, by construction, all the parameters and variables and the relations among them have specific meanings. In that regard, a judgement as a logical notion is not adequate to apprehend an assertion on the model. Actually, in this case, a judgement is understood as an empirical notion—the Kantian notion of judgment—seeing that it is a mental process in response to an empirical activity. Hence, the judging agent, in the later case, is said to be an empirical (psychological) ego.

Therefore, a judgement as a logical notion can only be understood from a first person perspective while a judgement as an empirical (psychological) notion can only be apprehended from a third person perspective, which, in turn, unveils a duality of the judging agent as a transcendental and as an empirical (psychological) ego. So, it now seems that psychology on its own was not the culprit of my confusion but not having a suitable account for the interplay between logical and psychological aspects of the model was tantamount to that.

But, how can I perform a systematic evaluation procedure of a phenomenological mathematical model? In fact, the logical notion of judgment in Frege's theory is an assertion to which the truth-value 'true' is assigned, that is, an assertion that a determinate object falls under a concept. As there are concepts whose definitions are dependent on other concepts—complex concepts—then there must be concepts that are true by themselves, which cannot be defined, or better, which cannot be reduced to other concepts. The later claim can be thought to be predicated upon the presupposition that it is inconceivable that a concept would be apprehended by an infinite entailment of notions. Thereby, such indefinable notions-true in themselves-are known as primitive concepts—opposed to complex concepts.

Therefore, if we succeed in conceptualizing 'something' related to the model that stands for an observation of the ontological counterpart, and if we can find the ones falling under the concept of that 'something' playing the role of primitive notions, then we have a method enabling us to perform a discrete search in the parameter space of the model. In fact, if I call that "something" a *scenario* in the model standing for an *observation* of the ontological system then the idea is that any *scenario* of the model can be reduced to a *primitive scenario*. Therefore, if I

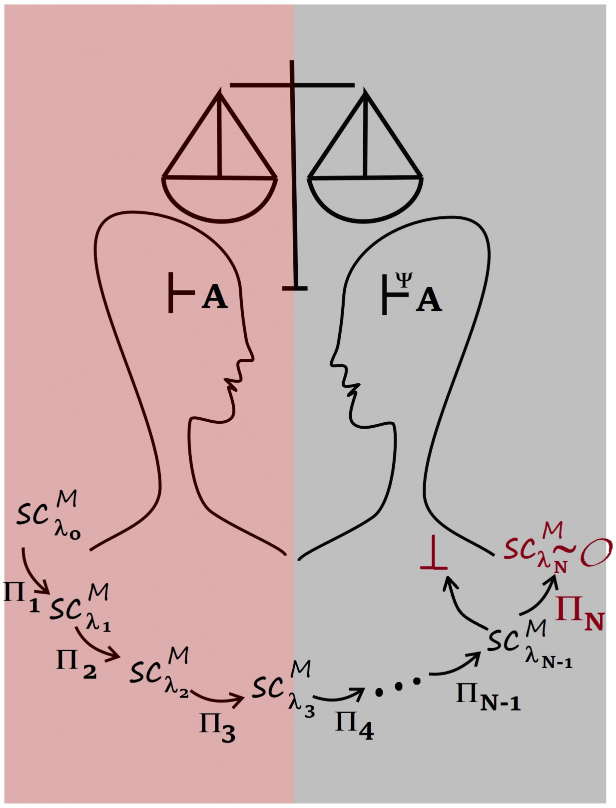


Figure 1: Here we see the illustration of the "justice symbol" above the two faces. The later ones stand for the duality of the judging agent as an empirical ego and as a transcendental ego, being represented by the judgement symbols $\vdash^{\Psi} A$ and $\vdash A$ respectively. Below the two faces, one sees the decomposition of the primitive scenario $sc^{M}_{\lambda_0}$, which starts by applying a suitable judgment Π_1, leading to the scenario $sc^{M}_{\lambda_1}$, which, in turn, by applying judgement Π_2, is shifted to the scenario $sc^{M}_{\lambda_2}$. The respective decomposition process stops when scenario $sc^{M}_{\lambda_N}$-similar to the observation $\mathcal{O}$-is found, or when a contradiction $\perp$ is found.

can find the primitive scenarios of the model then I can potentially find any scenario of the model, that is, I can tell whether or not an observation can be 'found' in the model. However, finding those primitive scenarios is not an easy task as we shall see through this thesis. The latter schema is depicted in the Figure 1.

I would like to thank my advisor Dr. Sander Hille for his kindness and patience during the course of this thesis. His superior thinking skills helped me to develop another way of looking at the interplay between equation and phenomenon. Furthermore, the realization of this thesis has only been possible due to the richness of his precious insights; I am deeply thankful for that and for all the analytical tools that he has taught to me. I would like to thank my co-advisor Dr. Stefan Semrau for having provided me with a project with which I believe that I have developed myself from several perspectives. His remarkable thinking fluidity and predisposition to think pragmatically about finding a solution for a problem were essential for me to reach a rather deep understanding of the phenomenon being modelled.

I would like to thank Moos Laurens Nogueira and Benjamin Abel Nogueira

for their existence. When you both come to an age at which you will be able to understand the underlying feelings of these notes, then you will know that you have helped me a lot through this process though paradoxical it might sound. If you both have goals in your life then try hard to find the way in which you can achieve them; neither will you internalize when someone tells you or makes you feel like you cannot reach them nor will you accept the idea that you are inherently supposed not to attain them. You must find the way in which you can disprove that, because the burden of proof might be on you.

Milton Nogueira da Silva Junior
Leiden, March 15, 2019

Chapter 1

Introduction

An ongoing problem in Biology can be formulated into the question of how can a complex organism come from a single cell? Or equivalently, how can a *zygote*[1] become an *embryo*? In fact, a *zygote* undergoes *mitosis*, that is, a process through which a cell gives rise to two identical cells with respect to the genetic material [*DNA*]; and *cell differentiation*, which is a process by which cells become specialized ones, resulting into a *multicellular organism*, or better, an *embryo*. What is fundamental so as to go from an *unicellular organism* to a *multicellular organism*? Further in this chapter, we will be giving an answer to the latter question.

Regarding the main goal of this thesis, we rely upon an argumentative approach based on the order of *conceptual priority*, which will reveal a *rational strategy* to evaluate *Huang's model* of *cell differentiation*, and an extension thereof: *Semrau-Huang's model*. But, how will we do it? In fact, we will first project our analysis onto the realm of the *philosophy of logic* by exploring the primitive nature of the concept of *knowledge* and *judgment*, turning our attention toward *perspectivalness*. The latter will then point us out to the necessity of a better clarification of the role of the *first-person perspective* in the *evaluation* of a *phenomenological mathematical model*.

Lastly, as for the wording, we will emphasize by italics all the important concepts throughout this thesis. Moreover, we will use "( )" in the text whether we judge that a better clarification ought to be provided. Next, unless we say otherwise, one has that the brackets "[ ]" will be used for synonyms, antonyms or examples assumed to reinforce the apprehension of an elucidation. Later in Chapter 2, one has that the symbol "[ ]" will also be taken to represent an important action in *natural deduction*.

1.1 The importance of the order of conception priority in understanding gene regulation

The notion of the order of *conceptual priority* was introduced by Dr. Per Martin-Löf in [55]. In fact, a *concept* precedes another one if the *definition* of the later one is dependent upon the definition of the former. Having defined that, if we draw upon the *epistemic status* of cell activity then we can say that we know that there are specific *molecules* within the cell that catalyze *biochemical reactions* which, in fact,

[1]The first *cell* in the living cycle of an *organism* resulting from the fusion of the *ovum* and the *sperm*.

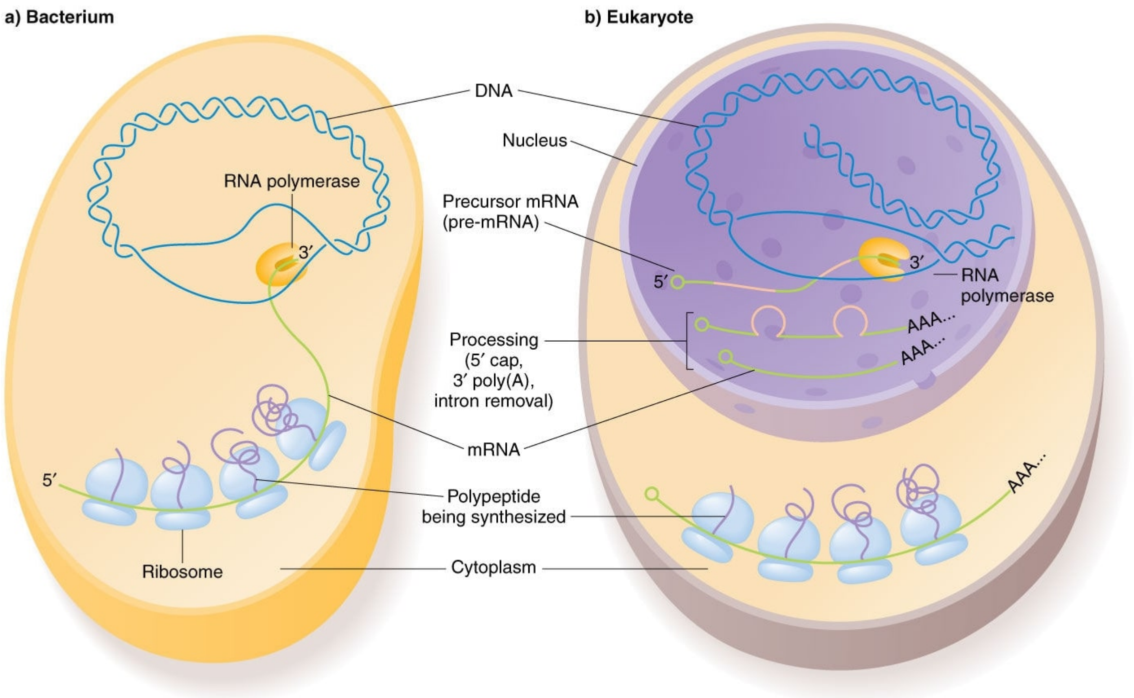


Figure 1.1: This cartoon has been taken from [78]. Here, one sees the depiction of the process of transcription and translation in a prokaryotic cell (bacterium) and in a eukaryotic cell. In contrast to a prokaryotic cell, in which translation presumably begins right after transcription, the eukaryotic apparatus is much more complex involving at least three levels of regulation prior to translation: mRNA capping, polyadenylation and RNA splicing.

are involved in a variety of cellular processes including *cell growth*, *cell division*, *cell proliferation* and *cell death*. In light of their particular function, those *molecules* actually receive a more sophisticated name, that is, they are known as *enzymes*. The latter *concept*, i.e. being an *enzyme* is solely functional and structural determined.

In order to unveil an entanglement of *notions* paved by the order of *conceptual priority*, we must ask ourselves questions regarding the synthesis of an *enzyme* in the cell environment. Or equivalently, How is an *enzyme* produced in the cell? In fact, if we rely upon the *epistemic status* of the concept of an *enzyme* (see [15]) then we can say that an *enzyme* is a *protein* or a *ribozyme*. Furthermore, the set of enzymes, which are proteins, and the set of enzymes, which are ribozymes, are mutually exclusive. But, what is a *protein*? And, what is a *ribozyme*? Actually, both of them are considered as a *gene-product*. Now, we know that the concept of an *enzyme* is conceptually dependent on the notions of a *protein* and a *ribozyme*, which, in turn, are conceptually dependent on the notion of a *gene*. However, what is a *gene*? Despite the controversy over the concept of a *gene* (see [27] and [68]), we adopt a definition that serves the purpose of our analysis. In fact, according to Gerstein et al [27], a *gene* is a *DNA coding sequence* or a *DNA functional non-coding sequence*. But, the latter concepts are conceptually dependent on the concept of a *DNA*. So, what is a *DNA*? In fact, a *DNA* is a *double-stranded polymeric macromolecule* that contains *genes* carrying instructions for the whole *life cycle* of a *living organism*. What is intriguing about their proposed concept of a *gene*? It is a *circular definition*, due to the fact that it depends on the concept of a *DNA* which, in

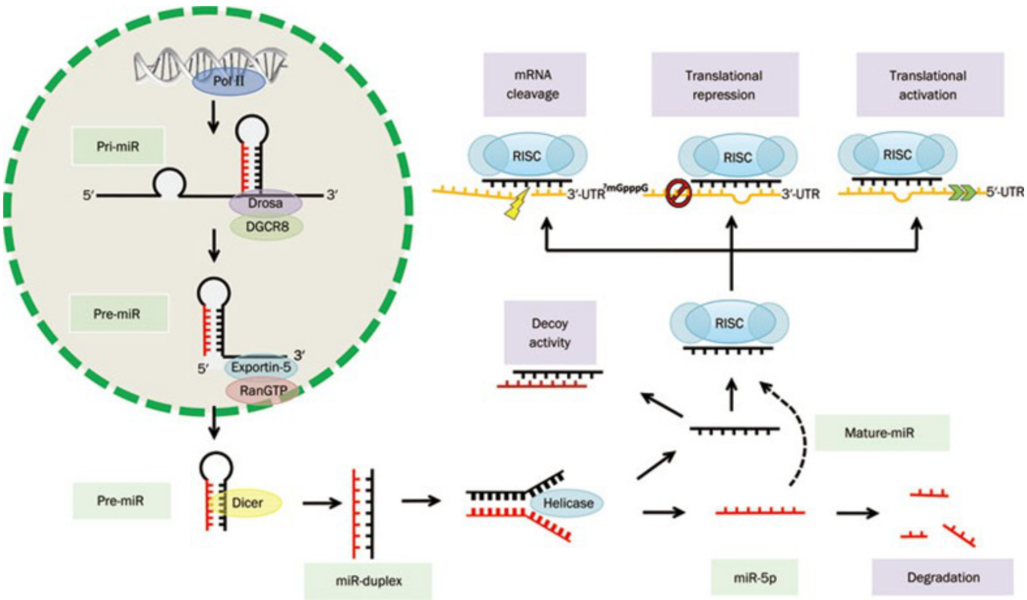


Figure 1.2: This cartoon has been taken from [51]. MicroRNA mechanism: RNAp II (RNA polymerase II: a ribozyme) transcribes pri-miR (primary microRNA); DGCR8-Drosha complex (DGCR8: a protein; Drosha: a RNase III: a RNA enzyme, that is, a ribozyme that catalyzes degradation of RNAs in small fragments) processes pri-miR into pre-miR; Exportin 5-RanGTP complex (Exportin 5: a protein; RanGTP: a protein) transports pre-miR out of the cell nucleus to the cytoplasm; Dicer (a RNase III) processes pre-miR into mature miR; RISC (a multiprotein complex) binds to miR to provoke repression of the translation of mRNA; RISC binds to miR to cleave mRNA; RISC can promote translation of mRNA by binding to its $5'$ *untranslated region (*$5'$ *UTR).*

turn, refers back to the concept of a *gene*. The latter circularity suggests that there might be something essential about trying to capture the notion of a *gene*. In fact, one has that the concept of a *gene* seems to be a *primitive notion*, or equivalently, a notion that cannot be defined in terms of previously well-defined notions whose definitions do not depend conceptually upon the notion being defined. However, how can we understand such a primitive notion then? Further in this thesis, we shall appropriately turn ourselves toward the latter question.

If we want to apprehend their proposed definition of the concept of a *gene* then we need to clarify the notions of a *DNA coding sequence* and a *DNA functional non-coding sequence*. But, such a clarification amounts to answering the following questions. How can a *gene* give rise to a *protein* or a *ribozyme*? How is this synthesis regulated then? Or rather, how does *gene regulation*, that is, the control of the turning on and off of a *gene*, occur? In order to cast light on the latter questions, we need to invoke the *central dogma*, or rather, the central hypothesis of molecular biology as illustrated in Figure 1.1. Indeed, the *central dogma* is a *dogmatic mechanism* for *gene regulation* that comprises a finite set of *regulatory proteins*, that is, the *transcription factors (TFs)*, which bind specific sites of *DNA* in the surroundings of a *gene* of interest. Thereby, those specific sides in *DNA* bound by *TFs* gives rise to the concept of an *operator*. What do *TFs* bind an *operator* for? In fact, when bound to *DNA*, *TFs* change *DNA-conformation* so they can either repress the activity of the respective *RNA polymerase* (*RNAP*) or facilitate

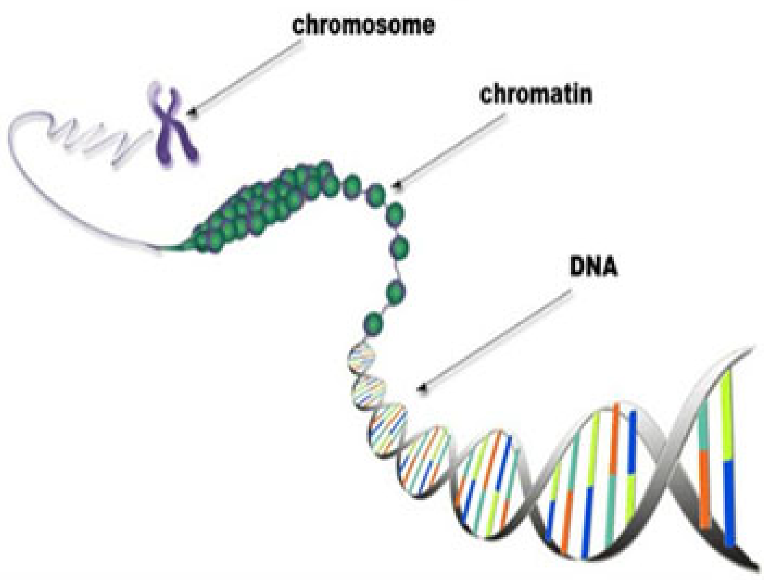


Figure 1.3: This cartoon has been taken from [30]. A chromosome as a compacted chromatin, or equivalently, a compacted structure consisting of DNA wrapped around histone proteins.

its binding to a fixed DNA sequence, which is defined as the *promoter*. Regarding the later case, *RNAP* will thereupon initiate the process of *transcription* of *DNA* into a *RNA*. In this regard, we identify *TFs* involved in the *repression* of *RNAP* as the *repressor* whereas *TFs* involved in the facilitation of *RNAP* are thought to be the *activator*. Hence, in this hypothetical mechanism[2], the *promoter* can be thought as being in one of the states: *active* or *inactive*.

But, what is a *RNAP*? It is a *RNA enzyme*, or equivalently, a *ribozyme*. More specifically, *RNAP* catalyzes the *transcription* of *DNA* into *RNA*. So, the concept of *RNA polymerase* is conceptually dependent upon the concepts of *RNA* and *enzyme*. But, what is a *RNA*? According to the *central dogma*, a *RNA* is a *polymeric molecule* synthesized during the process of *transcription*. If a *RNA* can be translated into a *protein* then it is said to be a *coding RNA*. On the other hand, if a *RNA* is already functional, such as *RNAP*, and cannot be translated into any *protein* then it is defined to be a *functional non-coding RNA*. But, what do we mean with a *RNA* being translated into a *protein*? In fact, in this case, a *RNA* is regarded as a messenger *RNA*−a *mRNA*.

Mainly driven by diffusion[3], that is, by performing a *random walk*, one has that a *mRNA* will be transported to the cytoplasm wherein it will be bound by a *ribosome*. But, what is a *ribosome*? It is a *complex molecule* consisting of *non-coding RNAs*, known as *ribosomal RNAs* or *rRNAs*, and lots of distinct *proteins*. The latter will perform the *translation* of a *mRNA* into an *amino acid sequence (polypeptide)* which,

[2]It might be misleading to use *hypothetical mechanism* in this context if we rely upon several papers in which one can find irrefutable evidences supporting the *falsifiable status* of the *central dogma*, but as the author of this thesis is not able to argue to what extent the central dogma is "true" and if the question is relevant in some "complex organism", he chooses to assign the *hypothetical status* to it.

[3]Not necessarily true for *prokaryotes*, seeing that there is no *membrane-bound nucleus* so *DNA* is already floating loosely in the cytoplasm.

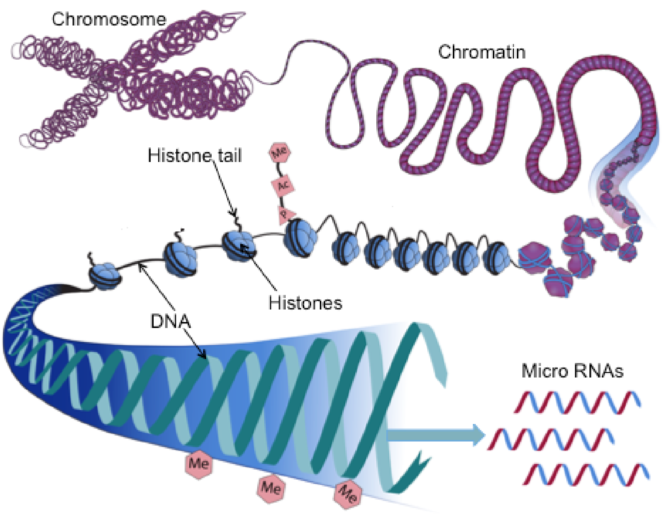


Figure 1.4: This cartoon has been taken from [20]. Here, one sees an illustration of the process of histone acetylation and cytosine methylation. In fact, HAT enzymes introduce an acethyl group to histone proteins which causes DNA to uncoil itsef. That allows TFs to bind target DNA sequences culminating in the transcription process performed by RNAPs, while HDACs enzymes removes the acethyl group from histone proteins what abrogates TFs due to the coiling of DNA.

in turn, will thereafter fold into a three-dimensional functional molecular structure defined as a *protein*. Now, if we assume that there is a one-to-one correspondence between the set of *DNA coding sequences* and *coding RNAs*; and between *DNA non-coding sequences* and *non-coding RNAs* then we can, in so doing, capture the essence of the definition of the concept of a *gene* introduced by Gerstein et al [27].

What guarantees that a *RNA* really suits the purpose? Or better, how can a *RNA* be correctly transcribed by a *RNAP*? In fact, if an error occurs during the process of *transcription* then *RNA polymerase* can pause *transcription* so as to cleave the error away from that sequence. So, *RNA polymerase* can fluctuate between an *active state* and a *backtracked state*. The latter mechanism of fidelity in the *transcription* process gives rise to the notion of *proofreading* [82, 36, 13], as illustrated in Figure 1.6. How can we conveniently apprehend *RNAs* at the conception level? In fact, *RNAs* can be regarded as the union of two mutually exclusive sets, that is, the one consisting of *coding RNAs*, such as *mRNAs*, and the one formed by *non-coding RNAs*. The latter can be categorized in *non-coding functional RNAs* and *non-coding non-functional RNAs*. As for *non-coding functional RNAs*, one can reefer to *RNAPs* and to *microRNAs* (*miRNA*; *miR*) as genuine examples. In fact, *microRNAs* are small *non-coding functional RNAs*, as reported in [31], which bind target *messenger RNAs* preventing them from being bound by *ribosomes*. So, it results in *mRNA-degradation* what corroborates the *repression*[4] of the related *gene* as illustrated in Figure 1.2. The latter process leads to the notion of *gene silencing*. Therefore, in the introduced conceptual framework, one has that the *concept* of a *microRNA* suggests a *stratification* of the notion of *gene regulation* so it can be divided into

[4]However, it has been also reported that *microRNAs* can promote translation of a mRNA by binding to its 5′ untranslated region (5′ *UTR*) as one can verify in [98].

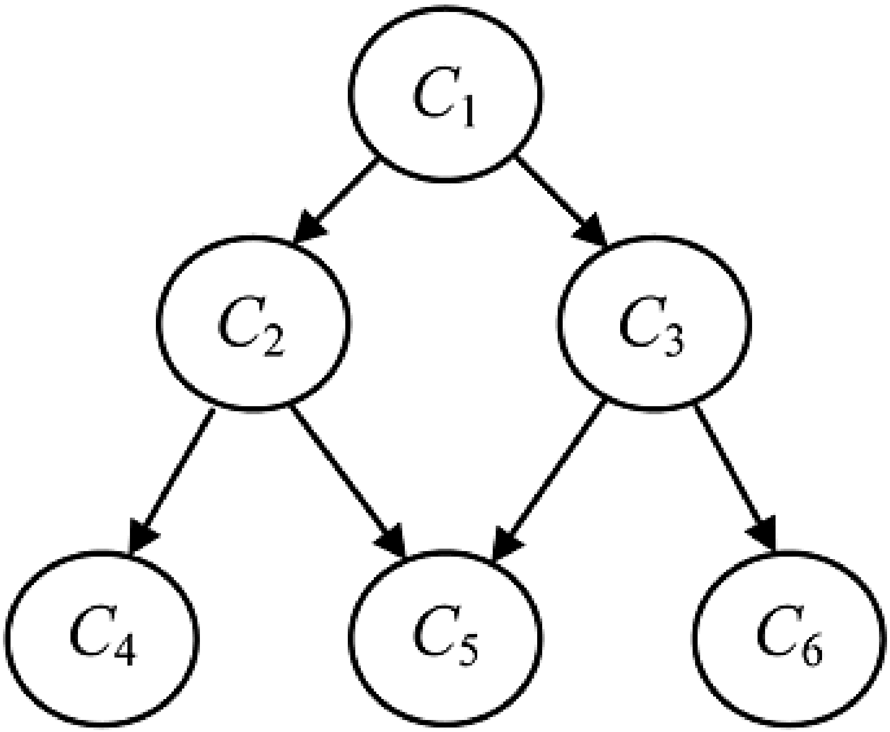


Figure 1.5: This cartoon has been taken from [102]. Here, one sees a "directed graph" in which the "nodes" represent the concepts. The direction of each "edge" is determined by the "conception order" which means that the concept C_1 is conceptually dependent upon the concepts C_2 and C_3 and so forth. However, the concepts C_2 and C_3 are not conceptually related to each other. That means that $\{C_1, C_2, C_3, C_4, C_5, C_6\}$ is "partially ordered". Furthermore, the concepts C_4, C_5 and C_6 can be thought as the most fundamental notions or as the irreducible ones, that is, the primitive ones. Therefore, at the conceptual level, one might regard gene regulation as a partially-ordered hierarchical graph.

pre-transcriptional one and *post-transcriptional* one.

In *eukariotic cells*, if we want to be a little bit more specific as to *post-transcriptional regulation* then we can also tell that a transcribed piece of *coding RNA* primarily consists of *introns*, that is, *DNA sequences* of a *gene* not used for *translation*, and *axons*, which, in turn, are defined as DNA sequences of a *gene* that will be definitely used for *translation*. Thus, the latter concepts of *axons* and *introns* give rise to the notion of a *pre-mRNA*[5], that is, a *coding RNA* containing *introns* and *axons*. In order to prevent a *pre-mRNA* from being clove by *RNases*, which are ribozymes specialized in catalyzing the *degradation* of *RNAs*[6], one has that a *pre-mRNA* undergoes physico-chemical modifications right after *transcription*. In fact, those modifications include the addition[7] of a *cap tail* to its *five-prime end* $\left(5' cap\right)$, and the annexation of a *poly(A) tail* to its *three-prime end* $\left(3' poly(A)\right)$ as shown in Figure 1.1. In this regard, one has the emergence of the concepts of *mRNA capping*

[5]It is fundamental to noting that *introns* are not necessarily wrong sequences. In fact, *introns* and *axons* in a transcribed sequence, are defined in relation to a specific protein what the respective *gene* code for. Actually, an unique *gene* can encode many proteins as reported in [99, 46].

[6]Such as RNA viruses.

[7]For biochemical details see [10].

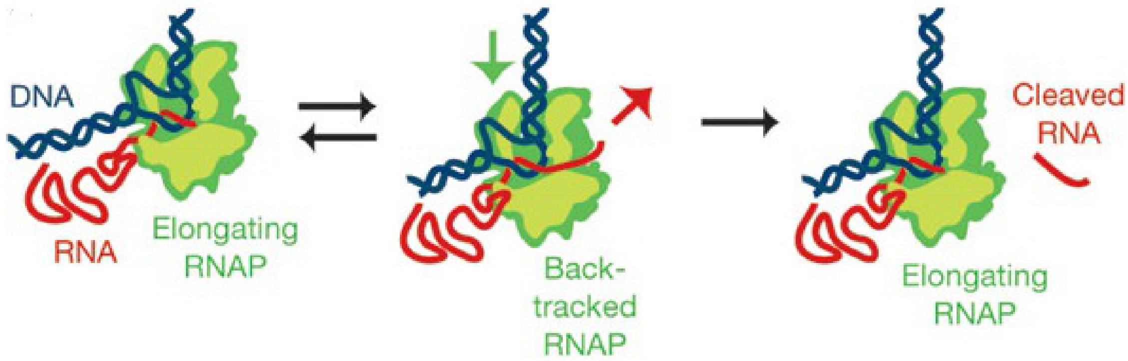


Figure 1.6: This cartoon has been taken from [88]. Here, one sees an illustration of the proofreading process through which RNAP can fluctuate between backtracked and active state.

and *polyadenylation*, respectively. Next, that modified *mRNA* undergoes another physico-chemical modification through which its *introns* get extracted by highly complex *macromolecules* made of several *proteins* and *RNAs*. Those molecules are known as *spliceosomes*. So, the latter dwindling process gives rise to the concept of *RNA splicing*. In light of that process, a *pre-mRNA* becomes a *mature mRNA*, that is, a *messenger RNA* ready for *translation*. In sum, in *eukaryotic cells*, one has that a necessary condition for *translation* to occur is that a pre-mRNA goes through *mRNA capping*, *polyadenylation* and *RNA splicing*. Therefore, *stratification* of *gene regulation* flows rationally in the direction of the *conceptual order*. Moreover, one might also assert that the notion of *stratification* of *gene regulation* is actually equivalent to the concept of *layers of gene regulation* introduced by Dr. Stefan Semrau in [87].

Likewise, if we appeal to the *central dogma* to deepen our understanding about the changes in *DNA-conformation* caused by *TFs* then we can assert that *gene regulation* can be separated into *pre-translational* one and *post-translational* one as well. Indeed, for instance, how can a target site of *DNA* become accessible for *TFs*? This is actually controlled by *epigenetic mechanisms*. But, what are epigenetic mechanisms? Those are mechanisms of *gene regulation* that cause *DNA* to change its conformation without altering *DNA-sequence*. So far, we have brought up the notion of *DNA-conformation* without explaining it sufficiently. So, what do we mean with *DNA-conformation*? It is defined as any feasible spatial arrangement that *DNA* can have. In order to understand it intuitively, we might build upon the order of *conception priority* by invoking the concept of a *chromosome*, which is a compact structure carrying *DNA*. But, how is that compact structure organized? That consists of a coiled *DNA* wrapped around *histone proteins*, which, in turn, gives rise to the concept of a *chromatin*. Hence, a *chromosome* can be defined as a compacted *chromatin* as illustrated in Figure 1.3. Therefore, consistently, the concept of a *chromosome* is conceptually dependent upon the concept of a *chromatin* which, in turn, is conceptually dependent upon the concepts of a *DNA* and a *protein*.

As an example of such epigenetic mechanisms, one has *histone acetylation* and *cytosine methylation*. As for the former, it consists of the insertion of an *acetyl group* by specific *enzymes*, that is, *Histone Acetyltransferases (HATs)*, to *lysine aminoacids* on *histone proteins*. Hence, a *post-translational* protein modification, that is, *acetylation* of *histone proteins*, cause *DNA* to uncoil itself which creates physical accessibility for *TFs* to bind target *operators* enabling *RNAPs* to access the *activator* so as to initiate the process of *transcription* as illustrated in Figure 1.4. As for the latter, it is described as the inclusion of a *methyl group* to *cytosines*[8] in the *DNA sequence*, causing *DNA* to get condensed what abrogates DNA-binding proteins *(TFs)* as depicted in Figure 1.4. Moreover, concerning the respective reversal mechanisms, one has *histone deacetylation* and *cytosine demethylation*. In fact, *histone deacetylation* is the removal of an *acetyl group* from *histone proteins* by *Histone Deacetylases (HDACs)* inducing coiling in *DNA* while *cytosine demethylation* is the extraction of a *methyl group* from *cytosines*, that is, the removal of a barrier switching off *DNA target sequences*[9].

If it is true that most of the *DNA* is useless then it is reasonable to know how genes are actually distributed in the *DNA*. As reported in [24], genes are not randomly distributed in the *DNA*, but they form clusters of genes that are likely to be coexpressed without having necessarily any functional relation. That means that genes belonging to the same cluster in the *DNA* are highly likely to be related to each other at the *transcriptional level* but not necessarily at the *translational level*. Although it seems to be counter-intuitive that neighboring genes might be functionally unrelated to each other, they argue in [24] that a plausible explanation for that is based on *natural selection*, which is the underlying *mechanism* of *evolution*. Indeed, this cluster organizational structure observed in the distribution of genes in the *DNA* has been achieved by fine-tuned evolutionary processes so as to reduce *gene expression noise*.

But, what was the purpose in reducing *gene expression noise*? In fact, a high *noise* in *gene expression* can have a negative effect on *cell fitness*[10]. In order to give an argument for that, we might draw upon the molecular morphology of *ribosomes* and its important roll in the process of *translation*. Indeed, as we described earlier, one has that *ribosomes* are highly *complex macromolecules* consisting of *RNAs* and many different *proteins*. Besides that, according to [48], the 'total number of ribosomes' in a *mammalian cell (eukariotic cell)* is around 10^7, which, for example, amounts[11] to 0.00002% of the total volume of an *egg cell*. So, if one regard the latter percentage as a significant one then it might be used as a reasonable justification for an eventual use of the notion of *concentration* in an argument referring to the 'level of ribosomes in the cell'. If not then one can also use "the total number of ribosomes" instead. In fact, in no way will the latter choice alter the conclusion of our argument.

However, as we shall see, even though our argument is not contingent upon the notion of the 'level of ribosomes in the cell' being used, it offers a suitable occasion to bring up the issue of *ribosomal heterogeneity* in the control of *gene expression*. To begin with, also according to [48], the number of *ribosomal proteins*[12], in each *ribosome*, amounts to 80. So, it is reasonable to imagine that ribosomes

[8] *Cytosine, adenine, guanine* and *thymine (uracil)* are the four *bases* found in *DNA*.

[9] Or equivalently, *DNA coding sequences* or *DNA functional non-coding sequences*.

[10] A measure of the *health state* of a cell concerning its ability of reproducing itself.

[11] This estimation was calculated by the author of this thesis by using that the diameter of an *egg cell* is approximately equal to $1.0mm$ and of an *ribosome* is around $25nm$. Moreover, he has been also predicated upon the assumption that their volumes might be approximated by the volume of a sphere.

[12] In a *mammalian cell*.

might be selective in translating *mRNAs*. In fact, it has been hypothesized that the *translation* process does depend on the interactions among *mRNAs* and *ribosomal RNAs and proteins*, or equivalently, cells presumably build specialized ribosomes for the synthesis of proteins. The later hypothesis is known as the *ribosome filter hypothesis* as broadly discussed in [41]. But, is there an evidence for that? Yes, there is indeed. In [92], it was shown that the variability in the total number of specific *ribosomal proteins* in *mouse embryonic cells (mESCs)* correlates with *cell fitness*.

So, an argument for the current question reads as follows. As the 'total number of ribosomes' must be maintained *stable* in the *cytoplasm* for a normal cellular function then a low *noise* in the expression of their respective *DNA coding sequences* and *DNA non-coding sequences* is a favourable condition for *cellular growth, division* and *proliferation* [21], which, in fact, are essential processes for *embryogenesis*. On the other hand, another *via positiva* argument for that, can be given from a *mechanical perspective* given that *gene expression* involves changes in *DNA-conformation* [60] caused by the binding and unbinding of *TFs*, which, in turn, embroils the *stress-strain*[13] relationship in that. Thereby, a high *expression noise* could potentially increase the chance of damage in the *DNA* structure, causing certain *mutations* to occur, that is, alterations in a *DNA coding sequence* or *DNA non-coding sequence*. Those *mutations* in *DNA* would presumably lead to severe implications for a normal cellular function which, in turn, would impair *embryonic development*.

If the latter arguments are plausible then we should ask ourselves what is fundamental to understanding them as a whole? It is irrefutable that knowing the meaning of the involved *concepts* is a necessary condition for that. However, we argue that apprehension of the *notions* might not be sufficient to know how the above arguments are interlocked with each other. In fact, the order of *conceptual priority* enables us to make such an connection between them seeing that the concept of a *ribosome* is conceptually dependent on the concept of a *rRNA* and on the concept of a *protein* which, in turn, are both reducible to the concept of a *gene*. How can we connect the above arguments then? In fact, the definition of the concept of a *gene* has been given in terms of the notions of *DNA coding sequence* and *DNA non-coding sequence*. The latter concepts have been clarified in terms of *transcription*, which entails changes in *DNA-conformation*, and *translation*, which involves the binding of *ribosomes* to a target *mRNA*, that is, one cannot understand the respective arguments without invoking the *central dogma* in which *translation* and *transcription* are crucial notions. Therefore, the conceptual order is essential to putting the latter arguments into perspective to each other.

Are there *non-primitive concepts* in *gene expression* that are non-comparable, or rather, that are conceptually independent upon one another? Yes, the concept of a *mRNA* and the concept of a rRNA are both dependent upon the concept of a *RNA*, but their definitions do not refer back to none of them, which is illustrated in Figure 1.5. So far, we have argued that understanding how possible *events* in *gene expression* are interrelated to each other requires knowledge of the involved concepts and of their *conceptual order* in relation to one another. Is knowing the concepts and their *conception order* a sufficient condition for us to know *events* in *gene expression* as a whole? No, it is not; and an argument for that relies upon the fact that the notion of *knowledge* is a *primitive concept*. In fact, if *knowledge*

[13] Or better, force and deformation.

is understood as a justified true belief then, intuitively, we cannot conceive of the idea that *knowledge* of all *events* in *gene expression* as a whole can be logically deduced at the conceptual level. That can be done if we know all the phenomena related thereto, that is, if we know all mechanisms involved in *gene expression*, and their agents, which, in this case, are supposed to have been properly conceptualized. Hence, the latter elucidation points us out to the primitiveness of the concept of *knowledge*.

Withal, from a *mechanistic perspective*, we assert that if we know the concepts and their *conception order* in relation to one another then we can potentially know *events* in *gene expression* as a whole. Why? Because "*actuality* precedes *potentiality*" [*Actus est prior potentia*] as categorically stated by Dr. Martin-Löf in [55]. In fact, if one claims that "something" is potentially doable then it means that it can actually be done. But, what do we mean with knowing *events* in *gene expression* as a whole? The answer for this question is implicit in the aforementioned *mechanistic perspective* of *gene expression*, that is, a *dynamical system perspective* thereof, from which one has that a *behaviour* of a *system* is strictly determined by the interaction among its parts. So, knowing the *conceptual order* of its parts can provide access to the way in which their interaction actually occurs in the *system*. Therefore, this view presumably gives us a systematic approach to get information about the *underlying mechanisms* in *gene expression* by solely using *analytical thought*. Furthermore, it perhaps offers a *rational recipe* to model *gene expression*.

What is essential in this view? Finding the *entailment of notions* with respect to a set of *events* of interest is of utmost importance. This process will unveil the most fundamental notions and, of course, if feasible, the primitive ones. That gives a thinking directionality completely determined by the *conceptual order*. Can we give an example for that? Yes, we can refer to the *birth-death model* of *gene expression* as described in [90]. In that model, it is essential to knowing that the notion of *transcription* precedes *translation* and that the concepts of *protein*, *mRNA* and *promoter* are entailed with each other in this respective order with regard to the *conceptual order* so that the notion of a *promoter* is the most fundamental one in that sequence of concepts. In the next section, we shall see that the aforesaid *mathematical model*, to some extent, enables us to understand *gene regulation*.

1.2 Noise in gene expression as a tool to understand gene regulation

A striking feature of *gene expression*, as evidenced in genuine experiments reported in the literature [107, 6, 9, 17], is that the number of *proteins* produced by a *gene* of interest varies in a *isogenic cell population*[14] and over time within a single cell. What is the cause of this *variability*? To give an argument for *single-cell variability* over time, we could limit ourselves to a *hypothetical event* in which the process of *transcription-translation* happens instantaneously and *mRNA* degrades on the same time scale as the translated proteins which amounts to assuming that they have the same *lifetime*[15], that is, $\tau_{mRNA} = \tau_{protein}$.

In [107], see Figure 1.7, the authors developed a technique with which they

[14] A *cell population* consisting of cells with the same *DNA*.

[15] The *lifetime* of a *protein* or *mRNA* is the average time that it takes to degrade.

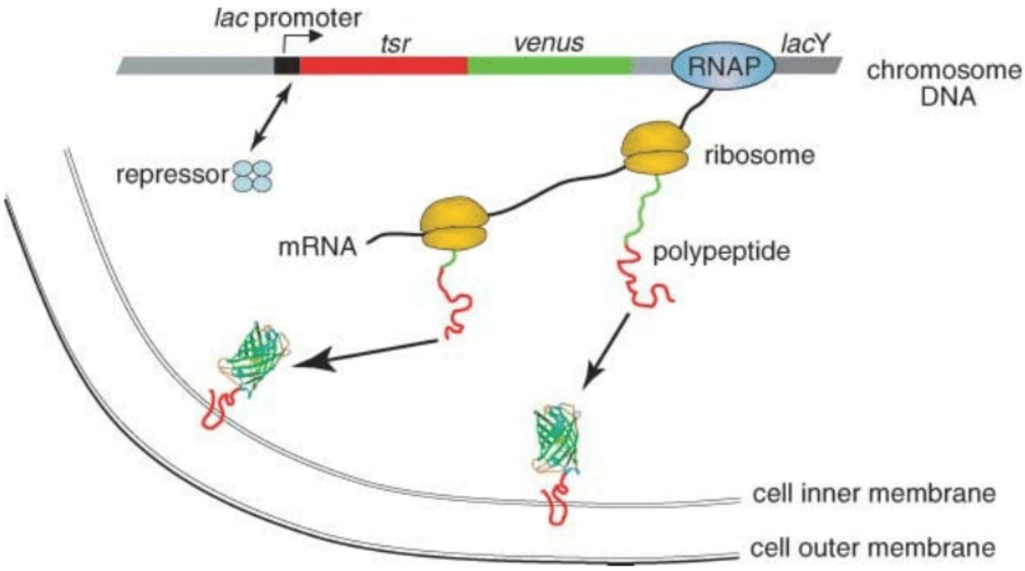


Figure 1.7: Cartoon taken from [107]. So, upon unbinding of repressor, RNAP initiates transcription with subsequent binding of several ribossomes to mRNA, which folds into a polypeptide and undergoes localization to the inner cellular membrane and after maturation of fluorophore, it can be easily detected by a fluorescence microscope.

quantified *gene expression* of a *chimeric gene*, i.e. *tsr-Venus gene*, incorporated in a *E.coli strain SX*4 (*Bacterium*: *prokaryotic cell*) replacing the *lacZ-gene*. From the *statistical analysis* of their *data*, they concluded that the *lifetime* of a *tsr-Venus mRNA* is $\tau_{mRNA} = 1.5 \pm 0.2$ min whereas the *lifetime* of a *tsr-Venus protein* is $\tau_{protein} = 29 \pm 8$ min. Does the latter difference of one-order magnitude in lifetime for a prokaryotic cell suffice to argue the unsoundness of the proposed hypothetical event with respect to eukaryotic cells? In fact, the logic of biological sciences is inherently inductive so that the *knowledge* acquisition process is driven by empirical extrapolation [50] from a model organism to a target organism. The principles underlying the inferential rule are either circumstantial evidence or phylogeny-based generalization, i.e. we either assume that the model and target organisms share enough relevant causal similarities or we appeal to shared ancestry. Having said that, arguably, we could say that the proposed hypothetical event is compatible with the known structural complexity of eukaryotic cells if we start assuming the existence of highly improbable mechanisms. Therefore, it must be highly unlikely that the proposed hypothetical event can occur within an eukaryotic cell.

So, under the aforesaid highly improbable hypothetical event, one has that if we assume that a transcription-translation occurred at time $t_1 > 0$ and that there has occurred another one at time $t_2 > \tau_{mRNA} = \tau_{protein} > t_1$ before *cell division*[16], that is, $\tau_{cell} > t_2$ so that one has the same cell, then it is highly improbable that the same amount of proteins will be produced at time t_2. In fact, under *randomness* of biochemical reactions, it cannot be the case that *mRNA* molecules are bound by the same amount of *ribosomes* at each *mRNA-protein lifetime*. So far, this suffices as an explanation for *single-cell variability* in *gene expression* over time.

[16]In [107], it was reported that the *cell cycle* of *E.coli strain* amounts to $\tau_{cell} = 55 \pm 10$ min.

To argue cell-to-cell variability, we can draw on the reasoning provided in [62] and limit ourselves to a less likely scenario wherein a cell underwent division and produced two genetically identical daughter cells carrying the same amount of copies of transcription factors with respect to a gene of interest. In order to promote *translation*, those *transcription factors* must perform a *random walk* (uncorrelated) toward the respective *operator* within each daughter cell causing these cells to have different levels of expression of the corresponding gene. Therefore, the latter argument serves the purpose to explain cell-to-cell variability.

Hence, the source of *noise* generated by this cascade of *biochemical reactions* from *translation* to *transcription* is said to constitute the *intrinsic noise* . Moreover, *RNA polymerase* is an *enzyme*, a *protein*, a *gene-product* so it also carries *noise*, which, in turn, is said to be extrinsic[17]. In this regard, the total noise has two components: *intrinsic* and *extrinsic*, as detailed in [97, 44].

But, how can we put this *variation* [*fluctuation*] in *gene expression* into perspective with *embryogenesis* [*embryonic formation*]? To answer the latter question, we quote from Semrau *et al.* in [87]:

> (...) Here, we review attempts to understand lineage decision-making as the interplay of single-cell heterogeneity and gene regulation. Fluctuations at the single-cell level are an important driving force behind cell-state transitions and the creation of cell-type diversity. Gene regulatory networks amplify such fluctuations and define stable cell types. They also mediate the influence of signaling inputs on the lineage decision.

Hence, according to Semrau *et al.*, one has that *embryonic formation* can be seen as an interplay between *single-cell heterogeneity*, caused by *fluctuations* in *gene expression*, and gene regulation, which, in turn, heavily augments the corresponding fluctuations. In fact, we draw upon the latter paradigm so as to suitably give an answer to the question: what is fundamental so as to go from a *unicellular organism* to a *multicellular organism*? Indeed, one has that *randomness* in *gene expression* underlying the *single-cell heterogeneity* in a genetically identical cell population together with a network of regulatory interaction encrypted in the DNA, is essential for the formation of a multitude of *cell types* comprising the *embryo*. Moreover, understanding *randomness* in *gene expression* can shed light on gene regulation mechanisms.

But, what is the probability distribution of the number of protein molecules per cell in the cell population ? To answer this question, we refer to an article published by Dr. V. Shahrezaei and Dr. P. Swain in 2008 (see [90]). In fact, grounded in the results of single-cell experiments, including [107] and [6], the authors applied the *time scale separation technique*[18] to the stochastic counterpart of the birth-death model for gene expression which enabled them to arrive at an expression for the probability distribution of the number of protein molecules per cell in the cell population.

So, in the two stage model, as shown in Figure 1.9(A), the promoter is assumed to be always in the active state. Drawing upon the inherent Markov process in

[17]Not only the respective *RNAP* carries *extrinsic noise* but all the cellular components which interact with the *stochastic system* comprising the regulation of a *gene* of interest, and that are not directly involved into the *transcription* and *translation* thereof.

[18]The reduction of the dimension of a dynamical system based on differences in time scales.

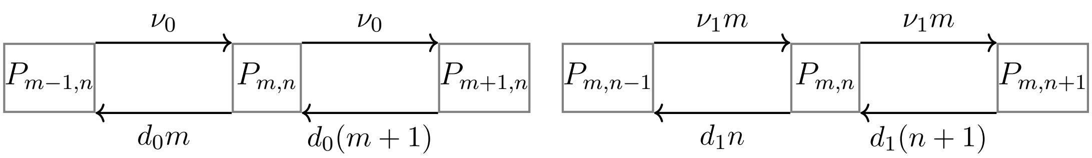


Figure 1.8: The Markov chain showing the transition probabilities regarding the two stage model.

this model, displayed in Figure 1.8, leads us to the birth-death master equation describing the evolution of the probability distribution of having m *mRNA molecules* and n *protein molecules* at time t

$$\begin{aligned}\frac{dP_{m,n}}{dt} &= \nu_0(P_{m-1,n} - P_{m,n}) + \nu_1 m(P_{m,n-1} - P_{m,n}) \\ &+ d_0[(m+1)P_{m+1,n} - mP_{m,n}] \\ &+ d_1[(n+1)P_{m,n+1} - nP_{m,n}],\end{aligned} \tag{1.1}$$

for $m, n \geq 1$, with ν_0 and ν_1 being the probability per unit time of *transcription* and *translation* respectively, whereas d_0 and d_1 denote the probability per unit time of *mRNA* and *protein* degradation. The same equation holds for all $m, n \geq 0$, if we take the convention that

$$P_{m',n'} \equiv 0, \tag{1.2}$$

if $m' < 0$ or $n' < 0$.

Next, by introducing the generating function

$$F(z', z) = \sum_{m,n} z'^m z^n P_{m,n}, \tag{1.3}$$

with $P_{m,n}$ representing the joint probability mass distribution of the number of mRNAs and proteins in the cell, one reduces the infinity system (1.1) of coupled ordinary differential equations to a single dimensionless partial differential equation

$$\frac{\partial F}{\partial \nu} - \gamma \left[b(1+u) - \frac{u}{\nu} \right] \frac{\partial F}{\partial u} + \frac{1}{\nu}\frac{\partial F}{\partial \tau} = a\frac{u}{\nu}F, \tag{1.4}$$

wherein

$$a = \nu_0/d_1,\ b = \nu_1/d_0,\ , \tag{1.5}$$

and with

$$\gamma = d_0/d_1 \tag{1.6}$$

being the parameter carrying the difference in time scale. Next, one has that

$$\tau = d_1 t \tag{1.7}$$

is the time in protein life time, while

$$u = z' - 1 \tag{1.8}$$

and

$$\nu = z - 1 \tag{1.9}$$

are thought to be the variables carrying the dynamics of mRNA and protein molecules respectively. Here, one must notice that the parameters of interest naturally emerges from the model with the intended meaning, i.e., a and b, or rather, the *burst frequency* and the *burst size*.

But, how can we derive the equation (1.4)? In fact, if we multiply the left-hand side and right-hand side of the equation (1.1) by $z'^m z^n$, and if we sum it in m and n, then we arrive at

$$\begin{aligned}\frac{\partial}{\partial t}\sum_{m,n=0}^{+\infty} z'^m z^n P_{m,n} &= \nu_0 \sum_{m,n=0}^{+\infty} z'^m z^n (P_{m-1,n} - P_{m,n}) + \nu_1 \sum_{m,n=0}^{+\infty} m z'^m z^n (P_{m,n-1} - P_{m,n}) \\ &+ d_0 \sum_{m,n=0}^{+\infty} z'^m z^n [(m+1)P_{m+1,n} - mP_{m,n}] \\ &+ d_1 \sum_{m,n=0}^{+\infty} z'^m z^n [(n+1)P_{m,n+1} - nP_{m,n}].\end{aligned} \tag{1.10}$$

Next, if we now draw upon the definitions (1.3), (1.7) then one has that the term on the left-hand side of equation (1.10) reads

$$\frac{\partial}{\partial t}\sum_{m,n=0}^{+\infty} z'^m z^n P_{m,n} = \frac{\partial F}{\partial \tau} d_1, \tag{1.11}$$

and if we also build on definitions (1.8) then one has that the first term on the right-hand side of equation (1.10) reads

$$\nu_0 \sum_{m,n=0}^{+\infty} z'^m z^n (P_{m-1,n} - P_{m,n}) = \nu_0 \left(z' \sum_{m=-1,n=0}^{+\infty} z'^m z^n P_{m,n} - \sum_{m=-1,n=0}^{+\infty} z'^m z^n P_{m,n}) \right). \tag{1.12}$$

So, if we bear in mind that $P_{m=-1,n} = 0$ for all $n \in \mathbb{N}$ then one has that

$$\nu_0 \sum_{m,n=0}^{+\infty} z'^m z^n (P_{m-1,n} - P_{m,n}) = \nu_0 (z' - 1) \sum_{m,n=0}^{+\infty} z'^m z^n P_{m,n} = \nu_0 u F. \tag{1.13}$$

Now, if we use (1.9) and that $P_{m,n=-1} = 0$ for all $m \in \mathbb{N}$, then one has that the second term on the right-hand side of equation (1.10) reads

$$\begin{aligned}\nu_1 \sum_{m,n=0}^{+\infty} mz'^m z^n (P_{m,n-1} - P_{m,n}) &= \nu_1 \left(z \sum_{m=0,n=-1}^{+\infty} mz'^m z^n P_{m,n} - \sum_{m,n=0}^{+\infty} mz'^m z^n P_{m,n}) \right) \\ &= \nu_1 (z-1) \sum_{m,n=0}^{+\infty} mz'^m z^n P_{m,n} \\ &= \nu_1 (z-1) z' \sum_{m,n=0}^{+\infty} mz'^{m-1} z^n P_{m,n} \\ &= \nu_1 \nu (1+u) \sum_{m,n=0}^{+\infty} mz'^{m-1} z^n P_{m,n} \\ &= \nu_1 \nu (1+u) \frac{\partial F}{\partial u},\end{aligned}$$

wherein the last equality is derived from the fact that

$$\frac{\partial F}{\partial u} = \sum_{m,n=0}^{+\infty} mz'^{m-1} z^n P_{m,n}, \tag{1.14}$$

so we have that

$$\nu_1 \sum_{m,n=0}^{+\infty} mz'^m z^n (P_{m,n-1} - P_{m,n}) = \nu_1 \nu (1+u) \frac{\partial F}{\partial u}. \tag{1.15}$$

Now, one has that the third term on the right-hand side of equation (1.10) can be written as

$$\begin{aligned}d_0 \sum_{m,n=0}^{+\infty} z'^m z^n [(m+1) P_{m+1,n} - m P_{m,n}] &= d_0 \sum_{m=1,n=0}^{+\infty} mz'^{m-1} z^n P_{m,n} \\ &\quad - d_0 \sum_{m,n=0}^{+\infty} mz'^m z^n P_{m,n} \\ &= d_0 \sum_{m=1,n=0}^{+\infty} mz'^{m-1} z^n P_{m,n} \\ &\quad - d_0 z' \sum_{m=1,n=0}^{+\infty} mz'^{m-1} z^n P_{m,n} \\ &= d_0 (1-z') \sum_{m=1,n=0}^{+\infty} mz'^{m-1} z^n P_{m,n} \\ &= -d_0 u \frac{\partial F}{\partial u},\end{aligned}$$

so we have that

$$d_0 \sum_{m,n=0}^{+\infty} z'^m z^n [(m+1) P_{m+1,n} - m P_{m,n}] = -d_0 u \frac{\partial F}{\partial u}. \tag{1.16}$$

Further, if we build on the same aforementioned argument and on the equality

$$\frac{\partial F}{\partial \nu} = \sum_{m=0,n=1}^{+\infty} n z'^m z^{n-1} P_{m,n}, \tag{1.17}$$

then one has that the fourth and last term on the right-hand side of equation (1.10) reads as

$$\begin{aligned} d_1 \sum_{m,n=0}^{+\infty} z'^m z^n [(n+1)P_{m,n+1} - nP_{m,n}] &= d_1 \sum_{m,n=0}^{+\infty} (n+1) z'^m z^n P_{m,n+1} \\ &\quad - d_1 \sum_{m,n=0}^{+\infty} n z'^m z^n P_{m,n} \\ &= d_1 \left(\sum_{m=0,n=1}^{+\infty} n z'^m z^{n-1} P_{m,n} - z \sum_{m=0,n=1}^{+\infty} n z'^m z^{n-1} P_{m,n} \right) \\ &= d_1 (1-z) \sum_{m=0,n=1}^{+\infty} n z'^m z^{n-1} P_{m,n} \\ &= -d_1 \nu \sum_{m=0,n=1}^{+\infty} n z'^m z^{n-1} P_{m,n} \\ &= -d_1 \nu \frac{\partial F}{\partial \nu}, \end{aligned}$$

so we have that

$$d_1 \sum_{m,n=0}^{+\infty} z'^m z^n [(n+1)P_{m,n+1} - nP_{m,n}] = -d_1 \nu \frac{\partial F}{\partial \nu}. \tag{1.18}$$

Therefore, if we multiply the terms (1.11), (1.13), (1.15), (1.16), and (1.18) by $\frac{1}{d_1 \nu}$, then we arrive at equation (1.4).

But, how can we solve (1.4)? To answer this question, we will rewrite (1.4) in a convenient way. In fact, if we multiply the left-hand side and the right-hand side of (1.4) by ν, then we arrive at

$$\frac{\partial F}{\partial \tau} + \nu \frac{\partial F}{\partial \nu} - \gamma \left[b\nu(1+u) - u \right] \frac{\partial F}{\partial u} = auF. \tag{1.19}$$

Now, if we define the scalar function

$$r(u,\nu) = au + 1 - \gamma(b\nu - 1), \tag{1.20}$$

and the vector-field

$$\mathbf{V}(u,\nu) = \left(V_1(u,\nu), V_2(u,\nu) \right), \tag{1.21}$$

with

$$V_1(u,\nu) = -\gamma \left[b\nu(1+u) - u \right] \tag{1.22}$$

and

$$V_2(u,\nu) = \nu, \tag{1.23}$$

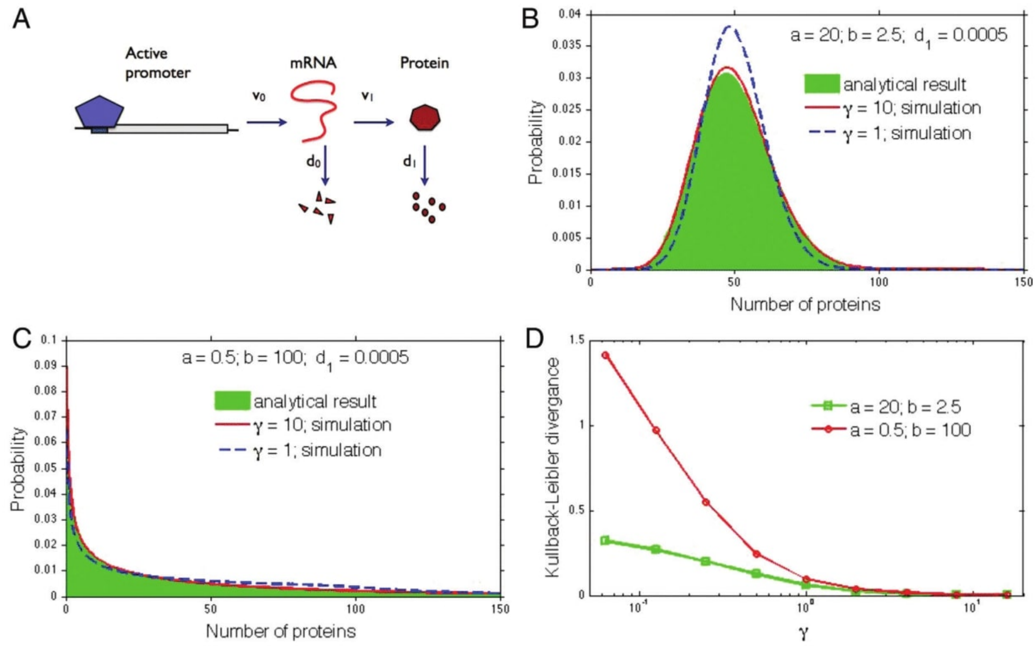


Figure 1.9: A) The cartoon and the plots have been taken from [90]. Two stage model with the promoter being always active. B) A simulation comparing the analytical solution of the two stage model and the numerical simulation of the master equation by using the Gillespie algorithm. Here, we see that the higher is γ, the better is the fitting. Moreover, for $a > 1$, one has that the distribution is peaked at a positive number. C) Here, for $\gamma = 10$, one sees a "perfect match". Moreover, as $a < 1$, with a high $b = 100$, ones sees that it is peaked at 0. D) Here, the Kullback–Leibler divergence quantifies the effects of small γ. As we see, for γ around 10, one has a perfect match, whereas for $\gamma < 1$, one sees a high divergence. This high divergence is due to the fact that, in this case, proteins are being degraded while being produced during the lifetime of a mRNA, so the probability distribution describing the number of proteins per mRNA cannot be geometrically distributed which, in turn, implies that the probability distribution of the number of proteins in the cell cannot be the negative binomial what is reflected in this high divergence effects for lower γ.

then one has that (1.19) can be rewritten as

$$\frac{\partial F}{\partial \tau} + \nabla \cdot (F\mathbf{V}) = r(u,\nu)F, \tag{1.24}$$

in which

$$\nabla \cdot (F\mathbf{V}) = \nabla F \cdot \mathbf{V} + F\nabla \cdot \mathbf{V} \tag{1.25}$$

with

$$\nabla F = \left(\frac{\partial F}{\partial u}, \frac{\partial F}{\partial \nu}\right) \tag{1.26}$$

and

$$\nabla \cdot \mathbf{V} = \frac{\partial V_1}{\partial u} + \frac{\partial V_2}{\partial \nu}. \tag{1.27}$$

Hence, one has that the equation (1.19) is indeed a transport equation in non-conservative form, while its conservative form is shown in (1.24).

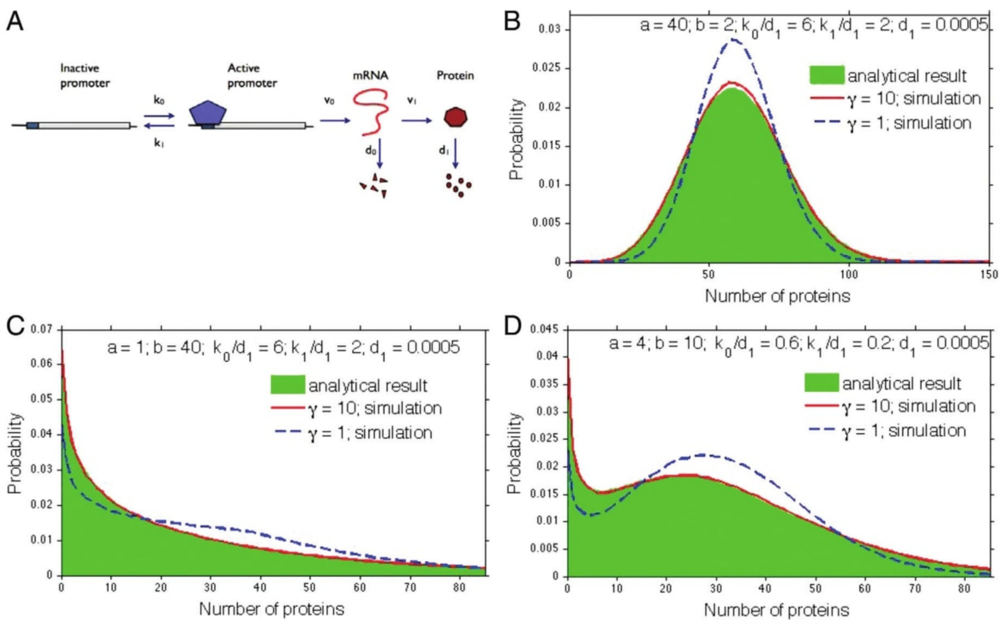


Figure 1.10: A)The cartoon and the plots have been taken from [90]. The three stage model including the transition of promoter between active and inactive states. B) and C) Similar results (unimodal behavior) for slightly high transition rates. D)Here, for small transition rates, one sees bimodality.

Further, drawing upon the method of characteristics presented in [18, p. 97–115], one can rewrite (1.19) as

$$\left(\frac{\partial F}{\partial u}, \frac{\partial F}{\partial \nu}, \frac{\partial F}{\partial \tau}, -1\right)\begin{pmatrix} -\gamma[b\nu(1+u)-u] \\ \nu \\ 1 \\ auF \end{pmatrix} = 0 \tag{1.28}$$

and one has that the vector field

$$\left(\frac{\partial F}{\partial u}, \frac{\partial F}{\partial \nu}, \frac{\partial F}{\partial \tau}, -1\right) \tag{1.29}$$

is normal to the surface solution $F = F(u, \nu, \tau) \in \mathbb{R}^4$ of (1.19). So, one can deduce that the vector field

$$(-\gamma[b\nu(1+u)-u], \nu, 1, auF) \tag{1.30}$$

is tangent to the surface solution $F = F(u, \nu, \tau) \in \mathbb{R}^4$ of (1.19) at each point $(u, \nu, \tau, F(u, \nu, \tau))$ for which (1.28) holds. So, intuitively, one can think about forming a curve on the surface solution for which the vector field in (1.30) coincides with tangent vector of the respective curve. But, How can we find such a curve? In fact, one can find such a curve by solving the following system of ordinary differential equations

$$\begin{cases} \frac{d\tau}{ds} = 1; \\ \frac{d\nu}{ds} = \nu; \\ \frac{du}{ds} = -\gamma[b\nu(1+u) - u]; \\ \frac{dF}{ds} = auF; \end{cases} \tag{1.31}$$

in which s denotes a parametrization by the arc length. Thus, one has that the union of all such curves [characteristics, integral curves] satisfying (1.31) amounts to the surface solution of (1.19). Thereby, $\tau = s$, which, in turn, implies that one can rewrite (1.31) as

$$\begin{cases} \frac{d\nu}{d\tau} = \nu; \\ \frac{du}{d\nu} = -\gamma[b(1+u) - \frac{u}{\nu}]; \\ \frac{dF}{d\tau} = auF. \end{cases} \tag{1.32}$$

But, how can we solve (1.32) under $\gamma \gg 1$? In fact, one has that

$$e^{\int \gamma\left(b - \frac{1}{\nu}\right) d\nu} = \frac{e^{\gamma b \nu}}{\nu^{\gamma}} \tag{1.33}$$

is the integrating factor of the differential equation $(1.32)_2$. Thus, if we multiply the left-hand side and the right-hand side of $(1.32)_2$ by the term (1.33) then we arrive at

$$\int \left(e^{\gamma b \nu} \frac{1}{\nu^{\gamma}} u \right)' d\nu = -\gamma b \int \frac{e^{\gamma b \nu'}}{\nu'^{\gamma}} d\nu', \tag{1.34}$$

which, in turn, implies that

$$u(\nu) = e^{-\gamma b \nu} \nu^{\gamma} \left(R - \gamma b \int \frac{e^{\gamma b \nu'}}{\nu'^{\gamma}} d\nu' \right) \tag{1.35}$$

with $R \in \mathbb{R}$ being a constant. Drawing upon [90], one can use the identity

$$e^{\gamma b \nu} = \sum_{n=0}^{+\infty} \frac{(\gamma b \nu)^n}{n!}, \tag{1.36}$$

in the integral on the right-hand side of (1.35) so as to arrive at

$$\begin{aligned} u(\nu) &= e^{-\gamma b\nu}\nu^{\gamma}\left(R-\gamma b\int\frac{e^{\gamma b\nu'}}{\nu'^{\gamma}}d\nu'\right) \\ &= e^{-\gamma b\nu}\nu^{\gamma}\left(R-\gamma b\int\frac{\sum_{n=0}^{+\infty}\frac{(\gamma b\nu')^n}{n!}}{\nu'^{\gamma}}d\nu'\right) \\ &= e^{-\gamma b\nu}\nu^{\gamma}\left(R-\gamma b\sum_{n=0}^{+\infty}\frac{(\gamma b)^n}{n!}\int\nu'^{n-\gamma}d\nu'\right) \\ &= e^{-\gamma b\nu}\nu^{\gamma}\left(R-\gamma b\sum_{n=0}^{+\infty}\frac{(\gamma b)^n}{n!}\frac{\nu^{n-\gamma+1}}{n-\gamma+1}\right) \\ &= e^{-\gamma b\nu}\left(\nu^{\gamma}R-\nu^{\gamma}\sum_{n=0}^{+\infty}\frac{(\gamma b\nu)^{n+1}}{n!}\frac{\nu^{-\gamma}}{n-\gamma+1}\right) \\ &= e^{-\gamma b\nu}\left(\nu^{\gamma}R-\sum_{n=0}^{+\infty}\frac{(\gamma b\nu)^{n+1}}{n!(n-\gamma+1)}\right). \end{aligned} \tag{1.37}$$

But, how to take care of the sum

$$\sum_{n=0}^{+\infty}\frac{(\gamma b\nu)^{n+1}}{n!(n-\gamma+1)} \ ? \tag{1.38}$$

In fact, if one denotes the n-th term of the series (1.38) by

$$A_n := \frac{(\gamma b\nu)^{n+1}}{n!(n-\gamma+1)} \tag{1.39}$$

then, under the assumption that $\gamma \gg 1$, one has that

$$\begin{aligned} \frac{A_{n-1}}{A_n} &= \left(\frac{n-\gamma+1}{n-\gamma}\right)\frac{n}{\gamma b\nu} \\ &\approx \frac{n}{\gamma b\nu} \end{aligned} \tag{1.40}$$

and one concludes that the sum in (1.38) is dominated by $A_n's$ with n close to $\gamma b\nu$. So, if one applies the following change of variables

$$s = n - \gamma b\nu \tag{1.41}$$

and if one uses the Stirling's approximation

$$n! \approx \sqrt{2\pi n}e^{-n}n^n \tag{1.42}$$

then, according to the authors of [90], one can show that

$$n! \approx (\gamma b\nu)^n e^{-\gamma b\nu}e^{\frac{(n-\gamma b\nu)^2}{2\gamma b\nu}}\sqrt{2\pi\gamma b\nu}, \tag{1.43}$$

or better,

$$n! \approx (\gamma b\nu)^n e^{-\gamma b\nu} e^{\frac{s^2}{2\gamma b\nu}} \sqrt{2\pi\gamma b\nu}. \tag{1.44}$$

Hence, drawing upon the approximation (1.44), one can evaluate the sum in (1.38) as an integral in s, from $-\infty$ to $+\infty$, seeing that, under the assumption that $\gamma \gg 1$, the respective integral is supposed to be dominated by terms $|s| \approx 0$, or equivalently, the sum in (1.38), under the assumption that $\gamma \gg 1$, is dominated by n-th terms with $n \approx \gamma b\nu$. In fact, if one uses that

$$\int_{-\infty}^{+\infty} \frac{e^{\frac{s^2}{2\gamma b\nu}}}{\sqrt{2\pi\gamma b\nu}} ds = 1 \tag{1.45}$$

then

$$\begin{aligned}
\sum_{n=0}^{+\infty} \frac{(\gamma b\nu)^{n+1}}{n!(n-\gamma+1)} &\approx \int_{-\infty}^{+\infty} \frac{e^{\frac{s^2}{2\gamma b\nu}}}{\sqrt{2\pi\gamma b\nu}} \left[\frac{\gamma b\nu e^{\gamma b\nu}}{\gamma(b\nu-1)+s+1}\right] ds \\
&= \int_{-\infty}^{+\infty} \frac{e^{\frac{s^2}{2\gamma b\nu}}}{\sqrt{2\pi\gamma b\nu}} \frac{b\nu e^{\gamma b\nu}}{(b\nu-1)} \left[1+\gamma^{-1}\left(\frac{s+1}{b\nu-1}\right)\right]^{-1} ds \\
&= \int_{-\infty}^{+\infty} \frac{e^{\frac{s^2}{2\gamma b\nu}}}{\sqrt{2\pi\gamma b\nu}} \frac{b\nu e^{\gamma b\nu}}{(b\nu-1)} \left\{1+\sum_{i=1}^{+\infty}(-1)^i \left[\gamma^{-1}\left(\frac{s+1}{b\nu-1}\right)\right]^i\right\} ds \\
&= \frac{b\nu e^{\gamma b\nu}}{(b\nu-1)} \int_{-\infty}^{+\infty} \frac{e^{\frac{s^2}{2\gamma b\nu}}}{\sqrt{2\pi\gamma b\nu}} ds + \frac{b\nu e^{\gamma b\nu}}{(b\nu-1)} \int_{-\infty}^{+\infty} \frac{e^{\frac{s^2}{2\gamma b\nu}}}{\sqrt{2\pi\gamma b\nu}} \sum_{i=1}^{+\infty}(-1)^i \left[\gamma^{-1}\left(\frac{s+1}{b\nu-1}\right)\right]^i ds \\
&= \frac{b\nu e^{\gamma b\nu}}{(b\nu-1)} + O\left(\gamma^{-1}\right) \\
&\approx \frac{b\nu e^{\gamma b\nu}}{(b\nu-1)},
\end{aligned} \tag{1.46}$$

or better,

$$\sum_{n=0}^{+\infty} \frac{(\gamma b\nu)^{n+1}}{n!(n-\gamma+1)} \approx \frac{b\nu e^{\gamma b\nu}}{(b\nu-1)} \tag{1.47}$$

which, in turn, by invoking (1.35), implies that

$$u(\nu) \approx Re^{-\gamma b\nu}\nu^\gamma + \frac{b\nu}{(1-b\nu)}. \tag{1.48}$$

So, if one focuses upon a integral curve [characteristic] with initial conditions

$$\nu(\tau=0) = \nu_0, \tag{1.49}$$

and

$$u(\tau=0) = u_0, \tag{1.50}$$

which, in turn, consistently, implies that

$$u(\nu_0) = u_0, \tag{1.51}$$

then one has that

$$R = \left(u_0 - \frac{b\nu_0}{1 - b\nu_0}\right)\frac{e^{\gamma b\nu_0}}{\nu_0^{\gamma}}. \tag{1.52}$$

Thus, if one uses (1.52) in (1.48) then one arrives at

$$u(\nu) \approx \left(u_0 - \frac{b\nu_0}{1 - b\nu_0}\right) e^{-\gamma b(\nu-\nu_0)}\left(\frac{\nu}{\nu_0}\right)^{\gamma} + \frac{b\nu}{1 - b\nu}. \tag{1.53}$$

Therefore, drawing on the method of characteristics, one reduces the problem of finding a solution for the transport-equation in non-conservative form, shown in (1.19), to a problem of finding a solution for the system of ordinary differential equations (1.31). Thereby, under[19] $\gamma \gg 1$, or rather, that the protein lifetime $1/d_1$ is much longer than the mRNA lifetime $1/d_0$, one can capitalize upon

$$\lim_{\gamma\to+\infty} e^{-\gamma b(\nu-\nu_0)}\left(\frac{\nu}{\nu_0}\right)^{\gamma} = 0$$

to conclude that

$$u(\nu) \approx \frac{b\nu}{1 - b\nu}, \tag{1.54}$$

and that

$$\nu = \nu_0 e^{\tau}. \tag{1.55}$$

Furthermore, we do have a situation amenable to the quasi steady-state approximation. In fact, for the most part of a protein lifetime, under $\gamma \gg 1$, one has that the variable u carrying the dynamics of mRNA molecules is at steady state, so $u(\nu)$ is given by (1.54) and the mRNA probability mass distribution is at steady state and peaked around zero, which, in fact, under $\gamma \gg 1$, implies that

$$P_{0,n} \approx P_n, \tag{1.56}$$

and that

$$P_{m,n} \ll 1, \tag{1.57}$$

for all $m \in \mathbb{N}\setminus\{0\}$ and $n \in \mathbb{N}$, which, in turn, implies that (1.3), under $\gamma \gg 1$, reads

$$F(z,\tau) = \sum_{n=0}^{+\infty} P_n(\tau) z^n. \tag{1.58}$$

Next, if one "eliminates" the fast variable u from $(1.32)_3$ and using $(1.32)_1$, then one arrives at the ordinary differential equation

$$\frac{dF}{d\nu} \approx \frac{ab}{1 - b\nu}F. \tag{1.59}$$

with the expression for F, under $\gamma \gg 1$, being approximated by (1.58). Thereby, by invoking the initial conditions (1.49), (1.50) and (1.51), if we draw upon the argument given in [90] then we suppose that there are initially $n_0 \in \mathbb{N}$ proteins. Hence, under the latter assumption, one has that

[19] This assumption is consistent with empirical evidences. In fact, as we mentioned earlier in this essay, tsr-Venus mRNA lifetime was estimated to be ≈ 1.5 min while a protein lifetime in *E.coli* is ≈ 30 min.

$$P_n(\tau = 0) = 0, \tag{1.60}$$

for all $n \in \mathbb{N} \setminus \{n_0\}$, and that

$$P_{n_0}(\tau = 0) = 1. \tag{1.61}$$

So, relying upon (1.60) and (1.61), if we recall (1.9) then one can evaluate (1.58) at ν_0 so as to arrive at

$$\begin{aligned} F(\nu_0) = F(z = \nu_0 + 1, \tau = 0) &= \sum_{n=0}^{+\infty} P_n(\tau = 0) z^n \\ = P_{n_0}(\tau = 0)(1 + \nu_0)^{n_0} + &\sum_{n \in \mathbb{N} \setminus \{n_0\}} P_n(\tau = 0)(1 + \nu_0)^n \\ &= (1 + \nu_0)^{n_0}, \end{aligned} \tag{1.62}$$

and one has that

$$F(\nu_0) = (1 + \nu_0)^{n_0}. \tag{1.63}$$

Next, if we integrate (1.59) then we have that

$$\int_{F(\nu_0)}^{F(\nu)} \frac{1}{F} dF = \int_{\nu_0}^{\nu} \frac{ab}{1 - b\nu'} d\nu' = a \int_{\nu_0}^{\nu} \frac{b}{1 - b\nu'} d\nu', \tag{1.64}$$

which implies that

$$\ln\left(\frac{F(\nu)}{F(\nu_0)}\right) = -a \ln\left(\frac{1 - b\nu}{1 - b\nu_0}\right), \tag{1.65}$$

which, in turn, implies that

$$\ln\left(\frac{F(\nu)}{F(\nu_0)}\right) = \ln\left(\frac{1 - b\nu_0}{1 - b\nu}\right)^a. \tag{1.66}$$

Now, from the equality (1.66), one concludes that

$$F(\nu) = F(\nu_0)\left(\frac{1 - b\nu_0}{1 - b\nu}\right)^a, \tag{1.67}$$

and if draw upon (1.9), (1.55) and (1.63), then we arrive at

$$F(z, \tau) = \left[1 + (z - 1)e^{-\tau}\right]^{n_0} \left[\frac{1 - b(z - 1)e^{-\tau}}{1 + b - bz}\right]^a, \tag{1.68}$$

which, in turn, taking $n_0 = 0$, implies that

$$F(z, \tau) = \left[\frac{1 - b(z - 1)e^{-\tau}}{1 + b - bz}\right]^a. \tag{1.69}$$

Further, we will build on the formulation provided in [34] to introduce necessary definitions and provide some results concerning the concept of *hypergeometric function*. The latter notion is essential to deriving the expression for $P_n(\tau)$ as shown in [90].

Definition 1.2.1. For $\tilde{a} \in \mathbb{R}$ and $n \in \mathbb{N}$, one has that

$$(\tilde{a})_n = \tilde{a}(\tilde{a}+1)(\tilde{a}+2)\dots(\tilde{a}+n-1) \tag{1.70}$$

represents the Pochhammer symbol with

$$(\tilde{a})_0 = 1. \tag{1.71}$$

Definition 1.2.2. Let $p, q \in \mathbb{N}$, $a_1, \dots, a_p \in \mathbb{R}$, and $b_1, \dots, b_q \in \mathbb{R} \setminus \mathbb{Z}_{\leq}$ wherein $\mathbb{Z}_{\leq} := \{n \in \mathbb{Z} : n \leq 0\}$. A hypergeometric function ${}_pF_q\,(a_1; a_2; \dots; a_p; b_1; b_2; \dots; b_q; w)$ is defined as

$${}_pF_q\left(\begin{matrix} a_1; & a_2; & \dots; & a_p \\ b_1; & b_2; & \dots; & b_q \end{matrix}; w\right) = \sum_{n=0}^{+\infty} \frac{(a_1)_n (a_2)_n \dots (a_p)_n}{(b_1)_n (b_2)_n \dots (b_q)_n} \frac{w^n}{n!}. \tag{1.72}$$

However, in this thesis, it suffices to consider the special case

$${}_2F_1\left(\begin{matrix} \tilde{a}, \tilde{b} \\ \tilde{c} \end{matrix}; w\right) = \sum_{n=0}^{+\infty} \frac{(\tilde{a})_n (\tilde{b})_n}{(\tilde{c})_n} \frac{w^n}{n!}, \tag{1.73}$$

with $\tilde{a}, \tilde{b} \in \mathbb{R}$, and $\tilde{c} \in \mathbb{R} \setminus \mathbb{Z}_{\leq}$ for all $n \in \mathbb{N}$. The latter is known as the concept of Gauss hypergeometric function.

Further, if we apply the *ratio test* to the series in (1.73) then we have that

$$\lim_{n \to +\infty} \frac{|c_{n+1}|}{|c_n|} = |w|, \tag{1.74}$$

with

$$c_n = \frac{(\tilde{a})_n (\tilde{b})_n}{(\tilde{c})_n} \frac{w^n}{n!} \tag{1.75}$$

for all $n \in \mathbb{N}$. Hence, from (1.75), one has that (1.73) converges on $|w| < 1$. Moreover, by Raabe's test (see [34, p. 94]), one has that if $Re(c - a - b) > 0$ then (1.73) converges on $|w| = 1$.

Lemma 1.2.1. *If* $\tilde{a} = -N$ *, with* $N \in \mathbb{N}$*, then*

$${}_2F_1\left(\begin{matrix} \tilde{a}, \tilde{b} \\ \tilde{c} \end{matrix}; w\right) = \sum_{n=0}^{N} \frac{(-N)_n (\tilde{b})_n}{(\tilde{c})_n} \frac{w^n}{n!}. \tag{1.76}$$

Proof. In fact, one has that

$$(\tilde{a})_n = (-N)_n = (-N)(-N+1)(-N+2)(-N+3)\dots(-N+n-2)(-N+n-1) = 0 \tag{1.77}$$

for $n \in \mathbb{N} \setminus \{0, 1, 2, 3, \dots, N\}$, which, in turn, implies that

$$\begin{aligned} {}_2F_1\left(\begin{matrix} \tilde{a}, \tilde{b} \\ \tilde{c} \end{matrix}; w\right) &= \sum_{n=0}^{+\infty} \frac{(\tilde{a})_n (\tilde{b})_n}{(\tilde{c})_n} \frac{w^n}{n!} \\ &= \sum_{n=0}^{N} \frac{(-N)_n (\tilde{b})_n}{(\tilde{c})_n} \frac{w^n}{n!}, \end{aligned} \tag{1.78}$$

and the proof is complete. □

Remark. As we have shown in Lemma 1.2.1, if $\tilde{a}$ is a negative integer then one has that (1.73) converges for all $w \in \mathbb{R}$.

By construction, under the assumption $\gamma \gg 1$, one has that the generating function in (1.3) can be written as (1.58), which, in turn, implies that

$$P_n(\tau) = \frac{1}{n!} \frac{\partial^n F}{\partial z^n} \bigg|_{z=0}, \tag{1.79}$$

for all $n \in \mathbb{N}$ and $\tau \geq 0$. To proceed with the derivation of the expression of $P_n(\tau)$, one must draw upon the solution (1.69) for the ordinary differential equation in (1.59). Conveniently, one can write the solution under $\gamma \gg 1$ shown in (1.69) as

$$F(z,\tau) = \left(\frac{1+be^{-\tau}}{1+b}\right)^a \frac{\left[1 - \frac{b}{(1+b)} z\right]^{-a}}{\left[1 - \frac{b}{(e^\tau + b)} z\right]^{-a}}. \tag{1.80}$$

Further, if we define

$$w_1(z) := \left[1 - \frac{b}{1+b} z\right]^{-a} \tag{1.81}$$

and

$$w_2(z) := \left[1 - \frac{b}{e^\tau + b} z\right]^{-a} \tag{1.82}$$

then, drawing on [91, 72], one has that

$$\frac{\partial^n}{\partial z^n} [1 - tz]^{-a} \bigg|_{z=0} = \frac{\Gamma(a+n)}{\Gamma(a)} t^n \tag{1.83}$$

with $t \in \mathbb{R}$, from which one can derive that

$$\frac{\partial^{n-k}}{\partial z^{n-k}} w_1(z) \bigg|_{z=0} = \frac{\Gamma(a+n-k)}{\Gamma(a)} \left(\frac{b}{1+b}\right)^{n-k}, \tag{1.84}$$

and that

$$\frac{\partial^{\tilde{k}}}{\partial z^{\tilde{k}}} (w_2(z))^j \bigg|_{z=0} = \frac{\Gamma(aj+\tilde{k})}{\Gamma(aj)} \left(\frac{b}{e^\tau + b}\right)^{\tilde{k}}, \tag{1.85}$$

for all $n, j, \tilde{k} \in \mathbb{N}$ and $k \in \{0, 1, 2, 3, \ldots, n\}$, with

$$\Gamma(s) = \int_0^{+\infty} x^{s-1} e^{-x} dx \tag{1.86}$$

for all $s \in \mathbb{R} \setminus \mathbb{Z}_{\leq}$. In fact, the integral in (1.86) does not converge for $s < 0$ so, in this case, $\Gamma(s)$ is obtained by analytic continuation. Moreover, by definition, one has that

$$\Gamma(n) = (n-1)! \tag{1.87}$$

for all $n \in \mathbb{N} \setminus \{0\}$. Now, before we proceed with the derivation of $P_n(\tau)$, it is convenient that we show the following identities.

Lemma 1.2.2. *If $a > 0$ and $k \in \mathbb{N} \setminus \{0\}$ then one has that*

$$(a)_k = \frac{\Gamma(a+k)}{\Gamma(a)}. \tag{1.88}$$

Proof. In fact, by induction on k, if one uses definition (1.86) then, for $k = 1$, one has that

$$\begin{aligned}\frac{\Gamma(a+1)}{\Gamma(a)} &= \frac{\int\limits_0^{+\infty} y^a e^{-y} dy}{\int\limits_0^{+\infty} x^{a-1} e^{-x} dx} \\ &= \frac{(-y^a e^{-y})\Big|_0^{+\infty} + a\int\limits_0^{+\infty} y^{a-1} e^{-y} dy}{\int\limits_0^{+\infty} x^{a-1} e^{-x} dx} \\ &= a,\end{aligned} \tag{1.89}$$

so the identity (1.88) is true for $k = 1$. Next, suppose that (1.88) is true for k, with $k \in \mathbb{N} \setminus \{1\}$ then one has that

$$(a)_k = \frac{\Gamma(a+k)}{\Gamma(a)}, \tag{1.90}$$

and if one multiplies both sides of (1.90) by the factor $(a + (k+1) - 1)$ then one arrives at

$$\begin{aligned}(a)_{k+1} &= \frac{\Gamma(a+k)}{\Gamma(a)} \cdot (a+k) \\ &= \frac{\Gamma((a+k)+1)}{\Gamma(a+k)},\end{aligned} \tag{1.91}$$

in which one capitalizes on the equality

$$(a+k) = \frac{\Gamma((a+k)+1)}{\Gamma(a+k)}, \tag{1.92}$$

and the identity (1.88) has been shown. □

Corollary 1.2.3. *If $a > 0$ and $k \in \mathbb{N} \setminus \{0\}$ then one has that*

$$\Gamma(a+1) = (-1)^k (-a)_k \Gamma(a-k+1). \tag{1.93}$$

Proof. The proof will be by induction on k. In fact, if one draws upon Lemma 1.2.2 then, for $k = 1$, one has that

$$\begin{aligned}\Gamma(a+1) &= (-1)^1 (-a)_1 \Gamma(a-1+1) \\ &= a\Gamma(a)\end{aligned} \tag{1.94}$$

is true. Now, suppose that (1.93) is true for $k \in \mathbb{N} \setminus \{1\}$. So, if we multiply both sides of (1.93) by the term $(-a + (k+1) - 1)$ then we arrive at

$$\begin{aligned}\Gamma(a+1)(-a+(k+1)-1) &= (-1)^k (-a)_k (-a+(k+1)-1)\Gamma(a-k+1) \\ &= (-1)^k (-a)_{k+1} \Gamma(a-k+1),\end{aligned} \tag{1.95}$$

which, in turn, by recalling Lemma 1.2.2, implies that

$$\begin{aligned}\Gamma(a+1) &= (-1)^{k+1}(-a)_{k+1}\frac{\Gamma(a-k+1)}{(a-(k+1)+1)} \\ &= (-1)^{k+1}(-a)_{k+1}\frac{\Gamma((a-(k+1)+1)+1)}{(a-(k+1)+1)} \\ &= (-1)^{k+1}(-a)_{k+1}\Gamma(a-(k+1)+1),\end{aligned} \tag{1.96}$$

with

$$\Gamma(a-(k+1)+1) = \frac{\Gamma((a-(k+1)+1)+1)}{(a-(k+1)+1)}. \tag{1.97}$$

Therefore, one has that the identity (1.93) has been shown. □

In addition to the latter identities, the authors of [90] assert that the following identity can be verified:

$$\sum_{j=1}^{k} \frac{(-1)^j\Gamma(aj+k)}{\Gamma(aj)(j+1)!(k-j)!} = \frac{(-1)^k\Gamma(a+1)}{\Gamma(a-k+1)(k+1)!}, \tag{1.98}$$

with $a > 0$ and $k \in \mathbb{N}\setminus\{0\}$ such that $a-k \in \mathbb{R}\setminus\mathbb{Z}_<$. But, can we give an argument for that ? In fact, if we draw upon (1.91) and (1.93) then we can rewrite (1.98) as

$$\begin{aligned}\sum_{j=1}^{k} \frac{(-1)^j\Gamma(aj+k)}{\Gamma(aj)(j+1)!(k-j)!} &= \sum_{j=1}^{k}\frac{(-1)^j(aj)_k}{(j+1)!(k-j)!} \\ &= \sum_{j=1}^{k}(-1)^j\frac{(aj)_k}{(j+1)!(k-j)!} \\ &= \sum_{j=1}^{k}(-1)^j\frac{\Gamma(a+1)}{\Gamma(a+1)}\frac{(aj)_k}{(j+1)!(k-j)!} \\ &= \sum_{j=1}^{k}(-1)^j\frac{\Gamma(a+1)}{(-1)^k(-a)_k\Gamma(a-k+1)}\frac{(aj)_k}{(j+1)!(k-j)!} \\ &= \sum_{j=1}^{k}(-1)^j\frac{(-1)^k\Gamma(a+1)\times(k+1)!}{(-1)^k(-1)^k(-a)_k\Gamma(a-k+1)\times(k+1)!}\frac{(aj)_k}{(j+1)!(k-j)!} \\ &= \frac{(-1)^k\Gamma(a+1)}{\Gamma(a-k+1)(k+1)!}\sum_{j=1}^{k}(-1)^j\frac{(k+1)!}{(j+1)!(k-j)!}\frac{(aj)_k}{(-a)_k},\end{aligned} \tag{1.99}$$

so the identity (1.98) is true if and only it is true that

$$\sum_{j=1}^{k}(-1)^j\frac{(k+1)!}{(j+1)!(k-j)!}\frac{(aj)_k}{(-a)_k} = 1. \tag{1.100}$$

Thus, one might try to verify (1.100) instead of directly proving (1.98). As this is not our main aim in this thesis, then we go further with the derivation of the expression of the probability distribution of the number of proteins in the cell. However, as we shall see later, the identity (1.98) is an essential proof step in the derivation process, which demands that one must be entirely convinced of its truth so as to go on with that.

Having done that, if we now use (1.81) and (1.82) in (1.80), then we have that

$$F(z,\tau) = \left(\frac{1+be^{-\tau}}{1+b}\right)^a \frac{w_1(z)}{w_2(z)}, \tag{1.101}$$

which, in turn, by invoking (1.79), implies that

$$P_n(\tau) = \left(\frac{1+be^{-\tau}}{1+b}\right)^a \frac{1}{n!}\frac{\partial^n}{\partial z^n}\left(\frac{w_1}{w_2}\right)\Bigg|_{z=0}. \tag{1.102}$$

So, as we see in (1.102), it is necessary that one works out the n-th derivative of the quotient $\frac{w_1(z)}{w_2(z)}$ so as to deduce an expression for $P_n(\tau)$. In fact, as argued in [90], if one builds upon [72, 106, 26, 91] then one has that

$$\frac{\partial^n}{\partial z^n}\frac{w_1(z)}{w_2(z)} = n!\sum_{k=0}^{n}\frac{\partial^{n-k}}{\partial z^{n-k}}w_1(z)\cdot\sum_{j=0}^{k}\frac{(-1)^j(k+1)\,(w_2(z))^{-j-1}}{(j+1)!(n-k)!(k-j)!}\frac{\partial^k}{\partial z^k}\left(w_2(z)\right)^j, \tag{1.103}$$

which, in turn, by invoking (1.82), (1.84), and (1.85), implies that

$$\begin{aligned}\frac{\partial^n}{\partial z^n}\left(\frac{w_1(z)}{w_2(z)}\right)\Bigg|_{z=0} &= n!\sum_{k=0}^{n}\frac{\Gamma(a+n-k)}{\Gamma(a)}\left(\frac{b}{1+b}\right)^{n-k}\\ &\times\sum_{j=0}^{k}\frac{(-1)^j(k+1)}{(j+1)!(n-k)!(k-j)!}\frac{\Gamma(aj+k)}{\Gamma(aj)}\left(\frac{b}{e^\tau+b}\right)^k\\ &= n!\sum_{k=0}^{n}\frac{\Gamma(a+n-k)}{\Gamma(a)}\left(\frac{b}{1+b}\right)^{n-k}\left(\frac{b}{e^\tau+b}\right)^k\frac{(k+1)}{(n-k)!}\\ &\times\sum_{j=0}^{k}\frac{(-1)^j\Gamma(aj+k)}{\Gamma(aj)(j+1)!(k-j)!}.\end{aligned} \tag{1.104}$$

Next, if one draws upon Definition 1.2.1 and Lemma 1.2.2 then one concludes that

$$\lim_{\epsilon\to0^+}\frac{\Gamma(a\epsilon+k)}{\Gamma(a\epsilon)} = 0, \tag{1.105}$$

which, in turn, by invoking (1.87) and the identity 1.98, implies that $(1.104)_2$ can be worked out as

$$\begin{aligned}
\frac{\partial^n}{\partial z^n}\left(\frac{w_1(z)}{w_2(z)}\right)\bigg|_{z=0} &= n!\sum_{k=0}^{n}\frac{\Gamma(a+n-k)}{\Gamma(a)}\left(\frac{b}{1+b}\right)^{n-k}\left(\frac{b}{e^{\tau}+b}\right)^{k}\frac{(k+1)}{(n-k)!} \\
&\qquad\times\sum_{j=1}^{k}\frac{(-1)^j\Gamma(aj+k)}{\Gamma(aj)(j+1)!(k-j)!} \\
&= n!\sum_{k=0}^{n}\frac{\Gamma(a+n-k)}{\Gamma(a)}\left(\frac{b}{1+b}\right)^{n-k}\left(\frac{b}{e^{\tau}+b}\right)^{k}\frac{(k+1)}{(n-k)!} \\
&\qquad\times\frac{(-1)^k\Gamma(a+1)}{\Gamma(a-k+1)(k+1)!} \\
&= n!\left(\frac{b}{1+b}\right)^{n}\sum_{k=0}^{n}\frac{(-1)^k(k+1)k!}{k!(k+1)!}\left(\frac{1+b}{b}\right)^{k}\left(\frac{b}{e^{\tau}+b}\right)^{k} \\
&\qquad\times\frac{1}{((n-k+1)-1)!}\frac{\Gamma(a+n-k)}{\Gamma(a-k+1)}\frac{\Gamma(a+1)}{\Gamma(a)} \\
&= n!\left(\frac{b}{1+b}\right)^{n}\sum_{k=0}^{n}\frac{(-1)^k}{k!}\frac{\Gamma(a-k+n)}{\Gamma(n-k+1)\Gamma(a-k+1)}\frac{\Gamma(a+1)}{\Gamma(a)}\left(\frac{1+b}{e^{\tau}+b}\right)^{k}.
\end{aligned} \tag{1.106}$$

Further, if we invoke Definition 1.2.1 and the identity (1.87) then we can deduce that

$$\begin{aligned}
\frac{n!}{\Gamma(n-k+1)} &= \frac{n(n-1)(n-2)\ldots(n-k+1)(n-k)!}{((n-k+1)-1)!} \\
&= n(n-1)(n-2)\ldots(n-k+1) \\
&= (-1)^k(-n)_k.
\end{aligned} \tag{1.107}$$

Moreover, if we build upon Lemma 1.2.3 then we can also deduce that

$$\begin{aligned}
\Gamma((a+n-1)+1) &= (-1)^k(-a-n+1)_k\Gamma((a+n-1)-k+1) \\
&= (-1)^k(-a-n+1)_k\Gamma(a+n-k),
\end{aligned} \tag{1.108}$$

which, in turn, implies that

$$\Gamma(a+n-k) = \frac{\Gamma(a+n)}{(-1)^k(-a-n+1)_k}. \tag{1.109}$$

Hence, if we now draw upon Corollary 1.2.3, and the latter identities, that is, (1.107) and (1.109), then we can rewrite $(1.106)_4$ as

$$\begin{aligned}\frac{\partial^n}{\partial z^n}\left(\frac{w_1(z)}{w_2(z)}\right)\Bigg|_{z=0} &= n!\left(\frac{b}{1+b}\right)^n\sum_{k=0}^{n}\frac{(-1)^k}{k!}\frac{\Gamma(a-k+n)}{\Gamma(n-k+1)\Gamma(a-k+1)}\frac{\Gamma(a+1)}{\Gamma(a)}\\ &\quad\times\left(\frac{1+b}{e^\tau+b}\right)^k\\ &= n!\left(\frac{b}{1+b}\right)^n\sum_{k=0}^{n}\frac{(-1)^k}{k!}\frac{\Gamma(a-k+n)}{\Gamma(n-k+1)\Gamma(a-k+1)}\frac{(-1)^k(-a)_k\Gamma(a-k+1)}{\Gamma(a)}\\ &\quad\times\left(\frac{1+b}{e^\tau+b}\right)^k\\ &= n!\left(\frac{b}{1+b}\right)^n\frac{1}{\Gamma(a)}\sum_{k=0}^{n}\frac{1}{\Gamma(n-k+1)}(-a)_k\Gamma(a-k+n)\frac{1}{k!}\left(\frac{1+b}{e^\tau+b}\right)^k\\ &= \left(\frac{b}{1+b}\right)^n\frac{1}{\Gamma(a)}\sum_{k=0}^{n}\frac{n!}{\Gamma(n-k+1)}(-a)_k\frac{\Gamma(a+n)}{(-1)^k(-a-n+1)_k}\frac{1}{k!}\left(\frac{1+b}{e^\tau+b}\right)^k\\ &= \left(\frac{b}{1+b}\right)^n\frac{\Gamma(a+n)}{\Gamma(a)}\sum_{k=0}^{n}(-1)^k(-n)_k(-a)_k\frac{1}{(-1)^k(-a-n+1)_k}\frac{1}{k!}\left(\frac{1+b}{e^\tau+b}\right)^k\\ &= \left(\frac{b}{1+b}\right)^n\frac{\Gamma(a+n)}{\Gamma(a)}\sum_{k=0}^{n}\frac{(-n)_k(-a)_k}{(-a-n+1)_k}\frac{1}{k!}\left(\frac{1+b}{e^\tau+b}\right)^k.\end{aligned}\tag{1.110}$$

So, bearing in mind that

$$\frac{1}{n!}=\frac{1}{\Gamma(n+1)},\tag{1.111}$$

if one uses $(1.110)_6$ in (1.102) then one gets

$$P_n(\tau)=\frac{\Gamma(a+n)}{\Gamma(n+1)\Gamma(a)}\left(\frac{b}{1+b}\right)^n\left(\frac{1+be^{-\tau}}{1+b}\right)^a\times{}_2F_1\left(\begin{matrix}-n,\ -a\\1-a-n\end{matrix};\frac{1+b}{e^\tau+b}\right),\tag{1.112}$$

which describes the approximate temporal evolution of the probability distribution of proteins in the cell on protein time scale. The authors in [90, p. 17257] claim that the mean with the mean equal to $ab(1-e^{-\tau})$. Moreover, by drawing on Definition 1.2.2 and Lemma 1.73, one has that

$${}_2F_1\left(\begin{matrix}-n,\ -a\\1-a-n\end{matrix};w\right)=\sum_{k=0}^{n}\frac{(-n)_k(-a)_k}{(-a-n+1)_k}\frac{w^k}{k!}\tag{1.113}$$

is indeed a Gauss hypergeometric function defined, in particular, on $\mathbb{R}$. Further, we approach the derivation of the steady state distribution of the number of proteins in the cell. In fact, if it is true that, for $\tau\gg 1$, one has that

$$\left(\frac{1+b}{e^\tau+b}\right)\approx 0,\tag{1.114}$$

and that

$$\left(\frac{1+be^{-\tau}}{1+b}\right)^a\approx\left(\frac{1}{1+b}\right)^a,\tag{1.115}$$

then, by invoking Definition 1.2.1, if one expands the right-hand side of (1.113) at $w = \left(\frac{1+b}{e^{\tau}+b}\right)$ then one concludes that

$$
\begin{aligned}
{}_2F_1\left(\begin{matrix}-n, \ -a \\ 1-a-n\end{matrix}; \left(\frac{1+b}{e^{\tau}+b}\right)\right) &= \sum_{k=0}^{n} \frac{(-n)_k(-a)_k}{(-a-n+1)_k} \frac{\left(\frac{1+b}{e^{\tau}+b}\right)^k}{k!} \\
= \frac{(-n)_0(-a)_0}{(-a-n+1)_0} \frac{\left(\frac{1+b}{e^{\tau}+b}\right)^0}{0!} + \frac{(-n)_1(-a)_1}{(-a-n+1)_1} \frac{\left(\frac{1+b}{e^{\tau}+b}\right)^1}{1!} &+ \ldots + \frac{(-n)_n(-a)_n}{(-a-n+1)_n} \frac{\left(\frac{1+b}{e^{\tau}+b}\right)^n}{n!} \\
= 1 + \frac{(-n)_1(-a)_1}{(-a-n+1)_1} \frac{\left(\frac{1+b}{e^{\tau}+b}\right)^1}{1!} &+ \ldots + \frac{(-n)_n(-a)_n}{(-a-n+1)_n} \frac{\left(\frac{1+b}{e^{\tau}+b}\right)^n}{n!} \\
&\approx 1,
\end{aligned}
\tag{1.116}
$$

or better,

$$
{}_2F_1\left(\begin{matrix}-n, \ -a \\ 1-a-n\end{matrix}; \left(\frac{1+b}{e^{\tau}+b}\right)\right) \approx 1, \tag{1.117}
$$

which, in turn, implies that (1.112) can be rewritten as

$$
P_n(\tau) \approx \frac{\Gamma(a+n)}{\Gamma(n+1)\Gamma(a)} \left(\frac{b}{1+b}\right)^n \left(\frac{1}{1+b}\right)^a, \tag{1.118}
$$

for $\tau \gg 1$, or rather, the steady state distribution P_n indeed reads

$$
P_n = \frac{\Gamma(a+n)}{\Gamma(n+1)\Gamma(a)} \left(\frac{b}{1+b}\right)^n \left(\frac{1}{1+b}\right)^a. \tag{1.119}
$$

Therefore, if $a \in \mathbb{N} \setminus \{0\}$ then (1.119) can be conveniently rewritten as

$$
\begin{aligned}
P_n &= \frac{\Gamma(a+n)}{\Gamma(n+1)\Gamma(a)} \left(\frac{b}{1+b}\right)^n \left(\frac{1}{1+b}\right)^a, \\
&= \frac{(n+a-1)!}{n!(a-1)!} \left(\frac{b}{1+b}\right)^n \left(\frac{1}{1+b}\right)^a, \\
&= \binom{n+a-1}{n} \left(\frac{b}{1+b}\right)^n \left(\frac{1}{1+b}\right)^a,
\end{aligned}
\tag{1.120}
$$

which, indeed, is the negative binomial distribution $NB(a, \rho)$ with $\rho = \frac{b}{1+b}$ being thought to be the probability of "success", while $\frac{1}{1+b}$ defines the probability of "failure". This distribution has mean

$$
\frac{a\rho}{1-\rho} = ab \tag{1.121}
$$

as seen in [58, p. 90].

