C. R. Mecanique 347 (2019) 337–341

Contents lists available at ScienceDirect

Comptes Rendus Mecanique

www.sciencedirect.com

Patterns and dynamics, homage to Pierre Coullet / *Formes et dynamique : hommage à Pierre Coullet*

# The biological frontier of pattern formation

Leonid M. Pismen

*Technion, Israel Institute of Technology, Haifa 32000, Israel*

ARTICLE INFO

*Article history:*
Received 26 November 2018
Accepted after revision 1 December 2018
Available online 2 April 2019

*Keywords:*
Patterns
Symmetry breaking
Signalling
Morphogenesis

ABSTRACT

Morphogenetic patterns are highly sophisticated dissipative structures. Are they governed by the same general mechanisms as chemical and hydrodynamic patterns? Turing's symmetry breaking and Wolpert's signalling provide alternative mechanisms. The current evidence points out that the latter is more relevant, but reality is still far more complicated.



## 1. Dissipative structures: from hydrodynamics to chemistry

The end of the last, and the beginning of this century was the heyday of general theory of pattern formation far from equilibrium that brought under a common roof symmetry breaking phenomena that had always fascinated people, but only marginally touched earlier the mainstream science. One of the high points of this drive was the Advanced Study Institute PHYSBIO organized in 2002–2008 by Pierre Coullet together with the late Lorenz Kramer. As it is clear from this abbreviation, already at that time the centre of attention of nonlinear science was moving from general theories to the huge unexplored territory of biological patterns, of which morphogenesis presents the most challenging problems. This tendency has only strengthened since.

First scientific studies of what would be encompassed by the term "dissipative structures" [1], in Faraday's vibrating liquid layers [2] and Bénard convection [3], originated in fluid mechanics. This was an inspiration and example, but it was natural and non-controversial; after all, everybody is used to a play of sea waves and river currents. Perhaps it was only the regularity of patterns, the hexagonal tiling resembling the crystalline order that maintains itself far from equilibrium calmness, which was unusual and thought provoking?

It was not hydrodynamic but chemical structures that excited the pioneering work of the mid-20th century, since they promised to show the way to understanding the basic mechanism of morphogenesis [4] and evolution [5,6]. The paper bearing the ambitious title "The chemical basis of morphogenesis" [4] (winged by the fame of the Turing machine and Enigma Code but more cited than read) ends on a humble note: "It must be admitted that the biological examples which it has been possible to give in the present paper are very limited. This can be ascribed quite simply to the fact that biological phenomena are usually very complicated".

The rational message to be extracted from the 36 long pages is that the formation of chemical patterns requires, in the simplest setting, combining a slowly diffusing activator with a rapidly diffusing inhibitor. This principle, that can be established in a few lines by linear stability analysis of a two-component reaction–diffusion system, is prominent in model pattern-forming systems [7,8], and has been later clearly formulated in a general form [9]. General problems become easy

*E-mail address:* pismen@technion.ac.il.

https://doi.org/10.1016/j.crme.2019.03.001
1631-0721/

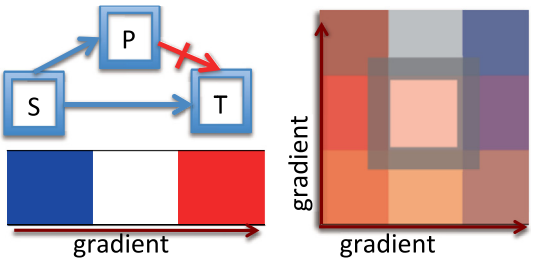


**Fig. 1.** On the left, the scheme of a feed-forward loop and Wolpert's "French flag". On the right, nine expression domains of the "Franco-German flag"; the frame indicates the zone of influence of the ligand produced in the middle square.

in retrospect; even nonlinear features of model patterns can be explored analytically using the scale separation between the diffusional lengths of the two species [10], but particular realisations of general principles overgrow by details like a ship's hull by barnacles.

Much attention has been paid to the Belousov–Zhabotinsky (BZ) reaction displaying dazzling chemical waves [11]; much later, stationary patterns have been also observed in precision experiments [12,13]. Patterns and waves on a finer scale, with added anisotropy observed on catalytic surfaces, earned the Nobel prize to Ertl [14]. Simple reaction-diffusion systems served as reasonable, if not quantitative, models of these patterns, even though the precise mechanism of the BZ reaction is disputed to this day. The same models were successfully applied to more complicated phenomena, such as desert vegetation patterns [15]. To be fair, generic models, such as the complex Ginzburg–Landau [16] or Swift–Hohenberg [17] equations reproduce qualitatively the same variety of patterns. Generalised models of this kind are equally applicable to chemical, hydrodynamical, optical, and population patterns [18]. But does the Turing mechanism indeed qualify as the chemical basis of morphogenesis?

