Causal isolation robustness analysis:

The combinatorial strategy of circadian clock research

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Abstract:

This paper distinguishes between *causal isolation* robustness analysis and *independent determination* robustness analysis and suggests that the triangulation of the results of different epistemic means or activities serves different functions in them. Circadian clock research is presented as a case of causal isolation robustness analysis: in this field researchers made use of the notion of robustness to isolate the assumed mechanism behind the circadian rhythm. However, in contrast to the earlier philosophical case studies on causal isolation robustness analysis (Weisberg and Reisman 2008, Kuorikoski et al. 2010), robustness analysis in the circadian clock research did not remain in the level of mathematical modeling, but it combined it with experimentation on model organisms and a new type of model, a synthetic model.

Keywords: Modeling, robustness analysis, causal isolation, synthetic biology, circadian clock

1. Introduction

Although biology and economics seem to deal with entirely different subject matters—one concentrating on the natural and the other on the social phenomena—there are some remarkable reminiscences between their theoretical practices. In both of them theory-building takes nowadays largely the form of modeling while the data in these fields is often discordant or equivocal, and the parameter values are difficult to estimate or measure. Models in biology and economics give often only qualitative results and due to the complexity of biological and social systems they are ridden with highly idealized assumptions making their unrealistic nature a constant worry. As a result, both biology and economics are populated by a multitude of theoretical models, which all approach only some selected aspects of the phenomena, often in a highly simplified fashion and even proceeding on different levels of organization and abstraction. The question, then, is whether this situation is just a predicament of the sciences studying complex phenomena, or are there any epistemic gains involved in such theoretical pluralism.

This question was famously posed by Richard Levins (1966), reflecting on the state of population biology in the 1960's. He tackled the problem of multiple models by introducing the notion of robustness (see also Weisberg 2006a). According to him, if several different models, each with different simplifications and idealizations, gave rise to similar results that would strengthen our confidence in those results. However, as we will argue, his classic article can be read in different ways, pointing at least to two different notions of robustness. More specifically, while his stress seems to be on the confirmatory weight the similar results, i.e. the "robust theorems", derived from different models might have, his article launches also a different concept of robustness. It is that of using the robustness of model results as a *means* of isolating and studying the core causal mechanism producing certain phenomena.[1]

The notion of robustness as a strategy to isolate and study the core causal mechanism behind some phenomenon is examined by two recent – and to our knowledge

[1] This strategy usually presumes that the phenomenon under study is robust itself, which creates a link between the epistemic uses of the notion of robustness and the causal robustness of the real phenomena (for this distinction, see Woodward 2006).

the only – philosophical case studies on robustness analysis. One of these cases is from population biology, examining the so-called Lotka-Volterra model on predator-prey dynamics (Weisberg and Reisman 2008), the other one is from geographical economics concerning the models of the spatial location of economic activity (Kuorikoski, Lehtinen and Marchionni 2010). However, the authors of the aforementioned articles do not discuss the relationship between modeling and empirical research, focusing solely on the kind of evidence mathematical modeling *alone* can provide. In the following we will present another case of robustness analysis, in which we will also take into account how mathematical modeling was triangulated with empirical research. Neither in this case did scientists primarily target the robustness of the results aiming rather at isolating the core causal mechanism behind them. The phenomenon of interest was remarkably robust in itself—the day and night rhythms of organisms.

The circadian clock research provides an interesting example of robustness analysis: Apart from studying a robust biological phenomenon, it has, with the emergence of molecular biology, assumed a strong and partially successful isolationist agenda as regards the mechanism that is supposed to produce the circadian rhythm. We will argue that this agenda could not have been accomplished by mathematical modeling alone. Instead, the researchers in the field triangulated the results from mathematical models and experiments. The results gained by the two activities informed each other, but their relationship was by no means unambiguous. This gave rise to a brand new kind of model: the Repressilator, a synthetic model, which probed the kinds of questions, which could not be answered either by mathematical modeling or by experiments on model organisms. Interestingly, the Repressilator also explored the kind of "low level confirmation" that has been identified by Weisberg (2006b) as the basis on which robustness analysis rests. Moreover, as we will show, it took the isolationist program to the stage in which it started to break down.

2. The notion of robustness and the varieties of robustness analysis

The notion of robustness has become frequently used term in a multitude of scientific contexts with varying meanings related to robustness as a characteristics of a process, a

design, or a system, for example. The notion of robustness is double-faced: on the one hand, it can be a property of real-world phenomena or the causal relationships underlying them, on the other hand, the notion of robustness has been used to characterize the results of various epistemic activities. Robustness is generally considered to be a "Good Thing" as Woodward (2006) has put it.[2] In the biological context, it refers to "the ability to maintain performance in the face of perturbations and uncertainty" (Stelling et al. 2004, see also Kitano 2004). This biological interpretation of robustness bears remarkable similarities to the notion of robustness in engineering, where "robustness is the state where the technology, product, or process performance is minimally sensitive to factors causing variability (either in the manufacturing or users' environment) […]" (Taguchi et al. 2000). More generally, at the heart of the idea of robustness is the dialectic between variance and invariance, stability and instability, or sensitivity and insensitivity, which dialectic has been put in science to many methodological uses serving simultaneously as a criteria for what is real or trustworthy (Wimsatt 2007, 38).

As regards the philosophical discussion on modeling, robustness as a search for the invariant in the midst of variability has usually concerned the similarity of the results derived from various models. According to Levins, if different models with different assumptions lead to similar results "we have what we can call a robust theorem that is relatively free of the details of the model" (1966, p. 423). Whether or not these models are supposed to be independent from each other has been a matter of controversy (see Orzak and Sober 1993, Levins 1993, Weisberg 2006b). Orzak and Sober (1993) attributed to Levins the claim that the robustness of the statement derived from independent models could provide some non-empirical confirmation for its truth. They contested this claim arguing that it is difficult to formulate clearly the idea of the independence of models. Given that Levins (1966) already clearly spelled out that he considered robustness in terms of the derivation of the same result from alternative models sharing "a common biological assumption", it is worthwhile to consider why Orzak and Sober came to interpret him differently.

It seems that at the heart of this misinterpretation was their perception that Levins's focus was solely on the *results* derived from models and our confidence in them.