But, what is the definition of the concept of negative binomial distribution ? In fact, the negative binomial distribution models a stochastic phenomenon in which one aims to find the probability distribution of the number of "successes" before a predefined number a of "failures" occur. However, elucidating the later description in relation to *gene expression* entails apprehending some important notions of probability theory.

To begin with, upon the transcription of one mRNA, one has that it can be bound several times by ribosomes before degrading, so one has an important event of gene expression which amounts to question of what is the probability distribution of the number of proteins produced upon transcription of one single mRNA ? To build a mental model hereof, one can think that mRNA has two states, that is, it either degrades (inactive state) or it is translated (active state) by a ribosome into one protein. From this perspective, one has that the notion of Bernoulli distribution models the aforementioned phenomenon with probability of success [translation] given by $\frac{\nu_1}{d_0+\nu_1}$, or equivalently, $\rho = \frac{b}{1+b}$, and probability of failure amounting to $(1-\rho)$, that is, $\frac{1}{1+b}$.

Now, suppose that the binding of ribosomes to one single mRNA is independent upon one another. In this regard, one has that the notion of geometric distribution suitably models the phenomenon of one mRNA being bound by ribosomes r times, resulting in the the translation of r proteins before the degradation of the respective mRNA. More specifically, one has that the probability of one mRNA producing r proteins before it degrades reads

$$P_{\frac{proteins}{mRNA}}(r) = \left(\frac{b}{1+b}\right)^r \left(1 - \frac{b}{1+b}\right), \tag{1.122}$$

so the number of bursts (proteins per MRNA) is geometrically distributed.

Having said that, we can now turn ourselves to the elucidation of the concept of binomial distribution with respect to gene expression. In fact, more specifically, protein production could be modelled as a sequence of independent Bernoulli trials in which two outcomes are possible: mRNA-translation or mRNA-degradation. In this regard, one could derive the probability of producing n proteins before a failures have occurred by noting that one can reformulate the later into the question of finding the probability of having failed translation $a-1$ times from $n+a-1$ trials ? Regarding the reformulated question, one has that the concept of binomial distribution suits the purpose to answer that , seeing that it models a stochastic phenomenon in which the number of Bernoulli trials is fixed $(n+a-1)$. So, according to this reasoning, one has that

$$P(W = a-1) = \binom{n+a-1}{n} \left(\frac{b}{1+b}\right)^n \left(\frac{1}{1+b}\right)^{a-1}, \tag{1.123}$$

wherein the random variable W symbolizes the number of failures within $n+a-1$ trials. Therefore,

$$\begin{aligned} P_n &= P(W = a-1) \times \left(\frac{1}{1+b}\right) \\ &= \binom{n+a-1}{n} \left(\frac{b}{1+b}\right)^n \left(\frac{1}{1+b}\right)^a, \end{aligned} \tag{1.124}$$

which, in turn, might be seen as an "ad hoc argument" to understand (1.118). Nonetheless, if one wants to fully understand the expression of the temporal evolution of the probability distribution of proteins in the cell on protein time scale given in (1.113) then one might need to elucidate why the probability of "failure" is

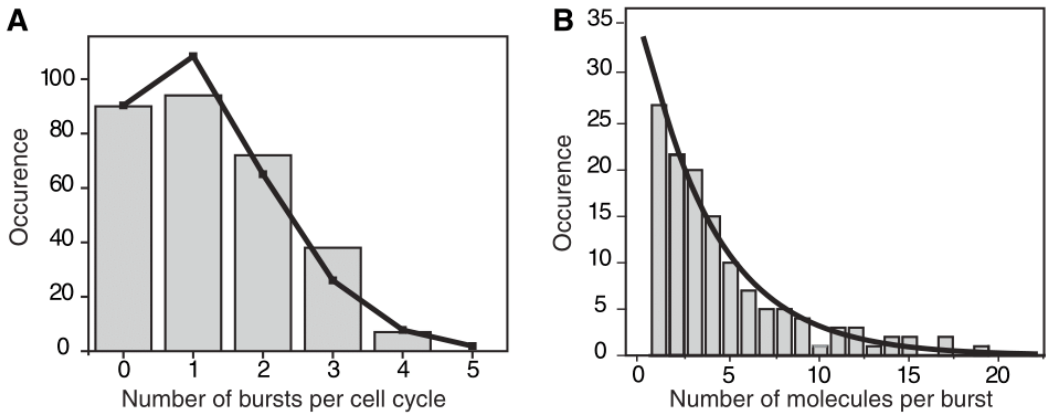


Figure 1.11: Histograms taken from [107]. A) Here we see that the histogram of the number of bursts per cell cycle fits a Poisson distribution with average $a \approx 1.2$. B)Here we see that the histogram of the number of proteins per burst event fits a exponential distribution with average $b \approx 4.2$.

proportional to $e^{-\tau}$, that is,

$$\left(\frac{1+be^{-\tau}}{1+b}\right)^n, \tag{1.125}$$

and what the Gauss hypergeometric series in (1.112) can tell us about gene regulation phenomenon. Concerning the latter point, one can perhaps wonder whether or not one can make a connection between the Gauss hypergeometric series in (1.112) and a corresponding concept of a probability distribution.

Now, if we turn ourselves to the publication [107], then we wonder whether or not it is possible that we could have guessed a negative binomial probability mass function as the steady state distribution for the number of tsr-Venus proteins per cell ? In fact, fitting a Poisson distribution for the number of bursts per cell cycle suggests that the bursts occur randomly in time and are independent of each other. Moreover, each burst event is geometric distributed with ribosome probability binding given by $\rho = \frac{b}{1+b}$, as show in the Figures 1.11(A) and (B). Therefore, if X_i and Y denote random variables representing the number of proteins per burst (i) and the number of proteins per cell, with X_i independently and identically distributed, then performing a convolution-based reasoning yields

$$\left(\bigwedge_{i=0}^{n+a-1} X_i \sim Geom(\rho)\right) \wedge \left(Y \sim \sum_{i=0}^{n+a-1} X_i\right) \Rightarrow Y \sim NB(a,\rho), \tag{1.126}$$

what means that the number of tsr-Venus protein molecules per cell in the cell population is supposed to be negative binomial distributed with parameters a and ρ. Why is it intuitive? Because a NB-distribution models a stochastic event in which one is interested in knowing the probability of a particular number of Bernoulli trials to have a fixed number of successes. Moreover, as the gamma distribution is the continuous analogue of the NB-distribution then a continuous version of (1.126) reads

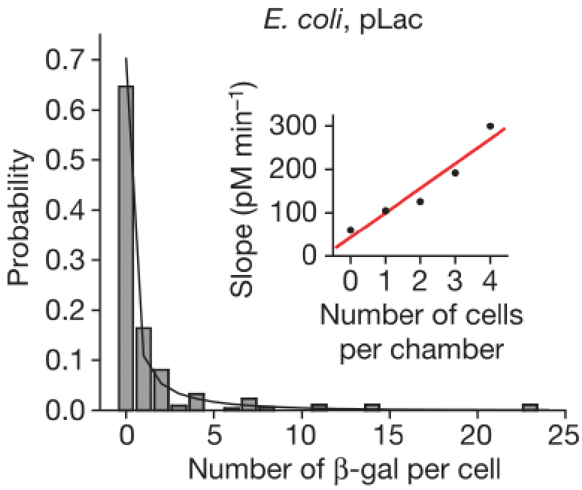


Figure 1.12: Histogram taken from [6], showing that the number of β-galactosidase per cell in the cell population fits a gamma distribution with $a = 0.16$ bursts per cell cycle and $b = 7.8$ proteins per burst.

$$\left(\bigwedge_{i=0}^{n+a-1} X_i \sim Exp(b) \right) \wedge \left(Y \sim \sum_{i=0}^{a+n-1} X_i \right) \Rightarrow Y \sim \Gamma(a, b), \tag{1.127}$$

with a clearer intuitive explanation. In fact, as the lifetime of a protein in *E.coli* cells, here conveniently denoted by $1/d_1$, is on the time scale of a cell cycle, i.e. $1/d_1 \approx 30$ min, then we add a bursts per life cycle with size b, which gives ab for the total number of proteins during the course of a cell division. However, in the cell population, we must add a-exponentially distributed bursts with length scale b, which amounts to a gamma distribution, as seen in Figure 1.12, with mean ab and variance ab^2.

Next, for completeness, we can give an argument for the probability distribution of the number of tsr-Venus mRNA molecules per cell in the cell population. In fact, it should be Poisson distributed as we want to know how many translations have occurred during the trs-Venus mRNA lifetime. In this scenario, if we take into account that there is degradation then a Poisson distribution is definitely a strong candidate. Now, we ask ourselves if there is an analytical framework in which one can derive the expression of the probability distribution for the number of tsr-Venus protein molecules per cell in the cell population? What about the probability distribution for the number of tsr-Venus mRNA molecules per cell in the cell population? From now to the end of this section, we will be entirely concerned with these questions.

Regarding the simulations, as we see in Figures 1.9 (B) and (C) in [90], the authors numerically implemented the Gillespie algorithm to the equation (1.1) and compared with the analytical solution in (1.118). If $\gamma \gg 1$ then the analytical solution accurately predicts the solution of (1.1). If $\gamma < 1$ then the divergence effect takes over, and the analytical solution provided in (1.118) is a poor approximation for the solution of (1.1), as seen in Figure 1.9 (D). But, how do they quantify the effects of small γ ? In fact, they drew upon the Kullback–Leibler divergence

$$D_{KL}(P||\tilde{P}) = \sum_j P(j) \ln \left(\frac{P(j)}{\tilde{P}(j)} \right) \tag{1.128}$$

with P, and $\tilde{P}$ representing two probability distributions defined on the same probability space, see [45]. So, by definition, one has that the *Kullback–Leibler divergence* is a measure that quantifies how close two distribution are to each other.

Next, they also considered a more realistic model, the three stage model, as seen in Figure 1.10 (A), in which the promoter can be active and inactive. By applying the same technique, they could derive an expression for the probability distribution of the number of proteins in the cell. More importantly, they showed in the simulations, as seen in Figure 1.10 (D), that a bimodality may emerge which is achieved by slow transitions between active and inactive states of the promoter.

But, what can this result tell us about gene regulation mechanisms? In fact, if we assume that the equation (1.1) is a reasonable representation for gene expression in prokaryotic organisms then their results states that if $\gamma \gg 1$, i.e., if a protein lifetime is much greater than a mRNA lifetime then the negative binomial distribution is an accurate approximation for the simulated distribution of (1.1). On the other hand, if $\gamma < 1$ then the analytic and simulated solution diverge significantly from each other with higher divergence effects for $\gamma \ll 1$. In fact, $\gamma \gg 1$ implies that all the proteins produced by a single mRNA, remain after mRNA degradation, what strongly suggests a geometric burst. On the other hand, if $\gamma < 1$ then some of the newly produced proteins are already degraded while others are being produced so it is not the case that a single mRNA leaves a geometric burst of proteins behind. Therefore, under the representation assumption, their approach sheds light on the mechanism of gene regulation in prokaryotic organisms what is supported by experimental data as we have seen in Figures 1.11 and 1.12.

However, with respect to eukaryotic organisms, complexity in the regulation process turns it into a very challenging target. Hence, regarding eukaryotes, it is very unlikely that the gamma distribution gives a good approximation for the steady state distribution [*equilibrium distribution*] of the number of proteins in the cell. Hence, it seems fair to claim that the use of noise as a tool for understanding gene expression is limited to prokaryotic species.

1.3 A framework for modelling cell differentiation

Considering that *TFs* are *gene-products* [*proteins*], one can conclude from the *central dogma* that genes influence the expression (repression) of one another which gives rise to the concept of a *gene regulatory network (GRN)* as illustrated in Figure 1.13(A). Moreover, the set consisting of all *genes*, that is, *DNA coding sequences* and *DNA functional non-coding sequences*, is defined as the *genome*. Hence, one has that the *genome* $\mathbf{S}$ of a *living organism* can be represented as

$$\mathbf{S} := (x_1, x_2, x_3, \ldots, x_{k-1}, x_k, x_{k+1}, \ldots, x_{m-1}, x_m)\,, \tag{1.129}$$

with $m \in \mathbb{N}$, and x_j representing a *gene* of the *genome* for all $j \in \{1, 2, 3, \ldots, m\}$. In this way, one has that a *gene regulatory network (GRN)*, at the conceptual level, can be seen as a *directed graph* whose *nodes* represent *genes* while its edges symbolize the interaction between two genes−(mutual) *repression* or (mutual) *activation*-as depicted in Figure 1.13(A).

But, what can we say about the structure of the *GRN* of a *living organism*? Actually, it is fully determined by the *genome* of a *living organism*. What is the role of *evolution* in this determination? In fact, the latter hypothesis asserts that the *genome* in a biological population was subject to several *physico-chemical* transformations over consecutive generations. Those transformations were driven by the demanding adaptation to environmental changes. Therefore, if we acknowledge that the genome of a living organism is still evolving, and that the time scale of the performed experiments is smaller than the time scale of evolutionary processes than one can assume that the structure of the *GRN* of a *living organism* is *invariant* on evolutionary life time. Thus far, we can conclude that the concept of *GRN* is conceptually dependent upon the concept of *genome*, which, in turn, is conceptually dependent upon the concept of *gene*.

But, if the *GRN* of a *living organism* can be regarded as *invariant* on evolutionary timescale and if we can say that, in light of its invariance, each cell in a *living organism* is genetically identical to one another then how can we account for different *cell types* such as a *skin cell*, *muscle cell*, a *nerve cell*, and a *white blood cell*? In order to answer this question, we have quoted from Semrau *et al* in [87]:

"(...) Here, we review attempts to understand lineage decision-making as the interplay of single-cell heterogeneity and gene regulation. Fluctuations at the single-cell level are an important driving force behind cell-state transitions and the creation of cell-type diversity. Gene regulatory networks amplify such fluctuations and define stable cell types. They also mediate the influence of signaling inputs on the lineage decision. In this review, we focus on insights gleaned from in vitro differentiation of embryonic stem cells. (...) "

In order to understand the latter quotation, we can draw upon an earlier argument. In fact, with respect to cell division, it is very unlikely that two genetically identical daughter cells contain the same number of proteins. Moreover, given that genes influence the expression of one another, one has that the respective single-cell heterogeneity gets accentuated by the gene regulatory network, which, in turn, leads cells to differentiate into different cell types.

Now, if we adopt a process perspective to answer the same question, then it is necessary that we briefly turn ourselves toward *embryonic formation* with emphasis on the differentiation process. So, how can we abridge the conceptual complexity of *embryonic formation* in a way in which we can adduce suitable facts to an argument for a answer to the latter question in line with the scope of this section? In fact, if we intend to concisely summarize *embryonic formation* then we might state that a *zygote* becomes an *embryo* by undergoing *cell division*, *proliferation*, *migration* and *differentiation*. In fact, a *zygote* undergoes *mitosis*, that is, a process through which a cell gives rise to two isogenic cells. Moreover, those cells divide further, causing the number of cells to increase. Thereby, the imbalance between the *upsurge in cells* (due to *cell division*) and *cell death* corroborates *cell migration*, placing the cells in the right position to undergo *cell differentiation*. The latter is a process through which cells become specialized ones, which, with respect to *embryonic formation*, will result in a *multicellular organism*-an *embryo*. So, from a process perspective, one has that *cellular differentiation* is a fundamental process so as to go from a *unicellular organism* [*zygote*] to a *multicellular organism* [*embryo*].

But, what is the definition of the notion of *specialized cell* or *cell type*? At the empirical level, it is a sort of cell with *specific characteristics and traits* that has a particular function to be carried out in a *living organism*. For example, a *skin cell* is a *cell type* that looks a bit elongated, whose function is to keep the permeability of the body of a *living organism*; *muscle cells* are long and tubular cells responsible for contraction, and *nerve cells*, which are indeed specialized in electrical activity, are cells consisting of a body surrounded by a branching dendritic tree, and a long axon, through which electrical signals are transmitted down from one *neuron* to another *neuron*; and white blood cells, consisting of round shaped cells, are indeed specialized in immunity. Having described the definition of the notion of *cell type*, it is however essential to emphasizing a distinguishing property of the latter cell types. In fact, *skin cells*, *muscle cells*, *nerve cells*, and *white blood cell* neither dedifferentiate nor do they differentiate further into other cell types, which, in turn, gives rise to the concept of *cell fate*.

Nonetheless, are there *cell types* that can either differentiate into other different *cell types* or, perhaps, dedifferentiate, for instance, by becoming *stem cells* again? In fact, with respect to the first part of the latter question, one has that during *embryonic formation*, which will be thoroughly described in a subsequent section, *trophoblasts cells*, forming the outer layer of the *blastocyst*, differentiate further in *syncitiotrophoblast* and *cytotrophoblast* during the process of *implantation* of the *blastocyst* in the *endometrium*. The latter two cell type, together with *mesenchymal cells* and *fetal vascular cells*, will give rise to the *placenta* as described in [104]. Furthermore, if we agree that differentiating into different cell types is a function in a living organism then it is logically true that *stem cells* are a cell type. So, as we shall also see later in this chapter, *stem cells* differentiate into *epiblast cells* and *hypoblast cells*. The latter cell types differentiate further into the three *germ layers*: *ectoderm, endoderm* and *mesoderm*. Moreover, the cells forming the three germ layers will also undergo *differentiation* resulting in other cell types which, in turn, will end up differentiating into the cell fates: *skin cells*, *muscle cells*, *nerve cells*, *white blood cells* and so forth; giving rise to the whole *embryo*. Therefore, in particular, one has that *trophoblasts cells* and *epiblast cells* are cell types that are not cell fates.

As for the second part of the ongoing question, it has been reported in [63] that mouse *epiblast cells* underwent dedifferentiation by the inhibition of *β-catenin*[20]. Hence, one has that *epiblast cells* differentiate further into other cell types with mouse *epiblast cells* being amenable to *dedifferentiation in vitro* upon the inhibition of *β-catenin*.

So, what can we conclude from the latter elucidations then? First, as for the definition of the notion of *cell type*, one has that it is a cell with *specific characteristics and traits*, carrying out a specific function in a *living organism*. On the other hand, a *cell fate* is a *cell type* that neither dedifferentiate nor differentiate further into other cell types. Therefore, one has that the notion of *cell fate* is conceptually dependent upon the notion of *cell type*, but not conceptually equivalent thereto.

Withal, how can we apprehend those "*specific characteristics and traits*" of a *cell*

[20]Further in this thesis, we will be talking about the role of β-catenin [protein] in differentiation.

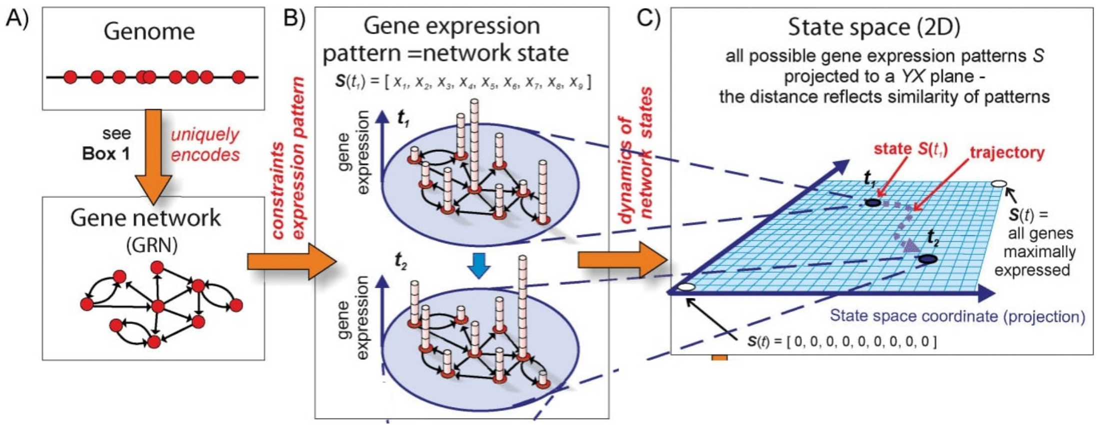


Figure 1.13: This cartoon has been taken from [37]. A) Genome and the respective GRN fixed on evolutionary timescale. B) Although the wiring of the GRN is invariant on evolutionary timescale, one has that the level of gene expression varies. C) Here, one sees the illustration of the GRN in the framework of the dynamical systems theory. In the context of cell differentiation, one can think of a highly dynamic GRN whose level of gene expression varies over time.

type from a gene regulatory network perspective ? In fact, by having differentiated, one has that genes have been switched on and off in each of those different *cell types*, which, by drawing upon the *central dogma*, is equivalent to saying that different proteins have been made in each of them. Now, if we acknowledge that the respective proteins determine the "*specific characteristics and traits*" [*phenotype*] of a cell, and more importantly, that they also stipulate the sort of function that must be carried out by each of the corresponding cell types, then one has that each *cell type* can be thought to be characterized by the *level of gene expression*, or equivalently, by the *gene expression pattern*, as illustrated in Figure 1.13 (B).

However, how can the respective *level of gene expression* be quantified? In fact, in this thesis, consistent with [86], the *level of gene expression* is the quantification of the *RNA* concentration of lots of genes of interest, which, in fact, is performed by a technique known as single-cell RNA-sequencing. So, the respective quantification is supposed to reveal which genes are actually active and how much of each of them is being transcribed in a single cell. The latter measurement is assumed to stipulate whether or not a gene is active within a cell type and to strongly indicate which proteins [e.g. *TFs*] are actually made.

Further, a characterization of each *cell type* based on the *level of gene expression* entails some notion of *stationarity*. Indeed, if we acknowledge that biological processes are inherently stochastic, then a *cell type* can be thought to be characterized by a *multidimensional stationary probability distribution* of *transcription factors (TFs)* that is robust to small perturbations as time progresses. Hence, one has that a *multidimensional stationary probability distribution* is essentially deterministic, which, in turn, suggests that a *dynamical system framework*, as an insightful primary approach, is suitable to model *cellular differentiation*. But, what is then the concept playing the role of a *multidimensional stationary probability distribution* in the latter framework? In fact, if the number of cells in the cell population is high enough then, drawing upon the law of large numbers [12, p. 185], one has that the *level of gene expression* characterizing a *cell type* ought to be close to the *mean*

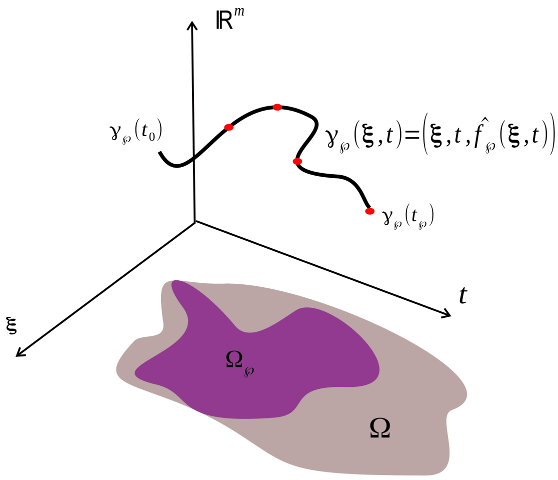


Figure 1.14: Here, one sees the developmental path $\gamma_\wp$ of a cell type $\wp$, which is essentially controlled by built-in functionals (the interplay between the transcriptional and epigenetic mechanisms) $\hat{f}_\wp$.

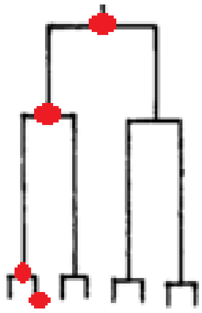


Figure 1.15: Here, one sees the illustration of the cell lineage of a cell type on the bottom of the developmental tree.

[*deterministic limit*] of the corresponding *multidimensional stationary probability distribution*. So, one has that a *cell type* can be seen as a *stable equilibrium*. Later in this thesis, we will properly present the definition of this fundamental concept within the dynamical system framework. The respective concept does capture the essence of the notion of *cell type*, seeing that the property of being robust to small perturbations is indeed inherited by the *deterministic limit*, and, as we shall see, this property is indeed in the description of the concept of *stable equilibrium*.

Moreover, if we draw upon Section 1.2 then we can at some level understand how *TFs* are distributed in the cell in a cell population. In fact, in Section 1.2, we give a concise description of an analytical framework with which Shahrezaei et al in [90] provided an expression for the *temporal evolution of the probability distribution*, see (1.112), and the *steady-state distribution*, see (1.118), of the number of proteins in the cell in a cell population. As we have argued therein, one has that the *negative binomial distribution* is a reasonable approximation of the *equilibrium distribution* of proteins in the cell of *prokaryotic organisms*, but, with respect to *eukaryotic organisms*, due to the complexity in the *gene regulation* process, it is very unlikely that the negative binomial distribution gives a judicious approximation of the *steady state distribution* of proteins in the cell population.

However, it is not clear that we can argue in the same way with respect to the notion of *cell fate*. In other words, does the concept of *stable equilibrium* captures the essence of the notion of *cell fate* ? In fact, as the notion of *cell fate* is conceptually dependent upon the notion of *cell type*, but not conceptually equivalent thereto, then it seems that the concept of *stable equilibrium* does contain an important property of the concept of *cell fate* but does not capture the essence thereof on its own. In fact, as far as the author of this thesis can see, the distinguishing property of the notion of *cell fate* can only be apprehended by appealing to the concept of *bifurcation*. The definition of the latter concept will be presented later in this thesis.

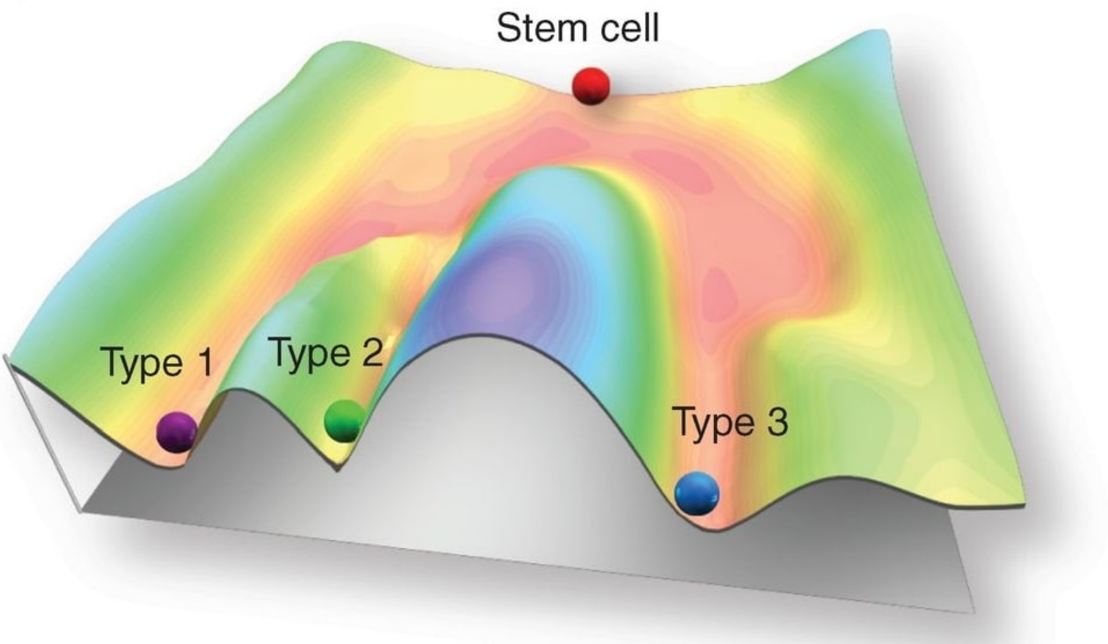


Figure 1.16: This cartoon has been taken from [23]. As we can envisage in the cartoon, noise in gene expression boosted by a gene regulatory network determines in which cell type stem cells will differentiate.

Next, if we apprehend *cell differentiation* from a gene regulatory network perspective, that is, as a *built-in process* through which the *gene expression pattern* of a cell is modified in time and space until it reaches a stable *level of gene expression*, then one can think about *cellular differentiation* as a path in $\mathbb{R}^{3+1+m}$ as depicted in Figure 1.14. As cell types can differentiate further into other cell types until they reach their ultimate fate, then it seems reasonable to wonder whether or not there is a pattern in the developmental history of a *cell fate*. If we presuppose that the pattern can be characterized by an invariant sequence of cell types leading to a specific *cell fate* then one might understand the notion of *cell lineage* through the respective sequence, as illustrated in Figures 1.14 and 1.15.

However, what can we say about the *mechanisms* through which genes are switched on and off during the course of the process of *cell differentiation*? In fact, as we described earlier in Section 1.1, epigenetic mechanisms controls where and when a target site of *DNA* become accessible to *TFs*, by changing *DNA-conformation* without altering *DNA-sequence* [*genome*]. So, at each *time* and *space*, during embryonic development, genes are switched off or on, e.g., are *methylated* or *acetylated*, which, in turn, gives rise to the concept of *epigenome*. Therefore, under the central hypothesis of molecular biology, if we appeal to the notion of *layers of gene regulation* introduced by Semrau *et al* in [87], then we might be entitled to make the claim that transcriptional and epigenetic activities presumably comprise the primary layer of gene regulation. If this is the case then, from a mechanistic perspective, it is logically true that the interplay between *transcriptional regulation* and *epigenome* is a necessary condition for *cell differentiation* to occur, but, of course, not sufficient. In fact, as categorically argued by Semrau et *al* in [87], provided that *cell differentiation* involves a network of molecular interactions, one has that characterizing it entails to understand "the interplay of internal factors (epigenetics, cell cycle, stochastic gene expression) and external factors (signaling molecules, cell-cell contacts, mechanical cues)"; see, e.g., [33] and [103].

But, can we provide a mental model of the concept of *epigenome* so as to grasp its definition? In fact, let t_{diff}^{embryo} be the average time for embryonic formation. So, the concept of *epigenome* with respect to *cell differentiation*, can perhaps be understood as a family

$$\mathcal{L}_{epi}^{diff}\left(\Omega \times \left[0, t_{diff}^{embryo}\right], 2^{\mathbf{S}}\right) = \left\{\hat{f}_{\wp}^{epi}|_{\Omega_{\wp}} : \wp\, is\ a\ cell\ type\right\}$$

of *built-in functionals*

$$\hat{f}_{\wp}^{epi}|_{\Omega_{\wp}} \colon \Omega_{\wp} \times [0, t_{\wp}] \subset \mathbb{R}^{3+1} \to 2^{\mathbf{S}}$$
$$(\xi, t) \mapsto \hat{f}_{\wp}^{epi}(\xi, t),$$

with ξ and t denoting the spatial and time variables, $\Omega \subset \mathbb{R}^3$ being thought to be the domain wherein *embryonic formation* takes place, whereas $\Omega_{\wp} \subseteq \Omega \subset \mathbb{R}^3$ betokens the sub-domain in which the cell type $\wp$ is formed. Moreover, $2^{\mathbf{S}}$ represents the set of all functions from $\{x_1, x_2, \ldots, x_{m-1}, x_m\}$ to $\{0, 1\}$, and $t_{\wp} \leq t_{diff}^{embryo}$ symbolizes the *average time* for the formation of the cell type $\wp$. Consistently, one has that

$$\hat{f}_{\wp_1}^{epi}|_{\Omega_{\wp_1}} \equiv \hat{f}_{\wp_2}^{epi}|_{\Omega_{\wp_2}} \tag{1.130}$$

on $\Omega_{\wp_1} \cap \Omega_{\wp_2}$. Thereby, one has that the functionals $\hat{f}_{\wp}^{epi}$ and $\hat{f}_{\wp}^{tr}$ indeed determines

$$\gamma_{\wp} \colon \Omega_{\wp} \times [0, t_{\wp}] \subset \mathbb{R}^{3+1} \to \mathbb{R}^{3+1+m}$$
$$(\xi, t) \mapsto \left(\xi, t, \hat{f}_{\wp}(\xi, t)\right),$$

which, in turn, as illustrated in Figure 1.13, describes the developmental history of the cell type $\wp$, with

$$\hat{f}_{\wp}(\xi, t) = \left(\hat{f}_{\wp}^{tr}\hat{f}_{\wp}^{epi}\right)|_{\Omega_{\wp}}(\xi, t) = \begin{cases} 1 & \text{if } \hat{f}_{\wp}^{epi}|_{\Omega_{\wp}}(\xi, t) = 1 \\ 0 & \text{otherwise} \end{cases} \tag{1.131}$$

representing the interplay between *transcriptonal regulation*, thought to be carried by $\hat{f}_{\wp}^{tr}|_{\Omega_{\wp}}(\xi, t) \in 2^{\mathbf{S}}$, and *epigenome*, thought to be carried by $\hat{f}_{\wp}^{epi}|_{\Omega_{\wp}}(\xi, t) \in 2^{\mathbf{S}}$.

Therefore, at each point of a cell type developmental history, that is, $(\xi, t) \in \Omega_{\wp} \times [0, t_{\wp}]$, one has that chemical changes [e.g. *methylation*, *acetylation*] occur in *DNA* and the *histone proteins* enabling [disabling] transcription factors to access target genes. For example, *muscle cells* presumably methylate lots of genes involved in the formation of *skin cells*, and vice versa. The later mental model is suitably envisaged in Weddington's epigenetic landscape shown in Figure 1.16. As a conclusion, one can say that $\gamma_{\wp}$ is indeed a precise description of a dynamical system. Hence, from a deterministic dynamical system perspective, one has that $\gamma_{\wp}$ is thought to be a solution of a system of differential equations

$$\frac{d\mathbf{S}^{cell}}{dt}(\xi, t) = \mathbf{F}(\mathbf{S}^{cell}(\xi, t)), \tag{1.132}$$

or better,

$$\frac{dx_j^{cell}}{dt}(\xi, t) = F_j(x_1^{cell}(\xi, t), x_2^{cell}(\xi, t), \ldots, x_{j-1}^{cell}(\xi, t), x_j^{cell}(\xi, t), x_{j+1}^{cell}(\xi, t), \ldots, x_m^{cell}(\xi, t)) \tag{1.133}$$

with the superscript "*cell*" meaning a "*cellular process*"[21] and with ξ and t denoting the spatial and time variables; whereas F_j, for all $j \in \{1, 2, 3, \ldots, m\}$, is the mathematical expression representing the interaction of a single *gene* x_j^{cell} with the remaining ones in the *GRN*. However, provided that we need to come up with a mathematical representation [*model*] for the right-handed side of (1.132) solely on our own, one has that the role of the *third-person perspective* and the *first-person perspective* in the evaluation of such a model will be of utmost importance. Indeed, we shall be giving a thorough account to that in Chapter 2. But, what about the *conception priority*? Does it play a role in the evaluation of such a model? And what about the formulation of such a model? Regarding the former question, we refer to Chapter 2. Now, to give an argument for the latter question, we refer to *Leibniz's Argument for Primitive Concepts* in [69]:

"Whatever is thought by us is either conceived through itself, or involves the concept of another. Whatever is involved in the concept of another is again either conceived through itself or involves the concept of another; and so on. So one must either proceed to infinity, or all thoughts are resolved into those which are conceived through themselves. If nothing is conceived through itself, nothing will be conceived at all. For what is conceived only through others will be conceived in so far as those others are conceived, and so on; so that we may only be said to conceive something in actuality when we arrive at those things which are conceived through themselves. "

So, Dr. Gottfried Wilhelm Leibniz argues that an appropriate apprehension of a complex concept must only be possible if there are concepts underlying it and that are understood through themselves. But, what does Leibniz's argument have to do with the role of the *conception priority* in the formulation of a mathematical representation for a biological process, such as *cell differentiation*? To answer the latter question, we quote from Dr. Dennis Plaisted in [69]:

" (...) And if the degree to which complex concepts are conceived is directly dependent upon the degree to which they are conceived down to their simple components (...), then complex concepts are conceived to the fullest degree when they are analyzed into their simple components. "

Hence, if we draw upon the latter quotation then we can argue that if it is true that the closer the mathematical representation is to the underlying mechanisms, the higher is the degree of similarity between the actual dynamics and the one produced by the model itself; and if it is true that the *conception order* reflects the intrinsic order in the set of mechanisms underpinning cell differentiation[22], then it is logically true that the conceptual priority plays an important role in the formulation of such a model.

[21] Although cell differentiation, mechanistically speaking, involves the interplay between intracellular and intercellular factors, the product thereof, that is, the change in cellular identity with respect to the level of gene expression, manifests itself in the single cell.

[22] In fact, e.g., translation occurs after transcription, and without knowing the concept of transcription, one cannot understand the concept of translation.

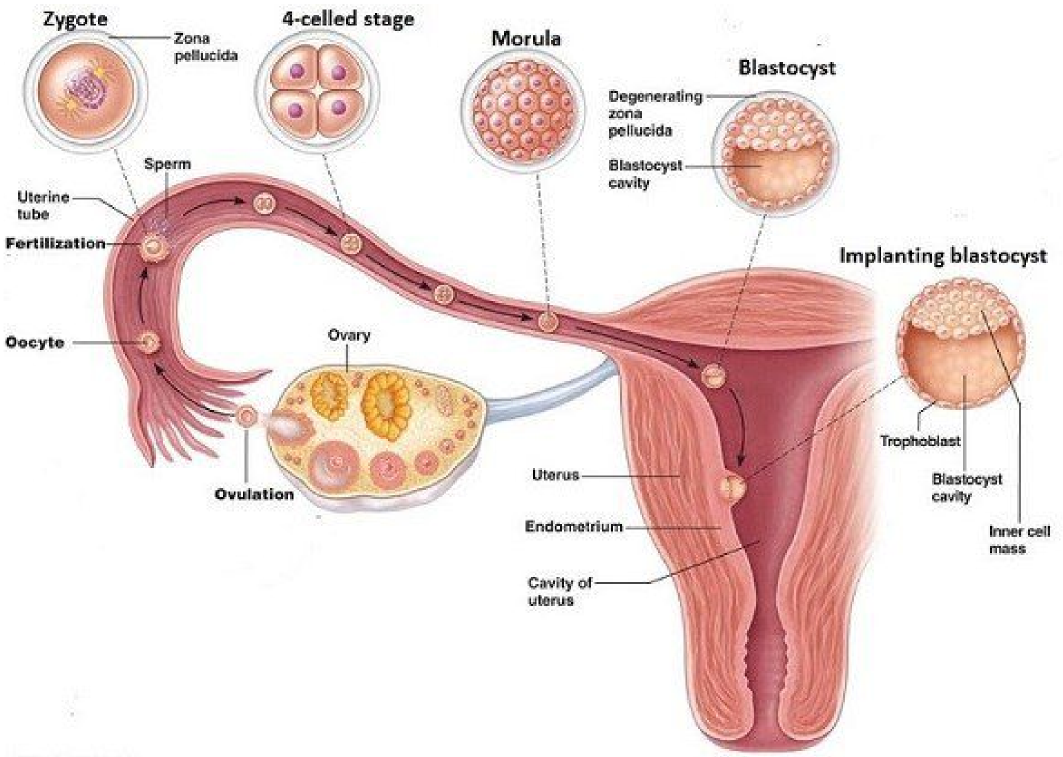


Figure 1.17: This cartoon has been taken from [43]. Here, one sees the illustration of fertilization, cleavage and blastulation.

1.4 Embryogenesis: a concise process perspective

Regarding *embryogenesis*, one can say that it initiates with the *fertilization*, that is, the fusion of the male *gamete* [*spermatozoon* or *sperm*] with the female *gamete* [*ovum* or *egg*]. But, what is a *gamete*? In the case of an *eukaryotic cell*, it is a *haploid cell*, that is, a cell containing 23 *chromosomes* instead of 23 pairs of *chromosomes*. Upon fertilization, one has the formation of a *zygote*, which, in fact, is a *diploid cell*, that is, a cell containing all the 23 pairs of *chromosomes*. But, what can we say about the structure of a *zygote*? Indeed, it is a *diploid cell* surrounded by an outer layer: the *zona pellucida*. The later is a layer consisting of cells that surround the *female gamete* supplying it with *nutrients*. Moreover, the *zona pellucida* is also responsible for the binding of the *sperm* to the *egg*, and for limiting the fertilized egg to a fixed domain as illustrated in Figure 1.17 and shown in Figure 1.19 (a).

However, if a single cell-the *zygote*-is supposed to become a multicellular organism-the *embryo*-then it needs to divide itself [*mitosis*] in numerous cells. In fact, one has that the process of multiplying itself occurs really fast in such a way that there is no time left for the daughter cells [*blastomeres*] to grow. The later process gives rise to the concept of *cleavage*, that is, dividing without growing. Besides, the respective daughter cells are called isogenic ones, that is, with the same *DNA*. So, with respect to the aforesaid aspect, the daughter cells are indeed identical to one another. But, when does *cleavage* stop? It stops by the end[23] of day 3 with the formation of a 32-cell zygote structure known as *morula*, as illustrated in Figure 1.17 and shown in Figure 1.19 (e).

[23] Here, days are counted from the day on which fertilization has been taken place.

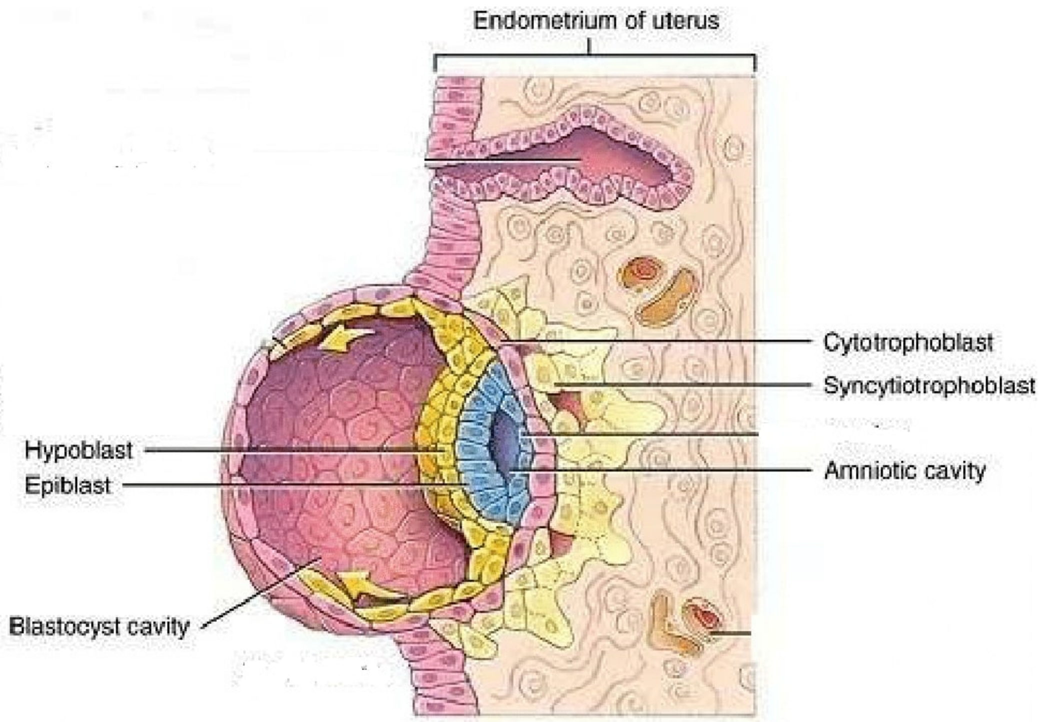


Figure 1.18: This cartoon has been taken from [43]. Here, one sees the beginning of the implantation process by which, the blastocyst invades the endometrium causing the trophoblasts to differentiate into cytotrophoblasts and syncytiotrophoblasts. Moreover, the embryoblasts are also being replaced by a bilaminar layer comprised by epiblasts and hypoblasts, which, in turn, gives rise to the amniotic cavity. Lastly, one can also see depicted in this cartoon that hypoblasts starting migrating toward the surface of the blastocyst cavity during implantation.

Now, it is important to bear in mind that *blastomeres* can form the whole organism, which, in turn, gives rise to the notion of *totipotent stem cells*. Now, upon the end of the *cleavage* process, one has that *blastomeres* start migrating toward each other forming a compact structure, with the outer cells thereof differentiating into *trophoblasts*. Furthermore, despite consisting of *blastomeres*, one has that the inner cells are now named *embryoblasts*. Upon the end of the *compactification* process, one has that *embryoblasts* start polarizing what culminates in a cluster of cells at one end, which, in turn, causes a fluid-filled cavity[24] to emerge at the other end. The respective cavity is known as the *blastocoel* [*blastocyst cavity*]. After the *polarization* process, one has that the resulting structure gives rise to the concept of *blatocyst*. So, by the end of day 5, one has the formation of the *blastocyst* as illustrated in Figure 1.17 and shown in Figure 1.19 (f). Hence, the process from *cleavage* to *polarization* leading to the formation of the *blastocyst*, gives rise to the concept of *blastulation*.

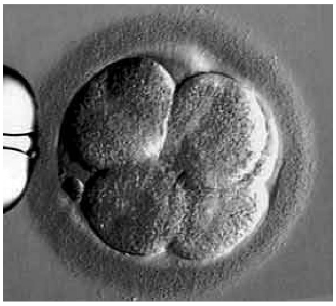
But, why is the structure of the *blastocyst* so important during development? In fact, the outer cell mass, the *trophoblasts* will cause the *placenta* to be formed whilst the inner cell mass, the *embryoblasts*, will generate the three germ layers [*ectoderm, mesoderm, endoderm*] from which all the tissues of the organism will develop. Now, invoking that *embryoblasts* are indeed undifferentiated cells, one has that the cells

[24]This cavity is used to store nutrients.

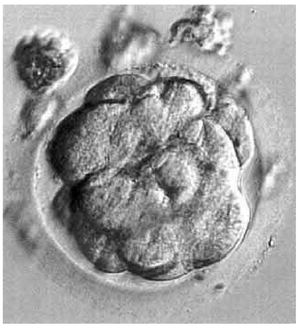
(a) A fertilized egg: a zygote.

(b) A 2-cell zygote.

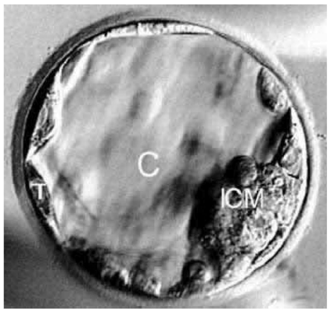
(c) A 4-cell zygote.



(d) A 8-cell zygote.



(e) By the end of day 3, a 32-cell zygote gives rise to the morula around which one still sees the zone pelucida enveloping it.



(f) By the end of day 5, one has the formation of the blastocyst. Moreover, it demarcates the degeneration and decomposition of the zona pelucida.

Figure 1.19: These figures have been taken from [2]. Here, one sees the stages of embryo development during in-vitro fertilization (ivf): from fertilization to the formation of the blastocyst.

forming the inner cell mass in the *blastocyst* right after *polarization* give rise to the concept of *pluripotent stem cells*. In fact, when those cells are removed and cultured, one has the emergence of the concept of *embryonic-like stem cells*. Unlike the *totipotent stem cells*, which can give rise to the whole organism, the *pluripotent stem cells* cannot generate the whole organism given that, for instance, they cannot form the *placenta*.

Further, immediately after *blastulation*, one has that the *zona pellucida*, still surrounding the *blastocyst*, starts to degenerate and decompose , which, in turn, demarcates the beginning of the *implantation* process, that is, the insertion of the *blastocyst* into the inner epithelial layer of uterus [*endometrium*] as clearly illustrated in Figure 1.17 and 1.18. So, insofar as the *blastocyst* (now free from the *zona pellucida*) invades the *endometrium*, one has that *trophoblasts* differentiate into an inner layer [*cytotrophoblasts*] and an outer layer [*syncytiotrophoblasts*]. Moreover, the *embryoblasts* [*pluripotent stem cells*] also differentiate into *epiblasts* and *hypoblasts* what gives rise to a bilaminar layer. The constitution of such a bilaminar layer forms a new fluid-filled cavity known as *amniotic cavity*. The *amniotic cavity* separates

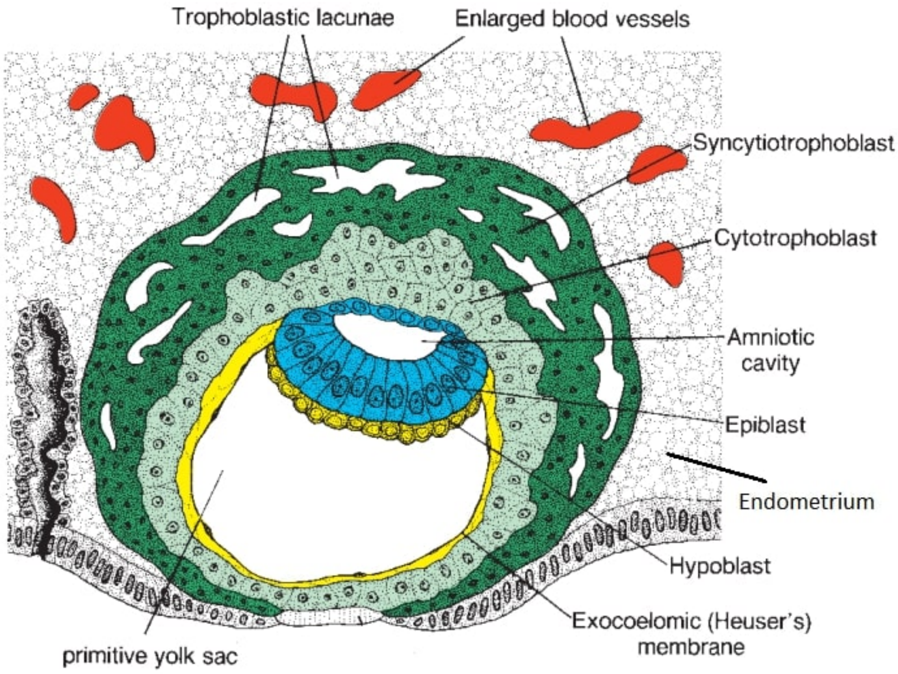


Figure 1.20: This cartoon has been taken from [79]. Here, one sees the bilaminar blastocyst completely implanted in the endometrium by the end of day 8. More specifically, one clearly sees the bilaminar layer setting two cavities apart from each other: the amniotic cavity and the primitive yolk sac. Moreover, one sees the appearance of the trophoblastic lacunae in the layer of syncytiotrophoblasts, the emergent exocoelomic membrane resulting from the migration of hypoblasts, and the approximation of endometrial capillaries (blood vessels) by the fast expansion of syncytiotrophoblasts and cytotrophoblasts.

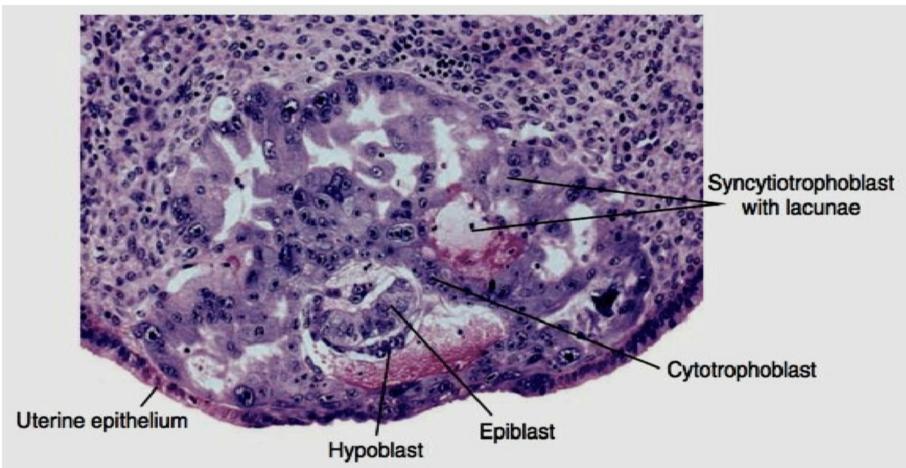


Figure 1.21: This cartoon has been taken from [79]. Here, one sees a section of a 7.5-day human blastocyst.

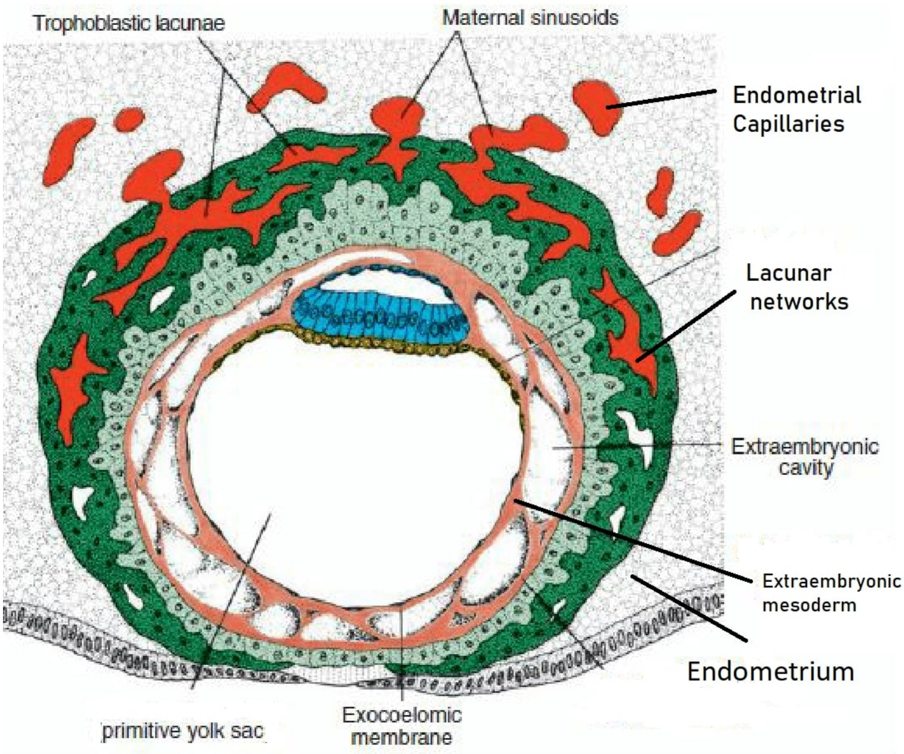


Figure 1.22: This cartoon have been taken from [79]. By the end of day 12*, one has that trophoblast lacunae have fused with each other forming the lacunar networks, through which the maternal blood will flow upon the erosion of the dilated maternal sinusoids caused by the fast expansion of syncytiotrophoblasts and cytotrophoblasts, which, in turn, will cause lacunae to form in the extraembryonic mesoderm.*

the bilaminar layer from the inner layer of *cytotrophoblasts* as illustrated in Figure 1.18.

Next, with the migration of *hypoblasts*, one has that a new layer is formed, that is, the *exocoelomic membrane*, which, in fact, surrounds the *primitive yolk sac* (it was however named the *blastocyst cavity* upon the end of *blastulation*) together with the hypoblasts constituting the bilaminar layer, as illustrated in Figure 1.20. So, by the end of day 9, the *bilaminar blastocyst* [25] is fully implanted in the *endometrium*.

Now, owing to a faster growth of *cytotrophoblasts* and *syncytiotrophoblasts* in relation to the cells composing the *bilaminar blastocyst* one has that small holes [*trophoblastic lacunae*] begin to emerge in the layer consisting of *syncytiotrophoblasts*. Inasmuch as the layer of *syncytiotrophoblasts* expands further, one has that *trophoblastic lacunae* fuse with one another giving rise to the *lacunar networks*, as illustrated in Figure 1.20 and shown in Figure 1.21.

Further, one has that *endometrial capillaries* surrounding the *bilaminar blastocyst* start to become wider as well, which causes them to turn into sinusoidal structures [*maternal sinusoids*]. So, provided that *syncytiotrophoblasts* will keep on expanding further then one has that *maternal sinusoids* will end up getting damaged owing thereto, which, in turn, will culminate in the flow of maternal blood through

[25] It is no longer the structure that has been named the *blastocyst* so we refer thereto as the bilaminar blastocyst.

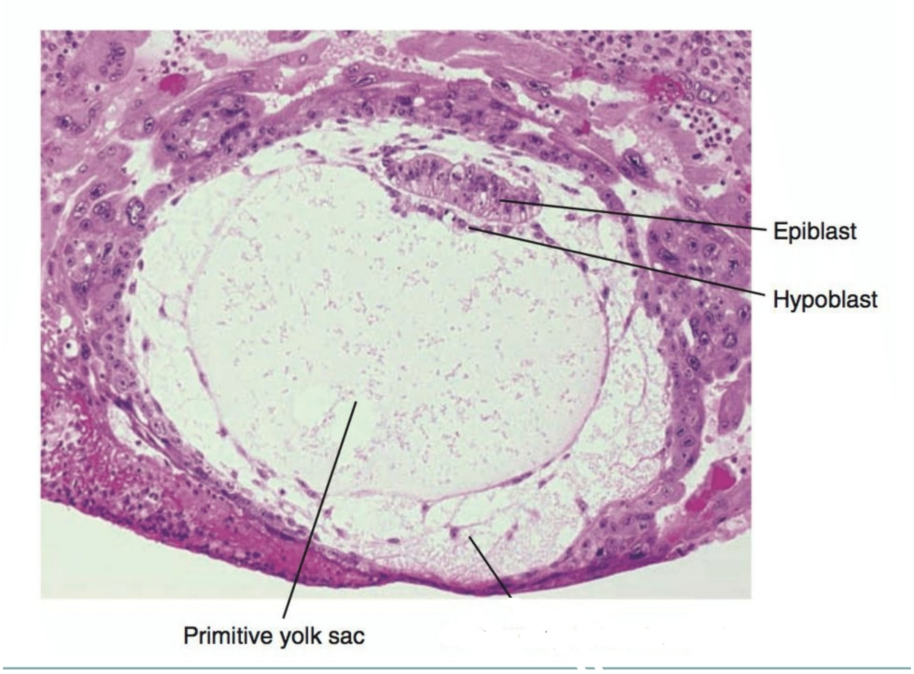


Figure 1.23: These cartoon has been taken from [79]. Here, one sees a fully implanted 12-day human bilaminar blastocyst.

the *lacunar networks*, which, indeed, allows the exchange of nutrients between the mother and the developing embryo; as illustrated in Figure 1.22 and shown in Figure 1.23.

Next, while *syncytiotrophoblasts* continue growing, as informed in [79, p.46], one has that *hypoblasts* composing the *exocoelomic membrane*, presumably begin to differentiate into another cell type-the *extraembryonic mesoderm*-which populates the layer that is formed between the outer layer of the *exocoelomic membrane* and the inner layer of *cytotrophoblasts*. Moreover, owing to the fast growth of *cytotrophoblasts* and *syncytiotrophoblasts*, one has the appearance of holes in the respective layer of *extraembryonic mesoderm* cells, see Figures 1.22 and 1.23, which will cause it to shrink down to a cylindrical structure still consisting of *extraembryonic mesoderm cells*, i.e. the *connecting stalk*. So, the *connecting stalk* [the *primitive umbilical chord*] connects the bilaminar embryonic structure [the *developing embryo*] to the *cytotrophoblasts*, which, as mentioned earlier, generate the *placenta* together with *syncytiotrophoblasts*. Moreover, the respective shrinking process will culminate in the formation of a new cavity, that is, the *chorionic cavity*, which, in turn, is delimited by the inner layer of *cytotrophoblasts* and by the outer layer of the *exocoelomic membrane*.

Now, by the end of day 13, one has that a part of the primitive yolk sac is removed, resulting in the secondary *yolk sac*, or simply, the *yolk sac*. the later is surrounded by the *exocoelomic membrane*, which, in turn, is now set apart from the inner layer of the *cytotrophoblasts* by the *chorionic cavity* as illustrated in Figure 1.24 and shown in Figure 1.25. Therefore, up to 2 weeks after *fertilization*, one has that the *developing embryo* is essentially comprised by a round structure, bound to the *cytotrophoblasts* by the *connecting stalk*, containing a bilaminar layer consisting of *epiblasts* and *hypoblasts*, and two cavities, the *amniotic cavity* and the *yolk sac*, which, in fact, are set apart from each other by the corresponding bilaminar

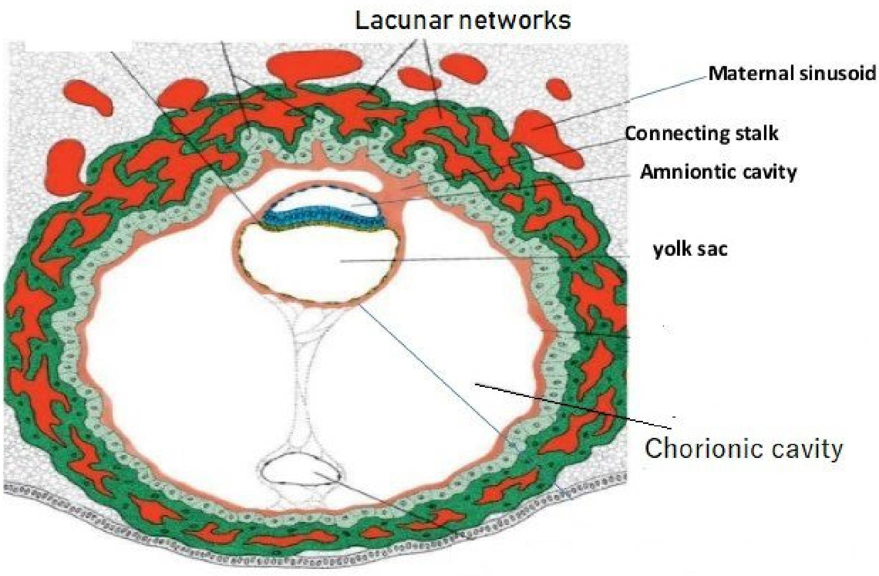


Figure 1.24: This cartoon has been taken from [79]. By the end of day 13, one has that maternal blood flows noticeably through the lacunar networks, the yolk sac is formed, and the chorionic cavity is constituted. Furthermore, the developing embryo is bound to the inner layer of cytotrophoblasts by the connecting stalk.

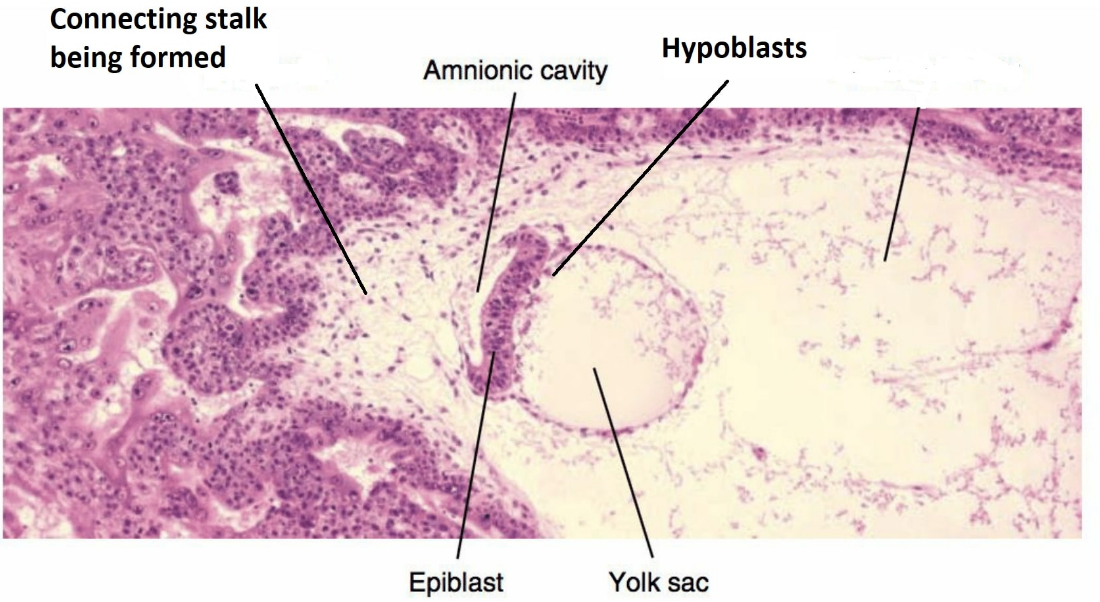


Figure 1.25: This cartoon has been taken from [79]. Here, one sees a section of a 13-day human bilaminar blastocyst. In fact, the yolk sac is visibly shrunk and the lacunar networks are mostly filled with blood.

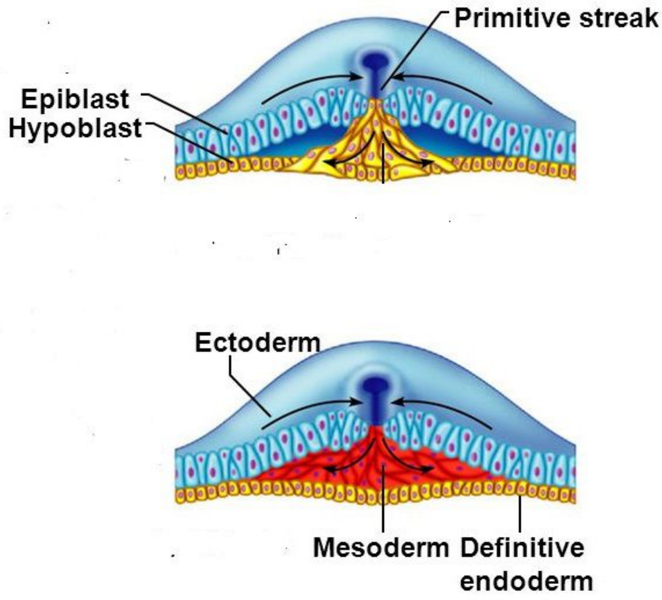


Figure 1.26: This cartoon has been taken from [53]. Here, one sees the illustration of the process of gastrulation. The latter initiates with the formation of the primitive streak through which migrating epiblasts invade the hypoblasts cells replacing them with endoderm cells by undergoing differentiation. The remaining epiblast cells differentiate into another cell type: mesoderm cells. Moreover, the layer of epiblast cells also undergoes differentiation, resulting in another cell type: ectoderm cells. So, by the end of day 16, one has the formation of the three germ layers.

layer. Moreover, the *developing embryo* is embedded into a cavity itself, that is, the *chorionic cavity*, which, as previously said, separates it from the inner layer of *cytotrophoblasts*.

However, how will the bilaminar embryonic structure [*primitive embryo*] develop itself further? In fact, on day 14, there is the formation of a linear band, the *primitive streak*, right in the middle of the epiblast-layer which extends itself as far as in the vicinity of the *connecting stalk*, giving rise to the tail while the other end forms the head of the *primitive embryo*. In fact, the primitive streak stipulates the main body axes of the primitive embryo. Next, right after the formation of the *primitive streak*, one has that epiblast cells start to migrate to it, through which they go toward the hypoblast cells, replacing them, when undergoing differentiation, by a new cell type, which, in turn, is known as *endoderm*. Moreover, the *epiblasts* that remain confined to the domain bounded from above by the epiblast-layer and from below by endoderm cells, differentiate into an another *cell type* known as *mesoderm*. Furthermore, in the meanwhile, epiblast cells composing the epiblast-layer, also undergo differentiation giving rise to a new *cell type* know as *ectoderm*, as illustrated in Figure 1.26. Hence, by the end of day 16, one has the formation of the three germ layers [*ectoderm, mesoderm, endoderm*], and the respective process is known as *gastrulation*.

Now, if one wants to characterize the developmental path through which pluripotent stem cells make their decisions, giving rise to the three germ layers, then it is necessary to know what happens with the dynamics of gene expression at the single-cell level down to the minute. Indeed, Semrau *et al* in [86] using mouse embryonic-like stem cells as a model, applied the technique known as *single-cell RNA-sequencing* to measure the amount of transcribed *RNAs* at the single cell level. The latter measurement is assumed to stipulate whether or not a gene is active within a cell type and to strongly indicate which proteins are actually made. Hence, their experiment quantified the dynamics of cell differentiation by unraveling which genes are involved in the differentiation of pluripotent stem cells and how their expression change through the time up to the decision making.

However, what does the dynamical system theory have to do with their experiment? Indeed, knowing the genes involved in the process of differentiation is not sufficient to characterize cell differentiation but answering the question of how they are wired together is of utmost importance. In this regard, as we have argued earlier in Section 1.3, the dynamical system framework offers either the possibility of conceptualizing the underlying mechanism or the possibility of actually modelling it.

1.5 Mathematical modelling of the experimental results

So far, we have argued why we can draw upon the dynamical system framework to understand *cell differentiation* and what the *conception order* has to do with that. Here, we will be summarizing the experimental results reported in [86] and, more importantly, we will present a minimal gene regulatory network and a extension thereof, which, in turn, will be conjectured as a conceptual mechanism for the observations.

1.5.1 Observations of the Experimental results

In [86], Semrau *et al*, aimed at characterizing the exit from *pluripotency* to *lineage commitment*, measured the *gene expression pattern* of *mouse embryonic-like stem cells*[mESC-like] undergoing *retinoic acid* [RA] driven differentiation. So, after 96h of exposition to *retinoic acid* [RA] a homogeneous cell population became morphologically heterogeneous, as seen in Figure 1.27.

But, how did the authors quantify the *gene expression pattern*? In fact, by applying the single-cell RNA-sequencing technique [RNA-seq] the authors quantified the *gene expression pattern* at the single cell level when measuring the RNA concentration of lots of genes of interest. In doing so, they could determine the exit from pluripotency and, subsequently, the bifurcation into *Ectoderm-like cells* [Ectoderm-like] and *Extra-embryonic endoderm-like cells* [XEN-like].

Consistent with Section 1.4, one might say that the authors in [86] focused on giving a characterization of the *developmental path*, see Figure 1.14, at the level of gene expression, from *blastocyst* to the three germ layers, or rather, from *stem cells* to the formation of the three germ-layers [*ectoderm, mesoderm, endoderm*]. However, as reported in [86], there was no observation of an upregulation of *mesodermal markers*[26] in the population of cells during the experiments, which, in fact, as ar-

[26] The concept of marker in the realm of gene expression might be concisely defined as a gene

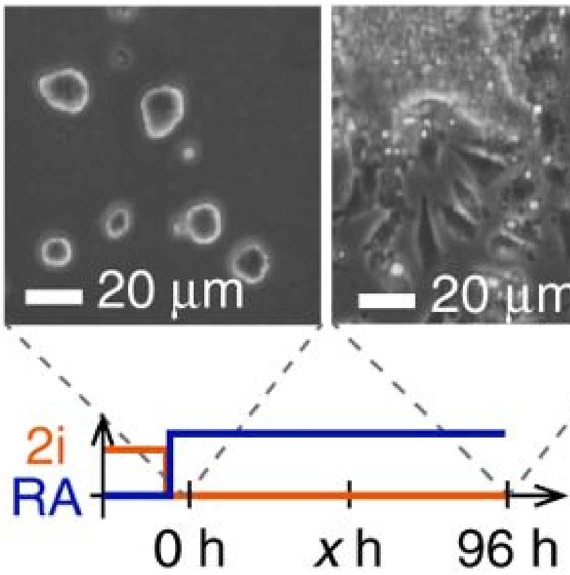


Figure 1.27: Figure taken from [86]. Here, on the left side, one sees pluripotent mECs (round cells) while, on the right side, after 96*h of exposition to retinoic acid RA, one sees elongated cells (differentiated cells).*

gued in [86], is consistent with other reports, see [73] , in which *retinoic acid* [RA] upregulated *neuroectoderm* and *XEN markers* while downregulating *mesodermal markers*.

Now, can we be rather specific as to the methodology used by the authors in [86] so as to determine the exit from pluripotency and the bifurcation into *Ectoderm-like cells* [Ectoderm-like] and *Extra-embryonic endoderm-like cells* [XEN-like]? In fact, for $n_P, n_X, n_E \in \mathbb{N}_{>0}$, we can regard a cell as the fuzzy network vector

$$\boldsymbol{cell}(t_\mu) = \left(TF_1^P(t_\mu), ..., TF_{n_P}^P(t_\mu), TF_1^X(t_\mu), ..., TF_{n_X}^X(t_\mu), TF_1^E(t_\mu), ..., TF_{n_E}^E(t_\mu)\right), \tag{1.134}$$

with

$$-4 \leq TF_{i_P}^P(t_\mu) \leq 2, \tag{1.135}$$

for $i_P \in \{1, 2, ..., n_P\}$, and

$$-4 \leq TF_{i_X}^X(t_\mu) \leq 2, \tag{1.136}$$

for $i_X \in \{1, 2, ..., n_X\}$, and

$$-4 \leq TF_{i_E}^P(t_\mu) \leq 2, \tag{1.137}$$

for $i_E \in \{1, 2, ..., n_E\}$, representing the expression of core *transcription factors* characterizing the *pluripotency*, the *XEN* and the *Ectoderm* networks respectively. As we have said earlier in this section, this expression is quantified by RNA-seq at each time point $t_\mu \geq 0$, but is being represented in (1.134) by pseudo-scores. That is, -4 stands for the lowest expression value while 2 represents the highest expression value so one says that right hand side of (1.134) characterizes the *transcriptional profile* of a cell at the time point t_μ.

Further, it has been reported in [86] that the authors quantified the transcriptional profile of over 2000 single cells during differentiation. The quantification was

whose expression is a necessary condition for the transcriptional profile of a certain cell type.

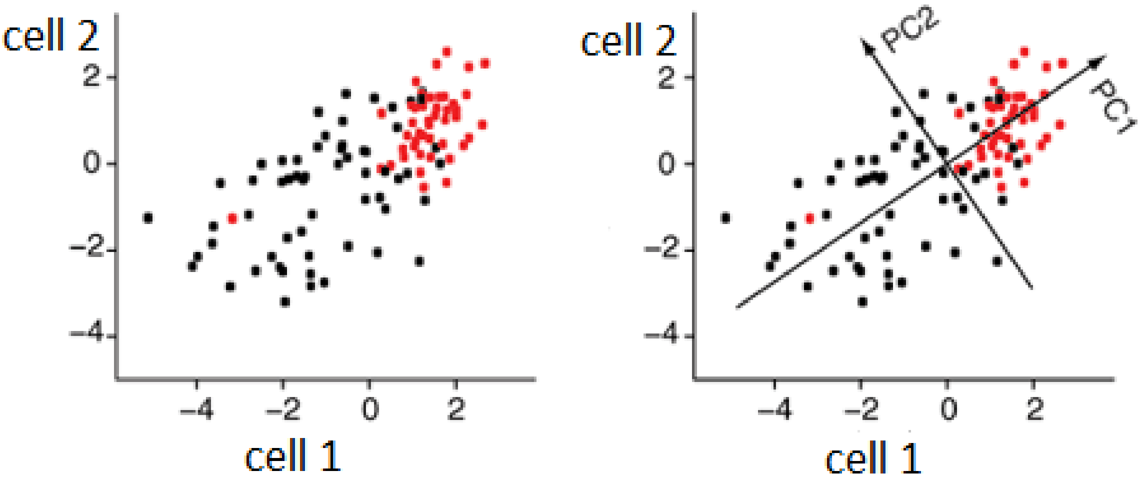


Figure 1.28: Figure taken from [74]. Here, on the left side, one sees the scatter plot of the gene expression pattern of $cell_1$ *versus the one of* $cell_2$*. On the right side, one sees the regression line showing that* $cell_1$ *and* $cell_2$ *are positively correlated. Furthermore, one sees how the first principal component* PC_1 *is defined as the one that spans the direction with the most variation and how the second principal component* PC_2 *is defined as the one that spans the direction of the second most variation. So, one sees that genes located close to the regression line and more grouped in the ends thereof have the most influence upon the variation in the first principal component* PC_1*. Similarly, one sees that genes located close to the line perpendicular to the regression line and more grouped together in the ends thereof have the most influence upon the variation in the second principal component* PC_2*.*

performed at 9 time points with an interval of 12 hours between a time point and its successor, which amounts to 96 hours in total. But, how did the authors plot this high-dimensional data? How did they make sense of it? In fact, it has been done by applying *PCA analysis* and *k-means clustering*.

And what is *PCA analysis* and *k-means clustering*? To begin with, the data can be thought as a collection of twelve matrices of dimension $2000 \times N$ with $N := n_P + n_X + n_E$. Indeed, if we denote

$$\boldsymbol{cell}_i(t_\mu) = \big(TF^P_{i1}(t_\mu), ..., TF^P_{in_P}(t_\mu), TF^X_{i1}(t_\mu), ..., TF^X_{in_X}(t_\mu), TF^E_{i1}(t_\mu), ..., TF^E_{in_E}(t_\mu)\big) \tag{1.138}$$

as the vector representing the transcriptional profile of the cell $\boldsymbol{cell}_i$ for $1 \leq i \leq 2000$, then one can define

$$\boldsymbol{cell}_i(t_\mu)[j] := \begin{cases} TF^P_{ij}(t_\mu), \text{ if } 1 \leq j \leq n_P \\ TF^X_{i(j-n_P)}(t_\mu), \text{ if } 1 + n_P \leq j \leq n_P + n_X \\ TF^E_{i(j-n_P-n_X)}(t_\mu), \text{ if } 1 + n_P + n_X \leq j \leq n_P + n_X + n_E \end{cases} \tag{1.139}$$

and

$$G^{(data)}(t_\mu) := (\boldsymbol{cell}_i(t_\mu)[j])_{i,j} \tag{1.140}$$

in which $\boldsymbol{cell}_i(t_\mu)[j]$ represents the j-th component of the vector $\boldsymbol{cell}_i(t_\mu)$ for all $1 \leq i \leq 2000$ and for all $1 \leq j \leq n_P + n_X + n_E$ at each time point $t_\mu \in \{0h, 12h, 24h, \ldots, 72h, 84h, 96h\}$.

Further, one can plot the gene expression pattern of $cell_2$ against the one of $cell_1$ as seen in Figure 1.28, in which each dot on the scatter plot represents the expression of a gene [transcription factor]. On the scatter plot on the right hand side of Figure 1.28, one sees a regression line showing that cell$_1$ and cell$_2$ are positively correlated. The respective regression line gives rise to the the first principal component PC_1-the one with the most variation-with respect to $cell_1$ and $cell_2$. Moreover, an orthogonal line thereto forms the second principal component PC_2-the one with the second of most variation-with respect to $cell_1$ and $cell_2$.

So, one sees that genes located close to the regression line and more grouped in the ends thereof have the most influence upon the variation in the first principal component PC_1. Similarly, one sees that genes located close to the line perpendicular to the regression line and more grouped together in the ends thereof have the most influence upon the variation in the second principal component PC_2.

But, how can we construct on the basis of the actual data a scatter plot on which each dot stands for a cell? In fact, drawing upon the influence of each gene on the variation in $PC1$ and $PC2$, at each time point t_μ, one can assign (see for more details *Abdi et al.* [1]) to each gene a weight [loading] in $PC1$ and $PC2$, that is,

$$\left[TF_r^k(t_\mu)\right]_{PC1} \tag{1.141}$$

and

$$\left[TF_r^k(t_\mu)\right]_{PC2} \tag{1.142}$$

with $k \in \{P, X, E\}$ and $1 \leq r \leq n_P + n_X + n_E$, which, in turn, gives rise to the eigenvectors $e_{PC1}(t_\mu)$ and $e_{PC2}(t_\mu)$ whose components are given by

$$e_{PC1}(t_\mu)[r] = \begin{cases} \left[TF_r^P(t_\mu)\right]_{PC1}, \text{ if } 1 \leq r \leq n_P \\ \left[TF_{r-n_P}^X(t_\mu)\right]_{PC1}, \text{ if } 1 + n_P \leq r \leq n_P + n_X \\ \left[TF_{r-n_P-n_X}^E(t_\mu)\right]_{PC1}, \text{ if } 1 + n_P + n_X \leq r \leq n_P + n_X + n_E \end{cases} \tag{1.143}$$

and by

$$e_{PC2}(t_\mu)[r] = \begin{cases} \left[TF_r^P(t_\mu)\right]_{PC2}, \text{ if } 1 \leq r \leq n_P \\ \left[TF_{r-n_P}^X(t_\mu)\right]_{PC2}, \text{ if } 1 + n_P \leq r \leq n_P + n_X \\ \left[TF_{r-n_P-n_X}^E(t_\mu)\right]_{PC2}, \text{ if } 1 + n_P + n_X \leq r \leq n_P + n_X + n_E \end{cases} \tag{1.144}$$

for all $1 \leq r \leq n_P + n_X + n_E$. Now, for each cell $\boldsymbol{cell}_i$ with $1 \leq i \leq 2000$, one can compute its principal components. In fact, if we draw upon (1.139), (1.143), and (1.144) then one can define

$$cell_i^{PC1}(t_\mu) := \sum_{r=1}^{n_P+n_X+n_E} \boldsymbol{cell}_i(t_\mu)[r] \left[TF_r^k(t_\mu)\right]_{PC1} \tag{1.145}$$

and

$$cell_i^{PC2}(t_\mu) := \sum_{r=1}^{n_P+n_X+n_E} \boldsymbol{cell}_i(t_\mu)[r] \left[TF_r^k(t_\mu)\right]_{PC2}. \tag{1.146}$$

Thus, one can write

$$\boldsymbol{cell}_i(t_\mu) = \left(cell_i^{PC1}(t_\mu), cell_i^{PC2}(t_\mu)\right) \tag{1.147}$$

for $1 \leq i \leq 2000$ and for each time point $t_\mu \in \{0h, 12h, \ldots, 84h, 96h\}$. Hence, one can reduce the *high-dimensional data* carried by the matrix G^{data}, defined in (1.140), to the 2-dimensional data in (1.147) as shown in Figure 1.31.

But, how can one then cluster cells at each time point $t_\mu \in \{0h, 12h, \ldots, 84h, 96h\}$ with respect to the 2-dimensional data in (1.147)? In fact, it can be done by applying *k-means clustering* [47, p. 289-294]. If we want to concisely summarize the latter method then we can start saying that k stands for the number of clusters that one wants to find. So, suppose that one wants to find two clusters $[k = 2]$ at $t_\mu = 96h$ then one chooses at random for two different data points $\boldsymbol{cell}_1(t_\mu)$ and $\boldsymbol{cell}_2(t_\mu)$, or better,

$$\boldsymbol{cell}_{i_1}(t_\mu) = \left(cell_{i_1}^{PC1}(t_\mu), cell_{i_1}^{PC2}(t_\mu)\right) \tag{1.148}$$

and

$$\boldsymbol{cell}_{i_2}(t_\mu) = \left(cell_{i_2}^{PC1}(t_\mu), cell_{i_2}^{PC2}(t_\mu)\right). \tag{1.149}$$

with $1 \leq i_1, i_2 \leq 2000$. Subsequently, for all $1 \leq q \leq 2000$ with $q \notin \{i_1, i_2\}$, one computes the distances

$$d_{q,i_2} := \sqrt{\left(cell_q^{PC1}(t_\mu) - cell_{i_2}^{PC1}(t_\mu)\right)^2 + \left(cell_q^{PC2}(t_\mu) - cell_{i_2}^{PC2}(t_\mu)\right)^2} \tag{1.150}$$

and

$$d_{q,i_1}\sqrt{\left(cell_q^{PC1}(t_\mu) - cell_{i_1}^{PC1}(t_\mu)\right)^2 + \left(cell_q^{PC2}(t_\mu) - cell_{i_1}^{PC2}(t_\mu)\right)^2}. \tag{1.151}$$

So, if it is true that

$$d_{q,i_2} < d_{q,i_1}$$

then we form the set

$$\{\boldsymbol{cell}_{i_2}(t_\mu), \boldsymbol{cell}_q(t_\mu)\},$$

but instead, If it is true that

$$d_{q,i_1} < d_{q,i_2}$$

then we form the set

$$\{\boldsymbol{cell}_{i_1}(t_\mu), \boldsymbol{cell}_q(t_\mu)\}.$$

Hence, we will have formed two clusters with $\boldsymbol{cell}_{i_2}(t_\mu)$ and $\boldsymbol{cell}_{i_2}(t_\mu)$ being the "leading representatives". What to do then? One computes, in each cluster, the *mean* in each principal component forming two new representatives of each cluster. Now, one computes the distance of each data point to each of the new representatives. As before, we joint the respective data point to the representative to which the minimum distance is assigned. So, we will have formed two new clusters for which we must compute the variance. Upon the computation of the variance, we repeat

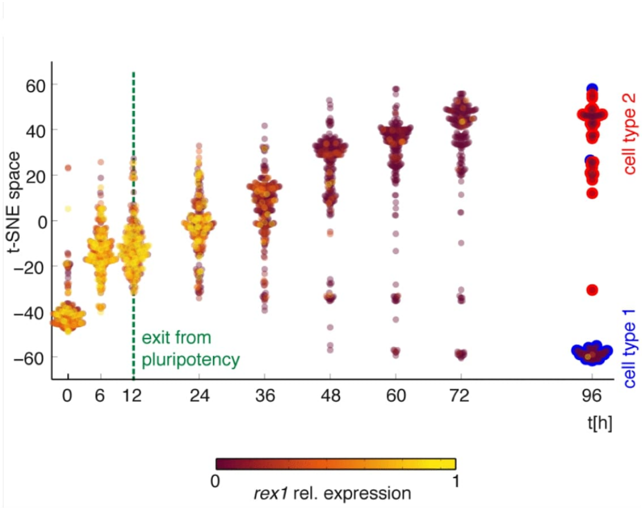


*Figure 1.29: Figure taken from [86]. Here, one sees the reduction of the high-dimensional single cell RNA-seq data to one dimensional tSNE-plot. Each data point corresponds to one single cell. The color of each data point corresponds to the expression of Rex*1 *(a pluripotency marker) in relation to the maximum expression across cells. So, as reported in [86], one sees that after* $24h$ *of retinoic acid exposition, the initial cluster splits up in two groups of cells that robustly clumped up together till the end of day* 4 *[*$96h$*]. Furthermore, the one- dimensional tSNE-data strongly suggests that the exit from pluripotency started between* $12h$ *and* $24h$ *and ended somewhere between* $48h$ *and* $60h$*.*

the whole procedure over and over again until we find a "satisfactory variance". But, how to determine the later? The latter depends upon the data analyst and her knowledge about the kind of data being analyzed. In Figure 1.31, one sees the scatter plots at each time point of the *in vitro differentiation* of *mESCs* resulting from *PCA* and *k-means clustering analysis* reported in [86].

Now, can one determine "key genes" from those scatter plots in Figure 1.31? In fact, in order to find the "key genes" of the *blue cluster* in Figure 1.31 for time point $t_\mu = 96h$, one must find the genes that have the most influence on the first principal component $PC1$, that is, the ones with the highest $PC1$ weights[loadings]. As reported in [86], the authors found that the respective set of genes

$$\{sparc, col4a1, lama1, dab2\}$$

consists of well-known markers of extraembrionic endoderm [XEN]. Similarly, in order to find the "key genes" of the *red cluster* in Figure 1.31 for time point $t_\mu = 96h$, one must find the genes that have the most influence on the second principal component $PC2$, that is, the ones with the highest $PC2$ weights[loadings]. As reported in [86], the authors found that the respective set of genes

$$\{prtg, mdk, fabp5, cd24\}$$

consists of well-known markers of ectoderm [Ecto]. Upon doing so, *Semrau et al.* in [86] conveniently called the cells forming the *blue* and red clusters shown in Figure 1.31, Xen-like cells and Ecto-like cells respectively. However, how did the authors in [86] determine the exit from pluripotency? In fact, it was done by applying a method which allows for the reduction of the high-dimensional data embodied in the matrix defined in (1.140) to 1-dimensional data points. In fact, as one can see in the scatter plot in Figure 1.29, the beginning of the exit from pluripotency lies somewhere between $12h$ and $24h$.

In order to understand the conditions under which Semrau *et al* in [86] performed the respective measurements, that is, the external signals to which *mESC-like* were subject during the experiments, it is fundamental to understanding how those signals ensure the stabilization of the *pluripotency network*. So, in order to build a mental model thereof, one can perhaps draw upon Dunn *et al* in [16], which, under 23 different culture conditions, analyzed the cross-regulatory interaction of 17 *transcription factors* [*Esrrb, Klf2, Klf4, Nanog, Oct4, Tbx3, Tfcp2l1, Stat3, Gbx2, Sall4, Tcf3, Sox2, Klf5, Nr0b1, Mbd3, Mi2b, Rex1*] involved in the maintenance of the pluripotent state of *mouse embryonic stem cells (mESCs)*. In fact, Dunn *et al* in [16] used the Pearson coefficient to measure the correlation between any two *transcription factors* of the aforesaid set. So, it allowed them to infer therefrom the sort of interaction (activation or suppression) between any pair of *TFs* comprising the hypothesized pluripotency network.

In so doing, Dunn *et al* in [16] arrived at a metamodel[27] of the pluripotency network. Next, they computationally unveiled a subset of sub-metamodels of the respective metamodel that satisfy all the 23 culture conditions. Further, they stipulated a suitable threshold for the Pearson correlation coefficient (0.792) so as to find the minimal set of interactions sufficient to satisfy all the 23 different culture conditions. Having done that, they found 70 possible interactions, from which, 11 were present in all the corresponding sub-metamodels. The latter interactions were conjectured as essential ones. Furthermore, they also found that 5 *transcriptional factors* [*Klf5, Rex1, Mbd3, Mi2b, Nr0b1*] are not necessary to satisfy all the 23 different culture conditions, so they were excluded. Therefore, they could avail themselves of the aforementioned knowledge to arrive at the minimal sub-metamodel of 16 interactions (functional but not necessarily direct) and 12 components [*Esrrb, Klf2, Klf4, Nanog, Oct4, Tbx3, Tfcp2l1, Stat3, Gbx2, Sall4, Tcf3, Sox2*] sufficient to simultaneously satisfy all the culture conditions, as shown in Figure 1.30.

Now, let P, E, and X denote the variables representing the changes in the *expression* of core *transcription factors (TFs)* of the corresponding cell types, or better, the *transcriptional profile* of *mESC-like, Ectoderm-like*, and *XEN-like*, respectively. But, what do we actually mean with *transcriptional profile*? In fact, intuitively, for $n(P), n(X), n(E) \in \mathbb{N}_{>0}$, we mean that the variables P, E, and X can be thought as *fuzzy network vectors*, that is,

$$\begin{cases} P = \left(TF_1^P, TF_2^P, TF_1^P, ..., TF_{n(P)}^P\right), \\ X = \left(TF_1^X, TF_2^X, TF_1^X, ..., TF_{n(X)}^X\right), \\ E = \left(TF_1^E, TF_2^E, TF_1^E, ..., TF_{n(E)}^E\right), \end{cases}$$

with

$$0 \leq TF_{i_P}^P \leq 1, \tag{1.152}$$

[27] A model of the hypothesized model.

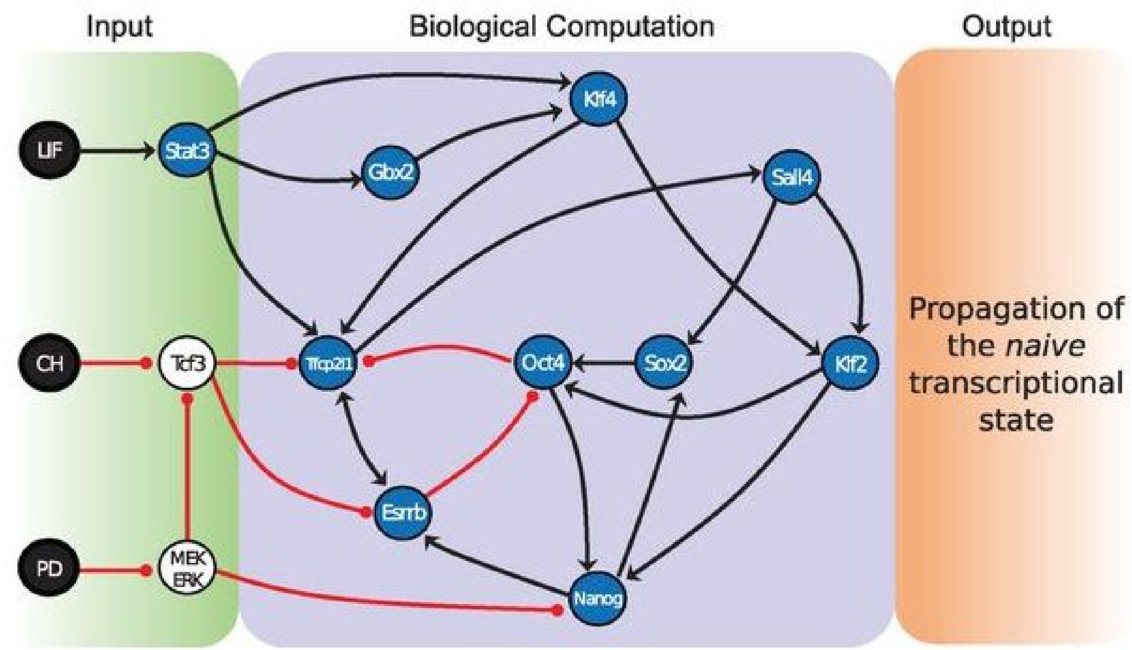


Figure 1.30: Figure taken from [16]. Here, one sees the minimal sub-metamodel of 16 *interactions (functional but not necessarily direct) and* 12 *components sufficient to simultaneously satisfy all the culture conditions reported in [16]. Moreover, one has that LIF, CH, and PD are abbreviations for leukemia inhibitory factor, Chiron99021, and PD0325901 respectively. As we see in the figure, the output of a combination of the later signaling inputs is the maintenance of the pluripotent state [the naive transcriptional state]. Regarding the interactions, an arrow in red with a small circle placed at one end betokens suppression, whilst, an arrow in black with a small pile at one end symbolizes activation. Consistently, an arrow in black with a small pile at both ends represents mutual activation.*

for $i_P \in \{1, 2, ..., n(P)\}$, and

$$0 \leq TF^X_{i_X} \leq 1, \tag{1.153}$$

for $i_X \in \{1, 2, ..., n(X)\}$, and

$$0 \leq TF^P_{i_E} \leq 1, \tag{1.154}$$

for $i_E \in \{1, 2, ..., n(E)\}$, representing the expression of core *transcription factors* [RNA-seq data] characterizing the *pluripotency network*, and the *XEN* and *Ectoderm* networks respectively.

Regarding the reported *observations* in [86], one has that the experiment performed by adding *PD0325901*, *Chiron99021* and *LIF* without *retinoic acid (RA)* favoured the *pluripotency network*, which we denote by the observation

$$O_P^{(CHIR^+, PD^+, LIF^+, RA^-)}, \tag{1.155}$$

and as reported in [86], one has that, the *transcriptional expression profile* of the cells concerning the *observation* (1.155), were very similar to the one characterizing

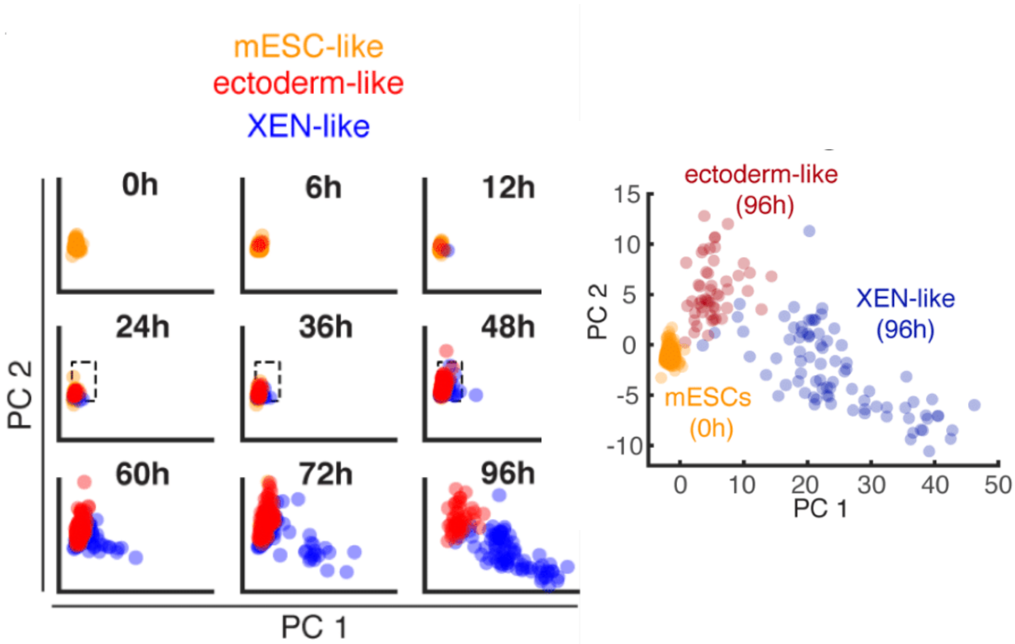


Figure 1.31: Figure taken from [86]. Here, one sees the result of PCA analysis together with k-means clustering for single-cell RNA-seq data resulting from the experiments of mESCs exposed to retinoic acid for 96*h. Initially, at time point* 0*h, one has a robust cluster of mESCs that around time point* 12*h begins to split up, which, in turn, demarcates the beginning of the exit from pluripotency. At the time point* 96*h, one sees that the cells belong to either of the two clusters in red [Ectoderm-like cells] or in blue [XEN-like cells], that is, the mESCs have made a decision between the two types of cells.*

the $E4.5$ *epiblast*[28] in *vivo* (see [70]); while adding neither of the latter ones favoured *Ectoderm-like cells*, which we denote by

$$O_E^{(CHIR^-,PD^-,LIF^-,RA^-)}, \tag{1.156}$$

as seen in Figure 1.32 (b). As reported in [86], the *transcriptional expression profile* of the cells concerning the *observation* (1.156), were indeed characterized by *neuroectodermal markers*. Further, adding *retinoic acid (RA)* without *PD0325901*, *Chiron99021* and *LIF* resulted in *symmetry breaking*, that is, a sub-population consisting of *extra-embryonic endoderm-like cells (XEN-like)*

$$O_X^{(CHIR^-,PD^-,LIF^-,RA^+)}, \tag{1.157}$$

with *transcriptional expression profile* being characterized by *extraembryonic endoderm markers*, and another sub-population consisting of *Ectoderm-like cells* (*Ectoderm-like*),

$$O_E^{(CHIR^-,PD^-,LIF^-,RA^+)}, \tag{1.158}$$

[28] If we want to be consistent with Section 1.4, then one has that $E4.5$ *epiblasts*, as reported in [70], cannot be stem cells [embryoblasts]. However, provided that $E4.5$ *epiblasts* as described in [70], will differentiate further and give rise to the three germ layers, then one can understand them as pluripotent cells. Anyway, the author of this thesis is not entitled to make any further judgment as to the notions of stem cells and pluripotency, what he tries to do is to prevent the flow of reasoning in this thesis from crossing the sharp borders of formal logic.