## 2. Wolpert *vs.* Turing

There is a strong general reason why the answer should be negative, at least as far as it concerns animal development. Observed chemical patterns, as well as patterns in model computations, are repetitive, and their apparent variety is caused either by a difference in initial conditions or by random inputs. On the contrary, the morphogenetic process starting from an undifferentiated eggshell is unique and precise, with variegated features emerging at precise locations. Repetitive patterns are common in plants where the symmetry-breaking scenario may be applicable [19], but even there common spiral patterns arise from self-organization in an iterative growth process [20] rather than Turing-like symmetry breaking. Superficially realistic animal coat patterns modelled by solving the FitzHugh–Nagumo equations [21] have no connection with the actual intricate three-layer mechanism [22].

A rival morphogenetic scenario, supported by the evidence accumulated during half a century [23–25], has been put forward by Wolpert [26]. Unlike Turing patterns emerging on a homogeneous background, patterns of animal development are governed by morphogens emanating from a certain source, thereby breaking the symmetry of a featureless background, and the positional information is provided by *morphogenetic gradients*.

The Turing and Wolpert scenarios do have common features and can be even combined [27]. Interaction of activating and repressing agents is necessary also in Wolpert's scenario, and the essential feature of acting genetic schemes is a *feed-forward* motif [28]. The simplest patterning scheme involves a single incoherent feed-forward loop $S \to P$, $S \to T$, $P \dashv T$ that includes two activating ($\to$) links with different thresholds initiated by the same signal $S$ (induced by a morphogen $M$), and an inhibiting ($\dashv$) link from the intermediate protein $P$ to the target. This scheme generates the classical "French flag" pattern [26], as in the left-hand panel of Fig. 1, with the target $T$ expressed in the middle ("white") interval, where the signal level is below the higher threshold of the link to the protein $P$ and above the lower threshold of the direct link to the target. Differences in diffusivities of morphogens also play a role in localising activating or repressing thresholds, although there is no reason for the latter being less diffusive. And, of course, all morphogenetic patterns are dissipative structures in a wide sense, as they are actively driven and sustained far from equilibrium.

Repetitive patterns do occur; for example, the formation of fingers has been reproduced by a rather realistic two-dimensional simulation [29]. Fingers have, however, to be generated at a proper location, which requires positional information, and in a predetermined number, which requires scale invariance. Even in development processes generating repetitive regular patterns, such us segmentation [30], hair follicle or feather formation [31,32], or the development of ommatidia in the *Drosophila* eye [33,34], these patterns do not emerge on a homogeneous background as a result of random fluctuations, but are generated by a morphogenetic wave propagating in a predetermined direction.

The "French flag" scheme can be straightforwardly extended to two-dimensional (2D) patterns under the combined action of crossed gradients. A common example of 2D signalling is found in combined anterior–posterior and dorsal–ventral gradients in a developing *Drosophila* eggshell [35]. A second signal, generated by a morphogen with the gradient in the direction normal to that of the first signal, may induce 2D patterning that can be presented, extending Wolpert's simile, as the nine-colour superposition of French and German flags, as in the right-hand panel of Fig. 1 [36]. For example, expression

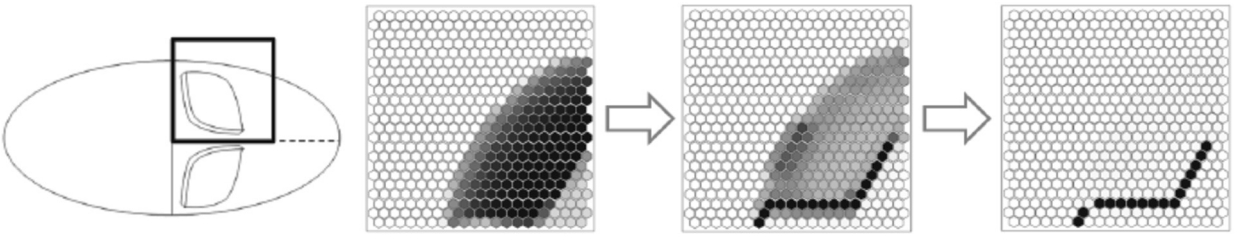

**Fig. 2.** On the left, the *Drosophila* eggshell with the computation domain outlined by the black square. On the right, the simulated development of the eggshell domain initiating the formation of dorsal appendages [36].

in the middle domain can be achieved by combining two incoherent feed-forward loops, provided both excitation links are necessary for the expression of the target $T$ (AND logic). A geometric approach of this type has been applied to modelling specific developmental features of *Caenorhabditis elegans* [37,38].

