REVIEW

# Mathematics and biology: a Kantian view on the history of pattern formation theory

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**Abstract** Driesch's statement, made around 1900, that the physics and chemistry of his day were unable to explain self-regulation during embryogenesis was correct and could be extended until the year 1972. The emergence of theories of self-organisation required progress in several areas including chemistry, physics, computing and cybernetics. Two parallel lines of development can be distinguished which both culminated in the early 1970s. Firstly, physico-chemical theories of self-organisation arose from theoretical (Lotka 1910–1920) and experimental work (Bray 1920; Belousov 1951) on chemical oscillations. However, this research area gained broader acceptance only after thermo-dynamics was extended to systems far from equilibrium (1922–1967) and the mechanism of the prime example for a chemical oscillator, the Belousov–Zhabotinski reaction, was deciphered in the early 1970s. Secondly, biological theories of self-organisation were rooted in the intellectual environment of artificial intelligence and cybernetics. Turing wrote his *The chemical basis of morphogenesis* (1952) after working on the construction of one of the first electronic computers. Likewise, Gierer and Meinhardt's theory of local activation and lateral inhibition (1972) was influenced by ideas from cybernetics. The Gierer–Mein-hardt theory provided an explanation for the first time of both spontaneous formation of spatial order and of self-regulation that proved to be extremely successful in elucidating a wide range of patterning processes. With the advent of developmental genetics in the 1980s, detailed molecular and functional data became available for complex developmental processes, allowing a new generation of data-driven theoretical approaches. Three examples of such approaches will be discussed. The successes and limitations of mathematical pattern formation theory throughout its history suggest a picture of the organism, which has structural similarity to views of the organic world held by the philosopher Immanuel Kant at the end of the eighteenth century.

**Keywords** Chemical oscillations · Spatial patterning · Dissipative structure · Turing pattern · Local activation · Lateral inhibition

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## From Kant to Driesch

A hallmark of Immanuel Kant's philosophy is its openness towards all developments of empirical sciences (Watkins 2001). In one of his earliest works, Kant presented a theory of the formation of the planetary system on the basis of Newton's laws of gravitation (*Universal natural history and the theory of heavens*, 1755). He reflected extensively about foundational questions of physics (*Metaphysical physical foundation of natural science*, 1786). Throughout his life, he absorbed all new developments in chemistry, although he states that the chemistry of his days is still far away from becoming a science. According to Kant, 'in every discipline of natural sciences there is present only so much real science, as there is mathematics' (Kant 1900ff Vol 4, 470). Contemporary chemistry clearly was not living up to this ideal. Kant also showed eager interest in biology (Löw 1980; Ginsborg 2001). His essays on the concept of the biological species (1775, 1785) were received as

important contributions to this topic by contemporary biologists (Roth 2008). Biology was even of great importance for the general architecture of Kant's philosophy reflected in his three *Critiques*. While the Newtonian laws were the prime example for a science in his first *Critique*, the *Critique of Pure Reason* (1781, 1787), biology represented a central topic in his third critique, the *Critique of Judgment* (1790). Here, Kant developed a theory of the organism, which would become influential for the life sciences of the nineteenth century.

This theory makes a strong claim about the way we analyse organisms as opposed to other objects of nature. Organisms, Kant believes, are not fully accessible for us through mechanistic explanations on the basis of general laws exemplified by Newton's principles. In a famous passage, he says: '…it would be absurd for humans even… to hope that there may yet arise a Newton who would make comprehensible even the generation of a blade of grass according to natural laws…' (Kant 1900ff Vol 5, 400). One may well rephrase this sentence by saying human beings are not able to mathematise biology. In spite of such a strong proposition, Kant supports mechanistic studies of organisms because '…without this no insight into the nature of things can be attained' (Kant 1900ff Vol 5, 410). Thus, Kant's attitude appears to be almost schizophrenic. On the one hand, he posits that organisms will always resist complete mechanistic and, in particular, mathematical explanations. The reason is that they have a certain goal-directedness (purposiveness), which humans can describe only in functional terms, i.e. in the same way as we describe a machine. He calls the type of judgment we use in analysing organisms and artefacts teleological judgement. On the other hand, biology as a science requires, according to Kant, a mechanistic approach using forward causal explanations, which ultimately need to be expressed in mathematical terms. Kant repeatedly stresses the point that the necessity of this dual approach for studying organisms is not a property of nature itself, but rather due to our limited faculties. He explicitly criticises all vitalistic approaches which ask for special laws of living matter.

Kant's third *Critique* was widely read by scientists in the nineteenth century and apparently had a significant impact on the research agenda of biologists. It freed the working biologist from the burden to explain every aspect of organisms on the scarce basis of the mechanical laws of the early nineteenth century—chemistry was still in its infancy—but at the same time motivated experimental approaches aiming for causal explanations. This attitude was termed *teleomechanism* (Lenoir 1989) because it combined mechanistic analyses with the idea of a given machine-like (purposive) structure that itself required no further theoretical grounding in known natural laws. We can still see this attitude at the end of the nineteenth century

in the work of Hans Driesch, who wrote: 'On the basis of this given structure, this machine, we gain a causal understanding of the functions with the help of chemistry and physics…. But the given structure of the living can only be understood in teleological terms' (Driesch 1894). This passage is from Driesch's *Analytical theory of development*, one of the great and influential books in the history of developmental biology. Inspired by ideas of Wilhelm Roux who had coined the term *Entwicklungsmechanik*, Driesch started his research with the goal to explain development on the basis of physics and mathematics. But soon, his experiments with sea urchins brought him into opposition to Roux who was a follower of Weismann's *Keimplasmtheorie* which held that differentiation in development results from the specific partitioning of the genetic material. Driesch, on the other hand, correctly assumed that the complete genetic material was present in each cell nucleus. 'Insofar as it contains a nucleus, every cell, during development, carries the totality of all primordia…' (Driesch 1894). This conclusion was in part based on Driesch's observation of self-regulation and scaling during development. When he separated the blastomeres of two-cell sea urchin embryos, each isolated blastomere gave rise to a complete embryo which was only smaller in size compared to a normal one. The experiments worked also with four-cell and partially with eight-cell embryos. How could it happen that a part of an embryo reconstituted the whole structure with all its elements correctly arranged, but just on a smaller scale? To explain the scaling behaviour, Driesch described the sea urchin embryo as a 'harmonious equipotential system'. The potential of a given cell to differentiate into a particular part of the body depends on its position within the embryo. Thus, Driesch introduced the concept of 'positional information' 75 years before it was re-introduced and refined by Lewis Wolpert (Wolpert 1969). Although Driesch first sought physical explanations for the 'harmonious equipotential system', further experimental studies convinced him that the phenomena of scaling and self-regulation could not be explained by the laws of physics and chemistry known at his time (Driesch 1899). Embryonic development seemed to require a form of self-organisation, which had not been observed in inanimate nature. Driesch postulated additional laws restricted to organismic life, and thus became one of the last famous vitalists in the history of biology. Now, he also explicitly broke with Kant's more cautious attitude. In *Kant und das Ganze*, Driesch states that Kant's use of teleological principles as mere methodological tools for analysing organisms was inconsistent and that Kant, on the basis of his own theory, should have adopted a vitalistic stance (Driesch 1924).

Driesch was harshly criticised by his colleagues, in particular by Wilhelm Roux in his reply *The self-regulation,*

*a characteristic and not necessarily vitalistic capacity of all organisms* (Roux 1914). However, when it comes to mechanisms of self-regulation, Roux could not offer much. He mentions phenomena in the inorganic world which seemed to be examples of self-regulation to him. One of these examples is the flame of a candle. The flame has metabolism, it exists only through the flow and consumption of material and it regulates its size according to the supply. The other example is the regeneration of defects in crystals, which inorganic chemists had observed (Przibram 1906). What these examples have in common is the highly inhomogeneous starting situation. They do not provide instances for spontaneous pattern formation emerging from unstructured initial conditions. Such examples were virtually non-existent when Driesch argued for his vitalistic view in 1899.

In later years, Driesch was confronted with attempts of colloid chemists to provide physicochemical analogues for life. In 1896, Raphael E. Liesegang had published a paper showing that in a solidified gel containing potassium dichromate, the local addition of a drop of silver nitrate leads to the formation of ring-like precipitation patterns of sliver dichromate. Subsequently, Liesegang described modifications of this experiment as 'Emulation of life processes' (Liesegang 1911a, b, c). Likewise, the French biologist Stéphane Leduc had shown that the interplay between precipitation of inorganic salts and osmotic growth produces complex morphologies. He called his experimental work 'synthetic biology' (Leduc 1912; for a famous description see Mann 1947, 22–24). While most of this early work on colloid chemistry left no significant traces in the history of pattern formation theory (Keller 2002, 15–49), Liesegang's periodic precipitation patterns continued to attract the attention of physicochemists. A satisfactory mechanistic account, though, required the fully developed formalism of chemical reaction diffusion theory which would become available only much later (Flicker and Ross 1974). To date, the complex interplay between gel matrix, ionic properties of the electrolytes and speed of precipitation producing not only rings but also spirals and complex branching patterns is not fully understood (Sultan and Sadek 1996; Karam et al. 2011). However, neither at the time of their discovery nor in subsequent years did the Liesegang patterns contribute much to an understanding of biological processes. They have mostly been linked to geochemical phenomena such as banding patterns in minerals (Krug and Kruhl 2000). At the time of Driesch, though, many biologists had the hope that colloid chemistry provided a key to the secrets of life. Driesch himself was impressed by the complex patterns and shapes generated in the experiments of Leduc and Liesegang, but doubted their importance for explaining embryonic development with two convincing arguments: they lacked the reproducible specificity of organic forms and the capacity to self-regulate (Driesch 1921, 80–82, 134).

Driesch was also fascinated by D'Arcy W. Thompson's monumental *On growth and form* (1917), a work which would become influential for all subsequent researchers addressing problems of morphogenesis and pattern formation. Thompson extensively applied mathematics to problems of biological form at all levels of organisation, but only in a descriptive and non-mechanistic way. 'My sole purpose is to correlate with mathematical statement and physical law certain of the simpler outward phenomena of organic growth and structure or form…' (Thompson 1917, 10). In Driesch's view, Thompson's geometric descriptions 'provided only the general type of an arrangement' (Driesch 1921, 80) or 'the general frame for the process' (Driesch 1921, 82). It could neither explain the specific arrangement nor the underlying generative forces. Had Driesch seen the later developments in molecular biology that addressed the problem of specificity and the rise of theories of self-organisation that provided models for self-regulation and scaling, I believe, he would not have voted for vitalism. However, these developments were only in their infancy in Driesch's lifetime.

## The rise of physicochemical theories of self-organisation

The history of biological pattern formation theory is indirectly linked to the discovery of self-organisation in chemical systems, which, in turn, is rooted in theoretical discussions and experimental findings on oscillating chemical reactions in homogeneous phase. This link, although indirect, has three ramifications. First, the most successful and influential early theories of biological pattern formation were based on chemical-reaction diffusion rather than physical (mechanical, hydrodynamic, electromagnetic) models. In the framework of these chemical approaches, there was no reason to believe that biomolecules within an organism would not obey the same laws of mass action and diffusion which apply to molecules in general. Therefore, whatever progress occurred in chemical kinetics could be used to compare and enrich biological theories. Second, conceptual problems to imagine how self-organising processes could occur on the basis of the known physical laws were not unique to biologists like Driesch. Many chemists in the first half of the twentieth century also believed that spontaneous emergence of temporal or spatial order in a homogeneous phase violated the second law of thermodynamics. Indeed, a broad acceptance of the possibility of self-organisation in biology was predated and prepared by the analysis of physical conditions allowing for temporal and spatial order to arise in chemical systems. Third, the communication between chemistry and biology was not

unidirectional, with chemistry providing the groundwork for biological theories. Rather the opposite applies: much of the work in chemistry was motivated by ideas and experimental observations from biology.

