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INVITED CONTRIBUTION

Fifty years after Alan M. Turing
An extraordinary theory of morphogenesis

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ABSTRACT. The publication of 'The Chemical Basis of Morphogenesis' by ALAN M. TURING in 1952 was a milestone for the development of mathematical biology and for many (biological) disciplines leaning on it. TURING provided an original solution to the problem of morphogenesis, by adapting a system of coupled differential equations to describe both chemical reaction and diffusion of morphogenetic substances in an initially homogeneous configuration. FOURIER'S analysis of the 'ring problem' in heat conduction, and the theory of spherical harmonics and their solution by (normalized) LEGENDRE'S associated functions form the mathematical backbone of TURING'S work on morphogenesis. TURING was up to more than providing a mathematical description of initial stages of embryonic development. Rather he was eager to unveil the mathematical foundations of living, biological organization. An investigation of the archival material of unpublished letters and manuscripts indicates that TURING was clearly determined to provide an argument for the generation of 'order-from-disorder'. Unfortunately, during his lifetime TURING remained unable to demonstrate the use of his model beyond the level of early embryonic stages. In the posthumously-published manuscripts several indications are found for further adaptation and improvement of TURING'S model to handle more accurately the reaction-diffusion processes in small organisms.

KEY WORDS : morphogenesis, reaction-diffusion theory, early embryonic development, spherical harmonics, normalized Legendre associated functions.

INTRODUCTION

In 1952 a paper was published that had a far-reaching impact not only for the application of the theory of reaction-diffusion mechanisms in biology, but also for developmental biology and embryology (TURING, 1952). Indirectly, genetics and the entire biological field were also affected. The author, however, was not a biologist but a mathematician. His 1952 paper was the only biological paper he published during his lifetime. ALAN MATHISON TURING (born 23 June 1912) unfortunately died under dramatic circumstances in June 1954, only two years after the publication of 'The Chemical Basis of Morphogenesis' (August 1952).

As early as 1953, the speculative value of TURING'S paper was recognised by J.W.S. PRINGLE (Department of Zoology, Cambridge) (1953), stating that TURING'S model of morphogenesis could 'provide a means of creating structure where no structure was initially present'. A system that is unstable with respect to its local concentrations of reacting molecules may be started on a course towards stability by a small event. However, if there is some initial heterogeneity due to factors other than the concentrations themselves, PRINGLE (1953) says this can provide 'the initial stimulus for morphogenesis if the heterogeneity has a component of its structure similar to the inherent tendency of the system'. The notion of an emerging self-explaining structure as well as the notion of the tendency of an unstable, homogeneous system towards a stable but heterogeneous system were both present in contemporary thought on morphogenesis. PRINGLE also refers to a personal communication of A.M. TURING with respect to the so-far unpublished work involving a model with non-linear differential equations for two morphogens.

The unpublished manuscripts and notes of ALAN TURING'S later research on 'The chemical theory of morphogenesis' have been collected at King's College Archive Centre (referred to as KCC). These were studied and pieced together by N.E. HOSKIN and B. RICHARDS and appeared – together with the 1952 paper – in the *Collected Works of A.M. TURING* (Volume *Morphogenesis* edited by P.T. SAUNDERS, 1992). In this posthumously published work, TURING's model indeed diverted from the linear case and also from geometrical constraints such as the ring of cells or the sphere. Moreover, an important conceptual role is reserved for the use of the mathematical theory of spherical harmonics and Legendre associated functions.

The extraordinary character of TURING'S work on morphogenesis links up with his outstanding achievements in fields such as mathematical logic, mechanical intelligence and pure mathematics. As a pinnacle in the twentieth century of mathematical and logical thought, he would rather work things out in a self-contained way than lean on others (see biography by SARA TURING, 1959, p. 119). As an example, in 1934 TURING proved the Central Limit Theorem independently of Lindeberg's proof of 1922 (HODGES,

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1983, p. 88). Also, TURING'S paper *'On Computable Numbers'* (1937) was independently provided but nevertheless antedated by Church's work in this field and published in 1936 (HODGES, 1983, p. 546). TURING was familiar with E. SCHRÖDINGER'S 1943 lecture *'What is Life?'*, deducing the crucial idea that genetic information must be stored at the molecular level (SCHRÖDINGER, 1944). But rather than follow up SCHRÖDINGER'S suggestion, TURING aimed at finding a parallel explanation of how a chemical soup of molecules could possibly give rise to a biological pattern, granted the transcription of genes into diffusible molecules (HODGES, 1983, p. 431).

However, TURING (1952) had some benefit of existing biological knowledge. The notion of diffusible molecules that affect embryological development and the discovery of the existence of chemical gradients that directed axis formation in the embryo, were especially elaborated by HÖRSTADIUS (1939, 1950, 1952, 1953). HÖRSTADIUS used eggs of the sea urchin *Paracentrotus lividus* (Echinodermata) as a model for the study of the development of an animal-vegetal gradient system in an initially spherical, symmetrical organism (for a review see BALINSKY, 1981). Moreover, since it became well known that also the cytoskeleton has a very important role in early embryogenesis, TURING'S conceptual basis of mere diffusible morphogenetic substances nowadays is considered too narrow to support the full range of embryological and developmental processes observed in biological species. The notion of 'positional information' (WOLPERT, 1969) – although more vague than TURING'S notion of morphogen (see HARRISON, 1987) – has regained popularity as a conceptual framework to embrace several biochemical mechanisms, acting in concert to direct morphogenesis (for a review see ALLAERTS & ROELANTS, 1993). Although mathematical contributions to the field of embryology have only occasionally been reported since the work of TURING (see e.g. GOODWIN & TRAINOR, 1980), mathematical modeling recently regained interest in the field of genome analysis (PERCUS, 2002).

We previously reported on the extraordinary position of TURING'S 1952 paper on morphogenesis with respect to the concept of positional information (ALLAERTS & ROELANTS, 1993), although we did not report in detail on the mathematical features of TURING'S work. In this study, we will focus on the mathematical core (section 1) and the origin and background of the 1952 paper (section 3). In section 2, TURING'S modifications of the reaction-diffusion model for small organisms are discussed, based on unpublished manuscripts and notes kept at King's College Archive Center and partly posthumously published in the *Collected Works of A.M. TURING* (SAUNDERS, 1992). Several examples have been found in the literature (biology as well as physics) to validate TURING'S theoretical findings. Finally, a few notes are added on TURING'S view on conscious living beings (section 4). An appendix is added for introduction into the mathematical techniques used by TURING to solve morphogenesis in a cylindrical (appendix a) and spherical configuration (appendix b). Also the mathematical fine-tuning of Turing's reaction-diffusion model as worked out by RICHARDS (see SAUNDERS, 1992), and the use of the technique of normalized Legendre associated functions to describe skeleton formation in radiolarian species are discussed (appendix c).

THE MATHEMATICAL CORE OF THE 1952 PAPER

ALAN TURING was not the first to use a mathematical model to describe complex dynamic systems in biology. Already VOLTERRA (1926), used a system of two coupled differential equations to describe the oscillatory behaviour of abundance numbers of prey and predator species. Later on, this model was referred to as the Lotka-Volterra system (MURRAY, 1989). But, in contrast to the Lotka-Volterra system, where an obvious relationship could be assumed between the prey species and the predator species feeding on it, the idea of morphogenetic substances that chemically reacted with each other, was an absolute *terra incognita.* The new feature of TURING'S work arose from the simultaneous consideration of diffusion as a factor influencing the concentrations in a region of space. In fact, TURING'S mathematical model of morphogenesis through chemical, diffusible substances shaped a new domain for mathematical modeling in biology, named reaction-diffusion theory (see also ALLAERTS & ROELANTS, 1993).