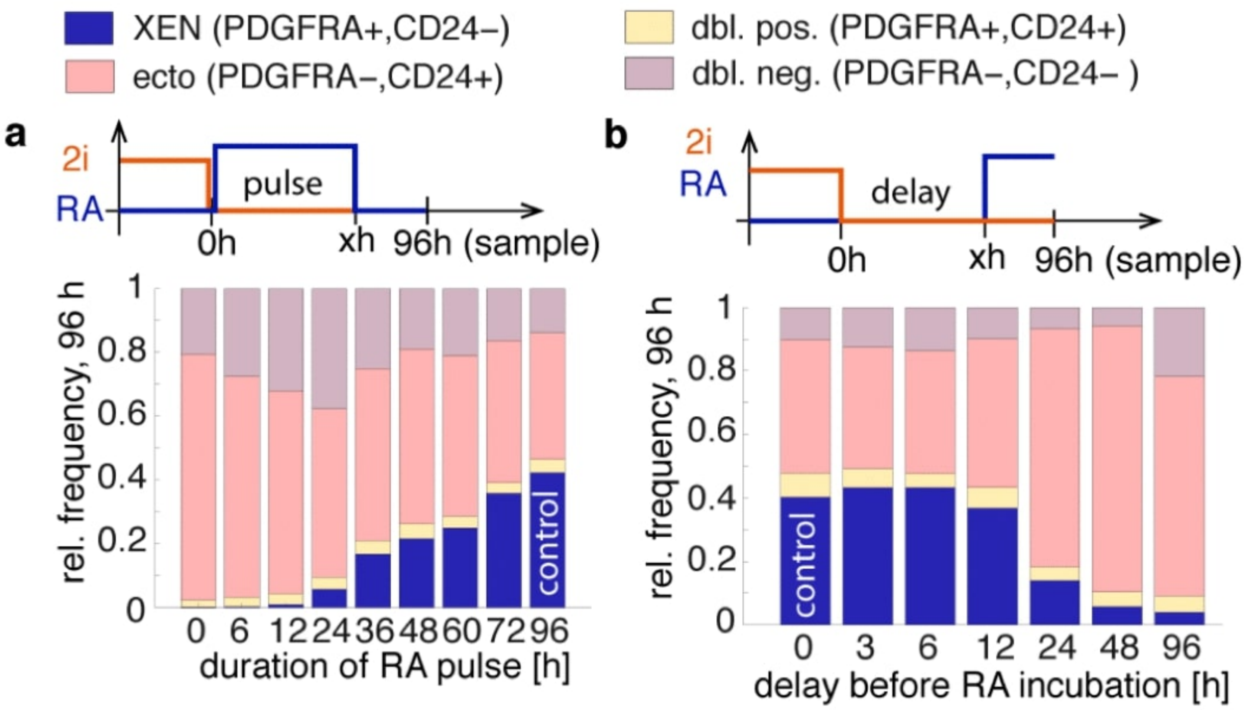


Figure 1.32: Figure taken from [86]. Here, one sees the frequency of cells either expressing $PDGFRA^+$, a marker for XEN cells, and suppressing $CD24^-$, a marker for Ecto cells, or expressing $CD24^+$ and suppressing $PDGFRA^-$; as well as cells either expressing both markers or repressing them. (a) When exposing mESCS cells to retinoic acid RA without adding LIF, PD, and $CHIR$, the histogram informs that, at time point 96h, the majority of the cells form two clusters, equally distributed, being characterized by the suppression and expression of one of the two markers: $PDGFRA$ and $CD24$. (b) When performing an experiment without LIF, PD, and $CHIR$, the histogram informs that the majority of the cells expresses $CD24^+$ and suppresses $PDFRA^-$.

as seen in Figures 1.32(a) and 1.31. Hence, the latter observation is conveniently denoted by

$$O_{X,E}^{(CHIR^-,PD^-,LIF^-,RA^+)}. \tag{1.159}$$

However, when performing an experiment by adding the *MEK inhibitor PD0325901* and *retinoic acid (RA)*, without *Chiron99021* and *LIF*, resulted in an intriguing outcome. In fact, there was a significant loss of *Extraembryonic endoderm-like cells* whereas the sub-population of *Ectoderm-like cells* was comparable with the latter two experiments as shown in Figure 1.33. Moreover, there was an increase of the number of cells with no *lineage marker* as seen in Figure 1.33. The latter has been hypothesized to be the *jammed state*, whose description is suitably worded in a quotation from Dr. Stefan Semrau in [85]:

“All in all, our observations showed that MEK inhibition suppressed the XEN lineage and instead resulted in a jammed state: a stable, strongly biased, undifferentiated state, transcriptionally close but functionally distinct from the ground state pluripotency.”

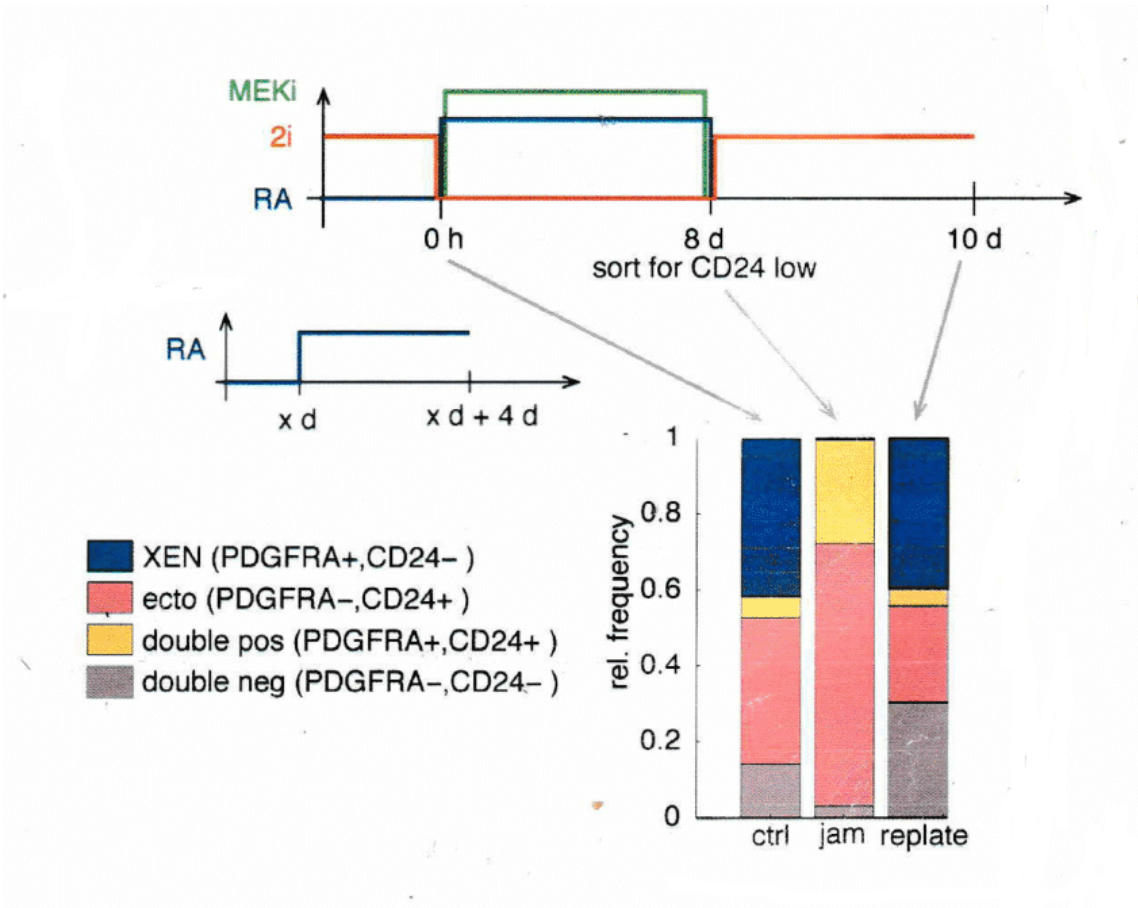


Figure 1.33: Figure taken from [86]. Here, one sees in the histogram the frequency of cells by performing an experiment with the MEK inhibitor PD0325901 and retinoic acid (RA), without Chiron99021 and LIF. So, one sees two significant population of cells, one expressing $CD24^+$ and suppressing $PDGFRA^-$ [Ecto cells], and the other one expressing both of the markers, that is, the hypothesized jammed population.

Thereby, we suitably denote the latter observation by

$$O_{J_E,E}^{(CHIR^-,PD^+,LIF^-,RA^+)}, \tag{1.160}$$

with J_E symbolizing the Jammed state.

Subsequently, as described in [85], they differentiated those *jammed cells* with *retinoic acid (RA)*, without *PD0325901*, *Chiron99021* and *LIF*, and they observed a strong bias toward *Ectoderm-like cells*. The later *observation* of the respective experiment can be conveniently symbolized as

$$O_{J_E}^{(CHIR^-,PD^+,LIF^-,RA^+)} \xrightarrow{PD0325901^-,\ RA^+} O_E^{(CHIR^-,PD^-,LIF^-,RA^+)}, \tag{1.161}$$

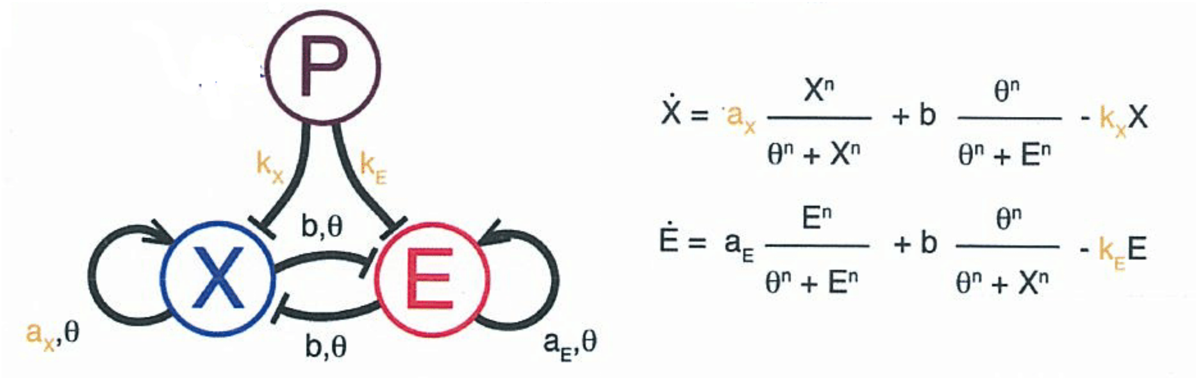


Figure 1.34: Cartoon taken from [85]. On the left side hereof, one sees a GRN initially proposed by Dr. Semrau with the pluripotency network repressing the Ecto and the Xen networks, while the Ecto and XEN networks mutually repress each other. On the right side hereof, one has the mathematical representation of the proposed GRN being given by the system of differential equation, with a_X and a_E being the respective autoactivation parameters, b being the mutual repression, k_X and k_E being the corresponding degradation rates, θ being the value at which each Hill function in the respective differential equations reaches half of its maximum value, and n represents the sigmoidicity at $X = \theta$ and at $E = theta$, as argued in [80]. Here, the parameters a_X, k_X, and k_E in orange are varied between conditions while the other ones are fixed.

and, for the sake of readability, let

$$\begin{aligned} &O_P^{(CHIR^+,PD^+,LIF^+,RA^-)},\\ &O_E^{(CHIR^-,PD^-,LIF^-,RA^-)},\\ &O_{X,E}^{(CHIR^-,PD^-,LIF^-,RA^+)},\\ &O_{J_E,E}^{(CHIR^-,PD^+,LIF^-,RA^+)},\\ O_{J_E,E}^{(CHIR^-,PD^+,LIF^-,RA^+)} \xrightarrow{PD0325901^-,\ RA^+} &O_E^{(CHIR^-,PD^-,LIF^-,RA^+)}, \end{aligned} \tag{1.162}$$

be denoted by

$$\mathcal{O}_{\mathcal{TS}},$$

meaning the set of all the *observations* of the *target system*, that is, "Retinoic acid driven mouse embryonic-like stem cells (mESC-like) differentiation in the presence of *PD0325901* or *Chiron99021* or LIF(Lekemia inhibitory factor), or in the absence of *PD0325901* or *Chiron99021* or LIF(Lekemia inhibitory factor)" as reported in [86].

1.5.2 Huang's model

Having done that, Dr. Stefan Semrau wondered whether or not he could come up with a *conceptual mechanism in silico* with which, notably, he could explain the *jammed state*, that is, the observation (1.160), as well as the other ones, i.e., the observations (1.155), (1.156), (1.157), (1.158) and (1.161).

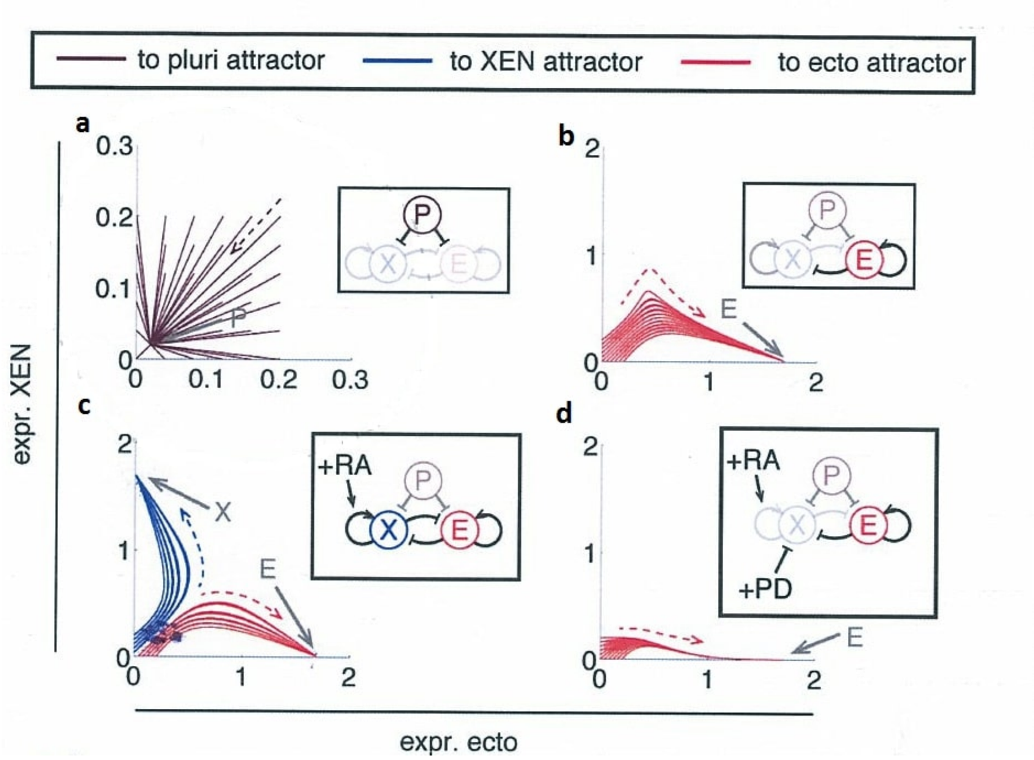


Figure 1.35: Cartoon taken from [85]. Here, one sees the results of the numerical experiments performed by Dr. Stefan Semrau when using Euler forward in Huang's model. The choice for the parameters were: $n = 4$, $\theta = 0.5$, $a_E = 0.7$; $a_X = [0, 0, 0.7, 0.7]$, $k_E = [50, 1, 1, 1]$, $k_X = [50, 1, 1, 5]$, $b = 1$. *As one sees, the model cannot generate an equilibrium that resembles the Jammed state.*

Toward this end, Dr. Semrau extended the *GRN model* proposed by Huang *et al* in [95] to model *lineage specification*. As we see in Figure 1.34, he initially considered an extension thereof in which the pluripotency network suppresses the Ecto and the Xen auto-activating transcriptional networks that mutually repress each other.

However, the mathematical representation for the respective GRN was the same one proposed by Huang *et al* in [95], that is, a model based on *Hill-function type interaction kinetics* as seen in Figure 1.34. Moreover, if we invoke the current consensus that "TFs regulate gene expression in a switch-like fashion", as stated in [80, p. 95], then one has that the corresponding regulation is thought to be highly sigmoidal. So, if this is the case then Huang's model is in line with that.

But, how did he model the conditions with respect to Huang's model? In fact, k_X and k_E model the maintenance of the pluripotency network. So, if the degradation rates k_X and k_E are sufficiently high-by means of treating mESCs with *PD0325901*, *Chiron99021* and *LIF*-then it potentially results in a low population of Xen and Ecto cells, so one has that the pluripotent state is indeed characterized by a steady state with sufficiently low values of the variables X and E.

Further, one has that exposition to retinoic acid *RA* was modelled by the autoactivation parameter a_X of the Xen network X. This means that a_X ought to be relatively higher to represent the treatment of mESCs with RA. Lastly, MEK inhibition was modelled by the degradation rate k_X of the Xen network X, which means that k_X ought to be relatively high which, in turn, intuitively, must result in a equilibrium with low expression of X and high expression of E as seen in Figure 1.35. But, the latter is indeed the Ecto equilibrium so the model cannot generate an equilibrium which might be interpreted as the Jammed state whatsoever. So, Dr. Stefan Semrau realized that he needed another extension.

1.5.3 Semrau-Huang's model

Due to the impossibility of Huang's model generating the Jammed state, Dr. Semrau has then proposed another GRN seen in Figure 1.36. But, how did he model the conditions with respect to Semrau-Huang's model? In fact, the autoactivation a_P of the variable P models the maintenance of the pluripotency network. So, if a_P is sufficiently high-by means of treating mESCs with *PD0325901*, *Chiron99021* and *LIF*-then it potentially results in a high expression of the variable P with a low expression for the variables X and E. The latter state indeed resembles the pluripotent state.

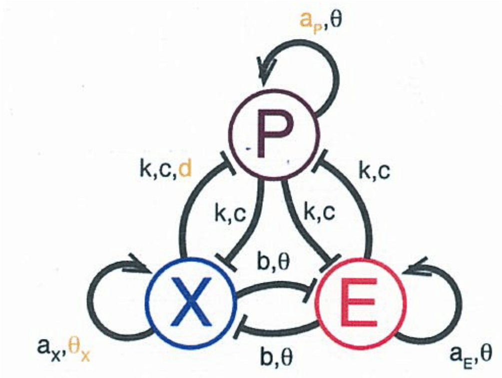
Further, one has that exposition to retinoic acid RA was modelled by the parameter θ_X of the Xen network X. This means that θ_X ought to be relatively lower to represent the treatment of mESCs with RA. Lastly, MEK inhibition was implicitly modelled by the dimensionless parameter d, which, in fact, models the suppression of Xen cells. This means that d should be sufficiently low.

As we see in Figure 1.37, the models seems to suit the purpose seeing that it generates a Jammed equilibrium. However, the behaviour of the latter one is inconsistent with observation $(1.162)_{4,5}$. In fact, if we switch from a sufficiently low d, for which we presuppose the existence of the Jammed state, to a sufficiently high d and if we subsequently choose for a sufficient low value of θ_X then one must have that the Jammed state will bifurcate. The latter can be thought as the action of removing the Meki inhibitor from the Jammed cells and adding subsequently retinoic acid RA to them.

So, from this numerical experiment of Dr. Semrau, actually for that specific choice of parameters, the model tells him that all the trajectories go toward the Xen equilibrium. Provided that all the cells differentiated further into a robust population of Ecto-like cells as reported in [86], one has that the respective numerical experiment contradicts the observation $(1.162)_{4,5}$. This drawback of this specific choice of parameters raises the question of whether or not the model suffices as a conception mechanism to explain the performed experiments, which, in turn, demands a suitable evaluation.

But, what do we mean with evaluating a phenomenological mathematical model? What do we exactly mean with finding the observations in the model? What is the role of the mathematician in that? What is the essence of the meaningfulness of the parameters of the model? Given that it is inconceivable to test all the feasible parameter settings, we are in need of deep thinking, that is, a suitable conceptual framework to elucidate ontological and epistemological aspects of the respective questions.

In Chapter II, we shall see how we can construct, on the basis of analytical thought, a systematic evaluation of phenomenological mathematical models that will be applied to Huang's model and to Semrau-Huang's model. Before doing that, we need to bear in mind the following assertions on Semrau-Huang's model which



$$\dot{P} = a_P \frac{P}{\theta + P} - kP(1 + c(E + dX))$$

$$\dot{X} = a_X \frac{X^n}{\theta_X{}^n + X^n} + b \frac{\theta^n}{\theta^n + E^n} - kX(1 + cP)$$

$$\dot{E} = a_E \frac{E^n}{\theta^n + E^n} + b \frac{\theta^n}{\theta^n + X^n} - kE(1 + cP)$$

Figure 1.36: Cartoon taken from [85]. On the left side hereof, one sees a GRN proposed by Dr. Semrau with the pluripotency network mutually repressing the Ecto and the Xen networks, as well as the Ecto and XEN networks mutually repressing each other. On the right side hereof, one has the mathematical representation of the proposed GRN being given by the respective system of differential equation. In contrast to the previous model, changes in the transcriptional profile of the pluripotency network is being explicitly modelled by the inclusion of a dynamical equation for the variable P. As for the parameters, one has that a_P represents the autoactivation of the pluripotency network thus modelling the maintenance of the pluripotent state; θ_X models the addition of retinoic acid RA; while Mek inhibition is being modelled by the parameter d. So, the parameters a_P, θ_X, and d in orange are varied between conditions while the other ones are fixed. How can we make sense of parameter c? So far, it can be regarded as a presupposition for the corresponding system of differential equation to make sense so it does not having any particular intention behind it.

will be invoked further in Chapter 4.

Assertion 1.5.1:

For a sufficiently high autoactivation a_P of the variable P, representing the changes in the expression of the transcriptional profile of the *pluripotency network*, one has that Semrau-Huang's model yields a stable equilibrium which resembles *pluripotent mouse embryonic-like stem cells.*

Assertion 1.5.2:

For a sufficiently low autoactivation a_P of the variable P, representing the changes in the expression of the transcriptional profile of the *pluripotency network*, one has that Semrau-Huang's model yields a stable equilibrium which resembles *Ectoderm like cells.*

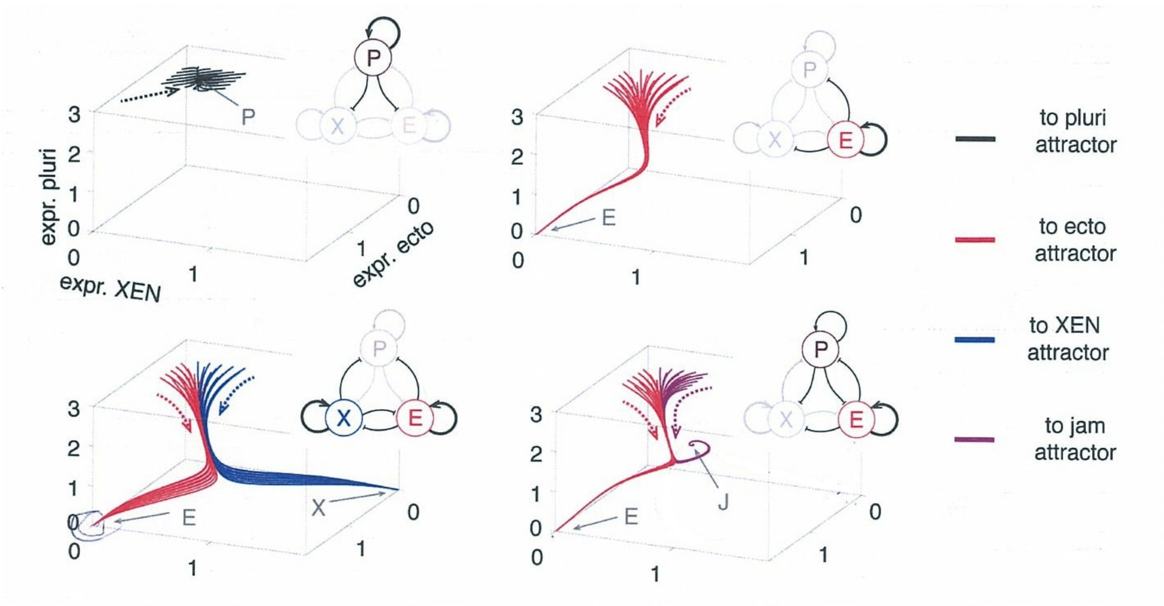


Figure 1.37: Cartoon taken from [85]. Here, one sees the results of the numerical experiments performed by Dr. Stefan Semrau when using Euler forward in Semrau-Huang's model. The choice for the parameters were: $n = 4$, $\theta = 0.5$, $\theta_X = [0.8, 0.8, 0.5, 0.5]$, $a_P = [5, 2, 2, 2]$, $a_E = 0.8$; $a_X = 0.8$, $k = 1$, $b = 1$, $c = 2$, $d = [1, 1, 1, 0]$. *As one sees, the model can generate an equilibrium that resembles the Jammed state, see lower right panel, but its behaviour in inconsistent with observation* $(1.162)_5$ *seeing that upon destabilization of the Jammed state, one has that all the trajectories go toward the Xen state. That is a harmful property of this choice of parameters. However, can we find a suitable choice of parameters consistent with all the observations and that suits the purpose?*

Assertion 1.5.3:

For a sufficiently low autoactivation a_P of the variable P, representing the changes in the expression of the transcriptional profile of the *pluripotency network*, and for a sufficiently low θ_X, which model the addition of retinoic acid RA, one has that Semrau-Huang's model yields two stable equilibria which resembles *Ectoderm like cells* and *Endoderm like cells*.

Assertion 1.5.4:

For a sufficiently low autoactivation a_P of the variable P, representing the changes in the expression of the transcriptional profile of the *pluripotency network*, for a sufficiently low θ_X, modelling the addition of retinoic acid RA, and for a sufficiently low d, modelling the suppression of Xen population, one has that Semrau-Huang's model yields a stable equilibrium which resembles the *Jammed cells* and another one which resembles the *Ectoderm like cells*.

Assertion 1.5.5:

For a sufficiently low autoactivation of the variable P, representing the changes in the expression of the transcriptional profile of the *pluripotency network*, if we presuppose that the Jammed- and the Ecto-state coexist for a choice of the parameters consistent with the intentions of the modelling agent then, for a sufficiently "high" d and for a sufficiently low θ_X, one has that the respective Jammed equilibrium of Semrau-Huang's model bifurcates, which culminates in all the trajectories going toward the Ecto equilibrium.

This thesis is further organized as follows. After having argued, in Chapter 1, that the notion of the order of conceptual priority is crucial in the apprehension of gene expression, in Chapter 2, we shall delve into the philosophy of logic by exploring the concepts of judgment from a Fregean and a Kantian perspective. Furthermore, we shall tell how the elucidations of Dr. Maria van der Schaar, as to the role of the first person in logic, reveals a systematic evaluation of a phenomenological mathematical model. Along with that, we explore what the notion of primitive concept has to do with that. In Chapter 3, we analyse Huang's model by applying the methodology introduced in Chapter 2. In that chapter, our main goal is to find the phase-portrait of the model and to demonstrate the (ins)stability of the steady states. In Chapter 4, we apply the methodology to evaluate Semrau Huang's model followed by a concise discussion and a succinct conclusion.

Chapter 2

A systematic approach to the evaluation of phenomenological mathematical models

> The key issue for me is finding the right definitions; finding the right definitions that really capture the essence of some mathematical phenomenon. I often have some vague vision of what I want to understand, but I am often missing the words to say that.
>
> *Dr. Peter Scholze*

Here, we will argue that, regardless the field of study, the concept of primitive notion is fundamental to *epistemology*. In fact, along with the *conceptual order*, it provides a way of defining concepts sequentially. Notwithstanding the importance of *primitive notions* in the realm of concepts as well as in *epistemology*, there is no *act of knowing* "something" without an agent-an *epistemic subject*-to assert, that is, to execute the cognitive activities; and this is closely related to the purpose of this thesis, considering that it requires a better understanding of the role of the *first-person perspective* in the evaluation and analysis of mathematical models. As a result, we will conclude that the *conception order*, the concept of *primitive notion*, the concept of *judgment* and the *first-person perspective*, in the realm of Frege's judgment theory, are fundamentally related to each other concerning the evaluation of phenomenological mathematical models, which, in turn, will unravel a rational strategy to perform such an evaluation.

2.1 The importance of primitive notions in epistemology and the role of the first-person in the evaluation of phenomenological mathematical models

What is so special about the notion of a *gene* proposed by Gerstein et al in [27]? As we have stated earlier, it is a *circular definition* seeing that it is dependent upon the notion of a *DNA* whose definition, in turn, draws on the concept of a *gene*. Having said that, one has that the concept of a *gene* seems to be a *primitive notion*, that is, an undefinable notion; which means that it cannot be reduced to a chain of previously well-defined notions in an independent way. What do we mean with "in an independent way"? That is supposed to mean that each element of such a chain has its meaning not referring back to the concept being defined, that is, the concept of a *gene*. Withal, how can we approach such a *metaphysical question*? Or better, how can we understand such a primitive notion then? In order to address this question, we go back to 1884 when Dr. Gottlob Frege started his logicist programme by publishing '*Die Grundlagen der Arithmetik*'. Therewith, he aimed to establish *logic* as a foundation for *arithmetic*, and, consequently, for *mathematics*. Or equivalently, he claimed that *mathematics* was reducible to *logic* by means of *axiomatization*, that is, all the theorems in *arithmetic* could be logically deduced from a set of axioms[1] with perfect accuracy. The latter properties mean *completeness* and *consistency* respectively. So, though conceptually different from one another, one can say that, in *axiomatization*, axioms play the same role as primitive notions. Nonetheless, in 1931, the logicist programme was forestalled by the publications [28, 29] of Dr. Kurt Gödel in which he demonstrated his famous two *incompleteness theorems*. In fact, he thwarted the logicist programme by showing that it is impossible to reduce *mathematics* to a consistent and complete set of *axioms*, seeing that such an *axiomatic system* cannot decide its own *consistency* and *completeness*.

What should we be considering as essential in his attempt to give a *foundation* to *mathematics* with regard to the scope of this thesis? First of all, with the purpose of understanding some phenomenon, one must be able to reduce it to certain concepts that are somehow known so it is not conceivable that we can keep performing a disentanglement of notions forever. So, if we want to assure that we have knowledge of some phenomenon then there must be notions that cannot be reduced to other ones. To quote from Dr. Eyal Shahar in [89]:

> "Primitive notions are essential in *epistemology* just as they are essential in *mathematics* and *logic*. They are the building blocks of sequential definitions (...). Without them we do not have the foundation upon which we can state the axioms of science, propose theories, and draw inference."

Withal, how can we get access to the meaning of a primitive notion then? Or equivalently, how can we know a primitive concept? In '*Begriffschrift*' [22], Dr.

[1] An *axiom* is a starting point, i.e. a *premise*; which is not supposed to be conceptually confused with *primitive notion*.

Gottlob Frege argues that the fundamental *laws of logic*[2] are unprovable. Yet, their *truth* can somehow be known in the sense that one can acknowledge their *truth*. Actually, "no mediation is needed for anyone to arrive at their *truth* because it should be an immediate understanding", as stated by Dr. Maria van der Schaar in some of her lectures. But, what does Dr. Maria van der Schaar mean with being an immediate understanding? In fact, if we cannot define a primitive notion in terms of previously well-defined concepts then we should be able to clarify its essence, or better, its meaning. Actually, we regard the latter process as a necessary condition to move through any *entailment of notions* with respect to the *conceptual order*. To quote from Dr. Gottlob Frege in [22]:

“Definitions proper must be distinguished from elucidations *'Erläuterungen'*. In the first stages of a science [*Wenn wir die Wissenschaft beginnen*] we cannot avoid the use of ordinary words [*die Wörter unserer Sprache*]. But these words are, for the most part, not really appropriate for scientific purposes, because they are not precise enough and fluctuate in their use. Science needs technical terms that have precise and fixed *Bedeutungen*, and in order to come to an understanding about these *Bedeutungen* and exclude possible misunderstandings, we provide elucidations. Of course in so doing we have again to use ordinary words, and these may display defects similar to those which the elucidations are intended to remove. So it seems that we shall then have to provide further elucidations. Theoretically one will never really achieve one's goal in this way. In practice, however, we do manage to come to an understanding about the *Bedeutungen* of words. Of course we have to be able to count on a meeting of minds [*ein verständnisvollen Entgegenkommen*], on other's guessing what we have in mind. But all this precedes the construction of a system and does not belong within a system. In constructing a system it must be assumed that the words have precise *Bedeutungen* and that we know what they are. Hence we can at this point leave elucidations out of account and turn our attention to the construction of a system.”

So, we can conclude from the latter quotation from Dr. Gottlob Frege that the meaning of primitive notions can only be accessed by *elucidations* [*'Erläuterungen'*], and we presume that it must be driven by *intuition*. But, what kind of intuition then? To answer this question, the author of this thesis would like to quote Dr. Peter Scholze [83]:

“The key issue for me is finding the right definitions; finding the right definitions that really capture the essence of some mathematical phenomenon. I often have some vague vision of what I want to understand, but I am often missing the words to say that.”

In his description, one can say that Dr. Peter Scholze is trying to understand some mathematical phenomenon that is solely taking place in his *mind* so he wants to grab it, or rather, he wants to apprehend the essence thereof. That apprehension can subsequently lead him to the formation of a new notion. So, he has knowledge of the mathematical phenomenon without being able to communicate it immediately. That sort of driving force in acquiring knowledge of such a mathematical

[2] The laws of identity, the excluded middle and the non-contradiction.

phenomenon is regarded as an *intellectual intuition* or *non-empirical intuition*. On the other hand, an elucidation of the concept of a *gene*, or better, of the notion of a *gene* is refined in response to an *empirical process*[3] so it is said to be driven by a *sensible intuition*, or better, an *empirical intuition*.

2.2 From Kant to Frege: the judging agent and the concepts of empirical and intellectual intuition

In which sense should we be understanding the latter notions of intuition? How is it essentially connected with the ultimate scope of this thesis? In order to answer the former question, we draw upon the *Kantian epistemology* as introduced in [42]. To begin with, as succinctly explained in [66], if we regard a *perspective* as any form of *epistemic access* to "something", that is, an *epistemic object*, then we say that "somebody", or better, an *epistemic subject* has always a *first-person perspective* or *first-person* experience of any *phenomenon* including or related to an *epistemic object*. However, if that access is independent upon the *epistemic subject* then we call it a *third-person perspective*. Now, if "somebody" relies on her own *first-person* experiences so as to understand someone else's *first-person* experience toward an *epistemic object* then we reefer to such an *epistemic access* as a *second-order perspective*. What do we mean with having a *first-person* experience of "something"? In fact, it means to be conscious of "something". But, what is necessary for someone to have a *subjective experience* of "something", or equivalently, to be conscious of "something"[4]? It entails that one perceives herself. Hence, being conscious, or equivalently, having *consciousness* involves the existence of a *self-concept*, a *self-identity* or better, an *ego*. Hence, as for the *Kantian epistemology*, the notion of an *ego* precedes the notion of *consciousness*.

As we have exhaustively argued so far, knowing "something" demands a decomposition of the definition of the concept of that "something" in well-known notions or sufficiently well-understood notions[5]. However, in the latter hypothetical decomposition, each well-known notion has been known at a certain point in *time* and *space* so that knowing the *entailment of notions* leading to the apprehension of that "something" necessitates that one has the ability of unifying them. But, what do we mean with "the ability of unifying them"? In fact, if we agree that the *act of knowing* "something" is preceded[6] by the *act of thinking* which, in turn, is preceded by the *act of being conscious of*[7] what one wants to understand, and, more importantly, if we acknowledge that *consciousness* changes direction in *time* and *space*, that is, we are always conscious of "one thing" at a time, then an *epistemic subject* needs to be endowed with built-in capabilities (*consciousness*, *thinking*, *judgment*, and so forth.) underpinning that unifying process in time and *space*, leading her to have knowledge of "something". The latter conception of a set of built-in capabilities defines *Locke's conception of mind* in [52]. Indeed, Locke's notion of *mind* is a

[3]An observation or an experiment.

[4]Here, so far, "something" is thought to be an *epistemic object* outside in the world.

[5]In the case of primitive notions.

[6]Here, preceding means going after the other one in *time* and *space*, but not necessarily at the conceptual level. In fact, as we mentioned earlier, the notion of *knowledge* is primitive. However, the *act of knowing* goes after manifold *acts*.

[7] Or equivalently, the *act of perceiving*.

distinct concept in *Kantian epistemology*.

Now, if it is true that manifold *acts* antecede the *act of knowing* according to a *temporal*, a *spacial* or a *conceptual* hierarchy then the *mind* is a multihierarchical set of built-in capabilities. Hence, the set of all built-in sequences of *acts* with respect to that multihierarchy gives rise to the concept of *cognition*. In fact, for instance, an *epistemic subject* perceives an *epistemic object*, that is, "something", at a certain point in *time* and *space*, followed by thinking about the properties of that "something", which, in turn, is followed by *reasoning* through the relation among those properties leading to a *judgment*, that is, the acknowledgement of the *truth* of a *claim* as to that "something" which, actually, results in having *knowledge* of that "something". Therefore, one can say that the Lockean *mind* is the set of all built-in capabilities of *cognition*. Moreover, consistently, one has that the concept of *mind* is conceptually dependent on the concept of *cognition* which, in turn, is conceptually dependent on the concept of *consciousness*.

So, what is an *empirical intuition* in the *Kantian epistemology*? It is the acquisition of *knowledge* through experience. In fact, it is grounded in the presumption that the *mind* possesses *a priori* forms of intuition[8] (*space* and *time*, *cause* and *effect*, and so forth) that give form to all the experiences of an *epistemic subject*. To quote from Dr. Immanuel Kant in [94]:

“All our knowledge is thus finally subject to time, the formal condition of inner sense. In it they must all be ordered, connected, and brought into relation.”

Clearly, one cannot account for Dr. Peter Scholze's acquisition of *knowledge* on the basis of an *empirical intuition*. Why not? First of all, in his description, the *epistemic object* is in his *mind* by means of an *act of thinking* and is not outside in the world whatsoever. Secondly, being aware of the fact that he is thinking about that "something" entails that he is conscious of his own consciousness which, in turn, demands the acknowledgement of a *self-ego*, or equivalently, an *non-empirical ego*, or better, a *transcendental ego*. In this regard, one has that such an acquisition of *knowledge* describes an *intellectual intuition*. Therefore, the *epistemic subject*, or better, the *judging agent* acknowledging the *truth* of a *thought* or a *claim*, leading to the accretion of *knowledge* can either be seen as an *empirical ego* or as a *non-empirical ego* (*transcendental ego*).

As we have stated in the *abstract* of this thesis, we analyze a *phenomenological model* based on Hill-function type interaction kinetics for *cell differentiation* so as to decide whether or not it adequately defines a *conceptual mechanism* for the performed experiments in [86]. What is a *phenomenological mathematical model*? To answer this question, we refer to the definition given in [75]:

“A traditional definition takes them to be models that only represent observable properties of their targets and refrain from postulating hidden mechanisms and the like.”

But, what is essential in the latter definition? In fact, it is crucial to acknowl-

[8]They are not in the world. So, they cannot be known by experience. Actually, as we have argued, they are built in the *mind* so as to enable any form of *consciousness*, or better, any sort of *first-person* experience.

edge the importance of the *modeling agent* who actually translates some *empirical process* into a *mathematical object*[9]. In that translation, she is subject to her own *first-person* experience so as to mathematically describe observable properties of the *target system*[10]. Hence, it means that each component of that *mathematical object*, as well as their relations in that mathematical representation, has an *intentionality*, which, in turn, rightly places our thoughts in the context of *Husserlian philosophy*. To quote from Dr. David W. Smith in [93]:

"In Husserl's own words, phenomenology is the science of the essence of consciousness(...). What, briefly, is the essence of consciousness? First, every experience, or act of consciousness, is conscious: the subject experiences it, or is aware of performing it. (...) Second, every act of consciousness is a consciousness of something: in perception I see such-and-such, in imagination I imagine such-and-such, in judgment I judge that such-and-such is the case, and so on. This property of consciousness, its being of or about something, Husserl called intentionality. Thus, we say an experience is intentional, or directed (...) toward some object."

As Dr. Edmund Husserl described in the latter quotation, a subjective experience is directed to an object, that is, it carries an intention. But, how are the *conception order*, the concept of a primitive notion, the concept of judgment and the *first-person perspective* essentially connected with the purpose of this thesis? In fact, *judging* is the activity through which we gain *knowledge*. To quote from Dr. Martin Löf in [55]:

"..., namely, to judge is the same as to know, more precisely, to get to know, which is to say that the act of judging is the very act of knowing, and that that which is judged is that which is known, that is, the object of knowledge. And knowing is of course to be taken here as a primitive concept; you can clarify it in various ways, but you cannot reduce it to any other kind of notion. "

As one sees in the latter quotation, Dr. Martin Löf identifies the notion of *knowledge* with the notion of *judgment*. So, the acquisition of knowledge depends on the *judging agent*. To quote from Prof. dr. Maria van der Schaar [81]:

"Logical questions are independent of psychological questions. But, as a theory of validity of inferences and rationality of our judgements, logic relates to what judging agents do. How can logic be objective if it takes its starting-point in the inferences and judgements we make? Is the judging agent perhaps a transcendental or some other kind of ideal subject?"

In order to understand the latter quotation from Dr. Maria van der Schaar, one needs to acknowledge that correct judgements about a *mathematical object* are certainly independent on the *judging agent*. To go further, we draw upon a quotation from Dr. Gottlob Frege in [81]:

[9] In this thesis, it refers to a system of differential equations.

[10] Or equivalently, the *ontological counterpart*. The latter represents the phenomenon being observed, that is, "Retinoic acid driven mouse embryonic stem cells (mESCs) differentiation in the presence of *PD0325901* or *CHIR99021* or LIF(Lekemia inhibitory factor), or in the absence of *PD0325901* or *CHIR99021* or LIF(Lekemia inhibitory factor)" as reported in [86].

“judging(acknowledging as true) is certainly an inner mental process; but that something is true is independent of the knowing agent, is objective.”

So, if we regard such a *phenomenological model* as a mere *mathematical object* then we need to consider the *Fregean* notion of *judgment*, or equivalently, the logical notion, that is, a *judgment* as an acknowledgement of the *truth* of a claim. In fact, the relation between *judgment* and *truth* is an essential elucidation in Frege's logic.

Drawing upon the *Fregean* notation in [22], one has that

$$\vdash A, \tag{2.1}$$

should represent a *judgment* that has been made, in which A is the *judgeable content (assertion)*, and the *judgment stroke* $\vdash$ can be interpreted as an *assertive force*, which together with the content A expresses the *judgment*, or better, the *act of judging* which, in turn, by invoking the elucidations of Dr. Martin Lőf in [55], is equivalent to the *act of knowing*. To quote from Dr. Maria van der Schaar in [81]:

“By putting the judgement stroke in front of an axiom, the agent claims not only that the Thought is true, but that anyone who understands the Thought thereby acknowledges it as true, and is thus entitled to use it as an axiom. By putting the judgement stroke in front of a theorem, the agent claims that anyone who knows the axioms and has made the relevant inference rules evident to himself or herself is entitled to use the theorem as a logical law. These judgements are thus made from a first-person perspective, but they are non-personal at the same time.”

So, acknowledging the truth of a mathematical assertion [*theorem*] requires a *first-person* perspective even though the *truth* is independent upon the *judging agent*. Having said that, one can conclude that Dr. Gottlob Frege vehemently believed that *logic* forms the foundation for all the sciences. To quote from Dr. Maria van der Schaar in [81]:

“Frege's elucidations of primitive terms differ in an important way from the a priori truths given in the phenomenological tradition. Whereas for Brentano and Husserl descriptive psychology or phenomenology is a science that precedes logic, for Frege logic is the foundational science. Primitive notions, such as judgement and truth, can only be understood by relating them to each other in elucidations. Frege's claim that judging is acknowledging the truth of a Gedanke is such an elucidation. What precedes logic is propaedeutic, consisting of elucidations, sharply to be distinguished from a priori truths, and from definitions as well, which do have a role within logic as science.”

So, according to Dr. Maria van der Schaar, elucidations of primitive notions are crucial for anyone who wants to acknowledge the *truth* of a *claim* by herself, but the *truth* of an *assertion* is independent on the *judging agent*. In this regard, the *judging agent* of such a *phenomenological model* can perhaps be understood as a *transcendental ego*, or equivalently, a *non-empirical ego*. However, in [81], Dr. Maria van der Schaar argues that such a *transcendental ego* needed in Frege's logic must differ from the *Kantian transcendental ego*, which, as we introduced earlier, accounts for *self-consciousness*. In fact, if a *judgment* in Frege's logic is an acknowledgment of the *truth* of a *claim* then no property is required from such an *ego* needed to assert. Moreover, *via negativa*, as it cannot be the *Kantian empirical-ego* and does not need to be the *Kantian transcendental ego* then she conjectures that such a *transcendental ego* can be regarded as a presupposition so as to enable the constitution of Frege's logic. But, can such a *ego* be conceived? To quote from David Carr in [8]:

"...A pure ego [transcendental ego] distinct from the empirical one would seem to be an ego without particular properties. Can such a thing exist?...In order even to think of it as a particular existent, don't we have to think of it as possessing properties?

To these questions a traditional answer has run as follows: What I am conscious of in pure apperception is not a particular, and it is for this reason that I do not need to attribute particular properties to it. Also, for this reason, it must be regarded as distinct from the empirical ego. What I am conscious of is not those particular properties which distinguish me from other persons, but rather those general properties which I share with any and all other egos, such as thinking as such. ...Such an ego is "transcendental" because it transcends all particular egos like you and me."

So, in Dr. David Carr's account of the *Kantian transcendental ego*, as far as the author of this thesis can understand it, such an *ego* is already the one presupposed by Prof. dr. Maria van der Schaar in [81]. In fact, Dr. David Carr argues that being conscious of thinking is not distinguishing, but rather reinforcing the *self-ego* transcendence. Nonetheless, we avoid going in the direction of the *metaphysics* of the *Kantian transcendental ego*[11] given that it deviates from the scope of this section. Actually, the author of this thesis regards such an account as a very difficult task and he doubts whether he would have the tools to do that. In sum, so far, he wants to emphasize that if a *phenomenological model* is merely regarded as a *mathematical object* then a logical notion of *judgment*-an acknowledgement of the *truth* of a thought- is required, considering that the correctness of mathematical judgements is objective. But, there is no assertion without an agent so, in this case, as she cannot be *psychological*, that is, *empirical*, then such a *judging agent* can be understood as a *transcendental ego*.

Now, if we consider that such a *phenomenological model* is inherently *psychological*, or equivalently, if we acknowledge that each element of such a *phenomenological model*, as well as their relation, has a specific *intention*. How do those *intentions* manifest themselves in the *model*? In fact, they are supposed to comply with the *epistemic status* of the *ontological counterpart* and, more importantly, to promote the reproduction of the observations thereof. So, we are in need of a psychological notion, or better, an empirical notion of *judgment*. In this regard, a *judgment* is a mental activity in response to an *empirical process*. To quote from Dr. Maria van der Schaar in [81]:

"When studying the act of judgement, one may distinguish two different points of view: one may study judgement from an empirical or from a logical point of view. From an empirical point of view, one understands judgement as an event in the world, to be represented by a predicate. Describing what John does, one may say

[11] See [54] for a thorough approach of the *metaphysics* of the *Kantian self-ego*.

'John judges that snow is white'. Judging is here understood as a relation obtaining between John and the thought that snow is white."

To understand the latter quotation, we can give an example. In fact, let

$$(a_P, a_E, a_X, \theta_X, \theta_E, b, c, d, n, k) \in \mathbb{R}^{10}$$

be a parameter setting of the dimensionless equations of Semrau-Huang's model-described in Section 1.5-and define

$$\tilde{a}_P \coloneqq \max\{a_X, a_E\}. \tag{2.2}$$

Next, Let

$$\mathcal{M}_{(a_P,a_E,a_X,\theta_X,\theta_E,b,c,d,n,k)} \tag{2.3}$$

represent Semrau-Huang's model with *parameter setting*

$$(a_P, a_E, a_X, \theta_X, \theta_E, b, c, d, k, n) \in \mathbb{R}^{10}.$$

Given that each of the latter *parameters* has an *intentionality* [*directionality*], the *judging agent* might claim that

$$\vdash^{\Psi} \quad a_P \gg \tilde{a}_P \to sc^{\mathcal{M}} \sim O_P^{\left(CHIR^+,PD^+,LIF^+,RA^-\right)}, \tag{2.4}$$

which means that choosing the *parameter* a_P much greater than a_X and a_E implies that the *model* gives rise to a *scenario* $sc^{\mathcal{M}}$ that is similar [$\sim$] to the *observation*

$$O_P^{\left(CHIR^+,PD^+,LIF^+,RA^-\right)}. \tag{2.5}$$

But, what is a *scenario*? Even though the definition of a *scenario* is only provided further in this chapter, one can, so far, say that it is indeed the *mathematical counterpart* of an *observation*, that is, a *description* of some of the model's properties with respect to a specific question. Furthermore, in (2.4), the sign $\vdash^{\Psi}$ must not be confused with the *Fregean judgment stroke* $\vdash$, which is an assertive force, acknowledging the *truth* [*objective*] of a *thought* [*claim*], but instead, $\vdash^{\Psi}$ ought to be interpreted as a sign representing a *mental activity*[12] that has occurred in *time* and *space* in response to an *empirical process*. Hence, in this case, one has that an empirical notion of *judgment* is the product of a mental process in response to experience so it is an event in the world characterized by a relation between the *thinker*, or better, the *agent* and a *thought*. In fact, asserting that the *parameter* $a_P \gg 1$ should correspond to an *observation*, in our case, is representing a property of the observable (*experiment*). Thereby, as an event in the world, one has that a *judgment* as an empirical phenomenon demands a *third-person perspective*.

As for the *evaluation* of such a *phenomenological model*, one still needs to decide whether or not a *scenario* is adequately similar [$\sim$] to an *observation*. In fact, owing to the fact that the *judging agent* and the *modelling agent* might not be the same *epistemic subject*, the author of this thesis conjectures that a proper answer for the latter question perhaps requires that one sheds light on the role of the *second-person perspective* in *mathematical models*.

[12] Here, Ψ should be regarded as the psychology symbol. In so doing, $\vdash^{\Psi}$ betokens that a *judgment* has been made in response to an empirical process.

In sum, the author of this thesis has purported to illustrate the *duality* of the *judging agent* as a *transcendental ego* and as a *psychological ego*, or rather, as a *non-empirical ego* and as an *empirical ego* respectively. In fact, in the analysis of such a *phenomenological model*, through which judging actions are made, the *judging agent* can be seen as the sum of two components, that is, two projections of herself entirely necessary for her to keep getting knowledge of such a *model.* Furthermore, this duality must be emphasized in the discrimination of the two involved perspectives, that is, the *first-person perspective* and the *third-person perspective*, along with the difference between a logical and an empirical notion of *judgment.* Ergo, undermining such a duality of the *judging agent* can presumably cause an impingement upon the *evaluation* of a proposed mathematical representation of a *target system.*

2.3 Frege on Truth

So, how are then the *conception order*, the concept of *primitive notion*, the concept of *judgment* and the *first-person perspective* fundamentally related to the aim of this thesis? First of all, if we acknowledge that, regardless the field of study, there is indeed an order among all our concepts, and that primitive notions are the elementary units of our *knowledge*, then this view seems to bestow a rational strategy that can be used to analyze and to evaluate the proposed phenomenological mathematical models. As this evaluation distinctively involves the formulation of '*statements about mathematical objects*' [*propositions*][13] then we need to apprehend the concept of 'truth'. Indeed, a *proposition* is defined as a statement (e.g. about a mathematical object) "for which it is reasonable to ask whether it can be proved *true* or *not true*[*false*] ", so it is conceptually dependent upon the notion of *proof*[14] [*demonstration*] and *truth.*

Now, considering that the concept of an *assertion* is defined as "a statement that one strongly believe to be true" then one has that an *assertion* is, by definition, a *proposition.* However, the converse may not be true for all *propositions.* Why not? The *judging agent* [*mathematician*] can definitely come up with a *proposition* without being overwhelmingly convinced that it is true. For instance, it might consist of a statement that is derived from some calculation during the mathematical analysis of the model, which, in this case, would not necessarily demand any *assertive force* in the *act of proposing.* Despite the latter remark, from now on, unless we explicitly consider otherwise, we shall only regard the *judgeable content* as an *assertion.*

But, what is the definition of the notions of *truth* and *proof* ? In fact, the notion of *proof* of a *judgement* "“is a chain of reasoning, and what it purports to do is to make the final *judgment* of that chain known, or evident”"; as literally formulated by Dr. Martin Löf in [56]. Or equivalently, it is a chain of *correct judgments* [*true assertions*] that shows the *correctness* [*truth*] of the last judgment[*assertion*]. So, it is conceptually dependent upon the notion of *truth*, which, in turn, propounds that the notion of *truth* might be in our conceptual foundation as a fundamental

[13]With the aim of determining whether or not the model suffices to adequately explain the observations.

[14]So far, we have been using the epistemic counterpart hereof, that is, a ' demonstration'. However, by means of simplicity, from now onward, we shall use 'proof' and 'demonstration' interchangeably.

one. But, how can we elucidate it? In order to do that, we must now turn our attention to the role of *logic* in the working of the *judging agent*[*mathematician*], which conspicuously encompasses the acts of reasoning and proving; culminating in the acquirement of knowledge. To quote from Solomon Feferman in [19]:

“The aim of the mathematician working in the mainstream is to establish truths about mathematical concepts by means of proofs as the principal instrument.(...)”

“(...) I am guided throughout by the simple view that what logic is to provide is all those forms of reasoning that lead invariably from truths to truths. The problematic part of this is how we take the notion of truth to be given.”

So, according to Dr. Solomon Feferman, the notion of *truth* is a prominent one as regards the working of the *judging agent* [*mathematician*]. Withal, what can we tell about the notion of *truth* in the *Fregean logic*? To answer the latter question, we quote from Dr. Maria van der Schaar in [81]:

“(...) Martin [Dr. Wayne M. Martin] brings out the notion of truth as a unique presupposition for logic: ‘logic presupposes and cannot explicate a pre-logical understanding of truth’ (...). This Heideggerian thesis Martin also finds in Frege’s writings: ‘Frege insists... that the most basic logical notion is neither concept nor judgment but truth ... Here, Frege effectively approaches the central claim of Heidegger’s mature philosophical logic’ (...). It is true that in early and later writings Frege claimed that the aim of logic is to know the laws of truth (...); the logical laws are a development of the content of the word ‘true’ (...). However, judgement seems to play an equally important role, as Frege characterizes the laws of logic both as the laws of truth and as the laws of judgement. ”

Hence, according to Dr. Gottlob Frege, the notion of truth is a primitive one, that is, it is indefinable. Why? To give an argument for that, we draw upon a quotation from "Über sinn und bedeutung" that can be found in [5, p. 159]: "“Judgments can be regarded as advances from a thought to a truth value"”. Hence, as far as the author of this thesis can see, in the Fregean logic, *truth* has a qualitative account, that is, it is a feature of an *assertion*. If this is the case then we can assign the feature "*true*" to an *assertion* that is thought to have it, and "*not true*" [*false*] to an assertion that is thought not to have it. Thereby, *truth* is then defined as "the quality of being true" so we end up in a circular definition. But, if it cannot be defined then how can we understand it in the context of the Fregean logic? As far as the author of this thesis can see, if we want to avoid giving the status of a presupposition to it, which seems to be evasive and not to capture the essence thereof, then we are in need of a suitable *elucidation* . In fact, we quote from Dr. Erich H. Reck in [71]:

“In Frege’s writings, the three notions mentioned in the title of this paper—truth, judgment, and objectivity—are all prominent and important. They are also closely related to each other, as is made explicit at various places. In "On Sinn and Bedeutung", Frege relates the first two as follows: "Judgments can be regarded as advances from a thought to a truth value" (...); at other places, including the late article "Thought", he also characterizes judging as "the acknowledgement of the truth of a thought" (...). Relating the second and third notions, he remarks in The Foundations of Arithmetic: "What is objective ... is what is subject to laws, what can be conceived and judged, what is expressible in words" (...). ”

So, in the latter quotation, Dr. Erich H. Reck provides a suitable elucidation for the conception of *truth*, by asserting that the essence of the notions of *truth* and *judgment*, in the Fregean logic, are entangled with each other. Therefore, one cannot apprehend those notions separately from one another. In fact, if we invoke the elucidation provided by Dr. Martin Lőf in [55], that is, the *act of judging*, namely

$$\vdash A, \tag{2.6}$$

as the very act of knowing what is being judged—the *assertion* A-then, as far as the author of this thesis can see, the *non-empirical notion of judgment* in the Fregean logic-an acknowledgement of the *truth* of an *assertion*-can also be understood as an *act of recognizing* an intrinsic assignment [*objective*] of a quality ["truth"] to an *assertion* A, or rather, as an *act of satisfying/fulfilling* a kind of wish ["that the assertion A is true"]. In sum, the judgeable content A as a *proposition* in the Fregean logic is either *true* or *not true* [*false*] as succinctly enlightened by Dr. Gőran Sundholm in [96]:

“Tradition is classical. Surely, nothing could be more pleonastic than that? The logical tradition, certainly, was squarely classical from Bolzano to Carnap, with, say, Frege, Moore, Russell and the Wittgenstein of the Tractatus as intermediaries. Propositions are construed as being in themselves true-or-false. Indeed, in this tradition, a declarative sentence S expresses a proposition (or is a proposition, depending on what version of the theory that is adopted) by being true-or-false. So the meaningfulness of a sentence consists in its being true-or-false. But S is true-or false, or so they say, only when S is true, or when S is false. On the classical account the presumption of bivalence is built into the very notion of meaningfulness: there is no difference between asserting that is a proposition and asserting that A is true-or-false.(...)”

Nonetheless, *truth* might not be a *primitive notion* in another logic as one can see below in the quotation from Dr. Martin Lőf in [55]:

“(...) Intuitionistically, truth of a proposition is analyzed as existence of proof: a proposition is true if there exists a proof of it. Now, I will not dwell upon the notion of proof of a proposition, because a proposition is defined precisely by explaining what a proof of it looks like; so, once we know the proposition, we certainly know what a proof of a proposition is. But look at the other component that I use to define the notion of truth, namely, the notion of existence. It is quite clear that the notion of existence that enters here is not the notion of existence that is expressed by means of the existential quantifier: rather, the notion of existence of an essence, if you prefer, where by saying that a concept has existence I mean that there exists an object which falls under the concept. So to say that a proposition is true is the same as to say that the concept proof of the proposition has existence in the traditional philosophical sense. ”

So, according to Martin Löf, the intuitionistic notion of *truth* is reducible to the primitive notion of *existence*, that is, the *existence* of a *conception of proof*. So, a *proposition* A is *true* if and only if there exists a *proof* of A. Therefore, one has that the latter elucidations of the notion of *truth* will help us to sharply demarcate the domain of the evaluation of the proposed models. But, which notion of *truth* should we be adopting then: the Fregean notion-as an assignment of a truth value- or the intuitionistic notion-as a construction of a *proof*? In order to answer the latter question, we need to clarify for ourselves which role logic exactly plays in our rational approach.

2.4 The role of logic in the acts of the judging agent

But, what is the role of logic in the working of the *judging agent* [*mathematician*] ? In fact, *logic* gives the rules of inference with which the *judging agent* can prove *mathematical assertions* on the model $\mathcal{M}$. Furthermore, according to Dr. Solomon Feferman in [19], such rules of inference are supposed to "“lead invariably from truths to truths”".

Now, if we bear in mind that we want to evaluate the *phenomenological mathematical model* $\mathcal{M}$, which intuitively means that we want to appraise its appropriateness as a conceptual mechanism that explains the *observations*; then we must be able to compare properties of the *target system* with the ones generated by the *mathematical assertions* on the model $\mathcal{M}$. But, how should we be thinking then in our rational approach? In fact, we cannot establish any containment relationship between those sets of properties [*true assertions*] in a proper way by simply using a *natural language* [e.g. *English*, *Dutch*, *German*, ...] so we are in need of a *formal language*.

2.5 Toward a rational understanding: a formal language for the proposed models

But, what is a *formal language*? In fact, it is a language with which the *judging agent* can properly formalize a *mathematical assertion* stated in a *natural language* so that, in our context, it does make sense to talk about a set of assertions containing another set of assertions. Furthermore, one has that the *true value* of an *assertion* can be formally established therein. In fact, let $\mathcal{L}^*$ denote such a *formal language*. So, as for the *alphabet* of $\mathcal{L}^*$, if we build upon the formulation given in [32, 11] then one has that 'nouns', 'compound nouns', and 'similar expressions' in a *natural language* (e.g. *critical auto-activation*, *critical threshold concentration*, *endoderm-like state*, *ectoderm-like state*, *pluripotent-like state* ...) are formalized as *constant* *symbols*. Hence, one assumes that $\mathcal{L}^*$ has infinitely many *constant symbols*, namely

$$\alpha,\ \beta,\ \gamma,\ \alpha_1,\ \beta_1,\ \gamma_1,\ \alpha_2,\ \beta_2,\ \gamma_2, \dots \ . \tag{2.7}$$

Next, in order to formalize 'nouns', 'compound nouns', and 'similar expressions' in a *natural language* that range over a set consisting of a particular type of object (e.g. *auto-activation*, *threshold concentration*, *mutual interaction*, *degradation parameter*, *pluripotent cells*, *ectoderm-like cells*, *extra-embryonic endoderm-like cells*, *time*, ...), one assumes that $\mathcal{L}^*$ has infinitely many *variable symbols*, namely

$$a_P,\ a_X,\ a_E,\ \theta_P,\ \theta_X,\ \theta_E,\ b,\ k,\ c,\ d,\ n,\ p,\ x,\ e,\ ,z\ ,t,\ \bar{a}_P,\ \bar{a}_X,\ \bar{a}_E,\ \bar{\theta}_P,\ \bar{\theta_X}, \dots \ . \tag{2.8}$$

Further, considering that we want to split a *sentence*, formulated in a *natural language*, in such a way that its formalization in the *formal language* provides a precise information as to which class, for instance, a noun belongs, and, more importantly, about the amount of it being considered (e.g. *for all values of the threshold concentration higher than the critical value, ...*), then one has that the *quantifiers*

$$\bigvee,\ \bigwedge, \tag{2.9}$$

meaning "there exists" and "for all" respectively, are also included in the formal language $\mathcal{L}^*$ so as to assure that. Moreover, so far, one considers *variables* that range over a particular set consisting of *non-logical objects*, which, in this case, describes a *first-order logic*. Nonetheless, it may be the case that variables range over a set consisting of *logical objects* (e.g. *m-place predicate letters*), which, in turn, configures a *higher-order logic*. Therefore, one has that we consider a translation of *mathematical assertions* on the model $\mathcal{M}$ into a *first-order language* $\mathcal{L}^*$.

Now, since English sentences can be combined to give rise to compound sentences in a *natural language* then one endows the formal language $\mathcal{L}^*$ with the connectives

$$\neg,\ \vee,\ \wedge,\ \rightarrow,\ \leftrightarrow, \tag{2.10}$$

which resembles, in particular, the English expressions "it is not the case that", "or", "and", "if ... then", and "if and only if" respectively[15].

Further, in contrast to *qualitative identity*, which, as we shall see further, can be formalized without introducing new symbols, *numerical identity*, wherein we are actually interested, is formalized with the addition of the symbol

$$= \tag{2.11}$$

to the formal language $\mathcal{L}^*$. Moreover, in order to enhance clarity and avoid ambiguity, one also adds the following auxiliary symbols to $\mathcal{L}^*$:

$$\top,\ \bot,\ :,\ (\ ),\ [\ ], \tag{2.12}$$

with : and ( ) being used for clarity and readability; with $\top$ and $\bot$ meaning a *tautology* and *contradiction* respectively; and with [ ] being used to symbolize a "discharged assumption" in the *formal language*, as it will be introduced later in this section.

[15] As long as no confusion can occur among the symbols: $\bigvee$; $\vee$; $\bigwedge$; $\wedge$.

Thus far, we have been talking about sentences in a natural language without having at least defined it informally. So, what is a sentence? In fact, the concept of *sentence* in a *natural language* [*English*] is defined as "a string of words expressing a *thought* and containing a verb". Hence, seeing that the concept of *statement* is defined as the expression of a *thought* in a *sentence*, and thus conceptually dependent upon the concept of *sentence*, one has that the concept of *assertion*—"a statement that one strongly believes to be true"—is conceptually dependent upon the concept of *sentence*. Therefore, if we know how to formalize a *sentence* in a *natural language* into a *sentence* in a *formal language* then we do know how to formalize an *assertion*. In order to do that, one endows the *formal language* $\mathcal{L}^*$ with infinitely many *m-place predicate letters*

$$P^m,\ Q^m,\ R^m,\ P_1^m,\ Q_1^m,\ R_1^m,\ldots,\ P_j^m,\ Q_j^m,\ R_j^m,\ldots, \tag{2.13}$$

for all $m, j \in \mathbb{N}$. Having introduced that, one has that a 0-*place predicate letter* stands for the formalization of an entire *sentence* in a *natural language*. Why do we need the latter definition to construe the conception of *sentence* in $\mathcal{L}^*$? To answer this question, we are in need of proper elucidations.

So far, we have introduced the *alphabet* of the *formal language* $\mathcal{L}^*$; but, how should we be intuitively understanding such an *alphabet* then? As far as the author of this thesis can see, the *constants* and *variables*, which have been defined in (2.7) and (2.8) respectively, play the role of "letters" in $\mathcal{L}^*$. Now, if we turn our attention to the first component of the definition of the concept of *sentence* in a *natural language* [*English*], that is, "a string of words", then one must define the notion of *word* in the *formal language* $\mathcal{L}^*$ so as to specify the notion of *sentence* in $\mathcal{L}^*$. In fact, one defines an *atomic formula*[*word*] in $\mathcal{L}^*$ as

$$\daleth = \gimel, \tag{2.14}$$

with $\daleth$ and $\gimel$ being a *variable* or a *constant*, and as

$$\Delta \gimel_1 \ldots \gimel_m, \tag{2.15}$$

with Δ being[16] a *m-place predicate letter* and each of $\gimel_1, \ldots, \gimel_m$ being a *variable* or a *constant*.

Next, bearing in mind that the notion of *sentence* in a *natural language* is defined as "a string of words expressing a *thought* and containing a verb" and considering that the notions of *clause*, *phrase*, and *expressions* in a *natural language* are defined as "a string of words containing a verb and a subject", as "a string of words, which is a part of a *sentence*", and as "a string of words" respectively, then one has that the notion of *sentence* in a *natural language* is conceptually dependent on the notion of *clause*, which, in turn, is conceptually dependent upon the notion of *expression*. Likewise, the notion of *sentence* in a *natural language* is conceptually dependent upon the notion of *phrase*, which, in turn, is conceptually dependent upon the notion of *expression*. Furthermore, though not being a primitive notion, one has that the notion of *expression* is the most fundamental one in the sequences (*sentence, clause, expression*) and (*sentence, phrase, expression*). Hence,

[16]Here, we are using the Greek letter "Δ" [*delta*] and the Hebrew letters "ג"[*gimel*] and "ד" [*daleth*] as metavariables, which, intuitively, are letters from a natural language [metalanguage] being used to communicate the formal language.

if one wants to conceptualize the notion of *sentence* in $\mathcal{L}^*$ then it is necessary to conceptualize the notion of *expression* in $\mathcal{L}^*$. Actually, one can coherently define the set of all *formulae* [*expressions*] in $\mathcal{L}^*$ by firstly including all *atomic formulae* [*words*] defined in (2.14) and (2.15). Next, by employing the *connectives* in (2.10) as "binding rules", one also includes

$$\neg\varpi,\ \varpi \wedge \vartheta,\ \varpi \vee \vartheta,\ \varpi \rightarrow \vartheta,\ \varpi \leftrightarrow \vartheta, \tag{2.16}$$

as formulae, with ϖ and ϑ being formulae in $\mathcal{L}^*$. In so doing, one sets the rules [*syntax*] under which one can construct a string of *atomic formulae* ["a string of words"] in $\mathcal{L}^*$. Moreover, by invoking (2.9), one also includes

$$\bigwedge \upsilon\varpi, \bigvee \upsilon\varpi, \tag{2.17}$$

as formulae in $\mathcal{L}^*$, with υ being a variable and ϖ being a formula in $\mathcal{L}^*$. Withal, how can a *sentence* be properly conceptualized in $\mathcal{L}^*$? To answer this question, we also turn our attention toward to the second component of the definition of the notion of *sentence*, that is, "a string of words expressing a *thought* (...)". So, we consider the following *clause* in a *natural language*[*English*]:

"For a sufficiently low auto-activation of the pluripotent network, one has that Semrau-Huang's model yields a stable equilibrium which resembles the ectoderm-like state."

Further, one can conveniently formalize the latter *clause* into a $\mathcal{L}^*$-*formula* as

$$\bigvee a_P(P^1 a_P \wedge Q^1 a_P) \rightarrow \bigvee z(R^1 z \wedge R^2 z\alpha), \tag{2.18}$$

with the following dictionary:

P^1: ... is an auto-activation of the pluripotent network;

Q^1: ... is sufficiently low;

R^1: ... is a stable equilibrium of Semrau-Huang's model;

R^2: ... resembles ... ;

α: the ectoderm like-state.

Now, if we consider the $\mathcal{L}^*$-*formula*

$$(R^1 z \wedge R^2 z\alpha), \tag{2.19}$$

taken from (2.18), then we have the following translation for (2.19):

"it is a stable equilibrium of Semrau-Huang's model and it resembles the ectoderm-like state".

Thereby, the latter is not a *sentence* in the respective *natural language* [*English*], seeing that there is no expression of a *thought* therein. Or rather, there is no *expression* in that, to which the pronoun "it" can refer. Intuitively, it is tantamount to not having anything to put in place of "it" about which one can think. Actually, one can think of it as if one had gotten stuck at the *act of reading*, being unable to perform the *act of thinking*, which is prior to, and necessary for the *act of judging*. So, one cannot judge the *phrase* "it is a stable equilibrium of Semrau-Huang's model and it resembles the ectoderm-like state" because one cannot advance any *truth-value* thereto. Therefore, there has been referred to no *structure* in (2.19) to which "it" belongs. In this regard, one has that the occurrence of the variable z-playing the role of "it"-in (2.19), is said to be *free*.

Now, if we draw upon the latter elucidations then we can conclude that the *clause*

"For a sufficiently low auto-activation of the pluripotent network, one has that Semrau-Huang's model yields a stable equilibrium which resembles the ectoderm-like state.",

is a *sentence* in *English*, given that it undoubtedly expresses a *thought*. Does the latter rationale shed light on the conceptualization of a *sentence* in a *formal language*? Intuitively, knowing the structure over which variables range, is a necessary condition for the *judging agent* to think about what is being stated, so that she can advance a *truth-value* to it. As we see in (2.18), the variables z, a_P do not occur freely therein. Actually, their occurrence in (2.18) is said to be bound, which, in turn, motivates the definition of a *sentence* in $\mathcal{L}^*$ as a $\mathcal{L}^*$*-formula* in which all *variables* occur boundedly. In so doing, one captures, with the latter formalized notion of *sentence*, the two essential components of the definition of the notion of *sentence* in a *natural language*, that is, "a string of words" with the "expression of a thought".

So, as we now know that the *clause*

"For a sufficiently low auto-activation of the pluripotent network, one has that Semrau-Huang's model yields a stable equilibrium which resembles the ectoderm-like state.",

is indeed a *sentence* then we can wonder whether or not it is an *assertion*. In fact, if we presuppose that the *judging agent* and the *modelling agent* of Semrau-Huang's model would assure that it is definitely the case then, bearing in mind the *empirical notion* of *judgment*, that is, a response to an *empirical process*, one has that

$$\vdash^{\Psi} A_{i_1}^{\mathcal{O}_E}, \tag{2.20}$$

with $i_1 \in \mathbb{N}$ and

$$A_{i_1}^{\mathcal{O}_\mathcal{E}}: \quad \bigvee a_P(P^1 a_P \wedge Q^1 a_P) \rightarrow \bigvee z(R^1 z \wedge R^2 z\alpha), \tag{2.21}$$

ought to be regarded as a property of the *observation* $\mathcal{O}_E$ being expressed in the formal language $\mathcal{L}^*$, and being solely stipulated by *empirical evidences*. Moreover, as we have said earlier in this thesis, $\vdash^{\Psi}$ symbolizes that a mental activity has occurred leading the *judging agent* to an *assertion*, which, in essence, is entirely predicated upon knowledge of the results of the performed experiments. Hence, if we intend to evaluate the model $\mathcal{M}$ with respect to the set of *observations* $\mathcal{O}$ then we must be able to determine whether or not the model $\mathcal{M}$ really generates the property $A_{i_1}^{\mathcal{O}_E}$ shown in (2.21). However, if we intend clarifying what we mean with the model $\mathcal{M}$ generating the property $A_{i_1}^{\mathcal{O}_E}$ then we need to understand how we can determine the *truth-value* of $A_{i_1}^{\mathcal{O}_E}$ in $\mathcal{L}^*$.

First of all, as *variables* and *constants* range over a set of *non-logical objects* then one has that the *structure* over which they range, does have an influence upon the *truth-value* of sentences in the *first-order language* $\mathcal{L}^*$, that is, a $\mathcal{L}^*$-*sentence* may be *true* in one *structure* and *false* in another one. So, the latter remark leads us to the conception of $\mathcal{L}^*$-*structure*, or rather, the *semantics* of the *formal language* $\mathcal{L}^*$. In this thesis, we consider the *structure*

$$(\mathbb{R}_+; 0, 1, +, \cdot, <), \tag{2.22}$$

with $0, 1, +, \cdot$ denoting the arithmetic relations, $\mathbb{R}_+ := \{w \in \mathbb{R} : w \geq 0\}$, and $<$ representing the *total order* in $\mathbb{R}$. Now, bearing in mind the *primitive notion* of *truth* in the *Fregean logic* as an assignment [*objective*] of a quality [*truth-value*] to an assertion A, and that *words*, in a natural language, are the primitive units of meaning, one has that if one stipulates how one interprets the *atomic formulae* in $\mathcal{L}^*$ [$\mathcal{L}^*$-*words*] then one knows how to convey meaning to all $\mathcal{L}^*$-*formulae*, and, in particular, to all $\mathcal{L}^*$-*sentences* [*formalized assertions*]. In so doing, one can naturally extend the *semantic assignment* on $\mathcal{L}^*$ to a *truth condition* on $\mathcal{L}^*$, which, in turn, enables us to semantically deduce the *truth-value* of a $\mathcal{L}^*$-*formula* and, in particular, of a $\mathcal{L}^*$-*sentence* [*formalized assertions*]. In fact, let $\mathcal{C}_{\mathcal{L}^*}$, $\mathcal{V}_{\mathcal{L}^*}$, $\mathcal{P}_{\mathcal{L}^*}^m$, $\mathcal{R}_m$, and $\mathcal{P}_{\mathcal{L}^*}^{(0)}$, denote the set of all *constants*, *variables*, *m-place predicate letters*, *m-ary relations*, with $m \in \mathbb{N}_{>0}$, and 0-*place predicate letters* respectively. So, one has that

$$\begin{aligned} \mathfrak{w}_c \colon \mathcal{C}_{\mathcal{L}^*} &\to \mathbb{R}_+ \\ \text{מ} &\mapsto \mathfrak{w}_c(\text{מ}), \end{aligned}$$

and

$$\begin{aligned} \mathfrak{w}_v \colon \mathcal{V}_{\mathcal{L}^*} &\to \mathbb{R}_+ \\ \text{צ} &\mapsto \mathfrak{w}_v(\text{צ}), \end{aligned}$$

and

$$\begin{aligned} \mathfrak{w}_{\mathcal{P},m} \colon \mathcal{P}_{\mathcal{L}^*}^m &\to \mathcal{R}_m \\ \text{ק} &\mapsto \mathfrak{w}_{P,m}(\text{ק}), \end{aligned}$$

and

$$\begin{aligned} \mathfrak{w}_{\mathcal{P},0} \colon \mathcal{P}_{\mathcal{L}^*}^0 &\to \{0, 1\} \\ \text{ק} &\mapsto \mathfrak{w}_{P,m}(\text{ק}), \end{aligned}$$

represent a *semantic assignment* on $\mathcal{L}^*$ by which $\text{ש}_{\mathcal{P},0}$ assigns a *truth-value* to each 0-*place predicate letter* in $\mathcal{P}^0_{\mathcal{L}^*}$, that is, 0 [*false*] or 1 [*true*]. As for the latter *semantic assignment*, for example, one has that the *Dedekind-Peano axioms* are formalized as 0-*place predicate letters*, to which 1 [*true*] is assigned. Having done that, one can extend such a *semantic assignment* to a *truth-condition* on $\mathcal{L}^*$ by describing it firstly on the $\mathcal{L}^*$-*primitive formulae*. In fact, one defines that

$$| \daleth = \gimel |_{\mathbb{R}_+} = 1 \tag{2.23}$$

if and only if,

$$\text{ש}_i(\daleth) = \text{ש}_j(\gimel) \tag{2.24}$$

with

$$\daleth, \gimel \in \mathcal{C}_{\mathcal{L}^*} \cup \mathcal{V}_{\mathcal{L}^*},$$

and with $i, j \in \{c, v\}$ being the respective indexes; and that

$$| \Delta \gimel_1 \gimel_2 \ldots \gimel_m |_{\mathbb{R}_+} = 1, \tag{2.25}$$

if and only if,

$$\langle \text{ש}_{i_1}(\gimel_1), \text{ש}_{i_2}(\gimel_2), \ldots, \text{ש}_{i_m}(\gimel_m) \rangle \in \text{ש}_{\mathcal{P},m}(\Delta), \tag{2.26}$$

with $\Delta \in \mathcal{P}^m_{\mathcal{L}^*}$,

$$\gimel_{i_1}, \gimel_{i_2}, \gimel_{i_3}, \ldots \gimel_{i_m} \in \mathcal{C}_{\mathcal{L}^*} \cup \mathcal{V}_{\mathcal{L}^*},$$

and with $i_1, i_2, \ldots, i_m \in \{c, v\}$ being the corresponding indexes. Let $\Sigma_{\mathcal{L}^*}$ denote the set of all $\mathcal{L}^*$-*formulae*. So, if one draws upon (2.25) and (2.26), then one can naturally extend such a *truth-condition* to $\Sigma_{\mathcal{L}^*}$ as shown in [32, p. 104]. Indeed, for all $\varpi, \vartheta \in \Sigma_{\mathcal{L}^*}$, one has that

$$| \ |_{\mathbb{R}_+} : \Sigma_{\mathcal{L}^*} \to \{0, 1\}$$

must satisfy that

$$| \neg\varpi |_{\mathbb{R}_+} = 1, \tag{2.27}$$

if and only if

$$| \varpi |_{\mathbb{R}_+} = 0; \tag{2.28}$$

and that

$$| \varpi \wedge \vartheta |_{\mathbb{R}_+} = 1, \tag{2.29}$$

if and only if,

$$| \varpi |_{\mathbb{R}_+} = 1 = | \vartheta |_{\mathbb{R}_+}; \tag{2.30}$$

and that

$$| \varpi \vee \vartheta |_{\mathbb{R}_+} = 1, \tag{2.31}$$

if and only if,

$$| \varpi |_{\mathbb{R}_+} = 1 \text{ or } | \vartheta |_{\mathbb{R}_+} = 1; \tag{2.32}$$

and that

$$| \varpi \to \vartheta |_{\mathbb{R}_+} = 1, \tag{2.33}$$

if and only if,

$$| \varpi |_{\mathbb{R}_+} = 0 \text{ or } | \vartheta |_{\mathbb{R}_+} = 1; \tag{2.34}$$

and that

$$\mid \varpi \leftrightarrow \vartheta \mid_{\mathbb{R}_+} = 1, \tag{2.35}$$

if and only if,

$$\mid \varpi \mid_{\mathbb{R}_+} = \mid \vartheta \mid_{\mathbb{R}_+}; \tag{2.36}$$

and that

$$\mid \bigwedge \upsilon \varpi \mid_{\mathbb{R}_+} = 1, \tag{2.37}$$

if and only if,

$$\mid \varpi \mid_{\mathbb{R}_+} = 1, \tag{2.38}$$

independent upon the variable assignment $\mathfrak{v}_v$; and that

$$\mid \bigvee \upsilon \varpi \mid_{\mathbb{R}_+} = 1, \tag{2.39}$$

if and only if,

$$\mid \varpi \mid_{\mathbb{R}_+} = 1, \tag{2.40}$$

for some variable assignment $\mathfrak{v}_v$. But, if we know how to determine the *truth-value* of a $\mathcal{L}^*$-*formula*, in particular, of a $\mathcal{L}^*$-*sentence* [e.g. *mathematical assertion*] then we can now clarify what we mean with the model $\mathcal{M}$ generating the property $A_{i_1}^{\mathcal{O}_E}$ shown in (2.21).

2.6 Models and the generation of observational properties

Firstly, rigorously speaking, the model $\mathcal{M}$ is indeed a set of model instances, i.e.

$$\mathcal{M} = \{\mathcal{M}_\lambda : \lambda \in \Lambda\}, \tag{2.41}$$

with Λ representing the *parameter space*. So, intuitively, relying upon (2.41), and invoking that

$$\vdash^{\Psi} A_{i_1}^{\mathcal{O}_E}, \tag{2.42}$$

one has that checking whether or not the model $\mathcal{M}$ generates the property $A_{i_1}^{\mathcal{O}_E}$ means that we must show whether or not there exists $\lambda[\mathcal{O}_E] \in \Lambda$ such that

$$A_{i_1}^{\mathcal{O}_E} \in \mathcal{A}\left[\mathcal{M}_{\lambda[\mathcal{O}_E]}\right], \tag{2.43}$$

and that

$$\mid A_{i_1}^{\mathcal{O}_E} \mid_{\mathbb{R}_+} = 1, \tag{2.44}$$

with $\mathcal{A}[\mathcal{M}_{\lambda[\mathcal{O}_E]}]$ denoting the set of all formalized mathematical assertions on the model $\mathcal{M}_{\lambda[\mathcal{O}_E]}$. In this regard, we are then entitled to write that

$$\vdash A_{i_1}^{\mathcal{O}_E}, \tag{2.45}$$

with "$\vdash$" symbolizing the Fregean's token for the assertoric force. Hence, one has that

$$\bigcup_{\lambda \in \Lambda} \mathcal{A}[\mathcal{M}_\lambda] \subset \Sigma_{\mathcal{L}^*}. \tag{2.46}$$

But, what is actually the essence of $\mathcal{M}_{\lambda[\mathcal{O}_E]}$ generating the property $A_{i_1}^{\mathcal{O}_E}$ for some $\lambda[\mathcal{O}_E] \in \Lambda$? If we draw upon (2.21) and upon the conditions (2.15), (2.33), (2.34), (2.39), and (2.40), then we need to decide whether

$$\mid P^1 a_P \wedge Q^1 a_P \mid_{\mathbb{R}_+} = 0 \tag{2.47}$$

independent upon the choice for [17] $\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(a_P) \in \mathbb{R}_+$, or

$$\mid R^1 z \wedge R^2 z\alpha \mid_{\mathbb{R}_+} = 1 \tag{2.48}$$

for some $\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(z) \in \mathbb{R}_+$. Or rather, one must decide whether

$$\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(a_P) \notin \mathfrak{w}_{\mathcal{P},1}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(P^1), \tag{2.49}$$

or

$$\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(a_P) \notin \mathfrak{w}_{\mathcal{P},1}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(Q^1), \tag{2.50}$$

independent upon the choice for $\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(a_P) \in \mathbb{R}_+$; or

$$\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(z) \in \mathfrak{w}_{\mathcal{P},1}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(R^1), \tag{2.51}$$

and

$$\left\langle \mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(z), \mathfrak{w}_c^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(\alpha) \right\rangle \in \mathfrak{w}_{\mathcal{P},1}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(R^2), \tag{2.52}$$

for some $\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(z), \mathfrak{w}_c^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}(\alpha) \in \mathbb{R}_+$. However, if we try to work it out from (2.49) to (2.52), by only building on the semantics of the formal language $\mathcal{L}^*$, then (2.47) is undecidable. But, why is it undecidable? In fact, For example, the *1-place predicate letter* Q^1 has an unary relation as its semantic value, but, whichever the variable assignment $\mathfrak{w}_v^{\mathcal{M}_{\lambda[\mathcal{O}_E]}}$ is, one cannot determine the truth-value of $Q^1 a_P$ ["the auto-activation of the pluripotent network is sufficiently low "] by working out the semantic values of connectives and quantifiers in the formal language $\mathcal{L}^*$, without having knowledge of the mathematical structure [Dynamical system] underlying it. Indeed, we must clarify what we mean with "being sufficiently low". To quote from Dr. Jules Molk in [61]:

> The definitions should be algebraic and not only logical. It does not suffice to say: 'A thing exists or it does not exist'. One has to show what being and not being mean, in the particular domain in which we are moving. Only thus do we make a step forward.

So, the latter quotation, actually taken from [3, p. 13], is fundamental to delimiting the domain of logic in our rational approach. In fact, *mathematical assertions* do have meaning on their own. Regarding the $\mathcal{L}^*$-formula $Q^1 a_P$ ["the auto-activation of the pluripotent network is sufficiently low "], one has to rescale the dynamical equations of the model $\mathcal{M}_{\lambda[\mathcal{O}_E]}$ so as to precisely define "sufficiently low". In sum, one cannot determine the truth-value of $A_{i_1}^{\mathcal{O}_E}$ with respect to $\mathcal{M}_{\lambda[\mathcal{O}_E]}$ by solely using a proof semantic-based argument[18] [*semantic proof*]. Hence, it means that *generation*

[17] Here, we adopt a notation for the variable assignment with which we accentuate that it is in relation to the model $\mathcal{M}_{\lambda[\mathcal{O}_E]}$.

[18] By means of working out the semantic values of connectives and quantifiers.

of the property $A_{i_1}^{\mathcal{O}_E}$ by the model $\mathcal{M}_{\lambda[\mathcal{O}_E]}$ must be shown by drawing upon the relevant properties of $\mathcal{M}_{\lambda[\mathcal{O}_E]}$, or equivalently, the property $A_{i_1}^{\mathcal{O}_E}$ must be constructed from $\mathcal{A}[\mathcal{M}_{\lambda[\mathcal{O}_E]}]$. So, the *generation of a property* by a model has an intuitionistic account rather than a classical account in our interpretation, that is,

$$A_{i_1}^{\mathcal{O}_E} \in \mathcal{A}\left[\mathcal{M}_{\lambda[\mathcal{O}_E]}\right] \tag{2.53}$$

holds, for some $\lambda[\mathcal{O}_E] \in \Lambda$, if and only if there exist

$$A_{i_2}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}},\ A_{i_3}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}},\ \ldots,\ A_{i_m}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}} \in \mathcal{A}\left[\mathcal{M}_{\lambda[\mathcal{O}_E]}\right], \tag{2.54}$$

with

$$\vdash A_{i_l}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}} \tag{2.55}$$

for all $l \in \{2, 3, \ldots, m\}$, such that

$$A_{i_2}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}},\ A_{i_3}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}},\ \ldots,\ A_{i_m}^{\mathcal{M}_{\lambda[\mathcal{O}_E]}} \vdash A_{i_1}^{\mathcal{O}_E}. \tag{2.56}$$

Now, what do we mean with (2.56)? It means that if we want to show that the property $A_{i_1}^{\mathcal{O}_E}$ is generated by the model $\mathcal{M}_{\lambda[\mathcal{O}_E]}$ then it is necessary and sufficient to show that there is a *formal proof* of $A_{i_1}^{\mathcal{O}_E}$ with assumptions [*sentences*] in $\mathcal{A}\left[\mathcal{M}_{\lambda[\mathcal{O}_E]}\right]$. In this case, if (2.56) holds then we are entitled to write that

$$\vdash A_{i_1}^{\mathcal{O}_E}, \tag{2.57}$$

or equivalently,

$$A_{i_1}^{\mathcal{O}_E} \to \top, \tag{2.58}$$

which, in turn, means that $A_{i_1}^{\mathcal{O}_E}$ is a *tautology* on the model $\mathcal{M}_{\lambda[\mathcal{O}_E]}$. Hence, under (2.56), if we invoke the definition of $\mathcal{A}\left[\mathcal{M}_{\lambda[\mathcal{O}_E]}\right]$ then we are entitled to write that

$$A_{i_1}^{\mathcal{O}_E} \in \mathcal{A}\left[\mathcal{M}_{\lambda[\mathcal{O}_E]}\right], \tag{2.59}$$

so one can say that *knowledge* of the model $\mathcal{M}$ with respect to the *observation* $\mathcal{O}_E$ has been gained indeed. Now, if for all $\lambda \in \Lambda$ one has that

$$\neg A_{i_1}^{\mathcal{O}_E} \in \mathcal{A}\left[\mathcal{M}_{\lambda}\right], \tag{2.60}$$

and that

$$\mid \neg A_{i_1}^{\mathcal{O}_E} \mid_{\mathbb{R}_+} = 1, \tag{2.61}$$

then $A_{i_1}^{\mathcal{O}_E}$ is said to be a *contradiction* on $\mathcal{M}$, that is,

$$\vdash \neg A_{i_1}^{\mathcal{O}_E}, \tag{2.62}$$

or equivalently,

$$\bot \to A_{i_1}^{\mathcal{O}_E}. \tag{2.63}$$

But, what is a *formal proof* [*syntactic proof*]? It is a chain of reasoning whose steps are fully determined by the rules of inferences introduced in [25, 40], which ones are described as follows.

$$\begin{array}{|l} \varpi \quad \vartheta \\ \hline \varpi \wedge \vartheta \end{array} \qquad \wedge\text{ -intro}$$

$$\begin{array}{|l} \varpi \wedge \vartheta \\ \hline \varpi \end{array} \qquad \wedge\text{ -Elim (1)}$$

$$\begin{array}{|l} \varpi \wedge \vartheta \\ \hline \vartheta \end{array} \qquad \wedge\text{ -Elim (2)}$$

$$\begin{array}{|l} [\varpi] \\ \hline \begin{array}{|l} \vartheta \\ \hline \begin{array}{|l} \varpi \to \vartheta \\ \hline \end{array} \end{array} \end{array} \qquad \to\text{ -Intro}$$

$$\begin{array}{|l} \varpi \quad \varpi \to \vartheta \\ \hline \vartheta \end{array} \qquad \to\text{ -Elim}$$

$$\begin{array}{|l} \varpi \\ \hline \varpi \vee \vartheta \end{array} \qquad \vee\text{ -Intro (1)}$$

$$\begin{array}{|l} \vartheta \\ \hline \varpi \vee \vartheta \end{array} \qquad \vee\text{ -Intro (2)}$$

$$\begin{array}{|l|l} [\varpi] & [\vartheta] \\ \hline \zeta & \zeta \quad \varpi \vee \vartheta \\ \hline \zeta & \end{array} \qquad \vee\text{ -Elim}$$

$$\begin{array}{|l|l} [\varpi] & [\varpi] \\ \hline \vartheta & \neg\vartheta \\ \hline \neg\varpi & \end{array} \qquad \neg\text{ -Intro}$$

$$\begin{array}{|l|l} [\neg\varpi] & [\neg\varpi] \\ \hline \vartheta & \neg\vartheta \\ \hline \varpi & \end{array} \qquad \neg\text{ -Elim}$$

$$\begin{array}{|l|l} [\varpi] & [\vartheta] \\ \hline \vartheta & \varpi \\ \hline \vartheta \leftrightarrow \varpi & \end{array} \qquad \leftrightarrow\text{ -Intro}$$

$$\begin{array}{|l} \varpi \quad \varpi \leftrightarrow \vartheta \\ \hline \vartheta \end{array} \qquad \leftrightarrow\text{ -Elim (1)}$$

$$\begin{array}{|l} \vartheta \quad \varpi \leftrightarrow \vartheta \\ \hline \varpi \end{array} \qquad \leftrightarrow\text{ -Elim (2)}$$

$$\begin{array}{|l} \varpi[\gimel/\upsilon] \\ \hline \bigwedge \upsilon\varpi \end{array} \qquad \bigwedge\text{ -Intro}$$

$$\begin{array}{|l} \bigwedge \upsilon\varpi \\ \hline \varpi[\gimel/\upsilon] \end{array} \qquad \bigwedge\text{ -Elim}$$

$$\begin{array}{|l} \varpi[\gimel/\upsilon] \\ \hline \bigvee \upsilon\varpi \end{array} \qquad \bigvee\text{ -Intro}$$

$[\varpi[\gimel/\upsilon]]$

$\vartheta \quad \bigvee \upsilon\varpi$

$\vartheta \quad \bigvee$ -Elim

$[\gimel = \gimel]$

$\vdots \quad =$ -Intro

$\varpi[\daleth/\upsilon] \quad [\daleth = \gimel]$

$\varpi[\gimel/\upsilon] \quad =$ -Elim (1)

$\varpi[\daleth/\upsilon] \quad [\gimel = \daleth]$

$\varpi[\gimel/\upsilon] \quad =$ -Elim (2)

Provided that $\daleth, \gimel \in \mathcal{C}_{\mathcal{L}^*}$, $\gimel$ occurs in no undischarged assumption in the proof of $\varpi[\gimel/\upsilon]$ in $\bigwedge$-Intro, and that $\gimel$ only occurs in the discharged assumption $[\varpi[\gimel/\upsilon]]$ in the proof of ϑ in $\bigvee$-Elim. So, one has that the *first-order language* $\mathcal{L}^*$ together with the aforesaid rules of inference is said to be a *formal system*. Furthermore, in order to eschew obfuscation[19], it must be clear that we are availing ourselves of the intuitionistic notion of truth so as to understand the generation of the property $A_{i_1}^{\mathcal{O}_E}$-solely stipulated by *empirical evidences*-by the model $\mathcal{M}_{\lambda[\mathcal{O}_E]}$, but we do not make any reference to intuitionistic logic in this thesis. Indeed, our rational approach is entirely based on the Fregean logic [*classical logic*]. However, are we aimed at providing *formal proofs* in this thesis? No, we are not. Actually, we have chosen a formal language to communicate our rational approach so as to avoid any misunderstanding and, more importantly, to be clear about the way in which we are reasoning through the composition of the proposed methodology. To quote from Dr. Christian S. Calude in [7]:

“ An informal (pen-on-paper) proof is a rigorous argument expressed in a mixture of natural language and formulae (for some mathematicians an equal mixture is the best proportion) that is intended to convince a knowledgeable mathematician of the truth of a statement, the theorem. Routine logical inferences are omitted. “Folklore” results are used without proof. Depending on the area, arguments may rely on intuition. Informal proofs are the standard of presentation of mathematics in textbooks, journals, classrooms, and conferences. They are the product of a social process.”

So, as described in the latter quotation, we will be mostly performing informal proofs throughout this thesis. In sum, as for the proposed models, let $\mathcal{O}$ denote an *observation*, so we need to determine whether or not

$$A^{\mathcal{O}} \in \mathcal{A}\left[\mathcal{M}_{\lambda[\mathcal{O}]}\right] \tag{2.64}$$

for some $\lambda[\mathcal{O}] \in \Lambda$, which, in turn, demands that we find a rational strategy that enables us to reduce a *continuous-based* search to a *discrete-based search*-an *algorithmic search*.

[19]Given that the author of this thesis is not an expert on the topics involved herein.

2.7 An algorithmic approach: the scenario space, the primitive scenario, the knowledge-transformation Π-functions, and the $\mathcal{M}$-qualitative graph

To begin with, although the *parameter space* Λ can be a subset of an *infinite-dimensional space*, we take $\Lambda \subset \mathbb{R}^N$ with $N \in \mathbb{N}_{>0}$. Next, denote the model $\mathcal{M}$ with fixed parameter setting $\lambda \in \Lambda$ by $\mathcal{M}_\lambda$. Let $A[\mathcal{M}_\lambda]$ denote a formalized mathematical *assertion*[20] on $\mathcal{M}_\lambda$ [e.g. "$\mathcal{M}_\lambda$ has 1 steady state"; "$\mathcal{M}_\lambda$ has 2 steady states"; ...; "$\mathcal{M}_\lambda$ has 18 steady states"; "$\mathcal{M}_\lambda$ has no periodic orbits"; "If the autoactivation of the endoderm-network (ectoderm network) divided by two times their respective degradation rates is greater or equal than their respective threshold concentrations then there is at least one stable steady state"], that is, $A[\mathcal{M}_\lambda]$ is a *sentence* in $\mathcal{L}^*$.

Let $\mathcal{A}[\mathcal{M}_\lambda]$ be the set of all assertions that can be made on the model instance $\mathcal{M}_\lambda$. Then, let $\mathcal{A}$ denote the set of *relevant aspects* of the model $\mathcal{M}$, i.e., the set of *mathematical assertions* on the model $\mathcal{M}$, which, in turn, are applicable to each model instance in the set

$$\{\mathcal{M}_\lambda : \lambda \in \Lambda\}.$$

Thereby, by definition, one has that

$$\mathcal{A} \subset \bigcap_{\lambda \in \Lambda} \mathcal{A}[\mathcal{M}_\lambda] \subset \Sigma_{\mathcal{L}^*}. \tag{2.65}$$

But, to whom is $\mathcal{A}$ relevant? Indeed, such a set of *relevant aspects* $\mathcal{A}$ of the model $\mathcal{M}$ is solely stipulated by the *judging agent*. In this case, one can naturally define a binary relation '$\sim_\mathcal{A}$' on $\Lambda \times \Lambda$. In fact, for $\lambda, \tilde{\lambda} \in \Lambda$, one has that

$$\lambda \sim_\mathcal{A} \tilde{\lambda} \tag{2.66}$$

if and only if

$$\mid A[\mathcal{M}_\lambda] \mid_{\mathbb{R}_+} = \mid A[\mathcal{M}_{\tilde{\lambda}}] \mid_{\mathbb{R}_+}, \tag{2.67}$$

for all $A \in \mathcal{A}$. Having defined that, we assert that it is not difficult to demonstrate that the *binary relation* defined in (2.66) is actually an *equivalence relation*, i.e. being *reflexive, symmetric* and *transitive*. In so doing, one denotes

$$[\lambda] := \{\tilde{\lambda} : \tilde{\lambda} \sim_\mathcal{A} \lambda\} \tag{2.68}$$

as the equivalence class of each $\lambda \in \Lambda$.