None of the players in the unfolding story of chemical self-organisation theories provides a better example for the mutual influence of chemical and biological concepts than the Austrian-American chemist Alfred Lotka (1880–1949). In 1910, Lotka published a paper entitled *Contribution to the theory of periodic reactions* in the Journal of Physical Chemistry (Lotka 1910). In this paper, he showed that under certain conditions, autocatalysis leads to chemical oscillations. He still assumed an inhomogeneous system in which a substance A is set free with a constant rate from a condensed phase and then reacts to produce a substance B. B influences autocatalytically its own production and decays with a constant rate. Lotka solved the coupled differential equations and showed that the system leads to damped oscillations. At the end of the paper, he states 'No reaction is known which follows the above law, and as a matter of fact the case here considered was suggested by the consideration of matter lying outside the field of physical chemistry' (Lotka 1910). In the footnote to this sentence, he mentions the work of Brailsford Robertson, who, motivated by Jacques Loeb, had measured growth curves of different organisms and had come to the conclusion: '…cell division has been shown by Loeb to be the expression of an autocatalytic synthesis of nuclear material…in all probability, cell growth or the synthesis of cytoplasm is also an autocatalytic reaction' (Brailsford Robertson 1908).

While the system Lotka had described in 1910 was producing damped oscillations, he later extended his work to systems with sustained, undamped oscillations and, in particular, also to homogeneous systems. In 1920, he published *Undamped oscillations derived from the law of mass action* (Lotka 1920a). In the introduction, he states that in the earlier work he believed that the relevant kinetic conditions could only be provided in heterogeneous systems. 'It is, therefore, somewhat contrary to his first expectations that the writer now finds the conditions for undamped oscillations may occur in the absence of any geometrical causes in a homogeneous system' (Lotka 1920a). His new system coupled two autocatalytic reactions, one consuming the product produced by the other reaction. Again, Lotka cannot point to a realistic example from chemistry and ends the article with a note on biology; 'Rhythmic phenomena are of particular interest in connection with biological systems (e.g. heart-beat)…' (Lotka 1920a). He also announces a more general extension of his work to biological problems. This extension, published in PNAS in the same year, contains the equations which were independently discovered by Volterra and today are called Lotka–Volterra equations used to model predator–prey relationships in population biology (Lotka 1920b). Lotka extensively cites Spencer's first principle; 'Every species of plants and animal is perpetually undergoing a rhythmic variation in number—now from the abundance of food and absence of enemies rising above its average, and then by a consequent scarcity of food and abundance of enemies being depressed below its average…' (Lotka 1920b). Biology at the population level or physiology seemed to offer applications of his chemically derived equations, while in chemistry, as he notes at the end of his article, oscillations are at best 'laboratory curiosities'. Later developments showed that neither realistic chemical oscillations (see below) nor predator–prey cycles could be described by Lotka's equations, although they remain extremely popular in ecology (Kot 2001).

The lack of applications in chemistry and the obvious affinity of his work to biology made Lotka more and more interested in a physical and mathematical foundation of biology. In particular, he became one of the first authors to point out the limits of the known laws of thermodynamics in explaining life processes; '…something more than the first and second laws of thermodynamics is required to predict the course of events' (Lotka 1922). The thermodynamics available to Lotka only dealt with reversible, i.e. equilibrium, states. However, '[r]eal phenomena are irreversible; and in particular, trigger action, which plays so important a role in life processes, is a typical irreversible process…To deal with these problems, requires the introduction of new principles' (Lotka 1922). Lotka envisages the development of a statistical mechanics for populations of organisms in 'which the units shall be, not simple material particles in ordinary reversible collision of the type familiar in the kinetic theory…the units in the new statistical mechanics will be energy transformers subject to irreversible collisions…' (Lotka 1922). These are very visionary comments; however, they seem to imply that an extension of thermodynamics is only required at the population level, while the highly ordered state of the individual organism poses no particular problem in the frame of the existing laws. In 1925, Lotka published *Elements of Physical Biology* which was reprinted in 1956 under the title *Elements of Mathematical Biology*. In his programme of a physical biology, he claims to capture all aspects of organismic life. Part I: *General remarks* deals with a physical theory of evolution; part II: *Kinetics* with growth; part III: *Statics* with populations and ecological interactions; and part IV: *Dynamics* with biochemistry, physiology, behaviour and even consciousness (Lotka 1925). In the introductory chapter *Regarding Definitions*, Lotka rejects attempts to define life by sharply distinguishing it from inorganic phenomena. He explicitly refers to Driesch's statement that no man-made machine exists which shows self-repair and self-reproduction and spec-

ulates that future technological developments will produce such things. Organisms are just highly structured physical systems. The aspect of structure and geometry has, however, been neglected in physical chemistry so far, 'In structured systems…geometrical and mechanical features may play the dominant role' (Lotka 1925, 15). 'The laws of the chemical dynamics of a structured system…will be precisely those laws, or at least a very important section of those laws, which govern the evolution of a system comprising living organisms' (Lotka 1925, 16). However, how structure arises from less-structured states is not at all addressed by Lotka. All concrete discussion of structural features of cells and organisms and their ontogeny are missing from his book. He jumped directly from chemistry to biology at the population level with some remarks on physiology of the adult organism. Curiously, this jump omitted precisely those parts of biology where a chemically motivated theory was most wanted among biologists. Later, Lotka shifted his mathematical work entirely to population growth and demography and, in 1934, published *Analytical Theory of Biological Populations* (Lotka 1934).

What had happened meanwhile in chemistry? In 1910, Lotka was unaware of any oscillating reaction and in 1920 he mentioned some not well-documented laboratory curiosities (Lotka 1920a). In 1921, however, William Bray at UC Berkeley published a paper which seemed to describe a reaction of the type Lotka was looking for (Bray 1921). Bray had worked on chemical reactions of hydrogen peroxide. In a solution containing iodate, iodine and hydrogen peroxide, the latter decomposes to oxygen and water. The rate of evolution of oxygen and iodine was found to vary periodically. Bray concludes 'the writer believes the present example to be the first instance of a periodic reaction in homogeneous solution' (Bray 1921). With his collaborators, Bray deciphered the basic reaction mechanisms (Bray 1921; Bray and Liebhafsky 1931). However, his work indeed remained a curiosity and most chemists and physicists believed that he had not really described a homogeneous reaction, but that the oscillations were an artefact due to dust particles or gas bubble formation. Theoretical arguments were put forward to show that chemical oscillations are not possible in a homogeneous phase. Thus, in 1969 a physicochemist could still publish a paper stating: 'There is no substantiated example of oscillation in a closed homogeneous chemical system and detailed balancing is sufficient to preclude it' (Swartz 1969). Only in the 1970s was the Bray oscillator reinvestigated and was shown to indeed represent a case of an oscillation in a homogeneous phase (Sharma and Noyes 1975; Epstein and Pojman 1998).

The generally hostile attitude towards the possibility of oscillating reactions in a homogenous phase also explains why the most famous and best studied case of an oscillating reaction had remained obscure for such a long time (Degn 1972; Winfree 1984). In 1950, the Russian chemist Boris Pavlovich Belousov (1893–1970) tried to find an inorganic version of the citrate cycle of biochemistry. He examined a variety of redox reactions with citrate, oxidating agents and metal ions as catalysts. A combination of citric acid, bromate and ceric ions showed oscillations in the redox state of the ceric ions and travelling waves in unstirred reaction cylinders. The manuscript he submitted in 1951 was rejected with the argument that 'the supposed discovery was impossible' (Winfree 1984). Belousov spent six more years of work on the system and submitted a revised manuscript which again was rejected. The editor insisted that the paper should be shortened to a letter. At that point, Belousov gave up publishing his work. Only one small abstract remains published in Russian in the proceedings of a conference on radiation biology (Belousov 1959). His original manuscript circulated among colleagues in Moscow, and in 1961, the biophysics student Anatol Zhabotinsky began systematically to analyse the mechanism which later would be called the Belousov–Zhabotinsky (BZ) reaction. Ten papers were published in Russian before experiments were taken up outside Russia and the first paper was published in English (Degn 1967).

Meanwhile, an increasing interest in non-equilibrium thermodynamics had emerged within physics. In 1922, Théophile De Donder had introduced the concept of affinity, which provided a connection between irreversible chemical reactions and entropy production (De Donder and van Rysselberghe 1936). In 1931, Lars Onsager had derived his famous reciprocal relations for couple irreversible processes near to equilibrium (Onsager 1931). In 1935, Ilya Prigogine started to study Chemistry and Physics at Brussels and later did his Master and Ph.D. thesis with De Donder. In his autobiography, Prigogine (1977) recalls that as an adolescent he was deeply impressed by Henri Bergson's *L' evolution creatrice* and that at Brussels, the French school of biochemistry and embryology (Brachet, Dalcq and Florkin) influenced his thoughts. By extending De Donder's approach to other macroscopic irreversible processes including transport phenomena such as diffusion and thermal conduction, Prigogine derived in the mid-1940s a general expression for entropy production and proved his celebrated theorem of minimum entropy production for non-equilibrium stationary states (Prigogine 1947). The phenomenon of thermodiffusion motivated Prigogine to write his first biological paper. An external temperature gradient applied to a mixture of two gases can lead to a situation in which one of the gases accumulates at the hot, and the other at the cold wall. In this simple case, the exchange of energy with the environment leads to an ordered state within the system and thus to a decrease of

entropy. In a small paper with the microbiologist Jean-Marie Wiame, Prigogine discusses the possibility that also biological systems evolve towards states of minimal entropy production corresponding to minimal metabolism and suggests that this tendency leads to an increase in complexity during phylogenetic evolution (Prigogine and Wiame 1946). Although this was a fairly speculative idea, it illustrates the strong interest in biological questions which distinguished Prigogine from his fellow physicists who provided major contributions to non-equilibrium thermodynamics such as Carl Eckart, Josef Meixner and Lars Onsager. Thus, it is not surprising that even Prigogine's early work on linear thermodynamic systems close to equilibrium was immediately discussed by biologists (Bertalanffy 1949; Spanner 1953).