Unfortunately, TURING himself gave very few hints to explain the mathematical origins of his model. The central role of the cylindrical case in TURING'S 1952 paper, suggests some affinity with the central 'ring problem' in FOURIER'S (1822) analysis of heat transfer (see appendix a). This was probably general knowledge to the trained mathematician TURING. On the other hand, TURING did refer to the work of JEANS (1927) on *'Electricity and Magnetism'*, concerning the application of spherical harmonic functions to the problem of morphogenesis in a sphere.

The cylindrical case of morphogenesis

The central problem in TURING'S model of morphogenesis through diffusion of two (later: two or three) chemical substances is a classical application of reaction-diffusion theory. TURING'S approach starts from a radially symmetric (cylindrical) system, such as a ring of cells or a continuous ring of tissue. In the continuous, cylindrical case the equations for diffusion of the substances X and Y are:

$$\left.\begin{aligned}\frac{\partial X}{\partial t} &= a(X-h)+b(Y-k)+\frac{\mu'}{\rho^2}\frac{\partial^2 X}{\partial \theta^2}\\ \frac{\partial Y}{\partial t} &= c(X-h)+d(Y-k)+\frac{\upsilon'}{\rho^2}\frac{\partial^2 Y}{\partial \theta^2}\end{aligned}\right\} \quad (1)$$

In this set of equations, the cylindrical notation for the basic reaction-diffusion model is recognised, namely:

$$\frac{\partial C}{\partial t} = f(C) + D\frac{\partial^2 C}{\partial \theta^2} \qquad \text{(see also ALLAERTS, 1992)}$$

with C the vector of chemical concentrations, $f(C)$ the vector representing the chemical reactions of these chemical substances, D the diffusion matrix and $\partial^2 C/\partial \theta^2$ the second-order derivative along the polar angle co-ordinate θ. TURING'S set of equations (1) is a limiting case of the equations for reaction diffusion in a ring of cells, the ring having radius ρ. In the continuous case of the ring the diameter of the cells is incorporated into the notations used for describing the diffusibilities μ' and ν', which are relat-

ed to the cell-to-cell diffusion constants μ and ν of the substances X and Y respectively :

$$\mu = \mu'(\frac{N}{2\pi\rho})^2 \ , \ \nu = \nu'(\frac{N}{2\pi\rho})^2$$

It is important to note that TURING considers the diffusion constants μ , ν, μ' and ν' as constants, a fact that has important consequences for the biological process of morphogenesis (ALLAERTS & ROELANTS, 1993).

The general solution of (1) proposed by TURING, using Fourier transformation, is of the form (see appendix a) :

$$X = h + \sum_{s=-\infty}^{\infty} (A_s e^{p_s t} + B_s e^{p_s' t}).e^{is\theta} \quad \}(2)$$

$$Y = k + \sum_{s=-\infty}^{\infty} (C_s e^{p_s t} + D_s e^{p_s' t}).e^{is\theta}$$

where p_s, p_s' are the roots of the equation:

$$(p - a + \frac{\mu' s^2}{\rho^2})(p - d + \frac{\nu' s^2}{\rho^2}) = bc \quad (3)$$

The constants A_s, B_s, C_s and D_s are not independent, but are restricted to satisfy the set of equations:

$$A_s(p_s - a + \frac{\mu' s^2}{\rho^2}) = bC_s \quad \} \ (4)$$

$$B_s(p_s' - a + \frac{\mu' s^2}{\rho^2}) = bD_s$$

It is important to consider the nature of the solutions to the equation set (2). These equations represent Fourier series in an exponential notation, describing the deviations from the equilibrium concentrations *h, k* of the respective morphogenetic substances *X,Y* (Fig.1.a). Depending on the value of the roots *p, p'* of the characteristic equation (see appendix a), and on the value of the diffusion constants μ and ν and reaction rates *a, b, c, d,* the resulting geometrical representations of these equations are stationary or oscillatory waves. TURING (1952) is very much concerned with the physical or biological importance of these wave functions, which after a lapse of time may result in patterns on the ring (Fig. 1.b). The wave-lengths of these patterns depend on the circumference of the ring and the chemical data set (a,b,c,d,μ,ν) (TURING, 1952, p. 51; see also ALLAERTS & ROELANTS, 1993). In his unpublished work, TURING himself indicated that the restriction to a ring of cells was *"altogether an unnecessary one (...) for the conclusions for the ring of cells could be directly taken over by any arrangement of cells"* (SAUNDERS, 1992, p. 90). This remark, however, points to the main mathematical assumption of TURING's 1952 paper, namely that the reaction rates are linear functions of the concentrations, which is considered *"reasonably valid so long as only small variations of concentrations are concerned"* (SAUNDERS, 1992, p. 90). In section 2, the characteristics of a more refined reaction-diffusion model devoted to the case of small organisms will be discussed, starting from TURING'S posthumously published work (SAUNDERS, 1992).

The spherical case of morphogenesis

In 1954 ROBIN O. GANDY, university lecturer at Leicester (UK) and inheritor of A.M. TURING'S articles and unpublished manuscripts, expressed his concern about the preservation of TURING'S work, in particular his work on the cylindrical case of morphogenesis. This remark was found in a letter to M.H.A. NEWMAN (KCC: A/8), a professor of topology who played an important role as teacher and mentor of A. M. TURING in the pre-war period at Cambridge (HODGES, 1983, pp. 90-93). In 1951, TURING himself had already brought his work on spherical structures to the attention of the neurophysiologist J.Z. YOUNG (KCC: K/1, nr. 78), but TURING considered this case rather 'more difficult and doubtful' than the cylindrical case. Therefore, it is

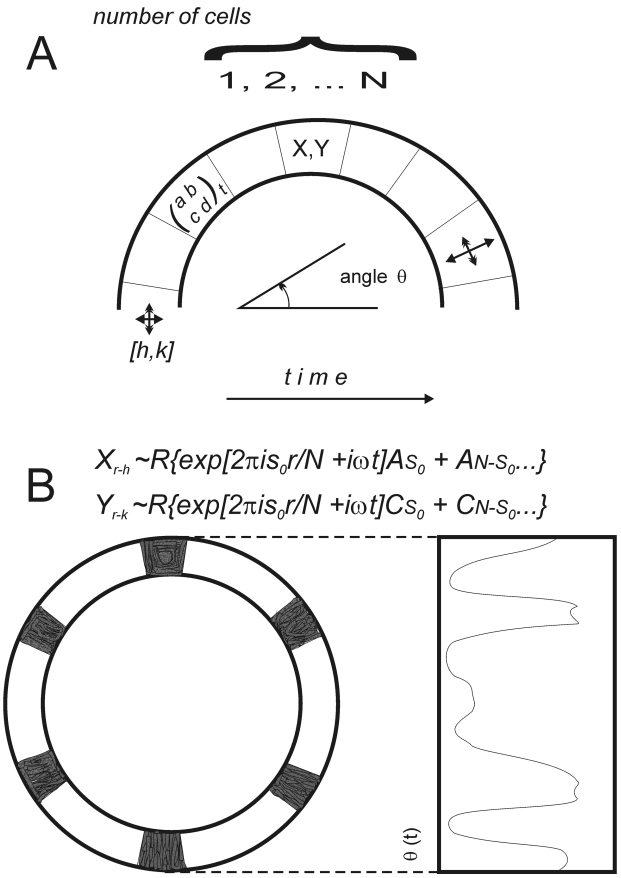

Fig. 1A. – Dimensions and parameters in TURING'S (1952) most simple model of morphogenesis, the reaction-diffusion system in a ring of cells (cylindrical case). *[h,k]* : equilibrium concentrations for morphogenetic substances *X,Y* ; $\begin{pmatrix} a & b \\ c & d \end{pmatrix}_t$: reaction rates at instant *t* ; double arrow: diffusibility; single arrow: deviation from equilibrium concentration after a lapse of time.