But, how can we suitably interpret $[\lambda]$? In fact, if we consider that we have intuitively defined a *scenario* as a description of some of the model's properties [*mathematical assertions*] with respect to a particular question [e.g. "Does the model generate five steady states?"] then the space $\mathcal{SC}^\mathcal{M}$ of all possible scenarios of the model $\mathcal{M}$ can be defined as

$$\mathcal{SC}^\mathcal{M} := {}^{\Lambda}\!/\!_{\sim_\mathcal{A}} \tag{2.69}$$

[20]Although some examples are being provided in a natural language [*English*], one has that $A[\mathcal{M}_\lambda]$ must be thought as a $\mathcal{L}^*$-sentence.

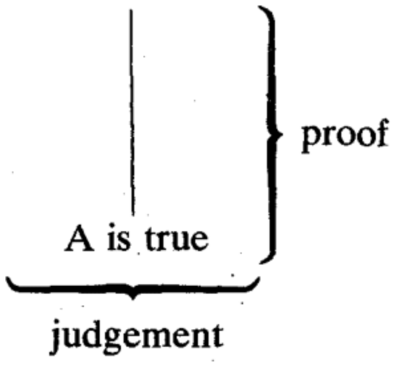


Figure 2.1: This figure has been taken from [57]. The act of proving[proof] precedes the act of judging the assertion as true. The non-empirical notion of judgment can only be understood from a first-person perspective. Indeed, anyone who has gone through the proof by herself, according to Dr. Gottlob Frege, will undoubtedly acknowledge the truth of the assertion.

with

$$\Lambda/\sim_{\mathcal{A}} := \{[\lambda] : \lambda \in \Lambda\} \tag{2.70}$$

representing the *quotient space*, that is, the set of all *equivalence classes* of Λ with respect to $\sim_{\mathcal{A}}$. Let

$$\begin{aligned} q\colon \Lambda &\to \Lambda/\sim_{\mathcal{A}} \\ \lambda &\mapsto [\lambda] \end{aligned}$$

denote the *canonical map*. Consistent with (2.69), one defines

$$sc^{\mathcal{M}}_{\lambda} := q(\lambda), \tag{2.71}$$

for all $\lambda \in \Lambda$. So, one has that a *scenario* $sc^{\mathcal{M}}_{\lambda}$ consists of all λ's for which the *relevant aspects* have the same *truth-values* on the respective $\mathcal{M}_\lambda$'s [e.g. "$\mathcal{M}_\lambda$ has five steady-states" is *true*; "$\mathcal{M}_\lambda$ has three stable steady states" is *false*; "$\mathcal{M}_\lambda$ has no periodic orbit" is *false*, ...]. Hence, one has that

$$\mathcal{SC}^{\mathcal{M}} = \left\{sc^{\mathcal{M}}_{\lambda} : \lambda \in \Lambda\right\} \tag{2.72}$$

is another representation for the *scenario space* $\mathcal{SC}^{\mathcal{M}}$.

By construction, one has that assertions on a specific model $\mathcal{M}_\lambda$ lead to assertions on a specific scenario $sc^{\mathcal{M}}_{\lambda}$. Moreover, if an assertion $A \in \mathcal{A}$ is *true* or *false* for a specific $\lambda_0 \in [\lambda]$, then it must be *true* or *false* for any representative $\hat{\lambda}_0 \in [\lambda]$, respectively. Therefore, for all $\lambda \in \Lambda$, one has that $A[sc^{\mathcal{M}}_{\lambda}]$ is well-defined for any $A \in \mathcal{A}$ used to define $\sim_{\mathcal{A}}$.

Now, let $\lambda_0 \in \Lambda$ and let $sc^{\mathcal{M}}_{\lambda_0}$ be the respective *scenario*, that is, the respective *equivalence class* in $\mathcal{SC}^{\mathcal{M}}$. So, let

$$\Xi[sc^{\mathcal{M}}_{\lambda_0}] := \left\{A[sc^{\mathcal{M}}_{\lambda_0}] : A \in \mathcal{A} \text{ and } A \to \top\right\} \tag{2.73}$$

denote the set of *relevant aspects* in $\mathcal{A}$ of $sc_{\lambda_0}^{\mathcal{M}}$ that are indeed *true*, and let

$$\widehat{\Xi[sc_{\lambda_0}^{\mathcal{M}}]} := \bigcap_{\lambda \in sc_{\lambda_0}^{\mathcal{M}}} \mathcal{A}[\mathcal{M}_\lambda] \tag{2.74}$$

symbolize the set of all formalized *mathematical assertions* on $sc_{\lambda_0}^{\mathcal{M}}$, while

$$\overline{\Xi[sc_{\lambda_0}^{\mathcal{M}}]} := \left\{ A \in \widehat{\Xi[sc_{\lambda_0}^{\mathcal{M}}]} : \bigvee_{\Gamma_A \subset \Xi[sc_{\lambda_0}^{\mathcal{M}}]} \Gamma_A \vdash A \right\} \tag{2.75}$$

represents a subset of $\widehat{\Xi[sc_{\lambda_0}^{\mathcal{M}}]}$ consisting of all formalized *mathematical assertions* on $sc_{\lambda_0}^{\mathcal{M}}$ that can be formally proved with assumptions in $\Xi[sc_{\lambda_0}^{\mathcal{M}}]$. Hence, by definition, one has that

$$A \to \top, \tag{2.76}$$

for all $A \in \overline{\Xi[sc_{\lambda_0}^{\mathcal{M}}]}$ and that

$$\Xi[sc_{\lambda_0}^{\mathcal{M}}] \subseteq \overline{\Xi[sc_{\lambda_0}^{\mathcal{M}}]} \subset \widehat{\Xi[sc_{\lambda_0}^{\mathcal{M}}]} \subset \Sigma_{\mathcal{L}^*}. \tag{2.77}$$

Next, for $q \in \mathbb{N}_{>0}$, let

$$A_{i_1}[sc_{\lambda_0}^{\mathcal{M}}], \;\; A_{i_2}[sc_{\lambda_0}^{\mathcal{M}}], \;\; \ldots, \;\; A_{i_q}[sc_{\lambda_0}^{\mathcal{M}}] \in \widehat{\Xi[sc_{\lambda_0}^{\mathcal{M}}]} \tag{2.78}$$

denote *assertions* on *scenario* $sc_{\lambda_0}^{\mathcal{M}}$ solely designed by the mathematical analysis of the model $\mathcal{M}$. Suppose that the assertions on $sc_{\lambda_0}^{\mathcal{M}}$ in (2.78) are indeed true. Consistent with the *Fregean notation*, one has that

$$\vdash A_{i_1}[sc_{\lambda_0}^{\mathcal{M}}], \;\vdash A_{i_2}[sc_{\lambda_0}^{\mathcal{M}}], \ldots, \;\vdash A_{i_q}[sc_{\lambda_0}^{\mathcal{M}}] \tag{2.79}$$

represent the respective correct judgements. But, mathematically speaking, what do we actually mean with (2.78) and (2.79)? As illustrated in Figure 2.1, it means that the *judging agent*, the one who mathematically analyses the model $\mathcal{M}$, proposes assertions on the *scenario* $sc_{\lambda_0}^{\mathcal{M}}$; and this *act of asserting* is followed by the *act of proving*, which ends up in the act of judging the assertions in (2.78) as true ones (or as false ones). Under a *non-empirical notion of judgment*, that is, an acknowledgement of the truth of an assertion, one has that 'the *act of judging* is the very *act of knowing*' as literally stated by Dr. Martin Löf in [55]. Hence, one can say that the correct judgements [*mathematical theorems*] in (2.79) are the objects of our knowledge as depicted in Figure 2.2. In this regard, we can say that the correct judgements in (2.79) constitute some of the properties of the *scenario* $sc_{\lambda_0}^{\mathcal{M}}$, and we are then entitled of making the claim that we have relevant knowledge[21] of *scenario* $sc_{\lambda_0}^{\mathcal{M}}$. But, why relevant? In fact, 'having relevant knowledge of *scenario* $sc_{\lambda_0}^{\mathcal{M}}$ ' can only be apprehended from the first-person perspective seeing that the *judging agent* is the one who performs the mathematical analysis of the model so he stipulates by himself what is relevant to know therefrom.

If we recall that we aim to provide a rational strategy for the *evaluation* of a phenomenological mathematical model and that a *scenario* is meant to be the *mathematical counterpart* of an *observation* provided by the *target system*, then we need

[21] We have defined 'knowledge' as a justified belief.

to clarify the rationale of such an *evaluation*. To begin with, to what extent can a model be regarded as a successful representation of the *target system*? In order to answer this question, we build on the analytical framework introduced in [39]. In fact, we regard *similarity* and *adequacy* as the two test-hypothesis so as to evaluate a model. As for *similarity*, we quote from Dr. Melissa Jacquart in [39]:

"For Weisberg, evaluation of the similarity-relation hypothesis is about assessing the "goodness of fit" between the model and the target system. The aspects of this evaluation are captured through the model's construal—the relevant intentions of the modeller. Recall, the construal of a model is composed of four parts: assignment, scope, and two kinds of fidelity criteria. Assignment and scope track how the real-world phenomena are intended to be represented in the model. The fidelity criteria provide the standards modellers use to evaluate a model's ability to represent the phenomena (...). On this view, similarity assessment is a central component to fit. For a model to fit, and therefore be successful, it must be grounded in the similarity relation."

Hence, we can conclude from the later quotation that Dr. Michael Weisberg's account of *similarity*, see [105], draws our attention to the *intentionality* and to the *third-person perspective*, as we discussed earlier. Why? Despite the fact that a phenomenological mathematical model is a product of a subjective experience of the *modeling agent*, an evaluation of some of the choices that she makes when creating such a mathematical representation, can only be apprehended from a *third-person perspective* by means of *empirical evidences*. Furthermore, as far as the author of this thesis can see, Dr. Michael Weisberg argues that the intentions of the *modeling agent* stipulates how the *similarity-hypothesis* ought to be tested. So, if the mathematical representation, that is, the *model* $\mathcal{M}$ itself, is similar enough to the *target system* 'for the purpose of explaining a set of observable properties thereof' then we say that the *similarity-hypothesis* is true. As for *adequacy*, we quote from Dr. Wendy Parker in [65]:

"In order to argue that we have confirmed or disconfirmed such an adequacy hypothesis, we will need to (i) determine what we are likely to observe if it is true that the model is adequate for the purpose(s) of interest and then (ii) check how well what is actually observed fits with what we are likely to observe if the model is adequate. If what is actually observed fits well enough, then the observation confirms the hypothesis that the model is adequate for the purpose(s) of interest."

So, we can draw from the later quotation that, if for each *observation* of interest generated by the *target sytem*, there is a *scenario* whose properties fit the properties of the respective *observation*, then we say that the model $\mathcal{M}$ is 'adequate for the purpose of explaining a given set of properties of the observations formed by the *target system*'. More specifically, being adequate means whether or not the model is able to yield correct information about the *target system* concerning specific phenomena of interest. So, if this criterion is fulfilled then we say that the *adequacy-hypothesis* is true. In this case, one has that such *scenarios* are said to be similar to the respective observations. Consistent with an early notation, for $\lambda \in \Lambda$ one has that

$$sc_{\lambda}^{\mathcal{M}} \sim \mathcal{O} \tag{2.80}$$

betokens that *scenario* $sc_{\lambda}^{\mathcal{M}}$ is similar [$\sim$] to *observation* $\mathcal{O}$. Moreover, if the *similarity-hypothesis* and the *adequacy-hypothesis* are true then we regard the *model* as a *reliable representation* of the *target system*. What should we be emphasizing in the later test-hypotheses? In fact, if we want to understand them, then we need to acknowledge the *empirical notion of judgment* as a necessary condition for us to do that. In this regard, we regard a *judgment* as a mental activity in response to an *empirical process*; which, in fact, can only be apprehended from a *third-person perspective* by means of *empirical evidences*.

Now, let

$$\Xi[\mathcal{O}] \tag{2.81}$$

denote the set of all relevant properties of an *observation* $\mathcal{O}$ imposed by the *modelling agent*. Thereby, one can 'translate'[22] the *adequacy-hypothesis* as the 'formula'

$$\bigwedge_{\mathcal{O}\in\mathcal{O}_{TS}} \bigvee_{sc_{\lambda}^{\mathcal{M}}\in\mathcal{SC}^{\mathcal{M}}} \Xi[\mathcal{O}] \subseteq \overline{\Xi[sc_{\lambda}^{\mathcal{M}}]} \leftrightarrow sc_{\lambda}^{\mathcal{M}} \sim \mathcal{O}, \tag{2.82}$$

with $\mathcal{O}_{TS}$ symbolizing the set of all observations of the *target system*. But, what do we mean with $\Xi[\mathcal{O}] \subseteq \overline{\Xi[sc_{\lambda}^{\mathcal{M}}]}$? In fact, as we have acknowledged earlier in this thesis, a *phenomenological mathematical model* is inherently *psychological* with each of its elements [e.g. *biochemical parameters*; *concentration thresholds*] and the relations among them having a specific *intention*. The latter manifests itself in the model with the expression of *judgments* [e.g. "If the concentration threshold of the endoderm-network decreases for a sufficiently low autoactivation of the pluripotent network, then the model has an equilibrium that resembles the endoderm-like state."]. Despite being expressed mathematically, those *judgements* are essentially *psychological* seeing that their *judgeable contents*[*assertions*] are solely stipulated by *empirical evidences*. Hence, one can write

$$\vdash^{\Psi} A_{i_1}^{O},\ \vdash^{\Psi} A_{i_1}^{O}, \ldots,\ \vdash^{\Psi} A_{i_{\tilde{q}}}^{O} \tag{2.83}$$

such that

$$\Xi[\mathcal{O}] = \left\{A_{i_1}^{O},\ A_{i_2}^{O},\ \ldots,\ A_{i_{\tilde{q}}}^{O}\right\}, \tag{2.84}$$

with $\tilde{q} \in \mathbb{N}_{>0}$, and $A_{i_h}^{O}$ being the respective *formalized mathematical assertion* for all $h \in \{1, 2, 3, \ldots, \tilde{q}\}$, that is, the corresponding *sentence* in $\mathcal{L}^*$. Moreover, by invoking an aforesaid elucidation, one has that, in contrast with the *Fregean judgment stroke* $\vdash$, which is an assertive force, acknowledging the *truth* [*objective*] of an *assertion*, the sign $\vdash^{\Psi}$ represents a *mental activity* in reaction to an *empirical process* being expressed in a *mathematical assertion*. In so doing, $\vdash^{\Psi}$ symbolizes that a *judgment* has been made based on *empirical evidences*.

So, if there exists $\lambda_0 \in \Lambda$ for which it is true that

$$\Xi[\mathcal{O}] \subseteq \overline{\Xi[sc_{\lambda_0}^{\mathcal{M}}]} \tag{2.85}$$

then

$$sc_{\lambda_0}^{\mathcal{M}} \sim \mathcal{O}. \tag{2.86}$$

[22] Here, in order to promote a better readability, we mix a natural language [English] with some logical symbols involving the sets $\Xi[\mathcal{O}]$, $\Xi[sc_{\lambda_0}^{\mathcal{M}}]$, $\overline{\Xi[sc_{\lambda_0}^{\mathcal{M}}]}$, and $\widehat{\Xi[sc_{\lambda_0}^{\mathcal{M}}]}$. Nonetheless, we only consider their elements [formalized mathematical assertions on the model $\mathcal{M}$] as being elements of the formal language $\mathcal{L}^*$.

However, if we want to understand the essence of (2.85), then we need to invoke the elucidations (2.53)-(2.63) with respect to the *generation of an observational property* by the model $\mathcal{M}$. In fact, (2.85) holds if and only if for each $h \in \{1, 2, 3, \dots, \tilde{q}\}$ there exists

$$A^{(h)}_{i_1}[sc^{\mathcal{M}}_{\lambda_0}],\ A^{(h)}_{i_2}[sc^{\mathcal{M}}_{\lambda_0}], \dots,\ A^{(h)}_{i_{q(h)}}[sc^{\mathcal{M}}_{\lambda_0}] \in \Xi[sc^{\mathcal{M}}_{\lambda_0}], \tag{2.87}$$

for which, by definition, one has that

$$\vdash A^{(h)}_{i_1}[sc^{\mathcal{M}}_{\lambda_0}],\ \vdash A^{(h)}_{i_2}[sc^{\mathcal{M}}_{\lambda_0}], \dots,\ \vdash A^{(h)}_{i_{q(h)}}[sc^{\mathcal{M}}_{\lambda_0}], \tag{2.88}$$

or equivalently,

$$A^{(h)}_{i_1}[sc^{\mathcal{M}}_{\lambda_0}] \to \top,\ \ A^{(h)}_{i_2}[sc^{\mathcal{M}}_{\lambda_0}] \to \top,\ \ \dots,\ \ A^{(h)}_{i_{q(h)}}[sc^{\mathcal{M}}_{\lambda_0}] \to \top, \tag{2.89}$$

such that

$$A^{(h)}_{i_1}[sc^{\mathcal{M}}_{\lambda_0}],\ A^{(h)}_{i_2}[sc^{\mathcal{M}}_{\lambda_0}], \dots,\ A^{(h)}_{i_{q(h)}}[sc^{\mathcal{M}}_{\lambda_0}] \vdash A^{O}_{i_h}. \tag{2.90}$$

with $q(h) \in \mathbb{N}_{>0}$. In this case, we are then entitled to write that

$$\vdash A^{O}_{i_h} \tag{2.91}$$

with

$$A^{O}_{i_h} \in \Xi[sc^{\mathcal{M}}_{\lambda_0}]. \tag{2.92}$$

In this regard, if

$$\mid \Xi[\mathcal{O}] \mid < \mid \overline{\Xi[sc^{\mathcal{M}}_{\lambda_0}]} \mid, \tag{2.93}$$

or equivalently, if

$$\overline{\Xi[sc^{\mathcal{M}}_{\lambda_0}]} \backslash \Xi[\mathcal{O}] \neq \emptyset \tag{2.94}$$

then the latter set, under the *similarity-hypothesis*, can be thought to account for the results of new *experiments*. Nonetheless, drawing upon (2.82), if there is $h_0 \in \{1, \dots, \tilde{q}\}$ with

$$\left(A_{i_{h_0}} \in \Xi[\mathcal{O}]\right) \wedge \left(A_{i_{h_0}} \notin \overline{\Xi[sc^{\mathcal{M}}_{\lambda_0}]}\right) \wedge \left(\Xi[\mathcal{O}] \backslash \left\{A_{i_{h_0}}\right\} \subseteq \overline{\Xi[sc^{\mathcal{M}}_{\lambda_0}]}\right), \tag{2.95}$$

then one has that $sc^{\mathcal{M}}_{\lambda_0}$ is not similar to the *observation* $\mathcal{O}$. In the latter case, how should we further proceed with the evaluation of the model? Or rather, how should we keep searching for a *scenario* in the *parameter space*, similar [$\sim$] to the *observation* $\mathcal{O}$? Intuitively, as one hypothetically has some relevant knowledge of the *scenario* $sc^{\mathcal{M}}_{\lambda_0}$ then, under the hypothesis that it does not have the property $A_{i_{h_0}}$, the *judging agent*, by means of mathematical analysis, might suitably construct a *function* Π_1 on the *parameter space* Λ, namely

$$\begin{aligned} \Pi_1 \colon \Lambda &\to \Lambda \\ \lambda &\mapsto \Pi_1(\lambda) \end{aligned}$$

where

$$\Pi_1(\lambda) = \begin{cases} \lambda & \text{if } \lambda \notin [\lambda_0], \\ \lambda_1 & \text{if } \lambda \in [\lambda_0], \end{cases} \tag{2.96}$$

with $\lambda_1 \in \Lambda$ being a choice determined by the performed mathematical analysis. Actually, in the framework of dynamical systems to which we shall turn ourselves

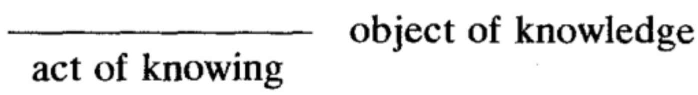


Figure 2.2: This figure has been taken from [57]. The act of knowing as the very act of judging so a correct judgment [mathematical theorem] is said to be the object of our knowledge.

later in Chapter 4, λ_1 ought to be thought as a point at which a bifurcation has occurred thus, consistently, one has that Π_1 must be conditioned upon

$$[\lambda_1] \neq [\lambda_0].$$

Next, drawing upon (3.246), such a Π_1-function on the *parameter space* Λ naturally gives rise to a $\bar{\Pi}_1$-function on the *scenario space*, namely

$$\begin{aligned} \bar{\Pi}_1 \colon \mathcal{SC}^{\mathcal{M}} &\to \mathcal{SC}^{\mathcal{M}} \\ sc_{\lambda}^{\mathcal{M}} &\mapsto \bar{\Pi}_1[sc_{\lambda}^{\mathcal{M}}] \end{aligned}$$

provided that

$$\bar{\Pi}_1[sc_{\lambda}^{\mathcal{M}}] := q \circ \Pi_1(\lambda), \tag{2.97}$$

for all $\lambda \in \Lambda$. Now, if the *judging agent* can show that the *scenario*

$$\Pi_1[sc_{\lambda_0}^{\mathcal{M}}] := sc_{\lambda_1}^{\mathcal{M}} \tag{2.98}$$

is similar [$\sim$] to the *observation* $\mathcal{O}$, that is,

$$\Pi_1[sc_{\lambda_0}^{\mathcal{M}}] \sim \mathcal{O}, \tag{2.99}$$

then the 'search' can stop at λ_1.

More generally, suppose that there is a subset

$$\{h_1, h_2, \dots, h_m\} \subset \{1, 2, \dots, \tilde{q}\} \tag{2.100}$$

with $h_m < \tilde{q}$ such that

$$\Xi[\mathcal{O}] \setminus \{A_{h_1},\ A_{h_2},\ \dots,\ A_{h_m}\} \subseteq \overline{\Xi[sc_{\lambda_0}^{\mathcal{M}}]}, \tag{2.101}$$

with

$$\vdash^{\Psi} A_{h_1},\ \vdash^{\Psi} A_{h_2},\ \dots,\ \vdash^{\Psi} A_{h_m}, \tag{2.102}$$

and

$$\bigwedge_{s \in \{1,2,\dots,m\}} (A_{i_{h_s}} \in \Xi[\mathcal{O}]) \wedge \left(A_{i_{h_s}} \notin \overline{\Xi[sc_{\lambda_0}^{\mathcal{M}}]}\right), \tag{2.103}$$

then, intuitively, for each $s \in \{1, 2, \dots, m-1\}$, the *judging agent*, by means of mathematical analysis, might construct a Π_s-function on the parameter space, namely

$$\begin{aligned} \Pi_s \colon \Lambda &\to \Lambda \\ \lambda &\mapsto \Pi_s(\lambda) \end{aligned}$$

being conditioned upon

$$[\lambda_s] \neq [\lambda_r]$$

for all $r \in \{0, 1, \ldots, s-1\}$, such that

$$\Pi_s(\lambda) = \begin{cases} \lambda & \text{if } \lambda \notin [\lambda_{s-1}], \\ \lambda_s & \text{if } \lambda \in [\lambda_{s-1}], \end{cases} \tag{2.104}$$

with $\lambda_s \in \Lambda$ being chosen according to the performed mathematical analysis, so that the *scenario*

$$\bar{\Pi}_s[sc^{\mathcal{M}}_{\lambda_{s-1}}] \coloneqq sc^{\mathcal{M}}_{\lambda_s} \tag{2.105}$$

satisfies

$$\Xi[\mathcal{O}] \backslash \left\{A_{h_{s+1}},\ A_{h_{s+2}},\ \ldots,\ A_{h_m}\right\} \subseteq \overline{\Xi[\bar{\Pi}_s[sc^{\mathcal{M}}_{\lambda_{s-1}}]]}, \tag{2.106}$$

with

$$\vdash^{\Psi} A_{h_{s+1}},\ \vdash^{\Psi} A_{h_{s+2}},\ \ldots,\ \vdash^{\Psi} A_{h_m}, \tag{2.107}$$

and

$$\bigwedge_{j \in \{s+1, \ldots, m\}} \left(A_{i_{h_j}} \in \Xi[\mathcal{O}]\right) \wedge \left(A_{i_{h_j}} \notin \overline{\Xi[\bar{\Pi}_s[sc^{\mathcal{M}}_{\lambda_{s-1}}]]}\right). \tag{2.108}$$

Thereby, by induction, for $s = m-1$, one has that

$$\Xi[\mathcal{O}] \backslash \{A_{h_m}\} \subseteq \overline{\Xi[\bar{\Pi}_{m-1}[sc^{\mathcal{M}}_{\lambda_{m-2}}]]}, \tag{2.109}$$

and that

$$\left(A_{i_{h_m}} \in \Xi[\mathcal{O}]\right) \wedge \left(A_{i_{h_m}} \notin \overline{\Xi[\bar{\Pi}_{m-1}[sc^{\mathcal{M}}_{\lambda_{m-2}}]]}\right), \tag{2.110}$$

with

$$\vdash^{\Psi} A_{i_{h_m}}. \tag{2.111}$$

So, by building upon the same argument, if we bear in mind that the *judging agent* hypothetically does know some relevant *aspects* of the *scenario* $sc^{\mathcal{M}}_{\lambda_{m-1}}$, then she might suitably construct a Π_m-function on the *parameter space* Λ, namely

$$\begin{aligned} \Pi_m \colon \Lambda &\to \Lambda \\ \lambda &\mapsto \Pi_m(\lambda) \end{aligned}$$

conditioned on

$$[\lambda_m] \neq [\lambda_r],$$

for all $r \in \{1, 2, \ldots, m-1\}$, such that

$$\Pi_m(\lambda) = \begin{cases} \lambda & \text{if } \lambda \notin [\lambda_{m-1}], \\ \lambda_m & \text{if } \lambda \in [\lambda_{m-1}], \end{cases} \tag{2.112}$$

with $\lambda_m \in \Lambda$ being chosen when performing the mathematical analysis, such that she can show that the *scenario*

$$\bar{\Pi}_m[sc^{\mathcal{M}}_{\lambda_{m-1}}] \coloneqq sc^{\mathcal{M}}_{\lambda_m} \tag{2.113}$$

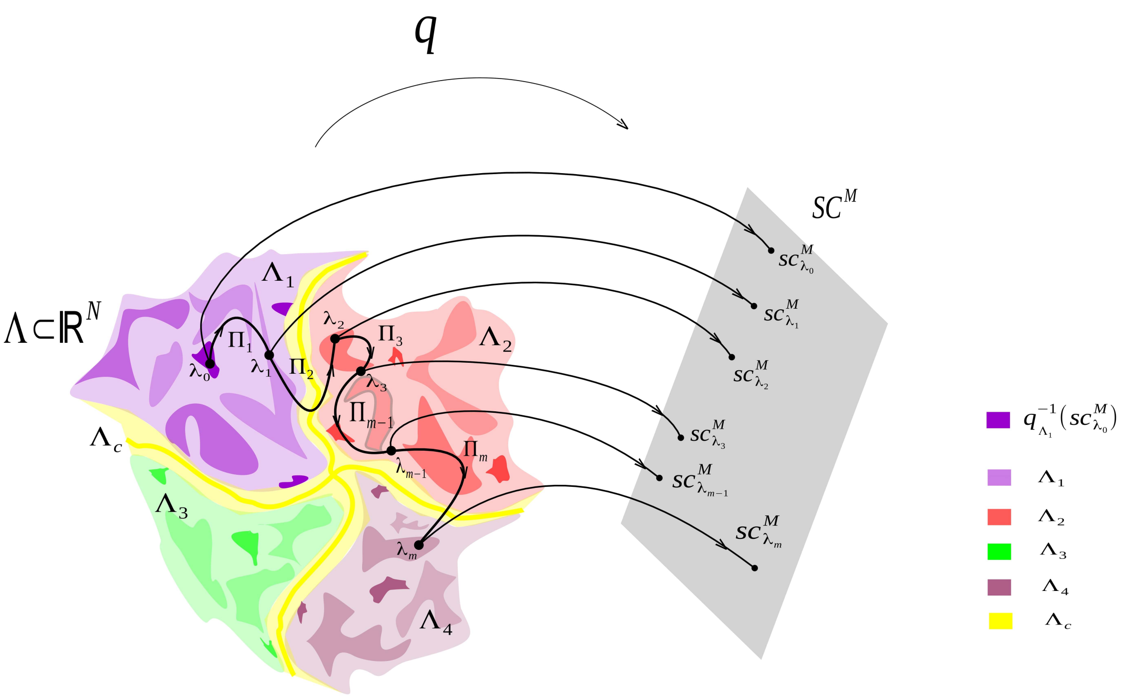


Figure 2.3: *Here, we have a cartoon in which one sees a suitable intuitive depiction of the canonical map* q*. In fact, by definition, one has that the scenario space* $\mathcal{SC}^{\mathcal{M}}$ *is discrete whereas* Λ_1, Λ_2, Λ_3, *and* Λ_4 *hypothetically represent the four main components of the parameter space* Λ*. The other component of the parameter space is given by a transition zone characterized by a lower dimensional layer* Λ_c *setting the frontier among the main components. Each of the main components is being illustrated as the union of mutually exclusive sets, which are thought to be entirely determined by the inverse image of* q^{-1}*. In fact, the subset* $q_{\Lambda_1}^{-1}(sc_{\lambda_0}^{\mathcal{M}}) = q^{-1}(sc_{\lambda_0}^{\mathcal{M}}) \bigcap \Lambda_1$ *consists of parameters which generate the primitive scenario* $sc_{\lambda_0}^{\mathcal{M}}$*, i.e. the equivalent class of* λ_0 *of which* λ_0 *is one of its representatives. As we see here,* $\lambda_0 \in q_{\Lambda_1}^{-1}(sc_{\lambda_0}^{\mathcal{M}})$*. Furthermore,* $q_{\Lambda_1}^{-1}(sc_{\lambda_0}^{\mathcal{M}})$ *is being illustrated as a subset of* Λ_1 *with the smallest volume so as to emphasize the primitiveness of* $q^{-1}(sc_{\lambda_0}^{\mathcal{M}})$*. How does this minimality in the volume representation of* $q_{\Lambda_1}^{-1}(sc_{\lambda_0}^{\mathcal{M}})$ *suit the intuitive purpose of expressing the primitiveness of* $q^{-1}(sc_{\lambda_0}^{\mathcal{M}})$ *? In fact, it accentuates its irreducibility and, more importantly, suffices to depict the fact that such a set in the parameter space is apprehended to be significantly constrained. Lastly, we see the qualitative path* $\Pi_m \circ \ldots \circ \Pi_1(\lambda_0)$ *going from* $\lambda_0 \in \Lambda_1$ *to* $\lambda_m \in \Lambda_4$ *in the parameter space.*

is similar [∼] to the *observation* $\mathcal{O}$, that is,

$$\bar{\Pi}_m[sc^{\mathcal{M}}_{\lambda_{m-1}}] \sim \mathcal{O}, \tag{2.114}$$

or equivalently,

$$\Xi[\mathcal{O}] \subseteq \overline{\Xi[sc^{\mathcal{M}}_{\lambda_m}]}. \tag{2.115}$$

Moreover, consistently, for each $s \in \{1, 2, \ldots, m\}$, one has that

$$\begin{aligned} \bar{\Pi}_s \colon \mathcal{SC}^{\mathcal{M}} &\to \mathcal{SC}^{\mathcal{M}} \\ sc^{\mathcal{M}}_{\lambda} &\mapsto \bar{\Pi}_s[sc^{\mathcal{M}}_{\lambda}] \end{aligned}$$

with

$$\bar{\Pi}_s[sc^{\mathcal{M}}_{\lambda}] \coloneqq q \circ \Pi_s(\lambda), \tag{2.116}$$

for all $\lambda \in \Lambda$ as depicted in Figure 2.4.

Now, what is the main hypothesis in the latter argument leading to the 'construction' of the *scenario* $sc^{\mathcal{M}}_{\lambda_m}$ similar [∼] to the observation $\mathcal{O}$? In fact, it has been assumed that the *judging agent* does know relevant *aspects* of *scenario* $sc^{M}_{\lambda_0}$. So, the essence of our argument is to know whether or not the scenario $sc^{M}_{\lambda_0}$ generates all the *observational properties* in $\mathcal{O}_{TS}$, which, in turn, immediately turns our attention to the ongoing question of this section, that is, how are the *conception order*, the concept of *primitive notion*, the concept of *judgment* and the *first-person perspective* fundamentally related to evaluation of *phenomenological mathematical models*? First of all, in our intuitive construction, if we know *scenario* $sc^{M}_{\lambda_0}$ then we can know *scenario* $sc^{M}_{\lambda_1}$, which, in turn, by induction, implies that we can know *scenario* $sc^{M}_{\lambda_m}$. So, such a pattern resembles the conceptual order reflected by a chain of concepts conceptually dependent upon each other. However, regarding our intuitive construction, for $l \in \{1, \ldots, m\}$, it must be emphasized that, in no way are we claiming that a necessary condition to have knowledge of scenario $sc^{\mathcal{M}}_{\lambda_l}$ is to have knowledge of $sc^{\mathcal{M}}_{l-1}$, but rather, if we have knowledge of $sc^{\mathcal{M}}_{l-1}$ then we can have knowledge of $sc^{\mathcal{M}}_{\lambda_l}$ with respect to a particular set of *observational properties* $\mathcal{O}_{TS}$.

Having said that, let $\mathcal{P}(\mathcal{A}) \coloneqq \{\Xi : \Xi \subset \mathcal{A}\}$ denote the set of all subsets of $\mathcal{A}$. Then, one has that $\mathcal{P}(\mathcal{A})$ is a partially ordered set with the partial order being given by the containment relationship, that is, $(\mathcal{P}(\mathcal{A}), \subseteq)$. Now, if one defines

$$\begin{aligned} \tilde{q} \colon \mathcal{SC}^{\mathcal{M}} &\to \mathcal{P}(\mathcal{A}) \\ sc^{\mathcal{M}}_{\lambda} &\mapsto \Xi[sc^{\mathcal{M}}_{\lambda}] \end{aligned}$$

then, under the presumption that the *judging agent* has knowledge of some relevant *aspects* of $sc^{\mathcal{M}}_{\lambda_0}$ and under the assumption that it is not true that

$$sc^{\mathcal{M}}_{\lambda_0} \sim \mathcal{O}, \tag{2.117}$$

and by drawing upon the same argument concerning the construction of the Π-functions, one can consider the mapping

$$\begin{aligned} \hat{\Pi}_1 \colon \tilde{q}(\mathcal{SC}^{\mathcal{M}}) &\to \tilde{q}(\mathcal{SC}^{\mathcal{M}}) \\ \Xi &\mapsto \hat{\Pi}_1\left[\Xi\right] \end{aligned}$$

with

$$\tilde{q}(\mathcal{SC}^{\mathcal{M}}) \coloneqq \left\{\Xi[sc^{M}_{\lambda}] \in \mathcal{P}(\mathcal{A}) : \lambda \in \Lambda\right\}, \tag{2.118}$$

such that

$$\hat{\Pi}_1(\Xi) = \begin{cases} \Xi & \text{if } \Xi \neq \Xi[sc^{\mathcal{M}}_{\lambda_0}], \\ \Xi_1 & \text{if } \Xi = \Xi[sc^{\mathcal{M}}_{\lambda_0}], \end{cases} \tag{2.119}$$

with

$$\Xi_1 \subseteq \Xi[sc^{\mathcal{M}}_{\lambda_1}], \tag{2.120}$$

provided that

$$\bar{\Pi}_1[sc^{\mathcal{M}}_{\lambda_0}] := q \circ \Pi_1(\lambda_0) = sc^{\mathcal{M}}_{\lambda_1}, \tag{2.121}$$

and that Π_1 is being conditioned upon

$$[\lambda_1] \neq [\lambda_0].$$

But, How should we apprehend the $\hat{\Pi}_1$-mapping? In fact, $\hat{\Pi}_1(\Xi)$ can only be understood from a *first-person perspective* seeing that it is stipulated by the Π_1-function, which, in fact, is constructed on the basis of the mathematical analysis performed by the *judging agent*. And what is the essence of the $\hat{\Pi}_1$-mapping? It is indeed a *knowledge-transformation mapping*. Further in this thesis, without loss of generality, we shall refer to the Π-functions as the actual *knowledge-transformation mappings*. How should we understand the inequality (2.120)? Or rather, what are the essential properties that such a mapping $\hat{\Pi}_1$ is thought to satisfy? In fact,

$$\hat{\Pi}_1(\emptyset) = \emptyset, \tag{2.122}$$

that is, if we have no knowledge of a *scenario* then we cannot extract any knowledge from that. Next, one has that $\hat{\Pi}_1$ is monotonically non-decreasing, that is, for $\Xi_1, \Xi_2 \in \tilde{q}(\mathcal{SC}^{\mathcal{M}})$, if

$$\Xi_1 \leq \Xi_2 \tag{2.123}$$

then

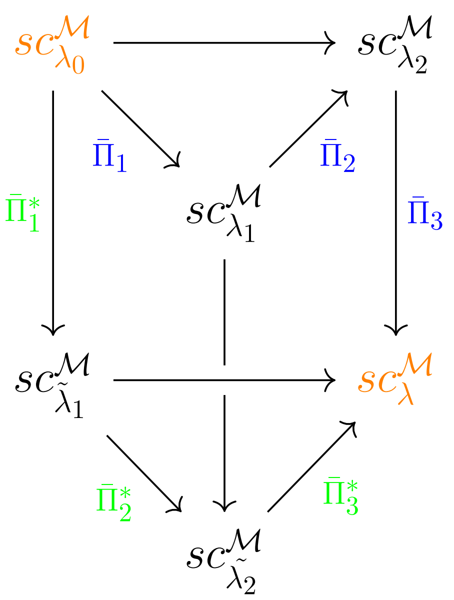
$$\hat{\Pi}_1(\Xi_1) \leq \hat{\Pi}_1(\Xi_2), \tag{2.124}$$

which means that one ought to apprehend the inequality (2.120) as follows. In fact, partial knowledge of the relevant *aspects* of $\Xi[sc^{\mathcal{M}}_{\lambda_0}]$ leads to partial knowledge of the relevant aspects of $\Xi[sc^{\mathcal{M}}_{\lambda_1}]$. So, (2.124) is an essential property to being acknowledged seeing that it enables the *judging agent* to build a chain of *knowledge-transformation*

$$\hat{\Pi}_1,\ \hat{\Pi}_2,\ \ldots,\ \hat{\Pi}_m \tag{2.125}$$

with respect to the set of *observational properties* $\mathcal{O}_{TS}$.

Hence, if she succeeds in finding Π-functions with which, at each step, she obtains close to total knowledge of the relevant *aspects* of the subsequent scenarios in the chain, then she will be likely to decide whether or not an *observational property* can be generated by the respective *scenarios* in the chain, which, in turn, reduces a continuous search to an algorithmic search. But, what do the Π-functions stand for in our philosophical approach? In fact, they are entirely constructed on the basis of the *judgements* made by the *judging agent*. In addition hereto, the latter chain of *knowledge-transformation* $\hat{\Pi}$-mappings can be visualized in the *parameter space* as a *qualitative path* generated by the respective Π-functions, as illustrated in Figure 2.3, or as a *qualitative graph* induced by the $\bar{\Pi}$-functions as shown in (2.126).



(2.126)

So, the burden of proof is upon the *judging agent* to build suitable $\bar{\Pi}$-functions which will unveil scenarios that preserve those matching properties, and annihilate those ones which contradict some of the properties describing the *observations* in $\mathcal{O}_{TS}$. Further, provided that we are under the assumption that the *judging agent* has total knowledge of the relevant *aspects* of *scenario* $sc^{\mathcal{M}}_{\lambda_0}$, one has that the *scenario* $sc^{\mathcal{M}}_{\lambda_0}$ seems to be more 'fundamental' than the *scenario* $sc^{\mathcal{M}}_{\lambda_1}$, of which, under Π_1 and in view of (2.120), the *judging agent* has at least a partial knowledge of its relevant *aspects*, and at most a total knowledge thereof. Therefore, one has that the role of the *scenario* $sc^{\mathcal{M}}_{\lambda_0}$ resembles the fundamental role of *primitive notions* in the *conceptual order*. In fact, if we can conveniently define the concept of a *primitive scenario* so that it somehow resembles the conceptual role of a *primitive notion*, that is, being somehow irreducible, then one has that the *scenario* $sc^{M}_{\lambda_0}$ can be regarded as a primitive one in our intuitive construction. Hence, intuitively, if an arbitrary *scenario* $sc^{\mathcal{M}} \in SC^{\mathcal{M}}$ may be represented [*decomposed*] as

$$sc^{\mathcal{M}} = \bar{\Pi}_m \circ \bar{\Pi}_{m-1} \ldots \circ \bar{\Pi}_2 \circ \bar{\Pi}_1[sc^{\mathcal{M}}_{\lambda_0}], \tag{2.127}$$

with

$$\bar{\Pi}_{r+1} \circ \bar{\Pi}_r \tag{2.128}$$

denoting the composition of $\bar{\Pi}$-functions for each $r \in \{1, 2, \ldots, m-1\}$, $m \in \mathbb{N}_{>0}$, $sc^{\mathcal{M}}_{\lambda_0}$ being a *primitive scenario*, and $\bar{\Pi}_r$ being defined in (2.116), then it seems that our rational strategy offers a way in which we could shrewdly walk through the *scenario space* $\mathcal{SC}^{\mathcal{M}}$ so as to test the *adequacy-hypothesis*. Moreover, consistent with the definition (2.116), one has that

$$sc^{\mathcal{M}} = sc^{\mathcal{M}}_{\lambda_m}. \tag{2.129}$$

However, considering that a *phenomenological mathematical model* is supposed to represent a *target system*, one has that the question is not whether or not one can find the representation [*decomposition*] proposed in (2.127) for an arbitrary *scenario* in $\mathcal{SC}^M$, but whether or not, one can find scenarios in the *scenario space* that are similar [$\sim$] to the *observations* of interest in the *target system*. So, in this case, if we know how to find a *primitive scenario* anywhere within the *scenario space* then we might suitably shift it therein. In so doing, the shifting process is thought to reveal *scenarios* which, in turn, might be regarded as the *mathematical counterparts* of the respective *observations*. Therefore, perhaps, a better representation of (2.127) reads

$$sc^{\mathcal{M}}_{\lambda_0} \xrightarrow{\bar{\Pi}_1} sc^{\mathcal{M}}_{\lambda_1} \xrightarrow{\bar{\Pi}_2} sc^{\mathcal{M}}_{\lambda_2} \xrightarrow{\bar{\Pi}_3} \dots \xrightarrow{\bar{\Pi}_{m-2}} sc^{\mathcal{M}}_{\lambda_{m-2}} \xrightarrow{\bar{\Pi}_{m-1}} sc^{\mathcal{M}}_{\lambda_{m-1}} \xrightarrow{\bar{\Pi}_m} sc^{\mathcal{M}}. \tag{2.130}$$

Lastly, as we see in the diagram (2.126), one has that

$$sc^{\mathcal{M}} = \bar{\Pi}_3 \circ \bar{\Pi}_2 \circ \bar{\Pi}_1[sc^{\mathcal{M}}_{\lambda_0}], \tag{2.131}$$

and that

$$sc^{\mathcal{M}} = \bar{\Pi}^*_3 \circ \bar{\Pi}^*_2 \circ \bar{\Pi}^*_1[sc^{\mathcal{M}}_{\lambda_0}], \tag{2.132}$$

which means that the representation [*decomposition*] in (2.127) is not necessarily unique.

2.8 A concise description of the proposed evaluation

But, how do we intend applying this method to our analysis? First of all, we need to understand the structure of the parameter space $\Lambda \in \mathbb{R}^N$ ($N \in \mathbb{N}_{>0}$). Indeed, if we now presuppose that two different parameter settings λ and $\tilde{\lambda}$ can presumably give rise to different model's properties, one has that there must be a lower dimensional layer $\Lambda_c \subset \Lambda$ to account for that. So, one has that there must exist $\Lambda_c \subset \Lambda$ which purportedly sets the frontier among *the main components* of the parameter space Λ. But, why does the layer $\Lambda_c \subset \Lambda$ have a lower dimension? Intuitively, Λ_c is thought to form the interface among the *main components* of the parameter space. Indeed, intuitively, for $m \in \mathbb{N}_{>0}$, one must have that

$$\Lambda = \Lambda_c \cup \bigcup_{j=1}^{m} \Lambda_j, \tag{2.133}$$

with Λ_c denoting the *lower dimensional layer* in Λ and with Λ_j representing the main components of Λ for all $j \in \{1, 2, \dots, m\}$ such that

$$\Lambda_r \cap \Lambda_s = \emptyset, \tag{2.134}$$

for $r \neq s$ and $r, s \in \{1, 2, \dots, m\}$.

But, what are the *main components* of the parameter space? How can we apprehend them? In fact, if we acknowledge that there are some *primary aspects* [*properties*] of the model $\mathcal{M}$ that are intrinsically determined by the mathematical formulation thereof [e.g *geometrical aspects*], then it is reasonable to think that those *primary aspects* cause the parameter space to form a kind of graphical structure whose nodes are thought to be given by the *main components* [$\Lambda_1, \dots, \Lambda_m$].

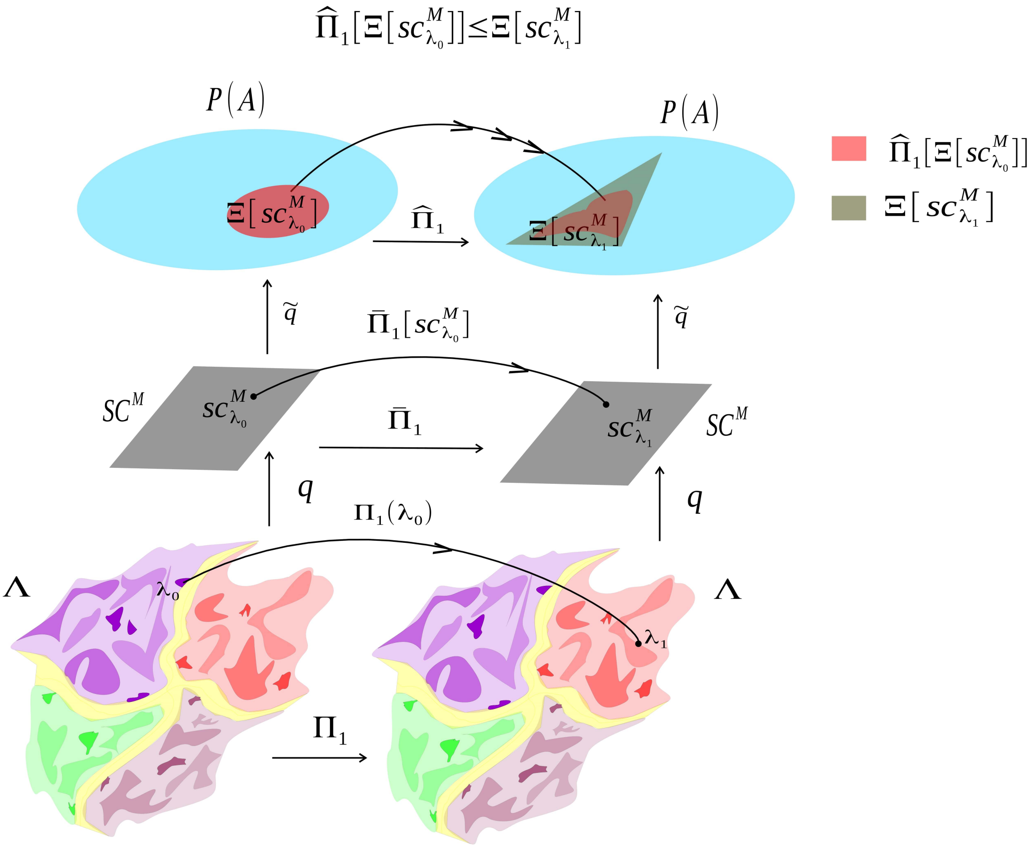


Figure 2.4: This cartoon shows how the judging agent performs the algorithmic search. In fact, with respect to some aspects that are prioritized, she stipulates the primitive scenario $sc^{\mathcal{M}}_{\lambda_0}$. Further, if $sc^{\mathcal{M}}_{\lambda_0}$ is not similar [$\sim$] to the target observation $\mathcal{O}$ then, by means of mathematical analysis, she tries to fully identify the missing aspects and the ones which contradict the target observation $\mathcal{O}$. Upon doing so, she performs a shift in the parameter space toward $\Pi(\lambda_0) = \lambda_1$, which corresponds to a shift in the scenario space toward $\bar{\Pi}[sc^{\mathcal{M}}_{\lambda_0}] = sc^{\mathcal{M}}_{\lambda_1}$, which, in turn, generates knowledge $\hat{\Pi}\left[\Xi[sc^{\mathcal{M}}_{\lambda_0}]\right]$ of $\Xi[sc^{\mathcal{M}}_{\lambda_1}]$.

Indeed, if we regard the main components in (2.133) as *equivalent classes* defined by the set of parameter settings $\lambda \in \Lambda$ for which those *primary aspects* have the same *truth-value*, then we have also justified that the *main components* $[\Lambda_1, \ldots, \Lambda_m]$ are actually *mutually exclusive*. Moreover, as we are interested in models that are *structural stable*, that is, models for which small perturbations of a parameter setting do not alter, in particular, the *truth-value* of those *primary aspects*, then we can justify why the *main components* ["equivalent classes with respect to those primary aspects"] in (2.133) must have non-empty *interior*, i.e.

$$\mathring{\Lambda}_j \neq \emptyset, \tag{2.135}$$

for all $j \in \{1, 2, \ldots, m\}$, which, in turn, implies that the structure arising from the equality (2.133) indeed gives rise to a *qualitative graphical representation* of the *parameter space* Λ that we conveniently call the $\mathcal{M}$-*qualitative graph* as illustrated in Figure 2.3.

In sum, to begin with, the *judging agent* must give a mathematical description of the *main components* of the parameter space with respect to the set of *primary aspects*. Thence, she must then consider *relevant aspects* of the model $\mathcal{M}$ so as to test the *adequacy-hypothesis*. Of course, if we draw upon the same aforementioned equivalence relation then the set of relevant aspects $\mathcal{A}$, which, in fact, is thought to contain the primary aspects, gives rise to other "sort of components" that we have then defined as scenarios. The latter ones form the scenario space $\mathcal{SC}^{\mathcal{M}}$ which, by definition, works as the counterpart of the set of target observations $\mathcal{O}_{\mathcal{TS}}$. How to proceed then? In fact, the *judging agent* must know which relevant aspects must be prioritized in her pursuit of a *scenario* in the *scenario space* $\mathcal{SC}^{\mathcal{M}}$ that is similar [$\sim$] to the target observation $\mathcal{O}$. For example, the relevant aspect being prioritized might be the number of steady states. In this case, a *scenario* with the maximal number of steady states will be the *primitive scenario* playing the role of a *primitive notion*, being irreducible, seeing that it cannot be reduced to any *scenario* with more steady states. So, finding a way in which one can fix such a *primitive scenario* in any of the *main components*

$$\Lambda_1,\ \Lambda_2,\ \Lambda_3,\ \ldots,\ \Lambda_m \tag{2.136}$$

of the parameter space is a challenge given to the *judging agent*. So, she must assure that she knows as many relevant aspects as possible of a *primitive scenario* in order to disregard it, or not, in her search for a *scenario* similar[$\sim$] to the target observation $\mathcal{O}$. Regarding a *primitive scenario*, how should we then proceed in case of missing aspects or aspects that contradict the target observation $\mathcal{O}$? In fact, the *judging agent* must suitably perform the mathematical analysis of the model $\mathcal{M}$ so as to know how to shift scenarios in the scenario space $\mathcal{SC}^{\mathcal{M}}$ until the search is finished, as illustrated in Figure 2.2. Lastly, it is important to stating that the efficiency of the method is predicated upon the presupposition that one does not need to be concerned about giving a detailed mathematical description of the lower dimensional layer provided that it is not generic, thus not observable.

2.9 Conclusion

In the respective chapter, we have introduced a *conceptual framework* in which

Frege's judgment theory can be applied to the evaluation of *phenomenological mathematical models.* We started discussing that, along with the conceptual order, the concept of primitive notion is fundamental to epistemology seeing that it provides a way of defining concepts sequentially. The latter essentially stipulates our rational strategy given that primitive scenarios play the role of primitive notions in our approach. So, intuitively, if scenarios are regarded as the counterparts of observations then knowing the primitive scenarios of the model can potentially lead us to know any scenario of the model, which means that we can potentially know whether or not an observation is actually generated by the model.

Withal, there is no act of knowing whether or not a matching scenario has been found on the *scenario space* without a judging agent to assert that. As the search throughout the *parameter space* is determined by the judgments made by the *judging agent* then one has that the *first-person perspective* is crucial to the evaluation process. These judgements are actually executed by the *knowledge-transformation* Π-*functions*, resulting in a chain of actions described by a *qualitative graph* on the scenario space and by a *qualitative path* on the *parameter space.* Moreover, in order to understand the essence of the *knowledge-transformation* Π-*functions*, one needs to acknowledge the duality of the judging agent as *a non-empirical ego* and as *an empirical ego* respectively. In fact, this duality must be accentuated when distinguishing the two involved perspectives, that is, the first-person perspective (the model as a mathematical object), and the third-person perspective (the model as description of observational properties) concerning the *logical* and the *empirical notion of judgment.*

Lastly, we would like to recognize that in the case of many models such a methodology is not necessary, but we do believe that our approach can have some value in the evaluation of *phenomenological mathematical models* with many parameters, in which structural properties are crucial to testing the adequacy of the model to explain the target observations.

Chapter 3

The analysis of Huang's model

"Among the thousand-and-one faces whereby form chooses to reveal itself to us, the one that fascinates me more than any other, and continues to fascinate me, is the structure hidden in mathematical things."

Alexander Grothendieck

In this chapter, we will provide an analysis of Huang's model by applying the procedure introduced in Chapter 2. We shall see that the key point of our analysis is to determine the possible qualitative behaviors of the nullclines of the model. In fact, the analysis is performed as follows. First, we will give a suitable description of the *scenario space* SC^H of Huang's model, which will enable us to give a graphical description of the parameter space of the model itself, which, in turn, will be of utmost importance to represent the qualitative graphical matrix of the model, or better, the Huang's qualitative graphical matrix.

Secondly, we will stipulate sufficient conditions so as to find the *primitive scenarios* of the model, wherein we will demonstrate the existence of the maximal number of *steady states* [*equilibria*]. Thirdly, we will perform *linear stability analysis* and we will appeal to topological arguments by drawing upon the Poicaré-Bendixon Theorem so as to determine the *(in)stability* of the found *steady states* in the respective *primitive scenarios*.

This chapter is organized as follows. Firstly, we will provide a thorough description of Huang's qualitative graphical matrix. Secondly, we will construct the *primitive scenarios* thereof, wherein we will find the maximal number of steady states that a *scenario* of the *scenario space* SC^H of the model can generate. Lastly, we will conclude this chapter by summarizing it and by providing a concise discussion thereof.

3.1 The qualitative graphical matrix of the model

Drawing upon Section 1.5, one has that the dimensionless dynamical equations of Huang's model read

$$\begin{aligned}\frac{dX}{dt} &= a_X\frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n} - kX,\\ \frac{dE}{dt} &= a_E\frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1+X^n} - kE,\end{aligned} \tag{3.1}$$

with

$$X = \hat{X}/\theta,\ \ E = \hat{E}/\theta,\ \ \theta_X = \hat{\theta}_X/\theta,\ \ \theta_E = \hat{\theta}_E/\theta, \tag{3.2}$$

and

$$t = \hat{t}/\tau,\ \ k = \hat{k}\tau,\ \ a_X = \tau\hat{a}_X/\theta,\ \ a_E = \tau\hat{a}_X/\theta, \tag{3.3}$$

where $\theta > 0$ and $\tau > 0$ are the respective *characteristic concentration* and *time*.

Now, note that

$$\frac{dX}{dt} \le a_X + b - kX, \tag{3.4}$$

so if

$$X > \frac{a_X}{k} + \frac{b}{k} \tag{3.5}$$

then

$$\frac{dX}{dt} < 0. \tag{3.6}$$

Similarly, one has that if

$$E > \frac{a_E}{k} + \frac{b}{k} \tag{3.7}$$

then

$$\frac{dE}{dt} < 0. \tag{3.8}$$

Therefore, the interesting dynamics is confined in the rectangle :

$$(X, E) \in \left[0, \frac{a_X}{k} + \frac{b}{k}\right] \times \left[0, \frac{a_E}{k} + \frac{b}{k}\right]. \tag{3.9}$$

3.1.1 The description of the nullclines $G_{\Psi_{1,n}}$ and $G_{\Psi_{2,n}}$

We now want to find the steady states of the model and perform Linear Stability Analysis. To begin with, we limit ourselves to the steady states with $X, E \ge 0$, as these are biologically relevant. Let $n \in \mathbb{N} \setminus \{0\}$. So, for the system (3.1), one can define

$$\begin{aligned} g_{1,n}(X) &\coloneqq kX - a_X\frac{X^n}{\theta_X^n + X^n},\\ g_{2,n}(E) &\coloneqq kE - a_E\frac{E^n}{\theta_E^n + E^n},\end{aligned} \tag{3.10}$$

and

$$h_n(Z) \coloneqq b\frac{1}{1+Z^n}, \tag{3.11}$$

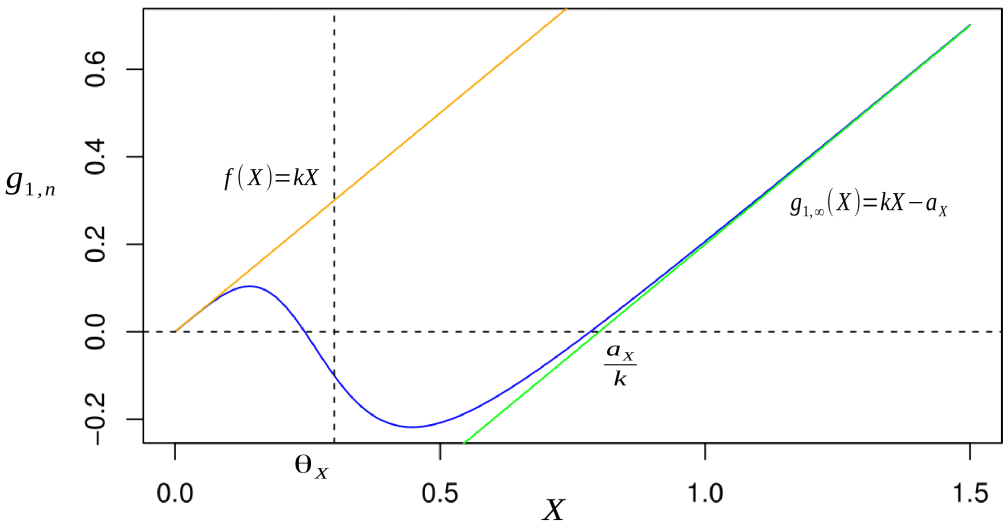


Figure 3.1: Here, one sees the plot of $g_{1,n}$ in blue for the choices $n = 4$, $\theta_X = 0.3$, $k = 1$ and $a_X = 0.8$; where $g_{1,x,\infty} = kX - a_X$.

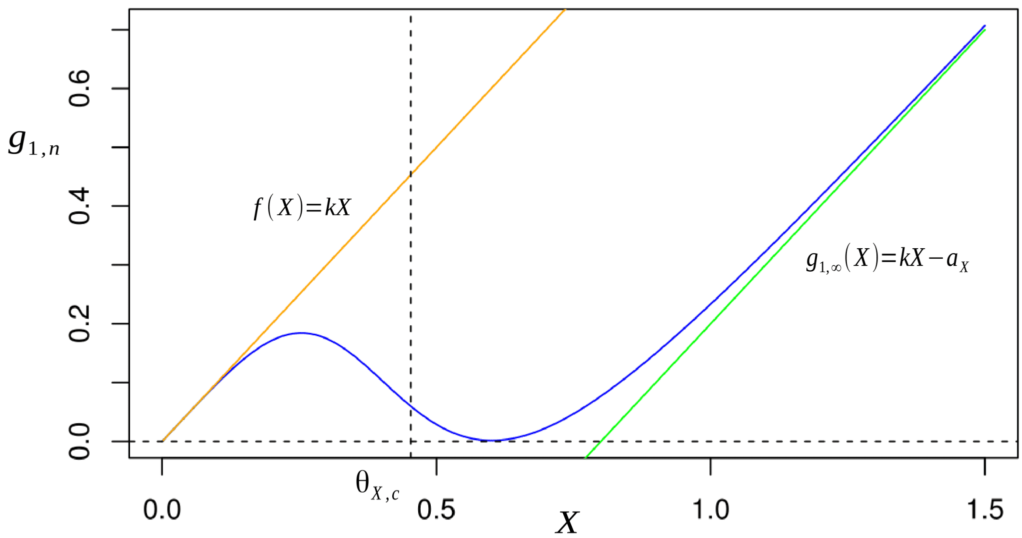


Figure 3.2: Now, we see the plot of $g_{1,n}$ in blue for the choices $n = 4$, $\theta_X = 0.4535$, $k = 1$ and $a_X = 0.8$.

for all $X, E, Z \geq 0$. If $b > 0$ then the function defined in (3.11) is invertible, whose inverse reads

$$h_n^{-1}(\tilde{Z}) = \left(\frac{b}{\tilde{Z}} - 1\right)^{\frac{1}{n}}, \tag{3.12}$$

for all $\tilde{Z} \in (0, b]$. Next, bearing in mind that a *steady state* of (3.1) must satisfy

$$\begin{aligned} 0 &= a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n} - kX, \\ 0 &= a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1+X^n} - kE, \end{aligned} \tag{3.13}$$

one can conveniently define

$$\Psi_{1,n} := h_n^{-1} \circ g_{1,n}, \tag{3.14}$$

and

$$\Psi_{2,n} := h_n^{-1} \circ g_{2,n}, \tag{3.15}$$

and

$$G_{\Psi_{1,n}} := \{(X, E) \in \mathbb{R}_+^2 : E = \Psi_{1,n}(X)\}, \tag{3.16}$$

and

$$G_{\Psi_{2,n}} := \{(X, E) \in \mathbb{R}_+^2 : X = \Psi_{2,n}(E)\}. \tag{3.17}$$

In this regard, one has that $(X^*, E^*) \in \mathbb{R}_+^2$ satisfies (3.13) if and only if

$$(X^*, E^*) \in G_{\Psi_{1,n}} \cap G_{\Psi_{2,n}}. \tag{3.18}$$

Hence, determining the *steady states* of (3.1) entails to know how the *nullclines* $G_{\Psi_{1,n}}$ and $G_{\Psi_{2,n}}$ behave, what boils down to the analysis of the functions $g_{1,n}(X)$ and $g_{2,n}(X)$.

3.1.2 Geometric aspects as primary aspects of the model: Huang's qualitative graphical matrix $(H_n\,[C_{i,X}, C_{j,E}])_{i,j}$

In fact, depending on $n \in \mathbb{N}$, and the relations among the parameters a_X, θ_X, and k, and analogously, among a_E, θ_E and k, one has that either $g_{1,n}$ and $g_{2,n}$ can exhibit the behaviors described in Figure 3.1, 3.2 and 3.3. That is, the graph is not monotonically increasing and has a region where it is below zero; it is not monotonically increasing but is non-negative, and the the case in which it is monotonically increasing.

As we see in Figure 3.1, for $n = 4$, one has that the choice of parameters satisfy

$$\theta_X < \frac{a_X}{2k}, \tag{3.19}$$

whilst, in the case of Figure 3.2, one has that

$$\frac{a_X}{2k} \leq \theta_X \leq \frac{a_X}{k}, \tag{3.20}$$

and, for Figure 3.3, one has that

$$\theta_X > \frac{a_X}{k}, \tag{3.21}$$

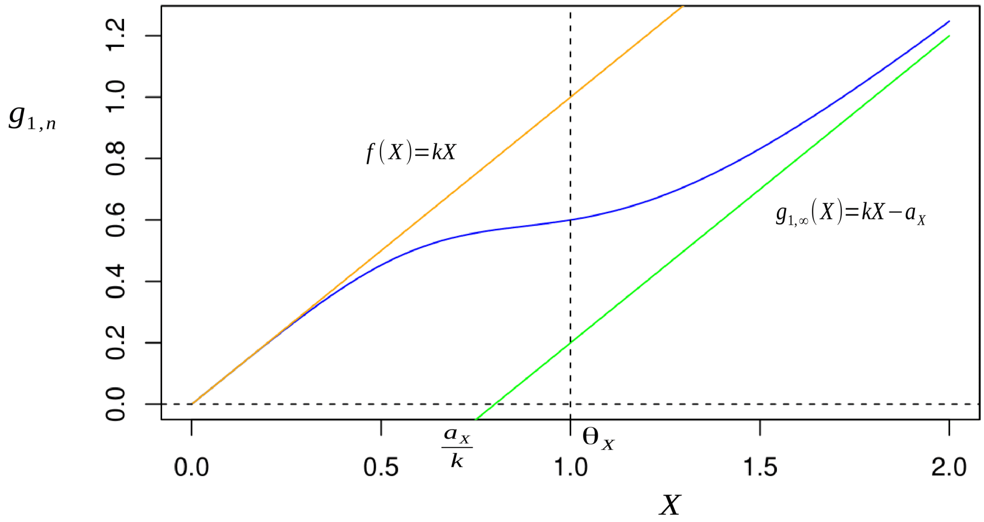


Figure 3.3: In this plot of $g_{1,n}$ in blue, we have chosen $n = 4$, $\theta_X = 1$, $k = 1$ and $a_X = 0.8$.

and these properties will be shown below.

However, Figures 3.1, 3.2 and 3.3 are representative for the behaviour of $g_{i,n}$ ($i = 1, 2$) when $n > 1$. For $n = 1$, the behaviour is different. These are depicted in Figures 3.4 and 3.5. In fact, one sees that in Figure 3.5, the parameters satisfy the relation (3.21), while, in Figure 3.4, the respective choices obey the condition

$$\theta_X \leq \frac{a_X}{k}. \tag{3.22}$$

But, how do we stipulate the conditions (3.19), (3.20), and (3.21) ? Are the respective conditions necessary and sufficient for the corresponding behaviours shown in Figures 3.1, 3.2, and 3.3 ? In order to answer the latter questions, one needs to have a more detailed look at the functions $g_{1,n}$ and $g_{2,n}$. Actually, we will analyse $g_{1,n}$ given that the case with $g_{2,n}$ is similar.

First, by construction, we note that for any $n \geq 1$, the graph of $g_{1,n}$ is below that of $f(X) = kX$, and has the line $Y = kX - a_X$ as an asymptotic limit as $X \to +\infty$. Conveniently, we denote the latter by $g_{1,\infty}(X)$. Moreover, for $n > 1$, one has that

$$g'_{1,n}(X) = k - n a_X \theta_X^n \frac{X^{n-1}}{(\theta_X^n + X^n)^2}, \tag{3.23}$$

which implies that

$$g'_{1,n}(0+) := \lim_{h \to 0^+} \frac{g_{1,n}(0+h) - g_{1,n}(0)}{h} = k, \tag{3.24}$$

so the graph of $g_{1,n}$ is tangent to the line $f(X) = kX$ at $X = 0$. If $n = 1$ then this no longer holds. We shall discuss this special case separately.

Lemma 3.1.1.

$$(i) \bigwedge_{n\geq 1}\left(\theta_X < \frac{a_X}{2k}\right) \Leftrightarrow (g_{1,n}(\theta_X) < 0)$$
$$(ii) \bigwedge_{n\geq 1}\left(\theta_E < \frac{a_E}{2k}\right) \Leftrightarrow (g_{2,n}(\theta_E) < 0) \tag{3.25}$$

Proof. (i) In fact, at $X = \theta_X$, one has that

$$g_{1,n}(\theta_X) = k\theta_X - \frac{a_X}{2}. \tag{3.26}$$

Thus,

$$\theta_X < \frac{a_X}{2k} \tag{3.27}$$

if and only if

$$g_{1,n}(\theta_X) < 0. \tag{3.28}$$

For (ii), on has that the proof is similar. □

Now, if we draw on the fact that $g_{1.n}$ is a continuous function on $(0, \infty)$ and right-continuous at $X = 0$, then for $\epsilon = \frac{|g_{1,n}(\theta_X)|}{2} > 0$ there exists $\delta(\epsilon) > 0$ such that if $X > 0$ and $|X - \theta_X| < \delta(\epsilon)$ then

$$\mid g_{1,n}(X) - g_{1,n}(\theta_X) \mid < \epsilon, \tag{3.29}$$

which implies that

$$g_{1,n}(X) < g_{1,n}(\theta_X) + \frac{\mid g_{1,n}(\theta_X) \mid}{2}, \tag{3.30}$$

which, in turn, implies that for $\theta_X < \frac{a_X}{2k}$

$$g_{1,n}(X) < 0. \tag{3.31}$$

So, there is a region in which $g_{1,n}(X) < 0$. Moreover, one can prove the following property.

Lemma 3.1.2.

$$(i) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \left(g'_{1,n}(\theta_X) < 0\right)$$
$$(ii) \bigwedge_{n\geq 2}\left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \left(g'_{2,n}(\theta_E) < 0\right) \tag{3.32}$$

Proof. (i)In fact, drawing on the expression (3.23), one has that for $n \geq 2$ and $\theta_X < \frac{a_X}{2k}$:

$$g'_{1,n}(\theta_X) = k - na_X\theta_X^n\frac{\theta_X^{n-1}}{(\theta_X^n + \theta_X^n)^2} < k - n\frac{a_X}{4\theta_X} < 0. \tag{3.33}$$

Similarly, one can show (ii). □

Corollary 3.1.3.

$$\begin{aligned} &(i) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \bigvee_{0<x_{max,n}<\theta_X} g'_{1,n}(x_{max,n}) = 0 \\ &(ii) \bigwedge_{n\geq 2}\left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \bigvee_{0<e_{max,n}<\theta_E} g'_{2,n}(e_{max,n}) = 0 \end{aligned} \tag{3.34}$$

Proof. (i) So, provided that $g'_{1,n}(X)$ is a continuous function on $(0,\infty)$ and right-continuous at $X=0$, if we draw upon the Intermediate Value Theorem [77, p. 93] then (3.313) and Lemma (3.1.2) imply that there exists $0 < x_{max,n} < \theta_X$ such that $g'_{1,n}(x_{max,n}) = 0$. Similarly, we can demonstrate (ii). □

So, the latter property is consistent with the Figure 3.1 under condition (3.19), seeing that $x_{max,n}$ is the candidate to be the local maximum, which, in turn, leads us to conjecture that the condition (3.19) is indeed sufficient for the behaviour of $g_{1,n}(X)$ shown in the Figure 3.1.

To demonstrate that it is indeed true, one can proceed as follows. Let $j \in \mathbb{R}_+ \setminus \{0\}$. So, we note that

$$g_{1,n}\left(\frac{\theta_X}{j}\right) > 0 \Leftrightarrow \frac{1+j^n}{j}\theta_X > \frac{a_X}{k}, \tag{3.35}$$

which guides us to the next result.

Lemma 3.1.4.

$$\begin{aligned} &(i) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \bigvee_{j_{0,X}>1}\left(\frac{1+j_{0,X}^2}{j_{0,X}}\theta_X > \frac{a_X}{k}\right) \\ &(ii) \bigwedge_{n\geq 2}\left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \bigvee_{j_{0,E}>1}\left(\frac{1+j_{0,E}^2}{j_{0,E}}\theta_E > \frac{a_E}{k}\right) \end{aligned} \tag{3.36}$$

Proof. (i) In fact, there exists $j_{0,X} > 1$ such that

$$\frac{1+j_{0,X}^2}{j_{0,X}}\theta_X > \frac{a_X}{k} \Rightarrow \theta_X j_{0,X}^2 - \frac{a_X}{k} j_{0,X} + \theta_X > 0. \tag{3.37}$$

Define $f(j) = \theta_X j_{0,X}^2 - \frac{a_X}{k} j_{0,X} + \theta_X$. So, under $\theta_X < \frac{a_X}{2k}$, one has that $f(j) > 0$ if and only if

$$j < j_{0,X}^{(-)} := \frac{\frac{a_X}{k} - \sqrt{\left(\frac{a_X}{k}\right)^2 - 4\theta_X}}{2\theta_X}, \tag{3.38}$$

and

$$j > j_{0,X}^{(+)} := \frac{\frac{a_X}{k} + \sqrt{\left(\frac{a_X}{k}\right)^2 - 4\theta_X}}{2\theta_X}. \tag{3.39}$$

Therefore, as $j_{0,X}^{(+)} > 1$ then one can choose $j_{0,X} > j_{0,X}^{(+)} > 1$ such that $f(j_{0,X}) > 0$. The proof of (ii) is similar. □

Corollary 3.1.5.

$$(i) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \bigvee_{\frac{\theta_X}{j_{0,X}}<x_{1,n}<\theta_X} g_{1,n}(x_{1,n}) = 0$$
$$(ii) \bigwedge_{n\geq 2}\left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \bigvee_{\frac{\theta_E}{j_{0,E}}<e_{1,n}<\theta_E} g_{2,n}(e_{1,n}) = 0 \tag{3.40}$$

Proof. (i) In fact, Let $j_{0,X} > 1$ satisfy Lemma (3.1.4). So, one has that

$$j_{0,X}^n > j_{0,X}^2 > 1, \tag{3.41}$$

which implies that

$$\frac{j_{0,X}^n + 1}{j_{0,X}} > \frac{j_{0,X}^2 + 1}{j_{0,X}}, \tag{3.42}$$

which implies that

$$\frac{j_{0,X}^n + 1}{j_{0,X}}\theta_X > \frac{a_X}{k}, \tag{3.43}$$

which, in turn, by invoking (3.35), implies that

$$g_{1,n}\left(\frac{\theta_X}{j_{0,X}}\right) > 0. \tag{3.44}$$

Therefore, if we invoke Lemma 3.1.1 and if we draw upon the Intermediate Value Theorem [77, p. 93], then we conclude that there exists $\frac{\theta_X}{j_{0,X}} < x_{1,n} < \theta_X$ such that $g_{1,n}(x_{1,n}) = 0$. The proof for (ii) is similar. □

Further, Let $j_{0,X} > 1$ satisfy Lemma 3.1.4. As we have worked out in the demonstration of Corollary 3.1.5, one has that

$$g_{1,n}\left(\frac{\theta_X}{j_{0,X}}\right) > 0. \tag{3.45}$$

So, by continuity, one has that given $\epsilon = \frac{g_{1,n}\left(\frac{\theta_X}{j_{0,X}}\right)}{2} > 0$ there exists $0 < \delta(\epsilon) < \frac{x_{1,n}-\frac{\theta_X}{j_{0,X}}}{2}$ such that if $X > 0$ and $\mid X - \frac{\theta_X}{j_{0,X}} \mid< \delta(\epsilon)$ then

$$\left| g_{1,n}\left(\frac{\theta_X}{j_{0,X}}\right) - g_{1,n}(X) \right| < \epsilon, \tag{3.46}$$

which implies that

$$g_{1,n}\left(\frac{\theta_X}{j_{0,X}}\right) - \frac{g_{1,n}\left(\frac{\theta_X}{j_{0,X}}\right)}{2} < g_{1,n}(X), \tag{3.47}$$

which implies that

$$\frac{g_{1,n}\left(\frac{\theta_X}{j_{0,X}}\right)}{2} < g_{1,n}(X), \tag{3.48}$$

which, in turn, by invoking (3.35), implies that

$$g_{1,n}(X) > 0. \tag{3.49}$$

So, there is a region in $(0, \theta_X)$ in which $g_{1,n}(X) > 0$, which, in turn, leads us to the next result.

Corollary 3.1.6.

$$\begin{aligned} &(i) \bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \bigvee_{\theta_X < x_{2,n} < \frac{a_X}{k}} g_{1,n}(x_{2,n}) = 0 \\ &(ii) \bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \bigvee_{\theta_E < e_{2,n} < \frac{a_E}{k}} g_{2,n}(e_{2,n}) = 0 \end{aligned} \tag{3.50}$$

Proof. (i) First, we note that

$$g_{1,n}\left(\frac{a_X}{k}\right) = a_X - a_X \frac{1}{\left(\frac{k\theta_X}{a_X}\right)^n + 1} > 0. \tag{3.51}$$

So, if we draw on Lemma 3.1.1 and upon the Intermediate Value Theorem [77, p. 93] then one has that there exists $\theta_X < x_{2,n} < \frac{a_X}{k}$ such that $g_{1,n}(x_{2,n}) = 0$. Similarly, one can prove (ii). □

Next, if we build upon the former argument then we can show that given $\epsilon = \frac{g_{1,n}\left(\frac{a_X}{k}\right)}{2}$ then there exists $0 < \delta(\epsilon) < \frac{\frac{a_X}{k} - x_{2,n}}{2}$ such that if $X > 0$ and $\mid X - \frac{a_X}{k} \mid < \delta(\epsilon)$ then

$$g_{1,n}(X) > 0. \tag{3.52}$$

So there is a region in $(x_{2,n}, \infty)$ in which $g_{1,n}(X) > 0$.

Further, one has that

$$g'_{1,n}\left(\frac{a_X}{k}\right) = k - na_x\theta_X^n \frac{\left(\frac{a_X}{k}\right)^{n-1}}{\left[\theta_X^n + \left(\frac{a_X}{k}\right)^n\right]^2}. \tag{3.53}$$

So, one has that $g'_{1,n}\left(\frac{a_X}{k}\right) \leq 0$ if and only if

$$2^n \left[\left(\frac{k\theta_X}{a_X}\right)^n + 1\right]^2 < \frac{\theta_X^n}{\left(\frac{a_X}{2k}\right)^n}, \tag{3.54}$$

which is a contradiction under (3.19), so one must have that

$$g'_{1,n}\left(\frac{a_X}{k}\right) > 0. \tag{3.55}$$

Corollary 3.1.7.

$$\begin{aligned} &(i) \bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \bigvee_{\theta_X < x_{min,n} < \frac{a_X}{k}} g'_{1,n}(x_{min,n}) = 0 \\ &(ii) \bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \bigvee_{\theta_E < e_{min,n} < \frac{a_E}{k}} g'_{2,n}(e_{min,n}) = 0 \end{aligned} \tag{3.56}$$

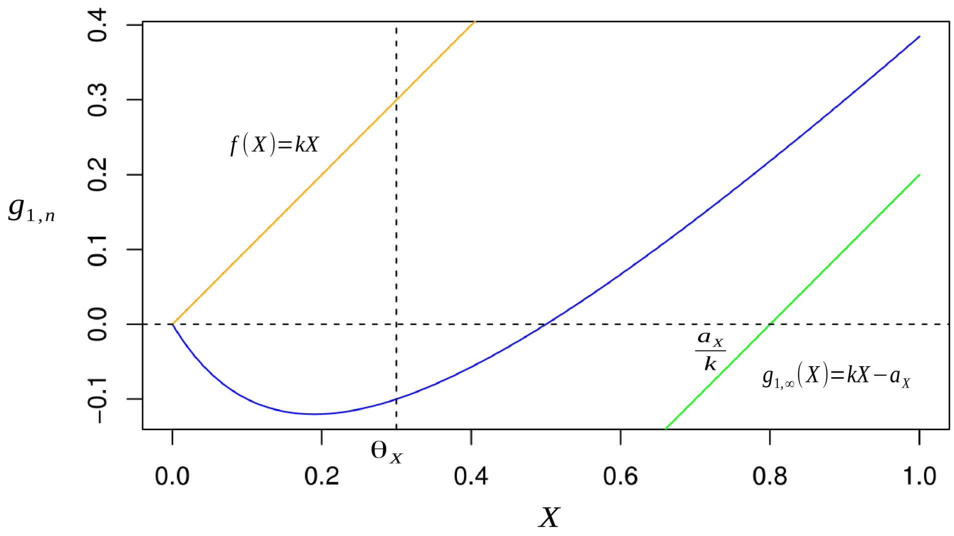


Figure 3.4: Here, we have plotted $g_{1,n}$ in blue for the choices $n = 1$, $\theta_X = 1$, $k = 1$ and $a_X = 3$.

Proof. (i) In fact, by continuity, if we draw on Lemma 3.1.2 and on the inequality (3.55), by invoking the Intermediate Value Theorem [77, p. 93], then we deduce that there exists $\theta_X < x_{min,n} < \frac{a_X}{k}$ such that $g'_{1,n}(x_{min,n}) = 0$. Similarly, we can demonstrate (ii). □

Thereby, the latter property of the function $g_{1,n}(X)$ is consistent with the Figure 3.1 under condition (3.19), so $x_{min,n}$ is indeed the candidate to be the local minimum, and indeed, as we shall see, the global minimum.

But, how can we give a logically valid argument for the characterization of the critical points $x_{max,n}$ and $x_{min,n}$ as the local maximum and minimum, respectively? And about the zeros of the function $g_{1,n}(X)$? To answer the latter questions, we draw upon Descartes' Theorem (see [101, p. 63]), which says that the number of positive real roots of a polynomial

$$p(x) = a_n x^n + a_{n-1}x^{n-1} + a_{n-2}x^{n-2} + \ldots + a_1 x + a_0, \tag{3.57}$$

with real coefficients, is less or equal to the the number of variations in sign of the sequence of coefficients

$$a_n,\ a_{n-1},\ a_{n-2},\ \ldots,\ a_1,\ a_0. \tag{3.58}$$

So, we can prove the following result.

Corollary 3.1.8.

$$\begin{aligned} &(i) \bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \{x \in \mathbb{R}_+ : g_{1,n}(X) = 0\} = \{0, x_{1,n}, x_{2,n}\} \\ &(ii) \bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \{E \in \mathbb{R}_+ : g_{2,n}(E) = 0\} = \{0, e_{1,n}, e_{2,n}\} \end{aligned} \tag{3.59}$$

Proof. (i) First, we note that

$$g_{1,n}(X) = 0 \tag{3.60}$$

if and only if

$$\frac{kX(\theta_X^n + X^n) - a_X X^n}{\theta_X^n + X^n} = 0 \tag{3.61}$$

if and only if

$$X(kX^n - a_X X^{n-1} + k\theta_X^n) = 0 \tag{3.62}$$

if and only if

$$X = 0 \tag{3.63}$$

or

$$p(X) = kX^n - a_X X^{n-1} + k\theta_X^n = 0. \tag{3.64}$$

So, drawing on Descartes' Theorem, one has that $p(X)$ has at most two positive real roots. Therefore, if we invoke Corollaries 3.1.5 and 3.1.6 then we have that

$$\{X > 0 : p(X) = 0\} = \{x_{1,n}, x_{2,n}\}, \tag{3.65}$$

and the Corollary has been proved. Similarly, one can prove (ii). □

Further, one can use the same argument to determine the zeros of the function $g'_{1,n}(X)$.

Corollary 3.1.9.

$$\begin{aligned} &(i) \bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \{x \in \mathbb{R}_+ : g'_{1,n}(X) = 0\} = \{x_{max,n}, x_{min,n}\} \\ &(ii) \bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \{E \in \mathbb{R}_+ : g'_{2,n}(E) = 0\} = \{e_{max,n}, e_{min,n}\} \end{aligned} \tag{3.66}$$

Proof. (i) First, we note that

$$g'_{1,n}(X) = 0 \tag{3.67}$$

if and only if

$$\frac{k}{(\theta_X^n + X^n)^2}\left[(\theta_X^n + X^n) + \gamma X^{\frac{n-1}{2}}\right]\left[(\theta_X^n + X^n) - \gamma X^{\frac{n-1}{2}}\right] = 0, \tag{3.68}$$

with $\sqrt{\frac{na_X\theta_X^n}{k}}$, if and only if

$$p_1(Y) = Y^{2n} + \gamma Y^{n-1} + \theta_X^n = 0, \tag{3.69}$$

or

$$p_2(Y) = Y^{2n} - \gamma Y^{n-1} + \theta_X^n = 0, \tag{3.70}$$

with $Y = X^{\frac{1}{2}}$. But, $p_1(Y)$ in (3.69) has no positive real solutions. On the other hand, drawing on Descartes' Theorem, one has that $p_2(Y)$ in (3.69) has at most two positive real roots. Therefore, if we invoke Corollaries 3.1.3 and 3.1.7 then we have that

$$\{Y > 0 : p_2(Y) = 0\} = \{X > 0 : X^n - \gamma X^{\frac{n-1}{2}} + \theta_X^n = 0\} = \{x_{max,n}, x_{min,n}\}, \tag{3.71}$$

and the Corollary has been proved. Similarly, one can show (ii). □

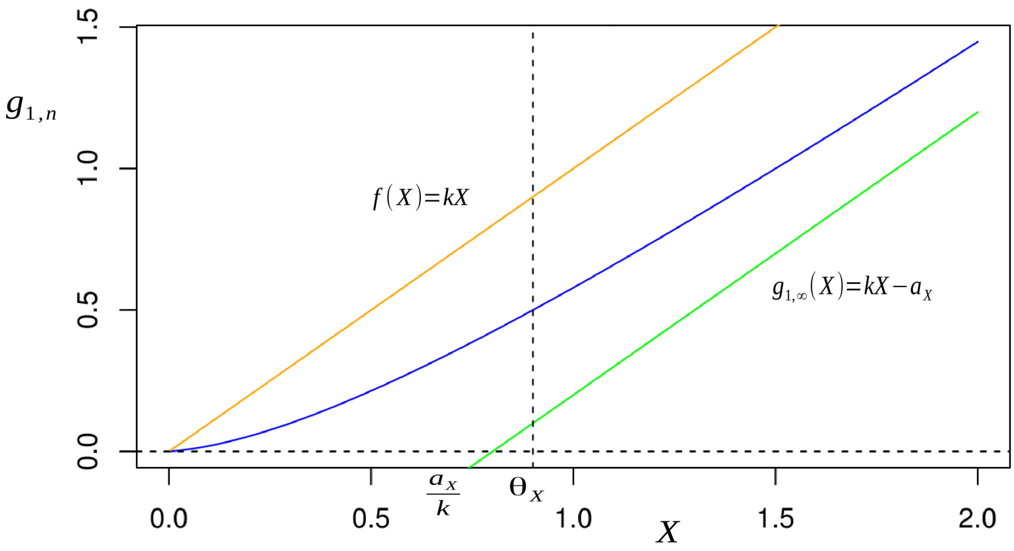


Figure 3.5: For this plot of $g_{1,n}$ in blue, one has made the choices $n = 1$, $\theta_X = 3.5$, $k = 1$ and $a_X = 3$.

Further,

$$g''_{1,n}(X) = -na_X \frac{\theta_X^n}{(\theta_X^n + X^n)^3} X^{n-2} \left[(n-1)\theta_X^n - (n+1)X^n\right] = 0 \tag{3.72}$$

if and only if

$$(n-1)\theta_X^n - (n+1)X^n = 0 \tag{3.73}$$

if and only if

$$X = x_{inf,n} = \left(\frac{n-1}{n+1}\right)^{\frac{1}{n}} \theta_X < \theta_X, \tag{3.74}$$

so one has that $g''_{1,n}(X) < 0$ for $X < x_{inf,n}$ and $g''_{1,n}(X) > 0$ for $X > x_{inf,n}$, that is, $g'_{1,n}(X) < 0$ is strictly decreasing on $[0, x_{inf,n})$ and strictly increasing on $(x_{inf,n}, \infty)$. Thereby, one has that the graph of $g_{1,n}(X)$ is concave down on $[0, x_{inf,n})$ and concave up [convex] on $(x_{inf,n}, \infty)$ wherein $x_{inf,n}$ denotes the inflection point.

As a conclusion, if we draw on (3.35), Lemma 3.1.4, and Corollary 3.1.8 then we have that $g_{1,n}(X) > 0$ on $(0, x_{1,n})$. Moreover, we deduce from (3.1.9) that $g'_{1,n}(X) > 0$ on $[0, x_{max,n})$ and $g'_{1,n}(X) < 0$ on $(x_{max,n}, x_{1,n}]$ so $x_{max,n}$ is a local maximum.

Likewise, if we build on Lemma 3.1.1 and Corollary 3.1.8 then we must have that $g_{1,n}(X) < 0$ on $(x_{1,n}, x_{2,n})$. In addition, we derive from Corollary 3.1.9 that $g'_{1,n}(X) < 0$ on $[x_{1,n}, x_{min,n})$ and $g'_{1,n}(X) > 0$ on $(x_{min,n}, x_{1,n}]$ so $x_{min,n}$ is a local minimum. Furthermore, we have shown in the demonstration of Corollary 3.1.6 that

$$g_{1,n}\left(\frac{a_X}{k}\right) > 0. \tag{3.75}$$

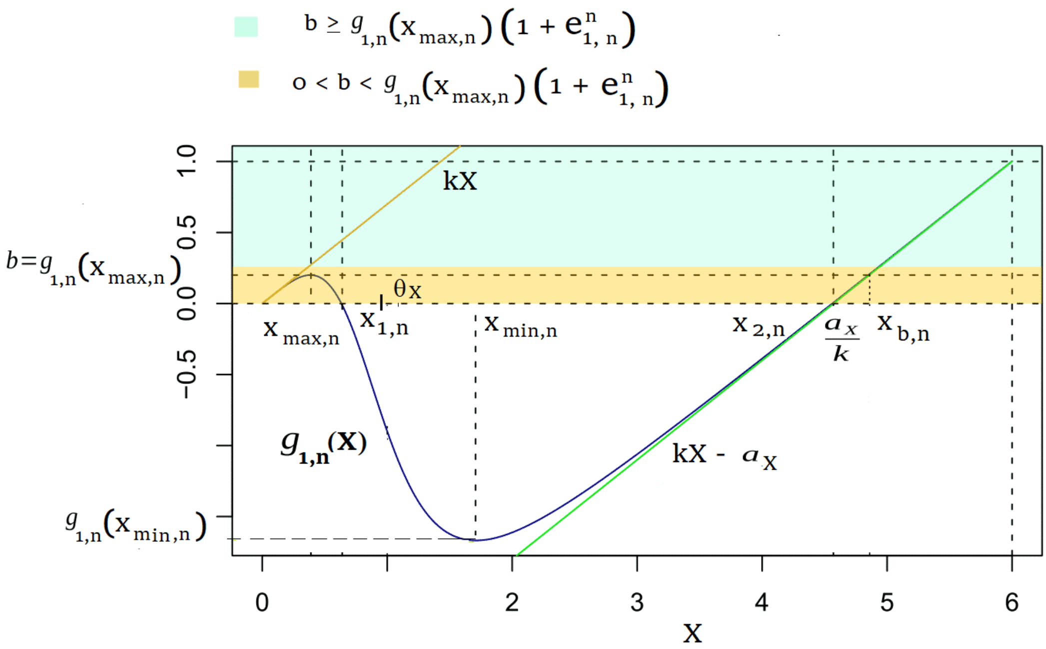


Figure 3.6: Here, we see the plot of the qualitative behavior of $g_{1,n}(X)$ in blue for the choices $n = 4$, $\theta_X = \theta_E = 1$, $k = 0.7$, $a_X = 3.2$ and $a_E = 3.2$. As a result, one has that $x_{1,n} = 0.64$, $x_{2,n} = 4.57$, $x_{max,n} = 0.39$, $x_{min,n} = 1.71$, $x_{b,n} = 4.85$, $g_{1,n}(x_{max,n}) = 0.2006438059$, and $g_{1,n}(x_{min,n}) = -1.6679341251$.

Thus, if we draw on the fact that $g'_{1,n}(X) > 0$ is, in particular, strictly increasing on $(x_{2,n}, \infty)$, and if we invoke Corollaries 3.1.8 and 3.1.9, then one has that $g_{1.n}(X) > 0$ on $(x_{2,n}, \infty)$. Therefore, for $n \geq 2$, under conditions

$$\theta_X < \frac{a_X}{2k} \tag{3.76}$$

and

$$\theta_E < \frac{a_E}{2k}, \tag{3.77}$$

one has that $g_{1,n}(X)$ has exactly the behavior shown in Figure 3.6. Moreover, one has that $x_{min,n}$ is indeed a global minimum.

Proposition 3.1.10.

$$\begin{aligned} &(i) \bigwedge_{n \geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \lim_{n \to +\infty} x_{1,n} = \theta_X \\ &(ii) \bigwedge_{n \geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \lim_{n \to +\infty} e_{1,n} = \theta_E \end{aligned} \tag{3.78}$$

Proof. (i) First, we note that

$$g_{1,n}\left(\frac{\theta_X}{n^{1/n}}\right) > 0 \tag{3.79}$$

if and only if

$$k\frac{\theta_X}{a_X} > \frac{n^{1/n}}{n+1}. \tag{3.80}$$

Now, bearing in mind that we are under

$$0 < \theta_X < \frac{a_X}{2k}, \tag{3.81}$$

if

$$k\frac{\theta_X}{a_X} \leq \frac{n^{1/n}}{n+1} \tag{3.82}$$

then

$$k\frac{\theta_X}{a_X} \leq 0, \tag{3.83}$$

seeing that

$$\lim_{n\to+\infty} n^{1/n} = 1 \tag{3.84}$$

as shown in [77, p. 57], and that

$$\lim_{n\to+\infty} \frac{1}{n+1} = 0. \tag{3.85}$$

However, (3.83) is a contradiction. Hence, one must have that

$$g_{1,n}\left(\frac{\theta_X}{n^{1/n}}\right) > 0, \tag{3.86}$$

which, by invoking (3.84), implies that

$$\frac{\theta_X}{n^{1/n}} < x_{1,n} < \theta_X, \tag{3.87}$$

which, in turn, implies that

$$\lim_{n\to+\infty} x_{1,n} = \theta_X. \tag{3.88}$$

The case (*ii*) can be shown with the same argument. □

Further, we define

$$H_{1,n}(X) := a_X\frac{X^n}{\theta_X^n + X^n}. \tag{3.89}$$

So, in particular, seeing that

$$H_{1,n}'(X) := na_X\theta^n X\frac{X^{n-1}}{(\theta_X^n + X^n)^2} > 0 \tag{3.90}$$

on $(0, +\infty)$, one must have that $H_{1,n}(X)$ is strictly increasing on $(0, +\infty)$. Moreover, it is not difficult to show that

$$\lim_{n\to+\infty} H_{1,n}(X) = \begin{cases} a_X, & X > \theta_X, \\ a_X/2, & X = \theta_X, \\ 0, & X < \theta_X, \end{cases} \tag{3.91}$$

in which the convergence notion is *pointwise convergence*.

Lemma 3.1.11.

$$
\begin{aligned}
&(i) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \lim_{n\to+\infty} g_{1,n}\left(\frac{a_X}{k}\right) = 0\\
&(ii) \bigwedge_{n\geq 2}\left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \lim_{n\to+\infty} g_{2,n}\left(\frac{a_E}{k}\right) = 0
\end{aligned} \tag{3.92}
$$

Proof. (i) In fact, if

$$\theta_X < \frac{a_X}{2k} \tag{3.93}$$

then

$$\left(\frac{k\theta_X}{a_X}\right)^n < \frac{1}{2^n}, \tag{3.94}$$

which implies that

$$a_X\frac{1}{\frac{1}{2^n}+1} < a_X\frac{1}{\left(\frac{k\theta_X}{a_X}\right)^n+1}, \tag{3.95}$$

which implies that

$$-a_X\frac{1}{\frac{1}{2^n}+1} > -a_X\frac{1}{\left(\frac{k\theta_X}{a_X}\right)^n+1}, \tag{3.96}$$

which, in turn, implies that

$$0 < g_{1,n}\left(\frac{a_X}{k}\right) < a_X - a_X\frac{1}{\frac{1}{2^n}+1}. \tag{3.97}$$

Thus,

$$\lim_{n\to+\infty} g_{1,n}\left(\frac{a_X}{k}\right) = 0. \tag{3.98}$$

(ii) The same argument can be used to demonstrate it. □

Proposition 3.1.12.

$$
\begin{aligned}
&(i) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \lim_{n\to+\infty} x_{2,n} = \frac{a_X}{k}\\
&(ii) \bigwedge_{n\geq 2}\left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \lim_{n\to+\infty} e_{2,n} = \frac{a_E}{k}
\end{aligned} \tag{3.99}
$$

Proof. (i) In fact, drawing on Corollary 3.1.6, one has that for $n_1 = 2$ there exists $\theta_X < x_{2,n_1} < a_X/k$ such that $g_{1,n_1}(x_{2,n_1}) = 0$. In view of (3.91), for $n_2 = 3 > n_1 = 2$, one has that

$$H_{1,n_1}(x_{2,n_1}) < H_{1,n_2}(x_{2,n_1}), \tag{3.100}$$

which, by invoking (3.75), implies that

$$g_{1,n_2}(x_{2,n_1}) < g_{1,n_1}(x_{2,n_1}) = 0 < g_{1,n_2}\left(\frac{a_X}{k}\right), \tag{3.101}$$

which, in turn, by drawing upon the Intermediate Value Theorem [77, p. 93], implies that there exists $x_{2,n_1} < x_{2,n_2} < a_X/k$ such that $g_{1,n_1}(x_{2,n_2}) = 0$.

Hence, by induction, we can construct a sequence $(x_{2,n_j})_{j=1}^{\infty}$ such that

$$\theta_X < x_{2,n_1} < x_{2,n_2} < \ldots < x_{2,n_q} < x_{2,n_{q+1}} < \ldots < a_X/k, \tag{3.102}$$

and that

$$\begin{cases} g_{1,n_{j+1}}(x_{2,n_j}) < 0, & X > \theta_X, \\ g_{1,n_j}(x_{2,n_j}) = 0, & X = \theta_X, \\ 0, & X < \theta_X, \end{cases} \tag{3.103}$$

with $j \in \{1, 2, 3, \ldots\}$. Therefore, if we draw upon Weierstraβ's Theorem, see [77, p. 40], then we deduce that there exists $\theta_X < \bar{x} \leq a_X/k$ such that

$$\lim_{j \to +\infty} x_{2,n_j} = \bar{x}. \tag{3.104}$$

However, by invoking (3.91), one has that

$$\lim_{j \to +\infty} H_{1,n_j}(\bar{x}) = a_X, \tag{3.105}$$

or equivalently,

$$\bigwedge_{\epsilon>0} \bigvee_{J(\epsilon)\geq 2} \bigwedge_{j \geq J(\epsilon)} \mid H_{1,n_j}(\bar{x}) - a_X \mid < k\frac{\epsilon}{2}, \tag{3.106}$$

which is true, if and only if

$$\bigwedge_{\epsilon>0} \bigvee_{J(\epsilon)\geq 2} \bigwedge_{j \geq J(\epsilon)} \mid g_{1,n_j}(\bar{x}) + a_X - k\bar{x} \mid < k\frac{\epsilon}{2}, \tag{3.107}$$

which implies that

$$\bigwedge_{\epsilon>0} \bigvee_{J(\epsilon)\geq 2} \bigwedge_{j \geq J(\epsilon)} \mid a_X - k\bar{x} \mid < k\frac{\epsilon}{2} + \mid g_{1,n_j}(\bar{x}) \mid. \tag{3.108}$$

which, in turn, implies that

$$\bigwedge_{\epsilon>0} \bigvee_{J(\epsilon)\geq 2} \bigwedge_{j \geq J(\epsilon)} \left| \frac{a_X}{k} - \bar{x} \right| < \frac{\epsilon}{2} + \frac{1}{k} g_{1,n_j}\left(\frac{a_X}{k}\right). \tag{3.109}$$

Now, without loss of generality, if we draw on Lemma 3.1.11 then we have that

$$\bigwedge_{\epsilon>0} \bigvee_{J(\epsilon)\geq 2} \bigwedge_{j \geq J(\epsilon)} g_{1,n_j}\left(\frac{a_X}{k}\right) < k\frac{\epsilon}{2}. \tag{3.110}$$

Therefore, (3.109) and (3.110) imply that

$$\bigwedge_{\epsilon>0} \left| \frac{a_X}{k} - \bar{x} \right| < \epsilon, \tag{3.111}$$

which, in turn, implies that

$$\bar{x} = \frac{a_X}{k}, \tag{3.112}$$

and we have just demonstrated (i). A similar argument can be used to show (ii). □

Lemma 3.1.13.

$$(i) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \bigwedge_{b>0}\bigvee_{x_{b,n}>x_{2,n}} (g_{1,n}(x_{b,n}) = b) \wedge \left(x_{b,n} < \frac{a_X}{k} + \frac{b}{k}\right)$$
$$(ii) \bigwedge_{n\geq 2}\left(\theta_X < \frac{a_E}{2k}\right) \Rightarrow \bigwedge_{b>0}\bigvee_{e_{b,n}>e_{2,n}} (g_{2,n}(e_{b,n}) = b) \wedge \left(e_{b,n} < \frac{a_E}{k} + \frac{b}{k}\right) \tag{3.113}$$

Proof. (i) Let $b > 0$. If we draw upon the Corollaries 3.1.6 and 3.1.8 then , under

$$\theta_X < \frac{a_X}{2k}, \tag{3.114}$$

we have that there exists $x_{2,n} > \theta_X$ such that $g_{1,n}(x_{2,n}) = 0$. Further, if we draw on Corollary (3.1.9) then we deduce that $g'_{1,n}(X) > 0$, that is, $g_{1,n}(X)$ is strictly increasing with $g_{1,n}(X) > 0$ for $X > x_{2,n}$. So, by invoking the Intermediate Value Theorem [77, p. 93], there exists $x_{b,n} > x_{2,n}$ for which

$$g_{1,n}(x_{b,n}) = b. \tag{3.115}$$

Moreover,

$$x_{b,n} < \frac{a_X}{k} + \frac{b}{k} \tag{3.116}$$

if and only if

$$kx_{b,n} - a_X < g_{1,n}(x_{b,n}) \tag{3.117}$$

if and only if

$$kx_{b,n} - a_X < kx_{b,n} - a_X\frac{x_{b,n}^n}{\theta_X^n + x_{b,n}^n} \tag{3.118}$$

if and only if

$$a_X\frac{x_{b,n}^n}{\theta_X^n + x_{b,n}^n} < a_X \tag{3.119}$$

if and only if

$$a_X\frac{1}{\frac{\theta_X^n}{x_{b,n}^n} + 1} < a_X. \tag{3.120}$$

(ii) Similarly, one can prove this case. □

Proposition 3.1.14.