Between 1947 and 1967, Prigogine and co-workers in Brussels attempted to extend non-equilibrium thermodynamics to situations far from equilibrium. Initially, they focussed on hydrodynamic instabilities like the Rayleigh–Bérnard convection, which was experimentally very well described (Glansdorff and Prigogine 1971). In parallel, the first papers on chemical systems were published. A crucial question was whether the current formalism could be applied to periodic phenomena. The first two papers addressing this question were written using the Lotka–Volterra mechanism as a kinetic example, with the aim to show that oscillations do not in principle violate the laws of thermodynamics (Prigogine and Balescu 1956; Prigogine and Balescu 1958). Balescu recalls that speaking of chemical oscillations 'was highly "politically incorrect" at that time' (Balescu 2007, 12). In the discussion of his 1958 paper by leading specialists in the field, one of the participants remarks '…I should like the authors to ask if actual systems have been observed whose behaviour approaches that of the model, or is there somewhere a contradiction in the reaction scheme proposed which makes such behaviour impossible' (Prigogine and Balescu 1958). However, in the following decade, the work of the Brussels school received increasing recognition. The term dissipative structure for states of coherent temporal or spatial order which are maintained far from equilibrium by the dissipation of energy became a trademark (Prigogine 1969). A consensus view emerged of how chemical oscillations, chemical waves or stable spatial patterns in a homogeneous system were compatible with an extended thermodynamic formalism (Nicolis and Prigogine 1977). In brief, chemical oscillations cannot occur as deviations around a chemical equilibrium state like the mechanical oscillations of a pendulum. Nor can they occur close to equilibrium in a steady-state situation characterised by minimum entropy production as Prigogine pointed out already in his earliest writing on irreversible thermodynamics (Prigogine 1947). Rather, the periodic changes in concentration require a situation far from equilibrium, in which chemical reactions need not be reversible and detailed balancing does not apply. In a closed container without exchange of matter with the surrounding environment, reactions have to be postulated which produce substances to perpetuate the oscillations. The oscillations continue as long as sufficient amounts of these substances are provided and cease when they are consumed. Thus, the situation in the homogeneous phase is energetically similar to the example of the flame where the flux of matter clearly transgresses distinct phases. The flame arises and is maintained in a highly structured environment while dissipative structures represent the spontaneous formation of order from an unstructured starting situation.

To develop a thermodynamic formalism which characterises the system behaviour far from equilibrium, Prigogine and his co-workers required chemical models for oscillating reactions or spatial patterning. The only existing model for chemical oscillations, the Lotka equations, turned out to be inappropriate for describing realistic situations (Nicolis and Portnow 1973). Chemical oscillations occurring in the laboratory should be characterised by a certain degree of structural stability, i.e. small variations in the starting concentrations should not affect the final oscillatory behaviour. Otherwise, it would be impossible to observe oscillations as a coherent behaviour of e.g. $10^{20}$ molecules in spite of naturally occurring fluctuations. However, exactly this feature of structural stability is lacking in the Lotka equations. They represent a conservative system more similar to the mathematical pendulum admitting an infinite number of different periodic solutions whose periods and amplitudes are fixed by the initial conditions. In search for a more realistic model for oscillations, the Brussels school could not work with any of the experimentally described cases. Neither the mechanism of the BZ reaction nor that of the Bray oscillator had been elucidated. The same applied for oscillations in biochemical systems which recently had been discovered (Ghosh and Chance 1964). In the absence of a model for oscillations, the Brussels school first investigated the Turing mechanism of spatial patterning, which, as we will see, was entirely based on biological reasoning (Prigogine and Nicolis 1967; Turing 1952). However, soon they presented a theoretical example of an oscillator which showed structural stability (limit cycle oscillations, Prigogine and Lefever 1968). It became subject of a large body of theoretical work and was later dubbed the *Brusselator* (Tyson 1973). In comparison to the Lotka mechanism, structurally stable limit cycle oscillations require at least three reactions, cross catalysis and non-linear autocatalysis; in the case of two-component systems (like the Brusselator), the autocatalytic reaction has to be trimolecular (Nicolis and Portnow 1973; Schnakenberg 1979). Prigogine and Lefever (1968) mention that 'this

reaction scheme is physically unrealistic because of the trimolecular step' and that it was mainly chosen for mathematical reasons. Because of its simplicity, the Brusselator is indeed still used as an example in mathematical treatments of pattern formation theory (Cross and Greenside 2009). As Prigogine and Lefever (1968) point out, systems with higher number of components can be devised easily that are based on more realistic kinetics and show limit cycle oscillations. In particular, enzyme kinetics offered a rich source for non-linear reaction schemes. In an influential paper, the new ideas on dissipative structures were applied to biological questions (Prigogine, et al. 1969). Albert Goldbeter, one of the authors of this paper, would become a leading figure in the analysis of rhythmic phenomena in biology (Goldbeter 1996).

Nevertheless, at the end of the 1960s there was no single case of an experimentally validated mechanism for an oscillating reaction (or a reaction producing spatial patterns). However, the theoretical work provided a strong motivation for experimental approaches. The Brussels school contributed to popularising the BZ reaction as the best experimental example of a chemical oscillator and motivated chemists to do research on its mechanism. In the early 1970s, R. Field and E. Körös in the laboratory of R. Noyes at the University of Oregon undertook a systematic investigation of the BZ reaction. When they started, the option was still discussed that the oscillatory behaviour and the chemical wave patterns were due to heterogeneous effects and would not occur in a completely homogeneous phase. Obviously, the concept of large-scale coherent phenomena far from equilibrium was still not easy to grasp for physical chemists. In 1972, Field, Körös and Noyes published the first successful and largely complete kinetic model of the BZ reaction which later was called the FKN (Field, Körös, Noyes) mechanism (Field, et al. 1972). To explain the oscillatory behaviour, they distinguished ten different reactions represented by detailed kinetic equations. From these, they later derived a model capturing the core kinetics which included only three variables for components and five equations (Field and Noyes 1974). This model was extensively used for computer simulations and largely reproduced the quantitative data from experiments. In analogy to the *Brusselator*, it was dubbed the *Oregonator*. Among biologists, the BZ reaction became very popular through the work of Arthur Winfree who, in particular, was interested in the mechanism of spiralwave formation (Winfree 1972).

Taken together in the early 1970s, both theoretical and experimental approaches had provided a thorough understanding of the spontaneous formation of order in homogeneous chemical systems. The Brussels school played a major role in clarifying the compatibility of these phenomena with the laws of thermodynamics and in defining the general prerequisites for self-organised patterning. The attempt to derive functions for entropy production which characterise temporal or spatial pattern formation far from equilibrium was later criticised (Landauer 1975) and does not play any role in modern treatments of this subject (Cross and Hohenberg 1993; Cross and Greenside 2009). It also should be noted that parallel to the Brussels school, which focused on chemistry, several groups studied the formation of ordered states in purely physical non-equilibrium systems. Hermann Haken developed his theory of synergetics based on his earlier work on laser theory (Haken 1977). The most impressive link between theory and experiments was probably achieved in studies of hydrodynamic phenomena (Swift and Hohenberg 1977; Cross and Hohenberg 1993). Ironically, Prigogine's hope for a deeper understanding and extension of non-equilibrium thermodynamics was not fulfilled by studying collective phenomena of self-organisation at the mesoscopic or macroscopic level, but rather through theories and experiments addressing single molecule behaviour at the nanoscale (Jarzynski 2011). Biology is providing prime examples also for these new approaches (Collin et al. 2005).

## Early theories of biological pattern formation

Despite the obvious interest of the Brussel's school in biology, its main goal was an extension of thermodynamics for which biology merely provided some of the nicest examples and applications. Most of the papers of the Brussels school addressed physicists and were not directly inspired by biological questions. However, in the meantime two papers had appeared, which were entirely motivated by problems of biological pattern formation. In 1952, Alan Turing had published *The chemical basis of morphogenesis* which, as already pointed out, was important for Prigogine's early work on dissipative structures (Prigogine and Nicolis 1967). However, Turing was not concerned with problems of chemistry or thermodynamics. Rather, he immediately addressed particular biological questions. Why was he interested in this topic at all? Before working on mathematical biology, Turing had made major contributions to mathematical logic, cryptology, computer science and artificial intelligence (Hodges 1992). None of these projects, with the exception of the last one, had links to the life sciences. However, Turing had kept a vivid interest in biology throughout his life (Saunders 1993). As a young boy, an American children's book *Natural wonders every child should know* (Brewster 1912) had stimulated his interest in science. This book is devoted entirely to living nature and contains detailed information about animal and plant development, reproducing many figures from original publications. These include accounts of chicken and frog

embryology, descriptions of starfish and planarian regeneration and examples of twinning and homeotic transformations, directly taken form Bateson's influential *Materials for the study of variation* (1894). In the 1930s, Turing had read D'Arcy Thompson's *On growth and form* and was particularly fascinated with the fact that leaf-arrangements (phyllotaxis) frequently follow the Fibonacci series. After the war, he decided to work on the construction of an electronic computer. He called this project 'building a brain'. In 1948, he wrote his first article on problems of artificial intelligence, which contains remarks on the relation between genes and brain structure (reprinted in Copeland 2004, 410–432). In the same year, he moved to Manchester where he played an important role in building one of the first computers able to perform massive calculations. He continued his philosophical reflections on the relation between human mind and computer (Turing 1950) and became involved in discussions with Michael Polanyi, a physicochemist turned philosopher who held a chair in social studies at Manchester. Polanyi criticised reductionist approaches not only regarding the human mind, but also for biology in general. For example, he claimed that morphogenesis cannot fully be explained on mathematical grounds (Roth 2011b). This might have provided additional incentives for Turing to formulate a mathematical theory of morphogenesis.

However, his main motivation certainly was the awareness that the relation between genetic information and morphology represented one of the central unsolved problems in biology. He clearly states that 'the purpose of this paper is to discuss a possible mechanism by which the genes of a zygote may determine the anatomical structure of the resulting organisms' (Turing 1952, 37). The genes are presumed to act purely as catalysts and 'influence the anatomical form of the organism by determining the rates of those reactions which they catalyse' (Turing 1952, 38). In describing the zygote, the early embryo or any developing tissue Turing initially takes chemical and mechanical properties of the cell into account. He omits only electrical properties and the internal structure of the cell because of insurmountable problems with complexity. However, also the 'interdependence of the chemical and mechanical data adds enormously to the difficulty' so that the paper then focuses on cases where 'the mechanical aspect can be ignored and the chemical aspect is the most significant' (ibid). This also provides a closer link to the primary action of the genes which he thought to be chemical. Turing then assumes the existence of 'chemical substances, called morphogens, reacting together and diffusing through a tissue'. These are initially uniformly distributed, but by virtue of their interactions, become distributed in spatial patterns. In a second step, these unequally distributed morphogens activate reactions in the tissue which produce the real shape. The word 'morphogen' was chosen by Turing 'to convey the idea of a form producer' (ibid). He compares his morphogens with Waddington's evocators, substances which induce the formation of a particular organ, and suggests that it is legitimate to separate the processes which lead to a particular 'distribution of the evocator in space and time' from the 'reactions set in train by it'. Here, Turing presages an idea, which later would become very important through the work of Lewis Wolpert, the separability of pattern formation and cell differentiation/morphogenesis.

With his paper, Turing wanted to reach a broad spectrum of readers including, in particular, biologists. Therefore, he uses sections 2 and 3 to provide the background of some mathematics and chemistry and in section 4 he presents his main idea: 'the break down of symmetry and homogeneity' in a system of interacting and diffusing chemicals, at a very elementary level. He chooses only two cells and two chemicals X and Y. The production of X depends on X itself (autocatalysis), while the production of Y depends on X (cross-catalysis). In addition, X moves slower than Y to the neighbouring cell, i.e. the diffusion rate of X is smaller than that of Y. The numerical values of the reaction constants and diffusion rates are chosen such that everybody can do the calculations by hand. Turing shows then that this system has an unstable homogenous equilibrium. It will accentuate any small differences in X and Y which may occur between the two cells just on the basis of random fluctuations. The final state will always be a stable difference of X and Y concentrations between the two cells, i.e. a stable pattern in space.