[2] Woodward in fact also criticizes this view, see also Stegenga 2009.

Now if this were the case, then supposedly our confidence on these results would be greater if they were not derived from models that are dependent from, or too similar to, each other. Otherwise, "the robustness comes too cheaply" (Orzak and Sober 1993, p. 540). Consequently, Orzak and Sober ascribed to Levins a notion of robustness that we call the *independent determination* robustness. This kind of robustness is important in the contexts of measurement and experimentation[3]. The independence of the different means of arriving to the same result shows that the result is not biased by the specific technique used or it is not an artifact of the particular experimental set-up. Yet theoretical models usually do not aim to measure, detect or intervene in real target systems but rather to describe and explore possible mechanisms giving rise to a certain phenomenon or function. Why should the same kind of reasoning that applies to measurement or experimentation apply to modeling?

In which situations would the robustness of a result derived from independent models increase our trust in it? First, the question of independence needs to be answered. One scientifically sensible criterion for the independence of models — though far from absolute one, and not among those that Orzak and Sober considered—is whether they depict different kinds of causal mechanisms. Suppose that we had reasons to believe that the same phenomenon could be brought about by different kinds of mechanisms. In this case the robustness of a result derived from independent models could raise our confidence in it. Indeed, this kind of redundancy is not uncommon in biological systems in which certain kinds of behavior can be brought about by different mechanisms, or where the mechanism lying behind a certain function can compensate for its failure or change of its parts by re-organization (see e.g. Mitchell 2008, Wagner 2007). Another possibility is that the mechanisms in question are operating on different levels in a "nested hierarchy" (Levins 1968, p. 8). This seems to be the possibility Levins (1966) had in mind in view of his examples: these models, apart from differing from each other substantially, describe also phenomena in different levels. This reading of Levins is also backed by the notion of "sufficient parameter", which he introduces in discussing his

[3] So far, there has not been much overlap between the philosophical literatures on experimentation and robustness analysis.

examples. With sufficient parameter he refers to an upper level parameter that contains "most of the important information about the events of lower levels" (1966, p. 428).

The importance of the independent means of determination has been stressed by Wimsatt (1981, 2007, see also Calcott in press). He approaches robustness analysis as the use of multiple independent means to "triangulate the existence and character of a common phenomenon, object or result" (2007, p. 43). Thus Wimsatt's notion of robustness does not limit itself to modeling alone applying to such diverse epistemic activities as observation, experimentation and modeling, and their combinations. Moreover, Wimsatt's vision stretches from evolution to the knowledge seeking strategies: Apart from being a "universal feature of sophisticated design" robustness provides powerful heuristics for problem solving and research. Robustness-driven heuristics are prone to systematic errors, however. Wimsatt's example of this is telling as regards the confirmatory weight of robust model results. He discusses a group of models in evolutionary biology that appeared to show that group selection could not be efficacious. This robust result turned out to be wrong, it was a consequence of biased and incorrect assumptions that the different models shared (2007, pp. 71-73).

Such systematic error seems to be the basic reason why Orzak and Sober questioned our confidence on robust model results. They suggested: "It is worth considering that robustness simply reflects something common among the frameworks and not something about the world those frameworks seek to describe" (1993, p. 539, see also Sugden 2000). To this critique Levins (1993) replied that he did not consider robustness analysis as "a way to truth independent of observation" (p. 554). Moreover, he argued that in targeting the common among the frameworks robustness analysis serves an important epistemic task: it separates "conclusions that depend on the common biological core of a model from simplifications, distortion and omissions introduced to facilitate the analysis, […]" (ibid.). This task, we suggest, points to a different conception of robustness, which is geared towards isolating and studying the common causal core of a group of models instead of assessing the credibility of their results. In such *causal isolation* robustness analysis the robust results function rather as *means* than *ends* in themselves: they have the function to isolate the common causal mechanism of a family of models and to assess its generality. The rationale behind this kind of robustness

analysis is due to the fact that modelers cannot be sure which idealizations, parameter values or tractability assumptions do the job, i.e. deliver the results sought for. Are the results really produced by the assumed causal core of the model? And if so, how general are they?

To find these things out modelers vary the idealizing assumptions and other features of the model to see whether they still get the same results. If the results are robust, then the modifications made to the basic model did not matter for the result. Weisberg and Reisman (2008) provide a nice classification of the kinds of activities such robustness analysis includes. In *parameter robustness analysis* the behavior of the model is studied under different parameter settings. This is often called sensitivity analysis. In *structural robustness analysis* the causal structure of the model is changed in order to isolate those causal components and interactions that are essential for the production of the robust result, while in engaging in *representational robustness analysis* scientists study whether the way the assumptions are represented make a difference (pp. 115-116, 120-121). Although it appears to us that in practice it is often difficult to separate the structural robustness analysis from the representational robustness analysis, this three-partite distinction is useful nevertheless.

Thus the robust results do not have the same epistemic status and role in the *independent determination* robustness analysis and in the *causal isolation* robustness analysis. Whereas the former targets the model results aiming to give more credence to them, the latter focuses on the causal mechanism giving rise to the robust results. In *causal isolation* robustness analysis the result is often already accepted on empirical grounds and the point of modeling is to specify the mechanism behind the phenomenon in question. Consequently, the goal of model construction is to produce a certain kind of result or behavior, e.g. day and night oscillations in the case of circadian rhythms. Whether or not the model succeeds to produce the anticipated results serves as an important criterion in its assessment. By contrast, in the *independent determination* robustness analysis the results themselves are not already empirically established and are thus in need of further consolidation.

In the following section we will study the circadian clock research as an instance of *causal isolation* robustness analysis. This research aimed at isolating the "sufficient

components" and their interactions lying behind the day and night rhythms of organisms. The models constructed were supposed to produce such oscillations that were already known on the basis of robust natural phenomena. The focus on "sufficient components" serves to show that generality was one important goal of this line of research. However, as we will argue, this isolationist research program could not proceed on the level of mathematical modeling alone, but called for a subtle triangulation of mathematical models, experiments on model organisms and synthetic models.