Fig. 1B. – Result of reaction-diffusion in a ring of cells after a lapse of time (according to TURING, 1952). The real parts *R* of the roots p_{S_0}, p'_{S_0} determine the wavelengths of the pattern resulting from equation (2) by the relation

$$X_{r-h} \approx \Re\left\{\exp\left[\frac{2\pi i S_0 r}{N} + i\varpi t\right] A_{S_0} + A_{N-S_0}...\right\},$$

the expression for the other morphogen *Y* being related through the relationship between the constants $A_S \sim C_S$,... Moreover, it can be easily shown that the roots p_{S_0} and p_{N-S_0} yield the same terms, using the relation :

$$\sin^2 \frac{\pi.(N - S_0)}{N} = \sin^2 \frac{\pi.S_0}{N}$$ (TURING 1952, p. 50).

somewhat surprising that GANDY did not mention TURING'S attempts to extend his model to spherical organisms, which in fact was already addressed to some degree in his 1952 paper. So, although in 1951 some doubt was expressed on the spherical extension of the model, TURING introduced the key mathematical features of this approach in 1952, as shown below (see also appendix b).

In the case of a hollow sphere of continuous tissue such as a blastula, the spherical notation for describing the diffusion of substances *X* and *Y* is needed. TURING uses the operator ∇^2 to indicate the superficial part of the Laplacian. ∇^2 is an abbreviation of the notation

$$\frac{1}{\rho^2}\frac{\partial^2 V}{\partial \phi^2}+\frac{1}{\rho^2 \sin^2\theta}\frac{\partial}{\partial\theta}(\sin\theta\frac{\partial V}{\partial\theta}) \ ,$$

where θ and ϕ are spherical polar co-ordinates on the surface of the sphere with radius ρ.

The equations corresponding to set (1) in the cylindrical case, may be written as:

$$\frac{\partial X}{\partial t}=a(X-h)+b(Y-k)+\mu'\nabla^2 X \qquad \} \ (5)$$

$$\frac{\partial Y}{\partial t}=c(X-h)+d(Y-k)+\nu'\nabla^2 Y$$

To solve this set of differential equations, TURING (1952) refers to JEANS (1927), stating that *"(almost) any function on the surface of the sphere can be expanded in spherical surface harmonics"*. This according to TURING means that solutions of (5) are to be found which are expressions of the form:

$$\sum_{n=0}^{\infty}[\sum_{m=-n}^{n}A_n^m P_n^m(\cos\theta)e^{im\phi}] \qquad (6).$$

Something curious happened with the introduction of this notation. First, TURING'S (1952) reference to JEANS (1927) was erroneously cited as *'The Mathematical theory of elasticity and magnetism'*, whereas JEANS' textbook, edited from 1908 onwards, was on *'electricity and magnetism'*. Herein, indeed a chapter on methods for the solution of spherical problems was included, in which the theory on spherical harmonics takes a very prominent place. This theory attempts to provide a general solution of Laplace's equation $\nabla^2 V = 0$ (see Appendix b)*.

Then, Legendre's associated functions expressed as $A_n^m P_n^m(\cos\theta)$, are introduced by TURING (1952) without much ado as a solution to Laplace's equation. The upper indices *m* indicate that here the associated Legendre functions are used, which are linked to the Legendre functions $P_n(\cos\theta)$ through the relation:

$$P_n^m(\cos\theta)=(-1)^m \sin^m\theta.\frac{d^m P_n(\cos\theta)}{d(\cos\theta)^m}$$

(HOBSON, 1931, p. 90).

In appendix b, a more elaborate explanation is given of TURING'S use of Legendre's associated functions in the 1952 paper. The fact that TURING here also uses Legendre's associated functions of degree *m = -1*, but without referring to the notation $\overline{P_n^m}(\cos\theta)$ for the normalized Legendre associated functions (see appendix c), is in favour of the view that TURING was still in a process of refinement of his mathematical techniques (see extension of TURING'S work by RICHARDS in SAUNDERS, 1992).

TURING emphasizes that the expression in the square bracket of (6) is a 'surface harmonic of degree *n*', and that *"its nearest analogue in the ring theory is a Fourier component"* (TURING, 1952, p. 70). Moreover, an essential property of a spherical harmonic of degree *n* is when the operator ∇^2 is applied to it the effect is the same as multiplication by $-(n+1)/\rho^2$. Preferentially, manageably low values of the degree *n* (such as 1 or 2) are chosen (see appendix b).

The analogy with the ring theory, in which Fourier expansions are an important method (see appendix a), brings TURING (1952) to the following solution of (5):

$$X=h+\sum_{n=0}^{\infty}\sum_{m=-n}^{n}(A_n^m e^{iq_n t}+B_n^m e^{iq_n' t})P_n^m(\cos\theta)e^{im\phi} \qquad \} \ (7)$$

$$Y=k+\sum_{n=0}^{\infty}\sum_{m=-n}^{n}(C_n^m e^{iq_n t}+D_n^m e^{iq_n' t})P_n^m(\cos\theta)e^{im\phi}$$

where q_n and q'_n are the two roots of:

$$(q-a+\frac{\mu'}{\rho^2}n(n+1))(q-d+\frac{\nu'}{\rho^2}n(n+1))=bc$$

and,

$$A_n^m(q_n-a+\frac{\mu'}{\rho^2}n(n+1)=bC_n^m$$

$$B_n^m(q_n'-a+\frac{\mu'}{\rho^2}n(n+1)=cD_n^m$$

indicating that also here A_n^m, B_n^m, C_n^m and D_n^m are arbitrary but not independent constants, resulting from the solution of the differential equation set (see appendix a for analogy).

As in the cylindrical case, TURING suggests that one particular form of wave (and wavelength) predominates, so reducing (7) into:

$$X-h=e^{iqn_0 t}\sum_{m=-n_0}^{n_0}A_{n_0}^m P_{n_0}^m(\cos\theta)e^{im\phi} \qquad \} \ (8)$$

$$b(Y-k)=(q_{n_0}-a+\frac{\mu'}{\rho^2}n(n+1))(X-h)$$

This brings TURING to the extraordinary conclusion that the two morphogens diffusing on the sphere have proportional concentrations, and both of them are described by surface harmonics of the same degree n_0. This degree n_0 is chosen to maximize the greater of the roots q_{n_0}, q'_{n_0} (TURING, 1952, p. 70).

In Fig. 2, some examples are shown of applications found in biology and chemistry, that validate the use of spherical harmonics in processes describing early embryogenesis (GOODWIN & TRAINOR, 1980) or that provide evidence for sustained non-equilibrium chemical patterns, called 'Turing structures' later on (CASTETS et al., 1990).

* This theory was to a large extent worked out by the French mathematician A.M. LEGENDRE (1752-1833), contemporary of Sir P.S. DE LAPLACE (1749-1827) (for an historical review see E.W. HOBSON, 1931, pp. 16-17).