(i) $\Psi_{1,n}(x_{b,n}) = 0$

(ii) $\Psi_{2,n}(e_{b,n}) = 0$

Proof. (i) In fact, recalling that

$$h_n(Z) := b\frac{1}{1 + Z^n}, \tag{3.121}$$

and that

$$\Psi_{1,n}(X) := h_n^{-1}(g_{1,n}(X)), \tag{3.122}$$

one has that

$$\Psi_{1,n}(X) = \left(\frac{b}{g_{1,n}(X)} - 1\right)^{1/n}. \tag{3.123}$$

Now, if we draw upon Lemma 3.1.13 then we know that $g_{1,n}(x_{b,n}) = b$. Therefore, one must have that

$$\Psi_{1,n}(x_{b,n}) = 0, \tag{3.124}$$

and the Corollary has been demonstrated. (ii) The same argument can be used to show this case. □

Proposition 3.1.15.

(i) $\bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \lim_{X\to 0^-} \Psi_{1,n}(X) = +\infty$

(ii) $\bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \lim_{X\to x_{1,n}^+} \Psi_{1,n}(X) = +\infty$

(iii) $\bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \Rightarrow \lim_{X\to x_{2,n}^-} \Psi_{1,n}(X) = +\infty$

(iv) $\bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \lim_{E\to 0^-} \Psi_{2,n}(E) = +\infty$

(v) $\bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \lim_{E\to e_{1,n}^+} \Psi_{2,n}(E) = +\infty$

(vi) $\bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \Rightarrow \lim_{E\to e_{2,n}^-} \Psi_{2,n}(E) = +\infty$

Proof. (i) In fact, if we draw on Corollary 3.1.8 then we have that

$$\{X \geq 0 : g_{1,n}(X) = 0\} = \{0, x_{1,n}, x_{2,n}\}. \tag{3.125}$$

Moreover, by construction, one has that

$$\Psi_{1,n}(X) = \left(\frac{b}{g_{1,n}(X)} - 1\right)^{1/n}, \tag{3.126}$$

which, in turn, implies that

$$\lim_{X\to 0^-} \Psi_{1,n}(X) = +\infty. \tag{3.127}$$

The items $(ii), (iii), (iv), (v)$, and (vi) can be shown in a similar way. □

Further, under $\theta_X < a_x/2k$, it is important to remark that

$$b \geq g_{1,n}(x_{max,n}) \tag{3.128}$$

if and only if

$$\Psi_{1,n}(x_{max,n}) = \left(\frac{b}{g_{1,n}(x_{max,n})} - 1\right)^{1/n} \geq 0. \tag{3.129}$$

So,

$$\Psi_{1,n}(x_{max,n}) = 0 \tag{3.130}$$

if and only if

$$b = g_{1,n}(x_{max,n}). \tag{3.131}$$

Moreover, if

$$b > g_{1,n}(x_{max,n}) \tag{3.132}$$

then there exists only one $x_{b,n} > 0$ such that $g_{1,n}(x_{b,n}) = 0$ and $\Psi_{1,n}(x_{b,n}) = 0$, and, in this case, consistent with Proposition 3.1.13, one must have that $x_{b,n} > x_{2,n}$. On the other hand, if

$$0 < b < g_{1,n}(x_{max,n}) \tag{3.133}$$

then, by invoking the Intermediate Value Theorem [77, p. 93], one has that

$$\{X \geq 0 : g_{1,n}(X) = b\} = \{x_{b,n}^{(-)}, x_{b,n}^{(+)}, x_{b,n}\}, \tag{3.134}$$

with $0 < x_{b,n}^{(-)} < x_{max,n}$, $x_{max,n} < x_{b,n}^{(+)} < x_{1,n}$, and $x_{b,n} > x_{2,n}$. Hence, one must have that

$$\Psi_{1,n}\left(x_{b,n}^{(-)}\right) = \Psi_{1,n}\left(x_{b,n}^{(+)}\right) = \Psi_{1,n}\left(x_{b,n}^{(-)}\right) = 0, \tag{3.135}$$

so that $\Psi_{1,n}(X)$ is not defined on the interval $\left(x_{b,n}^{(-)}, x_{b,n}^{(+)}\right)$. A similar reasoning can be performed under $\theta_E < a_E/2k$ and

$$0 < b < g_{2,n}(e_{max,n}), \tag{3.136}$$

for which one has that

$$\{E \geq 0 : g_{2,n}(E) = b\} = \{e_{b,n}^{(-)}, e_{b,n}^{(+)}, e_{b,n}\}, \tag{3.137}$$

and that

$$\Psi_{2,n}\left(e_{b,n}^{(-)}\right) = \Psi_{2,n}\left(e_{b,n}^{(+)}\right) = \Psi_{2,n}\left(e_{b,n}^{(-)}\right) = 0, \tag{3.138}$$

with

$$0 < e_{b,n}^{(-)} < e_{max,n}, \tag{3.139}$$

$$e_{max,n} < e_{b,n}^{(+)} < e_{1,n}, \tag{3.140}$$

and

$$e_{b,n} > e_{2,n}. \tag{3.141}$$

Proposition 3.1.16.

(i) $\bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \wedge (b \geq g_{1,n}(x_{max,n})) \Rightarrow \Psi_{1,n}(X)\Big|_{(0,x_{max,n}]}$ *is strictly decreasing;*

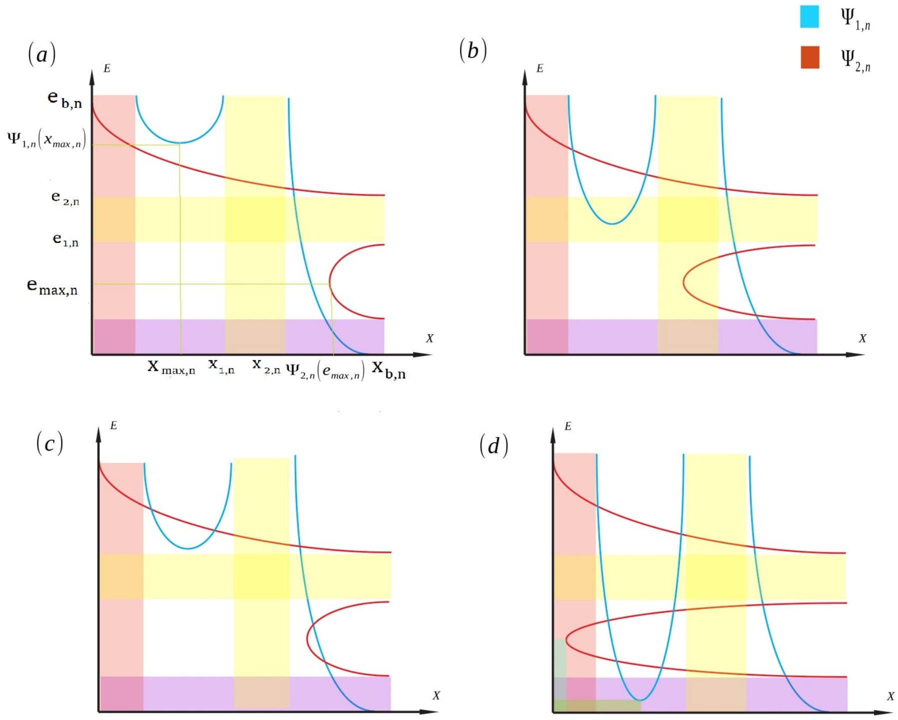


Figure 3.7: For $n \geq 2$, under conditions (3.149) and (3.150), one sees four possible geometric conformations of the graph of the functions $\Psi_{1,n}$ and $\Psi_{2,n}$, which must be intuitively apprehended since the feasible asymmetric cases are not being depicted therein. Moreover, (d) is the feasible geometric conformation with the maximal number of steady states.

(ii) $\bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \wedge (b \geq g_{1,n}(x_{max,n})) \Rightarrow \Psi_{1,n}(X)\Big|_{[x_{max,n},x_{1,n})}$ *is strictly increasing;*

(iii) $\bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \wedge (b \geq g_{1,n}(x_{max,n})) \Rightarrow \Psi_{1,n}(X)\Big|_{(x_{2,n},x_{b,n}]}$ *is strictly decreasing;*

(iv) $\bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \wedge (b \geq g_{2,n}(e_{max,n})) \Rightarrow \Psi_{2,n}(E)\Big|_{(0,e_{max,n}]}$ *is strictly decreasing;*

(v) $\bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \wedge (b \geq g_{2,n}(e_{max,n})) \Rightarrow \Psi_{2,n}(E)\Big|_{[e_{max,n},e_{1,n})}$ *is strictly increasing;*

(vi) $\bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \wedge (b \geq g_{2,n}(e_{max,n})) \Rightarrow \Psi_{2,n}(E)\Big|_{(e_{2,n},e_{b,n}]}$ *is strictly decreasing;*

Proof. (i) In fact, Let $0 < X_1 < X_2 < x_{max,n}$. If $\theta_X < \frac{a_X}{2k}$ then $g_{1,n}(X)\Big|_{(0,x_{max,n})}$ is strictly increasing. The latter implies that

$$g_{1,n}(X_1) < g_{1,n}(X_2) < g_{1,n}(x_{max,n}), \tag{3.142}$$

which, under

$$b \geq g_{1,n}(x_{max,n}), \tag{3.143}$$

implies that

$$\frac{b}{g_{1,n}(X_1)} > 1 \tag{3.144}$$

and that

$$\frac{b}{g_{1,n}(X_2)} > 1 \tag{3.145}$$

and that

$$\frac{b}{g_{1,n}(X_1)} > \frac{b}{g_{1,n}(X_2)}, \tag{3.146}$$

which, in turn, implies that

$$\left(\frac{b}{g_{1,n}(X_1)} - 1\right)^{1/n} > \left(\frac{b}{g_{1,n}(X_2)} - 1\right)^{1/n}, \tag{3.147}$$

or equivalently,

$$\Psi_{1,n}(X_2) < \Psi_{1,n}(X_1). \tag{3.148}$$

To prove the other items, one can use a similar argument by drawing upon the known properties of $g_{1,n}$ and $g_{2,n}$ under conditions $\theta_X < a_X/2k$ and $\theta_E < a_E/2k$. □

As a result of Proposition 3.1.16, under conditions

$$C_{1,X} : \theta_X < \frac{a_X}{2k} \tag{3.149}$$

and

$$C_{1,E} : \theta_E < \frac{a_E}{2k}, \tag{3.150}$$

one can sketch possible qualitative behaviors of the graphs of the functions $\Psi_{1,n}$ and $\Psi_{2,n}$, as seen in Figure 3.7. Furthermore, one can clearly see in Figure 3.7(d) that there might be a feasible geometric conformation of the graphs of the functions $\Psi_{1,n}$ and $\Psi_{2,n}$ with the maximal number of intersections, that is, with the maximal number of steady states. The latter indeed amounts to 9 steady states.

Corollary 3.1.17.

(i) $\bigwedge_{n\geq 2} \left(\theta_X < \frac{a_X}{2k}\right) \wedge (b \geq g_{1,n}(x_{max,n})) \Rightarrow \Psi_{1,n}(x_{max,n}) = \min_{X\in(0,x_{1,n})} \Psi_{1,n}(X)$

(ii) $\bigwedge_{n\geq 2} \left(\theta_E < \frac{a_E}{2k}\right) \wedge (b \geq g_{2,n}(e_{max,n})) \Rightarrow \Psi_{2,n}(e_{max,n}) = \min_{E\in(0,e_{1,n})} \Psi_{2,n}(E)$

Proof. In fact, (i) and (ii) follow directly from Proposition 3.1.16 (i), Proposition 3.1.16 (ii), Proposition 3.1.16 (iv) and Proposition 3.1.16 (v), respectively. □

However, under $\theta_X < a_X/2k$, if

$$0 < b < \min\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\} \tag{3.151}$$

then, by invoking (3.189) and (3.204), one can demonstrate that

$$\Psi_{1,n}(X)\Big|_{\left(0,x_{b,n}^{(-)}\right]} \tag{3.152}$$

and that

$$\Psi_{2,n}(E)\Big|_{\left(0,e_{b,n}^{(-)}\right]} \tag{3.153}$$

are strictly decreasing, while

$$\Psi_{1,n}(X)\Big|_{\left[x_{b,n}^{(+)},x_{1,n}\right)} \tag{3.154}$$

and

$$\Psi_{2,n}(E)\Big|_{\left[e_{b,n}^{(+)},e_{1,n}\right)} \tag{3.155}$$

are strictly increasing. The latter properties of $\Psi_{1,n}$ and $\Psi_{2,n}$ can be shown by constructing an argument that is similar to the one that has been used to show (3.1.16) (i).

But, what can we conclude from Proposition 3.1.16 ? In fact, one has that the conditions $\theta_X < a_X/2k$ and $\theta_E < a_E/2k$ are sufficient for the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$ to have the qualitative behavior displayed in Figure 3.7. Hence, any steady state $(X^*, E^*) \in G_{\Psi_{1,n}} \bigcap G_{\Psi_{1,n}}$must lie in the rectangle

$$\left\{(X,E) \in \mathbb{R}_+^2 : 0 \le X \le \frac{a_X}{k} + \frac{b}{k},\ 0 \le E \le \frac{a_E}{k} + \frac{b}{k}\right\}. \tag{3.156}$$

Furthermore, one has that Figure 3.7 (d) points us to the feasibility of having a geometric conformation of the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$ with the maximal number of steady states, for which, of course, it is necessary to stipulate additional conditions.

Lemma 3.1.18.

$$\begin{aligned} &(i) \bigwedge_{n\ge 2}\left(\theta_X > \frac{a_X}{k}\right) \Rightarrow \bigwedge_{X\ge 0} g_{1,n}(X) \ge 0 \\ &(ii) \bigwedge_{n\ge 2}\left(\theta_E > \frac{a_E}{k}\right) \Rightarrow \bigwedge_{E\ge 0} g_{2,n}(E) \ge 0 \end{aligned} \tag{3.157}$$

Proof. (i) First, by construction, one has that

$$g_{1,\infty}(X) \le g_{1,n}(X) \le f(X) = kX \tag{3.158}$$

for all $X \ge 0$, wherein $g_{1,\infty}(X) = kX - a_X$. So,

$$g_{1,\infty}(X) = kX - a_X \ge 0 \tag{3.159}$$

if and only if

$$X \geq \frac{a_X}{k}. \tag{3.160}$$

Thereby, under $\theta_X > \frac{a_X}{k}$, if we draw on (3.158) then we arrive at

$$g_{1,n}(X) > 0, \tag{3.161}$$

for all $X \geq \theta_X$. Now, under $\theta_X > \frac{a_X}{k}$, suppose that there exists $\bar{X} > 0$ such that

$$g_{1,n}(\bar{X}) < 0, \tag{3.162}$$

if and only if

$$k\bar{X} < a_X \frac{\bar{X}^n}{\theta_X^n + \bar{X}^n}, \tag{3.163}$$

which implies that

$$\bar{X} < \frac{a_X}{k}. \tag{3.164}$$

On the other hand,

$$k\bar{X} < a_X \frac{\bar{X}^n}{\theta_X^n + \bar{X}^n} \tag{3.165}$$

implies that

$$(\theta_X^n + \bar{X}^n) < \frac{a_X}{k}\bar{X}^{n-1}, \tag{3.166}$$

which implies that

$$\left(\frac{a_X}{k}\right)^n < \frac{a_X}{k}\bar{X}^{n-1}, \tag{3.167}$$

which, in turn, implies that

$$\bar{X} > \frac{a_X}{k}, \tag{3.168}$$

which is a contradiction. Therefore, under $\theta_X > \frac{a_X}{k}$, one must have that

$$g_{1,n}(\bar{X}) \geq 0 \tag{3.169}$$

for all $X \geq 0$. □

Further, if we recall that $g_{1,n}(X)$ has an inflection point at

$$x_{inf,n} = \theta_X \left(\frac{n-1}{n+1}\right)^{1/n} < \theta_X \tag{3.170}$$

then we have that $g''_{1,n}(X) < 0$ for $X < x_{inf,n}$ and $g''_{1,n}(X) > 0$ for $X > x_{inf,n}$, that is, $g'_{1,n}(X)$ is strictly decreasing on $[0, x_{inf,n})$ and strictly increasing on $(x_{inf,n}, \infty)$. Hence, one has that the graph of $g_{1,n}(X)$ is concave down on $[0, x_{inf,n})$ and concave up [convex] on $(x_{inf,n}, \infty)$.

So, if $g'_{1,n}(x_{inf,n}) \geq 0$ then $g_{1,n}(X)$ is strictly increasing on $[0, \infty)$. The latter condition amounts to

$$k - \frac{a_X}{\theta_X}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n} \geq 0, \tag{3.171}$$

that is,

$$\theta_X \geq \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}. \tag{3.172}$$

As an illustration of such a behavior, we refer to the Figure 3.3. On the other hand, under $\theta_X > a_X/k$, there exist $x_{max,n}$ and $x_{min,n}$ satisfying

$$g_{1,n}(x_{max,n}) = \max_{X\in(0,\theta_X]} g_{1,n}(X) \tag{3.173}$$

and

$$g_{1,n}(x_{min,n}) = \min_{X\in[\theta_X,\infty)} g_{1,n}(X), \tag{3.174}$$

if and only if

$$\theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}. \tag{3.175}$$

Further, one has that

$$g_{1,n}(\theta_X) = k\theta_X - \frac{a_X}{2}, \tag{3.176}$$

and

$$g'_{1,n}(\theta_X) = k - \frac{na_X}{4\theta_X}, \tag{3.177}$$

So, under $\theta_X > a_X/k$ and (3.175), that is, in case $x_{max,n}$ and $x_{min,n}$ exist, one has that the line Y_{θ_X} tangent to the graph of $g_{1,n}(X)$ at $X = \theta_X$ satisfies

$$g_{1,n}(X) \leq Y_{\theta_X}(X) = g_{1,n}(\theta_X) + g'_{1,n}(\theta_X)(X-\theta_X) \tag{3.178}$$

for all $0 \leq X \leq \theta_X$, wherein

$$g_{1,n}(\theta_X) = k\theta_X - \frac{a_X}{2}, \tag{3.179}$$

and

$$g'_{1,n}(\theta_X) = k - \frac{na_X}{4\theta_X}, \tag{3.180}$$

which implies that the inequality (3.178) can be rewritten as

$$g_{1,n}(X) \leq kX + \rho X + \frac{a_X}{2}\left(\frac{n}{2}-1\right), \tag{3.181}$$

with

$$\rho = -\frac{na_X}{4\theta_X}. \tag{3.182}$$

Therefore, one has that the lines $Y^{(\theta_X)}$ and $Y = kX$ intersect each other at the point $((1-2/n)\theta_X, k(1-2/n)\theta_X) \in \mathbb{R}^2$, which implies that

$$g_{1,n}(x_{max,n}) < k\left(1-\frac{2}{n}\right)\theta_X. \tag{3.183}$$

Likewise, one has that the lines $Y^{(\theta_X)}$ and $g_{1,\infty}(X) = kX - a_X$ intersect each other at the point $((1+2/n)\theta_X, k(1+2/n)\theta_X - a_X) \in \mathbb{R}^2$, which implies that

$$g_{1,n}(x_{min,n}) > k\left(1+\frac{2}{n}\right)\theta_X - a_X > 0, \tag{3.184}$$

as illustrated in Figure 3.8. Regarding the later, we denote

$$\theta_X^{(+)} = \left(1+\frac{2}{n}\right)\theta_X \tag{3.185}$$

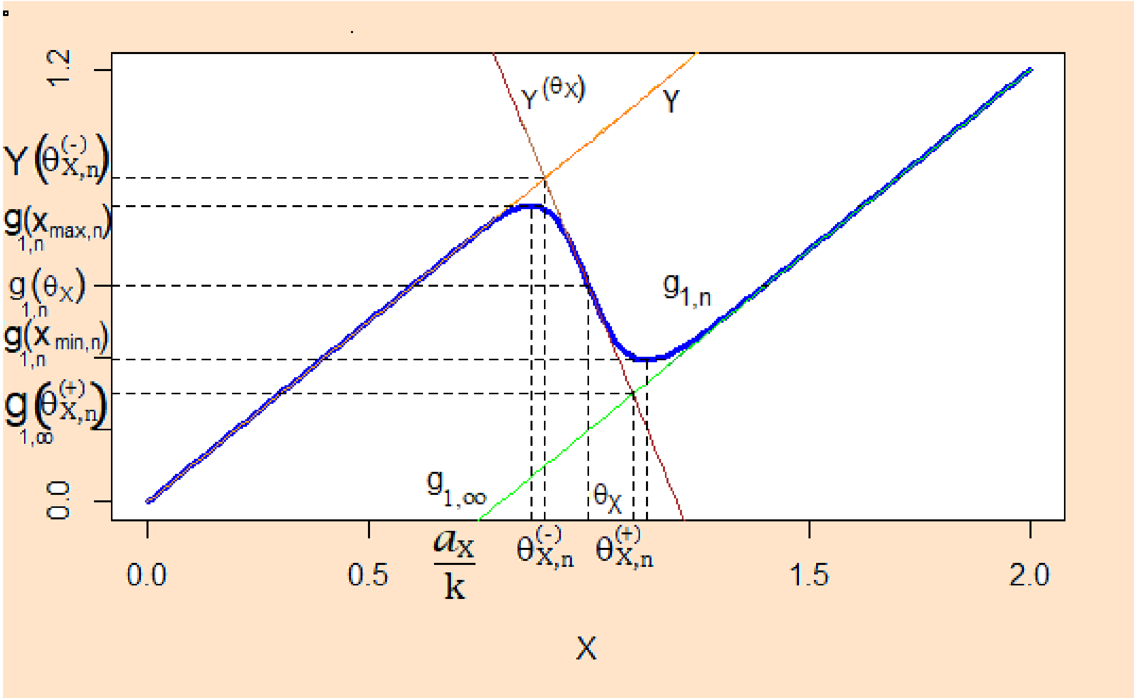


Figure 3.8: Here, we see the plot of the qualitative behavior of $g_{1,n}(X)$ in blue for the choices $n = 20$, $\theta_X = 1$, $k = 1$, $a_X = 0.8$. As a result, one has that $x_{max,n} = 0.87$, $x_{min,n} = 1.13$, $\theta_X^{(-)} = 0.9$, $\theta_X^{(+)} = 1.1$, $g_{1,n}(x_{max,n}) = 0.8234985$, $g_{1,n}(x_{min,n}) = 0.3951435$, $g_{1,n}(\theta_X) = 0.6$, $Y(\theta_X^{(-)}) = 0.9$, and $g_{1,\infty}(\theta_X^{(+)}) = 0.3$.

and

$$\theta_X^{(-)} = \left(1 - \frac{2}{n}\right)\theta_X. \tag{3.186}$$

Proposition 3.1.19.

(i) $\bigwedge_{n\geq 2} \left(\theta_X > \frac{a_X}{k}\right) \wedge (b \geq g_{1,n}(x_{max,n})) \wedge \left(\theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{1,n}(X)\Big|_{(0,x_{max,n}]}$ *is strictly decreasing;*

(ii) $\bigwedge_{n\geq 2} \left(\theta_X > \frac{a_X}{k}\right) \wedge (b \geq g_{1,n}(x_{max,n})) \wedge \left(\theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{1,n}(X)\Big|_{[x_{max,n},x_{min,n}]}$ *is strictly increasing;*

(iii) $\bigwedge_{n\geq 2} \left(\theta_X > \frac{a_X}{k}\right) \wedge (b \geq g_{1,n}(x_{max,n})) \wedge \left(\theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{1,n}(X)\Big|_{[x_{min,n},x_{b,n}]}$ *is strictly decreasing;*

(iv) $\bigwedge_{n\geq 2} \left(\theta_E > \frac{a_E}{k}\right) \wedge (b \geq g_{2,n}(e_{max,n})) \wedge \left(\theta_E < \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{2,n}(E)\Big|_{(0,e_{max,n}]}$ *is strictly decreasing;*

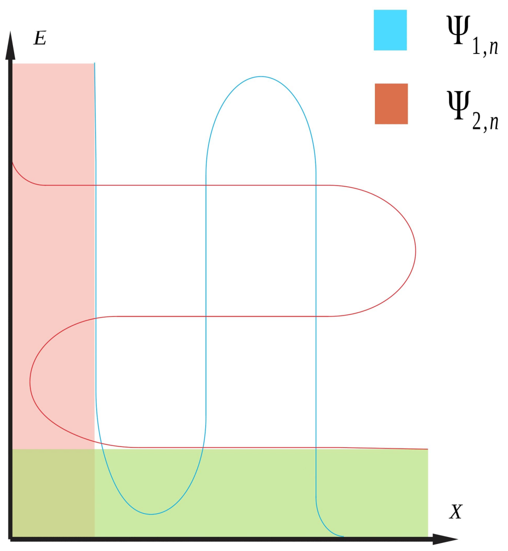


Figure 3.9: For $n \geq 2$, under conditions (3.209) and (3.210), one sees a geometric conformation of $\Psi_{1,n}(X)$ and $\Psi_{2,n}(X)$ with the maximal number of steady states.

(v) $\bigwedge_{n\geq 2} \left(\theta_E > \frac{a_E}{k}\right) \wedge \left(b \geq g_{2,n}(e_{max,n})\right) \wedge \left(\theta_E < \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{2,n}(E)\Big|_{[e_{max,n},e_{min,n}]}$ *is strictly increasing;*

(vi) $\bigwedge_{n\geq 2} \left(\theta_E > \frac{a_E}{k}\right) \wedge \left(b \geq g_{2,n}(e_{max,n})\right) \wedge \left(\theta_E < \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{2,n}(E)\Big|_{[e_{min,n},e_{b,n}]}$ *is strictly decreasing;*

Proof. We can build on a similar argument used to show Proposition 3.1.16. □

Corollary 3.1.20.

(i) $\bigwedge_{n\geq 2} \left(\theta_X > \frac{a_X}{k}\right) \wedge \left(b \geq g_{1,n}(x_{max,n})\right) \wedge \left(\theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{1,n}(x_{max,n}) = \min_{X\in(0,\theta_X]} \Psi_{1,n}(X)$

(ii) $\bigwedge_{n\geq 2} \left(\theta_X > \frac{a_X}{k}\right) \wedge \left(b \geq g_{1,n}(x_{max,n})\right) \wedge \left(\theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{1,n}(x_{min,n}) = \max_{X\in[\theta_X,x_{b,n}]} \Psi_{1,n}(X)$

(iii) $\bigwedge_{n\geq 2} \left(\theta_E > \frac{a_E}{k}\right) \wedge \left(b \geq g_{2,n}(e_{max,n})\right) \wedge \left(\theta_E < \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{2,n}(e_{max,n}) = \min_{E\in(0,\theta_E]} \Psi_{2,n}(E)$

(iv) $\bigwedge_{n\geq 2} \left(\theta_E > \frac{a_E}{k}\right) \wedge \left(b \geq g_{2,n}(e_{max,n})\right) \wedge \left(\theta_E < \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}\right) \Rightarrow \Psi_{2,n}(e_{min,n}) = \max_{E\in[\theta_E,e_{b,n}]} \Psi_{2,n}(E)$

Proof. It follows directly from Proposition (3.1.19). □

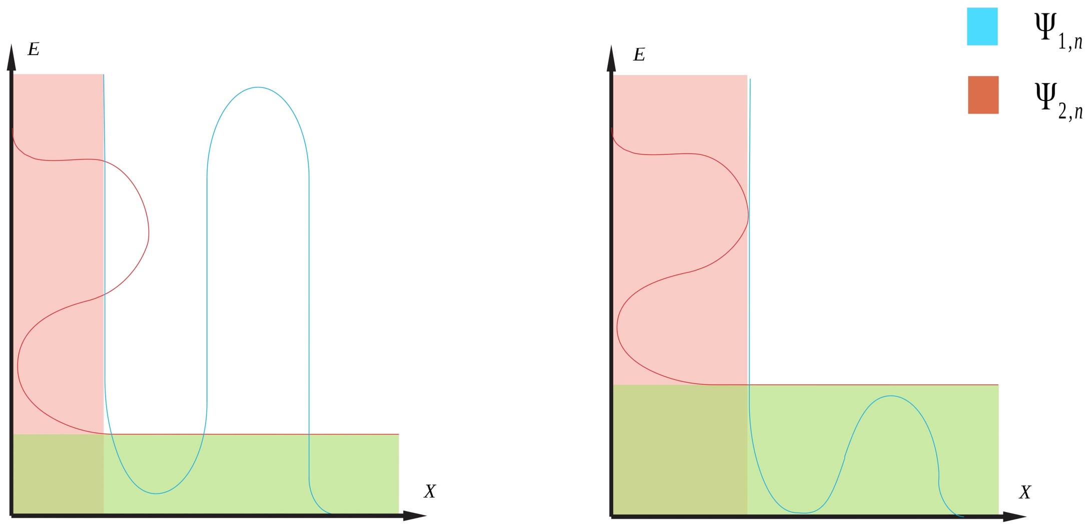


Figure 3.10: For $n \geq 2$, under conditions (3.209) and (3.210), one sees the geometric conformations of $\Psi_{1,n}(X)$ and $\Psi_{2,n}(X)$. As we see here, the respective conditions are not sufficient to give rise to geometric conformations with the maximal number of steady states.

However, if

$$\begin{aligned} \theta_X &> \frac{a_X}{k}, \\ \theta_X &< \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \end{aligned} \tag{3.187}$$

and

$$\max\{g_{1,n}(x_{min,n}), g_{2,n}(e_{min,n})\} \leq b < \min\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\}, \tag{3.188}$$

then, by invoking the Intermediate Value Theorem [77, p. 93], one has that

$$\{X \geq 0 : g_{1,n}(X) = b\} = \left\{x_{b,n}^{(-)}, x_{b,n}^{(+)}, x_{b,n}\right\}, \tag{3.189}$$

with $0 < x_{b,n}^{(-)} < x_{max,n}$, $x_{max,n} < x_{b,n}^{(+)} < x_{min,n}$, and $x_{b,n} > x_{min,n}$. Hence, one must have that

$$\Psi_{1,n}\left(x_{b,n}^{(-)}\right) = \Psi_{1,n}\left(x_{b,n}^{(+)}\right) = \Psi_{1,n}\left(x_{b,n}^{(-)}\right) = 0, \tag{3.190}$$

so that $\Psi_{1,n}(X)$ is not defined on the interval $\left(x_{b,n}^{(-)}, x_{b,n}^{(+)}\right)$. A similar reasoning can be performed under

$$\begin{aligned} \theta_E &> \frac{a_E}{k}, \\ \theta_E &< \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \end{aligned} \tag{3.191}$$

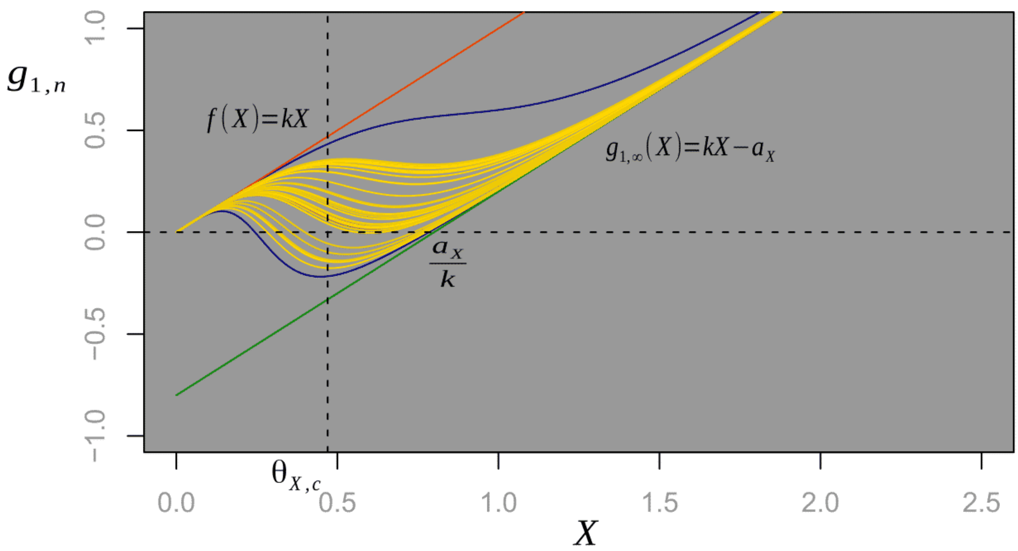


Figure 3.11: Here, one sees the transition zone $H_n[C_{2,X}, C_{1,E}] \cup H_n[C_{1,X}, C_{2,E}] \cup H_n[C_{2,X}, C_{2,E}] \cup H_n[C_{3,X}, C_{2,E}] \cup H_n[C_{2,X}, C_{3,E}]$ *in yellow. The idea being illustrated in this plot is that of a mutual transition between 4 subspaces of the parameter space, namely,* $H_n[C_{1,X}, C_{1,E}]$, $H_n[C_{3,X}, C_{1,E}]$, $H_n[C_{1,X}, C_{3,E}]$, *and* $H_n[C_{3,X}, C_{3,E}]$*. The transition occurs by varying* θ_X *in* $\left(\frac{a_X}{2k}, \frac{a_X}{k}\right)$ *with which one gets different plots for* $g_{1,n}$ *in blue. In this transition zone, one sees behaviors of both subspaces. Here, we vary* θ_X *in* $[0.42, 0.5]$*, and we choose* $n = 4$, $a_X = 0.8$*, and* $k = 1$*, with which we find that* $\theta_{X,c}^{layer} = 0.4535$*. The latter indicates the critical geometric conformation in the non-generic critical layer.*

and

$$\max\{g_{1,n}(x_{min,n}), g_{2,n}(e_{min,n})\} \leq b < \min\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\}, \tag{3.192}$$

respectively. In fact, in this case, one has that

$$\{E \geq 0 : g_{2,n}(E) = b\} = \{e_{b,n}^{(-)}, e_{b,n}^{(+)}, e_{b,n}\}, \tag{3.193}$$

and that

$$\Psi_{2,n}\left(e_{b,n}^{(-)}\right) = \Psi_{2,n}\left(e_{b,n}^{(+)}\right) = \Psi_{2,n}\left(e_{b,n}^{(-)}\right) = 0, \tag{3.194}$$

with $0 < e_{b,n}^{(-)} < e_{max,n}$, $e_{max,n} < e_{b,n}^{(+)} < e_{min,n}$, and $e_{b,n} > e_{min,n}$.

Therefore, one can show that

$$\Psi_{1,n}(X)\Bigg|_{\left(0,x_{b,n}^{(-)}\right]} \tag{3.195}$$

and that

$$\Psi_{2,n}(E)\Bigg|_{\left(0,e_{b,n}^{(-)}\right]} \tag{3.196}$$

are strictly decreasing, while

$$\Psi_{1,n}(X)\Big|_{\left[x_{b,n}^{(+)},x_{1,n}\right)} \tag{3.197}$$

and

$$\Psi_{2,n}(E)\Big|_{\left[e_{b,n}^{(+)},e_{1,n}\right)} \tag{3.198}$$

are strictly increasing. By the same token, one can show that

$$\Psi_{1,n}(X)\Big|_{\left[x_{min,n},x_{b,n}\right]} \tag{3.199}$$

and that

$$\Psi_{2,n}(E)\Big|_{\left[e_{min,n},e_{b,n}\right]} \tag{3.200}$$

are strictly decreasing.

Further, if

$$\begin{aligned} \theta_X &> \frac{a_X}{k}, \\ \theta_E &> \frac{a_E}{k}, \\ \theta_X &< \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \\ \theta_E &< \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \end{aligned} \tag{3.201}$$

and

$$0 < b < \min\{g_{1,n}(x_{min,n}), g_{2,n}(e_{min,n})\}, \tag{3.202}$$

then, by invoking the Intermediate Value Theorem [77, p. 93], one can show that

$$\{X \geq 0 : g_{1,n}(X) = b\} = \{x_{b,n}^{(+)}\} \tag{3.203}$$

and that

$$\{E \geq 0 : g_{2,n}(E) = b\} = \{e_{b,n}^{(+)}\}, \tag{3.204}$$

which implies that

$$\Psi_{1,n}\left(x_{b,n}^{(+)}\right) = 0, \tag{3.205}$$

and that

$$\Psi_{2,n}\left(e_{b,n}^{(+)}\right) = 0, \tag{3.206}$$

so

$$\Psi_{1,n}(X)\Big|_{\left(0,x_{b,n}^{(+)}\right]} \tag{3.207}$$

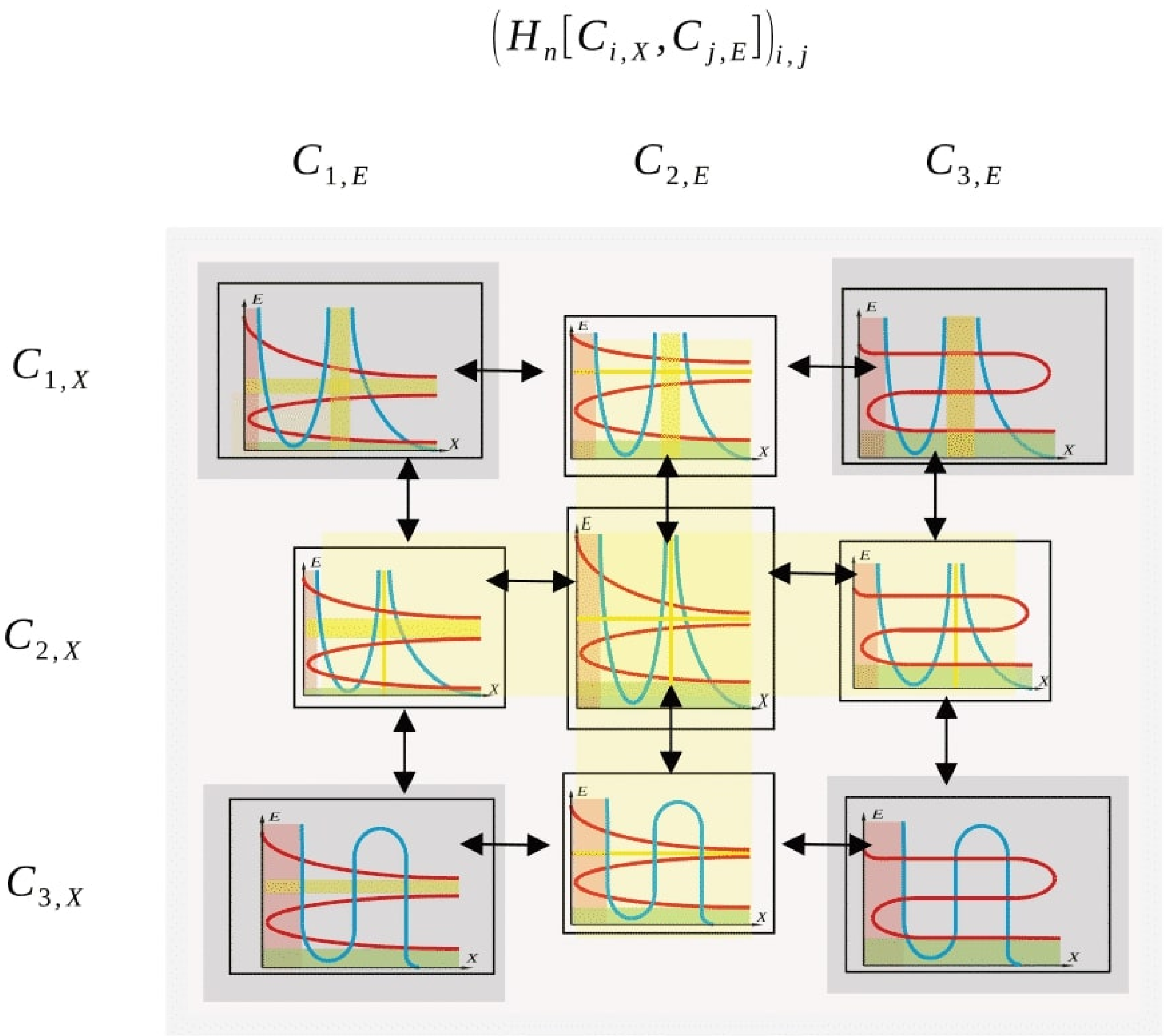


Figure 3.12: For $n \geq 2$, one sees Huang's qualitative graphical matrix $(H_n[C_{i,X}, C_{j,E}])_{1,j}$ whose entries are being illustrated with the respective geometric conformations of the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$ with the maximal number of steady states. Furthermore, in the transition zone, one has the occurrence of both conformations though not in the same parameter setting. Hence, the matrix consists of 4 principal components $H_n[C_{1,X}, C_{1,E}] \cup H_n[C_{3,X}, C_{1,E}] \cup H_n[C_{1,X}, C_{3,E}] \cup H_n[C_{3,X}, C_{3,E}]$ in gray and a transition zone $H_n[C_{2,X}, C_{1,E}] \cup H_n[C_{1,X}, C_{2,E}] \cup H_n[C_{2,X}, C_{2,E}] \cup H_n[C_{3,X}, C_{2,E}] \cup H_n[C_{2,X}, C_{3,E}]$ in yellow, in which the critical non-generic layer is being indicated with the critical geometric conformation.

and that

$$\Psi_{2,n}(E)\Bigg|_{\left(0,e^{(+)}_{b,n}\right]} \tag{3.208}$$

are strictly decreasing. Therefore, $\Psi_{1,n}(X)$ and $\Psi_{2,n}(E)$ are indeed strictly decreasing on $(0,+\infty)$.

But, what can we conclude from Proposition 3.1.19 ? In fact, under the conditions

$$C_{3,X} : \theta_X > \frac{a_X}{k} \tag{3.209}$$

and

$$C_{3,E} : \theta_E > \frac{a_E}{k}, \tag{3.210}$$

one has that the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$, for example, can have one of the qualitative behaviors displayed in Figures 3.9 and 3.10. In addition, any steady state $(X^*, E^*) \in G_{\Psi_{1,n}} \bigcap G_{\Psi_{1,n}}$ must lie in the rectangle

$$\left\{(X,E) \in \mathbb{R}^2_+ : 0 \leq X \leq \frac{a_X}{k} + \frac{b}{k},\ 0 \leq E \leq \frac{a_E}{k} + \frac{b}{k}\right\}. \tag{3.211}$$

Furthermore, one has that the qualitative behavior shown in Figure 3.9 is similar to the one displayed in Figure 3.7 (d), which, consistently, indicates the feasibility of having a geometric conformation of the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$ with the maximal number of steady states. Regarding the latter, one must accordingly stipulate additional conditions so as to fix such suitable geometric conformations.

Next, under the condition

$$C_{2,X} : \frac{a_X}{2k} \leq \theta_X \leq \frac{a_X}{k}, \tag{3.212}$$

one sees in Figure 3.11 that $g_{1,n}(X)$ shows behaviours under both conditions $\theta_X < a_X/2k$ and $\theta_X > a_X/k$. A similar argument can be used for $g_{2,n}(E)$ under the condition

$$C_{2,E} : \frac{a_E}{2k} \leq \theta_E \leq \frac{a_E}{k}, \tag{3.213}$$

respectively.

But, how can we understand the structure of the parameter space $\Lambda_H := \mathbb{R}^6_{\geq 0} \times \mathbb{N}_{\geq 0}$ of Huang's model? In fact, if we conveniently define

$$\hat{\Lambda}_H := \mathbb{R}^6_{\geq 0} \times \mathbb{N}_{\geq 2} \tag{3.214}$$

and

$$\Lambda^{(1)}_H := \mathbb{R}^6_{\geq 0} \times \{1\}, \tag{3.215}$$

then one has that

$$\Lambda_H = \hat{\Lambda}_H \cup \Lambda^{(1)}_H. \tag{3.216}$$

Upon doing so, we first intend understanding the structure of $\hat{\Lambda}_H$, whilst the structure of $\Lambda^{(1)}_H$ will be addressed later in Chapter 3.

Now, if we invoke Section 2.8, then we can regard

$$\hat{\mathcal{A}} \coloneqq \{C_{1,X}, C_{1,E}, C_{2,X}, C_{2,E}, C_{3,X}, C_{3,E}\} \tag{3.217}$$

as the set of the *primary aspects*, which, in fact, is fully determined by the mathematical formulation of Huang's model. Hence, for $\lambda, \tilde{\lambda} \in \hat{\Lambda}_H$, one can define

$$\lambda \sim_{\hat{\mathcal{A}}} \tilde{\lambda} \tag{3.218}$$

if and only if

$$\mid A[H_\lambda] \mid_{\mathbb{R}_+} = \mid A[H_{\tilde{\lambda}}] \mid_{\mathbb{R}_+}, \tag{3.219}$$

for all $A \in \hat{\mathcal{A}}$, with $\mid A \mid_{\mathbb{R}_+}$ denoting the truth-value of a mathematical assertion A. As H_λ betokens Huang's model with a fixed parameter setting $\lambda \in \hat{\Lambda}_H$, then $A[H_\lambda]$ symbolizes a formalized mathematical *assertion* on H_λ indeed.

Next, as we have argued in Chapter 2, one has that the binary relation defined in (3.218) is indeed an equivalence one. Thereby, for each $n \geq 2$, one must have that the sets

$$H_n[C_{i,X}, C_{j,E}] \coloneqq \left\{\lambda \in \mathbb{R}^6 \times \{n\} : \vdash C_{i,X}[H_\lambda] \wedge \vdash C_{j,E}[H_\lambda]\right\}, \tag{3.220}$$

with $i, j \in \{1, 2, 3\}$, are equivalent classes, among which $H_n[C_{1,X}, C_{1,E}]$, $H_n[C_{1,X}, C_{3,E}]$, $H_n[C_{3,X}, C_{1,E}]$, and $H_n[C_{3,X}, C_{3,E}]$, are consistently said to form the *main components*, while $H_n[C_{2,X}, C_{1,E}]$, $H_n[C_{1,X}, C_{2,E}]$, $H_n[C_{2,X}, C_{3,E}]$, $H_n[C_{3,X}, C_{2,E}]$, and $H_n[C_{2,X}, C_{2,E}]$, are said to generate the *transition zone*.

Hence, for each $n \geq 2$, one has that the equivalent classes in (3.220) give rise to a matrix structure , that is,

$$(H_n[C_{i,X}, C_{j,E}])_{i,j} \coloneqq \bigcup_{i,j \in \{1,2,3\}} H_n[C_{i,X}, C_{j,E}], \tag{3.221}$$

which, by construction, implies that

$$\hat{\Lambda}_H /\!\sim_{\hat{\mathcal{A}}} = \bigcup_{n \geq 2} (H_n[C_{i,X}, C_{j,E}])_{i,j}, \tag{3.222}$$

and we can now understand a large part of the parameter space of the model, as illustrated in Figure 3.13. However, we have not answered the question concerning the dimension of the critical layer, which, so far, has been intuitively apprehended. So, how large is this critical layer ? Which conception of measure can be used to access it ? Although knowing the critical layer entails answering the later questions, one has that such a task deviates from the scope of this thesis.

So, for each $n \geq 2$, if we slice $\hat{\Lambda}_H$ at the respective level n and if we look into it modulo the equivalence relation defined in (3.218), then we see the matrix structure defined in (3.221). The later can be envisaged through the qualitative behaviour of the graphs [nullclines] of $\Psi_{1,n}(X)$ and $\Psi_{2,n}(E)$, as illustrated in Figure 3.12. In light of that, we name the matrix structure $(H_n[C_{i,X}, C_{j,E}])_{i,j}$ defined in (3.221) *Huang's qualitative graphical matrix*.

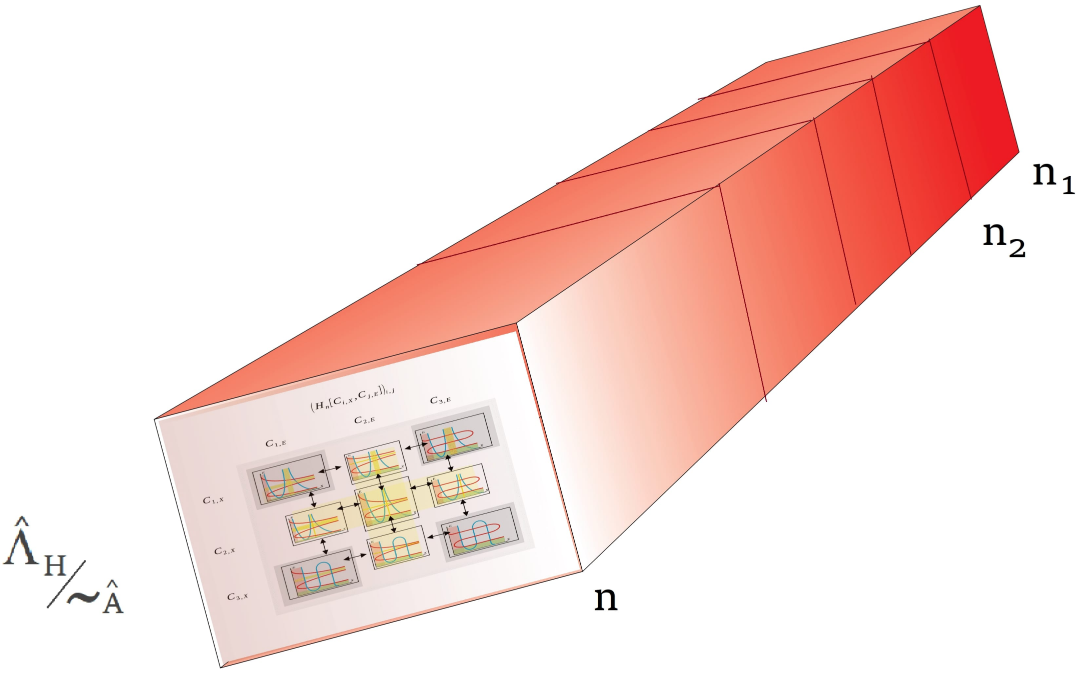


Figure 3.13: Here, one sees an illustration of $\hat{\Lambda}_H/\sim_{\hat{A}}$ *with* $2 \leq n_1 \leq n_2 \leq \ldots \leq n$*, which, for* $n \geq 2$*, amounts to Huang's qualitative graphical matrix* $(H_n[C_{i,X}, C_{j,E}])_{1,j}$*. Thereby, consistently,* $n_1 \geq 2$*, one has that* $H_{n_1} = (H_{n_1}[C_{i,X}, C_{j,E}])_{i,j}$*. Likewise, for* $n_2 \geq 2$*, one has that* $H_{n_2} = (H_{n_2}[C_{i,X}, C_{j,E}])_{i,j}$*.*

Further, without loss of generality, we make no explicit distinction among the equivalent classes defined in (3.220) as elements of

$$\hat{\Lambda}_H/\sim_{\hat{A}},$$

the components of Huang's qualitative graphical matrix and the sets on the left-hand side of definition (3.220).

3.2 The scenario space and the determination of primitive scenarios

So, for $n \geq 2$, if we invoke Proposition 3.1.16 and if we want to fix the geometric conformation of $\Psi_{1,n}$ and $\Psi_{2,n}$ shown in Figure 3.7(d), then it is sufficient to have that the conditions

$$\begin{aligned} C_{1,X} &: \theta_X < \frac{a_X}{2k}, \\ C_{1,E} &: \theta_E < \frac{a_E}{2k}, \\ C_{0,n} &: b \geq \max\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\}, \\ C_{1,n} &: 0 \leq \Psi_{2,n}(e_{max,n}) < \Psi_{1,n}^{-1}|_{(0,x_{max,n})}\left(\frac{a_E}{k} + \frac{b}{k}\right), \\ C_{2,n} &: 0 \leq \Psi_{1,n}(x_{max,n}) < \Psi_{2,n}^{-1}|_{(0,e_{max,n})}\left(\frac{a_X}{k} + \frac{b}{k}\right), \end{aligned} \tag{3.223}$$

hold, as illustrated in the Figure 3.14; with $x_{max,n} \in [0, x_{1,n}]$ satisfying

$$g_{1,n}(x_{max,n}) = \max_{X \in [0,x_{1,n}]} g_{1,n}(X), \tag{3.224}$$

and with $e_{max,n} \in [0, e_{1,n}]$ satisfying

$$g_{2,n}(e_{max,n}) = \max_{E \in [0,e_{1,n}]} g_{2,n}(E), \tag{3.225}$$

as seen in the Figure 3.6. So, by construction, for any $n \geq 2$, there is

$$(a_X, a_E, k, \theta_X, \theta_E, b, n) \in \hat{\Lambda}_H$$

for which the conditions in (3.223) hold. In fact, if a_X, k, θ_X satisfies $C_{1,X}$ then one chooses for $a_E = a_X$, $\theta_X = \theta_E$, and $b = g_{1,n}(x_{max,n}) = g_{2,n}(e_{max,n})$. In so doing, one has that $\Psi_{1,n}(x_{max,n}) = 0 = \Psi_{2,n}(e_{max,n})$. As a conclusion, one has that such a geometric conformation of the graphs of $\Psi_{1,n}(X)$ and $\Psi_{2,n}(E)$ can always be found in $H_n[C_{1,X}, C_{1,E}]$ independent upon $n \geq 2$. Furthermore, it leads us to one of the most important results of our analysis.

Theorem 3.2.1. *If $\lambda = (a_X, a_E, k, \theta_X, \theta_E, b, n) \in \hat{\Lambda}_H$ satisfies the conditions*

$$C_{1,X},\ C_{1,E},\ C_{0,n},\ C_{1,n},\ C_{2,n} \tag{3.226}$$

then

$$G_{\Psi_{1,n}} \cap G_{\Psi_{2,n}} = \{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}, z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,7}, z^0_{ss,8}, z^0_{ss,9}\}, \tag{3.227}$$

as illustrated in Figure 3.14.

Proof. It follows directly from Proposition 3.1.16 under conditions $C_{1,n}$ and $C_{2,n}$. □

In fact, for $n = 4$ and $(a_X = 3.2, a_E = 3.2, k = 0.7, \theta_X = 1, \theta_E = 1)$, one has that the choice $b = 0.2007054 = \max\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\}$ guarantees the satisfaction of the conditions $(3.223)_{4,5}$, which, indeed, can be verified in the numerical simulations shown in Figure 3.15 and 3.16. However, for $b = 0.209$, one loses information due to the destruction of 4 steady states as shown in Figure 3.17 and 3.18. In fact, for the later choice, one has that the conditions $(3.223)_{4,5}$ no longer hold.

But, can we estimate $b > 0$ for which it happens? In fact, by inspection of the Figure (4.1), a sufficient condition is that

$$e_{1,n} > \Psi_{1,n}(x_{n,max}) > \Psi_{2,n}^{-1}(x_{max,n}), \tag{3.228}$$

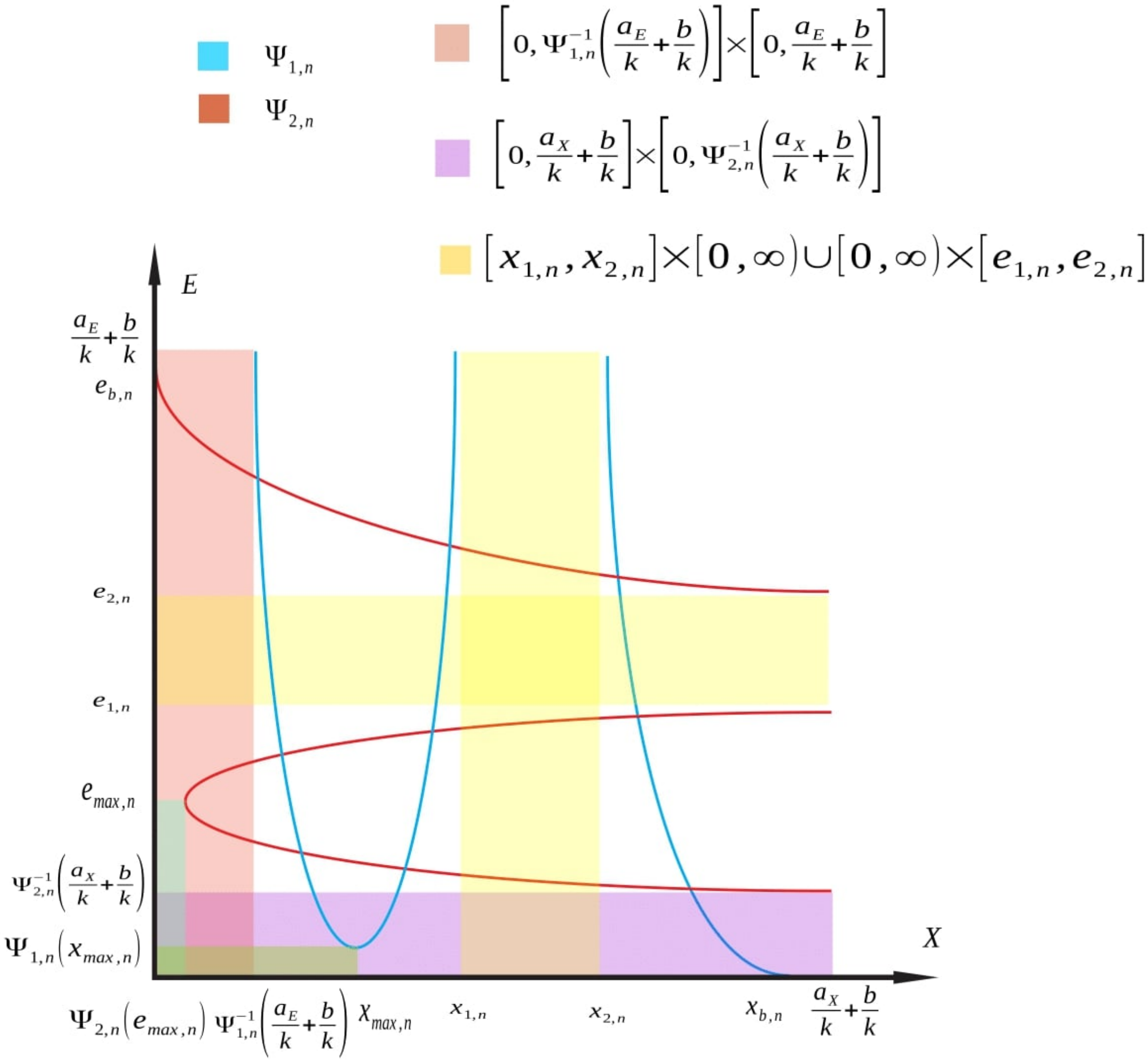


Figure 3.14: Here, for $n \geq 2$, we see the qualitative behavior of the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$, under (3.223).

which implies that

$$b < g_{1,n}(x_{max,n})[1 + e_{1,n}^n], \tag{3.229}$$

which, in turn, gives an upper bound for b. Hence, the critical value b_c of b must satisfy

$$\max\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\} < b_c < \min\{g_{1,n}(x_{max,n})[1+e_{1,n}^n], g_{2,n}(e_{max,n})[1+x_{1,n}^n]\}. \tag{3.230}$$

For instance, for $n = 4$ and ($a_X = 3.2, a_E = 3.2, k = 0.7, \theta_X = 1, \theta_E = 1$), one has that b_c must be in the interval $(0.2007054, 0.2343782)$. Consistently, if

$$b \geq \max\{g_{1,n}(x_{max,n})[1 + e_{1,n}^n], g_{2,n}(e_{max,n})[1 + x_{1,n}^n]\} \tag{3.231}$$

then one must have that the destruction of the 4 steady states, as shown in Figure 3.18, has already occurred. The inequalities (3.230) and (3.231) are clearly illustrated in Figure (3.8).

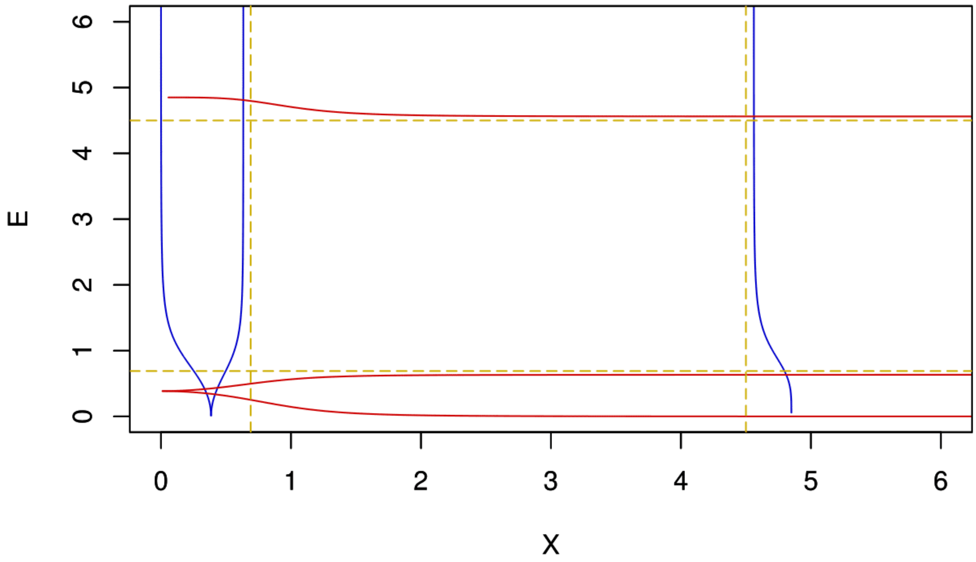


Figure 3.15: Here, we have plotted the graphs of $\Psi_{1,n}$ (in blue) and $\Psi_{2,n}$ (in red) for the choices $n = 4$, $b = 0.2007054$, $\theta_X = \theta_E = 1$, $k = 0.7$, $a_X = 3.2$ and $a_E = 3.2$. The latter choices shows correctness in our strategy to fix the geometric conformation showed in Figure 4.1.

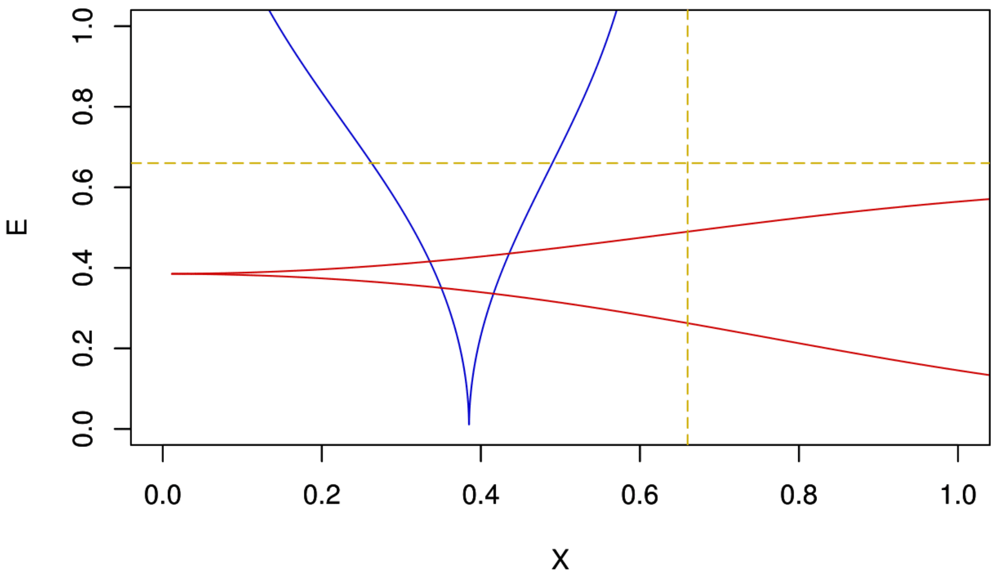


Figure 3.16: For the choices $n = 4$, $b = 0.2007054 = g_{1,n}(x_{max,n}) = g_{2,n}(e_{max,n})$, $\theta_X = \theta_E = 1$, $k = 0.7$, $a_X = 3.2$ and $a_E = 3.2$, one sees a better look at the region wherein one has 4 steady states.

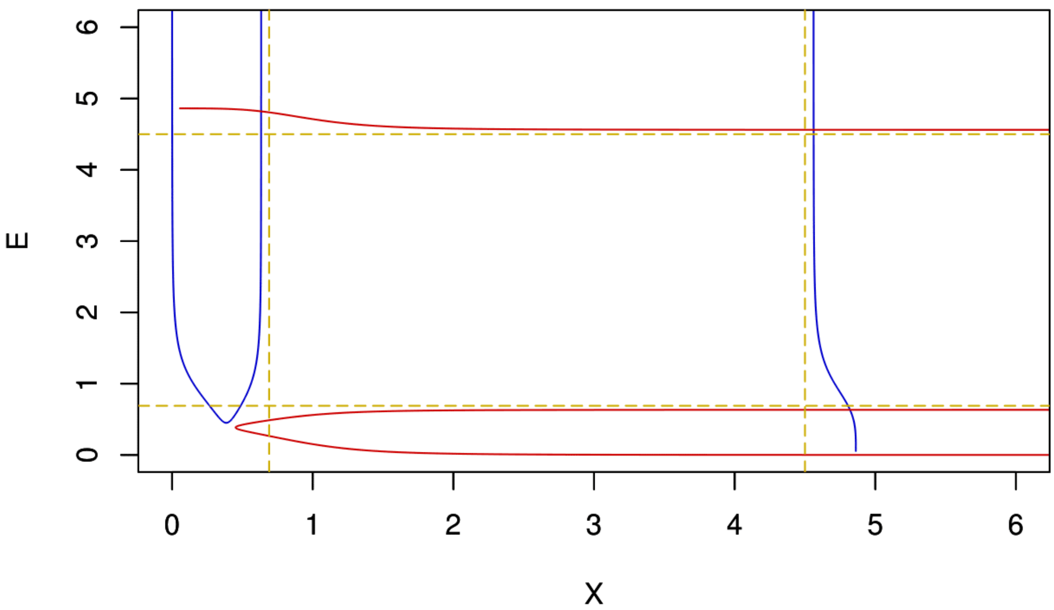


Figure 3.17: Here, we have plotted the graphs of $\Psi_{1,n}$ (in blue) and $\Psi_{2,n}$ (in red) for the choices $n = 4$, $b = 0.209$, $\theta_X = \theta_E = 1$, $k = 0.7$, $a_X = 3.2$ and $a_E = 3.2$. With this choice, we dismantle the geometric conformation in Figure (3.15) by destroying 4 steady states thereof.

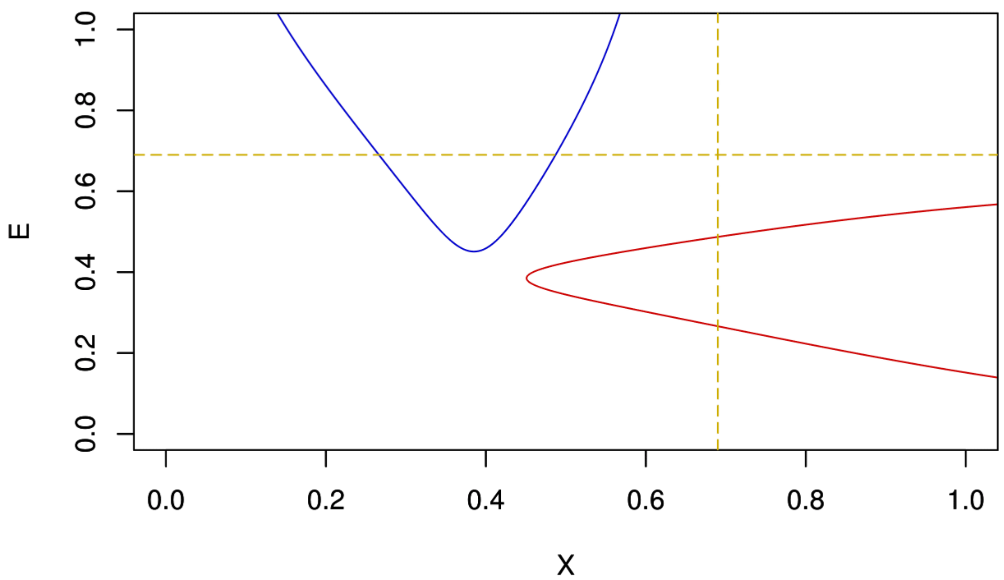


Figure 3.18: For the choices $n = 4$, $b = 0.209$, $\theta_X = \theta_E = 1$, $k = 0.7$, $a_X = 3.2$ and $a_E = 3.2$, one sees the region wherein one loses 4 steady states.

But, what about the other main components of Huang's qualitative graphical matrix ? In fact, it is sufficient to consider $H_n[C_{3,X}, C_{3,E}]$. So, by invoking Proposition 3.1.19, if the conditions

$$\begin{aligned} C_{3,X} &: \theta_X > \frac{a_X}{k}, \\ C_{3,E} &: \theta_E > \frac{a_E}{k}, \\ C_{0,n} &: b \geq \max\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\}, \\ C_{1,n} &: 0 \leq \Psi_{2,n}(e_{max,n}) < \Psi_{1,n}^{-1}|_{(0,x_{max,n})}\left(\frac{a_E}{k} + \frac{b}{k}\right), \\ C_{2,n} &: 0 \leq \Psi_{1,n}(x_{max,n}) < \Psi_{2,n}^{-1}|_{(0,e_{max,n})}\left(\frac{a_X}{k} + \frac{b}{k}\right), \\ C_{3,n} &: \theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \\ c_{4,n} &: \theta_E < \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \\ C_{5,n} &: \Psi_{1,n}(x_{min,n}) > \Psi_{2,n}^{-1}|_{(e_{min,n},+\infty)}(x_{min,n}), \\ C_{6,n} &: \Psi_{2,n}(e_{min,n}) > \Psi_{1,n}^{-1}|_{(x_{min,n},+\infty)}(e_{min,n}), \end{aligned} \tag{3.232}$$

hold, then one has that the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$ behave as illustrated in the Figure 3.19, with $x_{max,n} \in [0, x_{1,n}]$ satisfying (3.224) and (3.225). The latter amounts to the following result.

Theorem 3.2.2. *If $\lambda = (a_X, a_E, k, \theta_X, \theta_E, b, n) \in \hat{\Lambda}_H$ satisfies the conditions*

$$C_{3,X},\ C_{3,E},\ C_{0,n},\ C_{1,n},\ C_{2,n},\ C_{3,n},\ C_{4,n},\ C_{5,n},\ C_{6,n}, \tag{3.233}$$

then

$$G_{\Psi_{1,n}} \cap G_{\Psi_{2,n}} = \{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}, z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,7}, z^0_{ss,8}, z^0_{ss,9}\}, \tag{3.234}$$

with an illustration similar to the one displayed in Figure 4.1.

Proof. It follows directly from Proposition (3.1.19) under conditions $C_{1,n}$, $C_{2,n}$, $C_{5,n}$, and $C_{6,n}$. □

As an illustration of Theorem 3.2.2 one can look at the Figures 3.20 and 3.21. Further, if the conditions

$$\begin{aligned} C_{1,X}: \theta_X > \frac{a_X}{k}, \\ C_{3,E}: \theta_E > \frac{a_E}{k}, \\ C_{0,n}: b \geq \max\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\}, \\ C_{1,n}: 0 \leq \Psi_{2,n}(e_{max,n}) < \Psi_{1,n}^{-1}|_{(0,x_{max,n})}\left(\frac{a_E}{k} + \frac{b}{k}\right), \\ C_{2,n}: 0 \leq \Psi_{1,n}(x_{max,n}) < \Psi_{2,n}^{-1}|_{(0,e_{max,n})}\left(\frac{a_X}{k} + \frac{b}{k}\right), \\ c_{4,n}: \theta_E < \frac{a_E}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \\ C_{6,n}: \Psi_{2,n}(e_{min,n}) > \Psi_{1,n}^{-1}|_{(x_{min,n},+\infty)}(e_{min,n}), \end{aligned} \tag{3.235}$$

hold, then one has that the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$ behave as illustrated in the main component $H_n[C_{1,X}, C_{3,E}]$ illustrated in Figure 3.12, with $x_{max,n} \in [0, x_{1,n}]$ satisfying (3.224) and (3.225). In that regard, one can prove the following result.

Theorem 3.2.3. *If $\lambda = (a_X, a_E, k, \theta_X, \theta_E, b, n) \in \hat{\Lambda}_H$ satisfies the conditions*

$$C_{1,X},\ C_{3,E},\ C_{0,n},\ C_{1,n},\ C_{2,n},\ C_{4,n},\ C_{6,n}, \tag{3.236}$$

then

$$G_{\Psi_{1,n}} \cap G_{\Psi_{2,n}} = \{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}, z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,7}, z^0_{ss,8}, z^0_{ss,9}\}, \tag{3.237}$$

with an illustration similar to the one displayed in Figure 4.1.

Proof. It follows directly from Propositions (3.1.16) and (3.1.19) under conditions $C_{1,n}$, $C_{2,n}$, and $C_{6,n}$. □

Likewise, if the conditions

$$\begin{aligned} C_{3,X}: \theta_X > \frac{a_X}{k}, \\ C_{1,E}: \theta_E > \frac{a_E}{k}, \\ C_{0,n}: b \geq \max\{g_{1,n}(x_{max,n}), g_{2,n}(e_{max,n})\}, \\ C_{1,n}: 0 \leq \Psi_{2,n}(e_{max,n}) < \Psi_{1,n}^{-1}|_{(0,x_{max,n})}\left(\frac{a_E}{k} + \frac{b}{k}\right), \\ C_{2,n}: 0 \leq \Psi_{1,n}(x_{max,n}) < \Psi_{2,n}^{-1}|_{(0,e_{max,n})}\left(\frac{a_X}{k} + \frac{b}{k}\right), \\ C_{3,n}: \theta_X < \frac{a_X}{k}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n}, \\ C_{5,n}: \Psi_{1,n}(x_{min,n}) > \Psi_{2,n}^{-1}|_{(e_{min,n},+\infty)}(x_{min,n}), \end{aligned} \tag{3.238}$$

hold, then one has that the graphs of $\Psi_{1,n}$ and $\Psi_{2,n}$ behave as illustrated in the main component $H_n[C_{3,X}, C_{1,E}]$ illustrated in Figure 3.12, with $x_{max,n} \in [0, x_{1,n}]$ satisfying (3.224) and (3.225). As a consequence, one has the following result.

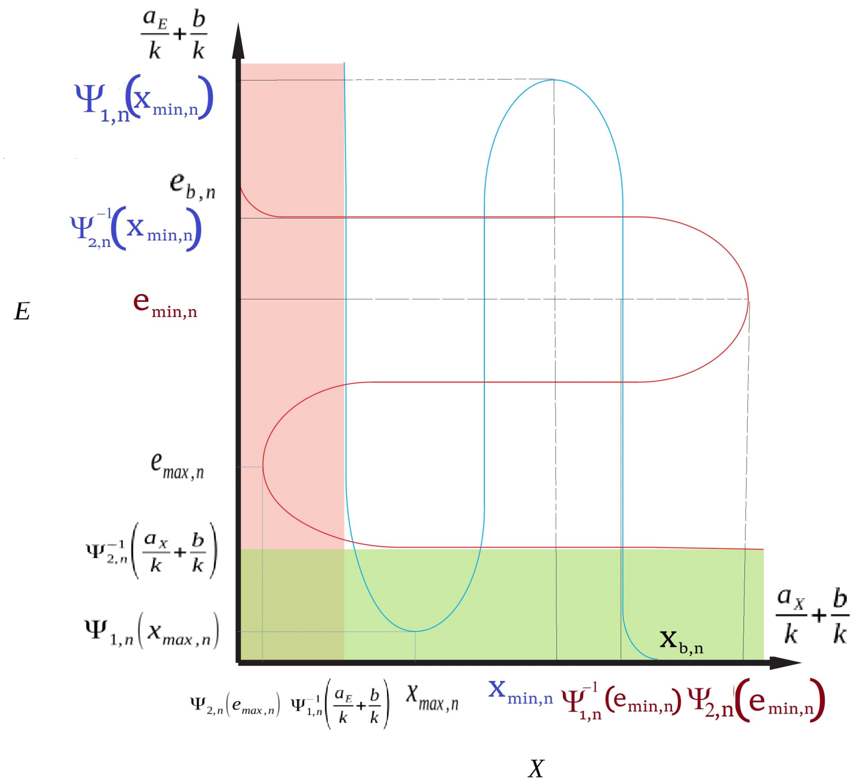


Figure 3.19: For $n \geq 2$, under conditions (3.232) and (3.210), one sees a geometric conformation of $\Psi_{1,n}(X)$ (in blue) and $\Psi_{2,n}(X)$ (in red) with the maximal number of steady states.

Theorem 3.2.4. *If $\lambda = (a_X, a_E, k, \theta_X, \theta_E, b, n) \in \hat{\Lambda}_H$ satisfies the conditions*

$$C_{3,X},\ C_{1,E},\ C_{0,n},\ C_{1,n},\ C_{2,n},\ C_{3,n},\ C_{5,n}, \tag{3.239}$$

then

$$G_{\Psi_{1,n}} \cap G_{\Psi_{2,n}} = \{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}, z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,7}, z^0_{ss,8}, z^0_{ss,9}\}, \tag{3.240}$$

with an illustration similar to the one displayed in Figure 4.1.

Proof. It follows directly from Propositions (3.1.16) and (3.1.19) under conditions $C_{1,n}$, $C_{2,n}$, and $C_{5,n}$. □

However, how can we define the *scenario space* of Huang's model ? Can we tell which scenarios are the primitive ones ? In fact, bearing in mind that the relevant aspect being prioritized here concern the number of steady states, if we anew invoke Section 2.8 then we can now regard

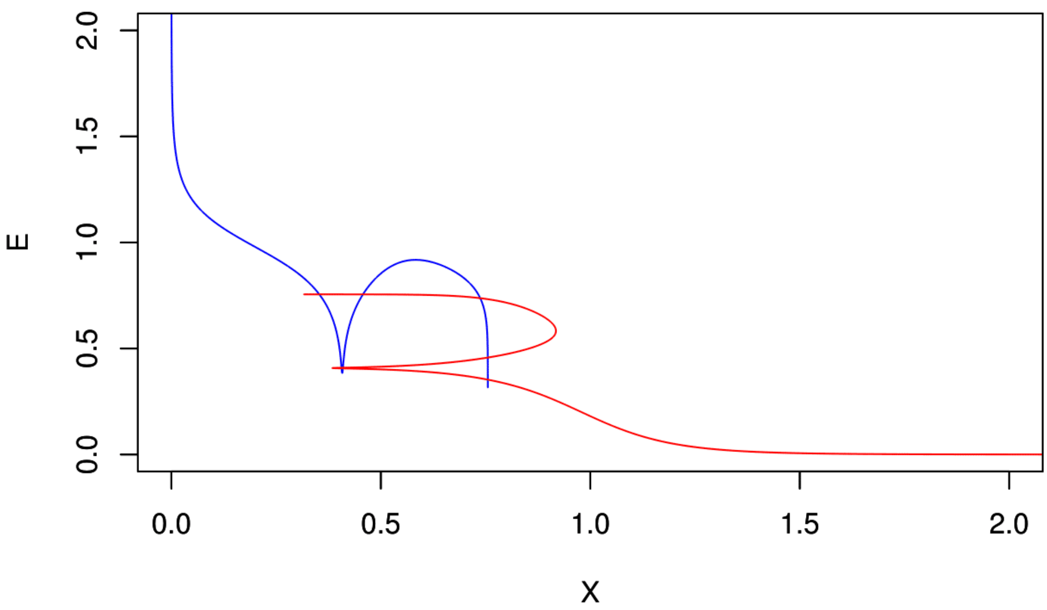


Figure 3.20: In this plot, we have the graphs of $\Psi_{1,n}$ (in blue) and $\Psi_{2,n}$ (in red) for the choices $n = 10$, $b = 0.36173 = g_{1,n}(x_{max,n}) = g_{2,n}(e_{max,n})$, $\theta_X = \theta_E = 0.5$, $k = 1$, $a_X = 0.4$ and $a_E = 0.4$. With these choices, we can see a geometric conformation with the maximal number of steady states.

$$\mathcal{A} := \{C_{1,X}, C_{1,E}, C_{2,X}, C_{2,E}, C_{3,X}, C_{3,E}, C_{0,n}, C_{1,n}, C_{2,n}, C_{3,n}, C_{4,n}, C_{5,n}, C_{6,n}\} \tag{3.241}$$

as the set of the *relevant aspects*, which, in fact, is fully determined by the mathematical analysis and formulation of Huang's model. By drawing on (3.217), one has that $\hat{\mathcal{A}} \subset \mathcal{A}$, so the *relevant aspects* contain the *primary aspects*. Hence, for $\lambda, \tilde{\lambda} \in \hat{\Lambda}_H$, one can define

$$\lambda \sim_{\mathcal{A}} \tilde{\lambda} \tag{3.242}$$

if and only if

$$\mid A[H_\lambda] \mid_{\mathbb{R}_+} = \mid A[H_{\tilde{\lambda}}] \mid_{\mathbb{R}_+}, \tag{3.243}$$

for all $A \in \mathcal{A}$, with $\mid A \mid_{\mathbb{R}_+}$ denoting the truth-value of a mathematical assertion A. Recalling that H_λ represents Huang's model with a fixed parameter setting $\lambda \in \hat{\Lambda}_H$, so $A[H_\lambda]$ symbolizes a formalized mathematical *assertion* on H_λ.

Hence, the space $\mathcal{SC}^H$ of all possible scenarios of Huang's model is defined as

$$\mathcal{SC}^H := {}^{\hat{\Lambda}_H}\!/\!_{\sim_{\mathcal{A}}} \tag{3.244}$$

with

$$^{\hat{\Lambda}_H}\!/\!_{\sim_{\mathcal{A}}} := \{[\lambda] : \lambda \in \hat{\Lambda}_H\} \tag{3.245}$$

representing the *quotient space*, that is, the set of all *equivalent classes* of $\hat{\Lambda}_H$ with

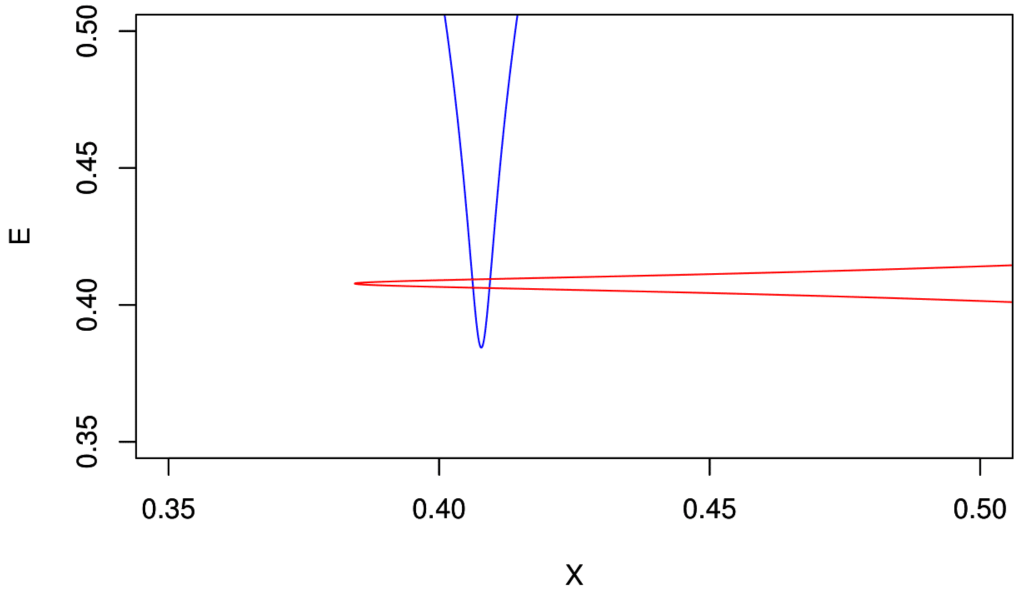


Figure 3.21: For the choices $n = 10$, $b = 0.36173 = g_{1,n}(x_{max,n}) = g_{2,n}(e_{max,n})$, $\theta_X = \theta_E = 0.5$, $k = 1$, $a_X = 0.4$ and $a_E = 0.4$, one has a better look at the region wherein 4 equilibria are located close to each other.

respect to $\sim_{\mathcal{A}}$. So, recalling from Chapter 2 that

$$q\colon \Lambda \to \hat{\Lambda}_H/\sim_{\mathcal{A}}$$
$$\lambda \mapsto [\lambda]$$

denotes the *canonical map*, and that

$$sc_\lambda^H := q(\lambda), \tag{3.246}$$

for all $\lambda \in \Lambda$; one has that a *scenario* $sc_\lambda^{\mathcal{M}}$ consists of all λ's for which the *relevant aspects* $\mathcal{A}$ have the same *truth-values* on the respective H_λ's. Hence, one has that

$$\mathcal{SC}^H = \left\{ sc_\lambda^H : \lambda \in \hat{\Lambda}_H \right\}. \tag{3.247}$$

Therefore, we have shown in Theorems (3.2.1), (3.2.1), (3.2.1), and (3.2.1) that the *scenario*

$$H_n\left[C_{1,X}, C_{1,E}, C_{0,n}C_{1,n}, C_{2,n}\right] \in \mathcal{SC}^H \tag{3.248}$$

defined by the set of all $\lambda \in \hat{\Lambda}_H$ for which

$$\vdash C_{1,X}[H_\lambda] \wedge \vdash C_{1,E}[H_\lambda] \wedge \vdash C_{0,n}[H_\lambda] \wedge \vdash C_{1,n}[H_\lambda] \wedge \vdash C_{2,n}[H_\lambda], \tag{3.249}$$

and that the *scenario*

$$H_n\left[C_{3,X}, C_{3,E}, C_{0,n}, C_{1,n}, C_{2,n}, C_{3,n}, C_{4,n}, C_{5,n}, C_{6,n}\right] \in \mathcal{SC}^H \tag{3.250}$$

defined by the set of all $\lambda \in \hat{\Lambda}_H$ for which

$$\vdash C_{1,X}[H_\lambda] \wedge \vdash C_{1,E}[H_\lambda] \wedge \vdash C_{0,n}[H_\lambda] \wedge \vdash C_{1,n}[H_\lambda] \wedge \vdash C_{2,n}[H_\lambda] \\ \wedge \vdash C_{3,n}[H_\lambda] \wedge \vdash C_{4,n}[H_\lambda] \wedge \vdash C_{5,n}[H_\lambda] \wedge \vdash C_{6,n}[H_\lambda], \tag{3.251}$$

and that the *scenario*

$$H_n\left[C_{1,X}, C_{3,E}, C_{0,n}, C_{1,n}, C_{2,n}, C_{4,n}, C_{6,n}\right] \in \mathcal{SC}^H \tag{3.252}$$

defined by the set of all $\lambda \in \hat{\Lambda}_H$ for which

$$\vdash C_{1,X}[H_\lambda] \wedge \vdash C_{3,E}[H_\lambda] \wedge \vdash C_{0,n}[H_\lambda] \wedge \vdash C_{1,n}[H_\lambda] \wedge \vdash C_{2,n}[H_\lambda] \\ \wedge \vdash C_{4,n}[H_\lambda] \wedge \vdash C_{6,n}[H_\lambda], \tag{3.253}$$

and that the *scenario*

$$H_n\left[C_{3,X}, C_{1,E}, C_{0,n}, C_{1,n}, C_{2,n}, C_{3,n}, C_{5,n}\right] \in \mathcal{SC}^H \tag{3.254}$$

defined by the set of all $\lambda \in \hat{\Lambda}_H$ for which

$$\vdash C_{3,X}[H_\lambda] \wedge \vdash C_{1,E}[H_\lambda] \wedge \vdash C_{0,n}[H_\lambda] \wedge \vdash C_{1,n}[H_\lambda] \wedge \vdash C_{2,n}[H_\lambda] \\ \wedge \vdash C_{3,n}[H_\lambda] \wedge \vdash C_{5,n}[H_\lambda], \tag{3.255}$$

are all primitive ones. Conveniently, we denote

$$\begin{aligned} Prim_{sc}^{H_n} := \{ & H_n\left[C_{1,X}, C_{1,E}, C_{0,n}, C_{1,n}, C_{2,n}\right], \\ & H_n\left[C_{1,X}, C_{3,E}, C_{0,n}, C_{1,n}, C_{2,n}, C_{4,n}, C_{6,n}\right], \\ & H_n\left[C_{3,X}, C_{1,E}, C_{0,n}, C_{1,n}, C_{2,n}, C_{3,n}, C_{5,n}\right], \\ & H_n\left[C_{3,X}, C_{3,E}, C_{0,n}, C_{1,n}, C_{2,n}, C_{3,n}, C_{4,n}, C_{5,n}, C_{6,n}\right]\} \end{aligned} \tag{3.256}$$

as the set of all primitive scenarios with respect to the Huang's qualitative graphical matrix with $n \geq 2$. In fact, the elements of $Prim_{sc}^{H_n}$ correspond to the primitive scenarios in each of the four *main components* $H_n[C_{1,X}, C_{1,E}]$, $H_n[C_{1,X}, C_{3,E}]$, $H_n[C_{3,X}, C_{1,E}]$, and $H_n[C_{3,X}, C_{3,E}]$ of Huang's qualitative graphical matrix respectively.

However, it is essential to pointing out that the conditions $C_{3,n}$, $C_{4,n}$, $C_{5,n}$, and $C_{6,n}$ suggest that finding a primitive scenario in either of the main components $H_n[C_{1,X}, C_{3,E}]$, $H_n[C_{3,X}, C_{1,E}]$, and $H_n[C_{3,X}, C_{3,E}]$ might not be realizable for some representatives of the respective equivalence classes, given that $n \geq 2$ is highly constrained therein. This is in opposition to the equivalent class $H_n[C_{1,X}, C_{1,E}]$ in which one can always find primitive scenarios independent upon the parameter $n \geq 2$.

To argue the latter, suppose that $\varphi = (a_X, a_E, \theta_X, \theta_E, k, b, n) \in \hat{\Lambda}_H$ satisfy conditions $C_{3,X}$ and $C_{3,E}$. So, if φ satisfies condition $C_{5,n}$ then it entails that

$$b > \left\{ \left[\frac{b^n}{k^n} \frac{1}{(1 + x_{min,n}^n)^n} \right] + 1 \right\} g_{1,n}(x_{min,n}), \tag{3.257}$$

which, drawing upon (3.184), implies that

$$b > k \left(1 + \frac{2}{n} \right) \theta_X - a_X > 0, \tag{3.258}$$

which implies that

$$\frac{b}{k} + \frac{a_X}{k} > \left(1 + \frac{2}{n}\right)\theta_X, \tag{3.259}$$

which, in turn, given that $\theta_X \geq \frac{a_X}{k}$, implies that

$$n > \frac{k}{b}\theta_X, \tag{3.260}$$

and we have found a lower bound for such a feasible $n \geq 2$. Moreover, if we draw upon the illustration shown in the Figure 3.8 then we have that

$$k\left(1 + \frac{2}{n}\right)\theta_X > b, \tag{3.261}$$

which implies that

$$n < \frac{2}{\left(\frac{b}{k\theta_X} - 1\right)}, \tag{3.262}$$

which, in turn, might not be realizable.

So far we know that we have proposed a systematic evaluation of a *phenomenological mathematical model* grounded in *Frege's judgment theory*, wherein the concept of primitive notion is of great importance provided that it allows concepts to be defined sequentially. So, in the respective proposed evaluation (see Chapter 2), primitive scenarios play the role of primitive notions. In fact, in our analysis of Huang's model, one has that a *scenario* with the maximal number of steady states will be the *primitive scenario* playing the role of a *primitive notion*, being irreducible, seeing that it cannot be 'reduced' to any *scenario* with more steady states.

Hence, if we want to test the *adequacy-hypothesis*, that is, if we want to execute suitable judgements upon a primitive scenario so as to shift to a scenario similar [∼] to an observation of the ontological system then we need to know the stability of the steady states of such a primitive scenario.

3.3 The phase-portrait of Huang's model

Having gone through the Section 3.2, we are now in the position of asserting that, in each primitive scenario, the total number of steady states amounts to 9 as illustrated in Figure 3.23 for the primitive scenario

$$H_n\left[C_{1,X}, C_{1,E}, C_{0,n}, C_{1,n}, C_{2,n}\right].$$

However, can we give an intuitive description of the phase-portrait of the respective primitive scenario ? If this is true then what can we tell about the phase-portrait of each element of $Prim_{sc}^{H_n}$? In fact, as we shall demonstrate in Section 3.4 that the stability of the corresponding steady states does not vary with the elements of $Prim_{sc}^{H_n}$, then we can thus far conclude that the phase-portrait of the each primitive scenario is "equivalent" to each other. Therefore, we are entitled to make the claim that the Figure 3.22 is indeed the phase-portrait of the model.

But, which rational rules can we stipulate so as to deduce the phase-portrait depicted in Figure 3.22 ? In fact, drawing upon the approach in [38, p. 11-49], if

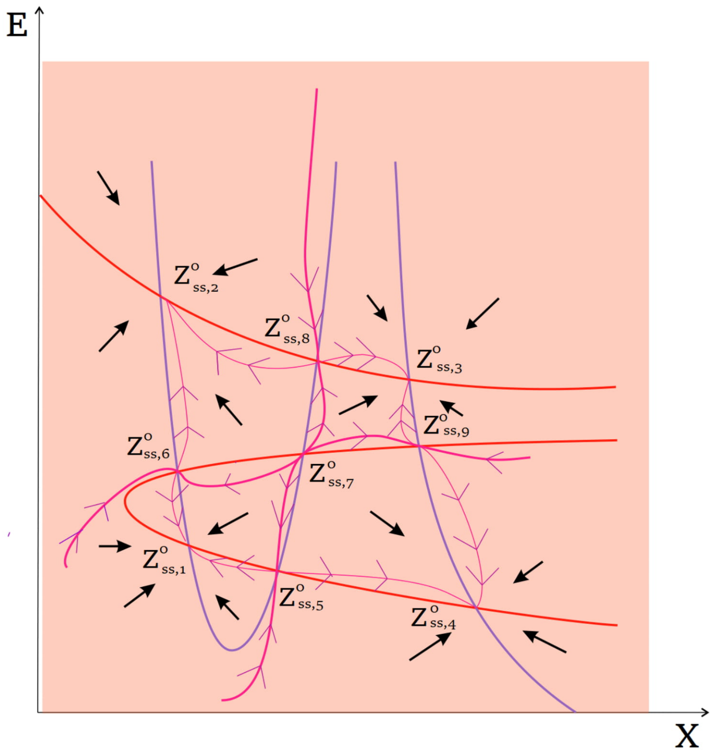


Figure 3.22: Here we see the illustration of the qualitative behavior of the nullclines defined by the graphs of $\Psi_{1,n}$ (in blue) and $\Psi_{2,n}$ (in red) (3.223), with the maximal number of steady states: $z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, \ldots, z^0_{ss,9}$. So, one has that the steady states $z^0_{ss,1}$, $z^0_{ss,2}$, $z^0_{ss,3}$, $z^0_{ss,4}$ are stable ones, while the steady states $z^0_{ss,5}$, $z^0_{ss,6}$, $z^0_{ss,8}$, and $z^0_{ss,9}$ are saddle ones. Finally, we have that the steady state $z^0_{ss,7}$ is an unstable one.

we invoke (3.1) then we denote the vector field of Huang's model by

$$\mathbf{F}^H(X,E) := \left(\frac{dX}{dt}, \frac{dE}{dt}\right) \tag{3.263}$$

for all $(X,E) \in \mathbb{R}^2_+$. By construction, one has that

$$\mathbf{F}^H(X,E) := \left(0, \frac{dE}{dt}\right) \tag{3.264}$$

on $G_{\Psi_{1,n}}$, and that

$$\mathbf{F}^H(X,E) := \left(\frac{dX}{dt}, 0\right) \tag{3.265}$$

on $G_{\Psi_{2,n}}$. So, one can then draw the respective horizontal and vertical arrows of the vector field $\mathbf{F}^H(X,E)$ on the corresponding nullclines[1], and subsequently, one can sketch the resultant of the vector field $\mathbf{F}^H(X,E)$ in the vicinity of each steady state. Upon doing so, one can then draw trajectories-to which the arrows of the resultant

[1]Actually, as we want to be pragmatic then we omit it in the illustration 3.22.

of the vector field are tangent-in the neighbourhood of the steady states , which, in turn, allows one to infer the stability and instability of the steady states.

Therefore, if we apply the aforementioned rational steps then we arrive at the sketch of the vector field depicted in Figure 3.22, which enables us to deduce that the steady states $z^0_{ss,1}$, $z^0_{ss,2}$, $z^0_{ss,3}$, $z^0_{ss,4}$ are stable ones; and that the steady states $z^0_{ss,5}$, $z^0_{ss,6}$, and $z^0_{ss,8}$, and $z^0_{ss,9}$ are saddle ones; and that the steady state $z^0_{ss,7}$ is an unstable one.

Nonetheless, how did we arrive at the sketch of the *stable and unstable manifolds* illustrated in Figure 3.22 ? First, how can we understand the essence of the concept of stable and unstable manifold ? Such manifolds form the four main trajectories-no eigenvalue of the Jacobian Matrix $DF(X^*, E^*)$ has real part equal to zero- from which it is possible to infer the qualitative behaviour of any trajectory in the vicinity of a steady state. For example, in the case of $Z^0_{ss,7}$-an unstable equilibrium-one has that one of the unstable trajectories of $Z^0_{ss,7}$ in the zone $[0, X^{*,0}_{ss,7}] \times [0, E^{*,0}_{ss,7}]$ cannot be above the graph of $\Psi_{2,n}$ on $[E^{*,0}_{ss,6}, E^{*,0}_{ss,7}]$ seeing that it would be inconsistent with the stipulated rational rules. In fact, if it was located slightly close or above the graph of $\Psi_{2,n}$ on $[E^{*,0}_{ss,6}, E^{*,0}_{ss,7}]$ then, consistent with the provided rational rules, one has that the arrow of vector field tangent to the respective unstable trajectory would not be pointing outwards. Hence, the respective unstable trajectory must be sufficiently under $\Psi_{2,n}$ on $[E^{*,0}_{ss,6}, E^{*,0}_{ss,7}]$ and sufficiently close to $\Psi_{1,n}$ on $[X^{*,0}_{ss,6}, X^{*,0}_{ss,1}]$ and to $\Psi_{2,n}$ on $[X^{*,0}_{ss,5}, X^{*,0}_{ss,1}]$ to be consistent with the corresponding dynamics.

As a conclusion hereof, one has that there is no closed orbit in the phase portrait of any element of $Prim^{H_n}_{sc}$. In the next section, we will give the proofs of the stability of the respective steady states.

3.4 Stability of steady states of the primitive scenarios in $Prim^{H_n}_{sc}$

Without loss of generality, we will adopt the same notation to refer to the set of all steady states in each element of $Prim^{H_n}_{sc}$, that is, the set

$$z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}, z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,7}, z^0_{ss,8}, z^0_{ss,9} \tag{3.266}$$

symbolizes all the nine steady states found in each of the primitive scenarios in $Prim^{H_n}_{sc}$ as illustrated in Figure 3.23 for the primitive scenario

$$H_n\left[C_{1,X}, C_{1,E}, C_{0,n}, C_{1,n}, C_{2,n}\right].$$

But, how shall we demonstrate the (in)stability of the steady states in (3.266) ? First of all, we need to decide which concept of stability suits the purpose of our analysis[2]. If we recall Section 1.3 then we have used the predicate "being robust to small perturbations" so as to intuitively describe the essence of stability. The latter is indeed captured by the notion of *linear stability* as defined in [67, p.128-129]. In fact, we will draw upon the Hartman-Grobman Theorem [67, p. 119-123], that is, *linearization*, which, indeed, essentially states that, in the case of a hyperbolic equilibrium, one has that the dynamics of a non-linear system is qualitatively

[2]There are many concepts depending on the nature of the dynamical system as seen in [59].