Turing's deep concern about problems of real biology is apparent from his digression into left-right asymmetry (section 5) before he enters into a serious mathematical analysis of his reaction-diffusion model. The occurrence of left–right asymmetric organisms can be explained as the result of an early event of symmetry breaking. However, left-handed and right-hand organisms should occur with equal frequencies since the laws of physics and chemistry underlying his reaction-diffusion theory do not possess an intrinsic preference for left- or right-handedness. Turing admits that the prevalence of only one type of handedness in a given species poses a serious problem for his model. He sees, however, one possible solution; the early symmetry breaking processes might be influenced by the handedness of organic molecules present in the embryo. Within modern developmental biology left–right asymmetry indeed remained enigmatic for a long time. Only recently has it become clear that for vertebrates, the symmetry-breaking step depends, as Turing suggested, on the chirality of a macromolecular complex (the cilium) which influences a reaction-diffusion mechanism (Tabin 2006).

The main body of Turing's paper is an in-depth mathematical analysis of generalised reaction-diffusion equations for two components acting in a ring of cells. To make the problem analytically tractable, Turing focuses on the emergence of the instability, which allows him to assume linearity for the reaction rates. He shows that depending on diffusion and reaction constants the system shows six different behaviours. (1) A stationary case, in which all cells tend to acquire the same temporally stable concentration. This is the most boring case. However, considering stochasticity effects, Turing shows that even this situation might be interesting for biology since it can produce dappled patterns in two dimensions and thus might account for phenomena like surface coloration. (2) Synchronous oscillation of all cells. Chemical oscillations are thus a subcategory within Turing's theory. He was obviously not aware of Lotka's or Volterra's work and claims that he also does not know any experimental example. The BZ reaction was yet unknown outside Russia and the Bary oscillator was a real laboratory curiosity. (3) Two types of spatiotemporal patterns including travelling waves, which Turing thought require at least a three-component system. He did not analyse these cases in depth. Travelling chemical waves, too, would become of interest only after they had been experimentally observed in a BZ reaction. This lead to the rediscovery of mathematical papers which indeed had treated the problem of spatiotemporal patterning even before Turing, albeit in the context of population genetics. Fisher had provided the partial solution of an equation describing the spreading of a beneficial gene within a population (Fisher 1937). The full solution was given in a paper by Kolmogoroff, Petrovsky and Piscounoff (1937). Exactly the same formalism can be applied to the diffusion wave produced by an autocatalytic substance (an early suggestion along this line had already been published in 1906 by R. Luther). However, Turing was not aware of this early work. (4) Two types of stationary spatial patterns. These were the most interesting cases for Turing, especially the case which allows stationary waves with a different wave-length depending on the difference in diffusion and reaction rates of the two components. It is ironic that both for oscillations and travelling waves, excellent chemical examples became accessible for experimental studies in the 1960s while it took almost 40 years until a stable spatial wave pattern was observed in a chemical system and named after Turing (Castets et al. 1990). In recent times, systematic strategies have been developed to produce beautiful Turing patterns in purely chemical systems (Horvath, et al. 2009).

In his paper, Turing spends most of his analysis and discussion on the stationary waves forming in a ring of cells. In particular, he uses the Manchester computer, which was partially built with his help, to perform numerical calculations on a realistic, fully non-linear example. He chooses a ring of 20 cells with realistic single cell diameters. The diffusion rates are derived from permeability studies of biological membranes and the reaction scheme is justified with concrete chemical considerations. Stochasticity effects are introduced for both the transport and the reaction kinetics. The computer simulations show how a homogenous starting situation evolves via a transitory pattern into a stable series of concentration peaks. Turing's analysis represents the world premier for the application of a digital computer to a reaction-diffusion system. He ends his paper with the visionary note that the use of digital computers will be indispensible for analysing non-linear reaction-diffusion systems.

As biological examples for pattern formation on a ring-like structure, Turing considers certain leaf arrangements (Woodruff) and tentacle formation in hydra. He also provides a linear stability analysis for a reaction-diffusion system confined to the surface of a sphere because he is interested in how symmetry-breaking takes place during gastrulation. Further, he announces a future paper on phyllotaxis. The work on this paper occupied the last 2 years of his life and remained unfinished when he died in 1954 (now published in Saunders 1992; Swinton 2004).

With his paper, Turing had clearly entered an uncharted terrain both in mathematics (at least with regard to numerical analysis) and biology. This becomes clear from his small list of references which comprises only six papers of which five were written by biologists. (1) C.M. Child's *Patterns and problems of development* (Child 1941). In this work, Child had introduced the concept of gradients controlling development. However, he mainly assumed metabolic gradients (e.g. oxygen consumption). (2) A book on the permeability of biological membranes (Davson and Danielli 1943). (3) Michaelis and Menten's paper on enzyme kinetics (Michaelis and Menten 1913). (4) D'Arcy Thompson's *On growth and form*. (5) C. H. Waddington's *Organizers and genes* which provided Turing with a state of the art developmental genetics background (Waddington 1940).

One month after *The chemical basis of morphogenesis* had appeared in print, Waddington sent a letter to Turing containing some prophetic remarks. Waddington characterises the work as 'extremely interesting and suggestive' (Waddington 1952). However, he mentions two problems. First, he believes that there are no homogenous starting situations in biology, 'The newly fertilized egg is, I think, never homogenous in this way, but always possesses some element of pattern of its own…' (ibid). Second, Waddington mentions an experiment with hydra to illustrate the problem of size regulation. He thinks that a chemical wave theory will be unable to explain scaling. Waddington suggests that Turing's mechanism will be most relevant for patterns

forming 'in apparently uniform areas such as the wings of butterflies, the shells of molluscs, the skin of tigers, …' (ibid). He ends his letter by describing the question in embryology which he finds most pressing to be 'tackled from a mathematical point of view' (ibid): the occurrence of 'a finite number of definitely distinct tissues and organs…' (ibid). How is it avoided that intermediate states emerge? How does the selection between discrete states take place? Waddington alludes here to a type of genetic network theory, a theory which does not explain patterning in physical space, but within the state space of a network of interacting genes. From the remarks in his letter, it is apparent that he had started to work on such a theory. In 1954, he published a paper where he proposed a system of two autocatalytic gene products that consume the same substrates and thus enter into a competition resulting in distinct stable states. He also considers a direct interaction of the autocatalytic gene products and compares the resulting system with 'two populations of animals which compete with one another for a limited food supply' (Waddington 1954). Lotka's work is cited for the mathematical treatment of this situation. In the following years, Brain Goodwin did his Ph.D. thesis in Waddington's laboratory and extended the theory to systems with many components and stochastic fluctuations. He called his ideas epigenetic thermodynamics (for a brief summary see Waddington 1962, 45–50).

Despite his criticism, Waddington was one of the few biologists who did take notice of Turing's work. He summarised Turing's results in his writings (e.g. Waddington 1956, 422; Waddington 1962, 125–130) and thereby made it known to other scientists. Another exception was John Maynard Smith, who used Turing's idea of chemical waves to explain modal variations in populations (i.e. the occurrence of fixed integers of anatomical structures like the five fingers of the hand, Maynard Smith 1960). In 1968, he wrote a small book on *Mathematical Ideas in Biology* which ends with a beautifully written, lucid account of Turing's ideas. Nevertheless, in the first 10 years after its publication, Turing's paper was cited fewer than two times per year (Nanjundiah 2003) and it remained largely unknown throughout the 1960s, even to developmental biologists. In part, this was due to a shift of research focus in modern biology. The discovery of the DNA structure in 1953 immediately led to a number of central questions such as deciphering the genetic code, understanding translation, etc. and thus opened up completely new research areas which were evidently crucial for an understanding of life. Physicists and chemists preferred to enter the new field of molecular biology rather than working on theoretical and mathematical modelling of biological phenomena which were not understood at the cellular or molecular level. In addition, there was a high demand for scientists with a physics and mathematics background in the early days of molecular biology both with regard to their experimental (e.g. for X-ray crystallography) and their conceptual (e.g. for deciphering the genetic code) skills.

There were also intrinsic reasons, however, for why Turing's work played only a marginal role even among the few developmental biologists who continued their work in the shadow of the molecular revolution. In his letter to Turing, Waddington had already pointed out what later would be viewed as the most significant deficit of Turing's work: the fact that it did not explain self-regulation (see also Waddington 1956, 422f). A general belief was formed that reaction-diffusion mechanisms are unable to account for such phenomena. Wolpert's introduction of the concept of positional information had spurred a renewed interest in self-regulation and scaling (Wolpert 1969). A typical feature of patterns produced by a positional information mechanism was size invariance. Wolpert used the French flag analogy to illustrate this point: the relative sizes of the blue, white and red areas of a French flag remain the same irrespective of the absolute size of a particular flag. He found Turing's chemical waves 'not very satisfactory' even to explain the pattern of bristles on the insect cuticle, and much less so to account for positional information in general (Wolpert 1969). Rather, he was attracted by the highly complex theory of Goodwin and Cohen (1969), who stated: 'Turing-type periodic waves remain a possibility, but the problem of regulation for such a model is severe'. Their own proposal was based on very specific, but largely unsupported biological assumptions. They derived positional information from the phase difference between coupled cellular oscillators. Nevertheless, their phase-shift model was more popular among theoretically inclined biologists than Turing's ideas, largely because it offered a solution to the mysterious problem of scaling.

In the mid-1960s, an outline of the basic molecular mechanisms of life (replication, transcription, translation) had been attained and some of the pioneers of the molecular revolution were looking for new challenges. More complex biological phenomena such as multicellular development and neurobiology attracted their attention. Alfred Gierer was one of these pioneers. He had received a Ph.D. in theoretical physics with work on proton transfer across hydrogen bonds and the theory of liquids. Pauling's book *The nature of the chemical bond* and the work on the α-helix had motivated Gierer to enter biology. In 1954, he started to work in Tübingen at the Max-Planck-Institute of Virus Research on the RNA of the Tobacco Mosaic Virus and in the following years demonstrated that not only DNA, but also RNA can be a carrier of genetic information. After some work on protein synthesis in the early 1960s, his group shifted to developmental biology, focusing on hydra as a model system which, in particular, allowed them

to study regeneration and self-regulation. In 1971, Hans Meinhardt, who had also received a Ph.D. in physics, joined the group. Influenced by ideas from cybernetics, they formulated their theory of local self-activation and lateral inhibition. From work in the neighbouring institute of Biological Cybernetics, they were aware that during contrast enhancement in the visual system, a local activation by the stimulus is linked to an inhibitory effect in the surroundings (Kirschfeld and Reichardt 1964). The external stimuli provide a pattern, which is enhanced through lateral inhibition. Inhibitory processes had also been invoked in developmental biology (Spiegelman 1945; Rose 1952). Also here, like in the sensory system, they could account for the spatial refinement of an inhomogeneous starting situation. However, in experiments with hydra, the Gierer group had observed axis formation emerging from a uniform aggregate of cells lacking an apparent prepattern. How could one explain such a situation? Inspiration came again from the context of cybernetics. In an account of the intellectual roots of their theory, Meinhardt and Gierer (2000) cite the work of Maruyama (1963), who was stressing the importance of positive feedback in nature and society. He pointed out that classical cybernetics was primarily concerned with deviation-counteracting feedback networks, like thermostats. However, many processes in the universe from the weathering of rocks, the fixation of single mutations in a population, to the growth of cities and economic inequality represent deviation-amplifying systems. They are governed by positive instead of negative feedback. Maruyama envisages a 'second cybernetics' in which deviation-amplifying processes are of central importance. Positive feedback provides the ingredient which causes a self-organising process to start. By combining a local deviation-amplifying process with lateral inhibition, Gierer and Meinhardt could explain how structure formation was possible starting from a homogeneous state. Lateral inhibition was realised either through substrate depletion by the local activation process or by the production of an inhibitor. Key aspects of the kinetics were non-linear autocatalysis of the activator and, as in Turing's model, differences in the diffusion rates of the components. The short-range action of the activator resulted from a small diffusion constant, the long-range effect of substrate depletion or inhibitor action resulting from a large diffusion constant. When they submitted their first paper (Gierer and Meinhardt 1972), they had not read Turing's work. A referee of the paper pointed out its existence (Meinhardt 2006).