TURING himself suggested that the forms of 'various, nearly spherical structures', such as radiolarian skeletons (Fig. 2.c), were closely related to spherical harmonic patterns, an idea that has been elaborated further by B. RICHARDS (see SAUNDERS, 1992; appendix c). The best application of his theory, however, according to TURING (1952), seemed to be the gastrulation of the blastula. TURING referred to the early stage in the development of an embryo, characterized as a hollow spherical aggregation of cells which still are morphologically identical. As long as the size of the blastula is not more than the dimensionless diffusibility *(μ')*, the system is considered 'quite stable'. Near this point, however, TURING thinks the harmonics of degree 1 begin to develop, bringing the Legendre's associated functions (P_1^1) into play (TURING, 1952, p. 71; see appendix b). At his untimely death however, this idea remained unexplored.

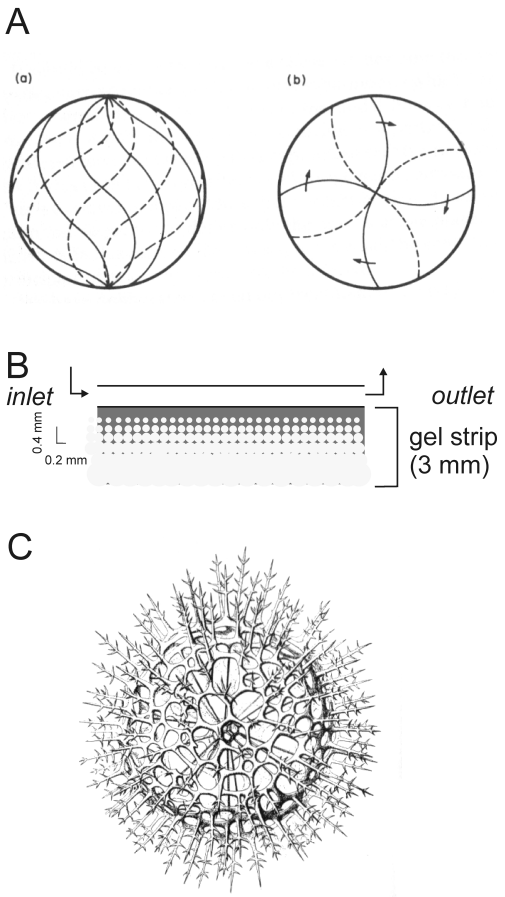

Fig. 2. – Applications of Turing structures in biological or chemical pattern formation:
A. – Spiral nodal lines derived from spherical harmonic functions have been used by GOODWIN & TRAINOR (1980) to describe the cleavage process in embryogenesis. Spiral nodal lines on the sphere from the side (left) and cleavage patterns up to third cleavage defined by spiral nodal lines seen from the animal pole (right) (after GOODWIN & TRAINOR, 1980, p. 766). Arrows show the movement of the blastomeres after cleavage.
B. – Evidence of sustained standing nonequilibrium chemical pattern in a single-phase open reactor suggested to be the first unambiguous evidence of a Turing structure (drawn after CASTETS *et al.*, 1990). The reactor is made of a chemically inert polyacrylamide gel, chemicals diffuse from the edges into the gel where the actual reactions take place.
C. – One of the examples, suggested by TURING (1952), of the spherical case of morphogenesis was the formation of the radiolarian skeleton (siliceous skeleton of *Trypanosphaera regina* after BARNES, 1974, p. 30). RICHARDS (see SAUNDERS, 1992) completed the mathematical solutions for the spherical case, making use of the normalized Legendre associated functions (see appendix c). The geometrical representations of these mathematical results revealed spheroid bodies with increasing numbers of fine radiant spines, when spherical harmonics of increasing degrees were introduced in the mathematical solution.

REFINEMENT OF THE MODEL FOR SMALL ORGANISMS

According to N.E. HOSKIN & B. RICHARDS (in SARA TURING, 1959, p. 137-144), at least two major modifications were adopted by TURING in his late, unpublished work on morphogenesis. These are: (1) the incorporation of quadratic terms in the differential equations in order to take account of a 'larger departure from a state of homogeneous equilibrium'; (2) the consideration that for small organisms the concentration function of the so-called growth-retarder or 'poison' substance (symbol V_j), were independent of position. The latter assumption requires that the organism is so small that the growth-retarder is uniformly diffused through it.

Today, it is a well-known fact that at least in the animal species studied so far, concentrations of morphogenetic substances with stimulatory effects and substances with inhibitory (or 'growth'-retarding) capacities do exist, and both occur in gradient-like or discrete distribution patterns. But this molecular biological knowledge obviously was not available in the early fifties, when TURING published his linear model for morphogenesis (1952) and the double helix strand model for DNA (WATSON & CRICK, 1953) was just discovered. For comparison, the homeobox-gene concept (and the idea of genes that regulate the patterned expression of morphogens) was proposed only in 1984 (see ALLAERTS, 1998 for references).The linear differential equations used in the 1952 paper were of the form $\frac{\partial X_i}{\partial t} = f_i(X_1,...,X_n) + \mu\nabla^2 X_i$ with *(i = 1,...,n)* for n different morphogens (9)and f_i the reaction function giving the rate of growth of X_i and $\mu\nabla^2 X_i$ the rate of diffusion of X_i (compare with equation 1 in section 1). In his 1952 paper, Turing considered the X_i's as variations from a homogeneous equilibrium, and, if the departures from equilibrium were only small, it would be permissible to linearize the f_i's and thus the differential equations. These conditions were assumed to be fulfilled in the 'initial' state of the morphogenetic system, where a homogeneous equilibrium state was present. Embryological studies afterwards have shown that the fertilized egg, although seemingly homogeneous at the macroscopic or even microscopic level, nevertheless has to be considered as a very dynamic and (in biochemical terms) far from equilibrium system.

In order to account for a larger departure from the initial, presumed homogeneous state, Turing introduced quadratic terms in the reaction functions. In the second part of the posthumously published manuscript *'Chemical Theory of Morphogenesis'* (SAUNDERS, 1992, pp. 88-

118), TURING gives the following general differential equation for the morphogen concentration function *U (t)* :

$$\frac{dU_j}{dt} = \left[\phi(-\nabla^2)U\right]_j GU_j^2 - HU_jV_j \qquad (10)$$

(SAUNDERS, 1992, p. 98),

where $\phi\,(-\nabla^2)$ denotes a function of the Laplacian of U_j, which has its maximum near the maximum wavelength, and V_j is the concentration function of the 'poison' substance. For small organisms, TURING considers two types of wavelengths of importance, namely the 'optimum' wavelength and the wavelength with zero root, i.e. the uniform distribution. According to TURING, the latter condition may be fulfilled in small and 'connected' organisms, where it is called a poison or growth-retarder (SAUNDERS, 1992, p. 98). *G* and *H* denote constants that are related to the use of spherical harmonics as solutions (see appendix b), and will appear to be solvable using the normalized Legendre associated functions and some reiteration procedure developed by RICHARDS (see SAUNDERS, 1992, p. 109; appendix c).