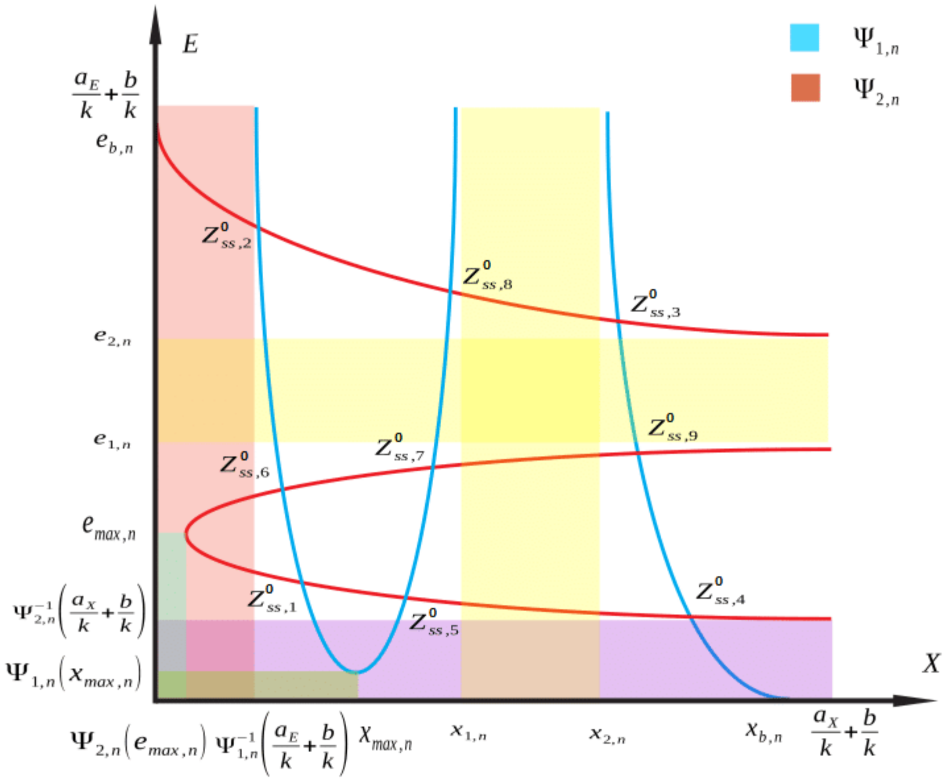


Figure 3.23: Here we see the illustration of the qualitative behavior of the $\Psi_{1,n}$ and $\Psi_{2,n}$ (3.223), with the maximal number of steady states: $z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, \dots, z^0_{ss,9}$.

equivalent to the dynamics of the corresponding linearized system. As we will see in this section, the later approach will suffice to determine the instability of a subset of the steady states in (3.266). To demonstrate the stability and instability of the remaining ones, we will appeal to topological arguments by drawing upon Poincaré-Bendixson Theorem.

In fact, if we invoke (3.1) then we can define

$$\begin{aligned} F_1(X,E) &\coloneqq a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n} - kX, \\ F_2(X,E) &\coloneqq a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1+X^n} - kE, \end{aligned} \tag{3.267}$$

which, by recalling (3.10) and (3.11), can be written as

$$\begin{aligned} F_1(X,E) &= h_n(E) - g_{1,n}(X), \\ F_2(X,E) &= h_n(X) - g_{2,n}(E). \end{aligned} \tag{3.268}$$

Further, let $(X^*, E^*) \in G_{\Psi_{1,n}} \cap G_{\Psi_{2,n}}$. So, one has that the Jacobian matrix reads

$$D\mathbf{F}^H(X^*, E^*) = \begin{bmatrix} DF_{11}(X^*, E^*) & DF_{12}(X^*, E^*) \\ DF_{21}(X^*, E^*) & DF_{22}(X^*, E^*) \end{bmatrix}, \tag{3.269}$$

which implies that

$$\begin{aligned} \det(D\mathbf{F}^H - \lambda \mathbf{I})(X^*, E^*) &= \begin{vmatrix} DF_{11} - \lambda & DF_{12} \\ DF_{21} & DF_{22} - \lambda \end{vmatrix} \\ = \lambda^2 - (DF_{11}(X^*, E^*) + DF_{22}(X^*, E^*))\lambda &+ DF_{11}(X^*, E^*)DF_{22}(X^*, E^*) \\ &- DF_{12}(X^*, E^*)DF_{21}(X^*, E^*), \end{aligned} \tag{3.270}$$

which, in turn, implies that the characteristic polynomial of the Jacobian matrix indeed reads

$$\begin{aligned} p(\lambda) = \lambda^2 - (DF_{11}(X^*, E^*) + DF_{22}(X^*, E^*))\lambda + (DF_{11}(X^*, E^*)DF_{22}(X^*, E^*) \\ - DF_{12}(X^*, E^*)DF_{21}(X^*, E^*)), \end{aligned} \tag{3.271}$$

or equivalently,

$$p(\lambda) = \lambda^2 - \operatorname{Tr} D\mathbf{F}^H(X^*, E^*)\lambda + \operatorname{Det} D\mathbf{F}^H(X^*, E^*), \tag{3.272}$$

with

$$\operatorname{Tr}\left(D\mathbf{F}^H(X^*, E^*)\right) = DF_{11}(X^*, E^*) + DF_{22}(X^*, E^*), \tag{3.273}$$

denoting the trace of the Jacobian matrix $DF(X^*, E^*)$, while

$$\operatorname{Det}\left(D\mathbf{F}^H(X^*, E^*)\right) = DF_{11}(X^*, E^*)DF_{22}(X^*, E^*) - DF_{12}(X^*, E^*)DF_{21}(X^*, E^*) \tag{3.274}$$

expresses the determinant of the Jacobian matrix. Hence, one has that the roots of (3.271) are given by the following formula

$$\lambda_{\pm} = \frac{(DF_{11} + DF_{22}) \pm \sqrt{(DF_{11} - DF_{22})^2 + 4DF_{12}DF_{21}}}{2}, \tag{3.275}$$

so

$$\operatorname{Tr}\left(D\mathbf{F}^H(X^*, E^*)\right) = \lambda_-(X^*, E^*) + \lambda_+(X^*, E^*), \tag{3.276}$$

and

$$\operatorname{Det}\left(D\mathbf{F}^H(X^*, E^*)\right) = \lambda_-(X^*, E^*)\lambda_+(X^*, E^*). \tag{3.277}$$

Next, by drawing on (3.268), one has that

$$\begin{aligned} DF_{11}(X^*, E^*) &= -g'_{1,n}(X^*), \\ DF_{22}(X^*, E^*) &= -g'_{2,n}(E^*), \\ DF_{12}(X^*, E^*) &= h'_n(E^*), \\ DF_{21}(X^*, E^*) &= h'_n(X^*), \end{aligned} \tag{3.278}$$

which implies that

$$\operatorname{Tr}\left(D\mathbf{F}^H(X^*, E^*)\right) = -g'_{1,n}(X^*) - g'_{2,n}(E^*), \tag{3.279}$$

and that

$$\text{Det}\left(D\mathbf{F}^H(X^*,E^*)\right) = g'_{1,n}(X^*)g'_{2,n}(E^*) - h'_n(E^*)h'_n(X^*). \tag{3.280}$$

Furthermore, by construction $h'_n < 0$, so one has that

$$DF_{12}(X^*,E^*)DF_{21}(X^*,E^*) = h'_n(E^*)h'_n(X^*) > 0, \tag{3.281}$$

which entails that

$$(DF_{11}(X^*,E^*) - DF_{22}(X^*,E^*))^2 + 4DF_{12}(X^*,E^*)DF_{21}(X^*,E^*) > 0, \tag{3.282}$$

and we conclude that $\lambda_\pm(X^*,E^*) \in \mathbb{R}$.

Therefore, concerning the stability of the steady states, one has that Huang's model can only yield saddle ones, for which

$$(\lambda_-(X^*,E^*) > 0 \wedge \lambda_+(X^*,E^*) < 0) \vee (\lambda_-(X^*,E^*) < 0 \wedge \lambda_+(X^*,E^*) > 0),$$

or stable ones, for which

$$\lambda_-(X^*,E^*) < 0 \wedge \lambda_+(X^*,E^*) < 0$$

or unstable ones, for which

$$\lambda_-(X^*,E^*) > 0 \wedge \lambda_+(X^*,E^*) > 0.$$

Moreover, as the eigenvalues are real, no Hopf bifurcations can occur.

Further, recalling definitions (3.310) and (3.311), if we note that

$$\Psi'_{i,n}(\cdot) = \frac{d\left(h_n^{-1}\right)}{dZ}\Bigg|_{Z=g_{i,n}(\cdot)} g'_{i,n}(\cdot), \tag{3.283}$$

and that

$$\frac{d\left(h_n^{-1}\right)}{dZ}(z) = \frac{1}{h'_n(h_n^{-1}(z))}, \tag{3.284}$$

with $i \in \{1,2\}$, then we have that

$$\Psi'_{i,n}(\cdot) = \frac{g'_{i,n}(\cdot)}{h'_n(\Psi_{i,n}(\cdot))}, \tag{3.285}$$

and we can prove the following results.

Definition 3.4.1. The sign function is defined as

$$\text{sgn}(x) := \begin{cases} -1 & if \;\; x < 0; \\ 0 & if \;\; x = 0; \\ 1 & if \;\; x > 0. \end{cases}$$

Proposition 3.4.1. *Let* $(X^*,E^*) \in G_{\Psi_{1,n}} \cap G_{\Psi_{1,n}}$. *One has that :*

(i) $\Psi'_{1,n}(X^*) < 0 \wedge \Psi'_{2,n}(E^*) < 0 \Rightarrow \text{Tr}\left(D\mathbf{F}^H(X^*,E^*)\right) < 0$ *(required for a steady state to be a stable one);*

(*ii*) $\Psi'_{1,n}(X^*) > 0 \wedge \Psi'_{2,n}(E^*) > 0 \Rightarrow \mathrm{Tr}\left(D\mathbf{F}^H(X^*, E^*)\right) > 0 \Rightarrow (X^*, E^*)$ *is a saddle or an unstable equilibrium;*

Proof. In fact, if we use (3.285) in (3.279) then we arrive at

$$\mathrm{Tr}\left(D\mathbf{F}^H(X^*, E^*)\right) = -h'_n(E^*)\Psi'_{1,n}(X^*) - h'_n(X^*)\Psi'_{2,n}(E^*), \tag{3.286}$$

which, in turn, implies that

$$\mathrm{sgn}\left(\mathrm{Tr}\left(D\mathbf{F}^H(X^*, E^*)\right)\right) = -\,\mathrm{sgn}\left(h'_n(E^*)\Psi'_{1,n}(X^*) + h'_n(X^*)\Psi'_{2,n}(E^*)\right). \tag{3.287}$$

Since $h'_n < 0$, we get that (*i*) and (*ii*) follow immediately from (3.289) and the proposition has been proved. □

Proposition 3.4.2. *Let* $(X^*, E^*) \in G_{\Psi_{1,n}} \cap G_{\Psi_{1,n}}$. *One has that :*

(*i*) $\mathrm{Det}\left(D\mathbf{F}^H(X^*, E^*)\right) = h'_n(E^*)h'_n(X^*)\left[\left(\Psi'_{1,n}(X^*)\Psi'_{2,n}(E^*)\right) - 1\right]$*;*

(*ii*) $\mathrm{sgn}\left(\mathrm{Det}\left(D\mathbf{F}^H(X^*, E^*)\right)\right) = \mathrm{sgn}\left(\left[\left(\Psi'_{1,n}(X^*)\Psi'_{2,n}(E^*)\right) - 1\right]\right)$*;*

(*iii*) $\left(\Psi'_{1,n}(X^*) < 0 \wedge \Psi'_{2,n}(E^*) > 0\right) \vee \left(\Psi'_{1,n}(X^*) > 0 \wedge \Psi'_{2,n}(E^*) < 0\right) \Rightarrow (X^*, E^*)$ *is a saddle equilibrium;*

Proof. In fact, if we use (3.285) in (3.280) then we get that

$$\begin{aligned}\mathrm{Det}\left(D\mathbf{F}^H(X^*, E^*)\right) &= g'_{1,n}(X^*)g'_{2,n}(E^*) - h'_n(E^*)h'_n(X^*)\\ &= h'_n(E^*)\Psi'_{1,n}(X^*)h'_n(X^*)\Psi'_{2,n}(E^*) - h'_n(E^*)h'_n(X^*)\\ &= h'_n(E^*)h'_n(X^*)\left[\left(\Psi'_{1,n}(X^*)\Psi'_{2,n}(E^*)\right) - 1\right],\end{aligned} \tag{3.288}$$

which, in turn, implies that

$$\mathrm{sgn}\left(\mathrm{Det}\left(D\mathbf{F}^H(X^*, E^*)\right)\right) = \mathrm{sgn}\left(\left[\left(\Psi'_{1,n}(X^*)\Psi'_{2,n}(E^*)\right) - 1\right]\right), \tag{3.289}$$

and (*i*) and (*ii*) have been proved. Now, as $h'_n < 0$ then one has that if $\Psi'_{1,n}(X^*) < 0$ and $\Psi'_{2,n}(E^*) > 0$ then $\Psi'_{1,n}(X^*)\Psi'_{2,n}(E^*) < 0$, which entails that

$$\mathrm{sgn}\left(\mathrm{Det}\left(D\mathbf{F}^H(X^*, E^*)\right)\right) < 0,$$

which, in turn, drawing on (3.277), implies that $\lambda_-(X^*, E^*) > 0$ or $\lambda_+(X^*, E^*) < 0$, or equivalently, (X^*, E^*) is a saddle equilibrium. Similarly, one can show (*iii*) for $\Psi'_{1,n}(X^*) > 0$ and $\Psi'_{2,n}(E^*) < 0$, what completes the proof. □

Theorem 3.4.3. *Let $n \geq 2$. With respect to each primitive scenario in*

$$Prim_{sc}^{H_n},$$

one has that the steady states

$$z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,8}, z^0_{ss,9}$$

are saddle ones.

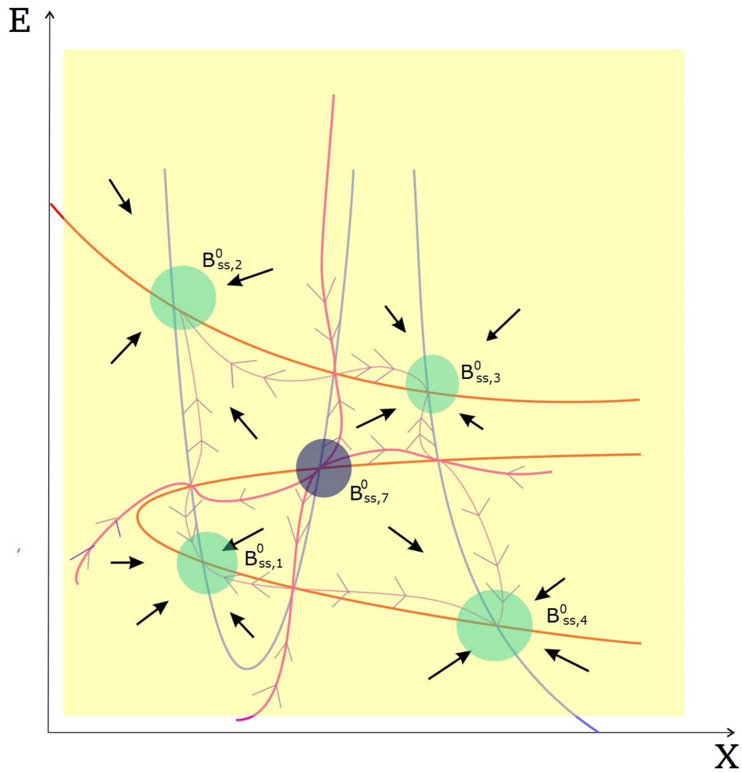


Figure 3.24: Here we see the phase-portrait of the primitive scenario $H_n[C_{1,X}, C_{1,E}, C_{0,n}, C_{1,n}, C_{2,n}]$. To apply the Poicaré-Bendixon Theorem, we choose convenient "trap regions" $B^0_{ss,1}$, $B^0_{ss,2}$, $B^0_{ss,3}$, and $B^0_{ss,4}$, and $B^0_{ss,7}$, i.e. a sufficiently small neighbourhood of the steady states $z^0_{ss,1}$, $z^0_{ss,2}$, $z^0_{ss,3}$, and $z^0_{ss,4}$ wherein all the positive orbits are bounded. Similarly, we choose a convenient "trap region" $B^0_{ss,7}$, i.e. a sufficiently small neighbourhood of the steady states $z^0_{ss,7}$, in which all the negative orbits are bounded.

Proof. Let $Z_{saddles} := \{z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,8}, z^0_{ss,9}\}$. If we draw upon Propositions 3.1.16 and 3.1.19 then we conclude that for all $z^* = (X^*, E^*) \in Z_{saddles}$, one has that

$$\Psi'_{1,n}(X^*) < 0 \wedge \Psi'_{2,n}(E^*) > 0,$$

or

$$\Psi'_{1,n}(X^*) > 0 \wedge \Psi'_{2,n}(E^*) < 0,$$

which, by building on Proposition 3.4.2 (iii), implies that $z^* = (X^*, E^*)$ is a saddle equilibrium, which completes the proof. □

Proposition 3.4.4. *Let $n \geq 2$ and $Z_{stable} := \{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}\}$. With respect to each primitive scenario in*

$$Prim^{H_n}_{sc},$$

one has that

$$\bigwedge_{z^* \in Z_{stable}} \lambda_-(z^*) < 0,$$

and the steady states in Z_{stable} are possibly stable.

Proof. In fact, by drawing on Section 1.2, for $z^0_{ss,1} = (X^0_{ss,1}, E^0_{ss,1})$, one knows that

$$\begin{aligned} 0 &< \frac{b}{1+\left(\frac{b}{k}+\frac{a_E}{k}\right)^n} < X^0_{ss,1} < x_{max,n}, \\ 0 &< \frac{b}{1+\left(\frac{b}{k}+\frac{a_X}{k}\right)^n} < E^0_{ss,1} < e_{max,n}, \end{aligned} \tag{3.290}$$

which, by drawing on (3.267), implies that

$$\begin{aligned} DF_{11}(z^0_{ss,1}) &= -g'_{1,n}(X^0_{ss,1}) < 0, \\ DF_{22}(z^0_{ss,1}) &= -g'_{2,n}(E^0_{ss,1}) < 0, \end{aligned} \tag{3.291}$$

given that $g_{1,n}(X)\Big|_{[0,x_{max,n}]}$ and $g_{2,n}(E)\Big|_{[0,e_{max,n}]}$, under $\theta_X < a_X/2k$ and $\theta_E < a_E/2k$, are strictly increasing. Therefore, one has that

$$\lambda_-(z^0_{ss,1}) = \frac{(DF_{11}+DF_{22}) \pm \sqrt{(DF_{11}-DF_{22})^2 + 4DF_{12}DF_{21}}}{2} < 0$$

Likewise, one can provide a similar argument for $z^0_{ss,2}$, $z^0_{ss,3}$, and $z^0_{ss,4}$. □

Hence, with respect to Theorem 3.4.4, if we want to draw upon *linearization* so as to prove that the steady states in $Z_{stable} := \{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}\}$ are indeed stable ones, then it is sufficient to demonstrate that, for all $z^* \in Z_{stable}$, it is true that

$$\lambda_+(z^*) = \frac{(DF_{11}+DF_{22}) + \sqrt{(DF_{11}-DF_{22})^2 + 4DF_{12}DF_{21}}}{2} < 0. \tag{3.292}$$

However, a priori, the right-hand side of (3.292) can be equal to zero for some representative [parameter setting] of a primitive scenario, which, in turn, implies that, in general, these equilibria might not be hyperbolic. Thereby, we might not be able to draw upon *linearization*, that is, the Hartman-Grobman Theorem [67, p. 119-123] so as to deduce the stability of the steady states in Z_{stable}.

Moreover, if we adopt the approach used to show Theorem 3.4.3 then it is not difficult to demonstrate that

$$\mathrm{Tr}\left(D\mathbf{F}^H(z^*)\right) < 0$$

for all $z^* \in Z_{stable}$. So, according to Proposition 3.4.2, it is sufficient to prove that

$$\Psi'_{1,n}(X^*)\Psi'_{2,n}(E^*) > 1 \tag{3.293}$$

so as to conclude that $z^* = (X^*, E^*) \in Z_{stable}$ is a stable equilibrium. Nonetheless, proving (3.293) is equivalent to proving (3.292). Similarly, we can prove that

$$\lambda_+(z^0_{ss,7}) > 0,$$

and that

$$\mathrm{Tr}\left(D\mathbf{F}^H(z^*)\right) > 0,$$

which, in turn, implies that it is sufficient to either prove that

$$\lambda_-(z^0_{ss,7}) > 0,$$

or that

$$\Psi'_{1,n}(X^0_{ss,7})\Psi'_{2,n}(E^0_{ss,7}) > 1,$$

so as to demonstrate that the equilibrium $z^0_{ss,7}$ is an unstable one. Thus, it seems that such an algebraic approach is neither suitable to prove the stability of the equilibria in Z_{stable} nor suitable to deduce that $z^0_{ss,7}$ is an unstable steady state.

Following the approach of [14, p. 141–142], we shall build on a topological argument so as to determine the stability of the steady states $Z_{stable} := \{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}\}$ and the instability of the steady state $z^0_{ss,7}$. Let (3.1) be denoted by

$$\dot{\mathbf{u}} = \mathbf{F}^H(\mathbf{u}), \tag{3.294}$$

with $\mathbf{u}(t) := (X(t), E(t))$ and $\mathbf{F}^H := \left(\frac{dX}{dt}, \frac{dX}{dt}\right)$. So, one has that, by definition, $\mathbf{F}^H \in C^1(\mathbb{R}^2_+)$. Now, let $\varphi(\cdot, \mathbf{u}_0) : \mathbb{R} \to \mathbb{R}^2_+$ be the solution of the initial value problem

$$\begin{aligned} \dot{\mathbf{u}} &= \mathbf{F}^H(\mathbf{u}) \\ \mathbf{u}(0) &= \mathbf{u}_0 \end{aligned} \tag{3.295}$$

so one has that the mapping $\varphi_t : \mathbb{R}^2_+ \to \mathbb{R}^2_+$ defined by

$$\varphi_t(\mathbf{u}_0) := \varphi(t, \mathbf{u}_0) \tag{3.296}$$

is said to be the *flow of the differential equation* in (3.1).

Next, let the set

$$\gamma_{\mathbf{u}_0} := \left\{\mathbf{u} \in \mathbb{R}^2_+ : \mathbf{u} = \varphi(t, \mathbf{u}_0), \ \ t \in \mathbb{R}\right\} \tag{3.297}$$

denote the orbit passing through $\mathbf{u}_0$. In that regard, one defines the positive orbit as the set

$$\gamma^+_{\mathbf{u}_0} := \left\{\mathbf{u} \in \mathbb{R}^2_+ : \mathbf{u} = \varphi(t, \mathbf{u}_0), \ \ t \geq 0\right\}, \tag{3.298}$$

while the negative orbit is defined by the set

$$\gamma^-_{\mathbf{u}_0} := \left\{\mathbf{u} \in \mathbb{R}^2_+ : \mathbf{u} = \varphi(t, \mathbf{u}_0), \ \ t \leq 0\right\}. \tag{3.299}$$

Having defined $\gamma^+_{\mathbf{u}_0}$, the positive orbit passing through $\mathbf{u}_0$, and $\gamma^-_{\mathbf{u}_0}$, the negative orbit passing through $\mathbf{u}_0$, one defines the ω-limit set of the positive orbit $\gamma^+_{\mathbf{u}_0}$, denoted by $\omega\left(\gamma^+_{\mathbf{u}_0}\right)$, as the set of all points $p \in \mathbb{R}^2_+$ for which there exists a sequence $(t_n)_{n=1}^{+\infty}$ satisfying

$$\lim_{n\to+\infty} t_n = +\infty, \tag{3.300}$$

such that

$$\mathbf{p} = \lim_{t_n\to+\infty} \varphi(t_n, \mathbf{u}_0). \tag{3.301}$$

In a similar way, one defines the $\alpha-$limit set of the negative orbit $\gamma^-_{\mathbf{u}_0}$, denoted by $\alpha\left(\gamma^-_{\mathbf{u}_0}\right)$, as the set of all points $p \in \mathbb{R}^2_+$ for which there exists a sequence $(t_n)_{n=1}^{+\infty}$ satisfying

$$\lim_{n\to+\infty} t_n = -\infty, \tag{3.302}$$

such that

$$\mathbf{p} = \lim_{t_n\to-\infty} \varphi(t_n, \mathbf{u}_0). \tag{3.303}$$

Theorem 3.4.5 (Poicaré-Bendixson). *If γ^+ is a bounded positive orbit of the system (3.294) then its $\omega-$limit set $\omega(\gamma^+)$ is either:*

1. *a steady state;*
2. *a periodic orbit;*
3. *a set consisting of steady-states and orbits having these steady-states as their $\alpha-$ and $\omega-$limit.*

Proof. See [100, p. 38-47]. □

Therefore, the essence of Poicaré-Bendixson's Theorem is that a bounded positive orbit can either converge to a steady state or to a periodic orbit or is already itself a periodic orbit. Moreover, an analogous result can be demonstrated to the case of a negative bounded orbit. But, how will we use this theorem then ? In fact, as we illustrate in Figure 3.24, we will find suitable small neighbourhoods ["trap regions"] around the respective equilibria which suffice to apply Poicaré-Bendixon's Theorem.

Theorem 3.4.6. *Let $n \geq 2$. With respect to each primitive scenario in*

$$Prim_{sc}^{H_n},$$

one has that the steady states

$$z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}$$

are stable ones.

Proof. Claim 1: It is true that $z^0_{ss,1}$ is a stable equilibrium. In fact, choose $0 < r_1 < \min\{x_{max,n}, e_{max,n}\}$. Consistent with our topological approach, we claim that there is no periodic orbit in $B_r(z^0_{ss,1}) := \{z = (X,E) \in \mathbb{R}^2 : d(z, z^0_{ss.1}) < r_1\}$. Let d denote the Euclidian distance on $\mathbb{R}^2$. If there is a periodic orbit in $B_{r_1}(z^0_{ss,1})$ then, by drawing upon the Bendixson's criterion [100, p. 38], one has that the divergence of the vector field $\nabla \cdot \mathbf{F}^H$, that is,

$$\nabla \cdot \mathbf{F}^H(X,E) = -g'_{1,n}(X) - g'_{2,n}(E) \tag{3.304}$$

changes sign in $B_{r_1}(z^0_{ss,1})$, which is a contradiction for all elements of $Prim_{sc}^{H_n}$. Hence, one must have that there is no periodic orbit in $B_{r_1}(z^0_{ss,1})$. Now, we claim that

$$\bigvee_{0<r^0_{ss,1}<r_1} \bigvee_{t^0_{ss,1}>0} \bigwedge_{t\geq t^0_{ss,1}} d(\varphi(t,\mathbf{u}_0), z^0_{ss,1}) < r^0_{ss,1}. \tag{3.305}$$

In fact, in view of the phase-portrait of Huang's model depicted in 3.22, if the claim (3.306) was not true then

$$\bigwedge_{0<\delta<r_1} \bigwedge_{n>1} \bigvee_{t_{n,\delta}>n} d(\varphi(t_{n,\delta}, \mathbf{u}_0), z^0_{ss,1}) > \delta, \tag{3.306}$$

which would imply that the vector field would not be pointing inward the small ball $B_\delta(z^0_{ss,1})$ contradicting the phase-portrait of Huang's model in the vicinity of the steady state $z^0_{ss,1}$. So, one has that all the positive orbits in $B_{r^0_{ss,1}}(z^0_{ss,1})$ are bounded, which, in turn, by drawing upon Poincaré-Bendixson Theorem, implies that all the positive orbits in $B^0_{ss,1} := B_{r^0_{ss,1}}(z^0_{ss,1})$ converge to the equilibrium $z^0_{ss,1}$. Therefore, one has that the steady state $z^0_{ss,1}$ is a stable one.

Claim 2: It is true that the steady states $z^0_{ss,2}$, $z^0_{ss,3}$, and $z^0_{ss,4}$ are stable ones. In fact, we essentially use the same argument to arrive at the "trap regions" $B^0_{ss,2}$, $B^0_{ss,3}$, and $B^0_{ss,4}$ so as to apply Poincaré-Bendixson Theorem, as illustrated in Figure 3.24. □

Theorem 3.4.7. *Let $n \geq 2$. With respect to each primitive scenario in*

$$Prim^{H_n}_{sc},$$

one has that the steady state $z^0_{ss,7}$ is an unstable one.

Proof. In fact, let $\tilde{t} = -t$ denote a parametrization by the reverse time. Let $B^0_{ss,7}$ be a sufficiently small open ball centered at $z^0_{ss,7}$. If we use the same argument to demonstrate the Theorem 3.4.6 then we arrive at the conclusion that the α-limit of all bounded negative orbits in $B^0_{ss,7}$ is equal to $z^0_{ss,7}$. Therefore, if we remove the parametrization by the reverse time then we have that all the orbits in $B^0_{ss,7}$ diverge from $z^0_{ss,7}$, which, in turn, implies that $z^0_{ss,7}$ is an unstable equilibrium. □

But, could we have used the same topological argument to determine the instability of the saddle equilibria ? No, we could not. In fact, as we see in the phase-portrait of Huang's model 3.22, for all sufficiently small neighbourhood of a saddle equilibrium there exists an orbit which is not bounded to the trapping region, so we are not in condition to apply Poicaré-Bendixon's Theorem. As an illustration of the results in this Section, one can have a look at the Figures 3.22, 3.25, and 3.26. The latter illustration and simulations provide us with a suitable picture of the phase portrait of a primitive scenario in each of the four *main components* $H_n[C_{1,X}, C_{1,E}]$, $H_n[C_{1,X}, C_{3,E}]$, $H_n[C_{3,X}, C_{1,E}]$, and $H_n[C_{3,X}, C_{3,E}]$ of Huang's qualitative graphical matrix.

3.5 The subclass $\left(H_1\left[C^{(1)}_{i,X}, C^{(1)}_{j,E}\right]\right)_{i,j}$ of models

If we invoke Subsection 1.1.1, then we can conveniently define

$$\begin{aligned} g_1(X) &:= kX - a_X \frac{X}{\theta_X + X}, \\ g_2(E) &:= kE - a_E \frac{E}{\theta_E + E}, \end{aligned} \tag{3.307}$$

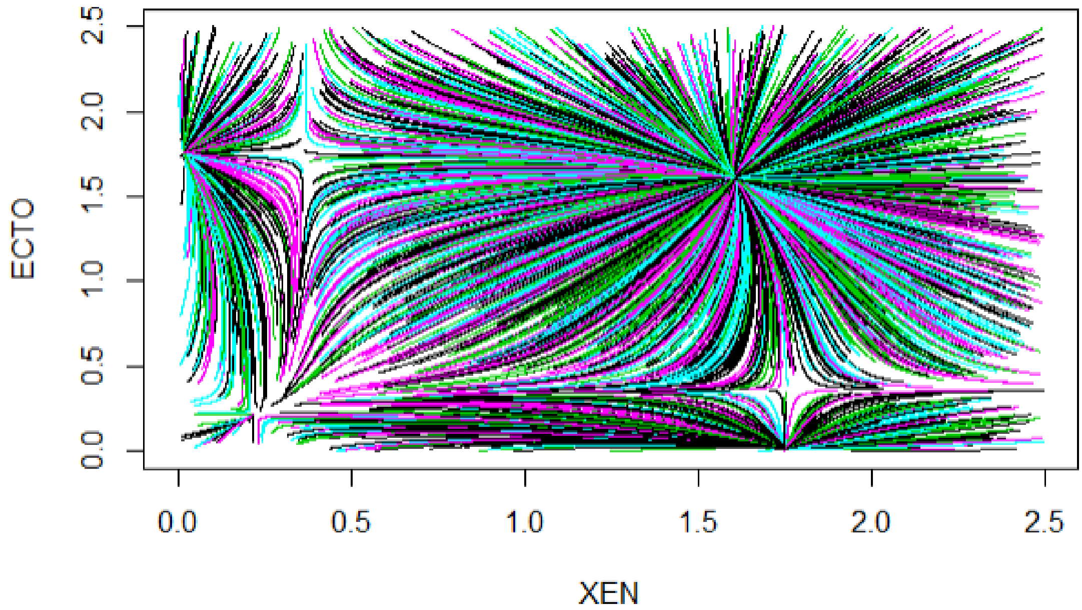


Figure 3.25: Here we see a simulation of the trajectories for the primitive scenario $[a_X = 3.2, a_E = 3.2, k = 0.7, \theta_X = 1, \theta_E = 1, b = 0.2007054, n = 4]$*, which numerically illustrate the phase-portrait of Huang's model illustrated in Figure 3.22.*

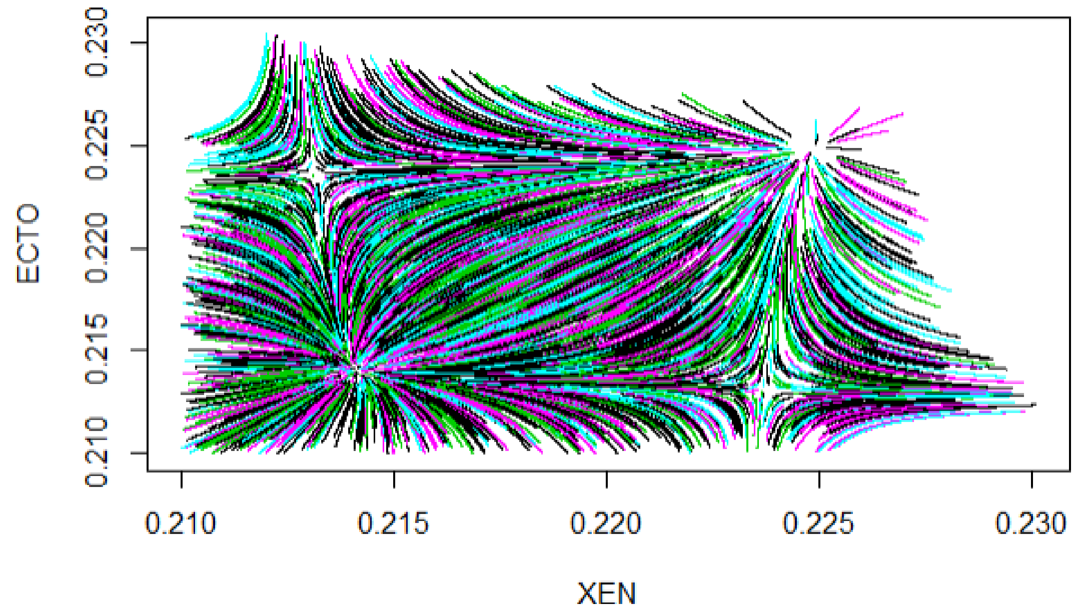


Figure 3.26: Here, we see a simulation of the trajectories of the primitive scenario $[a_X = 3.2, a_E = 3.2, k = 0.7, \theta_X = 1, \theta_E = 1, b = 0.2007054, n = 4]$ *in which we zoom in on the region with the steady states* $z^0_{ss,1}, z^0_{ss,5}, z^0_{ss,6}$*, and* $z^0_{ss,7}$*. In fact, we might infer therefrom that the steady state* $z^0_{ss,1}$ *is a stable one, and that the steady state* $z^0_{ss,7}$ *is an unstable one while the steady states* $z^0_{ss,5}$ *and* $z^0_{ss,6}$ *are saddle ones.*

and

$$h(Z) := b\frac{1}{1+Z}, \tag{3.308}$$

for all $X, E, Z \geq 0$. If $b > 0$ then the function defined in (3.308) is invertible, whose inverse reads

$$h^{-1}(\tilde{Z}) = \left(\frac{b}{\tilde{Z}} - 1\right), \tag{3.309}$$

for all $\tilde{Z} \in (0, b]$. So, following the same strategy, one defines

$$\Psi_1 := h^{-1} \circ g_1, \tag{3.310}$$

and

$$\Psi_2 := h^{-1} \circ g_2. \tag{3.311}$$

Lemma 3.5.1.

$$\begin{aligned} (i)\theta_X < \frac{a_X}{k} &\Rightarrow g_1'(0+) < 0 \\ (ii)\theta_E < \frac{a_E}{k} &\Rightarrow g_2'(0+) < 0 \end{aligned} \tag{3.312}$$

Proof. In fact, one has that

$$g_1'(0+) := \lim_{h \to 0^+} \frac{g_{1,n}(0+h) - g_{1,n}(0)}{h} = k - \frac{a_X}{\theta_X} < 0, \tag{3.313}$$

so the graph of g_1 is not tangent to the line $f(X) = kX$ at $X = 0$. Similarly, one can prove (ii). □

Lemma 3.5.2.

$$\begin{aligned} (i)\theta_X < \frac{a_X}{k} &\Rightarrow (g_1(\theta_X) < 0 \wedge g_1'(\theta_X) < 0) \wedge \left(g_1\left(\frac{a_X}{k}\right) > 0 \wedge g_1'\left(\frac{a_X}{k}\right) > 0\right) \\ (ii)\theta_E < \frac{a_E}{k} &\Rightarrow (g_1(\theta_E) < 0 \wedge g_1'(\theta_E) < 0) \wedge \left(g_1\left(\frac{a_E}{k}\right) > 0 \wedge g_1'\left(\frac{a_E}{k}\right) > 0\right) \end{aligned} \tag{3.314}$$

Proof. (i) In fact, at $X = \theta_X$, one has that

$$g_1(\theta_X) = k\theta_X - \frac{a_X}{2} < 0, \tag{3.315}$$

and at $X = \frac{a_X}{k}$ one has that

$$g_1\left(\frac{a_X}{k}\right) = a_X - a_X \frac{1}{\left(\frac{k\theta_X}{a_X}\right) + 1} > 0. \tag{3.316}$$

Next, one has

$$g_1'(X) = k - a_X \frac{\theta_X}{(\theta_X + X)^2}, \tag{3.317}$$

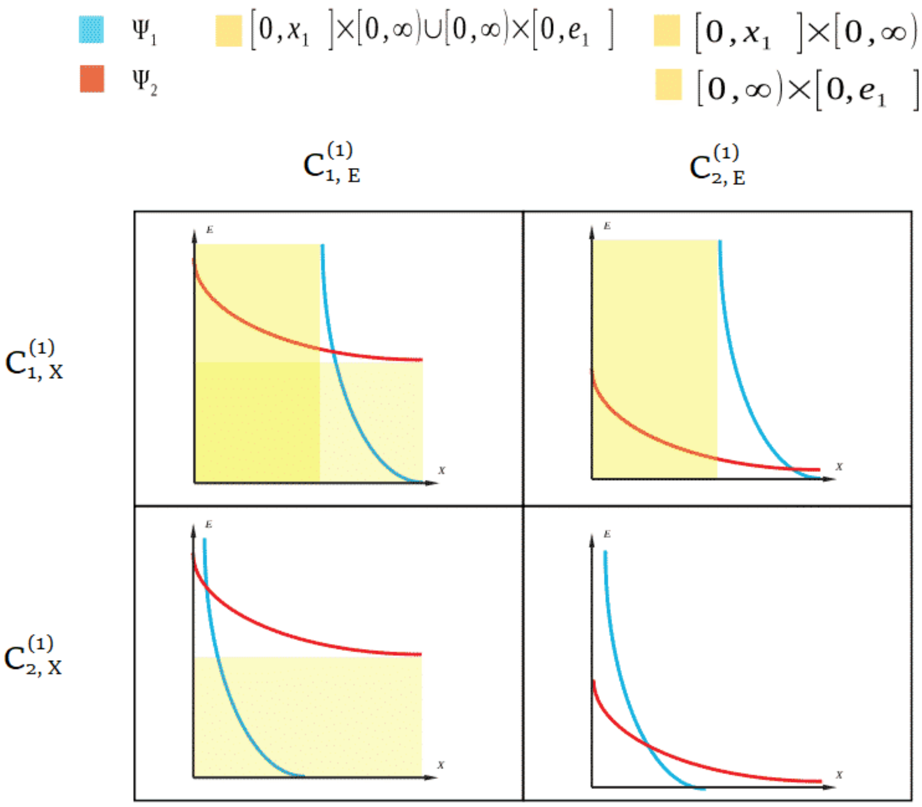


Figure 3.27: For $n = 1$, one has that Huang's qualitative graphical matrix $\left(H_1[C^{(1)}_{i,X}, C^{(1)}_{j,E}]\right)_{i,j}$ is a square matrix with a similar qualitative behavior of the nullclines in each of its entries. Furthermore, it has exactly one stable steady state regardless of the component of the matrix.

which implies that

$$g_1'(\theta_X) = k - \frac{a_X}{4\theta_X} < 0, \tag{3.318}$$

and that

$$g_1'\left(\frac{a_X}{k}\right) = k\left[1 - \frac{a_X}{k\theta_X}\frac{1}{\left(1+\frac{a_X}{k\theta_X}\right)^2}\right] > 0. \tag{3.319}$$

For (ii), on has that the proof is similar. □

So, drawing upon the Intermediate Value Theorem [77, p. 93] and Lemma 3.5.2, one has that there exists $\theta_X < x_1 < \frac{a_X}{k}$ such that $g_1(x_1) = 0$. In fact, $g_1(X) = 0$ if and only if

$$X\left[kX + (k\theta_X - a_X)\right] = 0, \tag{3.320}$$

that is, if and only if $X = 0$ or $X = \frac{a_X}{k} - \theta_X$. So,

$$x_1 = \frac{a_X}{k} - \theta_X > 0 \tag{3.321}$$

and, under $\theta_E < \frac{a_E}{k}$, one can similarly prove that $g_1(E) = 0$ if and only if $g_1(0) = 0$ or $g_1(e_1) = 0$ with

$$e_1 = \frac{a_E}{k} - \theta_E > 0. \tag{3.322}$$

Moreover, under $\theta_X < a_X/k$, there exists $\theta_X < \hat{X} < \frac{a_X}{k}$ such that $g_1'(\hat{X}) = 0$. In fact, $g_1'(\hat{X}) = 0$ if and only if

$$k(\theta_X + \hat{X})^2 - a_X\theta_X = 0, \tag{3.323}$$

if and only if

$$\hat{X} = \sqrt{\frac{a_X}{k}\theta_X} - \theta_X > 0. \tag{3.324}$$

In a similar way, under $\theta_X < a_X/k$, one can prove that $g_2'(\hat{E}) = 0$ if and only if

$$\hat{E} = \sqrt{\frac{a_E}{k}\theta_E} - \theta_E > 0, \tag{3.325}$$

with $\theta_E < \hat{E} < \frac{a_E}{k}$.

Further, one has that

$$g_1''(X) = 2a_X\frac{\theta_X}{(\theta_X + X)^3} > 0, \tag{3.326}$$

which implies that $g_1'(X)$ is strictly increasing on $[0, \infty)$, and that $g_1(X)$ is concave up [convex] on $[0, \infty)$. Therefore, for $n = 1$, under conditions

$$C_{1,X}^{(1)} : \theta_X < \frac{a_X}{k} \tag{3.327}$$

and

$$C_{1,E}^{(1)} : \theta_E < \frac{a_E}{k}, \tag{3.328}$$

one has that $g_1(X)$ has exactly the behaviour shown in Figure 3.4.

Now, with a similar argument used in Subsection 1.1.1, for $b > 0$ there exists $x_1 < x_b$ such that $g_1(x_b) = 0$. So, if

$$b > g_1\left(\frac{a_X}{k}\right) = a_X\left[1 - \frac{1}{\left(\frac{k\theta_X}{a_X}\right) + 1}\right] \tag{3.329}$$

then $x_b > a_X/k$; if

$$b \le g_1\left(\frac{a_X}{k}\right) = a_X\left[1 - \frac{1}{\left(\frac{k\theta_X}{a_X}\right) + 1}\right] \tag{3.330}$$

then $x_1 < x_b \le a_X/k$. In particular, if

$$b = g_1\left(\frac{a_X}{k}\right) = a_X\left[1 - \frac{1}{\left(\frac{k\theta_X}{a_X}\right) + 1}\right] \tag{3.331}$$

then $x_b = a_X/k$. So, by construction, under (3.327) and (3.328), one has that $\Psi_1(x_b) = 0$, $\Psi_1(e_b) = 0$,

$$\lim_{X \to x_1^+} \Psi_1(X) = +\infty, \tag{3.332}$$

and that

$$\lim_{E \to e_1^+} \Psi_2(E) = +\infty, \tag{3.333}$$

which leads us to the following result:

Proposition 3.5.3.

(i) $\theta_X < \frac{a_X}{k} \Rightarrow \Psi_1(X)\Big|_{(x_1,x_b]}$ *is strictly decreasing;*

(ii) $\theta_E < \frac{a_E}{k} \Rightarrow \Psi_2(E)\Big|_{(e_1,e_b]}$ *is strictly decreasing;*

Proof. (i) In fact, under $\theta_X < \frac{a_X}{k}$, one has that $g_1(X)\Big|_{(x_1,+\infty)}$ is strictly increasing whilst $h^{-1}(Z)\Big|_{(0,+\infty)}$ is strictly decreasing which, in turn, implies that $\Psi_1(X)\Big|_{(x_1,+\infty)}$ is strictly decreasing. For (ii), one can give a similar argument. □

As a consequence of Proposition 3.5.3, one has that

$$G_{\Psi_1} \cap G_{\Psi_2} = \{z^*\} \tag{3.334}$$

with $z^* = (X^*, E^*)$ satisfying

$$\theta_X < x_1 < X^* < x_b < \frac{a_X}{k} + \frac{b}{k}, \tag{3.335}$$

and

$$\theta_E < e_1 < E^* < e_b < \frac{a_E}{k} + \frac{b}{k}. \tag{3.336}$$

Now, under the conditions

$$C_{2,X}^{(1)} : \theta_X \geq \frac{a_X}{k} \tag{3.337}$$

and

$$C_{2,E}^{(1)} : \theta_E \geq \frac{a_E}{k}, \tag{3.338}$$

one has that $g_1(X) \geq 0$ for $X \geq 0$. In fact, if $X > 0$ and $a_X \geq a_X/k$ then

$$\theta_X + X > \frac{a_X}{k}, \tag{3.339}$$

which implies that

$$kX > a_X \frac{X}{\theta_X + X}, \tag{3.340}$$

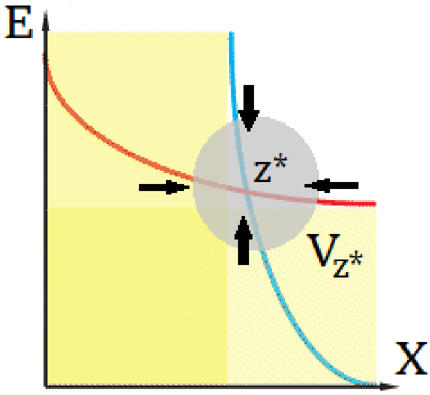


Figure 3.28: Here we see the phase-portrait in $H_1[C(1)_{1,X}, C^{(1)}_{1,E}]$. To apply the Poicaré-Bendixson Theorem, we choose a convenient "trap region" V_{z^*}, that is, a sufficiently small neighbourhood of the steady state z^* wherein all the positive orbits are bounded.

which, in turn, implies that

$$g_1(X) > 0. \tag{3.341}$$

In a similar way, one can show that if $X \geq 0$ and $\theta_X \geq a_X/k$ then

$$(\theta_X + X)^2 \geq \frac{a_X}{k}, \tag{3.342}$$

which implies that $g_1'(X) \geq 0$. Therefore, under the conditions (3.339) and (3.338), one has that $g_1(X)$ has precisely the behaviour shown in Figure 3.5. So, one has that

$$\lim_{X \to 0^+} \Psi_1(X) = +\infty, \tag{3.343}$$

and that

$$\lim_{E \to 0^+} \Psi_2(E) = +\infty, \tag{3.344}$$

and one can invoke the reasoning from (3.329) to (3.331) so as to demonstrate the following result.

Proposition 3.5.4.

(i) $\theta_X \geq \frac{a_X}{k} \Rightarrow \Psi_1(X)\Big|_{(0,x_b]}$ *is strictly decreasing;*

(ii) $\theta_E \geq \frac{a_E}{k} \Rightarrow \Psi_2(E)\Big|_{(0,e_b]}$ *is strictly decreasing;*

Proof. The same argument used in the proof of the Proposition 3.5.3. □

It is not difficult to see that Proposition 3.5.4 entails that

$$G_{\Psi_1\Psi_2} = \{z^* = (X^*, E^*)\}, \tag{3.345}$$

so one has that

$$\theta_X < x_1 < X^* < x_b < \frac{a_X}{k} + \frac{b}{k}, \tag{3.346}$$

and that

$$\theta_E < e_1 < E^* < e_b < \frac{a_E}{k} + \frac{b}{k}. \tag{3.347}$$

Next, by invoking (3.215), we intend understanding the structure of $\Lambda_H^{(1)}$. In fact, by drawing upon Section 2.8, then we can regard

$$\hat{\mathcal{A}}_1 := \left\{C_{1,X}^{(1)}, C_{2,X}^{(1)}, C_{1,E}^{(1)}, C_{2,E}^{(1)}\right\} \tag{3.348}$$

as the set of the *primary aspects*, so for $\lambda, \tilde{\lambda} \in \Lambda_H^{(1)}$, one can define

$$\lambda \sim_{\hat{\mathcal{A}}_1} \tilde{\lambda} \tag{3.349}$$

if and only if

$$\mid A[H_\lambda] \mid_{\mathbb{R}_+} = \mid A[H_{\tilde{\lambda}}] \mid_{\mathbb{R}_+}, \tag{3.350}$$

for all $A \in \hat{A}_1$. Hence, one can show that (3.349) is an equivalence relation. Thereby, one must have that the sets

$$H_1\left[C_{i,X}^{(1)}, C_{j,E}^{(1)}\right] := \left\{\lambda \in \mathbb{R}^6 \times \{1\} : \vdash C_{i,X}^{(1)}[H_\lambda] \wedge \vdash C_{j,E}^{(1)}[H_\lambda]\right\}, \tag{3.351}$$

with $i, j \in \{1, 2\}$, are equivalent classes.

So, for each $n = 1$, one has that the equivalent classes in (3.351) give rise to a matrix structure , that is,

$$\left(H_1\left[C_{i,X}^{(1)}, C_{j,E}^{(1)}\right]\right)_{i,j} := \bigcup_{i,j\in\{1,2\}} H_1\left[C_{i,X}^{(1)}, C_{j,E}^{(1)}\right], \tag{3.352}$$

which, by construction, implies that

$$\Lambda_H^{(1)} / \sim_{\hat{\mathcal{A}}_1} = \left(H_1\left[C_{i,X}^{(1)}, C_{j,E}^{(1)}\right]\right)_{i,j}, \tag{3.353}$$

and we can now understand the parameter space of the model for $n = 1$, as illustrated in Figure 3.27.

Theorem 3.5.5. *Let $i, j \in \{1, 2\}$. With respect to the scenario*

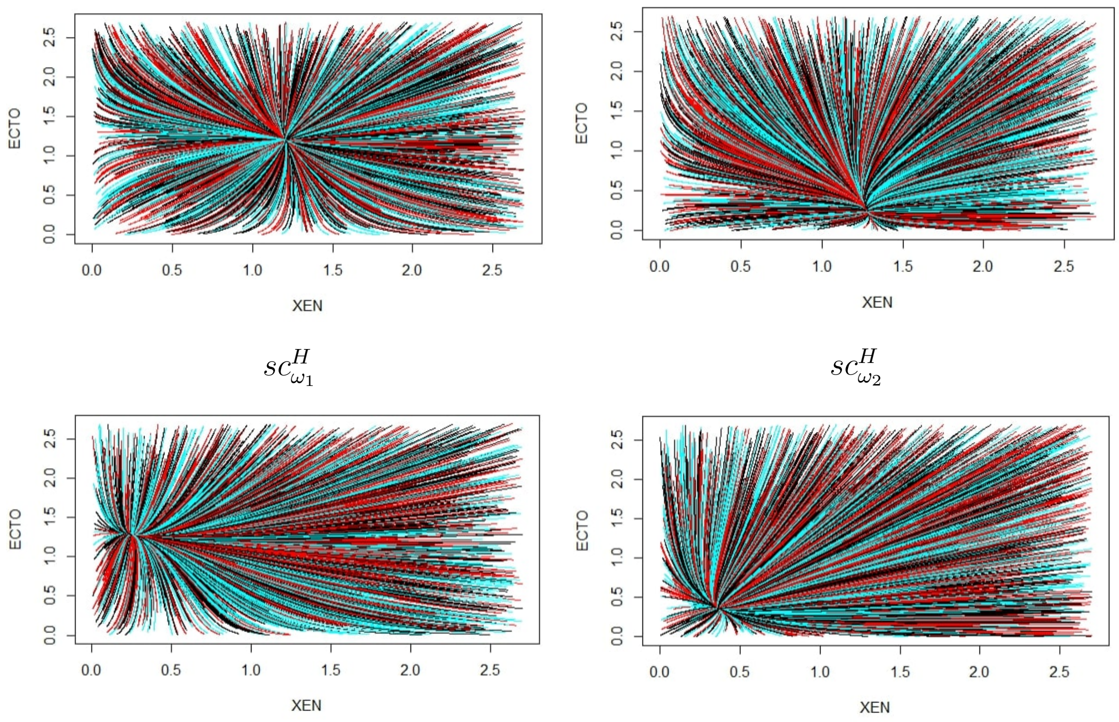
$$H_1\left[C_{i,X}^{(1)}, C_{j,E}^{(1)}\right],$$

one has that the unique steady state[3]

$$G_{\Psi_1} \cap G_{\Psi_2} = \{z^*\}$$

is a stable one.

[3]Up to a homeomorphism.



$sc^H_{\omega_1}$ $sc^H_{\omega_2}$

$sc^H_{\omega_3}$ $sc^H_{\omega_4}$

Figure 3.29: Here, one sees the simulation of the trajectories with respect to $\left(H_1\left[C^{(1)}_{i,X}, C^{(1)}_{j,E}\right]\right)_{i,j}$ *for the scenarios* $sc^H_{\omega_1} = [a_X = 0.8, a_E = 0.8, \theta_X = 0.5, \theta_E = 0.5, b = 0.0811, n = 1]; sc^H_{\omega_2} = [a_X = 0.8, a_E = 0.8, \theta_X = 0.5, \theta_E = 2, b = 0.0811, n = 1]; sc^H_{\omega_3} = [a_X = 0.8, a_E = 0.8, \theta_X = 2, \theta_E = 0.5, b = 0.0811, n = 1]$, *and* $sc^H_{\omega_4} = [a_X = 0.8, a_E = 0.8, \theta_X = 2, \theta_E = 2, b = 0.0811, n = 1]$.

Proof. First of all, for all $r > 0$ one has that the divergence of the vector field $\mathbf{F}^H$, that is,

$$\nabla \cdot \mathbf{F}^H(X, E) = -g'_{1,n}(X) - g'_{2,n}(E)$$

does not change sign in $B_r(z^*)$, which, by invoking Bendixson's criterion [100, p. 38], implies that there is no periodic orbit in $B_r(z^*)$. Next, by using the same argument of Theorem 3.4.6, there must exist a small neighbourhood V_{z^*} of z^*, as illustrated in Figure 3.28, in which all the positive orbits are bounded. Hence, by applying Poicaré-Bendixson Theorem, we conclude that all the positive orbits in V_{z^*} converge towards z^*. Therefore, z^* is a stable equilibrium. □

3.6 Discussion

In the respective chapter, we have analysed Huang's model by drawing upon the systematic approach introduced in Chapter 2. In fact, we have found that the parameter space of the model can be understood to some extent by using the Huang's qualitative graphical matrix. Indeed, as seen in 3.13, the parameter space can be imagined as an infinite rectangular cylinder parameterized by $n \in \mathbb{N}$, through which, for all $n \geq 2$, one has that any cylindric section amounts to the Huang's qualitative graphical matrix illustrated in 3.12, which, in turn, is characterized by a

3×3 squared matrix with four main components and a transition layer containing a critical layer. On the other hand, in the case of $n = 1$, one has that the corresponding cylindric section amounts to the Huang's qualitative graphical matrix characterized by a 2×2 squared matrix in which the transition layer has shrunk down to the critical layer.

Provided that a phenomenological model such as Huang's model is a simplified representation of a complex biological process [e.g. stem cell differentiation], which is inherently stochastic, it is of utmost importance for the model to be robust to small perturbations in the parameter setting of interest. But, what do we mean with that? To answer the later question, we turn ourselves towards the cornerstone notion of structural stability within the qualitative theory of dynamical system. In fact, if the phase-portrait of Huang's model H_λ for a given parameter setting of interest λ is invariant to small changes in the respective parameter setting, then H_λ is said to be structural stable. Having defined that, one has that structural stability is a crucial property to test the similarity-hypothesis.

Further, as we have observed through the course of the simulations, there are model instances for which the respective dynamical picture of the phase-space changes abruptly under small perturbations which are evidences for the claim that Huang's model is not structural stable. But how structural unstable is the model then? In fact, intuitively, if we think that such parameter instances, for which the phase portrait changes under small perturbations, form "surfaces" in $\mathbb{R}^6 \times \mathbb{N}_{\geq 2}$ separating locally two regions with non-empty interior in which the phase-portrait is qualitatively invariant, then we can definitely assert that Huang's model is in general more structural stable than unstable. However, an analytical assessment of the later claim seems to be a very challenging task in the case of Huang's model, which, a priori, deviates from the scope of this thesis.

Moreover, it is worth emphasizing that the demonstrations provided for the (in)stability of the steady states are solely dependent upon the qualitative properties of the functions defining the nullclines but not on their explicit expressions. Thereby, it means that the respective demonstrations are also valid for any functions having the same properties and for all the corresponding primitive scenarios.

However, we have not explored the limit as $n \to +\infty$ seeing that we do not intend to swerve from the main goal of this thesis. Neither have we tried to give a mathematical account for the claims that we had put forward concerning the dimension and the measure of the respective transition/critical layer. Furthermore, it is of utmost importance to emphasizing that, despite being able to envisage the parameter space as infinite rectangular cylinder parameterized by $n \in \mathbb{N}$, we do not mean whatsoever that $n \in \mathbb{N}$ must be seen as a generic parameter. In fact, we have seen through this section that there are conditions that are not realizable for some values of $n \geq 2$. More specifically, we have proved that, for all $n \geq 2$, we can find primitive scenarios in the first main component $H_n[C_{1,X}, C_{1,E}]$ of the Huang's qualitative graphical matrix, but it might not be true for the other main components in which the parameter $n \geq 2$ is highly constrained.

3.7 Conclusion

Throughout this chapter, we have stipulated how to find primitive scenarios of Huang's model with respect to the number of steady states. The latter essentially lays down our rational strategy given that primitive scenarios in our approach are the counterparts of primitive notions in *Frege's judgement theory*. In fact, if scenarios are regarded as the counterparts of observations then knowing the primitive scenarios of the model can potentially lead us to know any scenario of the model, which means that we can potentially know whether or not an observation is actually generated by the model. Now, we are in a position to extend the performed analysis to Semrau-Huang's model and apply the proposed evaluation procedure to test the adequacy-hypothesis.

Chapter 4

An application of the proposed evaluation to Semrau-Huang's model

In this chapter, we will perform an evaluation of an extension of Huang's model that has been proposed by Dr. Stefan Semrau, which is aimed at adequately explaining the *observations*

$$\begin{aligned} &O_P^{\left(CHIR^+,PD^+,LIF^+,RA^-\right)},\\ &O_E^{\left(CHIR^-,PD^-,LIF^-,RA^-\right)},\\ &O_{X,E}^{\left(CHIR^-,PD^-,LIF^-,RA^+\right)},\\ &O_{J_E,E}^{\left(CHIR^-,PD^+,LIF^-,RA^+\right)},\\ O_{J_E,E}^{\left(CHIR^-,PD^+,LIF^-,RA^+\right)} \xrightarrow{PD0325901^-,\ RA^+} &O_E^{\left(CHIR^-,PD^-,LIF^-,RA^+\right)}, \end{aligned} \tag{4.1}$$

with respect to the *experiments* described in Section 1.5, in which we found convenient to denote (4.1) by O_{TS}. See that section for notation and description.

In fact, if we draw upon the same approach used to mathematically analyze Huang's model then we shall be able to construct a *primitive scenario* which will enable us, by means of a rational strategy, to unveil *scenarios* of Semrau-Huang's model which, in turn, could be interpreted as the counterparts of the *observations* (4.1).

Furthermore, we will explore the causal relation implicit in the observations $(4.1)_{4,5}$. In fact, we shall see that the latter exploration will unravel a peculiar property of Semrau-Huang's model which, in turn, will be crucial in the evaluation of the model itself.

This chapter is organized as follows. Firstly, we build upon similar arguments displayed in Chapter 3 so as to construct such a *primitive scenario*. The latter will unravel all the steady states of the model. Secondly, we will find all the stable steady states of the respective *primitive scenario*. Thirdly, we shall test the adequacy-hypothesis by suitably decomposing a *primitive scenario* in scenarios similar to some of the observations. The latter will unveil a crucial property of Semrau-Huang's model. Afterwards, we will compute two Andronov-Hopf bifurcations in the model. Lastly, we will conclude this chapter by summarizing the evaluation of the model with respect to the similarity- and adequacy-hypothesis.

4.1 The qualitative graphical matrix of Semrau-Huang's model

As thoroughly described in Section 1.5, one has that the dimensionless dynamical equations of Semrau-Huang's model read

$$\begin{aligned} \frac{dX}{dt} &= a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n} - kX(1+cP), \\ \frac{dE}{dt} &= a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1+X^n} - kE(1+cP), \\ \frac{dP}{dt} &= a_P \frac{P}{1+P} - kP[1+c(E+dX)], \end{aligned} \tag{4.2}$$

with

$$P = \hat{P}/\theta, \ \ X = \hat{X}/\theta, \ \ E = \hat{E}/\theta, \ \ \theta_X = \hat{\theta}_X/\theta, \ \ \theta_E = \hat{\theta}_E/\theta, \tag{4.3}$$

and

$$t = \hat{t}/\tau, \ \ k = \hat{k}\tau, \ \ a_P = \tau\hat{a}_P/\theta, \ \ a_X = \tau\hat{a}_X/\theta, \ \ a_E = \tau\hat{a}_X/\theta, \ \ c = \theta\hat{c}, \tag{4.4}$$

and d being a dimensionless parameter. Here, we recall that the parameters a_P, θ_X, and d are highlighted in red because those are the varying parameters of the model.

Further, we will draw upon the same rationale of Chapter 2 to find the null-clines of (4.2), which, for this model, are two-dimensional curved surfaces in three-dimensional state space, typically.

First, note that at a fixed P level one has that

$$\frac{dX}{dt} \leq a_X + b - k_P X, \tag{4.5}$$

with

$$k_P := k(1+cP), \tag{4.6}$$

so if

$$X > \frac{a_X}{k_P} + \frac{b}{k_P} \tag{4.7}$$

then

$$\frac{dX}{dt} < 0. \tag{4.8}$$

Similarly, one has that if

$$E > \frac{a_E}{k_P} + \frac{b}{k_P} \tag{4.9}$$

then

$$\frac{dE}{dt} < 0. \tag{4.10}$$

Next, we can rewrite $(4.2)_3$ as

$$\frac{dP}{dt} = a_P \frac{P}{1+P} - kP - kPc(E+dX)), \tag{4.11}$$

so if

$$P > \frac{a_P}{k} - 1 \tag{4.12}$$

then

$$\frac{dP}{dt} < 0 \tag{4.13}$$

for any $X, E \geq 0$. So, if we define that

$$P_{sup} := a_P/k - 1 > 0,$$

and that

$$\tilde{I}_X := \left[\frac{a_X}{k_{P_{sup}}} + \frac{b}{k_{P_{sup}}}, \frac{a_X}{k} + \frac{b}{k}\right],$$

and that

$$\tilde{I}_E := \left[0, \frac{a_E}{k} + \frac{b}{k}\right],$$

and that

$$\alpha := -\frac{\frac{\left(\frac{a_P}{k}-1\right)}{\left(\frac{a_X}{k}+\frac{b}{k}\right)}}{\left[1 - \frac{\left(\frac{a_X}{k_{P_{sup}}}+\frac{b}{k_{P_{sup}}}\right)}{\left(\frac{a_X}{k}+\frac{b}{k}\right)}\right]}, \tag{4.14}$$

and that

$$\gamma := \frac{\frac{a_P}{k} - 1}{\left[1 - \frac{\left(\frac{a_X}{k_{P_{sup}}}+\frac{b}{k_{P_{sup}}}\right)}{\left(\frac{a_X}{k}+\frac{b}{k}\right)}\right]}, \tag{4.15}$$

and that

$$\mathbb{T} := \left\{(X, E, P) \in \mathbb{R}^3_+ : P = \alpha X + \gamma,\ (X, E) \in \tilde{I}_X \times \tilde{I}_E\right\}, \tag{4.16}$$

then we can conclude that the interesting dynamics is confined in the Trapezoidal prism

$$(X, E, P) \in \left[0, \frac{a_X}{k_{P_{sup}}} + \frac{b}{k_{P_{sup}}}\right] \times \left[0, \frac{a_E}{k_{P_{sup}}} + \frac{b}{k_{P_{sup}}}\right] \times \left[0, \frac{a_P}{k} - 1\right] \bigcup \mathbb{T}, \tag{4.17}$$

which, in turn, is contained in the rectangular [box] region:

$$(X, E, P) \in \left[0, \frac{a_X}{k} + \frac{b}{k}\right] \times \left[0, \frac{a_E}{k} + \frac{b}{k}\right] \times \left[0, \frac{a_P}{k} - 1\right]. \tag{4.18}$$

Furthermore, when denoting the vector field of the system of differential equations in (4.2) by $\mathbf{F}^{SH} := \left(\tilde{F}_1, \tilde{F}_2, \tilde{F}_3\right)$ with

$$\begin{aligned}
\tilde{F}_1(X, E, P) &= a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1 + E^n} - kX(1 + cP),\\
\tilde{F}_2(X, E, P) &= a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1 + X^n} - kE(1 + cP),\\
\tilde{F}_3(X, E, P) &= a_P \frac{P}{1 + P} - kP[1 + c(E + dX)],
\end{aligned} \tag{4.19}$$

one has that, at a level $P = \tilde{p} \geq 0$, the projection of the vector field (4.88) onto this plane is essentially a Huang's model with degradation rate $k_{\tilde{p}} = k(1 + c\tilde{p})$. However, it is important to remark that the dynamics of the Semrau-Huang's model by means of the (in)stability of the steady states might be different from the one generated by Huang's model as we shall see later in this chapter.

4.1.1 The description of the nullclines $\breve{G}_{\Psi_{1,n}}$, $\breve{G}_{\Psi_{2,n}}$ and $\breve{G}_{\Psi_3}$

To begin with, one has that a *steady state* of (4.2) must satisfy

$$
\begin{aligned}
0 &= a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n} - kX(1+cP),\\
0 &= a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1+X^n} - kE(1+cP),\\
0 &= a_P \frac{P}{1+P} - kP[1 + c(E + dX)].
\end{aligned}
\tag{4.20}
$$

However, $(4.20)_3$ is true if and only if

$$P = 0, \tag{4.21}$$

or

$$P = \frac{a_P}{k\left[1 + c(E + dX)\right]} - 1. \tag{4.22}$$

In fact, for the case $P = 0$, one has a reduction of (4.2) to Huang's model, which, in turn, has already been analyzed in Chapter 3. So, in this chapter, we will entirely focus on the case shown in (4.22). In fact, for $n \geq 1$ and $P > 0$, if we build upon Chapter 3 then we define

$$
\begin{aligned}
g_{1,n}^P(X) &:= k(1+cP)X - a_X \frac{X^n}{\theta_X^n + X^n},\\
g_{2,n}^P(E) &:= k(1+cP)E - a_E \frac{E^n}{\theta_E^n + E^n},
\end{aligned}
\tag{4.23}
$$

and

$$h_n(Z) := b\frac{1}{1+Z^n} \tag{4.24}$$

for all $X, E, Z \geq 0$. If $b > 0$ then the function defined in (4.24) is invertible, whose inverse reads

$$h_n^{-1}(\tilde{Z}) = \left(\frac{b}{\tilde{Z}} - 1\right)^{\frac{1}{n}}, \tag{4.25}$$

for all $\tilde{Z} \in (0, b]$. Furthermore, it is important to emphasize that both h_n and h_n^{-1} are strictly decreasing functions.

Hence, one can conveniently define

$$\Psi_{1,n}^P := h_n^{-1} \circ g_{1,n}^P, \tag{4.26}$$

and

$$\Psi_{2,n}^P := h_n^{-1} \circ g_{2,n}^P, \tag{4.27}$$

and

$$\Psi_3 := \frac{a_P}{k\,[1 + c(E + dX)]} - 1. \tag{4.28}$$

Further, given $P > 0$, define

$$G_{\Psi^P_{1,n}} := \{(X, E, P) \in \mathbb{R}^3_+ : E = \Psi^P_{1,n}(X)\}, \tag{4.29}$$

and

$$G_{\Psi^P_{2,n}} := \{(X, E, P) \in \mathbb{R}^3_+ : X = \Psi^P_{2,n}(E)\}. \tag{4.30}$$

So, one has that $G_{\Psi^P_{1,n}}$ and $G_{\Psi^P_{1,n}}$ are curves on the plane in $\mathbb{R}^3$ defined by a fixed $P > 0$. Therefore, the X, E, and P nullclines of (4.2) are then given by the sets

$$\breve{G}_{\Psi_{1,n}} = \bigcup_{P \geq 0} G_{\Psi^P_{1,n}}, \tag{4.31}$$

and

$$\breve{G}_{\Psi_{2,n}} = \bigcup_{P \geq 0} G_{\Psi^P_{2,n}}, \tag{4.32}$$

and

$$\breve{G}_{\Psi_3} = \{(X, E, P) \in \mathbb{R}^3_+ : P = \Psi_3(X, E)\}, \tag{4.33}$$

respectively. Therefore, consistently, one has that $(X^*, E^*, P^*) \in \mathbb{R}^3_+$ with $P^* > 0$ satisfies (4.20) if and only if

$$(X^*, E^*, P^*) \in \breve{G}_{\Psi_{1,n}} \cap \breve{G}_{\Psi_{2,n}} \cap \breve{G}_{\Psi_3}, \tag{4.34}$$

that is, a *steady state* of (4.2) is a point in $\mathbb{R}^3_+$ belonging to the intersection of the three nullclines (4.31), (4.32), and (4.33); or it has $P^* = 0$ and then it is a *steady state* of the Huang's model.

4.1.2 Geometric aspects as primary aspects of the model: Semrau-Huang qualitative graphical matrix $\left(SH_n[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{r,P}]\right)_{i,j,r}$

Drawing upon the systematic evaluation thoroughly described in Chapter 2 and concisely summarized in Section 2.8, it is essential to bearing in mind that the relevant aspects of (4.2) being prioritized concern the number of steady states. In this regard, a *scenario* with the maximal number of steady states will be a *primitive scenario*. But, how can we fix such a *primitive scenario* of (4.2) ? In fact, given $k, c, P > 0$ and $n \geq 2$, recall that

$$k_P = k(1 + cP). \tag{4.35}$$

So, under

$$\theta_X < \frac{a_X}{2k_P}, \tag{4.36}$$

and

$$\theta_E < \frac{a_E}{2k_P}, \tag{4.37}$$

if we draw upon Chapter 3 then one has that $x^P_{b,n} > a_X/k_P$ and $e^P_{b,n} > a_E/k_P$ are the unique solutions of the equations

$$\begin{aligned} b &= k_P X - a_X \frac{X^n}{\theta_X^n + X^n}, \\ b &= k_P E - a_E \frac{E^n}{\theta_E^n + E^n}, \end{aligned} \tag{4.38}$$

or equivalently, the unique positive real numbers satisfying

$$\begin{aligned} b &= g^P_{1,n}(x^P_{b,n}), \\ b &= g^P_{2,n}(e^P_{b.n}). \end{aligned} \tag{4.39}$$

Moreover, one has that

$$x^P_{b,n} < \frac{a_X}{k_P} + \frac{b}{k_P}, \tag{4.40}$$

and that

$$e^P_{b,n} < \frac{a_E}{k_P} + \frac{b}{k_P}, \tag{4.41}$$

with

$$\sup_n x^P_{b,n} = \frac{a_X}{k_P} + \frac{b}{k_P}, \tag{4.42}$$

and that

$$\sup_n e^P_{b,n} = \frac{a_E}{k_P} + \frac{b}{k_P}. \tag{4.43}$$

Now, if we denote the zeros of the functions $g^P_{1,n}(X)$ and $g^P_{2,n}(X)$ by

$$\{X \geq 0 : g^P_{1,n}(X) = 0\} = \{0; x^P_{1,n}; x^P_{2,n}\} \tag{4.44}$$

and

$$\{E \geq 0 : g^P_{2,n}(E) = 0\} = \{0; e^P_{1,n}; e^P_{2,n}\} \tag{4.45}$$

respectively, then

$$x^P_{1,n} < x^P_{2,n} < x^P_{b,n} < \frac{a_X}{k_P} + \frac{b}{k_P} \tag{4.46}$$

and

$$e^P_{1,n} < e^P_{2,n} < e^P_{b,n} < \frac{a_E}{k_P} + \frac{b}{k_P}. \tag{4.47}$$

Next, consistent with Chapter 3, let $x^P_{max,n} \in [0, x^P_{1,n}]$ denote the unique point in $[0, x^P_{1,n}]$ satisfying

$$g^P_{1,n}(x^P_{max,n}) = \max_{X \in \left[0, x^P_{1,n}\right]} g^P_{1,n}(X). \tag{4.48}$$

Likewise, one has that $e^P_{max,n} \in [0, e^P_{1,n}]$ is the unique point in $[0, e^P_{1,n}]$ satisfying

$$g^P_{2,n}(e^P_{max,n}) = \max_{E \in \left[0, e^P_{1,n}\right]} g^P_{2,n}(E). \tag{4.49}$$

So, as we have argued in Chapter 3, if one wants to fix a primitive scenario at level $P > 0$, under (4.36) and (4.36), then it is sufficient to have that

$$0 \leq \Psi^P_{2,n}(e^P_{max,n}) < \Psi^{P,-1}_{1,n}|_{(0, x^P_{max,n})} \left(\frac{a_E}{k_P} + \frac{b}{k_P} \right), \tag{4.50}$$

and that

$$0 \leq \Psi^P_{1,n}(x^P_{max,n}) < \Psi^{P,-1}_{2,n}|_{(0,e^P_{max,n})}\left(\frac{a_X}{k_P} + \frac{b}{k_P}\right), \tag{4.51}$$

as one sees in the Figure 4.1.

In order to understand the essence of the conditions (4.50) and (4.51), it is worth recalling that if $g^P_{1,n}$ has a local maximum at $x^P_{max,n}$ then $\Psi^P_{1,n}$ has a local minimum at $x^P_{max,n}$. Likewise, if $g^P_{2,n}$ has a local maximum at $e^P_{max,n}$ then $\Psi^P_{2,n}$ has a local minimum at $e^P_{max,n}$. Hence, by stipulating the conditions (4.50) and (4.51), we can sufficiently push downwards the graphs of $\Psi^P_{2,n}$ and $\Psi^P_{2,n}$ so as to give rise to a primitive conformation of the nullclines at each level $P > 0$.

Withal, if we intend fixing a *primitive scenario* of (4.2) then we need conditions that are independent upon $P > 0$. In fact, given that

$$\begin{aligned}\Psi_3(0,0) &= \sup_{X,E\geq 0} \Psi_3(X,E)\\ &= \frac{a_P}{k} - 1,\end{aligned} \tag{4.52}$$

one can define

$$\tilde{k} := k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right]$$

so that if the conditions

$$\breve{C}_{1,X} : \theta_X < \frac{a_X}{2\tilde{k}} \tag{4.53}$$

and

$$\breve{C}_{1,E} : \theta_E < \frac{a_E}{2\tilde{k}} \tag{4.54}$$

hold, then (4.34) and (4.36) hold for all $P > 0$. Analogously, if

$$\breve{C}_{1,n} : 0 \leq \Psi^P_{2,n}(e^P_{max,n}) < \Psi^{P,-1}_{1,n}|_{(0,x^P_{max,n})}\left(\frac{a_E}{k} + \frac{b}{k}\right) \tag{4.55}$$

and

$$\breve{C}_{2,n} : 0 \leq \Psi^P_{1,n}(x^P_{max,n}) < \Psi^{P,-1}_{2,n}|_{(0,e^P_{max,n})}\left(\frac{a_X}{k} + \frac{b}{k}\right) \tag{4.56}$$

hold then (4.50) and (4.51) hold for all $P > 0$. Moreover, define

$$b_P := \min\left\{g^P_{1,n}(x^P_{max,n}), g^P_{2,n}(e^P_{max,n})\right\} \tag{4.57}$$

for all $P \geq 0$. So, if we stipulate the condition

$$\breve{C}_{0,n} : 0 < b \leq \inf_{0\leq P\leq \frac{a_P}{k}-1} b_P \tag{4.58}$$

then we have that the conditions (4.50) and (4.51) hold for all $P > 0$. However, it is important to point that, in this case, one might have to consider an appropriate extension of $\Psi^P_{1,n}$ and $\Psi^P_{2,n}$ for which one would have that $\Psi^P_{1,n}(x^P_{max,n}) = 0$ and that $\Psi^P_{2,n}(e^P_{max,n}) = 0$, for all $P > 0$.

Further, we note that $X, E \geq 0$ satisfy

$$\Psi_3(X,E) = 0 \tag{4.59}$$

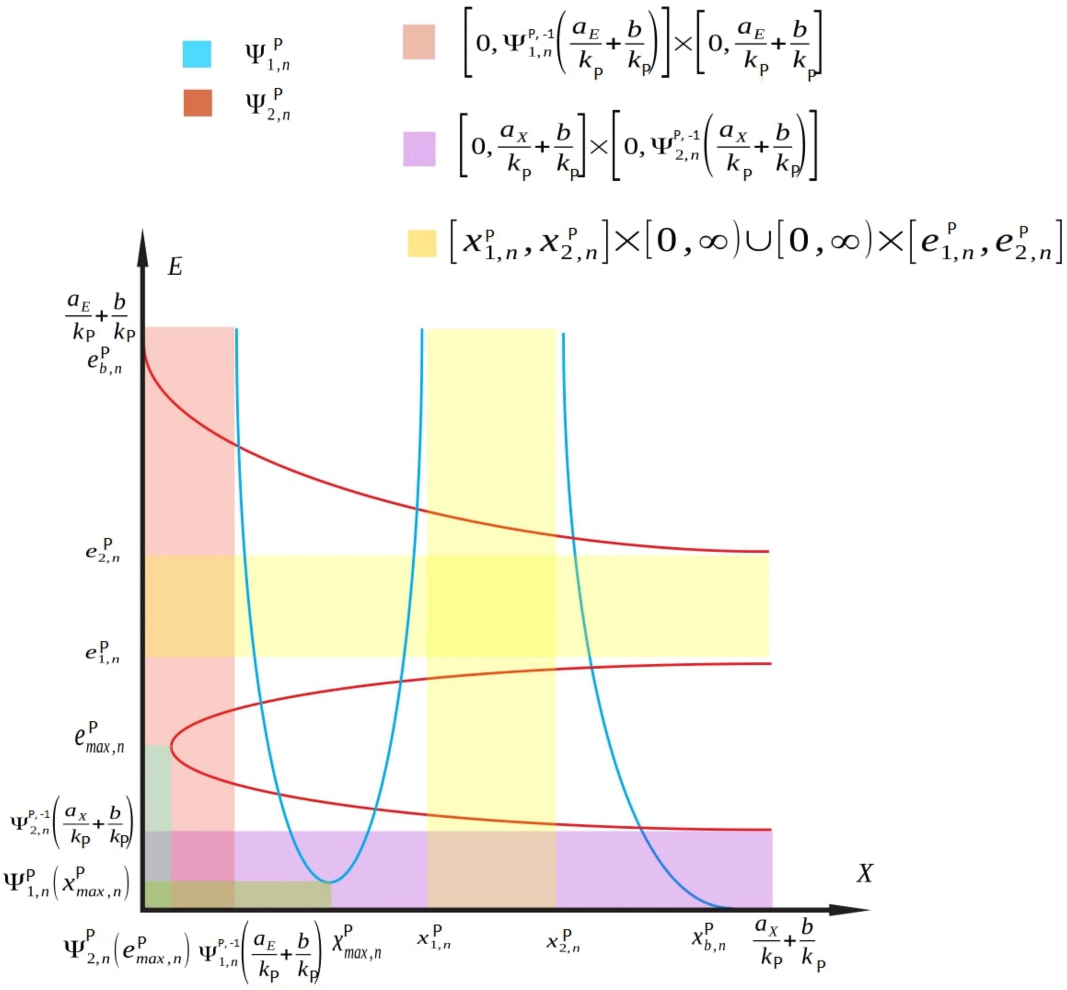


Figure 4.1: Here one sees the illustration of the qualitative behavior of the nullclines $\Psi^P_{1,n}$ (in blue) and $\Psi^P_{2,n}$ (in red) of a primitive scenario at level $P > 0$.

if and only if

$$\frac{1}{c}\left(\frac{a_P}{k} - 1\right) = E + dX, \tag{4.60}$$

so it is convenient to add the condition

$$\breve{C}_{1,P} : \frac{1}{c}\left(\frac{a_P}{k} - 1\right) > e^{(0)}_{b,n} + dx^{(0)}_{b,n}, \tag{4.61}$$

with $e^{(0)}_{b,n}$ and $x^{(0)}_{b,n}$ satisfying

$$\begin{aligned} b &= kx^{(0)}_{b,n} - a_X \frac{(x^{(0)}_{b,n})^n}{\theta^n_X + (x^{(0)}_{b,n})^n}, \\ b &= ke^{(0)}_{b,n} - a_E \frac{(e^{(0)}_{b,n})^n}{\theta^n_E + (e^{(0)}_{b,n})^n}, \end{aligned} \tag{4.62}$$

so that the intersection

$$\breve{G}_{\Psi_{1,n}} \cap \breve{G}_{\Psi_{2,n}} \cap \breve{G}_{\Psi_3} \tag{4.63}$$

is maximal.

By analogy with Chapter 3, one has that the conditions

$$\begin{aligned} \breve{C}_{1,X} : \theta_X < \frac{a_X}{2\tilde{k}}, \\ \breve{C}_{1,E} : \theta_E < \frac{a_E}{2\tilde{k}}, \\ \breve{C}_{2,X} : \frac{a_X}{2k} \leq \theta_X \leq \frac{a_X}{\tilde{k}}, \\ \breve{C}_{2,E} : \frac{a_X}{2k} \leq \theta_E \leq \frac{a_E}{\tilde{k}}, \\ \breve{C}_{3,X} : \theta_X > \frac{a_X}{k}, \\ \breve{C}_{3,E} : \theta_E > \frac{a_E}{k}, \\ \breve{C}_{1,P} : \frac{1}{c}\left(\frac{a_P}{k} - 1\right) > e^{(0)}_{b,n} + dx^{(0)}_{b,n}, \end{aligned} \tag{4.64}$$

can be regarded as the primary [geometrical] aspects of the model.

Thereby, by invoking Chapter 2 and by recalling that $\mathbb{N}_{\geq 2} := \{n \in \mathbb{N} : n \geq 2\}$, one can denote the set of the primary aspects of the model by :

$$\breve{\mathcal{A}} := \left\{\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{2,X}, \breve{C}_{2,E}, \breve{C}_{3,X}, \breve{C}_{3,E}, \breve{C}_{1,P}\right\}. \tag{4.65}$$

So, for $\lambda, \tilde{\lambda} \in \hat{\Lambda}_{SH} := \mathbb{R}^9_{\geq 0} \times \mathbb{N}_{\geq 2}$, one can define

$$\lambda \sim_{\breve{\mathcal{A}}} \tilde{\lambda} \tag{4.66}$$

if and only if

$$| \ A[SH_\lambda] \ |_{\mathbb{R}_+} = | \ A[SH_{\tilde{\lambda}}] \ |_{\mathbb{R}_+}, \tag{4.67}$$

for all $A \in \breve{\mathcal{A}}$, with $| \ A \ |_{\mathbb{R}_+}$ denoting the truth-value of a mathematical assertion A. Furthermore, one has that SH_λ represents Semrau-Huang's model with a fixed parameter setting $\lambda \in \hat{\Lambda}_{SH}$, while $A[SH_\lambda]$ betokens a formalized mathematical *assertion* on SH_λ. As we have argued in Chapter 2, the binary relation defined in (4.66) is indeed an equivalence relation.

Hence, for each $n \geq 2$, one must have that the sets

$$SH_n[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{1,P}] := \left\{\lambda \in \mathbb{R}^9_{\geq 0} \times \{n\} : \vdash \breve{C}_{i,X} \wedge \vdash \breve{C}_{j,E} \wedge \vdash \breve{C}_{1,P}\right\}, \tag{4.68}$$

with $i, j \in \{1, 2, 3\}$, are equivalence classes; which give rise to a cubic matrix structure, that is,

$$\left(SH_n[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{r,P}]\right)_{i,j,r} := \bigcup_{i,j,r} SH_n[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{r,P}], \tag{4.69}$$

with $i, j \in \{1, 2, 3\}$ as seen in Figure 4.3, which, by construction, implies that

$$\hat{\Lambda}_{SH} / \sim_{\breve{\mathcal{A}}} = \bigcup_{n \geq 2} \left(SH_n[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{r,P}]\right)_{i,j,r}, \tag{4.70}$$

and we will give an intuitive account in the next section for the description of this cubic matrix. But, what does the index r stand for ? It stands for a variety of reformulations of the condition $\breve{C}_{1,P}$ in (4.61).

So far, we have stipulated conditions [primary aspects] with which we have provided a mathematical description of main components of the parameter space of the model, that is,

$$\begin{aligned}
&\bigcup_{r\neq 1} SH_n[\breve{C}_{1,X},\breve{C}_{1,E},\breve{C}_{1,P}]\cup SH_n[\breve{C}_{1,X},\breve{C}_{1,E},\breve{C}_{r,P}],\\
&\bigcup_{r\neq 1} SH_n[\breve{C}_{3,X},\breve{C}_{1,E},\breve{C}_{1,P}]\cup SH_n[\breve{C}_{3,X},\breve{C}_{1,E},\breve{C}_{r,P}],\\
&\bigcup_{r\neq 1} SH_n[\breve{C}_{1,X},\breve{C}_{3,E},\breve{C}_{1,P}]\cup SH_n[\breve{C}_{1,X},\breve{C}_{3,E},\breve{C}_{r,P}],\\
&\bigcup_{r\neq 1} SH_n[\breve{C}_{3,X},\breve{C}_{3,E},\breve{C}_{1,P}]\cup SH_n[\breve{C}_{1,X},\breve{C}_{3,E},\breve{C}_{r,P}].
\end{aligned}\tag{4.71}$$

Furthermore, we have concisely constructed a primitive scenario in

$$SH_n[\breve{C}_{1,X},\breve{C}_{1,E},\breve{C}_{1,P}]$$

by augmenting it with the conditions $\breve{C}_{0,n}$, $\breve{C}_{1,n}$, and $\breve{C}_{2,n}$ provided in (4.58), (4.55), and (4.56).

Now, consistent with Chapter 3, let $x^P_{min,n}\in[\theta_X,\infty)$ denote the unique point satisfying

$$g^P_{1,n}(x^P_{min,n})=\max_{X\in[\theta_X,+\infty)} g^P_{1,n}(X).\tag{4.72}$$

Likewise, one has that $e^P_{min,n}\in[\theta_X,\infty)$ is the unique point satisfying

$$g^P_{2,n}(e^P_{min,n})=\max_{E\in[\theta_E,+\infty)} g^P_{2,n}(E).\tag{4.73}$$

So, if we invoke that $P_{sup}=a_P/k-1$ then, by analogy with the approach presented in Chapter 3, one can see that the conditions

$$\begin{aligned}
\breve{C}_{3,n}:\theta_X &< \frac{a_X}{\tilde{k}}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n},\\
\breve{C}_{4,n}:\theta_E &< \frac{a_E}{\tilde{k}}\frac{n^2-1}{4n}\left(\frac{n+1}{n-1}\right)^{1/n},\\
\breve{C}_{5,n}:\Psi^{P_{sup}}_{1,n}(x_{min,n}) &> \sup_{0\leq P\leq\frac{a_P}{k}-1}\Psi^{P,-1}_{2,n}|_{(e^P_{min,n},+\infty)}(x^P_{min,n}),\\
\breve{C}_{6,n}:\Psi^{P_{sup}}_{2,n}(e_{min,n}) &> \sup_{0\leq P\leq\frac{a_P}{k}-1}\Psi^{P,-1}_{1,n}|_{(x^P_{min,n},+\infty)}(e^P_{min,n}),
\end{aligned}\tag{4.74}$$

are sufficient to find primitive scenarios in the other main components as we shall see in the next section.

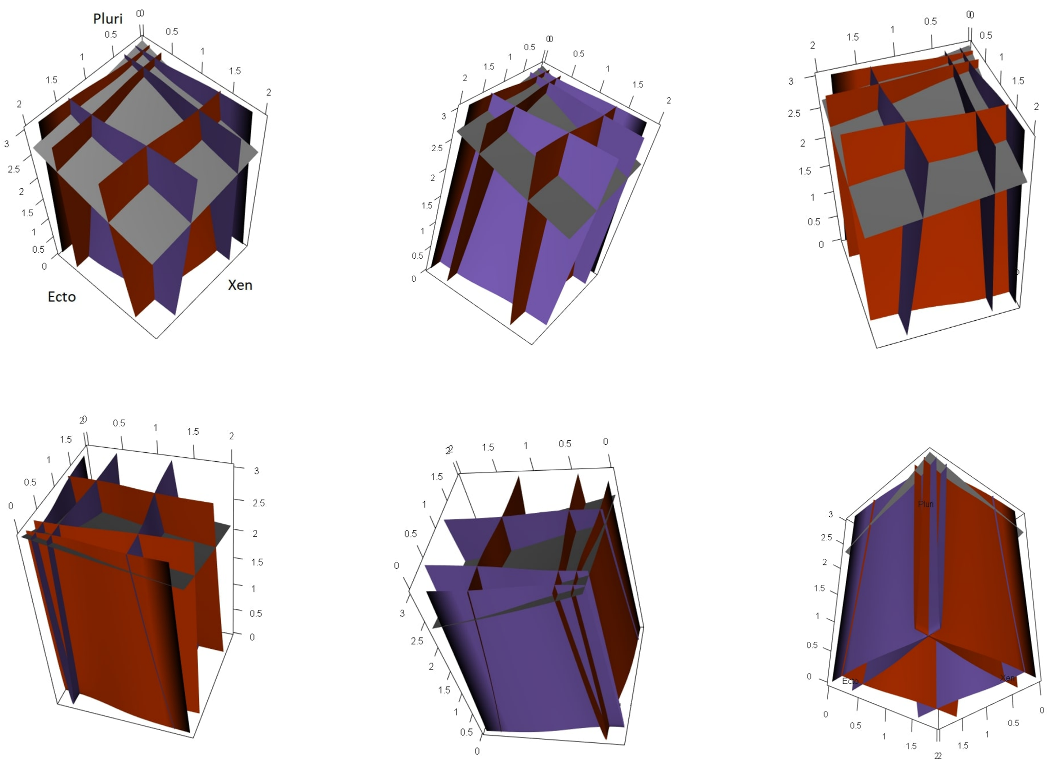


Figure 4.2: Here one sees the nullclines of the model for the primitive scenario $sc^{SH}_{\lambda_0}$ *with* $\lambda_0 = (a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 0.5, \theta_E = 0.5, b = 0.0811, c = 0.1, d = 0.5, k = 0.5, n = 4)$ *in six different perspectives.*

4.2 A concise description of the scenario space and the primitive scenarios of the model

Drawing upon the latter section, one has that

$$\breve{\mathcal{A}} := \left\{\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{2,X}, \breve{C}_{2,E}, \breve{C}_{3,X}, \breve{C}_{3,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}, \breve{C}_{3,n}, \breve{C}_{4,n}, \breve{C}_{5,n}, \breve{C}_{6,n}\right\} \tag{4.75}$$

can be regarded as the set of the relevant aspects of the model. Moreover, as we have argued in Chapter 2, one has that

$$SC^{SH} := \hat{\Lambda}_{SH} / \sim_{\breve{\mathcal{A}}} = \{[\lambda] : \lambda \in \hat{\Lambda}_{SH}\} \tag{4.76}$$

defines the scenario space of Semrau-Huang's model, with $[\lambda]$ representing an equivalence class.

So, if we build on the argumentation proposed in the latter section then we have that

$$SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}] := \{\lambda \in \hat{\Lambda}_{SH} : \vdash \breve{C}_{1,X} \wedge \vdash \breve{C}_{1,E} \wedge \vdash \breve{C}_{1,P} \wedge \vdash \breve{C}_{0,n} \wedge \vdash \breve{C}_{1,n} \wedge \vdash \breve{C}_{2,n}\}, \tag{4.77}$$

is a primitive scenario in $SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}]$ as shown in Figure 4.2 from several perspectives. Consistent with Chapter 2, we will denote the primitive scenario in (4.77) by $sc^{SH}_{\lambda_0}$ wherein λ_0 designates any representative of (4.77).

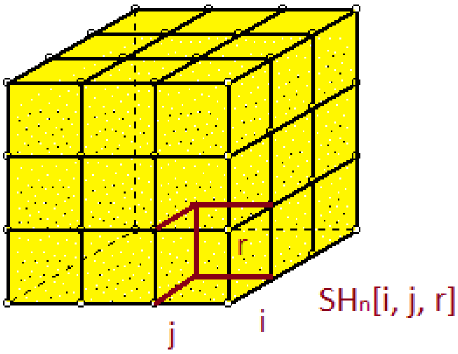


Figure 4.3: Cartoon taken from [84]. Here, one sees the illustration of $\left(SH_n[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{r,P}\right)_{i,j,r}$ *as a qualitative graphical cube matrix with* $SH_n[i,j,r] = SH_n\left[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{r,P}\right]$.

By analogy with the analysis performed in Chapter 3, one has that

$$\begin{aligned} Prim_{sc}^{SH_n} := \{ & SH_n\left[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}\right], \\ & SH_n\left[\breve{C}_{1,X}, \breve{C}_{3,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}, \breve{C}_{4,n}, \breve{C}_{6,n}\right], \\ & SH_n\left[\breve{C}_{3,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}, \breve{C}_{3,n}, \breve{C}_{5,n}\right], \\ & SH_n\left[\breve{C}_{3,X}, \breve{C}_{3,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}, \breve{C}_{3,n}, \breve{C}_{4,n}, \breve{C}_{5,n}, \breve{C}_{6,n}\right]\} \end{aligned} \tag{4.78}$$

is the set of all primitive scenarios in SC^{SH}. So, the elements of $Prim_{sc}^{SH_n}$ correspond to the primitive scenarios in each of the four *main components* of Semrau-Huang's qualitative graphical matrix as defined in (4.71).

But, why are the conditions (4.55), (4.56), and (4.61) sufficient to fix a primitive scenario in $SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}]$? And, why is the condition (4.61) essential to doing that? An 'ad hoc argument' for that reads as follows. First, one observes that if $P_2 > P_1 > 0$ then

$$g_{1,n}^{P_1}(x_{max,n}^{P_1}) \leq g_{1,n}^{P_2}(x_{max,n}^{P_2}), \tag{4.79}$$

which, in turn, implies that

$$\Psi_{1,n}^{P_2}(x_{max,n}^{P_2}) \leq \Psi_{1,n}^{P_1}(x_{max,n}^{P_1}), \tag{4.80}$$

so the conditions (4.55) and (4.56) guarantee that $\Psi_{1.n}^{P}$ and $\Psi_{1.n}^{P}$ are pulled downwards so as to enable their "primitive intersection" in the rectangle $[0, x_{1,n}^{\tilde{P}}] \times [0, e_{1,n}^{\tilde{P}}]$.

Moreover, given $\tilde{P} > 0$, one has that all the "potential steady-states" at the level $\tilde{P}$ are contained in the rectangle $[0, x_{b,n}^{\tilde{P}}] \times [0, e_{b,n}^{\tilde{P}}]$. But, what do we mean with "potential steady-states" at the $\tilde{P}$ level ? In fact, we mean the points in the set $G_{\Psi_{1,n}^{\tilde{P}}} \cap G_{\Psi_{2,n}^{\tilde{P}}}$ as illustrated in Figure 4.1. Furthermore, recalling (4.38), if $P_2 > P_1 > 0$ then

$$x_{b,n}^{\tilde{P}_2} < x_{b,n}^{\tilde{P}_1} \tag{4.81}$$

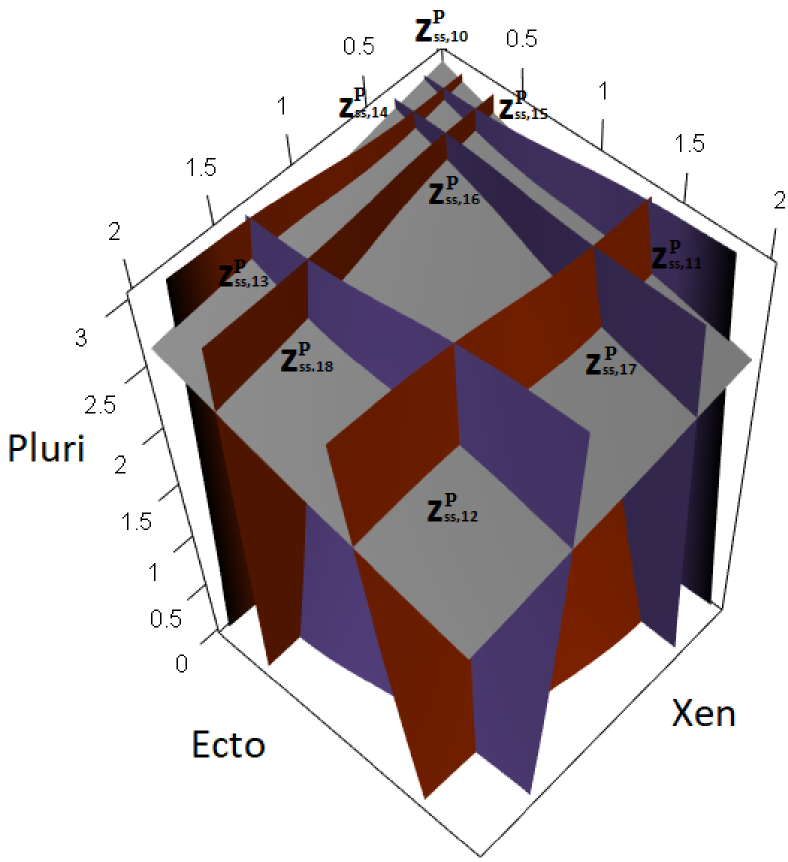


Figure 4.4: Here, one sees that the total number of steady states of the primitive scenario in (4.77), with P-component greater than zero, amounts to 9 : $z^P_{ss,10}$, $z^P_{ss,11}$, $z^P_{ss,12}$, $z^P_{ss,13}$, $z^P_{ss,14}$, $z^P_{ss,15}$, $z^P_{ss,16}$, $z^P_{ss,17}$, *and* $z^P_{ss,18}$.

and

$$e^{\tilde{P}_2}_{b,n} < e^{\tilde{P}_1}_{b,n}, \tag{4.82}$$

which, in turn, implies that the compact set $[0, x^{\tilde{P}}_{b,n}] \times [0, e^{\tilde{P}}_{b,n}]$ contracts as $P > 0$ increases. Therefore, recalling that Ψ_3 defined in (4.28) is strictly decreasing as a function of (X, E), one has that the condition (4.61) makes certain that the cardinality of $G_{\Psi^{\tilde{P}}_{1,n}} \cap G_{\Psi^{\tilde{P}}_{2,n}} \cap G_{\Psi^{\tilde{P}}_3}$ is maximal.