It is important to note the differences between Turing's approach and that of Gierer and Meinhardt. Firstly, the demonstration that chemical kinetics is sufficient to produce a spatial pattern from uniformity was not their main goal. They rather formulated two principles which in conjunction are sufficient for self-organised patterning. The molecular or cellular realisation was left open. 'The formalism of the theory is consistent with many…interpretations… Thus, production of components may be due to synthesis or release; removal to degradation or leakage; spreading to diffusion, convection, mechanisms involving transport along intracellular or intercellular fibres, and/or transducing effects across membranes. It is not adequate to limit considerations to molecular diffusion. What matters is that activation and inhibition effects spread from the place of origin in an attenuated manner' (Gierer 1981). Thus, the frequent characterisation of the Gierer–Meinhardt model as a chemical-reaction-diffusion theory is not entirely correct. Molecular kinetics and normal diffusion just provided the simplest case for writing down the equations.

Secondly, and most importantly, Gierer and Meinhardt showed that their theory could account for self-regulation and scaling even if they used only straightforward molecular kinetics. A simple restriction of the amount of activator which can be produced locally, e.g. by introducing a saturation term, leads to size regulation. Thus, the criticism that reaction-diffusion equations can only produce static chemical waves was not justified. The most important dynamic phenomenon in developmental biology was perfectly in the range of the theory. Had Driesch seen the dynamic aspect of Meinhardt's simulation, which by now could be followed easily at the computer, I suggest, he would have given up his vitalistic views.

Third, in contrast to Turing, Gierer and Meinhardt had a concrete experimental system in mind, the regeneration behaviour of hydra (Gierer, et al. 1972). Therefore, they introduced a number of features that were specifically derived from their experimental results, such as source densities which are influenced by the activator–inhibitor system, but change with different time scales. Their reference list contains 21 papers, almost half of them (10) dealing with hydra.

In the subsequent years, it was Hans Meinhardt in particular who extended the models and applied them to almost every dynamical problem in developmental biology. This resulted in his monograph *Models of biological pattern formation* (Meinhardt 1982), in which a bewildering multitude of complex phenomena from developmental biology were treated with clear analytical concepts. The work was much more than a stereotypic application of the idea of local activation and lateral inhibition. Meinhardt tried to work out the developmental logic underlying experimental findings and tested the validity of his ideas by computer simulations. Three examples might suffice to exemplify this point. First, in 1976, French and Bryant had formulated the polar coordinate model for appendage patterning (French, et al. 1976). This model worked with circumferential positional values, a shortest intercalation

and a complete circle rule. It could explain a number of complex regeneration experiments, but made assumptions which were hard to connect to known molecular mechanisms and simple morphogen models. Meinhardt realised that an organiser region defined by the crossing of an anterior–posterior and a dorsoventral compartment would provide the same explanatory power as the polar coordinate model and, in addition, could be linked to the recent discovery of compartments as clonally restricted groups of cells in *Drosophila* (Meinhardt 1983). Such insights were not dependent on particular local activation-lateral inhibition equations, but rather were derived from the attempt to find a minimal realistic model which could explain complex phenomena. The basic correctness of Meinhardt's ideas were acknowledged 12 years later after the molecular mechanisms of the predicted compartment interactions had been discovered (Vincent and Lawrence 1994).

The second example concerns stripe formation. Meinhardt and Gierer had recognised that certain types of patterns were difficult to simulate with a lateral inhibition mechanism (Meinhardt and Gierer 1980). In particular, this applied to stripe formation, an important prerequisite to understand segmentation. To produce stable stripes they introduced the idea of lateral activation of mutually exclusive states. Two cell states each producing an autocatalytic substance exchange signals which mutually promote their autocatalysis. Thus, they can only exist next to each other. However, they also stay separated since each cell state locally inhibits the other state. This model made stripe formation possible and thus, Meinhardt used it to simulate segmentation. Much later and without reference to the early work, this mechanism was rediscovered as the core kinetics of the *Drosophila* segment polarity network (von Dassow et al. 2000). It is of particular interest that the new simulations did not start with a specific kinetic model, but used model equations entirely based on experimental data and the assumption of robustness to identify relevant parameter sets. Thus, remarkably, an unbiased, data-driven approach discovered an intricate mechanism, which had been suggested entirely on theoretical grounds.

The third example refers to colour patterns on the surface of sea shells (Meinhardt and Klinger 1987). Here, it is not so much the particular logic of a mechanism which made the simulations attractive, but the sheer similarity, indeed frequently the complete accordance, of highly complex patterns with the computer-generated images (Meinhardt 2009). It seemed that an agreement between natural pattern and simulation, capturing so many details, could not be accidental. One was forced to assume that the theory has captured at least some correct aspects of the underlying patterning mechanisms. Recently, the same shell patterns have been simulated using a neurosecretory model supported by experimental findings on the anatomy of the

mollusc mantle, the shell-making machinery (Boettiger et al. 2009). The neural circuitries used in this model combine local activation and lateral inhibition and thus demonstrate the equivalence between different molecular and cellular realisations of the Gierer–Meinhardt model. However, they also provide a clue about how difficult it is to predict the actual nature of the mechanisms underlying a patterning process even if the simulations recover the output pattern with astonishing accuracy.

## The rise of *Drosophila* developmental genetics

In the early 1970s, when Meinhardt and Gierer formulated their model on the basis of, and in close contact with, the experiments on hydra conducted in Gierer's department, the theory seemed to be close to the biological phenomena. The members of the research group felt that it was only a matter of time until they had isolated the autocatalytic activator(s) and the broadly diffusible long-range inhibitor(s). The first candidates had been already purified (Schaller and Gierer 1973; Schaller 1973). A splendid validation of the theoretical work seemed imminent. However, this turned out to be much more difficult than expected. Even today, the regulatory behaviour of hydra is not fully understood at the molecular level, and the postulated diffusible activators and inhibitors have not been identified (Bode 2009). It transpired that biochemical methods were much less powerful for the dissection of developmental pathways than expected.

In the atmosphere of early enthusiasm about pattern formation theory and its molecular validation, Christiane Nüsslein-Volhard obtained her Ph.D. on bacteriophage transcription, not with Gierer, but in the neighbouring department of Gerhard Schramm (under the supervision of Heinz Schaller). In a contribution to a symposium in honour of the Gierer's 80th birthday, Nüsslein-Volhard recalls that she was fascinated by the work on hydra and that the theoretical work on morphogen gradients influenced her thoughts and her choice to pursue embryonic pattern formation as a postdoc (Nüsslein-Volhard, personal communication). Nüsslein-Volhard, however, chose a genetically accessible system, *Drosophila melanogaster*. She joined Walter Gehring's group in Basel, and in a short time characterised two maternal-effect mutants which were involved in setting up morphogen gradients in the early *Drosophila* embryo, *bicaudal* and *dorsal* (Nüsslein-Volhard 1979a; Nüsslein-Volhard 1979b). In the discussion of her first *Drosophila* paper, the description of the *bicaudal* mutant, the influence of the Tübingen theory group can clearly be felt. She favours the Gierer–Meinhardt model compared to other explanations for the *bicaudal* phenotypic series: 'Although this model probably does not account for

all experimental data, we feel that this or a similar gradient model maybe more adequate to explain the various *bic* phenotypes. Its main attraction is the ease with which mirror-image duplications of varying size and reversal of polarity are produced by lowering the concentration of a single component' (Nüsslein-Volhard 1977). After a short postdoc with Klaus Sander in Freiburg, Nüsslein-Volhard took, together with Eric Wieschaus, a junior group leader position at the EMBL in Heidelberg where they conducted their Nobel prize-winning screen for zygotic embryonic lethal mutations (Nüsslein-Volhard and Wieschaus 1980). In the long run, this work would completely change the basis for any theory of pattern formation. For the first time, complete, or almost complete genetic networks for particular patterning problems such as segmentation or neuroblast selection would become available and would provide test cases for modelling approaches. After returning to Tübingen, Nüsslein-Volhard also scientifically returned to the maternal morphogen gradients she had discovered as a postdoc in Gehring's and Sander's laboratories. The experience gained with the zygotic whole genome screens made it possible to conduct large-scale screens for maternal-effect mutations. Such screens were performed by Trudi Schupbach and Eric Wieschaus in Princeton (Schupbach and Wieschaus 1989, 1991) and by Ruth Lehmann, Kathryn Anderson, Gerd Jürgens and Nüsslein-Volhard in Tübingen. The analysis of mutant phenotypes together with transplantation experiments soon revealed that the AP morphogen gradients in the *Drosophila* egg emanate from localised determinants at the anterior and posterior egg poles. Later, these were shown to be localised mRNAs. At the level of the early embryo, gradient formation did not require self-organised patterning on which the egg asymmetries would only have a weakly orienting influence. Rather, embryogenesis already started with an amazingly high degree of spatial information present in the egg cell (St Johnston and Nüsslein-Volhard 1992). For a while, the dorsoventral (DV) axis seemed to be different. Apparently, DV axis formation did not require localised components within the egg, and thus appeared to be an example for a self-organised patterning system. However, the exciting observations of ectopic axis formation and axis inversions along the DV axis finally could be explained by local signals emanating from cues in the eggshell (Anderson et al. 1985; Stein et al. 1991; Roth 1993). The transition from eggshell cues, though, to the embryonic DV pattern occurs in a dynamic way which apparently has some self-organising properties (Meinhardt 2004; Moussian and Roth 2005; Roth and Schupbach 1994). However, the eggshell cues themselves provide fairly accurate information which can be traced back to a mRNA localised within the developing oocyte (Neuman-Silberberg and Schupbach 1993). Thus, for those instances where we had expected that the theory

would be most helpful, a completely different picture had emerged. As Waddington had already indicated in his response to Turing's paper, intracellular patterning was much more important than anticipated. It became necessary to study how the egg was constructed during oogenesis in order to understand axis formation. One might object that *Drosophila* and insects in general are unusual because they produce eggs that display bilateral symmetry presaging the two body axes before fertilisation. However, it seems that the lesson learned from *Drosophila* reflects a more general problem of the early theories of pattern formation. These theories systematically underestimated the potential and importance of intracellular patterning to provide spatial information for the multicellular level or to influence and constrain mechanisms of multicellular patterning. The description of intracellular patterning requires, however, at least in part, approaches different from the mechanisms employed in the original Gierer–Meinhardt model. Since every eukaryotic cell already represents a highly structured three-dimensional object, the spatial information contained in the cell can be used to initiate symmetry-breaking events. Thus, in *Drosophila*, DV asymmetry arises through asymmetric movement of the oocyte nucleus (Roth and Lynch 2009) or in amphibians, bilateral symmetry depends on rotation of the oocyte cell cortex with regard to the rest of the cell (Larabell et al. 1996; Marrari et al. 2004). These phenomena and other aspects of cell polarisation require dynamic changes of cytoskeletal elements. At this level, theory again becomes relevant. A deeper understanding of cytoskeletal dynamics requires biophysical modelling approaches (Howard 2001). The same applies for other aspects of intracellular patterning which increasingly become subject of mathematical modelling (Karsenti 2008). Meinhardt himself has contributed to some of this work addressing dynamic aspects of cell division in bacteria (Hale et al. 2001; Meinhardt and de Boer 2001) and of the chemotactic behaviour of eukaryotic cells (Meinhardt 1999, 2000).