Now, since only solutions with the optimum wavelengths have a significant contribution, and due to the effective equilibrium of the growth-retarders, also $\partial V_j / \partial t = 0$, the following reduction of equation (10) is obtained for small organisms:

$$\frac{dU_j}{dt} = (P - HV).U_j + G\left[\Im U^2\right]_j \qquad (11)$$

(SAUNDERS, 1992, p. 98),

A special linear operator $\Im\,(U^2)$ is introduced with the property to remove from a function on a sphere all spherical harmonics except those of a particular degree (exhibiting a particular wavelength). Moreover, solutions must be equivalent under rotation of the sphere, and therefore also the squared harmonics are selected based on a specific degree (HOSKIN & RICHARDS, in S. TURING, 1959). HOSKIN & RICHARDS remarked that in most of TURING'S unpublished manuscripts it was very difficult to discover the results as far as worked out by TURING himself. A number of numerical computations were carried out on one of the first electronic computers at Manchester University (S. TURING, 1959, p. 139). Important in this respect is TURING'S use of spherical harmonics (already introduced in the 1952 paper, see section 1,b) and his use of linear operators applied to these spherical harmonics. Nevertheless, HOSKIN and especially RICHARDS provided a prolific extension of TURING'S suggestions and first steps into a more general theory of morphogenesis. Moreover, RICHARDS was able to demonstrate some numerical examples giving rise to organisms resembling the morphology of a radiolarian skeleton, as suggested by TURING (1952) (appendix c).

REMARKS ON THE ORIGIN OF 'THE CHEMICAL BASIS OF MORPHOGENESIS'

Among pre-war British mathematicians, two branches could be discerned. On the one hand, there were those such as G.H. HARDY, one of TURING'S teachers, who considered real mathematics (in contrast to trivial, applied mathematics) as not useful and doing 'no good' to society (HARDY, 1940). On the other hand, there were men such as A.M. TURING, who assured his followers that mathematics applied to biology and digital computing were as esthetic and indulging as pure mathematics could be. Owing to ANDREW HODGES' biography (1983) it is well accepted nowadays that ALAN TURING was a genius of his own kind. His first biographer, Alan's mother SARA TURING, already quoted the words of ROBIN O. GANDY, *"that the mark of his genius was that even in the most abstract realms of thought he always bore in mind completely concrete ideas and examples"* (S. TURING, 1959). On another occasion, GANDY wrote that TURING was *"unmethodical, or his methods were so individual"*, that his work was hard to follow (KCC: A/18). GANDY was a PhD student and friend of TURING, and soon became university lecturer at Leicester (UK). Reading the collected typescripts and manuscripts at King's College Archive Centre, one is in no doubt about the creative forces of TURING's personality, as he was endowed equally with sound mathematical discernment and a most subtle sense of humour. The question is, however, why and how the post-war mathematician switched over to the study of a typically biological subject such as the problem of morphogenesis of animals and plants?

ALAN TURING indeed was very concerned with the problem of finding a chemico-mechanical process that would explain the origin of changing symmetry patterns in a developing embryo. Referring to the collected letters of Alan to his mother (KCC, AMT/K-1), HODGES pointed out that Turing was familiar with E.T. BREWSTER'S book '*Natural Wonders every Child should know*' from his childhood on (HODGES, 1983, p. 11). In BREWSTER'S book an illustration was given of the process of blastula formation and gastrulation in the early embryo. The fundamental question exemplified in the phenomenon of gastrulation was: if the fertilized eggs were symmetrical and the chemical equations describing the molecular reactions in these structures were symmetrical, without knowledge of right or left, down or up, where did the decision to adopt a different symmetry came from? This phenomenon inspired MICHAEL POLANYI (1958) to claim that some 'immaterial' force must be at work. For TURING it meant that in some way information was created at this point of development (HODGES, 1983, p. 431). POLANYI, a chemist who became a Christian philosopher at Manchester, was an intellectual opponent of TURING – although on friendly personal terms (HODGES, 1997). TURING told GANDY that his new ideas were intended to 'defeat the argument from design'. From the onset, TURING'S approach to the problem of morphogenesis was closely tied with the problem of defining the driving force in embryonic axis formation (see also ALLAERTS & ROELANTS, 1993).

On the other hand, SCHRÖDINGER'S view (1944) of a molecular basis for genetic information was definitely insufficient to explain the formation of pattern. SCHRÖDINGER put forward his viewpoint of a genuine 'order-from-order' principle (SCHRÖDINGER, 1944, p. 81) far ahead of WATSON and CRICK'S double stranded helix model for the DNA molecule (WATSON & CRICK, 1953). Despite, and to some extent because of considerations of statistical physics (genes are too big to follow the expected inaccuracy of physical laws, expressed by the $\sqrt{n}$ rule)[1],

1 The $\sqrt{n}$ rule is an expression of the degree of inaccuracy to be expected in any physical law (SCHRÖDINGER, 1944). If *n* are the number of molecules in a given compartment, the relative error according to this rule will be 10 % if *n* = 100, but only 0.1 % if *n* = one million.

SCHRÖDINGER concluded that the molecular basis of the biological hereditary mechanism was not in contrast with statistical physics, and that quantum indeterminacy played no relevant biological role, except perhaps by *"enhancing their purely accidental character in such events as meiosis, natural and X-ray induced mutations"* (SCHRÖDINGER, 1944, p. 83).

Determined to provide an argument for the generation of 'order-from-disorder', TURING did not await the publication of WATSON and CRICK'S model either. Rather than following up SCHRÖDINGER'S suggestion, TURING sought an explanation of how a chemical soup of molecules in an embryo could possibly give rise to a biological pattern. In fact, TURING considered the effects of genes to belong to the class of effects that are not normally distributed, but that show a Poisson distribution instead (SAUNDERS, 1992, pp. 100-101): *"In some applications of the theory, it may be important to consider seriously the possibility that there may be only one or two molecules present, or even none..."* The statistical nature of diffusion and chemical reactions (the reactants being small, manageable molecules, not genes) was considered more satisfactory than the variations of reactions from cell to cell or irregularities of cell pattern (SAUNDERS, 1992, pp. 101-102).

In 1931 the foundations of mathematics were questioned by KURT GÖDEL'S argument showing that arithmetic must be incomplete and that assertions existed that could neither be proved nor disproved (see HODGES, 1983). The discovery of GÖDEL swept away two of the three demands of DAVID HILBERT'S proposed finite scheme of formal (mathematical) systems, namely the terms of consistency and completeness (HODGES, 1983, p. 92). GÖDEL'S work, however, "*left outstanding Hilbert's third question of decidability, the Entscheidungsproblem, namely the question of whether there exists a definite method which, at least in principle, can be applied to a given proposition to decide whether that proposition is provable"* (HODGES, 1997, p. 8). This question had survived GÖDEL'S analysis because *"its settlement required a precise and convincing definition of method"* (HODGES,1997). When TURING studied at Princeton, he was clearly disappointed at not being able to contact GÖDEL (who had left Princeton earlier)[2]. It was here that TURING'S contribution to pure mathematics came into play (TURING, 1936, 1937), but also that he began his conceptual contribution to the development of the computer (HODGES, 1983, 1997). Moreover, his endeavour branched off to extend the 'computable' to the realm of biological organisms.

With respect to the biological problems that became his new interests in the post-war period, TURING probably decided already in 1941 that the uncomputable, the unprovable and the undecidable were irrelevant to the problem of the mind (HODGES, 1997). Unfortunately, TURING remained unable to demonstrate the use of chemico-mechanical models beyond the level of early embryonic stages (in his 1952 paper) in order to describe the ongoing development up to a conscious (human) being, or to an application into the biology of cancer (S. TURING, 1959, p. 106). His published work on morphogenesis through a model described by coupled linear differential equations - for a system that was considered linear only when close to the origin - obviously was but a starting point for further investigation.