4.3 An intuitive description of the qualitative matrix of the model

But, how many blocks does this cube matrix have then? Although the author of this thesis is not able to answer the later question, it is possible that we can at least understand some features thereof. In fact, if we limit ourselves to the number of ways in which we can reformulate the condition imposed in (4.61) with respect to the total amount of steady states of the primitive scenario (4.77) then we might reason as follows.

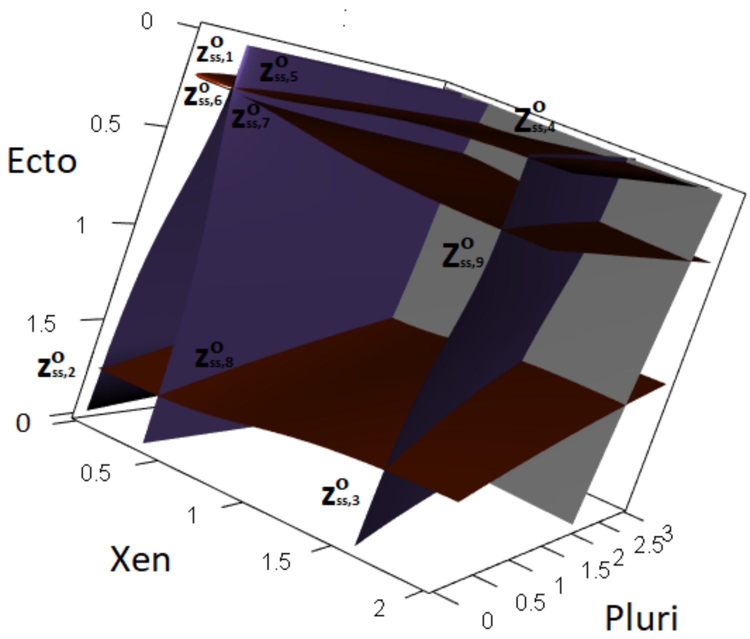


Figure 4.5: Here, one sees that the total number of steady states of the primitive scenario in (4.77) , with P-component equal to zero, amounts to 9 : $z^0_{ss,1}$, $z^0_{ss,2}$, $z^0_{ss,3}$, $z^0_{ss,4}$,$z^0_{ss,5}$,$z^0_{ss,6}$, $z^0_{ss,7}$, $z^0_{ss,8}$ *and* $z^0_{ss,9}$.

Indeed, as seen in Figure 4.4, if we acknowledge that the total amount of steady states of the primitive scenario (4.77), with P-component greater than zero, amounts to 9, then, given that Ψ_3 is strictly decreasing, one has that

$$2 \leq \# \bigcup_r SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{r,P}, \breve{C}_{1,n}, \breve{C_{2,n}}] \leq \sum_{m=0}^{9} \binom{9}{m} = 2^9, \tag{4.83}$$

with r indexing a reformulation of condition (4.61). So, for instance, $r = 0$ might index a hypothetical reformulation of condition (4.61) implying no intersection of the P-nullcline with the "potential steady states" with P-component greater than zero. Consistently, $r = 1$ must index the condition (4.61) itself, which, by construction, implies the maximal intersection between the X, E, P-nullclines (4.31), (4.32) and (4.33), with P-component greater than zero. Hence, one has that the number of blocks with respect to the first main component must be greater than 2, seeing that there is one block with the maximal number of steady states with P-component greater than zero, and there is another block with no steady state with P-component greater than zero.

On the other hand, $r = 2$ might be thought to represent a reformulation of the condition (4.61) that would result in

$$\breve{G}_{\Psi_{1,n}} \cap \breve{G}_{\Psi_{2,n}} \cap \breve{G}_{\Psi_3} = \{z^P_{ss,10}, z^P_{ss,14}\}, \tag{4.84}$$

while $r = 3$ would lead to a reformulation of (4.61), which, in turn, would imply that

$$\breve{G}_{\Psi_{1,n}} \cap \breve{G}_{\Psi_{2,n}} \cap \breve{G}_{\Psi_3} = \{z^P_{ss,10}, z^P_{ss,11}, z^P_{ss,12}\}, \tag{4.85}$$

and so on. Of course, by invoking (4.60), it can be the case that there are no parameter instances for which (4.53), (4.54), (4.55), and (4.56) hold and there exists a reformulation $\breve{C}_{r,P}$ of (4.61) resulting in

$$\breve{G}_{\Psi_{1,n}} \cap \breve{G}_{\Psi_{2,n}} \cap \breve{G}_{\Psi_3} = \{z^P_{ss,10}, z^P_{ss,12}\}, \tag{4.86}$$

which, in turn, strongly suggests that $\sum_{m=1}^{9} \binom{9}{m} = 2^9$ is a strict upper bound for the number of blocks in (4.87), that is,

$$2 \leq \# \bigcup_r SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{r,P}, \breve{C}_{1,n}, \breve{C_{2,n}}] < \sum_{m=0}^{9} \binom{9}{m} = 2^9. \tag{4.87}$$

As we have already asserted, the total number of steady states of the primitive scenario (4.77) equals 18 with respect to the cases (4.21) and (4.22) as shown in Figures 4.4 and 4.5. In the next section, we will give an argument for the determination of the (in)stability of the respective steady states.

4.4 Stability of the steady states

Recalling that $k_P := k(1 + cP)$ is the degradation rate at level $P \in \left[0, \frac{a_P}{k} - 1\right]$ and that $\mathbf{F}^{SH} := \left(\tilde{F}_1, \tilde{F}_2, \tilde{F}_3\right)$ represents the vector field of Semrau-Huang's model with

$$\begin{aligned}
\tilde{F}_1(X, E, P) &= a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1 + E^n} - kX(1 + cP), \\
\tilde{F}_2(X, E, P) &= a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1 + X^n} - kE(1 + cP), \\
\tilde{F}_3(X, E, P) &= a_P \frac{P}{1 + P} - kP[1 + c(E + dX)],
\end{aligned} \tag{4.88}$$

so if we denote the state vector by

$$\mathbf{S} = \begin{bmatrix} X \\ E \\ P \end{bmatrix}$$

then we have that

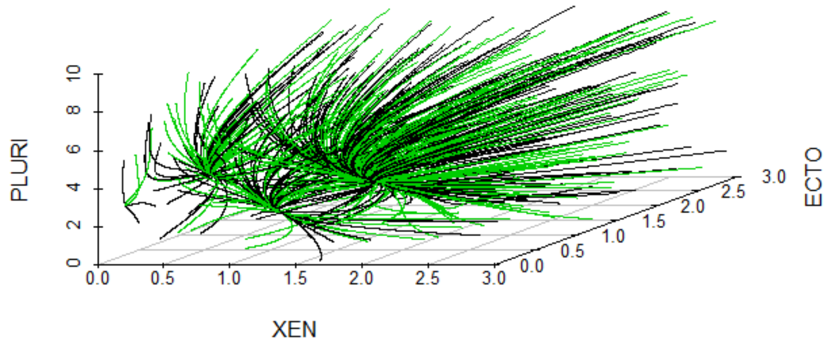
$$\frac{d\mathbf{S}}{dt} = \mathbf{F}^{SH}(\mathbf{S}) = \begin{bmatrix} \tilde{F}_1(\mathbf{S}) \\ \tilde{F}_2(\mathbf{S}) \\ \tilde{F}_3(\mathbf{S}) \end{bmatrix}$$

corresponds to the dynamical equations in (4.2). Further, if we define

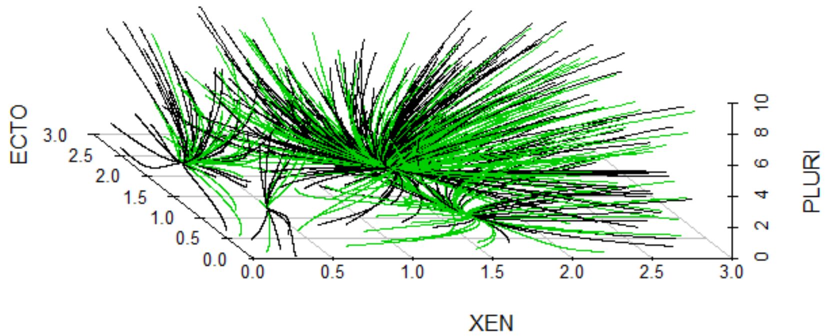
$$D\mathbf{F}^H_{k_P}(X, E) := \begin{bmatrix} D\tilde{F}_{11}(X, E, P) & D\tilde{F}_{12}(X, E, P) \\ D\tilde{F}_{21}(X, E, P) & D\tilde{F}_{22}(X, E, P) \end{bmatrix}$$

then we have that $D\mathbf{F}^H_{k_P}(X, E)$ stands for the Jacobian matrix of Huang's model with k replaced by k_P. If we define

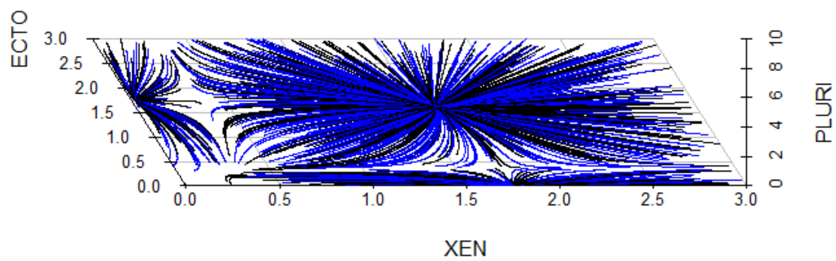
$$Z^0_{SH} := \left\{z^0_{ss,1}, z^0_{ss,2}, \ldots, z^0_{ss,8}, z^0_{ss,9}\right\}, \tag{4.89}$$



(a) Orbits strongly suggesting that the stable steady states of the primitive scenario $sc^{SH}_{\hat{\lambda}_0}$ *amount to* $z^P_{ss,10}$, $z^P_{ss,11}$, $z^P_{ss,12}$, *and* $z^P_{ss,13}$. *So, stable steady states on the* X, E*-plane with respect to Huang's model become unstable steady states with respect to Semrau-Huang's model.*



(b) Another perspective of the orbits going to the "potential stable steady states" $z^P_{ss,10}, z^P_{ss,11}, z^P_{ss,12}$, *and* $z^P_{ss,13}$.



(c) Orbits strongly suggesting a 2-dimensional stable manifold for each of the saddle steady states $z^0_{ss,1}$, $z^0_{ss,2}$, $z^0_{ss,3}$, *and* $z^0_{ss,4}$ *of Semrau-Huang's model, which, in turn, is consistent with the analysis of Huang's model, seeing that, in this case, the respective steady states are stable ones.*

Figure 4.6: Here, one sees the numerical simulation of the orbits of the primitive scenario $sc^{SH}_{\hat{\lambda}_0}$ *with* $\hat{\lambda}_0 = (a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 0.5, \theta_E = 0.5, b = 0.0811, c = 0.1, d = 0.5, k = 0.5, n = 4)$ *in 4 different perspectives.*

and

$$Z_{SH}^P := \left\{ z_{ss,10}^P, z_{ss,11}^P, \ldots, z_{ss,17}^P, z_{ss,18}^P \right\}, \tag{4.90}$$

then we have that

$$Z_{SH} := Z_{SH}^0 \cup Z_{SH}^P \tag{4.91}$$

consists of all the equilibria of a primitive scenario of Semrau-Huang's model.

So, what can we tell about the (in)stability of the steady states of Semrau-Huang's model at $P = 0$? Or equivalently, what happens to the (in)stability of the steady states of Huang's model with degradation rate $k_P = k$? In order to answer this question, we need to demonstrate the following proposition.

Proposition 4.4.1. *Let*

$$z^* = (X^*, E^*, 0) \in Z_{SH}^0$$

denote a hyperbolic steady state at $P = 0$ for any primitive scenario in $Prim_{sc}^{SH_n}$. Then it is true that:

1. *If $\frac{1}{c}\left(\frac{a_P}{k} - 1\right) < dX^* + E^*$ then (in)stability of $(X^*, E^*, 0)$ for Semrau-Huang's model is essentially the same as for Huang's model;*

2. *If $\frac{1}{c}\left(\frac{a_P}{k} - 1\right) > dX^* + E^*$ then $(X^*, E^*, 0)$ is a saddle or an unstable equilibrium for Semrau-Huang's model.*

Proof. To begin with, let $\mathbf{Id}$ denote the identity operator on $\mathbb{R}^3$. Next, one has that the Jacobian matrix of Semrau-Huang's model reads

$$D\mathbf{F}^{SH}(X^*, E^*, P^*) = \begin{bmatrix} D\tilde{F}_{11}(X^*, E^*, P^*) & D\tilde{F}_{12}(X^*, E^*, P^*) & D\tilde{F}_{13}(X^*, E^*, P^*) \\ D\tilde{F}_{21}(X^*, E^*, P^*) & D\tilde{F}_{22}(X^*, E^*, P^*) & D\tilde{F}_{23}(X^*, E^*, P^*) \\ D\tilde{F}_{31}(X^*, E^*, P^*) & D\tilde{F}_{32}(X^*, E^*, P^*) & D\tilde{F}_{33}(X^*, E^*, P^*) \end{bmatrix},$$

or better,

$$D\mathbf{F}^{SH}(X^*, E^*, P^*) = \begin{bmatrix} \begin{bmatrix} & \\ & D\mathbf{F}_{k_{P^*}}^{H}(X^*, E^*) \\ & \end{bmatrix} & \begin{matrix} -kcX^* \\ -kcE^* \end{matrix} \\ \begin{matrix} -kcdP^* & \quad -kcP^* \end{matrix} & a_P \frac{1}{(1+P^*)^2} - k[1 + c(E^* + dX^*)]. \end{bmatrix}$$

Thus, at $P = 0$, one has that

$$\begin{aligned} \text{Det}\left(D\mathbf{F}^{SH}(X^*, E^*, 0) - \lambda \mathbf{Id}\right) = \{a_P - k[1 + c(E^* + dX^*)] - \lambda\} \times \\ \text{Det}\left(D\mathbf{F}_{k_P}^{H}(X^*, E^*, 0) - \lambda \mathbf{Id}\right), \end{aligned} \tag{4.92}$$

which implies that the eigenvalues of $D\mathbf{F}^{SH}(X^*, E^*, 0)$ are $\lambda_0 := a_P - k[1 + c(E^* + dX^*)]$ and those of Huang's model, that is,

$$\lambda_{\pm} = \frac{(D\tilde{F}_{11} + D\tilde{F}_{22}) \pm \sqrt{(D\tilde{F}_{11} - D\tilde{F}_{22})^2 + 4D\tilde{F}_{12}D\tilde{F}_{21}}}{2}, \tag{4.93}$$

so if it is true that

$$\frac{1}{c}\left(\frac{a_P}{k}-1\right) < dX^* + E^* \tag{4.94}$$

then one has that $\lambda_0 < 0$, which, in turn, by invoking Theorems 3.4.3, 3.4.6, and 3.4.7, implies that the set $\{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}\}$ consists of stable steady states, whilst the set $\{z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,7}, z^0_{ss,8}, z^0_{ss,9}\}$ consists of saddle ones. So, under (4.94), (in)stability of $(X^*, E^*, 0)$ for Semrau-Huang's model is essentially as for Huang's model, except for $z^0_{ss,7}$ which, in this case, becomes a saddle equilibrium. On the other hand, if it is true that

$$\frac{1}{c}\left(\frac{a_P}{k}-1\right) > dX^* + E^* \tag{4.95}$$

then one has that $\lambda_0 > 0$, which, in turn, by invoking Theorems 3.4.3, 3.4.4, and 3.4.7, implies that the set $\{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}, z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,8}, z^0_{ss,9}\}$ consists of saddle equilibria, while $z^0_{ss,7}$ is an unstable equilibrium. So, under (4.95), (in)stability of $(X^*, E^*, 0)$ for Semrau-Huang's model is in general not the same as for Huang's model, except for $z^0_{ss,7}$ which stays unstable. That completes the proof. □

Hence, according to Proposition 4.4.1, the (in)stability of the steady states

$$\{z^0_{ss,1}, z^0_{ss,2}, z^0_{ss,3}, z^0_{ss,4}, z^0_{ss,5}, z^0_{ss,6}, z^0_{ss,7}, z^0_{ss,8}, z^0_{ss,9}\}$$

of Semrau-Huang's model is not necessarily the same as in Huang's model, as shown in the Figure 4.6. As we see in the respective simulation, as regards the primitive scenario

$$SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{1,n}, \breve{C}_{2,n}],$$

each steady state at level $P = 0$ has an unstable manifold emanating from the XE-plane with the vector field tangent thereto and pointing outwards from the XE-plane.

But, what about the steady states of Semrau-Huang's model at a level $P > 0$? Or equivalently, can we say something about the (in)stability of the equilibria in Z^P_{SH}?

In order to formulate an answer for this question, we will draw upon Routh-Hurwith's Theorem, see [76, p. 10-22], which stipulates necessary and sufficient conditions for the linear stability of an equilibrium of a dynamical system. Regarding the respective conditions, we will follow the approach presented in [64, p. 507-509].

Theorem 4.4.2 (Routh-Hurwith conditions). *Let $n = 2N-1$ with $N \in \mathbb{N} \setminus \{0\}$, $\{a_1, a_2, \ldots, a_{n-1}, a_n\} \subset \mathbb{R}$ with $a_n \neq 0$, and $\{\lambda_1, \lambda_2, \ldots, \lambda_{n-1}, \lambda_n\}$ be the solutions of the polynomial*

$$p(\lambda) = \lambda^n + a_1\lambda^{n-1} + a_2\lambda^{n-2} + \ldots + a_{n-1}\lambda + a_n.$$

Then, one has that

$$\bigwedge_{i\in\{1,2,\ldots,n\}} \Re\lambda_i < 0$$

if and only if

$$a_n > 0,$$

and

$$D_1 = a_1 > 0,$$

and

$$D_2 = \text{Det}\left(\begin{bmatrix} a_1 & a_3 \\ 1 & a_2 \end{bmatrix}\right) = a_1 a_2 - a_3 > 0$$

and

$$D_3 = \text{Det}\left(\begin{bmatrix} a_1 & a_3 & a_5 \\ 1 & a_2 & a_4 \\ 0 & a_1 & a_3 \end{bmatrix}\right) = a_1 a_2 a_3 + a_1 a_5 - a_1^2 a_4 - a_3^2 > 0$$

and

$$D_k = \text{Det}\, H_k = \sum_{\sigma \in S_k} \left(\text{sgn}(\sigma) \prod_{i=1}^{N} H^{(k)}_{i,\sigma(i)} \right) > 0$$

with

$$H_k := \begin{bmatrix} a_1 & a_3 & a_5 & \dots & \vdots \\ 1 & a_2 & a_4 & \dots & \vdots \\ 0 & a_1 & a_3 & \dots & \vdots \\ 0 & 1 & a_2 & \dots & \vdots \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \dots & a_k \end{bmatrix}$$

representing the k-nth Routh-Hurwith matrix for which $(H_k)_{i,j} = H^{(k)}_{i,j}$ *for all* $k = 1, 2, 3, \dots, n-1$. *with* $(H_k)_{i,j} = H^{(k)}_{i,j}$. *Moreover,* S_k *denotes the set of all* σ *permutations of the set* $k = \{1, 2, 3, \dots, n-1\}$.

Proof. For an elementary proof, See [35]. □

Hence, for a cubic polynomial

$$p(\lambda) = \lambda^3 + a_1 \lambda^2 + a_2 \lambda^3 + a_3$$

one has that the Routh-Hurwith conditions reads

$$\bigwedge_{i \in \{1,2,3\}} \Re\lambda_i < 0 \Leftrightarrow ((a_3 > 0) \wedge (a_1 > 0) \wedge (a_1 a_2 - a_3 > 0)) \,. \tag{4.96}$$

So, if we want to apply the respective conditions then we need to derive the characteristic polynomial of the Jacobian matrix $D\mathbf{F}^{SH}$ of Semrau-Huang's model. In fact, one has that

$$\begin{aligned}p^{SH}(\lambda) = \text{Det}\left(D\mathbf{F}^{SH} - \lambda\mathbf{Id}\right) &= \text{Det}\left(\begin{bmatrix} D\tilde{F}_{11}-\lambda & D\tilde{F}_{12} & D\tilde{F}_{13} \\ D\tilde{F}_{21} & D\tilde{F}_{22}-\lambda & D\tilde{F}_{23} \\ D\tilde{F}_{31} & D\tilde{F}_{32} & D\tilde{F}_{33}-\lambda \end{bmatrix}\right) \\ &= \left(D\tilde{F}_{33}-\lambda\right)\text{Det}\left(\begin{bmatrix} D\tilde{F}_{11}-\lambda & D\tilde{F}_{12} \\ D\tilde{F}_{21} & D\tilde{F}_{22}-\lambda \end{bmatrix}\right) \\ &\quad -D\tilde{F}_{32}\,\text{Det}\left(\begin{bmatrix} D\tilde{F}_{11}-\lambda & D\tilde{F}_{13} \\ D\tilde{F}_{22}-\lambda & D\tilde{F}_{23} \end{bmatrix}\right) \\ &\quad +D\tilde{F}_{31}\,\text{Det}\left(\begin{bmatrix} D\tilde{F}_{12} & D\tilde{F}_{13} \\ D\tilde{F}_{22}-\lambda & D\tilde{F}_{23} \end{bmatrix}\right),\end{aligned} \tag{4.97}$$

which implies that

$$p^{SH}(\lambda) = \lambda^3 + a_1^{SH}\lambda^2 + a_2^{SH}\lambda + a_3^{SH}, \tag{4.98}$$

wherein, by recalling (4.91), for $z^* = (X^*, E^*, P^*) \in Z_{SH}$, one has that

$$a_1^{SH}(z^*) = -\left(D\tilde{F}_{33}(z^*) + \text{Tr}\, DF_{k_P}^{H}(z^*)\right), \tag{4.99}$$

and that

$$\begin{aligned}a_2^{SH}(z^*) = \text{Det}\, DF_{k_P}^{H}(z^*) + D\tilde{F}_{33}(z^*)\,\text{Tr}\, DF_{k_P}^{H}(z^*) \\ +D\tilde{F}_{32}(z^*)D\tilde{F}_{23}(z^*) + D\tilde{F}_{13}(z^*)D\tilde{F}_{31}(z^*),\end{aligned} \tag{4.100}$$

and that

$$\begin{aligned}a_3^{SH} = -D\tilde{F}_{33}(z^*)\,\text{Det}\, DF_{k_P}^{H}(z^*) + D\tilde{F}_{11}(z^*)D\tilde{F}_{32}(z^*)D\tilde{F}_{23}(z^*) \\ -D\tilde{F}_{21}(z^*)D\tilde{F}_{32}(z^*)D\tilde{F}_{13}(z^*) - D\tilde{F}_{12}(z^*)D\tilde{F}_{31}(z^*)D\tilde{F}_{23}(z^*) \\ +D\tilde{F}_{22}(z^*)D\tilde{F}_{31}(z^*)D\tilde{F}_{13}(z^*),\end{aligned} \tag{4.101}$$

with

$$\begin{aligned}D\tilde{F}_{11}(X^*, E^*, P^*) &= -\frac{d}{dX} g_{1,n}^{P^*}(X^*), \\ D\tilde{F}_{12}(X^*, E^*, P^*) &= -b\frac{n(E^*)^{n-1}}{[1+(E^*)^n]^2}, \\ D\tilde{F}_{13}(X^*, E^*, P^*) &= -kcX^*, \\ D\tilde{F}_{21}(X^*, E^*, P^*) &= -b\frac{n(X^*)^{n-1}}{[1+(X^*)^n]^2}, \\ D\tilde{F}_{22}(X^*, E^*, P^*) &= -\frac{d}{dE} g_{2,n}^{P^*}(E^*), \\ D\tilde{F}_{23}(X^*, E^*, P^*) &= -kcE^*, \\ D\tilde{F}_{31}(X^*, E^*, P^*) &= -kcdP^*, \\ D\tilde{F}_{32}(X^*, E^*, P^*) &= -kcP^*, \\ D\tilde{F}_{33}(X^*, E^*, P^*) &= a_P\frac{1}{(1+P^*)^2} - k[1 + c(E^* + dX^*)].\end{aligned} \tag{4.102}$$

In order to have a clue as to the (in)stability of a steady state $z^* = (X^*, E^*, P^*) \in Z^P_{SH}$, we will either make assumptions about the order of the components X^*, E^*, and P^* or about the order of "key parameters" so as to arrive at suitable approximations. That approach , by means of formal logic, will enable us to access the stability of the steady states in $\{z^P_{ss,10}, z^P_{ss,11}, z^P_{ss,12}, z^P_{ss,13}\}$ by invoking the Routh-Hurwith Theorem 4.4.2.

Claim 1: $a_1^{SH}\left(z^P_{ss,10}\right) > 0$

By invoking Theorem 3.4.6, one has that

$$\mathrm{Tr}\, DF^H_{k_P}(z^P_{ss,10}) < 0. \tag{4.103}$$

Moreover, given that

$$\Psi^{P,-1}_{2,n}\left(\frac{a_X}{k_P} + \frac{b}{k_P}\right) > \frac{b}{k_P}\frac{1}{\left[1 + \left(\frac{a_X}{k_P} + \frac{b}{k_P}\right)^n\right]}, \tag{4.104}$$

if we draw on the formula for the inflection point in (3.170), then one has that

$$\frac{b}{k_P}\frac{1}{\left[1 + \left(\frac{a_X}{k_P} + \frac{b}{k_P}\right)^n\right]} < E^{*,P}_{ss,10} < \theta_X^n\left(\frac{n-1}{n+1}\right)^{1/n} < \theta_X, \tag{4.105}$$

and that

$$\frac{b}{k_P}\frac{1}{\left[1 + \left(\frac{a_E}{k_P} + \frac{b}{k_P}\right)^n\right]} < X^{*,P}_{ss,10} < \theta_E^n\left(\frac{n-1}{n+1}\right)^{1/n} < \theta_E. \tag{4.106}$$

But, we know that

$$\frac{b}{k_P}\frac{1}{\left[1 + \left(\frac{a_X}{k_P} + \frac{b}{k_P}\right)^n\right]} < 1, \tag{4.107}$$

and that

$$\frac{b}{k_P}\frac{1}{\left[1 + \left(\frac{a_E}{k_P} + \frac{b}{k_P}\right)^n\right]} < 1, \tag{4.108}$$

so if $\max\{\theta_X, \theta_E\} < 1$ then we have that

$$X^{*,P}_{ss,10} \approx O(10^{-N}), \tag{4.109}$$

and

$$X^{*,P}_{ss,10} \approx O(10^{-N}), \tag{4.110}$$

with $N > 2$, which implies that we can make the approximation

$$k\left[1 + c\left(E^{*,P}_{ss,10} + dX^{*,P}_{ss,10}\right)\right] \approx k,, \tag{4.111}$$

which, in turn, implies that

$$\begin{aligned} D\tilde{F}_{33}(X^{*,P}_{ss,10}, E^{*,P}_{ss,10}, P^*_{ss,10}) &= a_P\frac{1}{(1 + P^*_{ss,10})^2} - k[1 + c(E^* + dX^*)] \\ &\approx a_P\frac{1}{(1 + P^*_{ss,10})^2} - k. \end{aligned} \tag{4.112}$$

Now, in view of (4.109) and (4.110), one has that

$$P^*_{ss,10} \approx \frac{a_P}{k} - 1. \tag{4.113}$$

On one hand, by invoking (4.61), one has that

$$\frac{a_P}{k} - 1 > 0, \tag{4.114}$$

if and only if

$$k < a_P, \tag{4.115}$$

if and only if

$$\frac{k^2}{a_P} < k. \tag{4.116}$$

On the other hand,

$$P^*_{ss,10} = \frac{a_P}{k} - 1 \tag{4.117}$$

if and only if

$$(1 + P^*_{ss,10})^2 = \left(\frac{a_P}{k}\right)^2 \tag{4.118}$$

if and only if

$$\frac{k^2}{a_P} = \frac{a_P}{(1 + P^*_{ss,10})^2}, \tag{4.119}$$

which, by building upon (4.116), implies that

$$\frac{a_P}{(P^*_{ss,10} + 1)^2} < k, \tag{4.120}$$

which, in turn, implies that

$$D\tilde{F}_{33}(X^{*,P}_{ss,10}, E^{*,P}_{ss,10}, P^*_{ss,10}) = \frac{a_P}{(1 + P^*_{ss,10})^2} - k < 0, \tag{4.121}$$

and the argument has been raised.

Claim 2: $a_3^{SH}\left(z^P_{ss,10}\right) > 0$

In view of In view of (4.111), in which $E^{*,P}_{ss,10}$ and $X^{*,P}_{ss,10}$ are thought to be sufficiently small, one has that

$$\begin{aligned} a_3^{SH}(z^P_{ss,10}) = -D\tilde{F}_{33}(z^P_{ss,10}) \operatorname{Det} DF^H_{k_P}(z^P_{ss,10}) + D\tilde{F}_{11}(z^P_{ss,10}) D\tilde{F}_{32}(z^P_{ss,10}) D\tilde{F}_{23}(z^P_{ss,10}) \\ -D\tilde{F}_{21}(z^P_{ss,10}) D\tilde{F}_{32}(z^P_{ss,10}) D\tilde{F}_{13}(z^P_{ss,10}) - D\tilde{F}_{12}(z^P_{ss,10}) D\tilde{F}_{31}(z^P_{ss,10}) D\tilde{F}_{23}(z^P_{ss,10}) \\ +D\tilde{F}_{22}(z^P_{ss,10}) D\tilde{F}_{31}(z^P_{ss,10}) D\tilde{F}_{13}(z^P_{ss,10}) \\ \approx -D\tilde{F}_{33}(z^P_{ss,10}) \operatorname{Det} DF^H_{k_P}(z^P_{ss,10}) \\ = -D\tilde{F}_{33}(z^P_{ss,10}) D\tilde{F}_{11}(z^P_{ss,10}) D\tilde{F}_{22}(z^P_{ss,10}). \end{aligned} \tag{4.122}$$

Now, consistent with Theorem 3.4.6 and with the assumption that $E^{*,P}_{ss,10}$ and $X^{*,P}_{ss,10}$ are both sufficiently small, one can make the approximation

$$D\tilde{F}_{11}(z^P_{ss,10})D\tilde{F}_{22}(z^P_{ss,10}) \approx k_P^2 > 0, \tag{4.123}$$

with $k_P = k\left(1 + cP^*_{ss,10}\right)$, which, in turn, by consistently invoking (4.121), implies that

$$a_3^{SH}(z^P_{ss,10}) > 0, \tag{4.124}$$

and the argument has been raised.

Claim 3: $a_1^{SH}\left(z^P_{ss,10}\right)a_2^{SH}\left(z^P_{ss,10}\right) - a_3^{SH}\left(z^P_{ss,10}\right) > 0$

If we consistently draw upon (4.109), (4.110), and (4.123) then we arrive at the approximation

$$\begin{aligned} a_2^{SH}(z^P_{ss,10}) &= \mathrm{Det}\, DF^H_{k_P}(z^P_{ss,10}) + D\tilde{F}_{33}(z^P_{ss,10})\,\mathrm{Tr}\, DF^H_{k_P}(z^P_{ss,10}) \\ &+ D\tilde{F}_{32}(z^P_{ss,10})D\tilde{F}_{23}(z^P_{ss,10}) + D\tilde{F}_{13}(z^P_{ss,10})D\tilde{F}_{31}(z^P_{ss,10}), \\ &\approx D\tilde{F}_{11}(z^P_{ss,10})D\tilde{F}_{22}(z^P_{ss,10}) + D\tilde{F}_{33}(z^P_{ss,10})\,\mathrm{Tr}\, DF^H_{k_P}(z^P_{ss,10}), \end{aligned} \tag{4.125}$$

which, by invoking (4.276) and (4.122), implies that

$$\begin{aligned} a_1^{SH}\left(z^P_{ss,10}\right)a_2^{SH}\left(z^P_{ss,10}\right) - a_3^{SH}\left(z^P_{ss,10}\right) \approx -\left(D\tilde{F}_{33}(z^P_{ss,10})\right)^2 \mathrm{Tr}\, DF^H_{k_P}(z^P_{ss,10}) \\ -D\tilde{F}_{11}(z^P_{ss,10})D\tilde{F}_{22}(z^P_{ss,10})\,\mathrm{Tr}\, DF^H_{k_P}(z^P_{ss,10}) - D\tilde{F}_{33}(z^P_{ss,10})\left(\mathrm{Tr}\, DF^H_{k_P}(z^P_{ss,10})\right)^2, \end{aligned} \tag{4.126}$$

and if we now draw on (4.121), (4.103), and (4.123) then we conclude that

$$a_1^{SH}\left(z^P_{ss,10}\right)a_2^{SH}\left(z^P_{ss,10}\right) - a_3^{SH}\left(z^P_{ss,10}\right) > 0, \tag{4.127}$$

and the argument has been raised. Therefore, by invoking the Routh-Hurwith Theorem 4.4.2, we can assert that $z^P_{ss,10}$ is possibly stable.

Claim 4: $a_1^{SH}\left(z^P_{ss,13}\right) > 0$

By invoking Theorem 3.4.6, one has that

$$\mathrm{Tr}\, DF^H_{k_P}(z^P_{ss,13}) < 0. \tag{4.128}$$

Now, seeing that

$$\Psi^{P,-1}_{2,n}\left(\frac{a_X}{k_P} + \frac{b}{k_P}\right) > \frac{b}{k_P}\frac{1}{\left[1 + \left(\frac{a_X}{k_P} + \frac{b}{k_P}\right)^n\right]}, \tag{4.129}$$

and that $\theta_X < \frac{a_X}{k_P} + \frac{b}{k_P}$, one has that

$$\frac{b}{k_P}\frac{1}{\left[1 + \left(\frac{a_X}{k_P} + \frac{b}{k_P}\right)^n\right]} < E^{*,P}_{ss,13} < \Psi^{P,-1}_{2,n}(\theta_X) < \theta_X^n\left(\frac{n-1}{n+1}\right)^{1/n} < \theta_X. \tag{4.130}$$

But, by construction, taking a smaller d favours the existence of $z^P_{ss,13}$, so consistent with (4.111), one can assume that

$$k\left[1 + c\left(E^{*,P}_{ss,13} + dX^{*,P}_{ss,13}\right)\right] \approx k, \tag{4.131}$$

which implies that

$$D\tilde{F}_{33}(X^{*,P}_{ss,13}, E^{*,P}_{ss,13}, P^{*}_{ss,13}) = a_P\frac{1}{(1+P^{*}_{ss,13})^2} - k[1 + c(E^{*,P}_{ss,13} + dX^{*,P}_{ss,13})] \approx a_P\frac{1}{(1+P^{*}_{ss,13})^2} - k \tag{4.132}$$

which, in turn, by invoking (4.121) and assuming that

$$\frac{P^{*}_{ss,13}}{P^{*}_{ss,10}} = O(1),$$

implies that

$$D\tilde{F}_{33}(X^{*,P}_{ss,13}, E^{*,P}_{ss,13}, P^{*}_{ss,13}) = \frac{a_P}{(1+P^{*}_{ss,13})^2} - k < 0, \tag{4.133}$$

and the argument has been raised.

Claim 5: $a^{SH}_3\left(z^P_{ss,13}\right) > 0$

By invoking (4.131), in which $E^{*,P}_{ss,13}$ and $d > 0$ are thought to be sufficiently small, consistent with Theorem 3.4.6, one can make the approximation

$$\text{Det}\, DF^{H}_{k_P}(z^P_{ss,13}) \approx D\tilde{F}_{11}(z^P_{ss,13})D\tilde{F}_{22}(z^P_{ss,13}) > 0, \tag{4.134}$$

with $k_P = k\left(1 + cP^{*}_{ss,13}\right)$, which, in turn, by consistently invoking (4.133), implies that

$$\begin{aligned} a^{SH}_3(z^P_{ss,13}) &= -D\tilde{F}_{33}(z^P_{ss,13})\,\text{Det}\, DF^{H}_{k_P}(z^P_{ss,13}) \\ &+D\tilde{F}_{11}(z^P_{ss,13})D\tilde{F}_{32}(z^P_{ss,13})D\tilde{F}_{23}(z^P_{ss,13}) - D\tilde{F}_{21}(z^P_{ss,13})D\tilde{F}_{32}(z^P_{ss,13})D\tilde{F}_{13}(z^P_{ss,13}) \\ &-D\tilde{F}_{12}(z^P_{ss,13})D\tilde{F}_{31}(z^P_{ss,13})D\tilde{F}_{23}(z^P_{ss,13}) + D\tilde{F}_{22}(z^P_{ss,13})D\tilde{F}_{31}(z^P_{ss,13})D\tilde{F}_{13}(z^P_{ss,13}) \\ &\approx -D\tilde{F}_{33}(z^P_{ss,13})D\tilde{F}_{11}(z^P_{ss,13})D\tilde{F}_{22}(z^P_{ss,13}) - D\tilde{F}_{21}(z^P_{ss,13})D\tilde{F}_{32}(z^P_{ss,13})D\tilde{F}_{13}(z^P_{ss,13}) > 0, \end{aligned} \tag{4.135}$$

or better,

$$a^{SH}_3(z^P_{ss,13}) > 0, \tag{4.136}$$

and the argument has been raised.

Claim 6: $a^{SH}_1\left(z^P_{ss,13}\right) a^{SH}_2\left(z^P_{ss,13}\right) - a^{SH}_3\left(z^P_{ss,13}\right) > 0$

Consistently, by building on (4.131), in which $E^{*,P}_{ss,13} > 0$ and $d > 0$ are thought to be sufficiently small, one has that

$$\begin{aligned} a^{SH}_2(z^P_{ss,13}) &= \text{Det}\, DF^{H}_{k_P}(z^P_{ss,13}) + D\tilde{F}_{33}(z^P_{ss,13})\,\text{Tr}\, DF^{H}_{k_P}(z^P_{ss,13}) \\ &+D\tilde{F}_{32}(z^P_{ss,13})D\tilde{F}_{23}(z^P_{ss,13}) + D\tilde{F}_{13}(z^P_{ss,13})D\tilde{F}_{31}(z^P_{ss,13}), \\ &\approx D\tilde{F}_{11}(z^P_{ss,13})D\tilde{F}_{22}(z^P_{ss,13}) + D\tilde{F}_{33}(z^P_{ss,13})\,\text{Tr}\, DF^{H}_{k_P}(z^P_{ss,13}), \end{aligned} \tag{4.137}$$

which, by invoking (4.135), implies that

$$\begin{aligned} a^{SH}_1\left(z^P_{ss,13}\right) a^{SH}_2\left(z^P_{ss,13}\right) - a^{SH}_3\left(z^P_{ss,13}\right) &\approx -\left(D\tilde{F}_{33}(z^P_{ss,13})\right)^2 \text{Tr}\, DF^{H}_{k_P}(z^P_{ss,13}) \\ -D\tilde{F}_{11}(z^P_{ss,13})D\tilde{F}_{22}(z^P_{ss,13})\,\text{Tr}\, DF^{H}_{k_P}(z^P_{ss,13}) &- D\tilde{F}_{33}(z^P_{ss,13})\left(\text{Tr}\, DF^{H}_{k_P}(z^P_{ss,13})\right)^2 \\ &+D\tilde{F}_{21}(z^P_{ss,13})D\tilde{F}_{32}(z^P_{ss,13})D\tilde{F}_{13}(z^P_{ss,13}). \end{aligned} \tag{4.138}$$

So, if we draw on (4.128), (4.134), and (4.133) then we have that

$$-\left(D\tilde{F}_{33}(z^P_{ss,13})\right)^2 \operatorname{Tr} DF^H_{k_P}(z^P_{ss,13})$$
$$-D\tilde{F}_{11}(z^P_{ss,13})D\tilde{F}_{22}(z^P_{ss,13}) \operatorname{Tr} DF^H_{k_P}(z^P_{ss,13}) - D\tilde{F}_{33}(z^P_{ss,13}) \left(\operatorname{Tr} DF^H_{k_P}(z^P_{ss,13})\right)^2 > 0. \tag{4.139}$$

However, one has that the last term in (4.178), that is,

$$D\tilde{F}_{21}(z^P_{ss,13})D\tilde{F}_{32}(z^P_{ss,13})D\tilde{F}_{13}(z^P_{ss,13}) = -b\frac{n(X^{*,P}_{ss,13})^n}{\left[1+(X^{*,P}_{ss,13})^n\right]^2}k^2c^2P^*_{ss,13} < 0 \tag{4.140}$$

might be a problem to prove *Claim* 6. In fact, if $n > 2$ is sufficiently high then *Claim* 6 is possibly not valid. However, if we invoke the analysis of Huang's model performed in Chapter 3, one has that the parameter b can be chosen as close as possible to zero without changing the other representatives of the primitive scenario

$$SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C_{2,n}}],$$

and one would still have a representative for the respective primitive scenario with the new choice for $b > 0$ replacing the old one. Therefore, we can assume that $b > 0$ is compatible with

$$a_1^{SH}\left(z^P_{ss,13}\right) a_2^{SH}\left(z^P_{ss,13}\right) - a_3^{SH}\left(z^P_{ss,10}\right) > 0, \tag{4.141}$$

and the argument has been raised. Therefore, by invoking the Routh-Hurwith Theorem 4.4.2, we can assert that $z^P_{ss,13}$ is possibly stable. Nonetheless, as we have just seen in the argument for the Claim 6, under $d > 0$ sufficiently small, it can be the true that this stability can change for $n > 2$ sufficiently high in a scenario wherein b is highly constrained. In fact, as we will see in section 4.7, under $d > 0$ sufficiently small and $n > 2$ sufficiently high, one has that the stable equilibrium $z^P_{ss,13}$ will give rise to a *stable limit cycle* in the scenario

$$SH_n\left[\breve{C}_{3,X}, \breve{C}_{3,E}, \breve{C}_{-1,P}\right]$$

with

$$\breve{C}_{-1,P} : 0 < \frac{1}{c}\left(\frac{a_P}{k} - 1\right) < e^{(0)}_{b,n} + dx^{(0)}_{b,n}$$

as it will be clarified in Section 4.7.

<u>*Claim 7*</u>: $a_1^{SH}\left(z^P_{ss,11}\right) > 0$

By invoking Theorem 3.4.6, one has that

$$\operatorname{Tr} DF^H_{k_P}(z^P_{ss,11}) < 0. \tag{4.142}$$

Now, given that

$$\Psi^{P,-1}_{1,n}\left(\frac{a_E}{k_P} + \frac{b}{k_P}\right) > \frac{b}{k_P}\frac{1}{\left[1+\left(\frac{a_E}{k_P}+\frac{b}{k_P}\right)^n\right]}, \tag{4.143}$$

and that $\theta_E < \frac{a_E}{k_P} + \frac{b}{k_P}$, one has that

$$\frac{b}{k_P}\frac{1}{\left[1+\left(\frac{a_E}{k_P}+\frac{b}{k_P}\right)^n\right]} < X^{*,P}_{ss,13} < \Psi^{P,-1}_{1,n}\left(\theta_E\right) < \theta_E^n\left(\frac{n-1}{n+1}\right)^{1/n} < \theta_E, \tag{4.144}$$

so, consistent with $d > 0$ being sufficiently small in (4.131), one can assume that

$$k[1+c(E^{*,P}_{ss,11}+dX^{*,P}_{ss,11})] \approx k(1+cE^{*,P}_{ss,11}), \tag{4.145}$$

which implies that

$$\begin{aligned} D\tilde{F}_{33}(X^{*,P}_{ss,11}, E^{*,P}_{ss,11}, P^{*}_{ss,11}) &= \frac{a_P}{(1+P^{*}_{ss,11})^2} - k[1+c(E^{*,P}_{ss,11}+dX^{*,P}_{ss,11})] \\ &\approx \frac{a_P}{(1+P^{*}_{ss,11})^2} - k(1+cE^{*,P}_{ss,11}). \end{aligned} \tag{4.146}$$

So, if we now rely upon the symmetry of Huang's model then one can assume that

$$\frac{P_{ss,11}}{P_{ss,10}} = O(1),$$

which, in turn, by invoking (4.121), implies that

$$D\tilde{F}_{33}(X^{*,P}_{ss,11}, E^{*,P}_{ss,11}, P^{*}_{ss,11}) = \frac{a_P}{(1+P^{*}_{ss,11})^2} - k(1+cE^{*,P}_{ss,11}) < 0, \tag{4.147}$$

and the argument has been raised.

<u>*Claim 8*</u>: $a_3^{SH}\left(z^P_{ss,11}\right) > 0$

By invoking (4.145), in which $X^{*,P}_{ss,11}$ and $d > 0$ are thought to be sufficiently small, consistent with Theorem 3.4.6, one can make the approximation

$$\text{Det}\, DF^H_{k_P}(z^P_{ss,11}) \approx D\tilde{F}_{11}(z^P_{ss,11})D\tilde{F}_{22}(z^P_{ss,11}) > 0, \tag{4.148}$$

with $k_P = k\left(1+cP^{*}_{ss,11}\right)$, which, in turn, implies that

$$\begin{aligned} a_3^{SH}(z^P_{ss,11}) &= -D\tilde{F}_{33}(z^P_{ss,11})\,\text{Det}\, DF^H_{k_P}(z^P_{ss,11}) \\ &+D\tilde{F}_{11}(z^P_{ss,11})D\tilde{F}_{32}(z^P_{ss,11})D\tilde{F}_{23}(z^P_{ss,11}) - D\tilde{F}_{21}(z^P_{ss,11})D\tilde{F}_{32}(z^P_{ss,11})D\tilde{F}_{13}(z^P_{ss,11}) \\ &-D\tilde{F}_{12}(z^P_{ss,11})D\tilde{F}_{31}(z^P_{ss,11})D\tilde{F}_{23}(z^P_{ss,11}) + D\tilde{F}_{22}(z^P_{ss,11})D\tilde{F}_{31}(z^P_{ss,11})D\tilde{F}_{13}(z^P_{ss,11}) \\ &\approx -D\tilde{F}_{33}(z^P_{ss,11})D\tilde{F}_{11}(z^P_{ss,11})D\tilde{F}_{22}(z^P_{ss,11}) + D\tilde{F}_{11}(z^P_{ss,11})D\tilde{F}_{32}(z^P_{ss,11})D\tilde{F}_{23}(z^P_{ss,11}). \end{aligned} \tag{4.149}$$

Next, if $X^{*,P}_{ss,11}$ is sufficiently small then

$$D\tilde{F}_{11}(z^P_{ss,11}) \approx -k_P. \tag{4.150}$$

So, recalling that $\tilde{k} := k\left[1+c\left(\frac{a_P}{k}-1\right)\right]$, if we invoke (4.54), that is,

$$\theta_E < \frac{a_E}{2\tilde{k}}$$

and if we assume that $E^{*,P}_{ss,11} \approx \frac{a_E}{k_P}$ then we can make the approximation

$$\begin{aligned} D\tilde{F}_{22}(z^P_{ss,11}) &\approx -\left\{ k_P - na_E \frac{\theta_E^n}{\left(\frac{a_E}{k_P}\right)^{n+1}} \left[\frac{\left(\frac{a_E}{k_P}\right)^n}{\left(\theta_E^n + \left(\frac{a_E}{k_P}\right)^n\right)} \right]^2 \right\} \\ &= -\left\{ k_P - na_E \left(\frac{\theta_E}{\frac{a_E}{k_P}}\right)^n \frac{k_P}{a_E} \left[\frac{\left(\frac{a_E}{k_P}\right)^n}{\left(\theta_E^n + \left(\frac{a_E}{k_P}\right)^n\right)} \right]^2 \right\} \\ &\approx -\left[k_P - na_E \left(\frac{1}{2\tilde{k}}\right)^n \frac{k_P}{a_E}\frac{1}{4}\right], \\ &\approx -\left(k_P - n\frac{1}{2^{n+2}}\frac{k_P}{\tilde{k}^n}\right), \end{aligned} \tag{4.151}$$

with $k_P = k(1 + cP^*_{ss,11})$, which, in turn, seeing that

$$\frac{k_P}{\tilde{k}^n} < 1,$$

becomes

$$D\tilde{F}_{22}(z^P_{ss,11}) \approx -\left(k_P - \frac{n}{2^{n+2}}\right), \tag{4.152}$$

and, by using (4.150) and by recalling (4.148), we have that

$$D\tilde{F}_{11}(z^P_{ss,11})D\tilde{F}_{22}(z^P_{ss,11}) \approx k_P^2 - k_P\frac{n}{2^{n+2}} > 0, \tag{4.153}$$

which, by drawing on the inequality

$$n < 2^{n+2}$$

with $n \geq 2$, implies that

$$k_P = k(1 + cP^*_{ss,11}) > \frac{n}{2^{n+2}}, \tag{4.154}$$

or rather,

$$k_P > \frac{1}{8}. \tag{4.155}$$

So, under $X^{*,P}_{ss,11}$ and $d > 0$ sufficiently small, if we draw upon the approximation (4.153) then we arrive at

$$a_3^{SH}(z^P_{ss,11}) \approx \left[k(1 + cE^{*,P}_{ss,11}) - \frac{a_P}{(1 + P^*_{ss,11})^2}\right]\left(k_P^2 - k_P\frac{n}{2^{n+2}}\right) - k_P k^2 c^2 P^*_{ss,11} E^*_{ss,11}. \tag{4.156}$$

But, one has that

$$\begin{aligned} a_3^{SH}(z^P_{ss,11}) \approx \left[k(1 + cE^{*,P}_{ss,11}) - \frac{a_P}{(1 + P^*_{ss,11})^2}\right]\left(k_P^2 - k_P\frac{n}{2^{n+2}}\right) \\ -k_P k^2 c^2 P^*_{ss,11} E^*_{ss,11} \leq 0 \end{aligned} \tag{4.157}$$

if and only if

$$\left[k(1+cE^{*,P}_{ss,11})-\frac{a_P}{(1+P^*_{ss,11})^2}\right]\left(k_P^2-k_P\frac{n}{2^{n+2}}\right)\leq k_Pk^2c^2P^*_{ss,11}E^*_{ss,11}, \tag{4.158}$$

if and only if

$$\frac{1}{P^*_{ss,11}E^*_{ss,11}}\left[k(1+cE^{*,P}_{ss,11})-\frac{a_P}{(1+P^*_{ss,11})^2}\right]\frac{1}{k_Pk^2c^2}\left(k_P^2-k_P\frac{n}{2^{n+2}}\right)\leq 1, \tag{4.159}$$

which implies that or

$$\frac{1}{k_Pk^2c^2}\left(k_P^2-k_P\frac{n}{2^{n+2}}\right)\leq 1 \tag{4.160}$$

or

$$\frac{1}{P^*_{ss,11}E^*_{ss,11}}\left[k(1+cE^{*,P}_{ss,11})-\frac{a_P}{(1+P^*_{ss,11})^2}\right]\leq 1. \tag{4.161}$$

In fact, if it is true that

$$\frac{1}{k_Pk^2c^2}\left(k_P^2-k_P\frac{n}{2^{n+2}}\right)\leq 1, \tag{4.162}$$

then

$$k_P-\frac{n}{2^{n+2}}\leq kc, \tag{4.163}$$

but, one has that

$$\lim_{n\to+\infty}\frac{n}{2^{n+2}}=0, \tag{4.164}$$

which, implies that

$$k_P\leq kc, \tag{4.165}$$

and if $kc\leq\kappa\frac{1}{8}$ with $\kappa\leq 1$ then

$$k_P\leq\frac{1}{8}, \tag{4.166}$$

which, in view of (4.155), is a contradiction. On other hand, if it is true that

$$\frac{1}{P^*_{ss,11}E^*_{ss,11}}\left[k(1+cE^{*,P}_{ss,11})-\frac{a_P}{(1+P^*_{ss,11})^2}\right]\leq 1, \tag{4.167}$$

and if we recall that we are under the hypothesis that $X^*_{ss,11}$ and $d>0$ are sufficiently small, by drawing on the approximation

$$\begin{aligned}P^*_{ss,11}=\Psi_3(X^*_{ss,11},E^*_{ss,11})&=\frac{a_P}{k\left[1+c\left(E^*_{ss,11}+dX^*_{ss,11}\right)\right]}\\&\approx\frac{a_P}{k\left(1+cE^*_{ss,11}\right)},\end{aligned} \tag{4.168}$$

then one has that

$$\left[\frac{a_P}{P^*_{ss,11}}-\frac{a_P}{(1+P^*_{ss,11})^2}\right]\leq P^*_{ss,11}E^*_{ss,11}, \tag{4.169}$$

which implies that

$$\frac{a_P}{P^*_{ss,11}}\left[1-\frac{P^*_{ss,11}}{(1+P^*_{ss,11})^2}\right] \le P^*_{ss,11}E^*_{ss,11}, \tag{4.170}$$

which implies that

$$a_P\left[1-\frac{P^*_{ss,11}}{(1+P^*_{ss,11})^2}\right] \le \left(P^*_{ss,11}\right)^2 E^*_{ss,11}, \tag{4.171}$$

and using that the left hand side of (4.171) is at most a_P, one arrives at

$$a_P \le \left(P^*_{ss,11}\right)^2 E^*_{ss,11}. \tag{4.172}$$

Now, recalling that

$$\frac{P^*_{ss,11}}{P^*_{ss,10}} = O(1),$$

one can use the approximations

$$P^*_{ss,11} \approx \left(\frac{a_P}{k}-1\right)$$

and

$$E^*_{ss,11} \approx \frac{a_E}{k_P}$$

in the right hand side of (4.172) to arrive at

$$\begin{aligned} a_P &\le \left(\frac{a_P}{k}-1\right)\frac{a_E}{k_P} \\ &\le \left(\frac{a_P}{k}\right)^2\frac{a_E}{k_P} \end{aligned} \tag{4.173}$$

which, in turn, implies that

$$k_P \le \frac{a_P a_E}{k^2}. \tag{4.174}$$

So, by recalling (4.166), if it is true that

$$\frac{a_P a_E}{k^2} \le \kappa_1 \frac{1}{8}$$

with $\kappa_1 \le 1$ then we arrive at the contradiction

$$k_P < \frac{1}{8}, \tag{4.175}$$

which, in turn, implies that

$$a_3^{SH}(z^P_{ss,13}) > 0, \tag{4.176}$$

and the argument has been raised.

Claim 9: $a_1^{SH}\left(z^P_{ss,11}\right) a_2^{SH}\left(z^P_{ss,11}\right) - a_3^{SH}\left(z^P_{ss,11}\right) > 0$

Consistently, by building on (4.145), in which $X^{*,P}_{ss,11} > 0$ and $d > 0$ are thought to be sufficiently small, one has that

$$
\begin{aligned}
a_2^{SH}(z_{ss,11}^P) = \operatorname{Det} DF_{k_P}^H(z_{ss,11}^P) + D\tilde{F}_{33}(z_{ss,11}^P) \operatorname{Tr} DF_{k_P}^H(z_{ss,11}^P) \\
+D\tilde{F}_{32}(z_{ss,11}^P)D\tilde{F}_{23}(z_{ss,11}^P) + D\tilde{F}_{13}(z_{ss,11}^P)D\tilde{F}_{31}(z_{ss,11}^P), \\
\approx \operatorname{Det} DF_{k_P}^H(z_{ss,11}^P) \\
+D\tilde{F}_{33}(z_{ss,11}^P) \operatorname{Tr} DF_{k_P}^H(z_{ss,11}^P) + D\tilde{F}_{32}(z_{ss,11}^P)D\tilde{F}_{23}(z_{ss,11}^P),
\end{aligned}
\tag{4.177}
$$

which, by invoking (4.149) and (4.148), implies that

$$
\begin{aligned}
a_1^{SH}\left(z_{ss,11}^P\right) a_2^{SH}\left(z_{ss,11}^P\right) - a_3^{SH}\left(z_{ss,11}^P\right) \approx -\left(D\tilde{F}_{33}(z_{ss,11}^P)\right)^2 \operatorname{Tr} DF_{k_P}^H(z_{ss,11}^P) \\
-\operatorname{Det} DF_{k_P}^H(z_{ss,11}^P) \operatorname{Tr} DF_{k_P}^H(z_{ss,11}^P) - D\tilde{F}_{33}(z_{ss,11}^P)\left(\operatorname{Tr} DF_{k_P}^H(z_{ss,11}^P)\right)^2 \\
-D\tilde{F}_{33}(z_{ss,11}^P)D\tilde{F}_{32}(z_{ss,11}^P)D\tilde{F}_{23}(z_{ss,11}^P) - \operatorname{Tr} DF_{k_P}^H(z_{ss,11}^P)D\tilde{F}_{32}(z_{ss,11}^P)D\tilde{F}_{23}(z_{ss,11}^P) \\
-D\tilde{F}_{11}(z_{ss,11}^P)D\tilde{F}_{32}(z_{ss,11}^P)D\tilde{F}_{23}(z_{ss,11}^P).
\end{aligned}
\tag{4.178}
$$

So, if we draw on (4.142) and (4.147) then we have that

$$
a_1^{SH}\left(z_{ss,11}^P\right) a_2^{SH}\left(z_{ss,11}^P\right) - a_3^{SH}\left(z_{ss,11}^P\right) > 0, \tag{4.179}
$$

and the argument has been raised. Therefore, by invoking the Routh-Hurwith Theorem 4.4.2, we can assert that $z_{ss,11}^P$ is possibly stable. However, it is worth to recall that our reasoning has been consistently performed under the assumption that $d > 0$ is sufficiently small. Due to the symmetry of the degradation term of Semrau-Huang's model, that is,

$$
-kP[1 + c(E + dX)],
$$

if we had assumed that $c > 0$ was sufficiently small so as to eliminate the terms containing c, seeing that $c > 0$ favors the existence of $z_{ss,11}^P$, then we would have arrived at the conclusion that, for $n > 2$ sufficiently high, one has that this stability might change. In fact, as we will see in section 4.7, under $c > 0$ sufficiently small and $n > 2$ sufficiently high, one has that the stable equilibrium $z_{ss,11}^P$ will give rise to a *stable limit cycle*. So, it seems that our reasoning is consistent with the paradigm involving oscillations, that is, the interplay between a fast positive feedback and a slow negative feedback being outrageously manifested through the relationship enclosed by the parameters d and c.

Claim 10: $a_1^{SH}\left(z_{ss,12}^P\right) > 0$

By invoking Theorem 3.4.6, one has that

$$
\operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) < 0. \tag{4.180}
$$

So, consistently with $d > 0$ sufficiently small, one can assume that

$$
k[1 + c(E_{ss,12}^{*,P} + dX_{ss,12}^{*,P})] \approx k(1 + cE_{ss,12}^{*,P}), \tag{4.181}
$$

which, under the hypothesis

$$
\frac{P_{ss,12}^*}{P_{ss,10}} = O(1),
$$

by recalling (4.111), implies that

$$D\tilde{F}_{33}(X^{*,P}_{ss,12}, E^{*,P}_{ss,12}, P^{*}_{ss,12}) = \frac{a_P}{(1+P^{*}_{ss,12})^2} - k[1 + c(E^{*,P}_{ss,12} + dX^{*,P}_{ss,12})] \approx \frac{a_P}{(1+P^{*}_{ss,12})^2} - k(1 + cE^{*,P}_{ss,12}) < 0, \tag{4.182}$$

and the argument has been raised.

<u>*Claim 11*</u>: $a_3^{SH}\left(z^P_{ss,12}\right) > 0$

By invoking (4.182), in which $d > 0$ is thought to be sufficiently small, consistent with Theorem 3.4.6, one can make the approximation

$$\text{Det}\, DF^H_{k_P}(z^P_{ss,12}) \approx D\tilde{F}_{11}(z^P_{ss,12}) D\tilde{F}_{22}(z^P_{ss,12}) > 0, \tag{4.183}$$

with $k_P = k\left(1 + cP^{*}_{ss,12}\right)$, which, in turn, implies that

$$\begin{aligned} a_3^{SH}(z^P_{ss,12}) &= -D\tilde{F}_{33}(z^P_{ss,12})\,\text{Det}\, DF^H_{k_P}(z^P_{ss,12}) \\ &+ D\tilde{F}_{11}(z^P_{ss,12})D\tilde{F}_{32}(z^P_{ss,12})D\tilde{F}_{23}(z^P_{ss,12}) - D\tilde{F}_{21}(z^P_{ss,11})D\tilde{F}_{32}(z^P_{ss,12})D\tilde{F}_{13}(z^P_{ss,12}) \\ &- D\tilde{F}_{12}(z^P_{ss,12})D\tilde{F}_{31}(z^P_{ss,12})D\tilde{F}_{23}(z^P_{ss,12}) + D\tilde{F}_{22}(z^P_{ss,12})D\tilde{F}_{31}(z^P_{ss,12})D\tilde{F}_{13}(z^P_{ss,12}) \\ &\approx -D\tilde{F}_{33}(z^P_{ss,12})D\tilde{F}_{11}(z^P_{ss,12})D\tilde{F}_{22}(z^P_{ss,12}) \\ &+ D\tilde{F}_{11}(z^P_{ss,12})D\tilde{F}_{32}(z^P_{ss,12})D\tilde{F}_{23}(z^P_{ss,12}) - D\tilde{F}_{21}(z^P_{ss,11})D\tilde{F}_{32}(z^P_{ss,12})D\tilde{F}_{13}(z^P_{ss,12}). \end{aligned} \tag{4.184}$$

Next, recalling that $\tilde{k} := k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right]$, if we invoke (4.53) and (4.54), that is,

$$\theta_X < \frac{a_X}{2\tilde{k}}$$

and

$$\theta_E < \frac{a_E}{2\tilde{k}},$$

and if we assume that

$$E^{*,P}_{ss,11} \approx \frac{a_E}{k_P}$$

and that

$$X^{*,P}_{ss,11} \approx \frac{a_X}{k_P}$$

then we can make the approximation

$$D\tilde{F}_{11}(z^P_{ss,11}) D\tilde{F}_{22}(z^P_{ss,11}) \approx \left(k_P - n\frac{1}{2^{n+2}}\frac{k_P}{\tilde{k}^n}\right)^2, \tag{4.185}$$

with $k_P = k(1 + cP^{*}_{ss,11})$, which, in turn, seeing that

$$\frac{k_P}{\tilde{k}^n} < 1,$$

becomes

$$D\tilde{F}_{11}(z^P_{ss,11}) D\tilde{F}_{22}(z^P_{ss,11}) \approx \left(k_P - \frac{n}{2^{n+2}}\right)^2. \tag{4.186}$$

Hence, we can rely upon the same argument of *Claim* 8 so as to conclude that

$$a_3^{SH}(z_{ss,12}^P) > 0, \tag{4.187}$$

and the argument has been raised.

Claim12: $a_1^{SH}\left(z_{ss,12}^P\right) a_2^{SH}\left(z_{ss,12}^P\right) - a_3^{SH}\left(z_{ss,12}^P\right) > 0$

Consistently, by using the assumption that $d > 0$ is sufficiently small, one has that

$$\begin{aligned} a_2^{SH}(z_{ss,12}^P) &= \operatorname{Det} DF_{k_P}^H(z_{ss,12}^P) + D\tilde{F}_{33}(z_{ss,12}^P) \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) \\ &\quad + D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P) + D\tilde{F}_{13}(z_{ss,12}^P) D\tilde{F}_{31}(z_{ss,12}^P), \\ &\approx \operatorname{Det} DF_{k_P}^H(z_{ss,12}^P) + D\tilde{F}_{33}(z_{ss,12}^P) \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) + D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P), \end{aligned} \tag{4.188}$$

which implies that

$$\begin{aligned} a_1^{SH}\left(z_{ss,12}^P\right) a_2^{SH}\left(z_{ss,12}^P\right) - a_3^{SH}\left(z_{ss,12}^P\right) &\approx -\left(D\tilde{F}_{33}(z_{ss,12}^P)\right)^2 \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) \\ &\quad - \operatorname{Det} DF_{k_P}^H(z_{ss,12}^P) \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) - D\tilde{F}_{33}(z_{ss,12}^P) \left(\operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P)\right)^2 \\ &\quad - D\tilde{F}_{33}(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P) - \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P) \\ &\quad - D\tilde{F}_{11}(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P) + D\tilde{F}_{21}(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{13}(z_{ss,12}^P). \end{aligned} \tag{4.189}$$

Now, if we invoke Theorem 3.4.6 then we can assert that

$$\operatorname{Det} DF_{k_P}^H(z_{ss,12}^P) \geq 0, \tag{4.190}$$

so if we draw on (4.180) and (4.182) then we have that

$$\begin{aligned} &-\left(D\tilde{F}_{33}(z_{ss,12}^P)\right)^2 \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) \\ &- \operatorname{Det} DF_{k_P}^H(z_{ss,12}^P) \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) - D\tilde{F}_{33}(z_{ss,12}^P) \left(\operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P)\right)^2 \\ &- D\tilde{F}_{33}(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P) - \operatorname{Tr} DF_{k_P}^H(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P) \\ &- D\tilde{F}_{11}(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{23}(z_{ss,12}^P) > 0. \end{aligned} \tag{4.191}$$

Moreover, one has that the last term on the right hand side of the approximation (4.189), that is,

$$D\tilde{F}_{21}(z_{ss,12}^P) D\tilde{F}_{32}(z_{ss,12}^P) D\tilde{F}_{13}(z_{ss,12}^P) = -b \frac{n(X_{ss,12}^{*,P})^n}{\left[1 + (X_{ss,12}^{*,P})^n\right]^2} k^2 c^2 P_{ss,12}^* < 0 \tag{4.192}$$

seems not to be a problem. In fact, if we rely upon the argument for *Claim* 6 then we build upon the analysis of Huang's model performed in Chapter 3 to recall that the parameter b can be chosen as close as possible to zero without changing the other representatives of the primitive scenario

$$SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}],$$

and one would still have a representative for the respective primitive scenario with the new choice for $b > 0$ replacing the old one. Hence, we assume that $b > 0$ is compatible with

$$a_1^{SH}\left(z_{ss,12}^P\right) a_2^{SH}\left(z_{ss,12}^P\right) - a_3^{SH}\left(z_{ss,12}^P\right) > 0, \tag{4.193}$$

and the argument has been raised. Therefore, one has that $z_{ss,12}^P$ is possible stable.

Thereby, we have seen that the set $\{z_{ss,10}^P, z_{ss,11}^P, z_{ss,12}^P, z_{ss,13}^P\}$ possibly consists of stable steady states. In fact, the correctness of our reasoning can be strengthened by the numerical experiments shown in Figures 4.6a and 4.6b. But, what about the steady states in $\{z_{ss,14}^P, z_{ss,15}^P, z_{ss,16}^P, z_{ss,17}^P, z_{ss,18}^P\}$? Provided that our approach is mainly based on approximations of the components of each steady state, it is definitely not suitable to access the (in)stability of these steady states in the respective set. However, our numerical experiments in Figures 4.6a and 4.6b indicate that the set $\{z_{ss,14}^P, z_{ss,15}^P, z_{ss,16}^P, z_{ss,17}^P, z_{ss,18}^P\}$ is possibly comprised by unstable or saddle steady states. Therefore, with respect to the primitive scenario

$$SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{1,n}, \breve{C_{2,n}}], \tag{4.194}$$

one has that from 18 steady states, only 4 are stable ones. In fact, the set

$$\{z_{ss,10}^P, z_{ss,11}^P, z_{ss,12}^P, z_{ss,13}^P\}$$

consists of stable equilibria, whilst the set

$$\{z_{ss,1}^0, z_{ss,2}^0, z_{ss,3}^0, z_{ss,4}^0, z_{ss,5}^0, z_{ss,6}^0, z_{ss,7}^0, z_{ss,8}^0, z_{ss,9}^0, z_{ss,14}^P, z_{ss,15}^P, z_{ss,16}^P, z_{ss,17}^P, z_{ss,18}^P\}$$

consists of unstable or saddle equilibria.

4.5 An example of a rational decomposition of the primitive scenario $SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{1,n}, \breve{C}_{2,n}]$

In Chapter 2, we have introduced a systematic evaluation of a *phenomenological mathematical model* grounded in *Frege's judgment theory*. In the latter, one has that the concept of primitive notion is of uttermost importance owing to the fact that it provides a way of defining concepts sequentially. The latter essentially stipulates our rational strategy given that primitive scenarios play the role of primitive notions in our approach.

Bearing in mind that the relevant aspect in our evaluation of Semrau-Huang's model is the number of steady states, one has that knowing the primitive scenarios of the respective model, that is, the ones with the maximal number of steady states, can potentially lead us to know any scenario of the model, which means that we can potentially know whether or not the observations in (4.1) are actually generated by Semrau-Huang's model.

But, how will we execute suitable judgements upon the primitive scenario

$$sc_{\lambda_0}^{SH} = SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{1,n}, \breve{C_{2,n}}], \tag{4.195}$$

with λ_0 being any representative of (4.77), which, in turn, will unveil scenarios similar to the observations in (4.1)? Or rather, how can we shift the primitive

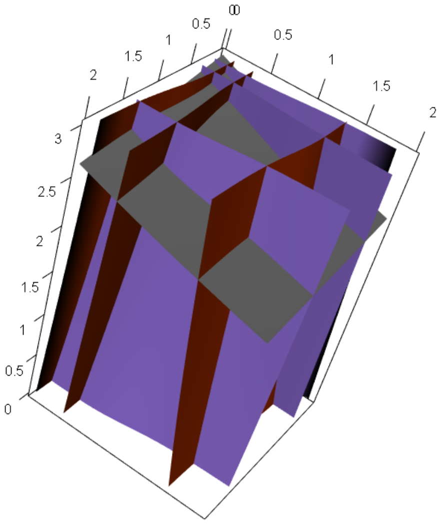


Figure 4.7: $sc^{SH}_{\hat{\lambda}_0}$

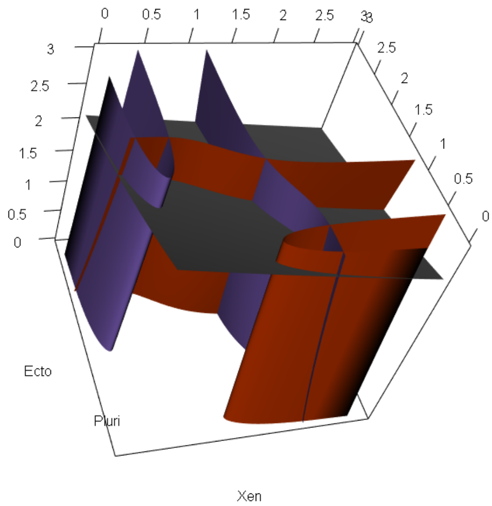


Figure 4.8: $sc^{SH}_{\hat{\lambda}_1}$

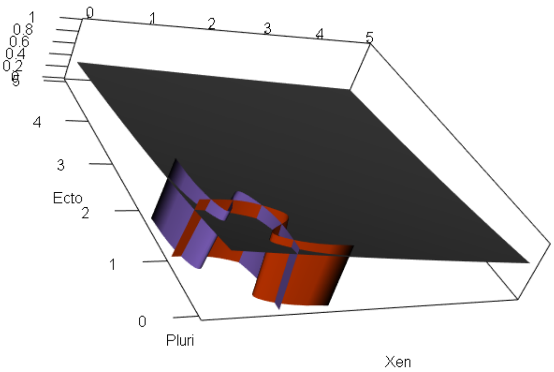


Figure 4.9: $sc^{SH}_{\hat{\lambda}_2}$

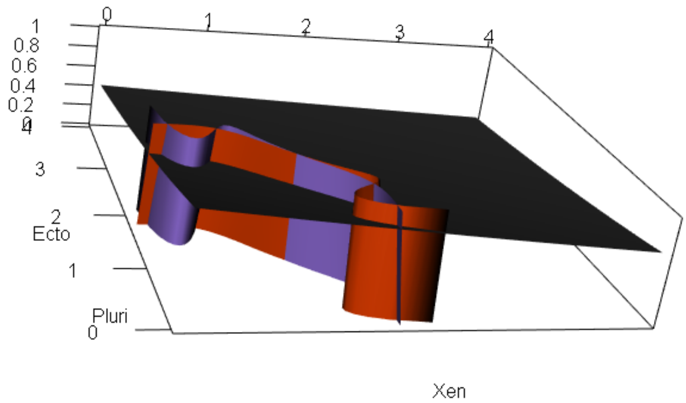


Figure 4.10: $sc^{SH}_{\hat{\lambda}_3}$

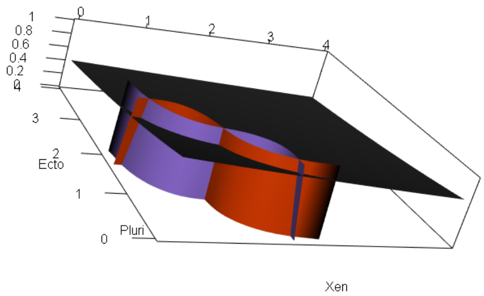


Figure 4.11: $sc^{SH}_{\hat{\lambda}_4}$

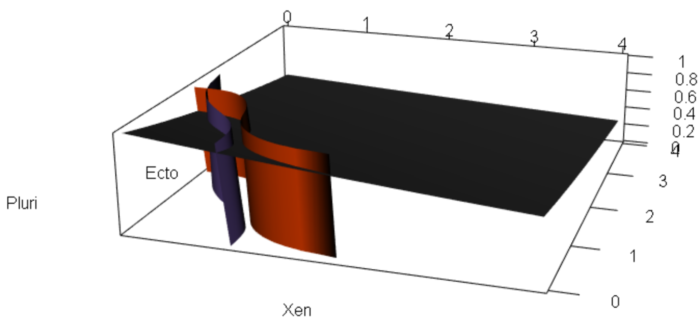


Figure 4.12: $sc^{SH}_{\hat{\lambda}_5}$

scenario (4.195) through the *scenario space* of Semrau-Huang's model in such a way that we can find scenarios that could be interpreted as the observations in (4.1)? In fact, we need to know how to construct $\bar{\Pi}_q-$functions, $q \in \{1, 2, 3, \ldots, m\}$, defined on SC^{SH} with which one has that

$$sc_{\lambda}^{SH} = \bar{\Pi}_m \circ \bar{\Pi}_{m-1} \ldots \circ \bar{\Pi}_2 \circ \bar{\Pi}_1[sc_{\lambda_0}^{\mathcal{M}}] \tag{4.196}$$

or that

$$sc_{\lambda_0}^{SH} \xrightarrow{\bar{\Pi}_1} sc_{\lambda_1}^{SH} \xrightarrow{\bar{\Pi}_2} sc_{\lambda_2}^{SH} \xrightarrow{\bar{\Pi}_3} \ldots \xrightarrow{\bar{\Pi}_{m-2}} sc_{\lambda_{m-2}}^{SH} \xrightarrow{\bar{\Pi}_{m-1}} sc_{\lambda_{m-1}}^{\mathcal{M}} \xrightarrow{\bar{\Pi}_m} sc_{\lambda}^{SH}, \tag{4.197}$$

such that

$$sc_{\lambda}^{SH} \sim O \tag{4.198}$$

for some $O \in O_{TS}$.

First of all, we need to discriminate the steady states of $sc_{\lambda_0}^{SH}$, that is, we need to indicate which steady states thereof are suitable to explain each of the observations in O_{TS}. In fact, by invoking the intentions of the modeling agent described in Section 1.5, if we carry out a closer inspection of the primitive scenario (4.195) then we can assert that it is sufficient to orientating ourselves towards steady states qualitatively similar to the steady states $z_{ss,2}^0, z_{ss,4}^0, z_{ss,10}^P$, and $z_{ss,11}^P$, seen in Figures 4.4 and 4.5, so as to explain the observations in (4.1). In fact, one has that $z_{ss,2}^0$ can be interpreted as the Ecto-like cells, $z_{ss,4}^0$ as the Xen-like cells, $z_{ss,10}^P$ as the Pluripotent cells, and $z_{ss,11}^P$ as the Jammed cells.

To begin with, consistent with the claim that we ought to direct our evaluation at scenarios containing steady states qualitatively similar to

$$z_{ss,2}^0, z_{ss,4}^0, z_{ss,10}^P, z_{ss,11}^P \tag{4.199}$$

then it seems that the first judgment concerns the destruction of the steady states

$$z_{ss,1}^0, z_{ss,5}^0, z_{ss,6}^0, z_{ss,7}^0, z_{ss,14}^P, z_{ss,15}^P, z_{ss,16}^P, \tag{4.200}$$

which, by inspecting Figure 4.1, can be executed by stipulating the sufficient condition

$$b >_{sc_{\lambda_0}^{SH}} \max_{0 \le P \le \left(\frac{a_P}{k}-1\right)} \left\{ g_{1.n}^P(x_{max,n}^P)[(e_{1,n}^P)^n + 1]; g_{2.n}^P(e_{max,n}^P)[(x_{1,n}^P)^n + 1] \right\}, \tag{4.201}$$

which, by invoking that

$$sc_{\lambda_0}^{SH} = [a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 0.5, \theta_E = 0.5, b = 0.0811, c = 0.1, d = 0.5, k = 0.5, n = 4], \tag{4.202}$$

implies that

$$b > 0.119628, \tag{4.203}$$

which, in turn, is satisfied by $\hat{b} = 0.3$. Moreover, one has that the symbol $>_{sc_{\lambda_0}^{SH}}$ betokens that the inequality $>$ must be verified by using all the components of the respective representative of the primitive scenario $sc_{\lambda_0}^{SH}$ except for the component

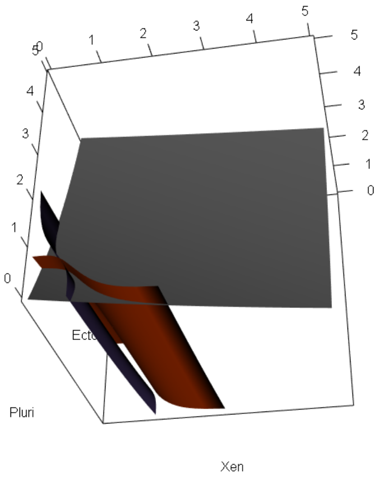


Figure 4.13: $sc^{SH}_{\hat{\lambda}_6}$

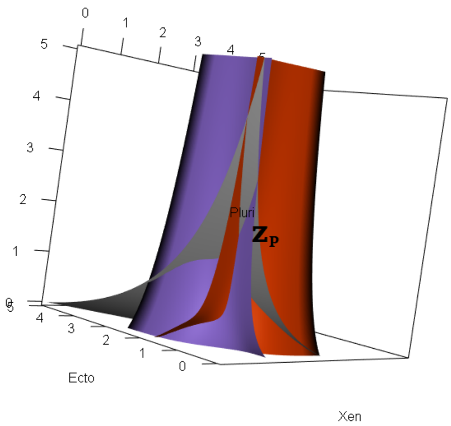


Figure 4.14: $sc^{SH}_{\hat{\lambda}_7}$

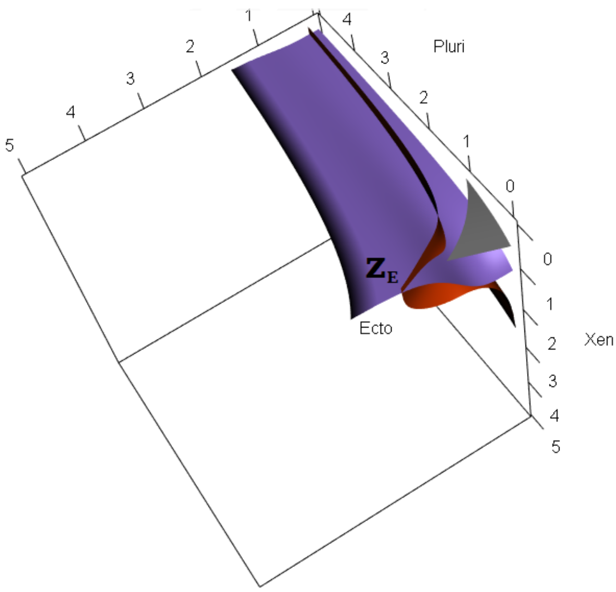


Figure 4.15: $sc^{SH}_{\hat{\lambda}_8}$

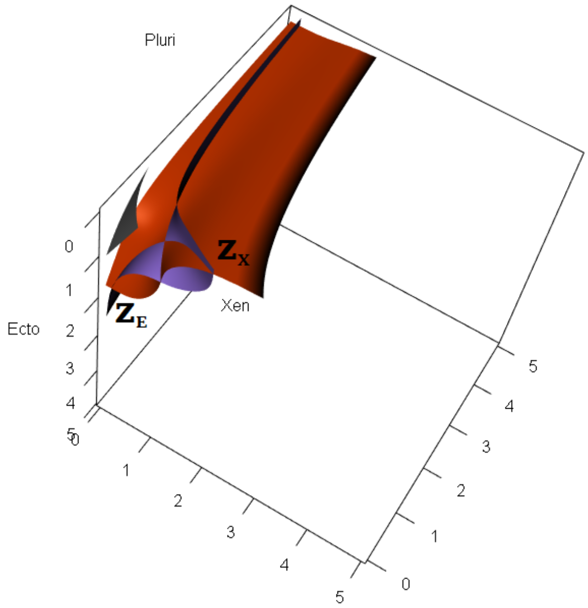


Figure 4.16: $sc^{SH}_{\hat{\lambda}_9}$

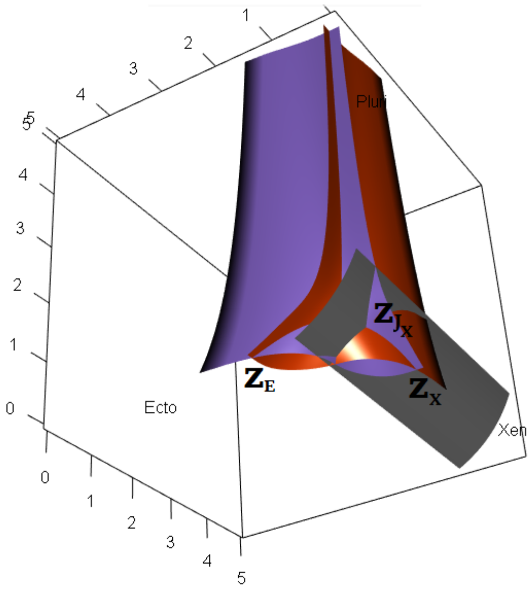


Figure 4.17: $sc^{SH}_{\hat{\lambda}_{10}}$

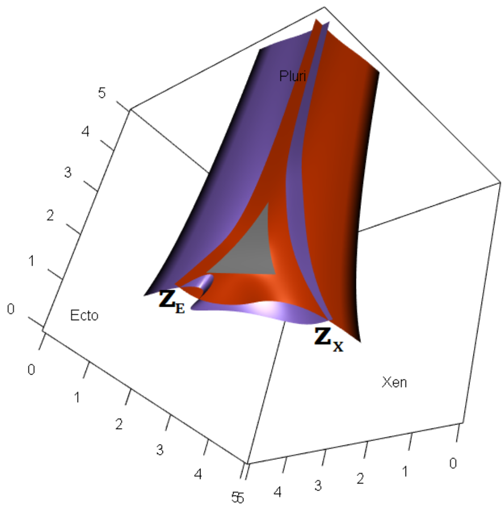


Figure 4.18: $sc^{SH}_{\hat{\lambda}_{11}}$

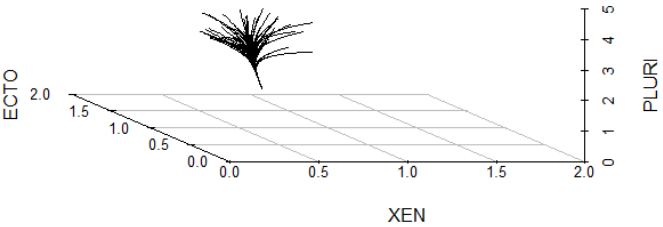


Figure 4.19: $sc^{SH}_{\hat{\lambda}_7} \sim O_P^{(CHIR^+,PD^+,LIF^+,RA^-)}$

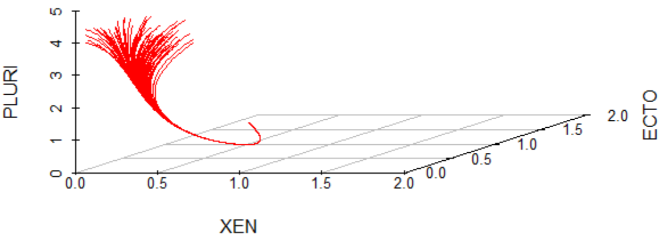


Figure 4.20: $sc^{SH}_{\hat{\lambda}_8} \sim O_E^{(CHIR^-,PD^-,LIF^-,RA^-)}$

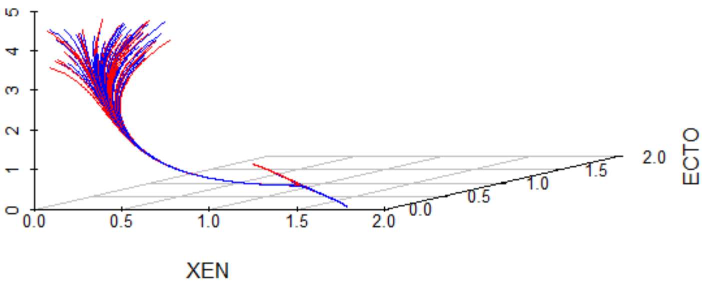


Figure 4.21: $sc^{SH}_{\hat{\lambda}_9} \sim O_{X,E}^{(CHIR^-,PD^-,LIF^-,RA^+)}$

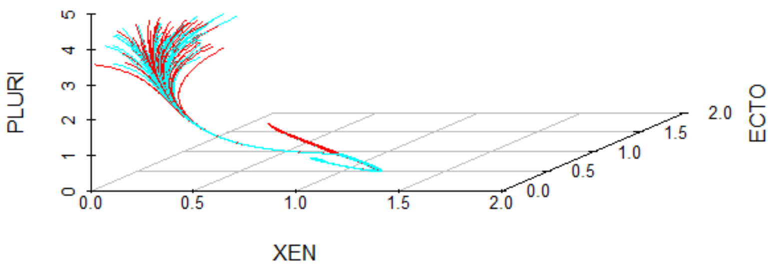


Figure 4.22: $sc^{SH}_{\hat{\lambda}_{10}} \nsim O_{J_E,E}^{(CHIR^-,PD^+,LIF^-,RA^+)}$

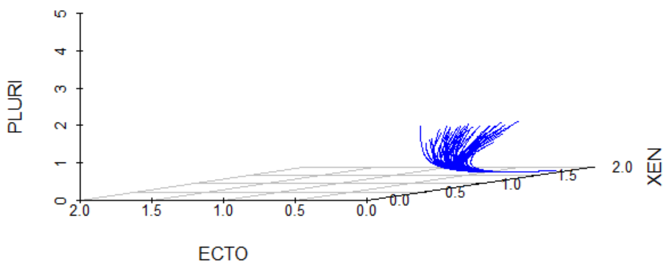


Figure 4.23: $sc^{SH}_{\hat{\lambda}_{11}} \nsim O_{J_E,E}^{(CHIR^-,PD^+,LIF^-,RA^+)} \xrightarrow{PD0325901^-,\ RA^+} O_E^{(CHIR^-,PD^-,LIF^-,RA^+)}$

being shifted, in this case, b. So, we see in Figures 4.7 and 4.8 that shifting from the primitive scenario $sc^{SH}_{\lambda_0}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_1}{=}[a_P{=}2{,}a_X{=}0.8{,}a_E{=}0.8{,}\theta_X{=}0.5{,}\theta_E{=}0.5{,}\hat{b}{=}0.3{,}c{=}0.1{,}d{=}0.5{,}k{=}0.5{,}n{=}4], \tag{4.204}$$

seems to be a suitable rational movement in the decomposition process. Nonetheless, one has that the steady state $z^P_{ss,10}$ has also been destroyed, what demands a convenient strategy throughout the decomposition process that will enable us to recover the counterpart of the respective steady state in some of the produced scenarios. In fact, such a state is suitable to explain the observation $(4.1)_1$ thus indispensable in our decomposition.

Next, as we will need to appeal to the curvature of the nullclines in the decomposition process, it is convenient to compute the second derivative of each of the defining maps. In fact, one has that

$$\begin{aligned}\frac{d^2}{dX^2}\Psi^P_{1,n}(X) &= \frac{d^2}{dX^2}h_n^{-1,P}\left(g^P_{1,n}(X)\right)\left(\frac{d}{dX}g^P_{1,n}(X)\right)^2 + \frac{d}{dX}h_n^{-1,P}\left(g^P_{1,n}(X)\right)\frac{d^2}{dX^2}g^P_{1,n}(X)\\ &= \frac{1}{n}\left(\frac{b}{g^P_{1,n}(X)}-1\right)^{-\left(1-\frac{1}{n}\right)}\frac{b}{(g^P_{1,n}(X))^3}\left[2-\frac{b}{g^P_{1,n}(X)}\frac{\left(1-\frac{1}{n}\right)}{\left(\frac{b}{g^P_{1,n}(X)}-1\right)}\right]\times\frac{d}{dX}g^P_{1,n}(X)\\ &\qquad -\frac{1}{n}\left(\frac{b}{g^P_{1,n}(X)}-1\right)^{-\left(1-\frac{1}{n}\right)}\frac{b}{(g^P_{1,n}(X))^2}\times\frac{d^2}{dX^2}g^P_{1,n}(X),\end{aligned} \tag{4.205}$$

with $g^P_{1,n}(X)$ being defined in (4.23), while

$$\frac{d}{dX}g^P_{1,n}(X) = k_P - na_X\theta_X^n\frac{X^{n-1}}{(\theta_X^n+X^n)^2} \tag{4.206}$$

and

$$\frac{d^2}{dX^2}g^P_{1,n}(X) = -na_X\frac{\theta_X^n}{(\theta_X^n+X^n)^3}X^{n-2}\left[(n-1)\theta_X^n-(n+1)X^n\right] \tag{4.207}$$

with $k_P = k(1+cP)$ being defined in (4.35). Likewise, we can derive a similar expression for $\frac{d^2}{dX^2}\Psi^P_{2,n}(X)$. Moreover, one has that

$$\frac{\partial^2}{\partial X^2}\Psi_3(X,E) = 2a_Pc^2d^2\frac{1}{k\left[1+c(E+dX)\right]^3} \tag{4.208}$$

and that

$$\frac{\partial^2}{\partial E^2}\Psi_3(X,E) = 2a_Pc^2\frac{1}{k\left[1+c(E+dX)\right]^3}. \tag{4.209}$$

Having done that, it now seems reasonable to shift the scenario $sc^{SH}_{\hat{\lambda}_1}$ to a scenario in which $\Psi^P_{1,n}$ and $\Psi^P_{2,n}$ are continuous functions for all $P \geq 0$. But, which parameter should we be changing then? In fact, by construction of $\left(SH_n[\breve{C}_{i,X},\breve{C}_{j,E},\breve{C}_{r,P}]\right)_{i,j,r}$, if it is true that

$$k > \max\left\{\frac{a_X}{\theta_X}, \frac{a_E}{\theta_E}\right\} \tag{4.210}$$

then $\Psi^P_{1,n}$ and $\Psi^P_{1,n}$ are both continuous in view of $(4.64)_{5,6}$. Hence, in the case of $sc^{SH}_{\hat{\lambda}_1}$, it is sufficient to choose $k \geq 1.6$. However, choosing $k > 1.6$ can lead us to a scenario in SC^{SH} wherein much of the existent information in $sc^{SH}_{\hat{\lambda}_1}$ would have been lost or altered drastically. But, what do we mean with the phrase "the existent information"? In fact, the latter refers to the number of steady states, their respective stability and location with respect to each other.

However, how can we choose for $k > 0$ with which one has that $\Psi^P_{1,n}$ and $\Psi^P_{2,n}$ will be both continuous without changing the information carried by the scenario $sc^{SH}_{\hat{\lambda}_1}$? In fact, if we now invoke the elucidations of Section 2.8 concerning the essence of the critical layer of the scenario space of a model, then we conjecture that we can find $0.5 < \hat{k} < 1.6$ which suits the purpose. Here, by invoking Chapter 2, we are implicitly asserting that, in the critical layer, one can find scenarios similar to the ones expected to be found in the main components of the parameter space of the model.

Hence, if we choose $\hat{k} = 1$ then shifting the scenario $sc^{SH}_{\hat{\lambda}_1}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_2} = [a_P=2, a_X=0.8, a_E=0.8, \theta_X=0.5, \theta_E=0.5, \hat{b}=0.3, c=0.1, d=0.5, \hat{k}=1, n=4], \tag{4.211}$$

has precisely the aforesaid description as seen in Figures 4.8 and 4.9, respectively. Consistently, by invoking (4.72) and (4.73) for the definition of $x^P_{min,n}$ and $e^P_{min,n}$, whichever the latter choice for $0.5 < \hat{k} < 1.6$ is, one has that it must be constrained to the condition

$$\hat{b} >_{sc^{SH}_{\hat{\lambda}_2}} \max_{0 \leq P \leq \left(\frac{a_P}{\hat{k}} - 1\right)} \left\{ g^P_{1.n}(x^P_{max,n})[(e^P_{min,n})^n + 1]; g^P_{2.n}(e^P_{max,n})[(x^P_{min,n})^n + 1] \right\}. \tag{4.212}$$

Otherwise, one would end up in a scenario containing the counterparts of the steady states $z^0_{ss,1}$, $z^0_{ss,5}$, $z^0_{ss,6}$, $z^0_{ss,7}$, $z^P_{ss,10}$, $z^P_{ss,14}$, $z^P_{ss,15}$, and $z^P_{ss,16}$, wherein $\Psi^P_{1,n}$ and $\Psi^P_{2,n}$ would be both continuous. In fact, for $\hat{k} = 1$, one has that

$$\max_{0 \leq P \leq \left(\frac{a_P}{\hat{k}} - 1\right)} \left\{ g^P_{1.n}(x^P_{max,n})[(e^P_{min,n})^n + 1]; g^P_{2.n}(e^P_{max,n})[(x^P_{min,n})^n + 1] \right\} \approx 0.2734148 < \hat{b} = 0.3, \tag{4.213}$$

so (4.212) is indeed satisfied for $\hat{k} = 1$.