## Modern theories of biological pattern formation

While the inadequate representation of intracellular patterning addresses the reference level of the theory, the more general problem of early pattern formation theories was the lack of molecular detail. Since none of the developmental processes which the theories described was understood at the molecular level, the theories could not work with realistic molecular assumptions. Activators and inhibitors remained abstract entities. As pointed out, this did not prevent the early theoreticians from gaining major insights which were validated by later research. However, these insights addressed a more generic level representing both

their strength—they could be applied to many cases, and their weakness—they could not capture any specific molecular process. With detailed molecular data, in particular from *Drosophila*, becoming available in the mid-1990s, a new generation of theoretical approaches became possible. Modelling was no longer restricted to general mechanism, such as local activation and lateral inhibition, but could incorporate genetic interaction data or even real measurements of concentrations of respective components. Now finally, one could hope, the real molecular processes could be simulated in the computer. This should lead to realistic models providing a deep understanding of multi-component systems. The step from the linear stability analysis to which Turing was largely restricted to the simulation of non-linear systems by Gierer and Meinhardt was linked to increased computer power. Likewise, the new simulations again were only possible by another large leap in simulation capacity. In particular, now it was possible to perform systematic explorations of parameter spaces or to apply complex algorithms of non-linear fitting (simulated annealing). The idea of robustness started to play an important role. By systematic variation of the parameters of given model equations, one could identify those conditions which provided the most robust mechanism.

In the following, three examples from recent work on *Drosophila* pattern formation will be discussed which reveal the power and limitations of the new approaches. Each of them demonstrates the need for mathematical modelling. They will be presented in a sequence of increasing closeness to quantitative experimental data: the first example deals with models for the formation of a BMP morphogen gradient in the embryo, the second with the gene circuit approach to explain the segmentation process, and the third with models for the formation of the Bicoid morphogen gradient.

(1) In *Drosophila*, the transcription factor Dorsal is responsible for establishing the entire dorsoventral axis (Moussian and Roth 2005). Dorsal forms a nuclear concentration gradient with peak levels along the ventral midline of the early embryo. Dorsal activates and represses target genes in a concentration-dependent manner and thereby specifies different gene expression domains along the DV axis. Interestingly, the Dorsal gradient is confined to the ventral half of the embryonic circumference, but nevertheless is also required for patterning the dorsal half. In particular, the Dorsal gradient indirectly establishes high levels of BMP signalling in a narrow, precisely defined stripe along the Dorsal midline (O'Connor et al. 2006). How is such a precisely controlled long-range influence possible? Mutant analysis showed that, paradoxically, a BMP inhibitor expressed ventrally under the control of Dorsal is required for establishing BMP peak levels at the

dorsal midline. This observation remained a conundrum until the suggestion was made that BMP–inhibitor complexes forming in ventral regions diffuse dorsally and thereby transport BMP to the dorsal side (Ashe and Levine 1999; Ferguson and Anderson 1991, 1992). Here, the inhibitor is cleaved and BMP is released. Since the protease cleaving the inhibitor is not confined to the dorsal midline, it was still hard to understand how this mechanism accounts for spatial precision. A general mathematical treatment of the reaction-diffusion system encompassing BMP, the inhibitor and the protease revealed that rather simple interactions are sufficient to reproduce the experimentally observed patterning capability (Eldar et al. 2002; Meinhardt and Roth 2002). The authors started with general model equations for the three-component reaction-diffusion system and calculated the steady-state BMP activation profile for thousands of randomly chosen parameter sets. The parameters included rate constants for production, decay and diffusion of the individual proteins and complexes. Parameter sets, which produced steady-state solutions mirroring the experimentally observed BMP activation pattern, were tested for robustness using the following rationale: Genetic experiments show that reducing the gene dosage of some network components by a factor of two does not significantly change the final BMP activation pattern. Thus, realistic parameter sets should also lead to solutions which are robust, given twofold changes in network components. Parameter sets fulfilling this condition were identified and suggested that free BMP does not significantly diffuse and the free inhibitor is not a good target for proteolysis. Thus, BMP should be predominantly transported in a complex with the inhibitor, and this complex should be the main target of proteolysis. This assumption led to simplified equations which could be treated analytically, providing precise mathematical arguments for the proposed mechanism. The entire approach seemed to be highly convincing (Meinhardt and Roth 2002). The derivation of an analytical expression capturing the mechanism of robustness could be almost regarded as a proof of the correctness of the proposed interactions.

However, several complications arose that revealed the intricateness and unpredictability of biological systems. First, one component of the network, the BMP ligand Dpp was known for a long time to be dosage-sensitive. Indeed, *dpp* is the only developmental gene in *Drosophila* which is haplolethal, meaning that a twofold reduction of its dose leads to lethality (Irish and Gelbart 1987). Irrespective of the particular implications for the proposed mechanism, this observation raises the general question of how valid robustness is as a universal criterion for identifying appropriate modelling parameters. In the case of *dpp*, one could argue that its employment in different patterning contexts leads to an evolutionary optimisation problem

which has no ideal robust solution. However, the mere fact that such exceptions exist brings into question the blind reliance on robustness for selecting appropriate parameter sets. It seems that assumptions about robustness have to be analysed in each particular case.

Second, biochemical studies and additional modelling approaches revealed different interaction networks, which could also produce sharp BMP signalling peaks. An interesting assumption was that Sog acts as a competitive inhibitor of BMP binding to its receptor and that receptor-bound BMP is degraded (Mizutani et al. 2005). Thus, Sog protects BMP from receptor-mediated degradation and thereby enhances its range of action. Another paper showed that the main signalling molecule is a heterodimer of two BMPs and that it is the heterodimer which is bound by the inhibitor, while mathematical modelling had suggested alternative interactions (Shimmi et al. 2005).

While the former two papers demonstrated that the interactions of even a small number of secreted proteins and their receptors can lead to several alternative models which can account for observed phenotypes, another publication opened up completely new aspects of the system. Eldar et al. (2002) could still state: 'No apparent transcriptional feedback, which might account for the robustness of dorsal patterning, has been identified so far'. However, 3 years later, precisely such a feedback loop was identified (Wang and Ferguson 2005). A transcription factor was shown both to be a target of, and to promote, dorsal BMP signalling. Apparently, this feedback loop promotes future BMP binding to its receptor as a function of previous signalling strength. This finding led to a completely new series of modelling approaches (Umulis et al. 2006). In addition, new precise measurements of BMP signalling in whole embryos provided the basis for organism-scale modelling using realistic geometries (Umulis et al. 2010). Unfortunately, until now the molecular basis of the positive feedback has remained elusive. The most recent publication introduces eight different models for possible positive feedback mechanisms encompassing a variety of cell biological and biochemical details as well as newly observed interactions with extracellular matrix molecules. The most important outcome of the paper is that positive feedback mechanisms are indeed producing the best fit to the data and that, among these mechanisms, a positive feedback involving a surface-bound BMP binding protein is slightly superior to other mechanisms. However, the modelling approaches veered away from elegant analytical results to numerical solutions of high dimensional systems of more than ten coupled partial differential equations and 17 parameters. Despite whole embryo 3D representations of BMP signalling in wild-type and mutant embryos at different developmental stages, and even comparisons of different *Drosophila* species, the discriminatory power of

modelling results appears to be weak with regard to the mechanistic alternatives. Even the most ardent aficionado of pattern formation theory might come to the conclusion that we have moved further away from understanding the system. The complexity of the embryo appears to evade full mathematical treatment.

(2) Segmentation in *Drosophila* is probably the paradigm for a complex hierarchical patterning process which generates fine-grained spatial information starting from broadly distributed morphogen gradients (Akam 1987). Within 2 h of embryonic development corresponding to the blastoderm stage, maternal gradients activate a cascade of transcription factors with increasingly refined expression domains. First, the gap genes are activated in broad domains and in turn control the pair-rule genes which represent the first level of periodic gene expression in the embryo, albeit with double-segment periodicity. Segmental periodicity is only reached at the next tier, the expression of the segment polarity genes, which receive their regulatory input largely from the pair-rule genes. At the end of the blastoderm stage, the patterning process has reached its highest possible resolution: single-cell wide stripes of segmental polarity gene expression defining the position of segment and compartment boundaries. The gap and pair-rule genes code for transcription factors which can diffuse between nuclei since patterning occurs before the cell membranes are formed. Thus, the system is basically an interaction network of factors which mutually regulate their expression. Besides nuclear divisions, no morphogenetic events take place, allowing a description of the process with one spatial coordinate representing the AP axis (or rather the position of the nuclei along the AP axis).

Inspired by models for neural networks (Hopfield 1984), a data-driven modelling approach for gene regulatory networks was devised called the connectionist gene circuit method and was specifically applied to the *Drosophila* segmentation cascade (Mjolsness et al. 1991; Reinitz and Sharp 1995). Usually, theoretical modelling is based on proposed interactions of the relevant components inferred from genetic and molecular experiments. The gene circuit method uses the opposite strategy and therefore was also characterised as a 'reverse-engineering' approach (Perkins et al. 2006). Only minimal a priori assumptions are made about potential regulatory interactions. A system of general model equations is formulated which describes the concentration change of each transcription factor in a particular nucleus as a function of gene expression, diffusion and decay. The expression of a particular transcription factor depends on regulatory inputs from other transcription factors. To capture these inputs, an interconnectivity matrix T is introduced, in which the regulatory effect of gene $a$ on gene $b$ is represented by the matrix element $T^{ab}$. Depending on whether this element is positive or negative, gene $a$

activates or represses gene *b*, respectively. The quantity of T$^{ab}$ defines the strength of the interaction; if it is zero, the two genes do not interact. The main point of the approach is that no a priori assumptions are made about the numerical values for the elements of the matrix or the diffusion and decay rate of the transcription factors. Instead, prior to modelling, a set of quantitative expression data is collected. In this particular case, antibodies were produced against most segmentation genes, allowing the measurement of the protein distribution in the entire embryo at different time points. The resulting highly accurate quantitative descriptions of the spatiotemporal expression changes were used to determine the numerical values of the matrix elements and the diffusion and decay rates of the transcription factors by procedures of non-linear fitting. Thus, the network topology was recovered from experimental data, rather than being implemented prior to the actual modelling work.