3. Mathematical models, model organisms, and synthetic models in isolating the circadian mechanism

When scientists like Brian Goodwin started working on the phenomena of temporal organization of biological systems in the 1960's their approach was primarily of theoretical nature consisting of modeling (Goodwin 1963). First 20 years later when empirical data from experiments on model organisms such as *Drosophila* became available the theoretical modeling activity became informed by experimental work. In this process theoretical models such as the Goodwin model were transformed by integrating in them details from the experimental studies such as for example time delays due to phosphorylation processes (Goldbeter 1996). Theoretical and experimental studies started to interact. Different models describing circadian mechanisms were suggested that raised the question whether it would be possible to empirically distinguish between different possible causal mechanisms, all able to produce oscillations typical of the circadian rhythm. Leibler and colleagues (Alon et. al 1999, Barkai and Leibler 2000) introduced the notion of robustness as a criterion that would help in distinguishing between different proposed mechanisms. The idea was to analyze whether a mechanism is robust against internal noise, that is, stochastic variations due to the low number of molecules in the cell. Only such mechanisms, they argued, would be able produce robust circadian rhythms. This criterion reduced the number of viable mechanisms but still it did not give any definite answer to the question of which of the possible mechanisms could have been implemented by actual biological systems.

In order to further reduce the gap between experiments on model organisms and the results gained from the different modeling activities Elowitz and Leibler constructed a synthetic model using biological components, such as genes and proteins, arranging them into an oscillatory network. This famous model, which was one of the first synthetic models, was called the *Repressilator* (Elowitz and Leibler 2000) because the three genes of the network were chosen and arranged in such a way that they repressed each others' activities. The Repressilator was constructed to study whether negative feedback loops, already familiar from mathematical modeling, could give rise to sustained regular oscillations in biological organisms. To find this out the Repressilator was designed in such a way that it used mathematical model as a blueprint for engineering a novel biological system.

In the following we will discuss in more detail the development described above. We concentrate on the Goodwin model and its successors. Another influential model was presented by Arthur Winfree (1967), who instead of concentrating on intracellular mechanisms studied the interactions between oscillators, and how they are able to synchronize.

3.1. The Goodwin model

A way before the empirical isolation of the components of the circadian clock had even started, Brian Goodwin already explored the dynamics of this system in terms of a feedback mechanism inspired by Jacob and Monod's (1961) operon model of gene regulation. Another source of inspiration was the work of the physicist Edward Kerner (1957). He had tried to formulate statistical mechanics for the Lotka- Volterra model, which prompted Goodwin also attempt to introduce statistical mechanics for biological systems. Central to his model was the idea that oscillations created by a feedback mechanism control day and night rhythms, physiological functions etc. Whereas in engineering such oscillations are usually regarded as unwanted and parasitic, in biology they got a functional status. Brian Goodwin described this point in his book in the following way: "The appearance of such oscillations is very common in feedback control systems. Engineers call them parasitic oscillations because they use up a lot of energy.

They are usually regarded as undesirable and the control system is nearly always designed, if possible, to eliminate them." (Goodwin 1963, 5). The basic structure of the network underlying the molecular mechanism of the Goodwin's model is represented in the following diagram:

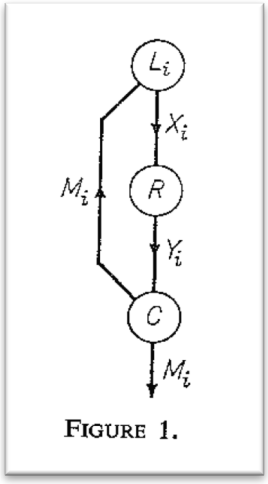

Fig. (1) An example of a circuit diagram taken from Goodwin (1963,6).

The main structure of the model amounts to a negative feedback loop. It consists of a genetic locus L_i, synthesizing *mRNA* in quantities represented by the variable X_i. The *mRNA* leaves the nucleus and enters the ribosome, which reads out the information from the *mRNA* and synthesizes proteins in quantities denoted by Y_i. The proteins are connected to metabolic processes. At the cellular locus C the proteins influence a metabolic state by for example enzyme action, which results in the production of metabolic species in quantity M_i. A fraction of the metabolic species is traveling back to the genetic locus L_i where it functions as a repressor.

This mechanism leads to oscillations in the protein level Y_i regulating the circadian rhythm. Goodwin described the mechanism by a set of differential equations,

which were due to the feedback mechanism of non-linear character. Such systems display complicated behavior and no analytical solutions exist for them. Goodwin explored the dynamics described by the differential equations by making simplifying assumptions such as leaving aside biochemical parameters of the process and by using numerical methods.

Goodwin was able to show by performing very basic computer simulations that the change in the concentration of proteins Y_i and concentration of mRNA form a closed trajectory. This means that the model system is able to perform regular oscillations. But the oscillations were not robust. Goodwin wrote: "The oscillations which have been demonstrated to occur in the dynamic system […] persist only because of the absence of damping terms. This is characteristic of the behavior of conservative (integrable) systems, and it is associated with what has been called weak stability." (Goodwin 1963, 53) He went on explaining that a limit-cycle dynamic would have been the desirable dynamic behavior. In this case after small disturbances the system moves back to its original trajectory. This is a characteristic of non-conservative systems. Biological systems are non-conservative systems: they are not closed systems because they exchange energy with the environment. Without going into further detail, such systems are in general very difficult to treat and analyze. Consequently, in order to make the model tractable Goodwin to had give up the desired limit-cycle dynamics and with it an all-encompassing robustness of the mechanism.

Goodwin's endeavor of establishing a general biological framework similar to statistical mechanics and thermodynamics in physics was not further developed by other scientists, yet the idea of approaching the circadian phenomena in terms of a feedback mechanism became a theme that was further explored in experiments as well as in other mathematical models.

3.2. Experiments exploring circadian rhythms

The first circadian clock gene was discovered in experiments performed by Seymour Benzer together with Ron Konopka (Konopka and Benzer 1971) in the beginning of the

1970's. They named the gene period (per). The experimental research on circadian rhythms in molecular biology and genetics progressed only slowly after Konopka and Benzer published their results. This was because the experiments were rather elaborate. A great challenge consisted in finding the protein related to the isolated gene (per), its function in the biochemical processes, and deciding whether the protein was part of the mechanism or an output of it regulating other functions in biological system. Only in the mid 1980's and 1990's with further advances in molecular biology and genetics did the circadian clock research start booming and more genes, proteins and possible mechanisms were discovered in experiments on model organisms such as *Drosophila, Neurospora* and *Arabidopsis*. In the beginning of 1990's Hardin et al. (1990) suggested that the circadian rhythm in *Drosophila* results from a negative feedback mechanism exerted by the PER protein on the synthesis of the per mRNA or on its translation. PER should act by binding to and removing a transcription activator. In this way the experimental research synthesized the results of the modeling endeavor with the emerging knowledge on the molecular basis of the assumed circadian clock mechanism. On the other hand, mathematical modeling was informed by empirical results, of which the work of Albert Goldbeter provides an illustrative example.