THE REALM OF CONSCIOUS LIVING BEINGS

In TURINGs correspondence with the neurophysiologist JOHN Z. YOUNG, he admits in a letter dated 8th February 1951 (KCC: K/1, Nr. 78), to be *"very far from the stage where I feel inclined to start asking anatomical questions"* (concerning the human brain). This would not occur, TURING said, until he had *"a fairly definite theory about how things were done"*. However, the organization of the brain seemed to be far more complicated than *"the polygonally symmetrical features of a starfish, flowers and leaf arrangements, or than the colour patterns on animals"*, although "*the formation of the brain structure should be one that could be achieved by the genetical embryological mechanism"* (TURING, KCC: K/1, Nr. 78). TURING is very confident that his work on reaction-diffusion theory will make clear *"what restrictions are really implicated"* (to the development of brain structure), and announces that he is interested in J.Z. YOUNG'S remarks on the stimulation under certain circumstances of neuron growth.

On another occasion, when comparing the activities of the human mind and the analytical properties of digital computers, TURING adopts a different stand on the phenomenon of human intelligence (TURING, 1950). Rather than examining the semantics of terms such as 'thinking' and 'machine', TURING argues that digital computatory algorithms and human intelligent activities can be completely matched or can be considered as perfect imitations of each other. That TURING considers the anatomical organization of the brain as of different order than the thinking performed by it, is indicated by the following premise of TURING'S approach: namely, putting forward the imitable properties of both systems *"has the advantage of drawing a fairly sharp line between the physical and intellectual capacities of a man"* (TURING, 1950, p. 434). Also *"there was little point in trying to make a 'thinking machine' more human by dressing it up in such artificial flesh (like a material which is indistinguishable from the human skin)"* (TURING, 1950, p. 434). According to Turing the realm of conscious human activities - being in no way performed by 'discrete state machines'-, cannot be apprehended without considering the 'educational aspect of learning'. For in contrast to the 'slow process of natural selection', the process of learning is much better to speed up the conditioning or teaching of intelligent human behaviour (TURING, 1950, p. 456). It is therefore not surprising that TURING showed hardly any interest in the anatomical characteristics of the brain (as he admits in the correspondence with J.Z. YOUNG), and nor did many of his followers in artificial intelligence and neural network theory.

Recently, attempts to describe the organization of the nervous system using an integration of neuro-anatomical and topological approaches were found in literature (Young et al., 1995; see also ALLAERTS, 1999). The integration of neuro-anatomical and topological approaches is well documented in the primate visual cortex, as shown by M.P. YOUNG and co-workers (YOUNG et al., 1995). Using

2 Later on, TURING provided a remarkable extension of GÖDEL'S theorem (see GANDY & YATES, 2001).

a topological approach, YOUNG succeeded in defining several topological characteristics of the visual system in the primate brain cortex, that correlated with known neuro-anatomical features. In other model systems of visual centers of the vertebrate brain, especially in the cat and the chicken, the role of learning and the biochemical processes underlying the adaptive capacities for learning of the visual system have been considerably well documented (see also ALLAERTS, 1999).

Contrary to the rather complicated visual system of the vertebrate brain – which indeed has served as a model system of the nervous system as a whole – , probably the best example in the nervous system for applying TURING'S reaction-diffusion theory is to be found in the regulation of axon growth. In agreement with TURING'S taste for manageably low numbers of key parameters in the model, the balance between neuron outgrowth stimulating and neuron outgrowth inhibiting factors in the regulation of axonal reconnection after spinal cord injury would be a suitable application field for TURING'S theory. In 1951, this was only a speculative idea, although TURING mentioned his interest on this point in his correspondence with J.Z. YOUNG (KCC: K/1, nr. 78).

CONCLUSIONS

The conclusion that TURING (1952) has worked out an extraordinary theory of morphogenesis is based on the following main arguments: (1) TURING worked out a complete mathematical model based on reaction-diffusion mechanisms in a very personal and self-contained way, no other template for such a model being available at his time; (2) the molecular, biological, and biochemical knowledge available to him was so scarce that his contribution to the conceptualization of the biological problem of morphogenesis and his pioneering role towards the development of theoretical biology can hardly be over-emphasized.

As pointed out by authors such as STEWART & GOLUBITSKY (1992) however, TURING'S model was in many ways imperfect and biologically inadequate. This was already recognised by TURING himself, who worked on a new theory with even greater mathematical complexity and incorporated several improvements on the original model. Unfortunately, this work was not finished at his death in 1954. We previously discussed a number of interpretations of TURING'S 1952 paper, such as the study of HARRISON (1987) (ALLAERTS & ROELANTS, 1993). Although HARRISON (1987) remarks that in TURING'S theory morphogens should be considered as diffusable cells (e.g. mesenchyme cells) rather than as chemical molecules, it can be doubted whether TURING would have agreed with this interpretation. Indeed, in his unpublished material he gave an even more restrictive meaning to the word morphogen, *"viz. chemical substance, the variation of whose concentration is described by a variable in the mathematical theory"* (KCC: AMT/C/26/5). One may regret the fact that, so far, TURING'S theory was at best exemplified in the formation of radiolarian skeletons, and not for instance in the gastrulation of the blastula, an event that better reflects the common sense of chemical processes influencing embryonic development. TURING must have been aware of this difference when stating that *"gastrulation was the most important application of his theory"* (TURING, 1952, p. 71), also because in this process the result of morphogenesis was a breakdown of the spherical symmetry to a lower degree (ALLAERTS & ROELANTS, 1993; ALLAERTS, 1999).

The importance of TURING'S mathematical theory of morphogenesis may very well extend beyond the latter questions related to embryonic development (such as the problem of symmetry breakdown), and provide genuine mathematical tools for remote biological questions. TURING'S 1952 paper was entitled a *'chemical **basis** for morphogenesis'*, whereas in the collected notes and manuscripts the title *'chemical **theory** of morphogenesis'* occurs (SAUNDERS, 1992). Although it can be doubted whether this title was chosen by TURING – the title on the manuscript shows a different handwriting, probably that of R.O. GANDY –, it is obvious that TURING in his last years mostly confined his morphogenetic studies to the domain of growth in plants, and especially the problem of phyllotaxis. However, in the same notes applications of morphogenetic theory in other domains, such as an application of this theory to the pathogenesis of cancer or to the spread of epidemics, are mentioned as well (SAUNDERS, 1992, p. 100). Anno 2002, it is needless to say that new mathematical approaches in these areas might be very valuable.

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APPENDIX

a) Use of Fourier's method for the cylindrical case of morphogenesis

An important mathematical technique used by TURING is the expansion of a function of angle θ into a Fourier series. In both the 1952 paper and in the collected unpublished works (SAUNDERS, 1992), TURING often uses a combination of a Fourier series (in one variable) and a Fourier integral (in the other variable) (SAUNDERS, 1992, p. 74).

TURING is rarely explicit regarding the biographical sources of his mathematical methods. In the case of Fourier's method, this is probably common mathematical knowledge. For most biological readers, however, this may not be easy to grasp, so some explanation of the techniques used by TURING (1952) is given below.

The problem of conduction or diffusion in a ring, in which the dependent variable depends only on one co-ordinate and the time t, is a classical example of FOURIER'S 'ring problem'. According to CARSLAW & JAEGER (1959), it was the first problem to which FOURIER applied his mathematical theory on series of periodical functions, and for which the results of his mathematical investigations were compared with the facts of experiment (FOURIER, 1822).

The expansion of a function X of angle θ into a Fourier series is of the form:

$$X(\theta) = \sum_{s=-\infty}^{\infty} G_s e^{is\theta} \qquad (1)$$

$X(\theta)$ being values of X at $t = 0$

provided that the first derivative of X is continuous. To denote its angular nature, θ is also commonly expressed in the form $\frac{2\pi}{\varpi} z$.

In the case of true periodical functions it is more convenient to expand to the goniometrical functions *sin* and *cos* because of the real periods, than to expand to exponential functions.