This is, however, far from sufficient to explain the rich variety of locations and shapes of expression domains. Notwithstanding the complexity of intracellular interaction schemes, the variety of persistent expression patterns cannot exceed the limit set by intersections of level sets of the signals. Modifying the form and location of a signal source, e.g., replacing a linear source by a point one, would change only the shape, but not the topology of the expression domain. Adding more initiating links with different thresholds may increase the number of subdivisions, but domain shapes will be always set by the signal level sets, making it difficult to explain less regular gene expression patterns, such us cusp-like or "eyebrow"-shape groups of cells in the *Drosophila* eggshell [39].

## 3. Localisation, scaling, and robustness

A possible way to creating variegated shapes of expression domains is combining external signals with *autocrine* morphogenetic signalling initiated within the embryonic tissue by ligands whose expression, in turn, is determined by local morphogen levels [36]. If there is a single target gene and a single ligand, there are altogether 16 combinations of target and ligand expression in the presence or absence of the autocrine signal. The diffusional range of the autocrine ligand is typically shorter than that of externally supplied morphogens. If, for example, a particular genetic network is built in such a way that in one of the nine domains (say, A) of the "Franco-German flag" the target is not expressed, but the ligand is expressed, while in a neighbouring domain B the ligand is not expressed and the target is expressed only in the presence of the autocrine signal, the target expression will be observed only in a narrow strip near the boundary where the level of the autocrine signal is sufficiently strong, as in the shaded frame in the right panel of Fig. 1. Conversely, if in the domain A both the target and the ligand are expressed, while in the domain B the ligand is not expressed and the target is expressed only at a low of the autocrine signal, the target will be expressed everywhere except in a narrow strip in the domain B near the boundary with the domain A. This helped to explain gene expression in domains of convoluted form, e.g., that involved in the formation of dorsal appendages in the *Drosophila* eggshell [40] (Fig. 2). The number of combinations grows exponentially as $2^{2(n+1)}$ with the number $n$ of autocrine ligands, leading to a great variety of expression domains for the same intrinsic genetic scheme.

The scaling and robustness problem is an Achilles heel of both Turing patterns and Wolpert's flags, and it is not helped at all by adding more signalling species. In both cases, the scale of a pattern is fixed by diffusional lengths of, respectively, reactants or morphogens, while in reality the development pattern is scaled by the size of an organism; as an extreme example, a mouse and a giraffe have the same number of vertebra. A variety of mechanisms were suggested to rectify this contradiction. Some naive attempts suggested doubling a signal by either a counter-propagating signal or a sink at the opposite edge – an arrangement becoming forbiddingly clumsy in the case of 2D patterning and in the presence of several morphogens. A more rational recipe [41] is to enhance the decay rates of morphogen at higher concentrations by making them dependent on the concentration levels. This adds robustness by buffering fluctuations at the sources. Other approaches involve interaction between expression domains [42].

The scaling problem can be solved by some kind of global control [43], which is also known to stabilise localised structures in model reaction–diffusion systems [44,45]. For example, having morphogen degradation dependent on some chemical species present in a fixed amount and uniformly distributed in a developing embryo would automatically make the morphogen gradients scale-invariant; it could also act in the same way on all morphogens diffusing in different directions. Global agents require, however, a fast mechanism for sustaining their uniform concentration, which is unlikely to exist in real tissues.

The efforts to solve all these problems in the diffusional framework may be eventually proven futile. Diffusion is too slow to ensure observed characteristic times of the establishment of morphogenetic gradients [46], and morphogens may be delivered in a more sophisticated way through cell extensions specialised in transporting them to cells [47]. This implies more intricate and specialised interactions that determine positional information.

Mechanical regulation is complementing chemistry in morphogenesis as well as in the functioning of living cells and tissues [48,49]. The perspective of application of general equations of continuous mechanics in their more sophisticated

forms, as in the active gel [50] and poroelastic [51] theories is certainly appealing to the physicists, and mechanical feedback may be an efficient agent of global regulation [52,53]. Chemo-mechanical interactions involve, however, complex specific mechanisms, and application of continuum theories is impeded by the crowded and irregular microstructure of living cells.