3.3. Mathematical and synthetic models of the circadian clock

Albert Goldbeter, a chemist by training, spent a large part of his scientific career on modeling oscillatory behavior in biological systems. Goldbeter described the procedure of discovering such molecular mechanisms accordingly: "Elucidating the molecular bases of biochemical and biological rhythms first requires the identification of the types of molecules involved and of their regular interactions. An isolated enzyme or receptor will never produce any oscillations. It is the network of molecular species, controlled by positive and negative feedback loops and driven by a flow of matter that gives rise to periodic or chaotic behaviour. The oscillatory mechanism is only clarified when a theoretical model based on these observations successfully accounts for the observed periodic or chaotic phenomenon." (Goldbeter 1996, 2). Goldbeter (1995) picked up the basic structure of Goodwin's model in using a feedback mechanism as the main control

element but he was also able to take it a step further by taking into account the results from experimental studies.

The model designed by Goldbeter accounting for the experimental findings of Hardin et al. (1990) on the behavior of the circadian clock gene per in *Drosophila* is depicted in Fig.(2).

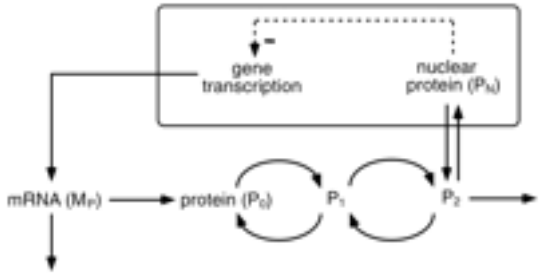

Fig. (2) A diagrammatic depiction of Goldbeter's model of the time delayed circadian mechanism.

The mechanism underlying the production of PER is a time delayed negative feedback mechanism. In the nucleus per mRNA is produced with the rate M. From per mRNA the protein PER is synthesized with a rate that is proportional to M. The observed time delay in the peak of per mRNA and PER is explained in the model by the twofold phosphorylation process in which PER transfers through three different states P_0, P_1, P_2. P_2 is then degraded or transferred back into the nucleus were it suppresses its own production. After laying out the basic structure of the gene regulatory network Goldbeter formulated a set of differential equations and explored the dynamics and steady states of the system. The mechanism depicted was able to produce regular oscillations.

3.3.1. Robustness as a criterion in distinguishing between different possible mechanisms

The network and the associated mechanism introduced by Goldbeter (1995) was not the only theoretical possibility for producing circadian oscillations. Different combinations of feedback loops can lead to similar network dynamics and usually it is difficult to distinguish between them by performing experiments on model organisms. This multiplicity of possible mechanisms might reflect the real situation since there is often

redundancy in biological systems, yet this does not necessarily be the case. Clearly, any theoretical mechanism, although able to produce the observed phenomena, might still be such that it cannot be realized in any biological systems. This problem was addressed by Leibler and his associates (Alon et. al. 1999, Barkai and Leibler 2000), who introduced the notion of *the robustness of the mechanism against stochastic fluctuations* as a criterion for distinguishing between different possible mechanisms.

Leibler and his associates started out from the observation that the differential equations introduced by Goldbeter (1995) are continuous and as such do not take into account the stochastic fluctuations due to the low number of molecules in some biological systems, which according to Leibler et al. have an effect on the dynamic behavior of such systems. By forming a source of internal noise these stochastic fluctuations introduce an interesting way to distinguish between different possible mechanisms. Namely, despite those fluctuations the mechanism has to function in a robust manner. Or, as Barkai and Leibler formulated it: "The ability to function reliably in the presence of internal noise may impose a further constraint on the oscillation mechanism. Internal noise in the operation of biochemical networks results from the stochastic nature of reaction events, and is particularly important when there are few molecules in the system, as is often the case in a cell." (Barkai and Leibler 2000). They suggested a different network design consisting of both a positive and a negative regulatory element activating and repressing gene expression. A positive element A increases its own expression and that of an negative element R. Strong binding of R to A inhibits its activity and so represses the expression of both elements.

The computer simulations (Monte Carlo simulations) showed that the model of Barkai and Leibler was more robust against stochastic fluctuations than that of Goldbeter. Also Barkai and Leibler's model was based on a very general template, that of hysteresis. Hysteresis is a phenomenon that can be observed in electric, magnetic, and elastic material. Those systems react with a time delay to the application and removal of a force or field. For example if a magnetic field is applied to a piece of iron and then removed from it, there remains some magnetization in the iron. For such systems it is not possible to predict the outcome knowing the input without knowing the current state of the system. They are not deterministic systems like the system described by Goldbeter but have a

memory. Barkai and Leibler suggested at the end of their article that "noise resistance may therefore help to uncover the design principles underlying circadian clocks" (Barkai and Leibler 2000, 268).

3.3.2. The Repressilator

Around the same time as Barkai and Leibler worked on their robust model a further strategy to study the various possible circadian clock mechanisms emerged. Neither mathematical modeling nor experiments on model organisms were able to decide whether mechanisms such as Goodwin's oscillator model, Goldbeter's time delayed oscillatory model, or Barkai and Leibler's hysteresis model, could in principle be realized in natural systems. This gave reason for the construction of a synthetic model, which was built from biological components. The model, called the *Repressilator*, was designed to study the question of whether a negative feedback loop in a genetic network is able to produce oscillations in the protein level.