Initially, this approach encountered doubts and scepticism from molecular biologists studying the transcriptional regulation of individual segmentation genes. The analysis of cis-regulatory elements should uncover those interactions which actually occur in the embryo. Why would one need a more global indirect approach? However, even early gene circuit modelling using still limited experimental data sets for fitting led to some highly non-trivial predictions. For example, a paper on the formation of pair-rule gene expression stripes predicted that stripe formation requires very low diffusion rates of the pair-rule gene products (Reinitz and Sharp 1995). This prediction later gained experimental support by the demonstration that the mRNA of pair-rule genes is tightly localised to the cell cortex above the nuclei. Translation of the localised mRNA at the cortical positions is likely to hinder the spreading of the protein to neighbouring nuclei, accounting for low diffusion rates. More importantly, later modelling of the gap gene network using improved data sets for fitting and more sophisticated optimisation programmes led to results not predicted by any experimental work and in addition, provided a deeper understanding of one of the central questions in developmental biology: how spatial precision emerges despite noisy starting conditions.

A careful analysis of the gap gene expression pattern showed that the gap gene domains undergo a coordinated anterior shift after their initial establishment under the control of maternal gradients (Jaeger et al. 2004b). This had not been noticed by experimentalists and implies that the readout of morphogen gradients, at least in this particular case, is not a static but rather a dynamic process. The positions of target gene domains are not ultimately fixed by particular morphogen concentrations. Rather, interactions among the target genes define the final coordinates of target gene expression. The data-fitting algorithms revealed the regulatory parameters responsible for the domain shifts and allowed an interpretation of the underlying mechanism (Jaeger et al. 2004a). The elaborate picture of the gap gene network which emerged from these studies was later expanded and used to address the problem of canalisation.

The term canalisation was introduced by Waddington who pointed out '…that developmental reactions, as they occur in organisms submitted to natural selection are in general canalised. That is to say, they are adjusted so as to bring about one definite end-result regardless of minor variations in conditions during the course of the reaction' (Waddington 1942). The segmentation cascade of *Drosophila* allows one to detect canalisation at the molecular level and to analyse its mechanism. The crucial maternal morphogen responsible for activating the gap genes is the gradient of the transcription factor Bicoid. Careful measurements were used to detect the embryo-to-embryo variability of the Bicoid gradient and of the early and the late gap domains. A comparison of the results revealed a progressive reduction of the variation with developmental time. The shape of the Bicoid gradient as well as the early expression domains of the gap genes were noisier than the late gap domains. By applying the gene circuit modelling approach, the specific regulatory interactions could be identified which are responsible for the noise reduction (Manu et al. 2009a). A combination of strong and weak mutual inhibition was shown to be crucial. The mathematical analysis of a precise dynamical model which contained the experimentally derived parameters revealed that the gap gene network possesses certain attractors to which the system trajectories converge, irrespective of small variations in the starting conditions (Manu et al. 2009b). Thus, in this particular case, a mathematical explanation of the stability, i.e. reproducibility of an actual developmental process was achieved. The fact that modelling in this case was an a posteriori analysis of interactions occurring in the embryo is a distinctive feature of this work. The stability of steady state solutions in the face of variable starting conditions had always been a strong motive in pattern formation theory. Turing already being aware of this fact, states in the context of introducing the idea of symmetry breaking: 'The variety of such new equilibria will normally not be so great as the variety of irregularities giving rise to them' (Turing 1952). Gierer and Meinhardt also considered the stability of the outcome of pattern formation processes in spite of system perturbations as one of the most essential aspects of their theory (Gierer 1981; Meinhardt 1982). However, the papers on the gap gene network represent probably the first case in which quantitative data, including an assessment of the actual noisiness of the system have been used as the basis for modelling.

Despite these seminal achievements and the obvious closeness to biological reality, the gene circuit approach

remains at the phenomenological level in one basic respect. The elements of the interconnectivity matrix are open to a number of molecular interpretations and they are also not free from a priori assumptions which might not fully reflect the complexity of the transcriptional process. For example, the model does not allow that a transcription factor activates and represses at low or high concentrations, respectively. Exactly this situation has been discussed for one of the key gap genes, *hunchback*, with regard to its regulation of *Krüppel* (Papatsenko and Levine 2008). The fact that the authors can produce a self-consistent model for the gap gene network without assuming more complex interactions may have three explanations. (1) These more complex interactions might in fact not exist and the experimental evidence provided by molecular biologists is based on artefacts. (2) The interactions exist, but are not relevant for the dynamics. (3) The derived network, in spite of recovering the quantitative dynamics of the system, is only a partial approximation of the reality and might even wrongly suggest interactions which have no molecular counterpart in the embryo.

A crucial question will be how the model fares when applied to the next level of the segmentation process: the emergence of the pair-rule gene expression pattern. At this level, several fundamental questions still need to be addressed, in particular the precise phase shift between partially overlapping pair-rule stripe patterns. This phase shift is crucial for initiating segment formation (Klingler and Gergen 1993; Warrior and Levine 1990), but we are almost completely ignorant with regard to the underlying molecular mechanisms. Here, the gene circuit model might indicate where to look at the molecular level, and thus could have a crucial heuristic role for the working molecular biologist. On the other hand, there is some indication that besides protein–DNA interactions, protein–protein interactions between the pair-rule gene transcription factors also contribute to the patterning process (Fitzpatrick et al. 1992). Such interactions have not been included in the gene circuit model equation to date, and it is doubtful whether they can be fully represented. Any mechanism which necessitates the elements of the interconnectivity matrix to become functions of space and time will probably render the optimisation problem unsolvable. Thus, it is possible that the gene circuit model in its current form has a very limited applicability. There might be few other instances in all developmental biology, which suit this approach as much as the first step in the *Drosophila* segmentation cascade, the gap gene network.

(3) The last example deals with a question which for a long time was assumed to be trivial and did not require particular modelling efforts: the formation of a simple morphogen gradient from a localised source. Crick (1970) had already provided a special solution to this problem. The

particular case in question is the aforementioned gradient of the transcription factor Bicoid, which provides the input for the gap gene pattern (Porcher and Dostatni 2010; Grimm et al. 2010). Bicoid mRNA is localised to the anterior pole of the egg where it is translated generating a local source of Bicoid protein which diffuses to more posterior regions of the embryo (Driever and Nüsslein-Volhard 1988a, b). Even intuitively, one can imagine how local production and diffusion are able to produce a long-range concentration gradient. This intuitive concept can be captured in a simple equation describing the spatiotemporal change of Bicoid concentration as a function of synthesis, decay and diffusion (SDD model). For SDD models, steady-state distributions can be calculated in which synthesis, diffusion and decay are balanced such that the resulting concentration profiles do not change in time (Gregor et al. 2005). A localised source and spatially uniform decay give rise to an exponential steady-state profile characterised by a length scale which only depends on the diffusion and decay rates. Measurements of the Bicoid gradient indeed showed an exponentially decaying concentration profile which was sufficiently stable for assuming a steady state (Driever and Nüsslein-Volhard 1988b; Gregor et al. 2005). Furthermore, measurements of the diffusion of fluorescent dextran molecules which had a molecular mass comparable to that of Bicoid were in agreement with the SDD model for gradient formation (Gregor et al. 2005).

However, one phenomenon suggested a more complex scenario. Closely related fly species show almost identical early segmentation gene expression. However, their egg size may vary over more than a factor of five in length (Gregor, et al. 2005). The scaling of the segmentation gene expression pattern can be traced back to the scaling of the Bicoid gradient, i.e. the gradients from different flies have the same relative concentration profiles irrespective of the egg size. Thus, in a large egg, the gradient has a proportionally larger length scale which might result either from an increased diffusion or a decreased decay rate of Bicoid protein. However, the Bicoid proteins of different fly species have a very similar structure and thus should have similar diffusion and decay rates implying similar length scales. This assumption was strongly corroborated by generating *Drosophila melanogaster* embryos expressing a Bicoid protein derived from a fly species with large eggs (Gregor et al. 2008). The heterologous Bicoid protein formed a gradient indistinguishable from the endogenous one. Thus, scaling seems to be a feature of the embryonic system rather than of the respective Bicoid protein. To explain this fact, a cellular property had to be found which also showed scaling behaviour. An obvious candidate was the number of nuclei, which is conserved in different fly species. Flies with large eggs have a lower nuclear density, and those with small eggs a higher one. As a transcription

factor, Bicoid acts within the nuclei. Thus, for its biological function the nuclear, as opposed to the cytoplasmic, concentrations are of crucial importance. If the degradation of Bicoid mainly occurs in the nuclei, then the scaling of the gradient could be explained because the length scale of the Bicoid gradient would depend on the nuclear density.

These ideas led to an increased interest in the precise measurement of Bicoid's diffusion, decay and nuclear shuttling rates in living embryos. The production of transgenic flies expressing fully functional GFP-tagged Bicoid protein partially allowed such measurements and produced two surprising results (Gregor et al. 2007). Firstly, during development, the nuclear concentration of Bicoid at a given position remains constant within a 10% margin, despite increasing nuclear densities (the number of nuclei increases 16-fold). This observation is in direct conflict with the explanation for scaling of the gradient in different flies because scaling requires that the gradient changes concomitantly with changing nuclear densities. In addition, nuclear stability by itself is not easy to explain given a steady state model of the gradient. Gregor et al. (2007) concluded: '…, rather than passively sampling a large excess of molecules in the cytoplasm, the nuclei *must* perturb the gradient significantly'. Several models were developed to address this question. One of them assumes that the gradient is not in a steady state, but that the total amount of Bicoid protein increases, and this increase balances the increase in nuclear volume so that the local nuclear concentrations remain constant (Coppey et al. 2007).

The second surprising finding concerned the measured diffusion constant of Bicoid, which was an order of magnitude too low to account for the length scale of the gradient (Gregor et al. 2007). Even assuming non-stationary models the measured rate of Bicoid diffusion cannot explain the shape of the gradient (Grimm et al. 2010). To tackle this problem, mathematical models were developed that paid more attention to the actual cellular complexity of the early *Drosophila* embryo. A coarse-grained model for the syncytial blastoderm was derived which combined cytoplasmic diffusion and nucleocytoplas-mic shuttling of Bicoid (Sample and Shvartsman 2010; Kavousanakis et al. 2010). The model uses a homogenisa-tion approach that is applied in physics and engineering for the description of heterogeneous materials. If materials are structured at two clearly distinct length scales, the structure at the larger length scale might result from the repetition of a small-scale unit with internal structural complexity (the reference cell). In the case of the syncytial blastoderm, the reference cell was assumed to be a nucleus with its surrounding cytoplasm. The homogenisation approach allowed the definition of an effective (large scale) diffusiv-ity for Bicoid as a function of the geometry of the nucleus

together with the surrounding cytoplasmic island, the diffusivity in the cytoplasm and nucleocytoplasmic shut-tling (Kavousanakis et al. 2010). However, these highly sophisticated approaches still did not lead to an explanation for gradient formation despite Bicoid's low diffusivity, or to an understanding of nuclear stability and scaling. In particular, the last point remained disconcerting since the explanations invoked for each phenomenon required conflicting assumptions.