## 4. The devil in details

The generalising attitude of 20th-century physicists gave way in the new century to focusing on details. Tens of thousand researchers apply their ingenuity and modern technology to study detailed genetic expression details of the famous *Drosophila*, with the attendance of "Fly" meetings exceeding those of the American Physical Society. Is it really justified? Details are essential when studying human development and physiology, even at the price of sacrificing our mammal relatives to save human lives. But does a study of the detailed of proteins involved in the formation mechanism, say, of a fly wing disk, contribute to either practical or existential knowledge?

Of course, detailed studies help to elucidate general principles as well; in particular, only in this way the relative role of diffusion and active transport in shaping morphogenetic patterns can be understood. More insight is promised by *in vitro* studies in controlled artificially engineered environment [54]. The detailed view is, however, infinitely complex. In the end, by Wolpert's [25] admission, "we still do not know the molecular basis of positional information [...], nor do we have convincing evidence of how positional values are specified or interpreted. Even the role of diffusion in morphogens is unclear." The other cited review [24] ends at a similar note.

It is necessary to know what do we really need to know. In Schrödinger's words [55], we really know only what we can make. Even this may cease to be true in the age of artificial intelligence (AI), but details certainly matter as manufacturing adopts biomimetic principles of pattern formation governed by signalling, replacing, or complementing the assembly methods, that also encompass, alongside traditional manufacturing, such modern processes as lithography or 3D printing. A promising direction is the use of stimuli-responsible soft materials, such as hydrogels and nematic elastomers that are distinguished by feedback interactions between chemical and environmental signals and mechanical properties – similar to natural materials but on a far lower level of complexity.

The BZ reaction in a hydrogel causes its mechanical oscillations [56,57]. The orientation texture of nematic elastomers can be prepared in such a way that they acquire a desired shape upon transition to the isotropic state [58–60]. Reshaping can be also induced in biomimetic fashion by externally imposed dopant gradients [61]. Repeated reshaping has been used to simulate [62] and reproduce in the laboratory [63] artificial crawlers.

One can expect that intelligent design, either by humans or AI, will come to simpler and more rational (if not superior) solutions than blind Darwinian search, notwithstanding the wonders of natural design.

## References

[1] P. Glansdorff, I. Prigogine, Thermodynamic Theory of Structure, Stability, and Fluctuations, Wiley, New York, 1971.
[2] M. Faraday, Philos. Trans. R. Soc. Lond. 121 (1831) 299–318.
[3] H. Benard, Ann. Chim. Phys. 7 (1900) 62.
[4] A.M. Turing, Philos. Trans. R. Soc. Lond., Ser. B 237 (1952) 37–72.
[5] M. Eigen, Naturwissenschaften 58 (1971) 465–523.
[6] I. Prigogine, G. Nicolis, A. Babloyantz, Phys. Today 25 (1972) 38–44.
[7] R. FitzHugh, Biophys. J. 1 (1961) 445–466.
[8] I. Prigogine, G. Nicolis, J. Chem. Phys. 46 (1967) 3542–3550.
[9] L.A. Segel, J.L. Jackson, J. Theor. Biol. 37 (1972) 545–559.
[10] L.M. Pismen, Patterns and Interfaces in Dissipative Dynamics, Springer, Berlin, 2006.
[11] A.M. Zhabotinsky, Proc. Acad. Sci. USSR 157 (1964) 392;
A.N. Zaikin, A.M. Zhabotinsky, Nature 225 (1970) 535.
[12] V. Castets, et al. 64 (1990) 2953.
[13] Q. Ouyang, H.L. Swinney, Nature 352 (1991) 610.
[14] G. Ertl, Science 254 (1991) 1750.
[15] E. Meron, Annu. Rev. Condens. Matter Phys. 9 (2018) 79–103.
[16] I.S. Aranson, L. Kramer, Rev. Mod. Phys. 74 (2002) 99–143.
[17] J.B. Swift, P.C. Hohenbelg, Phys. Rev. A 15 (1977) 319.
[18] M.C. Cross, P.C. Hohenberg, Rev. Mod. Phys. 65 (1993) 851–1112.
[19] S. Diguini, et al., Mol. Syst. Biol. 4 (2008) 217.
[20] S. Douady, Y. Couder, Phys. Rev. Lett. 68 (1992) 2098–2101.
[21] J.D. Murray, J. Theor. Biol. 88 (1981) 161–199.
[22] U. Irion, A.P. Singh, C. Nÿsslein-Volhard, Curr. Top. Dev. Biol. 117 (2016) 141–169.
[23] C. Nusslein-Volhard, Coming to Life: How Genes Drive Development, Kales Press, 2008.
[24] K.W. Rogers, A.F. Schier, Annu. Rev. Cell Dev. Biol. 27 (2011) 377–407.
[25] L. Wolpert, Curr. Top. Dev. Biol. 117 (2016) 597–608.
[26] L. Wolpert, J. Theor. Biol. 25 (1969) 1–47.
[27] A. Gierer, H. Meinhardt, Kybernetik 12 (1972) 30–39.
[28] U. Alon, Nat. Rev. Genet. 8 (2007) 450.
[29] J. Raspopovic, et al., Science 345 (2014) 566–570.
[30] O. Pourquie, Int. J. Dev. Biol. 47 (2003) 597.
[31] S. Sick, et al., Science 314 (2006) 1447.
[32] Lin, et al., Dev. Biol. 334 (2009) 369.