The first step in constructing the *Repressilator* was to design a mathematical model, which was used to explore the basic biochemical parameters and their interactions. Having constructed a mathematical model of a gene regulatory network Elowitz and Leibler performed computer simulations on the basis of it. They showed that there were two possible types of solutions: "The system may converge toward a stable steady state, or the steady state may become unstable, leading to sustained limit-cycle oscillations" (Elowitz and Leibler 2000, 336). Furthermore the numerical analysis of the model gave insights into the experimental parameters relevant for constructing the synthetic model in showing that "[...] oscillations are favoured by strong promoters coupled to efficient ribosome binding sites, tight transcriptional repression (low 'leakiness'), cooperative repression characteristics, and comparable protein and mRNA decay rates" (ibid, 336). Elowitz and Leibler also explored the continuous as well as the stochastic dynamics of the model in order to analyze the role of internal noise in the mechanism (see above).

The mathematical model functioned as a blueprint for the engineering of a novel biological system. The structure of the *Repressilator* is depicted in the following diagram:

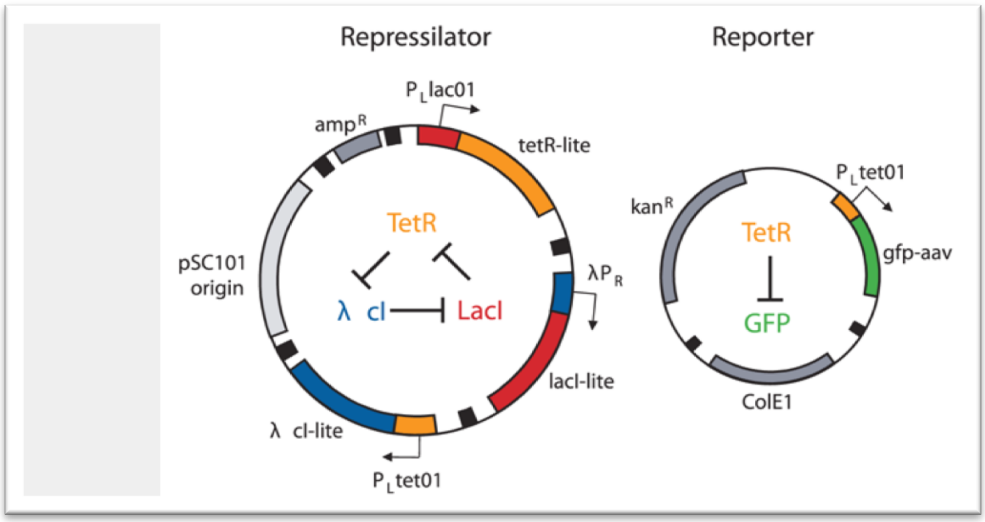

Fig.(3). The main components of the Repressilator (left hand side) and the Reporter (right hand side) (Elowitz and Leibler 2000, p. 336).

In the diagram the synthetic gene regulatory network, the *Repressilator,* is shown on the left hand side and it consists of two parts. The outer part is an illustration of the plasmid constructed by Elowitz and Leibler. The plasmid is an extra-chromosomal DNA molecule integrating the three genes of the *Repressilator*. Plasmids occur naturally in bacteria. In the state of competence, bacteria are able to take up extra chromosomal DNA from the environment. In the case of the *Repressilator,* this property allowed the integration of the specific designed plasmid into E.coli bacteria. The inner part of the illustration represents the dynamics between the three genes, *TetR*, *LacI*, and *λcl*. The three genes are connected by negative feedback loops. The left hand side of the diagram shows the *Reporter* consisting of a gene expressing a green florescent protein (GFP), which was fused to one of the three genes of the *Repressilator*. The GFP oscillations in the protein

level made visible the behavior of the transformed cells allowing researchers to study them over time by using fluorescence microscopy.

The construction of the *Repressilator* was enabled by the development of new methods and technologies, such as the construction of plasmids and Polymerase Chain Reactions (PCR). These new methods enabled physicists like Leibler and Elowitz to enter the biology lab and to perform wet lab experiments. Several fundamental questions were faced in engineering the synthetic model. For instance, would the *Repressilator* interfere with the rest of the cell or would it function autonomously, that is, would the engineered genetic network form a module in the cell? This is an important question regarding the possible applications of synthetic models as well as the use of them as tools for exploring various hypothetical design principles in biological systems. In both cases, it would be desirable that the synthetic model could function as independently as possible from the rest of the cell environment. As a consequence, the behavior of synthetic models in biological organisms would also provide evidence on to what degree biological systems are organized in a modular fashion.

Like the theoretical models discussed above, synthetic models such as the *Repressilator* are used in gaining understanding about what kind of components and interactions produce certain observed behavior. The goal is not to replicate any specific mechanism found in biological systems, the stress is rather on isolating the "sufficient components" able to account for a class of phenomena. For example the genes used in the *Repressilator* do not occur in such a combination in any biological system but are chosen and tuned in such a way, on the basis of the simulations of the underlying mathematical model and other background knowledge, that the resulting mechanism would allow for oscillations. At this point the synthetic approach is still rather limited because of the insufficient empirical knowledge on, for example, biochemical parameters. But it nevertheless goes beyond purely theoretical modeling activity by testing whether the possible mechanisms suggested by mathematical models could be realized in biological systems.

The *Repressilator* was a partial success. In contrast to the mathematical model underlying it, the *Repressilator* did not show the expected behavior: regular oscillations. Instead the oscillations turned out to be noisy. Computer simulations taking into account

stochastic fluctuations did show that such fluctuations could be the cause of the noisy behavior. But the noisy behavior could have also been caused by external noise coming from the cell environment. A new line of research emerged exploring the different sources of noise and their effects on biological systems. In the context of this research, and in line with the results gained by studying complex systems in physics and in neuroscience, noise based on stochastic fluctuations gained a functional status: molecular mechanisms in biological systems make use of internal noise, for example in decision processes such as cell division. As for the question of robustness this meant that robustness does not provide an all-encompassing desideratum – or unequivocal criteria for distinguishing between different theoretical mechanisms – as certain amount of noise might be functional for biological mechanisms.