Now, one has that the next judgment entails the destruction of the counterparts in the scenario $sc^{SH}_{\hat{\lambda}_2}$ of the steady states $z^0_{ss,8}$, $z^0_{ss,9}$, $z^P_{ss,17}$, and $z^P_{ss,18}$ in the primitive scenario $sc^{SH}_{\hat{\lambda}_0}$. In fact, as illustrated in Figures 4.24 and 4.25, by inspection of the scenario $sc^{SH}_{\hat{\lambda}_2}$, one has that it is sufficient to have that

$$\Psi_{1,n}(x_{min,n}) < \Psi^{-1}_{2,n}(x_{min,n}), \tag{4.214}$$

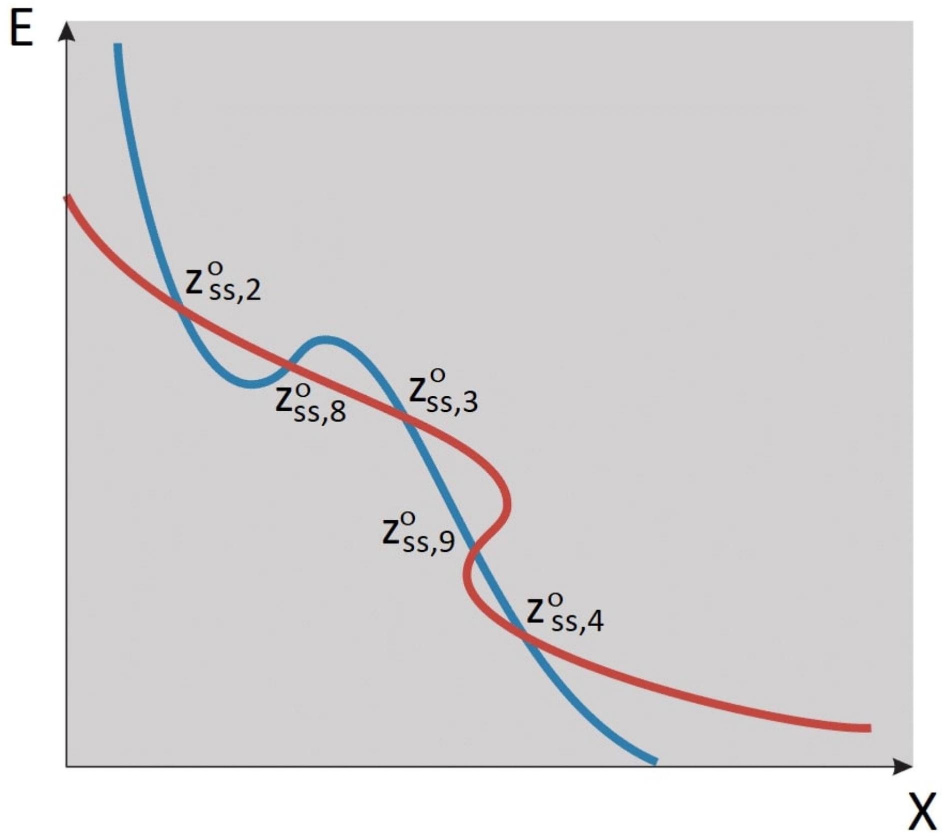


Figure 4.24: Here, one sees the scenario $sc^{SH}_{\hat{\lambda}_2}$ on the plane $P = 0$. How to destroy the steady states $z^0_{ss,8}$ and $z^0_{ss,9}$? In fact, one can do it by considering geometric aspects hereof. Likewise, one can destroy the steady states $z^P_{ss,17}$ and z^P_{18}.

and that

$$\boxed{\Psi_{2,n}(e_{min,n}) < \Psi^{-1}_{1,n}(e_{min,n}),} \tag{4.215}$$

in which, recalling Chapter 3, one has that

$$g_{1,n}(x_{min,n}) = \inf_{X \in [x_{max,n},\infty)} g_{1,n}(X), \tag{4.216}$$

and that

$$g_{2,n}(e_{min,n}) = \inf_{E \in [e_{max,n},\infty)} g_{2,n}(E). \tag{4.217}$$

To apprehend (4.214), one can work out the equality

$$\Psi_{1,n}(x_{min,n}) = \left(\frac{b}{g_{1,n}(x_{min,n})} - 1\right)^{\frac{1}{n}} = \Psi^{-1}_{2,n}(x_{min,n}), \tag{4.218}$$

which implies that

$$\left(\frac{b}{g_{1,n}(x_{min,n})} - 1\right)^{\frac{1}{n}} = \hat{E}, \tag{4.219}$$

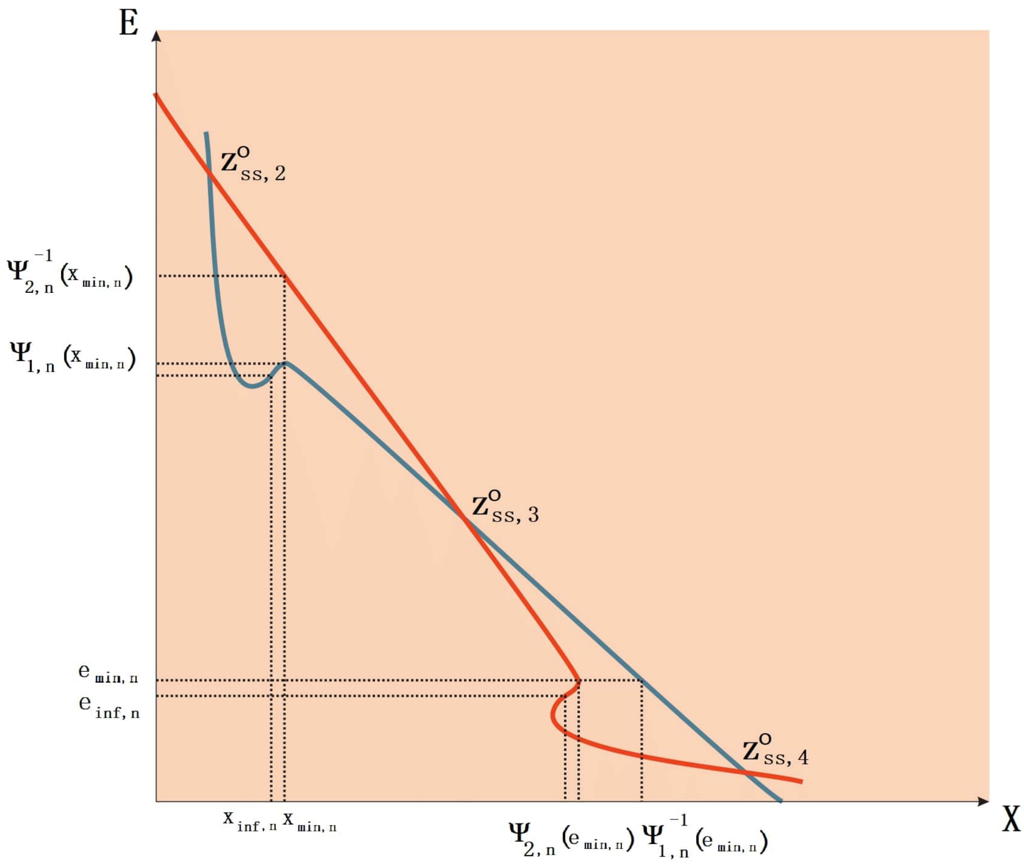


Figure 4.25: Here, one sees clearly that conditions (4.214) and (4.215) are sufficient to extirpate the steady states $z^0_{ss,8}$, $z^0_{ss,9}$, $z^P_{ss,17}$ and $z^P_{ss,18}$ disappear as well. But, how to change the stability of $z^0_{ss,3}$? In fact, we see in the illustration that accounting for the role of the inflection points, $x_{inf,n}$ and $e_{inf,n}$, in keeping the respective geometric aspect, is of utmost importance.

with $\hat{E} = \Psi^{-1}_{2,n}(x_{min,n})$ satisfying

$$k\hat{E} - a_E \frac{\hat{E}^n}{\theta_E^n + \hat{E}^n} = \frac{b}{1 + (x_{min,n})^n}, \tag{4.220}$$

which, implies that

$$\hat{E} > \frac{b}{k\left[1 + (x_{min,n})^n\right]}. \tag{4.221}$$

Hence, using (4.221) in (4.219) implies that

$$k\left[1 + (x_{min,n})^n\right]\left(\frac{b}{g_{1,n}(x_{min,n})} - 1\right)^{\frac{1}{n}} > b, \tag{4.222}$$

or equivalently

$$\boxed{\frac{1}{k^n\left[1 + (x_{min,n})^n\right]^n} b^n - \frac{b}{g_{1,n}(x_{min,n})} + 1 < 0,} \tag{4.223}$$

which, in turn, implies that

$$b > g_{1,n}(x_{min,n}). \tag{4.224}$$

By drawing on (4.215), one can work out a similar argument so as to arrive at

$$\boxed{\frac{1}{k^n\left[1+(e_{min,n})^n\right]^n}b^n - \frac{b}{g_{2,n}(e_{min,n})} + 1 < 0,} \tag{4.225}$$

which, in turn, implies that

$$b > g_{2,n}(e_{min,n}). \tag{4.226}$$

So, if we now define

$$f(b) = a_{0,n}b^n - a_{1,n}b + 1, \tag{4.227}$$

such that

$$a_{0,n} := \frac{1}{k^n\left[1+(x_{min,n})^n\right]^n}, \tag{4.228}$$

and

$$a_{1,n} := \frac{1}{g_{1,n}(x_{min,n})}, \tag{4.229}$$

then one has that

$$f'(b) = a_{0,n}nb^{n-1} - a_{1,n}, \tag{4.230}$$

and that

$$f''(b) = a_{0,n}n(n-1)b^{n-2} \geq 0. \tag{4.231}$$

Thereby, (4.231) implies that the graph of f is convex, while (4.230) implies that

$$f'(b) = 0, \tag{4.232}$$

if and only if

$$b = b_c = \left(\frac{1}{n}\frac{a_{1,n}}{a_{0,n}}\right)^{\frac{1}{n-1}}, \tag{4.233}$$

so, for $b < b_c$, one has that $f'(b) < 0$, while, for $b > b_c$, one has that $f'(b) > 0$, which, in turn, implies that

$$f(b_c) = \min_{b\geq 0} f(b). \tag{4.234}$$

In fact, with respect to the scenario $sc^{SH}_{\hat{\lambda}_2}$, one has that

$$b_c \approx 2.01, \tag{4.235}$$

and that

$$f(b_c) \approx -25.40, \tag{4.236}$$

which, in turn, by drawing upon Bolzano's theorem (see [77, p. 93]), given that f is a continuous function for $b \geq 0$, implies that there exist $b_1, b_2 > 0$ such that $f(b_1) = 0 = f(b_2)$ with $0 < b_1 < b_c$ and $b_c < b_2$. In fact, one has that

$$b_1 \approx 0.06, \tag{4.237}$$

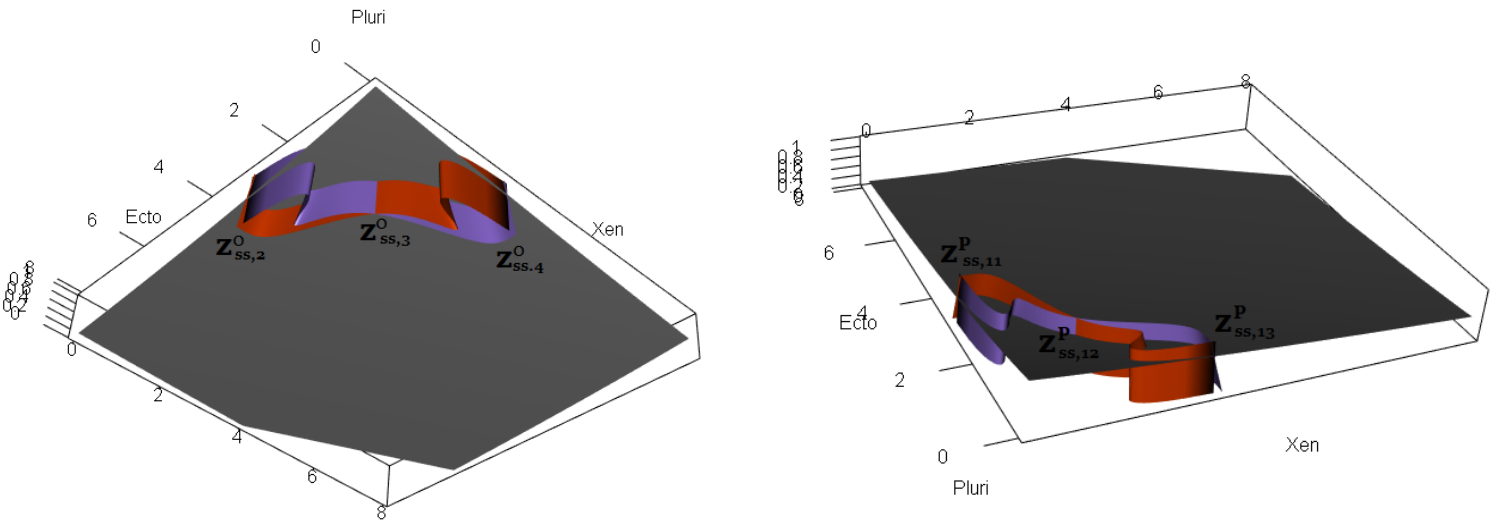


Figure 4.26: Here, one can clearly see that the steady states $z^0_{ss,7}$ and $z^0_{ss,8}$ have been destroyed in the scenario sc^{SH}_{φ}. Likewise, one sees that the steady states $z^P_{ss,17}$ and $z^P_{ss,18}$ are not generated by the scenario sc^{SH}_{φ} either.

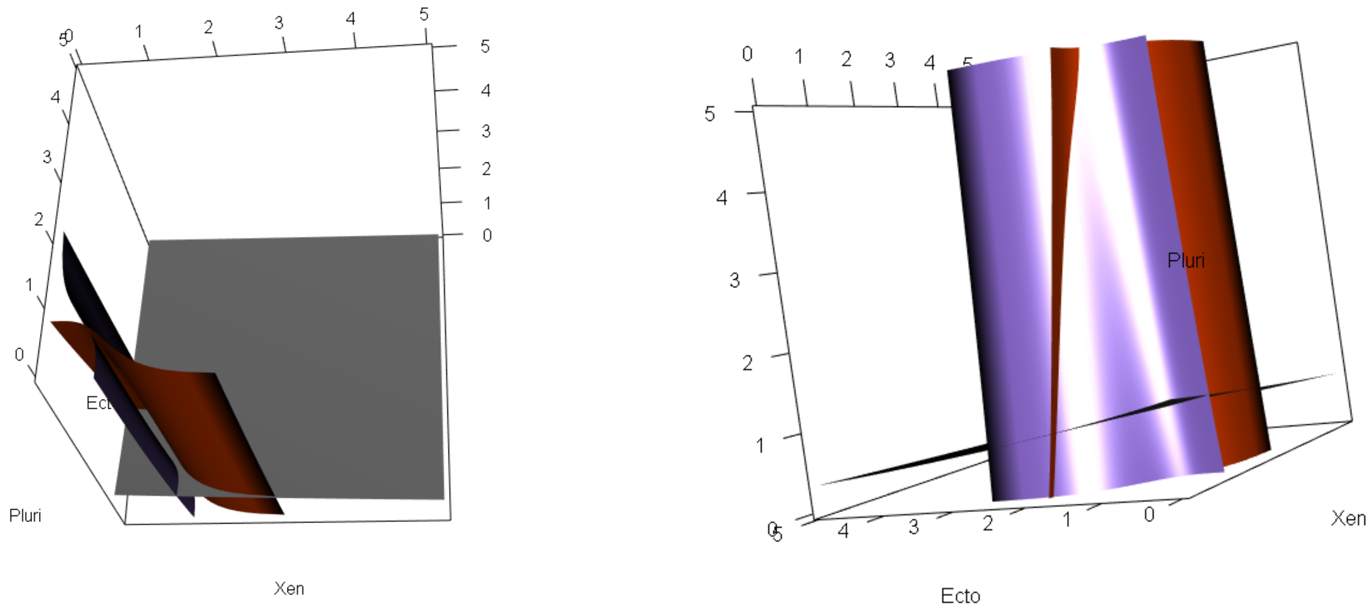


Figure 4.27: Here, one sees that $P_c \approx 5$, which, in turn, determines how one can perform the next shift toward a scenario wherein the steady state $z^P_{ss,10}$ will have been restored.

and that

$$b_2 \approx 3.07, \tag{4.238}$$

thus for all $0.06 < b < 3.07$ one has that $f(b) < 0$, that is,(4.223) holds. Moreover, whichever of the admissible values , that is, $0.06 < b < 3.$, must be constrained to the condition (4.212), so $b > 0.2734148$.

Therefore, as we have verified numerically, if we had chosen for $\check{b} = 2.5$ then we would have shifted from the scenario $sc^{SH}_{\hat{\lambda}_2}$ to the scenario sc^{SH}_{φ}, i.e.,

$$sc^{SH}_{\varphi}{=}[a_P{=}2,a_X{=}0.8,a_E{=}0.8,\theta_X{=}0.5,\theta_E{=}0.5,\check{b}{=}2.5,c{=}0.1,d{=}0.5,\hat{k}{=}1,n{=}4], \tag{4.239}$$

in which the counterparts of the steady states $z^0_{ss,8}$, $z^0_{ss,9}$, $z^P_{ss,17}$, and $z^P_{ss,18}$ found in the primitive scenario $sc^{SH}_{\hat{\lambda}_0}$ would have been wiped out, as seen in Figures 4.26. However, bearing in mind that our evaluation is predicated on the assumption that the parameters have the same order of magnitude unless one can argue otherwise, we choose for $\hat{\hat{b}} = 1$, which, in turn, leads us to shift from the scenario $sc^{SH}_{\hat{\lambda}_2}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_3}{=}[a_P{=}2,a_X{=}0.8,a_E{=}0.8,\theta_X{=}0.5,\theta_E{=}0.5,\hat{\hat{b}}{=}1,c{=}0.1,d{=}0.5,k{=}1,n{=}4], \tag{4.240}$$

in which the counterparts of the steady states $z^0_{ss,8}$, $z^0_{ss,9}$, $z^P_{ss,17}$, and $z^P_{ss,18}$ found in the primitive scenario $sc^{SH}_{\hat{\lambda}_0}$ are about to be destroyed, as seen in Figures 4.9 and 4.10. In fact, although we would have drawn the same conclusions from our evaluation by having chosen for $\check{b} = 2.5$, it would have been done by contradicting the aforementioned assumption with respect to the parameters θ_X and θ_E, which, indeed, can be numerically verified.

But, what can we tell about scenario $sc^{SH}_{\hat{\lambda}_3}$? In fact, we must know how we can change the stability of the counterpart-in the scenario $sc^{SH}_{\hat{\lambda}_3}$-of the stable steady state $z^P_{ss,12}$ in the primitive scenario $sc^{SH}_{\hat{\lambda}_0}$. In fact, recalling the analysis of Huang's model in Chapter 3, and capitalizing upon the illustration in Figure 4.25, if we draw on (4.207) then we have that

$$x_{inf,n} = \theta_X \left(\frac{n-1}{n+1}\right)^{\frac{1}{n}} < \theta_X, \tag{4.241}$$

and that

$$\frac{d^2}{dX^2} g_{1,n}(X) < 0 \tag{4.242}$$

for $0 \leq X < x_{inf,n}$, and that

$$\frac{d^2}{dX^2} g_{1,n}(X) > 0 \tag{4.243}$$

for $X > x_{inf,n}$, which, in turn, implies that $\frac{d}{dX} g_{1,n}(X)$ is strictly increasing for $X > x_{inf,n}$, and strictly decreasing for $0 \leq X < x_{inf,n}$. Therefore, if

$$\frac{d}{dX} g_{1,n}(x_{inf,n}) \geq 0 \tag{4.244}$$

then $g_{1,n}(X)$ is strictly increasing for $X \geq 0$, which, in fact, amounts to the condition

$$\boxed{\theta_X \geq \frac{a_X}{k} \frac{n^2-1}{4n} \left(\frac{n+1}{n-1}\right)^{\frac{1}{n}},} \tag{4.245}$$

and by the same token, if

$$\boxed{\theta_E \geq \frac{a_E}{k} \frac{n^2-1}{4n} \left(\frac{n+1}{n-1}\right)^{\frac{1}{n}},} \tag{4.246}$$

then $g_{2,n}(E)$ is strictly increasing for $E \geq 0$. Therefore, by construction, one has that (4.245) implies that $\Psi_{1,n}(X)$ is strictly decreasing for $X \geq 0$, while (4.246) implies $\Psi_{2,n}(E)$ is strictly decreasing for $E \geq 0$.

Thereby, with respect to the scenario $sc^{SH}_{\hat{\lambda}_3}$, one has that the conditions (4.245) and (4.246) read

$$\theta_X \geq 0.8521645 \tag{4.247}$$

and

$$\theta_E \geq 0.8521645. \tag{4.248}$$

So, if we choose for $\hat{\theta}_X = \hat{\theta}_E = 1$ then we can shift from the scenario $sc^{SH}_{\hat{\lambda}_3}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_4}=[a_P=2,a_X=0.8,a_E=0.8,\hat{\theta}_X=1,\hat{\theta}_E=1,\hat{\hat{b}}=1,c=0.1,d=0.5,\hat{k}=1,n=4], \tag{4.249}$$

with the counterpart of the steady state $z^P_{ss,12}$ being unstable[1] in $sc^{SH}_{\hat{\lambda}_4}$, as seen in Figures 4.10 and 4.11.

Further, by inspecting (4.206), one might agree that if $\theta_X \gg 1$ then

$$\frac{d}{dX} g^P_{1,n}(X) \approx k_P, \tag{4.250}$$

that is, the graph of $g^P_{1,n}(X)$ is approximately a straight line, and so is the graph of $\Psi^P_{1,n}(X)$. Moreover, if we draw upon $(4.38)_1$ and if we regard $x^P_{b,n}(\theta_X)$ as a function of θ_X, one has that $x^P_{b,n}(\theta_X)$ decreases as θ_X increases. Hence, there must exist $\theta^{(c)}_X$ so that for $\hat{\hat{\theta}}_X \in [\theta^{(c)}_X, \infty)$ one has that we will end up in scenarios wherein the counterparts of the steady states $z^0_{ss,3}$, $z^0_{ss,4}$, $z^P_{ss,12}$, and $z^P_{ss,13}$ will have been extirpated. In fact, by choosing $\hat{\hat{\theta}}_X = 2$, one can shift from the scenario $sc^{SH}_{\hat{\lambda}_4}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_5}=[a_P=2,a_X=0.8,a_E=0.8,\hat{\hat{\theta}}_X=2,\hat{\theta}_E=1,\hat{\hat{b}}=1,c=0.1,d=0.5,\hat{k}=1,n=4], \tag{4.251}$$

wherein the counterparts of the steady states $z^0_{ss,3}$, $z^0_{ss,4}$, $z^P_{ss,12}$, and $z^P_{ss,13}$ have been destroyed, as seen in Figures 4.11 and 4.12.

Now, if one consults the expression (4.205) then one might agree that

$$\frac{d^2}{dX^2}\Psi^P_{1,n}(X) = O\left(nk_P^{-\left(2+\frac{1}{n}\right)}\right), \tag{4.252}$$

which, in turn, implies that the curvature of $\Psi^P_{1,n}$ decreases as $P > 0$ or $k > 0$ increases. By the same token, one has that the curvature of $\Psi^P_{2,n}$ decreases as $P > 0$ or $k > 0$ increases.

Now, bearing in mind that we wish we could recover the counterpart of the steady state $z^0_{ss,10}$, suitable to explain observation $(4.1)_1$, if we draw upon the estimate (4.294) then there must exist $P_c > 0$ for which one has that

$$\frac{d}{dX} g^P_{1,n}(X) \approx k_P, \tag{4.253}$$

[1]This is numerically verified.

and that

$$\frac{d}{dE} g_{2,n}^P(E) \approx k_P, \tag{4.254}$$

for all $P \gg P_c$, which implies that both $g_{1,n}^P(X)$ and $g_{2,n}^P(E)$ are approximately a straight line, and so are $\Psi_{1,n}^P(X)$ and $\Psi_{2,n}^P(E)$ respectively. Thus, intuitively, if $P_c > 0$ is sufficiently large then $\Psi_{1,n}^{P_c}(X)$ and $\Psi_{2,n}^{P_c}(E)$ are strictly decreasing so they intersect each other in a single point with the X and E-coordinate of the respective point being significantly small, which, indeed, is in line with the description of the pluripotent state.

But, how can we proceed to recover $z_{ss,10}^P$? In fact, one can numerically determine $P_c > 0$, and then choose

$$\boxed{a_P \geq k(P_c + 1),} \tag{4.255}$$

which, in this case, amounts to choosing

$$a_P \geq 6, \tag{4.256}$$

given that $P_c \approx 5$, as seen in Figure 4.27.

Hence, if we choose $\hat{a}_P = 6$ then we can shift from the scenario $sc_{\hat{\lambda}_5}^{SH}$ to the scenario

$$sc_{\hat{\lambda}_6}^{SH} = [\hat{a}_P = 6, a_X = 0.8, a_E = 0.8, \hat{\hat{\theta}}_X = 2, \theta_E = 1, \hat{\hat{b}} = 1, c = 0.1, d = 0.5, \hat{k} = 1, n = 4], \tag{4.257}$$

and seemingly, we have strategically restored $z_{ss,10}$, as seen in Figure 4.13.

However, if one draws upon (4.208) and (4.209) then one can conclude that unravelling scenarios similar to all the observations in (4.1) entails to change the curvature of Ψ_3. In fact, by invoking Assertion 01 in Section 1.5 with respect to $sc_{\hat{\lambda}_6}^{SH}$, even though one might argue that the respective scenario is suitable to explain the observation $(4.1)_1$, by invoking Assertion 02 in Section 1.5, one would have to set $a_P = 0$ so as to end up in a scenario that could be used to explain the observation $(4.1)_2$, which, in turn, would impede us from finding a scenario in such a decomposition that might be used to explain the observation $(4.1)_4$, that is, the one concerning the *Jammed state* J_E.

But, how can we stipulate conditions to avoid it ? In fact, with respect to $sc_{\hat{\lambda}_6}^{SH}$, it amounts to the sufficient conditions

$$\boxed{\frac{1}{\bar{e}_{z_E}} \left(\frac{\hat{\hat{a}}_P}{\hat{k}} - 1 \right) < \hat{c},} \tag{4.258}$$

and

$$\boxed{\frac{1}{\hat{c} x_{b,n}^{(0)}} \left(\frac{\hat{\hat{a}}_P}{\hat{k}} - 1 \right) < \hat{d},} \tag{4.259}$$

with $\bar{e}_{z_E}$ being the E-coordinate of the counterpart of the steady state $z_{ss,2}$ in the scenario $sc_{\hat{\lambda}_6}^{SH}$, so $\bar{e}_{z_E} \approx 1.8$. By inspection of Figure 4.27, one sees that $x_{b,n}^{(0)} \approx 1$. But, how to stipulate $\hat{\hat{a}}_P$? In fact, by invoking Assertion 02, if we scrutinize the scenario $sc^{SH}_{\hat{\lambda}_6}$ in the Figure 4.27 then, as we will see further, it is evident that choosing $\hat{\hat{a}}_P = 2$ is sufficient to give rise to a scenario that can be used to explain the observation $(4.1)_2$. Thereby, one has that

$$\hat{c} > 0.56, \tag{4.260}$$

and

$$\hat{d} > 1.8. \tag{4.261}$$

Nonetheless, predicated upon the assumption that all the parameters are of the same order of magnitude unless one can argue otherwise, if we choose $\hat{c} = 1$ and $\hat{d} = 2$ then we can shift from the scenario $sc^{SH}_{\hat{\lambda}_6}$ to

$$sc^{SH}_{\hat{\lambda}_7}=[\hat{a}_P=6,a_X=0.8,a_E=0.8,\hat{\hat{\theta}}_X=2,\hat{\theta}_E=1,\hat{\hat{b}}=1,\hat{c}=1,\hat{d}=2,\hat{k}=1,n=4], \tag{4.262}$$

which, by invoking the Assertion 1.5.1 in Section 1.5, is indeed similar to observation $(4.1)_1$, or better,

$$sc^{SH}_{\hat{\lambda}_7} \sim O_P^{\left(CHIR^+,PD^+,LIF^+,RA^-\right)}, \tag{4.263}$$

as seen in Figures 4.14 and 4.19. In fact, $\hat{a}_P = 6 > a_X = 0.8 = a_E$ embodies the self-renewal property of *mESCs* when being cultured with *Chiron*99021 [CHIR], *PD0*325901 [PD], and *Leukemia inhibitory factor* [LIF]. Indeed, under the latter conditions, one has that all the *mESCs* are maintained in the pluripotent state, which, in turn, is described by the scenario $sc^{SH}_{\hat{\lambda}_7}$ given that all the orbits in a vicinity of z_P goes towards z_P, i.e. the Pluri-equilibrium.

Further, if we choose $\hat{\hat{a}}_P = 2$ then we can shift from the scenario $sc^{SH}_{\hat{\lambda}_7}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_8}=[\hat{\hat{a}}_P=2,a_X=0.8,a_E=0.8,\hat{\hat{\theta}}_X=2,\hat{\theta}_E=1,\hat{\hat{b}}=1,\hat{c}=1,\hat{d}=2,\hat{k}=1,n=4], \tag{4.264}$$

which, by invoking the Assertion 1.5.2 in Section 1.5, is indeed similar to observation $(4.1)_2$, or better,

$$sc^{SH}_{\hat{\lambda}_8} \sim O_E^{\left(CHIR^-,PD^-,LIF^-,RA^-\right)}, \tag{4.265}$$

as seen in Figures 4.15 and 4.20. In fact, choosing $\hat{\hat{a}}_P = 2 < \hat{a}_P = 6$ causes z_P to be wiped out, which describes the removal of *Chiron*99021 [CHIR], *PD0*325901 [PD], and *Leukemia inhibitory factor* [LIF], leading *mESCs* to differentiate into *Ecto-like* cells, which, in turn, is captured by the scenario $sc^{SH}_{\hat{\lambda}_8}$, seeing that all the orbits initiating within a neighbourhood of the point z_P^{bif} being[2] characterized by the coordinates of z_P, goes towards z_E, that is, the Ecto-equilibrium.

Now, by appealing to Assertion 1.5.3 in Section 1.5, if we choose $\hat{\hat{\hat{\theta}}}_X = 1$ then we can shift from the scenario $sc^{SH}_{\hat{\lambda}_8}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_9}=[\hat{\hat{a}}_P=2,a_X=0.8,a_E=0.8,\hat{\hat{\hat{\theta}}}_X=1,\hat{\theta}_E=1,\hat{\hat{b}}=1,\hat{c}=1,\hat{d}=2,\hat{k}=1,n=4], \tag{4.266}$$

which is indeed similar to observation $(4.1)_3$, or better,

[2]The superscript "bif" represents this point after bifurcation of the respective steady state.

$$sc^{SH}_{\hat{\lambda}_9} \sim O^{(CHIR^-,PD^-,LIF^-,RA^+)}_{X,E}, \tag{4.267}$$

as seen in Figures 4.16 and 4.21. In fact, decreasing the parameter θ_X captures the addition of *retinoic acid* [RA] which, in turn, culminates in symmetry breaking, that is, just as much orbits within a neighbourhood of z_P^{bif} go either towards the Ecto-equilibrium, z_E, or towards the Xen-equilibrium, z_X.

However, if we build upon the intentions of the model, and indeed upon Assertion 1.5.4 in Section 1.5, then unravelling a scenario similar to observation $(4.1)_4$ do entail to lower the parameter d, which, by construction, embodies the suppression of the Xen-like population when only adding *PD0*325901 [PD] together with *retinoic acid* [RA]. However, lowering d biases the appearance of the Xen-jammed equilibrium z_{J_X}, which, in turn, implies that such a scenario cannot be similar to the observation $(4.1)_4$ whatsoever. In fact, this is illustrated by choosing $d = 0$ what leads us to shift from the scenario $sc^{SH}_{\hat{\lambda}_9}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_{10}} = [\hat{\hat{a}}_P = 2, a_X = 0.8, a_E = 0.8, \hat{\hat{\hat{\theta}}}_X = 1, \hat{\theta}_E = 1, \hat{\hat{b}} = 1, \hat{c} = 1, \hat{d} = 0, \hat{k} = 1, n = 4], \tag{4.268}$$

which is not similar to observation $(4.1)_4$, or better,

$$sc^{SH}_{\hat{\lambda}_{10}} \not\sim O^{(CHIR^-,PD^+,LIF^-,RA^+)}_{J_E,E}, \tag{4.269}$$

as seen in Figures 4.17 and 4.22. In fact, one has that just as much orbits within a vicinity of z_P^{bif} either go towards the Ecto-equilibrium z_E or towards the Xen-Jammed equilibrium z_{J_X}.

Consequently, by drawing on Assertion 1.5.5, if we keep on invoking the intentionality of the Semrau-Huang's model, then we can choose $\hat{\hat{d}} = 2$ and $\hat{\hat{\hat{\theta}}}_X = 0.5$ to shift from the scenario $sc^{SH}_{\hat{\lambda}_{10}}$ to the scenario

$$sc^{SH}_{\hat{\lambda}_{11}} = [\hat{\hat{a}}_P = 2, a_X = 0.8, a_E = 0.8, \hat{\hat{\hat{\theta}}}_X = 0.5, \hat{\theta}_E = 1, \hat{\hat{b}} = 1, \hat{c} = 1, \hat{\hat{d}} = 2, \hat{k} = 1, n = 4], \tag{4.270}$$

which is not similar to observation $(4.1)_5$, or better,

$$sc^{SH}_{\hat{\lambda}_{11}} \not\sim O^{(CHIR^-,PD^+,LIF^-,RA^+)}_{J_E,E} \xrightarrow{PD0325901^-,\ RA^+} O^{(CHIR^-,PD^-,LIF^-,RA^+)}_{E}, \tag{4.271}$$

as seen in Figures 4.18 and 4.23. In contrast to the respective experiment, in which the Jammed cells were differentiated further with retinoic acid [RA] in the absence of *PD0*325901 showing an enormous bias towards *Ecto-like* cells, one has that in the scenario $sc^{SH}_{\hat{\lambda}_{11}}$, the majority of the orbits within the vicinity of the point $z_{J_X}^{bif}$ being characterized by the coordinates of z_{J_X} in the scenario $sc^{SH}_{\hat{\lambda}_{10}}$, go toward the Xen-equilibrium z_X, which, in turn, is detrimental to Semrau-Huang's extension with respect to the observations in (4.1).

Hence, we have just seen that the intentions of the model seem not to fully manifest themselves in the model itself, which, in turn, leads us to conjecture that Semrau-Huang's model is inadequate to explain the observations 4.1. However, can we find an argument for that ? Or rather, can we somehow argue that Semrau-Huang's model contains a contradiction with respect to the observations 4.1 ? In fact, we will be exclusively addressing the latter questions in the next section.

4.6 An argument for the inadequacy of the model

If we rely upon the mathematical structure of the model then we can assert that a choice of parameters $d \gg 1$ and $c \ll 1$ biases the model to have scenarios in which the Ecto-equilibrium z_E coexists with the Ecto-jammed equilibrium z_{J_E}. However, $d \gg 1$ and $c \ll 1$ seems to contradict the description of the observation

$$O_{J_E,E}^{\left(CHIR^-,PD^+,LIF^-,RA^+\right)}. \tag{4.272}$$

In fact, given that d models the suppression of the Xen-like cells observed in (4.272) whereby the addition of PD0325901-a $MEKi$ inhibitor-was performed, vitiating MAPK/Erk signaling pathway as thoroughly described in Section 1.5, one has that such a scenario, containing the property $d \gg 1$ and $c \ll 1$, seems utterly inadequate to explain the observation (4.272).

However, we are not entitled to make the assertion that all the admissible scenarios in SC^{SH} to explain the observation (4.272), that is, the ones wherein the Ecto-equilibrium z_E coexists with the Ecto-jammed equilibrium z_{J_E} do have the property

$$d \gg 1 \wedge c \ll 1. \tag{4.273}$$

If we could demonstrate that then, predicated upon the assumption that all the parameters should have the same order of magnitude unless there was a reason to be otherwise, it would irrefutably imply that the model is inadequate.

Therefore, we are in need of a better argument to evaluate the adequacy of Semrau-Huang's model with respect to the observations

$$O_{J_E,E}^{\left(CHIR^-,PD^+,LIF^-,RA^+\right)} \xrightarrow{PD0325901^-,\ RA^+} \begin{array}{l} O_{J_E,E}^{\left(CHIR^-,PD^+,LIF^-,RA^+\right)}, \\ O_{E}^{\left(CHIR^-,PD^-,LIF^-,RA^+\right)}, \end{array} \tag{4.274}$$

respectively.

In fact, Let $z = (X^*, E^*, P^*)$ be a steady state of Semrau-Huang's model. if we recall the polynomial characteristic of the Jacobian matrix of Semrau-Huang's model, namely

$$p^{SH}(\lambda) = \lambda^3 + a_1^{SH}\lambda^2 + a_2^{SH}\lambda + a_3^{SH} \tag{4.275}$$

with

$$a_1^{SH}(z^*) = -\left(D\tilde{F}_{33}(z^*) + \operatorname{Tr} DF_{k_P}^{H}(z^*)\right), \tag{4.276}$$

and

$$\begin{aligned} a_2^{SH}(z^*) = \operatorname{Det} DF_{k_P}^{H}(z^*) + D\tilde{F}_{33}(z^*) \operatorname{Tr} DF_{k_P}^{H}(z^*) \\ + D\tilde{F}_{32}(z^*)D\tilde{F}_{23}(z^*) + D\tilde{F}_{13}(z^*)D\tilde{F}_{31}(z^*), \end{aligned} \tag{4.277}$$

and

$$\begin{aligned} a_3^{SH} = -D\tilde{F}_{33}(z^*) \operatorname{Det} DF_{k_P}^{H}(z^*) + D\tilde{F}_{11}(z^*)D\tilde{F}_{32}(z^*)D\tilde{F}_{23}(z^*) \\ -D\tilde{F}_{21}(z^*)D\tilde{F}_{32}(z^*)D\tilde{F}_{13}(z^*) - D\tilde{F}_{12}(z^*)D\tilde{F}_{31}(z^*)D\tilde{F}_{23}(z^*) \\ +D\tilde{F}_{22}(z^*)D\tilde{F}_{31}(z^*)D\tilde{F}_{13}(z^*), \end{aligned} \tag{4.278}$$

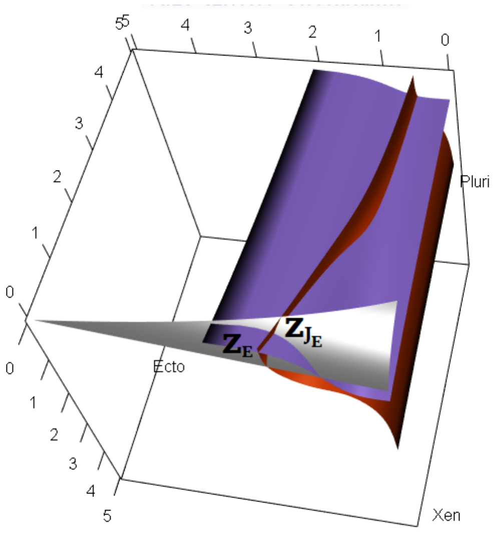


Figure 4.28: $sc^{SH}_{\nu_0}$

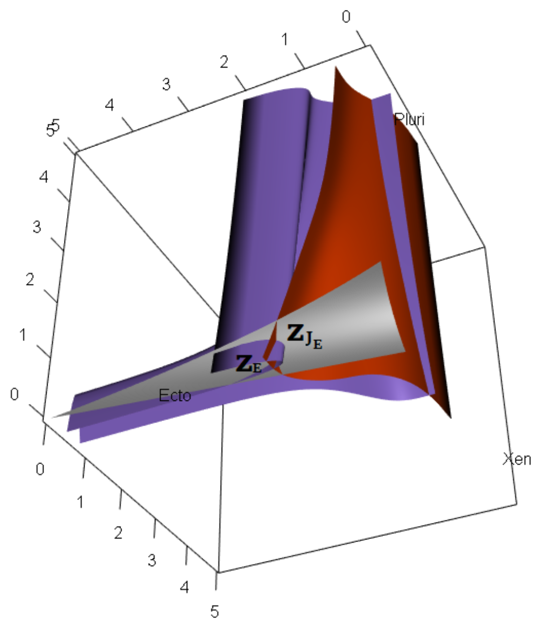


Figure 4.29: $sc^{SH}_{\nu_1}$

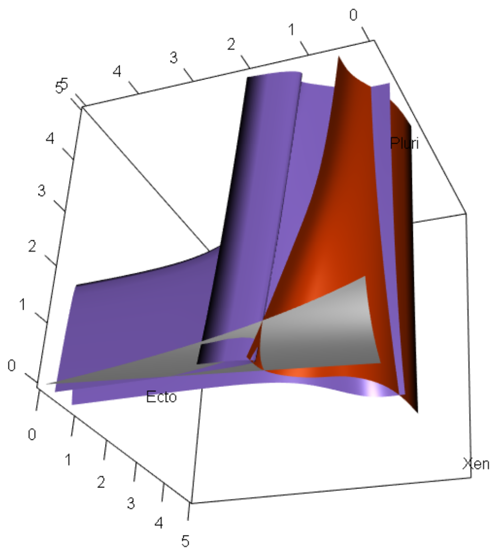


Figure 4.30: $sc^{SH}_{\nu_2}$

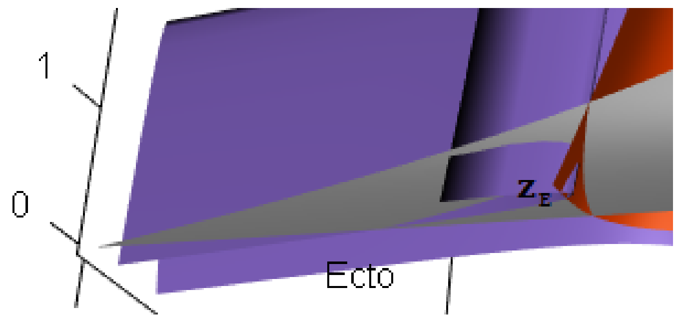


Figure 4.31: $sc^{SH}_{\nu_2}$

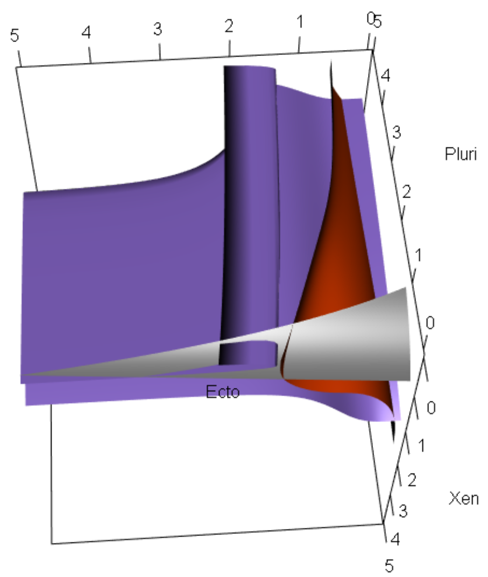


Figure 4.32: $sc^{SH}_{\nu_3}$

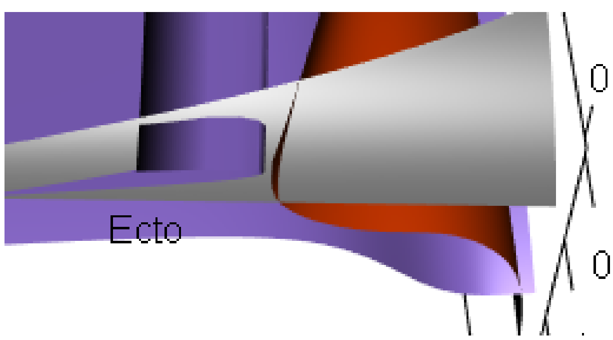


Figure 4.33: $sc^{SH}_{\nu_3}$

then, by drawing on (4.102), one has that the terms of the polynomial (4.275), that is, a_1^{SH}, a_2^{SH}, and a_3^{SH} are explicitly dependent upon θ_X through $D\tilde{F}_{11}$. In fact, if we recall that

$$D\tilde{F}_{11}(X,E,P) = -\frac{d}{dX}g^{P}_{1,n}(X) = -\left[k_P - na_X\theta_X^n\frac{X^{n-1}}{\left(\theta_X^n + X^n\right)^2}\right], \tag{4.279}$$

then we can conveniently rewrite $D\tilde{F}_{11}$ as

$$D\tilde{F}_{11}(X,E,P) = -\left\{k_P - a_X\left[\frac{nX^{n-1}}{(\theta_X^n + X^n)} - n\frac{X^{2n-1}}{(\theta_X^n + X^n)^2}\right]\right\}. \tag{4.280}$$

On the other hand, one has that

$$\begin{aligned} \tilde{F}_1(X,E,P) &= 0, \\ \tilde{F}_2(X,E,P) &= 0, \\ \tilde{F}_1(X,E,P) &= 0, \end{aligned} \tag{4.281}$$

in particular, implies that

$$a_X\frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n} - kX(1+cP) = 0, \tag{4.282}$$

which implies that

$$\frac{nX^{n-1}}{(\theta_X^n + X^n)} = \frac{n}{a_X X}\left[kX(1+cP) - b\frac{1}{1+E^n}\right], \tag{4.283}$$

and that

$$n\frac{X^{2n-1}}{(\theta_X^n + X^n)^2} = \frac{n}{a_X^2 X^3}\left[kX(1+cP) - b\frac{1}{1+E^n}\right]^2. \tag{4.284}$$

Now, if we use the equality's (4.283) and (4.284) in (4.285) then we arrive at

$$\begin{aligned} D\tilde{F}_{11}(X,E,P) = -k_P &+ \frac{n}{X}\left[kX(1+cP) - b\frac{1}{1+E^n}\right] \\ &- \frac{n}{a_X X^3}\left[kX(1+cP) - b\frac{1}{1+E^n}\right]^2, \end{aligned} \tag{4.285}$$

which means that $D\tilde{F}_{11}$ is indeed implicitly independent upon θ_X. Instead, one can say that the it depends upon the point at which the Jacobian matrix is being computed.

But, what can we conclude from the latter elucidation with respect to Semrau-Huang's model? In fact, it implies that the roots of the characteristic polynomial of the Jacobian matrix of Semrau-Huang's model (4.275) are implicitly independent on θ_X. Hence, according to the later elucidations, one has that z_E and z_{J_E} do coexist for the same range of admissible θ_X's. By the same token, one has that z_E and z_{J_E} do not exist for the same range of admissible θ_X's.

To understand the latter and the former claims, we have numerically simulated that. Indeed, as seen in Figures 4.28, 4.29, 4.30 and 4.31, one has that z_E and z_{J_E} do coexist in the scenarios

$$sc_{\nu_0}^{SH}=[a_P=2,a_X=0.8,a_E=0.8,\theta_X=10^4,\theta_E=1,b=1,c=0.2,d=7,k=1,n=4] \quad (4.286)$$

and

$$sc_{\nu_1}^{SH}=[a_P=2,a_X=0.8,a_E=0.8,\theta_X=0.4,\theta_E=1,b=1,c=0.2,d=7,k=1,n=4]. \quad (4.287)$$

Actually, z_E and z_{J_E} do coexist in the scenarios

$$sc_{\nu_s}^{SH}=[a_P=2,a_X=0.8,a_E=0.8,\theta_X=s,\theta_E=1,b=1,c=0.2,d=7,k=1,n=4] \quad (4.288)$$

with $s \in [0.4, \infty)$. On the other hand, z_E and z_{J_E} do not coexist in the scenario

$$sc_{\nu_2}^{SH}=[a_P=2,a_X=0.8,a_E=0.8,\theta_X=0.33,\theta_E=1,b=1,c=0.2,d=7,k=1,n=4], \quad (4.289)$$

wherein z_{J_E} has been destroyed, and do not exist in the scenario

$$sc_{\nu_3}^{SH}=[a_P=2,a_X=0.8,a_E=0.8,\theta_X=0.28,\theta_E=1,b=1,c=0.2,d=7,k=1,n=4], \quad (4.290)$$

in which both z_E and z_{J_E} have been destroyed, as seen in Figures 4.30, 4.31, 4.32 and 4.33 respectively. In fact, one has that z_E and z_{J_E} do not coexist in the scenarios

$$sc_{\nu_r}^{SH}=[a_P=2,a_X=0.8,a_E=0.8,\theta_X=r,\theta_E=1,b=1,c=0.2,d=7,k=1,n=4] \quad (4.291)$$

with $r \in [0.28, 0.33]$, and do not exist in the scenarios

$$sc_{\nu_q}^{SH}=[a_P=2,a_X=0.8,a_E=0.8,\theta_X=q,\theta_E=1,b=1,c=0.2,d=7,k=1,n=4] \quad (4.292)$$

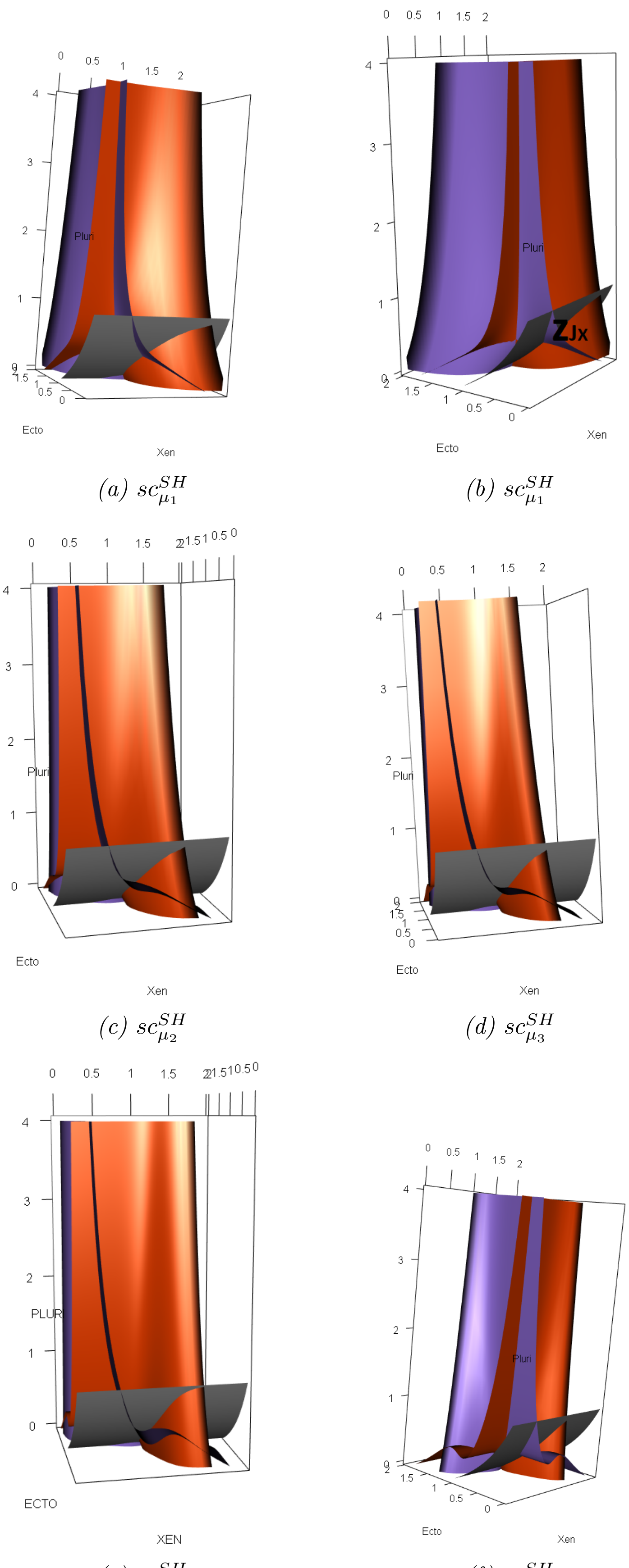
with $q \in [0, 0.28)$.

Therefore, based on the geometrical aspects of the model, if we acknowledge that for the main components of $\left(SH_n[\breve{C}_{i,X}, \breve{C}_{j,E}, \breve{C}_{r,P}]\right)_{i,j,r}$, one has that z_E and z_{J_E} do plausibly coexist in a closed region of $\mathbb{R}^3$, then, according to the latter elucidations, when varying parameter θ_X, one has that z_E and z_{J_E} do not coexist in the respective closed region of $\mathbb{R}^3$ for a tiny interval of admissible θ_X's.

Hence, the latter elucidation of Semrau-Huang's model strongly motivates us to rule out the model as a conceptual mechanism to explain the observation

$$O_{J_E,E}^{(CHIR^-,PD^+,LIF^-,RA^+)} \xrightarrow{PD0325901^-,\ RA^+} O_E^{(CHIR^-,PD^-,LIF^-,RA^+)}, \quad (4.293)$$

given that it leads us to conjecture that the model has indeed a contradiction. In fact, even though one could find d and c compatible with the intentionality of Semrau-Huang's model towards the observation $(4.274)_1$, that is, even though one could find d and c for which z_E and z_{J_E} do coexist in a respective scenario similar to the observation $(4.274)_1$, one has that z_E and z_{J_E} do bifurcate in the same parameter range what is harmful for the model itself. However, if we want to be entitled to rule out the model as a conceptual mechanism to explain $(4.274)_2$ then we need to give a formal proof for the latter conjecture.



(a) $sc^{SH}_{\mu_1}$ (b) $sc^{SH}_{\mu_1}$ (c) $sc^{SH}_{\mu_2}$ (d) $sc^{SH}_{\mu_3}$ (e) $sc^{SH}_{\mu_4}$ (f) $sc^{SH}_{\mu_4}$

Figure 4.34: The X, E, P-nullclines for $\mu_1 = (a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 1, d = 0, k = 1, n = 4), \mu_2 = (\ldots, n = 6), \mu_3 = (\ldots, n = 8)$, and $\mu_4 = (\ldots, n = 10)$.

4.7 The numerical computation of two Andronov–Hopf bifurcations in $SH_n[\tilde{C}_{3,X}, \tilde{C}_{3,E}, \tilde{C}_{-1,P}]$

Based on the paradigm that oscillations can be seen as a interplay between a fast positive feedback and a slow negative feedback, one can wonder whether or not Semrau-Huang's model contains stable limit cycles. To argue that it is indeed the case, we will be giving a geometric argument for that. First, we have that the interplay between a fast positive feedback and a slow negative feedback is embodied in the parameters c and d.

How will we proceed then ? In fact, if we recall that

$$\frac{d^2}{dX^2}\Psi^P_{1,n}(X) = O\left(nk_P^{-\left(2+\frac{1}{n}\right)}\right), \tag{4.294}$$

then we have that $\frac{d^2}{dX^2}\Psi^P_{1,n}(X)$ increases as $n > 1$ increases, and decreases as k_P increases. Therefrom, we conjecture that the curvature of the X-nullcline increases as n increases within a sufficiently large neighborhood of the Xen-jammed equilibrium z_{J_X} shown in Figures 4.34 (a) and (b) for the scenario

$$sc^{SH}_{\mu_1} = [a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 1, d = 0, k = 1, n = 4], \tag{4.295}$$

respectively. Regarding the respective scenario, one has that

$$\theta_X = 1 > \frac{a_X}{k} = 0.8, \tag{4.296}$$

and that

$$\theta_E = 1 > \frac{a_E}{k} = 0.8, \tag{4.297}$$

and that the condition

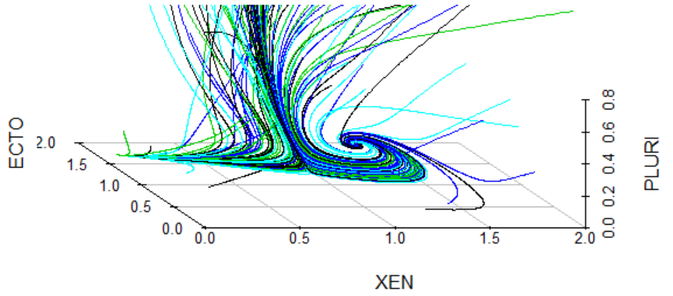
$$\breve{C}_{-1,P} : 0 < \frac{1}{c}\left(\frac{a_P}{k} - 1\right) < e^{(0)}_{b,n} + dx^{(0)}_{b,n} \tag{4.298}$$

is satisfied for the representative $\mu_1 = (a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 1, d = 0, k = 1, n = 4)$. So, we have that $sc^{SH}_{\mu_1}$ is actually the scenario

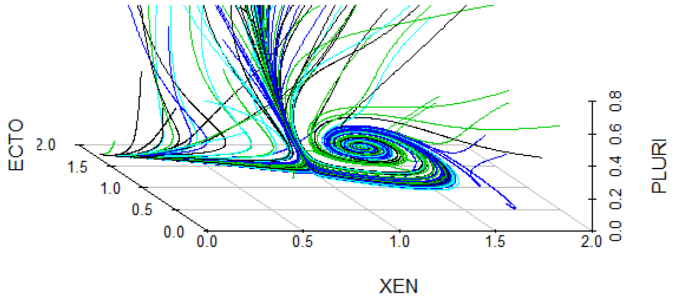
$$SH_4[\tilde{C}_{3,X}, \tilde{C}_{3,E}, \tilde{C}_{-1,P}].$$

But, what about the raised conjecture? In fact, we have numerically verified that the latter conjecture seems to be true as seen in Figures 4.34 (a)-(f). In fact, in Figures 4.34 (a) and (f), one clearly sees a significant increase in the curvature of the X, E-nullclines in the vicinity of the Xen-jammed equilibrium z_{J_X}. Likewise, we could find a similar expression for $\Psi^P_{2,n}$ leading to the similar assertion.

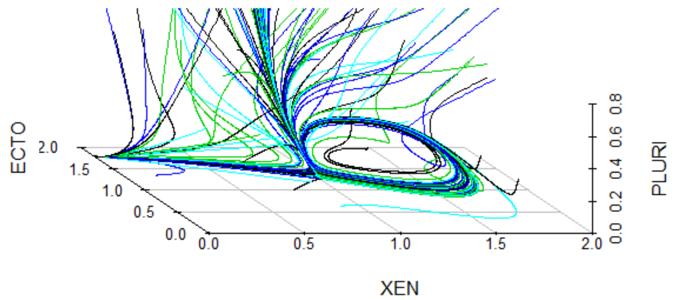
But, what does the change in the curvatures of the X, E-nullclines have to do with the emergence of an Andronov-Hopf bifurcation at the Xen-jammed equilibrium z_{J_X}? In fact, heuristically, a geometric change of the nullclines in the vicinity of a stable equilibrium can lead the respective equilibrium to become a stable spiral, which, in turn, owing to the attainment of a critical value by the varying parameter, can culminate in the emergence of a limit cycle, as numerically verified in Figures 4.35 (a)-(f). However, what is the compromise with the interplay between $c = 1$ and $d = 0$? In fact, drawing upon (4.208), one has that



(a) Orbits in $sc^{SH}_{\mu_1} = [a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 1, d = 0, k = 1, n = 4]$.



(b) Orbits in $sc^{SH}_{\mu_2} = [a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 1, d = 0, k = 1, n = 6]$.



(c) Orbits in $sc^{SH}_{\mu_4} = [a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 1, d = 0, k = 1, n = 8]$.

Figure 4.35: The Andronov-Hopf bifurcation of the Xen-jammed equilibrium.

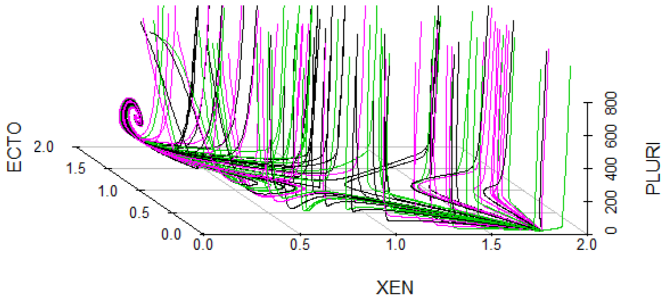
$$\frac{\partial^2}{\partial X^2}\Psi_3(X, E) \equiv 0, \tag{4.299}$$

and that

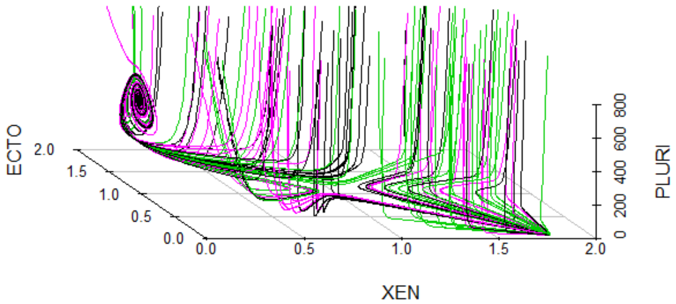
$$\frac{\partial^2}{\partial E^2}\Psi_3(X, E) \approx 2a_P c^2 \frac{1}{k} \tag{4.300}$$

in the vicinity of z_{J_X}, which, in turn, geometrically speaking, implies that the curvatures of the X, E-nullclines solely account for the emergence of the limit cycle seen in Figures 4.35 (a)-(c).

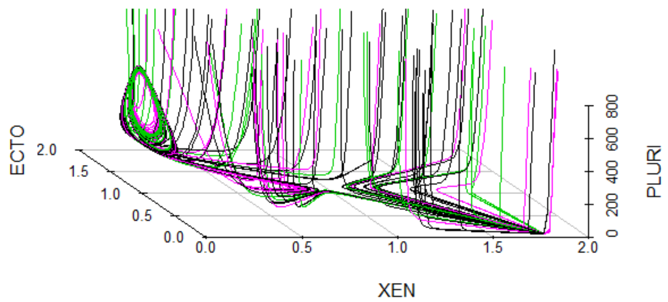
A similar reasoning can be used to explain the emergence of the Ecto-limit cycle related to the Ecto-jammed equilibrium z_{J_E}, seen in Figures (4.36) (a)-(c). Regard-



(a) Orbits in $sc^{SH}_{\sigma_1} = [a_P = 1000, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 0.001, d = 25000, k = 1, n = 10]$.



(b) Orbits in $sc^{SH}_{\sigma_2} = [a_P = 1000, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 0.001, d = 25000, k = 1, n = 20]$.



(c) Orbits in $sc^{SH}_{\sigma_3} = [a_P = 1000, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 0.001, d = 25000, k = 1, n = 30]$.

Figure 4.36: The Andronov-Hopf bifurcation of the Ecto-jammed equilibrium.

ing the simulations of the latter, one has that $c = 0.001$ and $d = 2.5 * 10000$, which implies that

$$\frac{\partial^2}{\partial X^2}\Psi_3(X, E) \approx 2a_P c^2 d^2 \frac{1}{k} \tag{4.301}$$

and that

$$\frac{\partial^2}{\partial E^2}\Psi_3(X, E) \approx O(10^{-6}), \tag{4.302}$$

in the vicinity of z_{J_E}.

But, can we give a better argument for the interplay between the parameters c, d and n? Or better, can we derive necessary conditions for the emergence of a Andronov-Hopf bifurcation at the Xen-Jammed equilibrium or/and at the Ecto-Jammed equilibrium? In fact, by recalling the expression of the polynomial characteristic of the Jacobian matrix of Semrau-Huang's model in (4.275), if we define

$$\lambda := t - \frac{a_1^{SH}}{3}$$

and if we invoke Cordano's method [4] to solve a cubic equation then we can eliminate the square term of the equation

$$\lambda^3 + a_1^{SH}\lambda^2 + a_2^{SH}\lambda + a_3^{SH} = 0, \tag{4.303}$$

which, in turn, becomes

$$t^3 + \vartheta t + \varpi = 0 \tag{4.304}$$

with

$$\vartheta = a_2^{SH} - \frac{\left(a_1^{SH}\right)^2}{3} \tag{4.305}$$

and

$$\varpi = a_3^{SH} - \frac{\left[2\left(a_1^{SH}\right)^2 - 9a_1^{SH}a_2^{SH}\right]}{27}. \tag{4.306}$$

As for the onset of the Andronov-Hopf bifurcation, one wants to find conditions for which the characteristic polynomial has complex roots. Indeed, if it is true that

$$27\varpi^2 + 4\vartheta^3 < 0 \tag{4.307}$$

then the characteristic polynomial (4.303) has two non-real complex conjugate roots and one real root, as shown in [4]. This follows from Cordano's method as we shall now explain. Hence, if t_0 represents the real root and if t_1 and t_2 symbolizes the two complex conjugate roots of (4.304) then one has that the expressions

$$\begin{aligned}\lambda_0 &= t_0 - \frac{a_1^{SH}}{3},\\ \lambda_1 &= t_1 - \frac{a_1^{SH}}{3},\\ \lambda_2 &= t_2 - \frac{a_1^{SH}}{3},\end{aligned} \tag{4.308}$$

provide the corresponding roots of the characteristic polynomial (4.303). But, how to determine t_0, t_1, and t_2 ? In fact, if σ_1 and σ_2 represent, under (4.307), the two complex conjugate roots of the quadratic equation

$$\sigma^2 + \varpi\sigma - \frac{\vartheta^3}{27} = 0 \tag{4.309}$$

then one has that

$$t_1 = \sigma_1 - \frac{\vartheta}{3\sigma_1} = 0, \tag{4.310}$$

and that

$$t_2 = \sigma_2 - \frac{\vartheta}{3\sigma_2} = 0, \tag{4.311}$$

with

$$\sigma_1 = \frac{-\varpi - i\frac{\sqrt{3}}{9}\sqrt{-\left(27\varpi^2 + 4\vartheta^3\right)}}{2} \tag{4.312}$$

and

$$\sigma_2 = \frac{-\varpi + i\frac{\sqrt{3}}{9}\sqrt{-\left(27\varpi^2 + 4\vartheta^3\right)}}{2}, \tag{4.313}$$

or better,

$$\sigma_1 = -\frac{\varpi}{2} - i\Sigma_0 \tag{4.314}$$

and

$$\sigma_2 = -\frac{\varpi}{2} + i\Sigma_0, \tag{4.315}$$

wherein

$$\Sigma_0 = \frac{\sqrt{3}}{18}\sqrt{-\left(27\varpi^2 + 4\vartheta^3\right)} > 0, \tag{4.316}$$

and we arrive at

$$\begin{aligned} \lambda_1 &= -\left[\frac{a_1^{SH}}{3} + \frac{\varpi}{2} - \frac{\varpi}{6}\frac{\vartheta}{\left(\frac{\varpi^2}{4} + \Sigma_0^2\right)}\right] - i\left[1 + \frac{\vartheta}{3\left(\frac{\varpi^2}{4} + \Sigma_0^2\right)}\right]\Sigma_0, \\ \lambda_2 &= -\left[\frac{a_1^{SH}}{3} + \frac{\varpi}{2} - \frac{\varpi}{6}\frac{\vartheta}{\left(\frac{\varpi^2}{4} + \Sigma_0^2\right)}\right] + i\left[1 + \frac{\vartheta}{3\left(\frac{\varpi^2}{4} + \Sigma_0^2\right)}\right]\Sigma_0, \end{aligned} \tag{4.317}$$

which are the expressions for the two complex conjugate roots of (4.303) respectively.

Now, if we consistently assume that

$$E_{J_X} \approx 0,$$

and that

$$P_{J_X} \approx \left(\frac{a_P}{k} - 1\right)$$

then, by invoking (4.134) and (4.153), we have that

$$\begin{aligned} a_1^{SH}(z_{J_X}) &= -\left(D\tilde{F}_{33}(z_{J_X}) + \operatorname{Tr} DF_{k_P}^H(z_{J_X})\right) \\ &\approx -\left[\frac{k^2}{a_P} - k(1 + cdX_{J_X}) - 2k_P + \frac{n}{2^{n+2}}\right] \\ &\approx -\left\{\frac{k^2}{a_P} - k(1 + cdX_{J_X}) - 2k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right] + \frac{n}{2^{n+2}}\right\}, \end{aligned} \tag{4.318}$$

and that

$$\begin{aligned} a_2^{SH}(z_{J_X}) &= \text{Det}\, DF_{k_P}^H(z_{J_X}) + D\tilde{F}_{33}(z_{J_X})\,\text{Tr}\, DF_{k_P}^H(z_{J_X}) \\ &\quad + D\tilde{F}_{32}(z_{J_X})D\tilde{F}_{23}(z_{J_X}) + D\tilde{F}_{13}(z_{J_X})D\tilde{F}_{31}(z_{J_X}), \\ &\approx D\tilde{F}_{11}(z_{J_X})D\tilde{F}_{22}(z_{J_X}) + D\tilde{F}_{33}(z_{J_X})\,\text{Tr}\, DF_{k_P}^H(z_{J_X}) \\ &\quad + D\tilde{F}_{13}(z_{J_X})D\tilde{F}_{31}(z_{J_X}) \\ &\approx k_P\left(k_P - \frac{n}{2^{n+2}}\right) + \left[\frac{k^2}{a_P} - k(1+cdX_{J_X})\right]\left(-2k_P + \frac{n}{2^{n+2}}\right) \\ &\quad + k^2c^2d\left(\frac{a_P}{k} - 1\right)X_{J_X} \\ &\approx k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right]\left\{k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right] - \frac{n}{2^{n+2}}\right\} \\ &\quad + \left[\frac{k^2}{a_P} - k(1+cdX_{J_X})\right]\left\{-2k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right] + \frac{n}{2^{n+2}}\right\} \\ &\quad + k^2c^2d\left(\frac{a_P}{k} - 1\right)X_{J_X}, \end{aligned} \tag{4.319}$$

and that

$$\begin{aligned} a_3^{SH}(z_{J_X}) &= -D\tilde{F}_{33}(z_{J_X})\,\text{Det}\, DF_{k_P}^H(z_{J_X}) + D\tilde{F}_{11}(z_{J_X})D\tilde{F}_{32}(z_{J_X})D\tilde{F}_{23}(z_{J_X}) \\ &\quad - D\tilde{F}_{21}(z_{J_X})D\tilde{F}_{32}(z_{J_X})D\tilde{F}_{13}(z_{J_X}) - D\tilde{F}_{12}(z_{J_X})D\tilde{F}_{31}(z_{J_X})D\tilde{F}_{23}(z_{J_X}) \\ &\quad + D\tilde{F}_{22}(z_{J_X})D\tilde{F}_{31}(z_{J_X})D\tilde{F}_{13}(z_{J_X}) \\ &\approx -\left[\frac{k^2}{a_P} - k(1+cdX_{J_X})\right]k_P\left(k_P - \frac{n}{2^{n+2}}\right) + b\frac{nX_{J_X}^n}{\left[1+X_{J_X}^n\right]^2}k^2c^2\left(\frac{a_P}{k} - 1\right) \\ &\quad - k_Pk^2c^2d\left(\frac{a_P}{k} - 1\right)X_{J_X} \\ &\approx -k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right]\left\{k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right] - \frac{n}{2^{n+2}}\right\}\left[\frac{k^2}{a_P} - k(1+cdX_{J_X})\right] \\ &\quad + b\frac{nX_{J_X}^n}{\left[1+X_{J_X}^n\right]^2}k^2c^2\left(\frac{a_P}{k} - 1\right) - k\left[1 + c\left(\frac{a_P}{k} - 1\right)\right]k^2c^2d\left(\frac{a_P}{k} - 1\right)X_{J_X} \end{aligned} \tag{4.320}$$

can be taken as suitable approximations so as to deduce meaningful necessary conditions. For convenience purposes, we have highlighted the terms containing cd in orange and the terms containing n in purple in the expression of the approximations (4.318), (4.319), and (4.320).

But, what do we mean with meaningful necessary conditions? In fact, in view of (4.307), one has that a necessary condition for the existence of complex roots is that

$$\vartheta < 0, \tag{4.321}$$

or rather,

$$a_2^{SH} < \frac{\left(a_1^{SH}\right)^2}{3}. \tag{4.322}$$

More specifically, (4.322) is a necessary condition for the roots of the characteristic polynomial $p^{SH}(z_{J_X})$ to be comprised by one real root and two complex conjugate roots. So, by having a look at the approximations (4.318) and (4.319) respectively, we claim that(4.322) can lead us to a condition on the interplay between c and d that might tell us how the stable equilibrium z_{J_X} becomes an stable spiral. Moreover, given that

$$\frac{n}{2^{n+2}} < 1$$

and that

$$\lim_{n\to+\infty} \frac{n}{2^{n+2}} = 0,$$

one expects that the terms containing n in the approximations (4.318) and (4.319) will not play an essential role in the condition (4.322), i.e. in the onset of a stable spiral at z_{J_X}.

However, where will the parameter n play an essential role then ? In fact, under (4.307), by invoking (4.317), a necessary condition for the emergence of a Andronov-Hopf bifurcation at z_{J_X} is that

$$\frac{a_1^{SH}(z_{J_X})}{3} + \frac{\varpi(z_{J_X})}{2} - \frac{\varpi(z_{J_X})}{6}\frac{\vartheta(z_{J_X})}{\left(\frac{\varpi^2(z_{J_X})}{4} + \Sigma_0^2(z_{J_X})\right)} = 0 \tag{4.323}$$

with

$$\vartheta(z_{J_X}) = a_2^{SH}(z_{J_X}) - \frac{\left(a_1^{SH}(z_{J_X})\right)^2}{3} < 0, \tag{4.324}$$

and

$$\varpi(z_{J_X}) = a_3^{SH}(z_{J_X}) - \frac{\left[2\left(a_1^{SH}(z_{J_X})\right)^2 - 9a_1^{SH}(z_{J_X})a_2^{SH}(z_{J_X})\right]}{27}, \tag{4.325}$$

and

$$\Sigma_0(z_{J_X}) = \frac{\sqrt{3}}{18}\sqrt{-\left(27\varpi^2(z_{J_X}) + 4\vartheta^3(z_{J_X})\right)} > 0, \tag{4.326}$$

and we might solve (4.323) for n.

Consistently, we turn our attention towards the term

$$\frac{nX_{J_X}^n}{\left[1 + X_{J_X}^n\right]^2} = O(n),$$

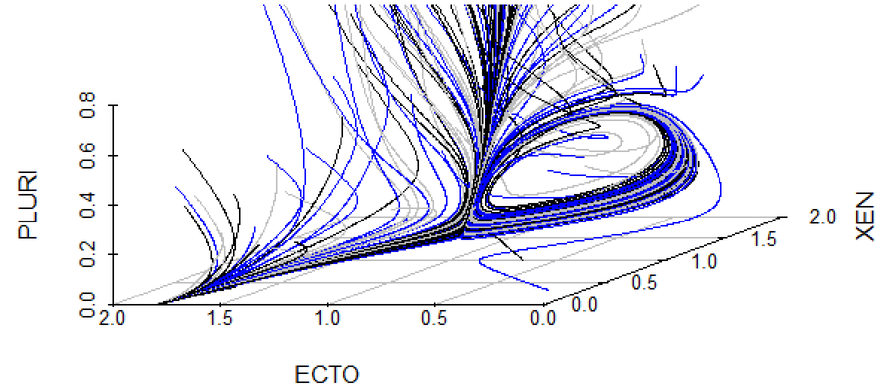
which is contained in the expression of $a_3^{SH}(z_{J_X})$. By invoking the Routh-Hurwith conditions (4.4.2), z_{J_X} is a stable equilibrium, or a stable spiral, if and only if

$$\begin{aligned} a_1^{SH}(z_{J_X}) &> 0, \\ a_3^{SH}(z_{J_X}) &> 0, \\ a_1^{SH}(z_{J_X})a_2^{SH}(z_{J_X}) - a_3^{SH}(z_{J_X}) &> 0, \end{aligned} \tag{4.327}$$

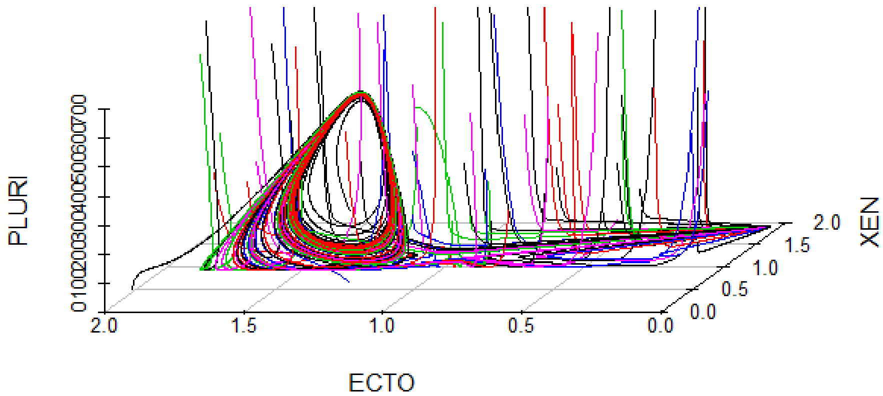
holds. If $E_{J_X} \approx 0$ and $d \ll 1$ is sufficiently small to hold $a_1^{SH} > 0$ and $a_3^{SH} > 0$ true, then a bifurcation occurs when

$$a_1^{SH}(z_{J_X})a_2^{SH}(z_{J_X}) - a_3^{SH}(z_{J_X}) \leq 0.$$

Therefore, by sufficiently increasing the parameter n, one has that a stable spiral at z_{J_X} undergoes a Hopf-Andronov bifurcation through which a stable limit cycle



(a) Orbits in $sc^{SH}_{\mu_4} = [a_P = 2, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 1, d = 0, k = 1, n = 8]$.



(b) Orbits in $sc^{SH}_{\sigma_2} = [a_P = 1000, a_X = 0.8, a_E = 0.8, \theta_X = 1, \theta_E = 1, b = 1, c = 0.001, d = 25000, k = 1, n = 30]$.

Figure 4.37: The two Andronov-Hopf bifurcation of the Xen-jammed and Ecto-jammed equilibria.

emerges. This is in line with our intuitive geometric argument and with the numerical experiments shown in Figure 4.37. Lastly, yet consistent with our geometric argument, we assert that there can occur no oscillations in the scenario

$$SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}],$$

seeing that, as n increases, the curvature of the nullclines in the vicinity of the jammed equilibria $[z^P_{ss,11}, z^P_{ss,13}]$ tend to zero.

4.8 Discussion

As we have seen through this chapter, we can apply the systematic procedure established in Chapter 2 so as to evaluate Semrau-Huang's model. Upon doing so, we found the primitive scenarios of the model which unravelled the maximal number of steady states that the model can generate.

Moreover, we have drawn upon Chapter 3 to access the (in)stability of the steady states of Semrau-Huang's model at the level $P = 0$, that is, the ones given by Huang's model. In order to have a clue as to the (in)stability of the steady states of Semrau-Huang's model at a level $P > 0$, we have invoked the Routh-Hurwith conditions with which, by means of suitable approximations of the coefficients of the characteristic polynomial of the Jacobian matrix of the model, we have understood the stability of key steady states produced by Semrau-Huang's model [$z^P_{ss,10}$, $z^P_{ss,11}$, $z^P_{ss,12}$, $z^P_{ss,13}$] verified by the numerical experiments.

Concerning the evaluation itself, we have provided a suitable decomposition of a primitive scenario unravelling scenarios similar to the observations of the experiments. Nonetheless, this decomposition strongly suggests that the model does have a contradiction with respect to the properties of the observations (4.1) described in Section 1.5.

In fact, we have argued that the Ecto equilibrium z_E [$z^0_{ss,2}$] and the Ecto-jammed equilibrium z_{J_E} [$z^P_{ss,11}$] do bifurcate in the same parameter range with respect to θ_X. Furthermore, we have argued that the coexistence of the Ecto equilibrium z_E [$z^0_{ss,2}$] and the Ecto-jammed equilibrium z_{J_E} [$z^P_{ss,11}$] can only occur under a condition contradicting one property of the observation $(4.1)_4$. In fact, the Ecto-jammed equilibrium, described in Section 1.5 and represented in $(4.1)_4$, emerges upon the abrogation of the *Mek-Erk pathway*, which, in turn, suppresses the population of *Xen-like* cells. This is captured by the parameter d in the model, which, in this case, should be sufficiently small $d \ll 1$.

However, with respect to the counterpart equilibrium generated by the model, that is, the Ecto-jammed equilibrium z_{J_E} [$z^P_{ss,11}$] , one has that finding a scenario wherein z_{J_E} [$z^P_{ss,11}$] and z_E [$z^0_{ss,2}$] coexist, is favoured by $d \gg 1$. So, if we assume that the described role of the parameter d is in line with the intentions of the modelling agent, then the latter property of the model is inconsistent with the observations $(4.1)_{4,5}$. Therefore, if we give a formal proof for the aforesaid claims about the properties of the model, then we will have proved that Semrau-Huang's model has a property that contradicts the observations $(4.1)_{4,5}$.

Further, we have found oscillations in Semrau-Huang's model. In fact, we have numerically shown that the Ecto and the Xen-jammed equilibria undergo Andronov-Hopf bifurcation when the parameter n increases under a suitable interplay of the parameters c and d. Those numerical experiments led us to conjecture that the onset of such a bifurcation at the jammed equilibria is essentially determined by the curvature of the X and E-nullclines at level $P > 0$, that is, $\frac{d^2}{dX^2}\Psi^P_{1,n}(X)$ and $\frac{d^2}{dE^2}\Psi^P_{2,n}(E)$.

Thereby, one has that oscillations are not to be found in the scenario

$$SH_n[\breve{C}_{1,X}, \breve{C}_{1,E}, \breve{C}_{1,P}, \breve{C}_{0,n}, \breve{C}_{1,n}, \breve{C}_{2,n}],$$

wherein the curvatures $\frac{d^2}{dX^2}\Psi^P_{1,n}(X)$ and $\frac{d^2}{dE^2}\Psi^P_{2,n}(E)$, in the vicinity of the Xen- and Ecto-jammed equilibria [$z^P_{ss,11}$, $z^P_{ss,13}$], tend to zero as n increases. Lastly, we have used the Cordano method to solve cubic equations so as to analytically understand how the interplay between c and d stipulates that z_{J_E} [$z^P_{ss,11}$] and z_{J_X} [$z^P_{ss,13}$] become stable spirals and what is the relation between n and the onset of the Andronov-Hopf bifurcation at the respective equilibria.

4.9 Conclusion

Regarding the similarity property, one has that Semrau-Huang's extension has a dynamical equation for the P variable representing the pluripotency network, that is,

$$\frac{dP}{dt} = a_P \frac{P}{\theta + P} - kP[1 + c(E + dX)]$$

which is not entirely in line with the current paradigm concerning the modelling of transcriptional regulation in gene expression. In fact, the general consensus is that transcription factors regulates gene expression in a switch-like fashion, as stated in [80, p. 95]. So, with regard to the phenomenon itself, one has that a suitable mathematical representation thereof is thought to be highly sigmoidal, which, in turn, is not the case of the regulatory function

$$a_P \frac{P}{\theta + P}$$

being formulated for the *pluripotency network* in Semrau-Huang's model.

Therefore, if transcriptional regulation in gene expression is supposed to be modelled in a switch-like fashion and if a suitable mathematical representation for that is thought to be highly sigmoidal then a Hill-function seems to be suitable for that. Indeed, it presumptively contains all the main properties of the phenomenon as argued in Section 1.5.

Having said that, one might agree that the representation

$$a_P \frac{P^n}{\theta^n + P^n} + b \frac{\theta^n}{\theta^n + X^n} + b \frac{\theta^n}{\theta^n + E^n}$$

of the regulatory part of the dynamical equation for the *pluripotency network* suffices to satisfy the similarity hypothesis, as well as the representations

$$a_X \frac{X^n}{\theta_X^n + X^n} + b \frac{\theta^n}{\theta^n + E^n} + b \frac{\theta^n}{\theta^n + P^n}$$

and

$$a_E \frac{E^n}{\theta_E^n + E^n} + b \frac{\theta^n}{\theta^n + X^n} + b \frac{\theta^n}{\theta^n + P^n}$$

of the regulatory part of the dynamical equations for the *endoderm* and *ectoderm network* respectively.

Regarding the adequacy hypothesis, due to the former elucidations, we have conjectured that Semrau-Huang's extension has a contradiction with respect to the observation $(4.1)_5$, which, if we provide a proof for that, rules out the model as a conceptual mechanism to explain the experiments in (4.1).

However, it is essential to state that Semrau-Huang's extension could be the model to explain other phenomena and, furthermore, that it is a model that allows for more complex behaviour, as evidenced by the generation of oscillations shown in Figures 4.37a and 4.37b.

Appendix A

Numerical integration methods

As we have seen through this thesis, the differential equations describing Semrau-Huang's model are too complex to allow an analytical solution thereof. Although our approach is analytical, we numerically verify the existence of the steady states so as to illustrate the analytical results. To numerically solve the corresponding equations, we use the forward Euler method which approximates the derivative at time t, that is,

$$\begin{cases} \dfrac{dP}{dt} \approx \dfrac{P(t+\Delta t) - P(t)}{\Delta t} & \text{(A.1)} \\ \dfrac{dX}{dt} \approx \dfrac{X(t+\Delta t) - X(t)}{\Delta t} & \text{(A.2)} \\ \dfrac{dE}{dt} \approx \dfrac{E(t+\Delta t) - E(t)}{\Delta t} & \text{(A.3)} \\ & \text{(A.4)} \end{cases}$$

in which accuracy is determined by the increment Δt, so the smaller is Δt, the more accurate is the method. In fact, I have implemented the Euler forward method for Semrau-Huang's model.

But, what can we tell about the drawbacks of the Euler forward method ? Are we in need of a better method to be used in this thesis ? In fact, following the approach of [49, p. 153–156], one has that the Euler forward method amounts to

$$\begin{cases} \dfrac{P(t+\Delta t) - P(t)}{\Delta t} = M_P P & \text{(A.5)} \\ \dfrac{X(t+\Delta t) - X(t)}{\Delta t} = M_X X & \text{(A.6)} \\ \dfrac{E(t+\Delta t) - E(t)}{\Delta t} = M_E E & \text{(A.7)} \end{cases}$$

in which the matrices M_P, M_X, and M_E are solely determined by the respective differential equations and by the discretization of the time interval being adopted.

Now, to which concepts of the theory of numerical analysis should we refer so as to answer the ongoing questions ? In fact, the key concept is *numerical stability*. If we want to understand the essence of the latter concept then we can limit ourselves to the equation

$$\begin{cases} w' = \lambda w & \text{(A.8)} \\ w(0) = w_0 & \text{(A.9)} \end{cases}$$

and the perturbed equation

$$\begin{cases} \tilde{w}' = \lambda \tilde{w} & \text{(A.10)} \\ \tilde{w}(0) = w_0 + \epsilon & \text{(A.11)} \end{cases}$$

with $\lambda \in \mathbb{C}$ and with $\epsilon > 0$ being small. If we apply Euler forward to (A.8) and (A.10) then we arrive at

$$\begin{aligned} w^{n+1} &= (1 + \lambda\Delta t)w^n = \ldots = (1 + \lambda\Delta t)^n w_0, \\ \tilde{w}^{n+1} &= (1 + \lambda\Delta t)\tilde{w}^n = \ldots = (1 + \lambda\Delta t)^n (w_0 + \epsilon), \end{aligned} \tag{A.12}$$

with $\{t_0, t_1, \ldots, t_n, \ldots, t_N\}$ being a partition of the time interval $[0, T]$ $(T > 0)$. Hence, one has that

$$\tilde{w}^{n+1} - w^{n+1} = (1 + \lambda\Delta t)^{n+1}\epsilon, \tag{A.13}$$

and dependent upon the increment Δt, one has that or it is true that

$$(1 + \lambda\Delta t) < 1, \tag{A.14}$$

or it is true that

$$(1 + \lambda\Delta t) \geq 1. \tag{A.15}$$

So, if $\mid 1 + \lambda\Delta t \mid < 1$ and $\delta \ll 1$ then $\mid \tilde{w}^{n+1} - w^{n+1} \mid \ll 1$. In the later case, one says that the numerical solution $\{w^n : n = 0, 1, 2, \ldots, N\}$ is said to be numerical stable. In fact, the Euler forward method is stable in the region

$$S_{EF} = \{z \in \mathbb{C} :\mid 1 + z \mid < 1\}, \tag{A.16}$$

that is, in the unitary ball centered at $z = -1$. Therefore, one has that the Euler forward method is conditionally stable.

Despite the existence of better methods, that is, with a wider stability region, such as the Trapezoidal method, whose stability region reads

$$S_{TM} = \{z \in \mathbb{C} : \Re(z) < 0\}, \tag{A.17}$$

and the Euler backward method with stability region given by

$$S_{EF} \coloneqq \{z \in \mathbb{C} :\mid 1 + z \mid > 1\}, \tag{A.18}$$

thus being unconditionally stable; it suffices to implement the Euler forward method provided that we choose a convenient increment Δt so that

$$z = \mu\Delta t \in S_{EF}, \tag{A.19}$$

for all $\mu \in \sigma(M_i)$ and for all $i \in \{P, X, E\}$, with

$$\sigma(M_i) \coloneqq \{\mu : \mu\mathbb{I} - M_i \ \textit{is not invertible} \ \}. \tag{A.20}$$

But, why is the later claim true ? In fact, as we have shown the existence and stability of the steady states then it suffices to choose for a method that sufficiently reproduces the qualitative behaviour guaranteed by the analytical approach.

Appendix B

Some important remarks about the numerical approach

Now, how can we observe the changes in the nullclines as we perform the judgments? In fact, if we turn our attention to the $X-$nullcline

$$a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n} - kX(1+cP) = 0, \tag{B.1}$$

then we have that

$$\tilde{\Psi}_{1,n}(X,E) = \frac{1}{c}\left[\frac{1}{kX}\left(a_X \frac{X^n}{\theta_X^n + X^n} + b\frac{1}{1+E^n}\right)\right]. \tag{B.2}$$

Likewise, with respect to the $E-$nullcline

$$a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1+X^n} - kE(1+cP) = 0, \tag{B.3}$$

one has that

$$\tilde{\Psi}_{2,n}(X,E) = \frac{1}{c}\left[\frac{1}{kE}\left(a_E \frac{E^n}{\theta_E^n + E^n} + b\frac{1}{1+X^n}\right)\right]. \tag{B.4}$$

Moreover, provided that the $P-$nullcline reads

$$\tilde{\Psi}_3(X,E) = \frac{a_P}{k\left[1+c\left(E+dX\right)\right]} - 1, \tag{B.5}$$

I have been able to write a script in R with which we neatly make plots of the nullclines of Semrau-Huang's model. But, how could we numerically verify the stability of the steady states of Semrau-Huang's model ? In fact, I have extended an existent script in R to neatly produce the trajectories of Semrau-Huang's model in 3D from different perspectives as seen in Appendix C.

To conclude, based upon a natural order of importance emerging from the numerical approach, I would like to let the reader know that I have decided to omit some details thereof. In case of any doubt, I am pleased to send all the respective codes and scripts in R.

Appendix C

Key codes of the numerical implementation

The Euler forward method for Huang's model:

```
```{r, tidy=TRUE, eval=TRUE}

Huang_model_Euler_Forward <- function(samp, X00, E00, X01,E01, a_E, a_X
    , theta_X, theta_E, K, n, B, delta_t, T_final){
  #This function numerically solves the network modeling
  #with Euler forward.

  #Args:

  #concentration and reaction rates;

  #Returns:

  #It returns a matrix whose columns stand for the evolution of the
    concentrations .
dt <- delta_t
T_f <- T_final
a_e <- a_E
a_x <- a_X
theta_x <- theta_X
theta_e <- theta_E
k <- K
b <- B
m <- n
x00 <- X00
e00 <- E00
x01 <- X01
e01 <- E01
N <- (T_f/dt)
s <- samp
tt <- seq(0, T_f - dt, dt)
tt <- as.vector(tt)
X0 <- runif(s, x00, x01)#sample initial conditions
E0 <- runif(s, e00, e01)#sample initial conditions
X <- matrix(rep(0, N*1), nrow = N, ncol = 1)
E <- matrix(rep(0, N*1), nrow = N, ncol = 1)
Points <- array(0, dim=c(N, 3, s))
for(j in 1:s){
  X[1,] <- X0[j]
```
```

```
  E[1, ] <- E0[j]
for(q in 1:(N-1)){
  X[q + 1, ] <- X[q, ] + dt*(a_x*((X[q, ])^{m}/((theta_x)^{m} + (X[q,])
    ^{m})) + b*((1/(1 + (E[q, ])^{m}))) - k*X[q, ])
  E[q + 1, ] <- E[q, ] + dt*(a_e*((E[q, ])^{m}/((theta_e)^{m} + (E[q,])
    ^{m})) + b*(1/(1 + (X[q, ])^{m})) - k*E[q, ])
Points[q, 1, j] <- tt[q]
Points[q, 2, j] <- X[q,1]
Points[q, 3, j] <- E[q,1]
Points[N, 1, j] <- tt[N]
Points[N, 2, j] <- X[N,1]
Points[N, 3, j] <- E[N,1]
}
}
Matrix_multidimensional <- Points
Mat_names <- c("time", "X", "E")
colnames(Matrix_multidimensional) <- Mat_names
Data <- Matrix_multidimensional
XEN<- Data[,2,]
ECTO<- Data[,3,]
plot_Data <- matplot(XEN, ECTO,type="l",col= c(1,2,5),lty=c(1,1))
Results <- list("Data"= Data, "plot_Data"=plot_Data)
return(Results)
}

ss <- Huang_model_Euler_Forward(samp=2500,X00=0.0, E00=0.0,X01=2.7,E01
    =2.7,a_E=0.8, a_X=0.8, theta_X=0.5, theta_E=0.5, K=0.5, n=4, B
    =0.08110187
, delta_t=0.01, T_final=50)
'''
```

The Euler forward method for Semrau-Huang's model:

```
'''{r, tidy=TRUE, eval=TRUE}

Semrau_Huang_model_Euler_Forward <- function(samp, X00, E00, P00, X01,
    E01,P01, a_P, a_E, a_X, theta_X, theta_E, K, C, D, n, B, delta_t,
    T_final){
  #This function numerically solves SH model
  #with Euler forward.

  #Args:

  #'concentrations' and reaction rates;

  #Returns:

  #It returns a matrix whose columns stand for the evolution of the '
    concentrations'.
dt <- delta_t
T_f <- T_final
a_p <- a_P
a_e <- a_E
a_x <- a_X
theta_x <- theta_X
theta_e <- theta_E
c <- C
d <- D
k <- K
```

```
b <- B
m <- n
p00 <- P00
x00 <- X00
e00 <- E00
x01 <- X01
e01 <- E01
p01 <- P01
N <- (T_f/dt)
s <- samp
tt <- seq(0, T_f - dt, dt)
tt <- as.vector(tt)
P0 <- runif(s, p00, p01)#sample initial conditions
X0 <- runif(s, x00, x01)#sample initial conditions
E0 <- runif(s, e00, e01)#sample initial conditions
P <- matrix(rep(0, N*1), nrow = N, ncol = 1)
X <- matrix(rep(0, N*1), nrow = N, ncol = 1)
E <- matrix(rep(0, N*1), nrow = N, ncol = 1)
Points <- array(0, dim=c(N, 4, s))
for(j in 1:s){
  X[1,] <- X0[j]
  E[1, ] <- E0[j]
  P[1,] <- P0[j]
for(q in 1:(N-1)){
  P[q + 1, ] <- P[q, ] + dt*((a_p*(P[q, ]/(1 + P[q, ]))) - k*P[q, ]*(1
    + c*(E[q, ] + (d)*(X[q, ]))))
  X[q + 1, ] <- X[q, ] + dt*(a_x*((X[q, ])^{m}/((theta_x)^{m} + (X[q,])
    ^{m})) + b*((1/(1 + (E[q, ])^{m}))) - k*X[q, ]*(1 + c*P[q,]))
  E[q + 1, ] <- E[q, ] + dt*(a_e*((E[q, ])^{m}/((theta_e)^{m} + (E[q,])
    ^{m})) + b*(1/(1 + (X[q, ])^{m})) - k*E[q, ]*(1 + c*P[q,]))
Points[q, 1, j] <- tt[q]
Points[q, 2, j] <- X[q,1]
Points[q, 3, j] <- E[q,1]
Points[q, 4, j] <- P[q,1]
Points[N, 1, j] <- tt[N]
Points[N, 2, j] <- X[N,1]
Points[N, 3, j] <- E[N,1]
Points[N, 4, j] <- P[N,1]
}
}
Matrix_multidimensional <- Points
Mat_names <- c("time", "X", "E", "P")
colnames(Matrix_multidimensional) <- Mat_names
Data <- Matrix_multidimensional
#Results <- list("Data"= Data, "plot_Data"=plot_Data)
return(Data)
}

```
```

A script to neatly plot the nullclines of Semrau-Huang's model with interactive perspective function:

```
```{r, tidy=TRUE, eval=TRUE}
SH_Plot_nullclines_3D <- function(XEN, ECTO, PLURI, Xrange, Yrange,
```

```
    Zrange, a_P, a_E, a_X, theta_X, theta_E, K, C, D, m, B){
  #This function plots the nullclines of SH model.

  #Args:

  #Parameters and range of the plot

  #Returns:

  #It returns an interative plot3D of the nullclines
a_p <- a_P
a_e <- a_E
a_x <- a_X
thetax <- theta_X
thetae <- theta_E
c <- C
d <- D
k <- K
b <- B
n <- m
xrange <-Xrange
yrange <-Yrange
zrange <-Zrange
xen <- XEN
ecto <- ECTO
pluri <- PLURI
X<- seq(0, xen, by = 0.025)
r <- length(X)
E <- seq(0, ecto, by = 0.025)
s <- length(E)
P <- seq(0, pluri, by = 0.025)
t <- length(P)
######################## Semrau-Huang Pluri-Nullcline
    #########################
SP <- matrix(rep(0, r*s), nrow=r, ncol=s)
for(i in 1:r){
  for(j in 1:s){
    SP[i,j] <- ((a_p/(k*(1 + c*(E[j] + d*X[i])))) - 1)
  }
}
######################## Semrau-Huang XEN-Nullcline
    #############################
Psi_P_1_n  <- matrix(rep(0, r*s), nrow=r, ncol=s)
for(i in 1:r){
  for(j in 1:s){
    Psi_P_1_n[i,j] <- (1/c)*(((1/(k*(X[i])))*((a_x*((X[i])^{n}/(thetax
    ^{n} + (X[i])^{n}))) + (b/(1 + (E[j])^{n})))) - 1)
  }
}
############################### Semrau-Huang ECTO nullcline
    ################################
Psi_P_2_n  <- matrix(rep(0, r*s), nrow=s, ncol=r)
for(i in 1:r){
  for(j in 1:s){
    Psi_P_2_n[i,j] <- (1/c)*(((1/(k*(E[j])))*((a_e*((E[j])^{n}/(thetae
    ^{n} + (E[j])^{n}))) + (b/(1 + (X[i])^{n})))) - 1)
  }
}
```

```
###############Plot nullclines##########################
library(rgl)
 persp3d(x = X, y = E, z= SP, col="gray47", aspect="iso", axes=TRUE,
    box=FALSE, xlim = xrange, ylim = yrange, zlim = zrange,
xlab = "Xen", ylab = "Ecto", zlab = "Pluri")

  persp3d( x = X, y = E, z = Psi_P_2_n, col="orangered3", xlim = xrange
    , ylim = yrange, zlim = zrange, add=TRUE)
  persp3d( x = X, y = E, z= Psi_P_1_n, col="mediumpurple3", xlim =
    xrange, ylim = yrange, zlim = zrange, add=TRUE)

  invisible()
}

######### Primitive Scenario ###############
library(rgl)
open3d()
sc_8_SH_4 <- SH_Plot_nullclines_3D (XEN=10, ECTO=10, PLURI=15, Xrange=c
    (0,2), Yrange=c(0,2), Zrange=c(0,2), a_P=2,a_E=0.8, a_X=0.8,
    theta_X=0.5, theta_E=0.5, K=0.5, C=0.1, D=0.5, m=4, B=0.0811)
rgl.postscript("sc_SH_4_optimal.pdf","pdf")
###########################################
```

A script in R to neatly produce the trajectories of Semrau-Huang's model in 3D from an 'arbitrary' perspectives:

```
```{r, tidy=TRUE, eval=TRUE}
trajectory_3D <- function (x, y, z, ang, type = "p", lty = 1:5, lwd =
    1, lend = par("lend"),
     pch = NULL, col = 1:6, cex = NULL, bg = NA, xlab = NULL,
     ylab = NULL, zlab = NULL, xlim = NULL, ylim = NULL, zlim= NULL, log
     = "", ..., add = FALSE,
     verbose = getOption("verbose"))
{
  #This function is a generalization of the Matplot Function-an
    existent script in R.
  #This generalized function perfectly plots the trajectories of Semrau
    -Huang's model in 3D.
  #We must emphasize that the most part of this code was already done
    to yield plots in 2D.
  #So the code for 2D has been extended to 3D so that it could produce
    the trajectories in 3D.
  #The idea is to substitute the interplay between plot and lines by
    something sufficient, in fact, by
  #scatterplot3d and trj$plot3d.


  #Args:

    # R-object: an array, or a dataframe, or a matrix; and graphical
    parameters

  #Return:
  #It returns the plot with the trajectories
```
```

```
    alpha_a <- ang
    paste.ch <- function(chv) paste0("\"", chv, "\"", collapse = " ")
    str2vec <- function(string) {
        if (nchar(string, type = "c")[1L] > 1L)
            strsplit(string[1L], NULL)[[1L]]
        else string
    }
    xlabel <- if (!missing(x))
        deparse(substitute(x))
    ylabel <- if (!missing(y))
        deparse(substitute(y))
    zlabel <- if (!missing(z))
        deparse(substitute(z))
    if (missing(x)) {
        if (missing(y))
          if(missing(z))
            stop("must specify at least one of 'x','y', or 'z' ")
        else x <- seq_len(NROW(y))
    }else if (missing(y)) {
        y <- x
        ylabel <- xlabel
        x <- seq_len(NROW(y))
        xlabel <- ""
    }else if (missing(z)) {
        z <- x
        zlabel <- xlabel
        x <- seq_len(NROW(z))
        xlabel <- ""
    }
    kx <- ncol(x <- as.matrix(x))
    ky <- ncol(y <- as.matrix(y))
    kz <- ncol(z <- as.matrix(z))
    n <- nrow(x)
    if (n != nrow(y))
        stop("'x' , 'y', and 'z' must have same number of rows")
    if (n != nrow(z))
        stop("'x' , 'y', and 'z' must have same number of rows")
    if (kx > 1L && ky > 1L && ky > 1L && ((kx != ky)|| (kx != kz) || (
ky != kz)))
        stop("'x' , 'y', 'z' must have only 1 or the same number of
columns")
    if (kx == 1L)
        x <- matrix(x, nrow = n, ncol = ky)
    if (ky == 1L)
        y <- matrix(y, nrow = n, ncol = kx)
    if (kz == 1L)
        z <- matrix(z, nrow = n, ncol = ky)
    k <- max(kx, ky, kz)
    type <- str2vec(type)
    if (is.null(pch)) {
        pch <- c(1L:9L, 0L, letters, LETTERS)
        if (k > length(pch) && any(type %in% c("p", "o", "b")))
            warning("default 'pch' is smaller than number of columns
and hence recycled")
    }
    else if (is.character(pch))
        pch <- str2vec(pch)
    if (verbose)
```

```
        message("matplot: doing ", k, " plots with ", paste0(" col= (",
            paste.ch(col), ")"), paste0(" pch= (", paste.ch(pch),
            ")"), " ...\n", domain = NA)
    xyz <- xyz.coords(x, y, z, xlabel, ylabel, zlabel, log = log)
    xlab <- if (is.null(xlab))
        xyz$xlab
    else xlab
    ylab <- if (is.null(ylab))
        xyz$ylab
    else ylab
    zlab <- if (is.null(zlab))
        xyz$zlab
    else zlab
    xlim <- if (is.null(xlim))
        range(xyz$x[is.finite(xyz$x)])
    else xlim
    ylim <- if (is.null(ylim))
        range(xyz$y[is.finite(xyz$y)])
    else ylim
    zlim <- if (is.null(zlim))
        range(xyz$z[is.finite(xyz$z)])
    else zlim
    if (length(type) < k)
        type <- rep_len(type, k)
    if (length(lty) < k)
        lty <- rep_len(lty, k)
    if (length(lend) < k)
        lend <- rep_len(lend, k)
    if (length(lwd) < k && !is.null(lwd))
        lwd <- rep_len(lwd, k)
    if (length(pch) < k)
        pch <- rep_len(pch, k)
    if (length(col) < k)
        col <- rep_len(col, k)
    if (length(bg) < k)
        bg <- rep_len(bg, k)
    if (is.null(cex))
        cex <- 1
    if (length(cex) < k)
        cex <- rep_len(cex, k)
    ii <- seq_len(k)
    dev.hold()
    on.exit(dev.flush())
    if (!add) {
        ii <- ii[-1L]
        library(scatterplot3d)
        scatterplot3d(x[, 1L], y[, 1L], z[, 1L], color = col[1L], pch =
     pch[1L], xlim = xlim, ylim = ylim, zlim= zlim, xlab = xlab,
            ylab = ylab, zlab = zlab, scale.y = 1, angle = alpha_a,
   grid = TRUE, box = FALSE, type = type[1L], highlight.3d = FALSE,
   mar = c(5, 3, 4, 3) + 0.1, bg = bg[1L], log = log, ...)

        trj <- scatterplot3d(x[, 1L], y[, 1L], z[, 1L], color = col[1L
   ], pch = pch[1L], xlim = xlim, ylim = ylim, zlim= zlim, xlab = xlab
   ,
            ylab = ylab, zlab = zlab, scale.y = 1, angle = alpha_a,
   grid = TRUE, box = FALSE, type = type[1L], highlight.3d = FALSE,
   mar = c(5, 3, 4, 3) + 0.1, bg = bg[1L], log = log, ...)
```

```
    }

     for (i in ii)  trj$points3d(x[, i], y[, i], z[, i], type = type[i
    ], col = col[i], lwd=1)

    invisible()
}

################# Trajectories of Semrau−Huang ###########
steady_states <− Semrau_Huang_model_Euler_Forward(samp=500,X00=0.0, E00
    =0.0, P00=0.0,X01=2,E01=2, P01=2, a_P=2,a_E=0.8, a_X=0.8, theta_X
    =0.5, theta_E=0.5, K=1, C=0.1, D=0.5, n=30, B=0.811, delta_t=0.01,
    T_final=150)
XEN<− steady_states[,2,]
ECTO<−steady_states[,3,]
PLURI <−steady_states[,4,]
trajectory_3D(XEN, ECTO,PLURI, ang= 120, type="l",col= c(1,3,6,9), xlim
     = c(0,2), ylim = c(0,2), zlim=c(0,2))
#################################################################
'''
```

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