Using the Taylor expansions of the complex exponential function and the goniometrical functions, the following important relation directly follows:

$$e^{is\theta} = \cos s\theta + i \sin s\theta \qquad (2)$$

Consequently, taking the real parts of the Fourier expansion, a Fourier series is obtained of the form:

$$f(x) = \sum_{s=-\infty}^{\infty} (b_s \cos s\theta + c_s \sin s\theta) \qquad (3)$$

Substituting the co-ordinates x_r, y_r (denoting a small but linear deviation of the equilibrium concentrations for $X = h$ and $Y = k$ in the r-th cell) by the new co-ordinates $\xi_0, \ldots \xi_r, \ldots \xi_{N-1}$ and $\eta_0, \ldots \eta_r, \ldots \eta_{N-1}$, according to

$$x_r = \sum_{s=0}^{N-1} \exp\left[\frac{2\pi irs}{N}\right].\xi_s \qquad (4)$$

$$y_r = \sum_{s=0}^{N-1} \exp\left[\frac{2\pi irs}{N}\right].\eta_s$$

and using the relation derived from equation (2) between the complex exponential functions:

$$e^{2is\theta} + e^{-2is\theta} = 2 - 4\sin^2 s\theta \qquad (5)$$

TURING (1952) obtains the following set of linear differential equations in ξ_s, η_s with constant co-efficients :

$$\frac{d\xi_s}{dt} = (a - 4\mu \sin^2 \frac{\pi s}{N})\xi_s + b\eta_s \qquad (6)$$

$$\frac{d\eta_s}{dt} = c\xi_s + (d - 4\nu \sin^2 \frac{\pi s}{N})\eta_s$$

In simplified notation this set is of the form:

$$\frac{dx_1}{dt} = a_{11}x_1 + a_{12}x_2 \qquad (6^*)$$

$$\frac{dx_2}{dt} = a_{21}x_1 + a_{22}x_2$$

It is important to note that the co-efficients $a_{11},..a_{22}$ are constants, depending on the diffusibilities μ, ν and chemical reaction rates (see section 1.a. of main text), so diffusion constants are considered constant throughout the morphogenetic system (ALLAERTS & ROELANTS, 1993). The solutions of set (6*) are of the form $x_1 = \alpha_1 . e^{kt}$, $x_2 = \alpha_2 . e^{kt}$, where α_1, α_2 and k are chosen so that the exponential functions fulfill the set of linear equations:

$$k\alpha_1.e^{kt} = (a_{11}\alpha_1 + a_{12}\alpha_2).e^{kt} \quad \text{(PISKOUNOV, 1980, p. 121)}$$

$$k\alpha_2.e^{kt} = (a_{21}\alpha_1 + a_{22}\alpha_2).e^{kt}$$

The latter set has to be resolved with respect to α_1, α_2, yielding:

$$(a_{11} - k)\alpha_1 + a_{12}\alpha_2 = 0$$

$$a_{21}\alpha_1 + (a_{22} - k)\alpha_2 = 0$$

If k is chosen so that the determinant of the set of equations is different from zero, the only solution is the trivial solution where $\alpha_1 = \alpha_2 = 0$. However, if the determinant $\Delta(k)$ is zero, non trivial solutions are obtained from solving the so-called 'characteristic equation' of the system (see PISKOUNOV, 1980, p. 122):

$$\Delta(k) = \begin{vmatrix} a_{11} - k & a_{12} \\ a_{21} & a_{22} - k \end{vmatrix} = 0$$

or $(a_{11} - k)(a_{22} - k) - a_{12}a_{21} = 0$ (7).

The characteristic equation given by TURING (1952, p. 48) for the set of linear differential equations (6) is as follows:

$$(p - a + 4\mu \sin^2 \frac{\pi s}{N})(p - d + 4\nu \sin^2 \frac{\pi s}{N}) - bc = 0 \qquad (8)$$

of which p_s and p'_s are called the roots of the characteristic equation in p. In the case that p_s and p'_s are two distinct roots (either real or complex), the solution of set (6) is of the form:

$$\xi_s = A_s.e^{p_s t} + B_s.e^{p'_s t} \qquad (9)$$

$$\eta_s = C_s.e^{p_s t} + D_s.e^{p'_s t}$$

where TURING (1952, p. 48) states that A_s,, B_s,, C_s and D_s are arbitrary but not independent co-efficients, which are restricted to satisfy :

$$A_s(p_s - a + 4\mu \sin^2 \frac{\pi s}{N}) = bC_s \qquad (10)$$

$$B_s(p'_s - a + 4\mu \sin^2 \frac{\pi s}{N}) = bD_s$$

This follows the matrix notation for multiplication of the matrix of set (6) with the column-matrix of the solutions of the characteristic equation (8). The interdependence of A_s,, B_s,, C_s and D_s therefore directly follows the mathematical procedure for solving the set of linear differential equations (see also PISKOUNOV, 1980, Vol. 2, pp. 593-598). This mathematical result is interpreted by TURING (1952) in such a way that also important biological conclusions regarding the diffusion of morphogens are inferred from it (see main text, section 1).

By substituting (9) back into (4) and replacing the variables x_r, y_r (departures from equilibrium) by X_r, Y_r (the actual concentrations), the expression similar to (2) in section 1 (main text) is obtained for reaction and diffusion in a ring of cells. For the continuous ring of tissue, the limiting case is considered where the Fourier series is summed from $-\infty$ to $+\infty$.

b) Use of Legendre's Associated Functions and spherical harmonics

In the appendix to Part II, entitled *'Chemical Theory of Morphogenesis'* (based on TURING'S drafts used by N. HOSKIN and B. RICHARDS for the *'Morphogen Theory of Phyllotaxis'*, SAUNDERS, *ibidem*, p. 117), the use of normalised Legendre associated functions is introduced (see also appendix c). In the latter manuscript, which was actually worked out (on a suggestion by TURING) by RICHARDS, one of TURING'S students, reference is given to the work of E.W. HOBSON (1931). This is a textbook on spherical and ellipsoidal harmonics, written for trained mathematicians. The Legendre's associated functions also occur in TURING'S (1952) paragraph 12, devoted to *'Chemical waves on spheres. Gastrulation'*, where they appear as solutions to the harmonic functions on the sphere. We here present some important features of the theory of spherical harmonics and LA-functions, in order to elucidate the biological inferences, that, according to TURING (1952) can be obtained from these mathematical techniques. For the mathematical deductions, a detailed survey is given in HOBSON (1931).

Starting from Laplace's equation in polar co-ordinates r, θ, ϕ:

$$\frac{\partial}{\partial r}(r^2 \frac{\partial V}{\partial r}) + \frac{1}{\sin\theta}\frac{\partial}{\partial \theta}(\sin\theta \frac{\partial V}{\partial \theta}) + \frac{1}{\sin^2\theta}\frac{\partial^2 V}{\partial \phi^2} = 0 \quad (11),$$

and substituting $V = R\,\Theta\,\Phi$, where R, Θ, Φ are functions of r, θ, ϕ, respectively, it can be shown that some terms of Laplace's equation can be separated in only one variable. This implies that the equation can only be satisfied if these are constant terms, resulting in the general form of the solution for $\Phi = C \cos m\phi + D \sin m\theta$. Again, substituting $\cos\theta = \mu$ and $\Theta = u$, equation (11) is transformed into:

$$\frac{d}{d\mu}\left\{(1-\mu^2)\frac{du}{d\mu}\right\} + \left\{n(n+1) - \frac{m^2}{1-\mu^2}\right\}u = 0 \quad (12),$$

where n is derived from the solution of the first term of Laplace's equation :

$R = Ar^n + Br^{-n-1}$.