4. Discussion: Circadian clock research as an instance of causal isolation robustness analysis

In the preceding sections we have argued that independent determination robustness analysis should be distinguished from causal isolation robustness analysis. Moreover, we have presented the circadian clock research as an instance of the latter kind of robustness analysis: it has aimed for the causal isolation of the sufficient components and interactions leading to circadian oscillations using the notion of robustness as a means for this end. To get more philosophical grasp of the issues involved let us first examine more closely the notion of isolation. It has featured prominently in the work of Uskali Mäki (e.g. 1992, 2009) and Nancy Cartwright (e.g. 1989, 1999). Both have drawn from philosopher and economist John Stuart Mill (1843) the idea that models abstract causally relevant factors, mechanisms or capacities, to study their behavior in particular controlled model environments. While Cartwright writes variably about abstraction, isolation and idealization, Mäki makes isolation the central concept of his account of modeling. According to him a theoretical model is an outcome of the method of isolation, in which a set of elements is theoretically removed from the influence of other elements through the use of a variety of unrealistic assumptions. These assumptions make use of various techniques such as abstraction, idealization and omission (Mäki 1992). For Mäki the

notion of isolation is part of his larger project of showing that the notion of truth has a proper place in the discussions on modeling (e.g. Mäki in press), but for our present purposes let us focus on two important features of his account.

First, the idea of models as outcomes of theoretical isolation justifies the use of simplified and highly idealized models by paying attention to how the unrealistic assumptions function as strategic falsehoods that isolate from other things whatever seems worthy of close attention (Mäki 2009). This seems to imply the idea, which Mäki does not spell out, but others like Strevens (2008) do, that if we aim to explain a certain phenomenon of interest, only those core causal factors, capacities or (parts of) causal mechanisms are taken into account that bring about it. Weisberg (2007) characterizes this strategy as "minimalist idealization" since according to it only such factors that "*make a difference* to the occurrence and essential character of the phenomenon in question" are considered (p. 642, italics of the original). Second, and closely related to the first point, theoretical isolation is understood in an analogy to material experiments, which also close a system of interest by neutralizing or "sealing off" a number of factors that might cause disturbances (Mäki 1992, 2005, 2009).

It seems indubitable that the method of isolation captures well the spirit of many scientific practices. However, especially as regards modeling the method of isolation has problems both in practice as well as in principle. The success of causal isolation is obviously linked to the causal structure of the world, which may not be modular allowing one to manipulate the assumedly separable causal factors somewhere without altering causal relationships elsewhere. This was also what the researchers following the strategy of causal isolation in the circadian clock research came across. It also shows that the applicability of the method of isolation is ultimately an empirical matter, and depends on the nature of the systems under study.

Another problem of the method of isolation is related to the nature of mathematical abstraction. Idealizing assumptions are often driven by the requirements of tractability rather than by those of isolation. Thus model assumptions do not merely neutralize the effect of the other causal factors but rather construct the modeled situation in such a way that it can be conveniently mathematically represented making it often unclear which assumptions are crucial for the results, or whether the results are model

dependent (i.e. dependent on the specific mathematical construction of the model, see e.g. Cartwright 1999, Morrison 2008, Knuuttila 2009). This feature of mathematical modeling is further enhanced by its use of cross-disciplinary computational templates that are in the modeling process adjusted to fit the field of application (for computational templates, see Humphreys 2002, 2004; Knuuttila and Loettgers in press). Such templates are often transferred from other disciplines, like in the case of the circadian clock research, where many models, formal methods and related concepts originate from physics and engineering (e.g. the concepts of oscillator, non-conservative system, feedback mechanism, and noise).

Causal isolation robustness analysis has been suggested precisely as a strategy to deal with the aforementioned chronic problem of modeling caused by the idealizing assumptions needed to make the model tractable. This idea was already present in Levins's classic article (see above) and it is reformulated and illustrated in the two recent case studies on robustness analysis. One of these cases examines the so-called Lotka-Volterra model on predator-prey dynamics (Weisberg and Reisman 2008), and the other one geographical economics models concerning the spatial location of economic activity (Kuorikoski, Lehtinen and Marchionni 2010). Weisberg characterizes this kind of robustness analysis as "simply a way of isolating a core structure which is common to multiple models and then determining the consequences of this core structure" (2006a, 643).

Weisberg's characterization of robustness analysis suits both aforementioned cases, although one case concerns population biology and the other one geographical economics. In both of the cases there was originally a simple core model with certain predictions or results, the robustness of which was then studied by changing some of its assumptions and associated mathematical representations. Although in both cases scientists were interested in the robustness of the results derived from a family of models, these (robust) results were not, per se, in doubt, or in need of further confirmation. Rather, the starting point for modeling was provided by an empirically observed phenomenon. For instance, Vito Volterra sought originally to account for surprising post First World War fisheries statistics. His main accomplishment was to show mathematically that the periodic fluctuations in fish populations could be produced by the

mere fact of the interaction between the predator and prey populations (see e.g. Volterra 1927). This was a novel result. Ecologists of his time were acquainted with fluctuations, but they tended to seek explanations from some external cause (e.g. Whittaker 1941).

Likewise Paul Krugman, on whose basic model the geographical economics models studied by Kuorikoski et al. (2010) are based, regarded the core-periphery pattern he was modeling as "among the most striking features of the real-world economies" (Krugman 1991, 483). To be sure, one important aspect of the modeling endeavor is also that of deriving other interesting results from the model, of which the so-called Volterra principle provides an excellent example (see Weisberg and Reisman 2008). In these aforementioned cases, then, robustness analysis functioned as a means for isolating the causal core of the model and studying the generality of the results derived (i.e. the conditions in which the robustness holds or fails).

We have discussed above how circadian clock research has made use of the notion of robustness in its aim to isolate the mechanism producing circadian rhythms. However, in contrast to the aforementioned cases, the robustness analysis in the circadian clock research did not remain in the realm of mathematical modeling alone, but instead combined mathematical modeling with experimentation and a new type of model, synthetic model. In this case it is perhaps misguided to talk about causal isolation when it comes to mere mathematical modeling of circadian rhythms, since the modeled feedback mechanisms were not originally isolated from anything else. Rather, as we have demonstrated above, the various models of circadian mechanism borrowed their underlying templates from already established models, originating even from other disciplines. This also shows that there are limits in how far such robustness analysis that relies on varying the assumptions of models can go. It is often not possible to decompose a model into its assumptions, since they follow frequently from the model template used. Idealizing assumptions are typically linked to certain mathematical abstractions, and there are limited ways in which such assumptions can be relaxed or corrected, constrained by the available mathematical methods. Moreover, in the case of circadian clock oscillations, the oscillatory phenomena have been studied by physicists already for long and there are many well-established ways of mathematically creating them. There exists a whole body of literature on this topic of which Steven Strogatz's book *Nonlinear*

Dynamics and Chaos (Strogatz 1994) provides a good example. Consequently, the modelers were not that uncertain on how their models were able to create the oscillations sought for. The problem was rather that the alternative models were too general and underdetermined by available data.