[33] S. Greenwood, G. Struhl, Development 126 (1999) 5795.
[34] J.P. Kumar, Nat. Rev. 2 (2001) 846.
[35] C.A. Berg, Tissue Eng., Part A 14 (2008) 1479.
[36] L.M. Pismen, D.S.A. Simakov, Phys. Rev. E 84 (2011) 061917.
[37] F. Corson, E.D. Siggia, Proc. Natl. Acad. Sci. USA 109 (2012) 5568–5575.
[38] F. Corson, E.D. Siggia, eLife 6 (2017) e3074.
[39] F. Peri, C. Bokel, S. Roth, Mech. Dev. 81 (1999) 75.
[40] D.S.A. Simakov, et al., Development 139 (2012) 2814–2820.
[41] A. Eldar, et al., Dev. Cell 5 (2003) 635–646.
[42] S. Vakulenko, et al., Phys. Rev. Lett. 103 (2009) 168102.
[43] D. Ben-Zvi, N. Barkai, Proc. Natl. Acad. Sci. USA 107 (2010) 6924–6929.
[44] K. Krischer, A. Mikhailov, Phys. Rev. Lett. 73 (1994) 3165–3168.
[45] L.M. Pismen, J. Chem. Phys. 101 (1994) 3135–3144.
[46] H. Teimouri, A.B. Kolomeisky, J. Phys. A, Math. Theor. 49 (2016) 483001.
[47] M. Kerszberg, L. Wolpert, Cell 130 (2007) 205–209.
[48] B.N. Belintsev, L.V. Beloussov, A.G. Zaraisky, J. Theor. Biol. 129 (1987) 369–394.
[49] L.A. Taber, Philos. Trans. R. Soc. A 367 (2009) 3555–3583.
[50] F. Jülicher, et al., Phys. Rep. 449 (2007) 3–28.
[51] E. Moeendarbary, et al., Nat. Mater. 12 (2013) 253–261.
[52] L. Hufnagel, et al., Proc. Natl. Acad. Sci. USA 104 (2007) 3835–3840.
[53] N. Hervieux, et al., Curr. Biol. 26 (2016) 1019–1028.
[54] A.P. McGuigan, S. Javaherian, Annu. Rev. Biomed. Engng. 18 (2016) 1–24.
[55] E. Schrödingier, What is Life?, Cambridge University Press, Cambridge, UK, 1962.
[56] V.V. Yashin, et al., Rep. Prog. Phys. 75 (2012) 066601.
[57] S. Métens, S. Villain, P. Borckmans, in: P. Borckmans, et al. (Eds.), Chemomechanical Instabilities in Responsive Materials, Springer, 2009.
[58] L.T. de Haan, A.P.H.J. Schenning, D.J. Broer, Polymer 55 (2014) 5885.
[59] C. Mostajeran, et al., Proc. R. Soc. A 472 (2016) 20160112.
[60] H. Aharoni, et al., Proc. Natl. Acad. Sci. USA 115 (2018) 7206–7211.
[61] A.P. Zakharov, L.M. Pismen, Soft Matter 13 (2017) 2886–2892.
[62] A.P. Zakharov, L.M. Pismen, Phys. Rev. E 93 (2016) 022703.
[63] M. Rogóż, et al., Adv. Opt. Mater. 4 (2016) 1689–1694.