Additional experimentation was required. In a recent paper, altered Bicoid proteins were analysed that had an impaired ability for nuclear transport or were lacking nuclear transport altogether (Grimm and Wieschaus 2010). Surprisingly, these Bicoid versions produced gradients indistinguishable from that of the wild-type protein. This is a striking observation since wild-type Bicoid concen-trations show huge local fluctuations depending on the nuclear cycle. Thus, one would assume intuitively that these local changes have an effect on the overall gradient. These new findings also rule out explanations for scaling that invoke nuclear density. According to a recent sugges-tion, scaling might result from the positive correlation between the amount of *bicoid* mRNA and egg size (Cheung et al. 2011). Finally, the new findings re-open the whole discussion on the mechanisms of gradient formation. No doubt, we are currently unable to provide a mechanistic explanation of one of the simplest patterning problems in biology, the formation of the exponentially decaying gradient of a single protein species emanating from a local source. This problem seems to be much simpler than that of self-organised patterning which motivated Turing or Gierer and Meinhardt; it is also much simpler than BMP gradient formation or the gap gene network, the two situations exemplified above. However, it touches on one key feature of development: the capability to produce the same pattern at different length scales. It was precisely this feature, in some of its extreme realisations, that Driesch believed could not be explained by physics and chemistry and for which he developed his harmonious equipotential system reflecting a type of lawfulness only found in organisms.

As we do not want to take refuge in vitalistic tenets, the question arises of where we could obtain additional physicochemical explanations to explain Bicoid gradient formation. The problem here is not the lack of potential candidates, but rather the multitude of options. (1) Bicoid translation might not exclusively occur at the anterior pole and Bicoid's rate of translation might not be constant over time as assumed in most models (Surdej and Jacobs-Lorena 1998; He et al. 2011). Indeed, *bicoid* mRNA is not tightly localised to the anterior cortex, but forms a steep gradient (Spirov et al. 2009; St Johnston et al. 1989; Weil et al. 2008; Little et al. 2011). Thus, Bicoid might emerge from a graded source. This might certainly help to explain the

discrepancy between low diffusivity and large length scale. (2) Bicoid movement might be more complex, including both space- and time-dependent changes in the diffusion constant as well as convective streaming. Diffusivity of Bicoid has so far been measured only at the surface of the syncytial blastoderm in embryos. It could be larger in earlier stages or in the inner part of the embryo as opposed to the cortex. Recent measurements of Bicoid diffusion using different biophysical methods have questioned previous data (Abu-Arish et al. 2010). In addition, a certain amount of cytoplasmic streaming occurs in the embryo which has been used to explain the length scale of the gradient (Hecht et al. 2009). (3) Bicoid is a target of post-translational modifications which may occur in a spatially controlled way. For example, the phosphorylation of Bicoid by the receptor tyrosine kinase Torso occurs only in a restricted anterior zone (Janody et al. 2000; Ronchi et al. 1993). Recently, a ubiquitinylation of Bicoid was demonstrated, which targets the protein for degradation (Liu and Ma 2011). Thus, many different protein forms of Bicoid might exist in the embryo, each with particular diffusion and decay rates or even with particular spatial distributions.

Taken together, organismic complexity impinges on multiple levels even for the simple process of forming a protein gradient. The most difficult problem for theory lies in the fact that no a priori physicochemical argument exists that allows us to decide which aspects are relevant and which can be neglected. Thus, for some time, *bicoid* mRNA distribution was not considered to be important for gradient formation since the mRNA is clearly more locally restricted than the protein. Given the suggested low diffusivity of Bicoid protein, the potential contribution of mRNA spreading becomes more important. Conversely, nucleocytoplasmic shuttling of Bicoid appeared to be of pivotal importance for understanding gradient stability and scaling, leading to very impressive theoretical approaches (Gregor et al. 2007). However, recent experimental evidence undermines the importance of this process (Grimm and Wieschaus 2010).

Evolutionary processes can affect and fine-tune each level of a developmental process. From the physical point of view, no simple process exists in an organism since not a single protein or RNA molecule can be treated separately from the organismic context. This is impressively shown by the example of Bicoid proteins from large eggs that form normal gradients within smaller eggs (Gregor et al. 2008). Since evolution can operate at each level (e.g. changing the localisation or stability of an mRNA, adding a modification to a protein, changing the size or the physical property of a compartment), multiple ways exist in which the organism can overcome physicochemical constraints. Bicoid shows us even more impressively than the other examples, how the organism evades the attempts of the theoretician.

## Conclusions: Kant revisited

The history of pattern formation theory presented here reveals the necessity of mathematical modelling already for simple chemical and even more so for biological systems. The oscillatory behaviour Lotka found by solving the differential equations is not evident if one just looks at the reaction scheme. It becomes plausible by using 'graphical' interpretations such as predator–prey relationships. However, understanding the important difference between conservative and dissipative systems, reflected in the difference between structurally unstable and structurally stable limit cycle oscillations, requires relatively abstract mathematical arguments. Nevertheless, this point is absolutely crucial; all interesting chemical and biological pattern-forming systems have the property of structural stability. The demonstration by Turing at the beginning of his paper that differences in diffusion rates can lead to unequal distributions of components starting from homogeneity is rather counter-intuitive. Is not diffusion a process that diminishes concentration differences? Finally, the rich pattern-forming capabilities of systems combining non-linear autocatalysis, inhibition and differential diffusion rates (Gierer–Meinhardt model) could have never been demonstrated without computer simulations. The need for mathematical modelling is even more apparent in modern data-driven approaches. Already data acquisition requires sophisticated algorithms. A large part of the efforts of the Reinitz group was devoted to the question of how to reliably retrieve expression data from microscopic images (Janssens et al. 2005; Myasnikova et al. 2009; Surkova et al. 2008). Non-linear fitting or screening parameter spaces require elaborate programming. But even in rather simple cases such as Bicoid gradient formation, a model is needed to decide whether the measured diffusion constant is appropriate for a postulated mechanism or whether a substantial deviation from the expectation indicates the existence of unknown processes. One can easily predict that with our increased ability to acquire highly accurate measurements of molecular properties in living systems, the need for model building will increase. Pattern formation theory seem thus to be a prime example for Kant's statement that '…there is present only so much real science, as there is mathematics' (Kant 1900ff Vol 4, 470).

However, in the face of organismic complexity, the work of the theoretician can appear to be a Sisyphean ordeal. In the examples we have described, successful and internally consistent modelling approaches were called into question by re-interpretations of underlying mechanisms or by new data sets, which contradicted previous assumptions. In the case of the gene circuit approach, a huge modelling effort was undertaken over more than a decade to produce one of the most impressive results in

all theoretical biology: a precise description of how canalisation takes place. However, the actual structure of the model might have only limited applicability beyond a particular stage of *Drosophila* development and despite its proclaimed closeness to experimental data cannot be easily linked to concrete transcriptional mechanisms. In the case of the Bicoid gradient, it even seems that after repeated rounds of modelling and experimentation, the theory currently has lost its object since it is not clear where the property responsible for scaling is to be found.

In the light of these observations, the question arises whether the relation between a mathematical model and its object is fundamentally different in biology than in other areas of science. An answer to this question requires careful consideration. Are not disagreements between theoretical modelling and experimental results anyway the normal situation with which theoreticians in all fields of science are confronted? Famous examples are attempts to simulate the processes in the earth's atmosphere from global climate changes to the local weather forecast. However, here we know very well that some of the underlying equations inherently lead to unpredictability (deterministic chaos). The impossibility of long-term predictions is mathematically understood. In other instances, this point is less clear. An impressive example is represented by the long-standing attempts to derive the properties of liquid water from computer simulations. The forces between two water molecules (the pair potential) include repulsion, electrostatic dipole interactions and hydrogen bonding. There is a surprising variety of how to formulate a pair potential based on these three contributions. Additionally, there are higher order interactions between more than two water molecules. In a comparison of simulations based on 45 different water models, Guillot summarises: 'The fact that no model potential is able to reproduce in every detail the properties of real water despite 30 years of active research leaves a taste of incompletion.' (Guillot 2002). From a biologist's point of view, these simulations, however, are pretty impressive. All of them recover basic properties of water and provide accurate predictions for at least some of the measured physical constants of water. The process of approximation to the real object seems to be much smoother than in the case of biology. Indeed, Guillot can precisely localise the weaknesses in the assumption of many models and suggest very specific improvements. With slightly relaxed demands on the quantitative accuracy, another expert in the field concludes '…, contrary to numerous statements in the literature, I am convinced that the behaviour of water… is reasonably well understood…' (Ben-Naim 2009).

Biological pattern formation theory is a relatively young field of research. Although its object of reference is infinitely more complex than liquid water, we cannot exclude that future modelling efforts will lead to a similar degree of approximative understanding. Beyond mere material complexity, however, organisms possess features not found in the inorganic world, which are likely to complicate every mathematical approach. Proteins and mRNAs, the types of molecules which are and will be the main target of modelling are not only huge macromolecules compared to water but they also exhibit features fundamentally different from any molecule of the inorganic world. Their sequence-based structure depends on an evolutionary process which combines adaptive (determinative) and non-adaptive (drift-like or stochastic) events. Evolution can target singular features (individual positions within the sequence) of these molecules. Therefore, their physical properties are to a large degree at the disposal of events buried deeply in the evolutionary past. For these events, it is not even clear whether they all have a straightforward physical grounding. As long as we are dealing with adaptive processes, physical causes have to be postulated which guide adaptive events. For example, the particular modes of early development in insects might have served as a patterning environment to which proteins (and their respective mRNAs) had to adapt if they were to be useful as morphogens in providing positional information to the embryo. For a particular morphogen, an evolutionary process can be imagined that leads to the fitting of the morphogen's molecular characteristics to the physical environment of the embryo. This process ought to be highly complex since its goal is the fit between the physical properties of a complex molecule to a highly structured cellular environment. Nevertheless, the adaptive, 'goal-directed' events driving this process should in principal be physically describable. However, in particular for multicellular organisms, drift-like processes might have a significant contribution to evolutionary change (Lynch 2007). The reducing theory for such processes is probability theory (McShea and Brandon 2010). Consequently, some of the molecular properties of proteins and RNAs might not have a physical cause linked to the biophysics of the particular process they are involved in, but rather result from collective statistical phenomena at the population level.

Thus, pattern formation theories working with functionally and physically motivated mechanisms are, already at the level of the single molecule, likely to face unexpected complications. However, patterning processes usually require coupled reactions of many components and frequently encompass several hierarchical levels (e.g. diffusion in the cytoplasm, transcription, morphogenesis). Accordingly, evolution has almost infinite possibilities to complicate and fine-tune patterning mechanisms and thus can overcome or undermine almost every physicochemical constraint postulated by a particular theory.

The picture of the organism emerging from these reflections has astonishing similarity to Kant's views outlined at the beginning of this essay. Although Kant speculated about evolution in his late writings, he had no mechanistic explanations for how the functional adaptations observed in the organic world could arise (Roth 2011a). Nevertheless, he assumed that the apparent goal-directedness or purposiveness of organisms is not in contradiction with the general laws of nature. Organisms just represent unique constellations of matter. From the perspective of general laws, their structure is extremely improbable, yet highly reproducible. They are, according to Kant, examples of the lawfulness of the contingent (Roth 2011b). This implies that we have no alternative for studying organisms to the mechanistic approach, despite its insufficiency for a full understanding of their contingent features. Thus, Kant clearly realised that his ideal of mechanistic understanding implying mathematics is faced with significant, indeed seemingly insurmountable problems when applied to living nature.

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