The isolation of the circadian clock mechanism could first start when empirical research localized the component genes and proteins of the circadian clock mechanism that informed consequent model building. This made it possible to develop more specific models. The modeling activity, in turn, had an impact on the experimental activity in that the results of the experiments were interpreted some underlying mechanisms (notably the negative feedback mechanism) in mind. This process of causal isolation was taken even further in the construction of the Repressilator. It was not only constructed from genetic components on the basis of a mathematical model, but it also focused on components and interactions that are "sufficient" for the production of oscillations characteristic of gene regulatory networks. In striving for the *general design principles* of circadian clock, the Repressilator provides thus a clear instance of minimalist idealization.

The most controversial question as regards causal isolation robustness analysis is whether it is able to provide confirmation for the model if it is performed on the level of modeling alone. To this question the answer of both Weisberg and Reisman (2008) and Kuorikoski et al. (2010) seems to be negative. Both Weisberg (2006b) and Kuorikoski et al. point out that robustness analysis assists in evaluating the different parts of models helping thus to guard against error. Kuorikoski et al. add also that robustness analysis in economics is "usually a special, degenerate form of general robustness analysis as Wimsatt defines it" (2010, 562). Although we agree with the general thrust of their analysis, this suggestion appears to be beside the point since, as we have argued above, the Wimsattian kind of independent determination robustness analysis targets the model results while the causal isolation robustness analysis focuses on the causal core of a group of models.

Weisberg (2006b) in turn puts forth an interesting suggestion that seems to reach into the very heart of mathematical modeling. Namely, he claims that it is not robustness analysis that ultimately confirms mathematical models but rather the low-level mathematical confirmation, which allows robust theorems to make claims about real-

world phenomena. Low-level confirmation is "confirmation of the fact that certain mathematical structures can adequately represent properties of target phenomena" (Weisberg 2006b, 740). The basic idea behind this proposal appears to be that if we are able to mathematically represent certain phenomena correctly *and* our models cover enough heterogeneous set of situations then successful robustness analysis can give some degree of confirmation to the explanations offered by our models.

An example Weisberg offers on low-level confirmation is: "If the population is growing logistically then the logistic model will continue to make accurate predictions about the population size in the future" (Weisberg 2006b, 741). Yet this was precisely the kind of information that the circadian clock modelers were lacking. What puzzled them was whether the negative feedback structure was the right way of representing the assumed mechanism producing circadian rhythms. The Repressilator was constructed for that very reason. The uncertainty was aggravated by the fact that the model templates and associated concepts used in modeling the circadian clock phenomena had largely been transferred from other disciplines, notably from physics and engineering. Moreover, this kind of knowledge concerning the underlying mechanism(s) producing circadian oscillations was needed also to interpret empirical findings. Consequently, in circadian clock research mathematical modeling, experiments on model organisms and synthetic modeling formed an interconnected *combinatorial* fabric on which any confirmation was tied. The case of circadian clock research also shows that although there are similarities between modeling and experimentation, in the actual scientific practice they have different, partly complementary functions[4], which is a point to which the present discussion on experimentation and modeling/ simulation has not paid enough attention (cf. also Parker 2009).

5. Conclusion

[4] Circadian clock research has been used recently by Bechtel and Abrahamsen (in press a, b) as an exemplary case of mechanistic research that has successfully combined the decompositional approach of finding the basic components of mechanisms to the study of their interactions by modeling. On our account this "recomposition" taking place between experimental results and modeling was far from seamless and synthetic models were partly designed to fill the gap between these two activities (cf. Loettgers 2007).

The case of the circadian clock research points to the limits of robustness analysis performed on the level of modeling alone. The scientists in this field did not take it for granted that mathematical models succeeding to produce oscillatory behavior would necessarily represent the kinds of mechanisms that operate in nature. Mere mathematical modeling remained in the level of depicting *possible* mechanisms and it would have also remained too unspecific as regards the molecular basis of the circadian phenomena had it not integrated results from the empirical work. Moreover, mathematical modeling was ridden by the problem of underdetermination. Consequently, in order to further isolate the components and the structure of the circadian mechanism researchers had to revert to experimentation and synthetic modeling, which were triangulated with mathematical modeling in subtle ways. In this case, then, empirical work was a necessary part of the causal isolation robustness analysis. Robustness was used in the circadian clock research as a criterion of model development and evaluation. The first goal of modeling was to build models that exhibited the kind of robust oscillation that is typical of circadian clock phenomena. Then robustness was used as a criterion for distinguishing between alternative models. Finally, the mathematically described hypothetical mechanisms producing robust oscillations were tested by a synthetic model to see whether they are realizable, that is, able to produce robust oscillations in biological systems.

One might ask whether the way in which modeling was informed by empirical work in the circadian clock research was due to the phenomenon studied. Namely, while the variants of the Lotka-Volterra model and geographical economics models remain in the population level, the circadian clock research was also digging into the basic biological components of the gene regulatory mechanisms. Yet this decompositional reductionist program led eventually to the conclusion that environmental influences and internal stochastic variations ("noise") are more important for the functioning of gene regulatory networks than what where originally assumed. While there is thus no doubt that the isolationist strategy provides a strong and fruitful heuristics for scientific research, its consistent application might lead to its own demise.

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References

Alon U, Surette M, Barkai N, Leibler S (1999) Robustness in bacterial chemotaxis. Nature 397(6715):168-171

Barkai N, Leibler S (2000) Circadian clocks limited by noise. Nature 403(20):267-268

Bechtel W, Abrahamsen A (in press a) Dynamic mechanistic explanation: Computational modeling of circadian rhythms as an examplar for cognitive science. Stud. in Hist. and Philos. of Sci. Part A. doi:10.1016/j.shpsa.2010.07.003

Bechtel W, Abrahamsen A (in press b) Complex biological mechanism: Cyclic, oscillatory, and autonomous. In: Hooker CA (ed) Philosophy of complex systems. Handbook of the Philosophy of Science, Volume 10. Elsevier, New York

Calcott B (in press) Wimsatt and the robustness family: Review of Wimsatt's Re-engineering Philosophy for Limited Beings. Biol. Philos.

Cartwright N (1989) Nature's capacities and their measurement. Clarendon Press, Oxford

Cartwright N (1999) The vanity of rigour in economics: Theoretical models and Galilean experiments. Centre for Philosophy of Natural and Social Science. Discussion paper series 43/99. (Published also in P. Fontaine and R. Leonard (eds), The Experiment in the History of Economics. Routledge, 2005)

Elowitz M, Leibler S (2000) A Synthetic Oscillatory Network of Transcriptional Translation. Nature 403 (6767):335-338

Goldbeter A (1995) A Model of Circadian Oscillations in the Drosophila Period Protein (PER). Proc. of the R. Soc. B 261, 319-324

Goldbeter A (1996) Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behavior. Cambridge University Press, New York

Goodwin B (1963) Temporal Organization in Cells. Academic Press, London and New York

Hardin PE, Hall JC and Rosbash M (1990) Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. Nature 343, 536–540.

Humphreys P (2002) Computational models. Philos. Sci. 69: 1-11

Humphreys P (2004) Extending ourselves: Computational science, empiricism, and scientific method. Oxford University Press, Oxford

Jacob F, Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3, 318–356

Kitano H (2004) Biological robustness. Nat. Rev. Genet. 5: 826-837

Kerner, E (1957) A Statistical Mechanics of Interacting Biological Species. Bul. of Math. Biophys. 19:121-146

Knuuttila T (2009) Isolating representations versus credible constructions? Economic modeling in theory and practice. Erkenntnis 70: 59-80

Knuuttila T, Loettgers A (in press) The productive tension: Mechanisms vs. templates in modeling the phenomena. In: Humphreys P, Imbert C (eds) Representations, Models, and Simulations. Routledge
Konopka RJ, Benzer S (1971) Clock Mutants of Drosophila melanogaster. Proc. Natl. Acad. Sci. 68(9): 2112-2116
Krugman P (1991) Increasing returns and economic geography. The Journal of Political Economy. 99(3): 483-499
Kuorikoski J. Lehtinen A, Marchionni, Caterina (2010) Economic modeling as robustness analysis. Br. J. Philos. Sci. 61: 541-567
Levins R (1966) The strategy of model building in population biology. Am. Sci. 54: 421-431
Levins R (1968) Evolution in changing environments; some theoretical explorations. Princeton University Press, Princeton, N.J.
Levins R (1993) A Response to Orzack and Sober: Formal analysis and the fluidity of science. Q. Rev. Biol. 68: 547-555
Loettgers, A (2007) Model organisms, mathematical models, and synthetic models in exploring gene regulatory mechanisms. Biol. Theor. 2:134-142
Mäki U (1992) On the method of isolation in economics. Poznan Studies in the Philosophy of the Sciences and the Humanities. 26: 319–354
Mäki, U (2005) Models are experiments, experiments are models. J. of Econ. Meth. 12: 303-315
Mäki U (2009) MISSing the world. Models as isolations and credible surrogate systems. Erkenntnis 70:29-43
Mäki U (in press) Models and the locus of their truth. Synthesis doi:10.1007/s11229-009-9566-0
Mill JS (1843) A System of Logic. Longman, Green, & co, London
Mitchell SD (2008) Exporting causal knowledge in evolutionary and developmental biology. Philos. Sci. 75: 697-706
Morrison M (2008). Fictions, representations, and reality. In: M. Suárez (ed), Fictions in science: Philosophical essays on modeling and idealization (pp 110-135). Routledge, New York & London
Orzack SH, Sober E (1993) A Critical assessment of Levins's The strategy of model building in population biology (1966). Q. Rev. Biol. 68: 533-546
Parker W (2009) Does matter really matter? Computer simulations, experiments and materiality. Synthesis 169(3): 483-96
Stelling J, Sauer U, Szallasi Z, Doyle III FJ, Doyle J (2004) Robustness of cellular functions. Cell 118: 675-685
Stegenga J (2009) Robustness, Discordance, and Relevance. Philos. Sci. 76: 650-661.
Strevens M (2008) Depth: An account of scientific explanation. Harvard University Press, Harvard
Strogatz S (1994) Nonlinear Dynamics and Chaos. Perseus Books Publiching, LLC.
Sugden R (2000) Credible worlds: The status of theoretical models in economics. J. of Econ. Meth. 7(1): 169-201
Taguchi, Genichi, Chowdhury S, Taguchi S (2000) Robust engineering. McGraw-Hill, New York
Volterra V (1927) Variations and fluctuations in the numbers of coexisting animal

species. In: Scudo FM and Ziegler JR (eds), 1978, The golden age of theoretical ecology: 1923-1940. Springer-Verlag, Berlin, pp 65-236
Wagner A (2007) Robustness & evolvability in living systems (Princeton Studies in Complexity). Princeton University Press, Princeton
Weisberg, M (2006a) Forty years of "The strategy": Levins on model building and idealization. Biol. Philos. 21: 623-645
Weisberg, M (2006b) Robustness analysis. Philos. Sci. 73: 730-742
Weisberg, M (2007) Three kinds of idealization. J. Philos. CIV(12):639-59
Weisberg M, Reisman K (2008) The robust Volterra principle. Philos. Sci. 75: 106-131
Whittaker, E (1941) Vito Volterra. Obituary Notices of Fellows of the Royal Society of London 3:691-729
Wimsatt WC (1981) Robustness, reliability, and overdetermination. In: Brewer MB, Collins B (eds) Scientific inquiry and the social sciences – A volume in honor of Donald T. Campbell. Jossey-Bass, San Francisco, pp 124-163
Wimsatt WC (2007) Re-engineering philosophy for limited beings: Approximations to reality. Harvard Univ. Press, Cambridge
Winfree AT (1967) Biological Rhythms and the Behavior of Populations of Coupled Oscillators. J. Theoret. Biol. 16: 15-42
Woodward, James (2006) Some varieties of robustness. J. Econ. Meth. 13: 219-240