In the particular case where $m = 0$, equation (12) becomes:

$$\frac{d}{d\mu}\left\{(1-\mu^2)\frac{du}{d\mu}\right\} + n(n+1)u = 0 \quad (13),$$

which is known as Legendre's equation (HOBSON, 1931, pp. 9-10). The complete solution of Legendre's equation (13), where n denotes a positive integer, is of the form:

$$u = AP_n(\mu) + BQ_n(\mu) \quad (14),$$

where $P_n(\mu)$ is called Legendre's polynomial or function of the n-th degree, $Q_n(\mu)$ is the Legendre's function of the second kind and n-th degree (which has both real and complex values), and A and B denote arbitrary constants (HOBSON, *ibidem*, p. 13). As explained below, the most important term is $P_n(\mu)$, which is an algebraic function of $\mu = \cos\theta$ of degree n, and is given by:

$$P_n(\mu) = \frac{1.3.5...(2n-1)}{1.2.3...n}\left\{\mu^n - \frac{n(n-1)}{2(2n-1)}\mu^{n-2} + \frac{n(n-1)(n-2)(n-3)}{2.4.(2n-1)(2n-3)}\mu^{n-4} - ...\right\} \quad (15)$$

The first values of the polynomial $P_n(\mu)$ are as follows:
$P_0(\mu) = 1$, $P_1(\mu) = \mu$, $P_2(\mu) = \frac{1}{2}(3\mu^2 - 1)$,
$P_3(\mu) = \frac{1}{2}(5\mu^3 - 3\mu)$, and so on, and :

$P_n(0) = 0$ for all n.

RODRIGUEZ (*see* HOBSON, 1931, p. 18) gave a more convenient expression for the calculation of Legendre's function in terms of $(\mu^2 - 1)$, namely:

$$P_n(\mu) = \frac{1}{2^n.n!}(\frac{\partial}{\partial\mu})^n(\mu^2-1)^n \quad (16)$$

Moreover, making use of Laplace's definite integral expression for $P_n(\mu)$ (HOBSON, p. 25):

$$P_n(\mu) = \frac{1}{\pi}\int_0^{\pi}(\mu \pm \sqrt{\mu^2-1}\cos\phi)^n\,d\phi \quad (17)$$

the following recursion formula is obtained between three consecutive Legendre's functions:

$$nP_n - (2n-1)\mu P_{n-1} + (n-1)P_{n-2} = 0 \quad (18).$$

For a proper understanding of TURING'S use of the above equations, the question of the geometrical relevance of these algebraic relations is not without importance. It can be shown that the expression $P_n(\cos\theta) = 0$ defines a system of nodal lines (see also Fig. 2A) on the sphere, perpendicular to the axis and symmetrical to the diametral plane $\theta = \pi/2$ (HOBSON, 1931, p. 19). Therefore, $P_n(\mu)$ is called a 'zonal' harmonic, dividing the spherical surface into zones[3].

The above properties of Legendre's polynomial $P_n(\mu)$ require some introduction into the theory of spherical harmonics. This theory dates back to the work of W. THOMSON (LORD KELVIN) in England and A. CLEBSCH in Germany (HOBSON, 1931, p. 119). According to JEANS (1927, p. 208), any solution of Laplace's equation (11) is called a spherical harmonic. The most important class of harmonics consists of rational integral functions of three independent variables, e.g. the polar co-ordinates r, θ, ϕ. For the physical interpretation of these harmonics, it is important to note that the value of any finite single-valued function of position on a spherical surface can be expressed as a series of rational integral harmonics, each of the form $r^n P_n$, provided the function has only a finite number of discontinuities and of maxima and minima on the surface (JEANS, 1927, p. 211). This rule is probably the one referred to by TURING (1952, p. 70), when stating that *"any function on the sphere, or at least any that is likely to arise in a physical problem, can be expanded in spherical surface harmonics"*. JEANS (1927) wrote his textbook for students in physics and engineering, interested in the application of advanced mathematical techniques to electricity and magnetism.

So far, only the Legendre's functions of the first kind $P_n(\mu)$ are considered. This is due to the fact that, if n = integer, one of the two Legendre polynomials in equation (14) is finite, the other is infinite. When dealing with complete spheres, it is impossible for the Legendre's function of the second kind $Q_n(\mu)$ to become finite. However, in cases where the infinities of the Q_n harmonic can be excluded, for instance by excluding certain parts of the sphere, it may be necessary to take both P_n and Q_n into account (JEANS, 1927, p. 237).

Another simplification so far was that only the solutions of Laplace's equation (12) have been considered where $m = 0$, giving rise to Legendre's equation (13). When considering the general solution of the form $\Phi = C \cos m\phi + D \sin m\phi$, with m = integer, then so-called 'tesseral' harmonics constitute the solution to Laplace's equation. These tesseral harmonics are expressed in terms of so-called Legendre's associated functions (shortly: LA-functions), with symbols $P_n^m(\mu)$, $P_n^m(\mu)$. The general solution of equation (14) now becomes:

$$u = AP_n^m(\mu) + BQ_n^m(\mu)$$

$$\text{with } P_n^m(\mu) = \frac{1}{2^n.n!}(1-\mu^2)^{m/2}.\frac{\partial^{m+n}}{\partial\mu^{m+n}}(\mu^2-1)^n \quad (19),$$

in which RODRIGUEZ' expression (16) for Legendre's functions in terms of $(\mu^2 - 1)$ is recognized. An alternative, shorter notation is given by:

$$P_n^m(\mu) = \sin^m\theta\,\frac{\partial^m P_n(\mu)}{\partial\mu^m} \quad (20) \text{ (JEANS, 1927, p. 239).}$$

Equation (19) vanishes if $m + n > 2n$, i.e. if $m > n$, so also here important simplifications can be obtained. Moreover, since $Q_n^m(\mu)$ cannot be a rational integral function of $\sin\theta$ and $\cos\theta$ it is concluded that from the solution of Laplace's equation only the part with $P_n^m(\mu)$ gives rise to rational integral harmonics (JEANS, 1927, p. 239). Therefore, the solution of Laplace's equation is of the form:

$$P_n^m(\mu)(C_m \cos m\phi + D_m \sin m\phi) \quad (21)$$

which is the equivalent - TURING uses exponential rather than goniometrical terms (see appendix a) - of the expression found in

3 The idea of determining harmonics by the position of the poles was suggested by C.F. GAUSS, but was first developed by J.C. MAXWELL, although the definiteness of this method was contested by others (see HOBSON, 1931, p. 132).

TURING'S solution for morphogenesis in the spherical case (TURING, 1950, p. 70). According to JEANS (1927, p. 239), there are *(2n+1)* tesseral harmonics of degree *n*, namely:

$$P_n(\mu)\ ,\ cos\ \phi\ \ P_n^1(\mu)\ ,\ sin\ \phi\ \ P_n^1(\mu)\ ,\ ...,$$

$$\cos n\phi\ \ P_n^n(\mu),\ sin\ n\phi\ \ P_n^n(\mu).$$

For manageably low values of *n*, these are also the examples of LA-functions used by TURING (1952, p. 71)**. For instance, for $n = 1$ only three tesseral harmonics are possible:
$cos\ \theta = \mu$ (since $P_1^0(\mu)$ is always a solution), $sin\ \theta\ cos\ \phi$ and $sin\ \theta\ sin\ \phi$ (JEANS, 1927, p. 239).

For higher values of *n*, LA-functions can be calculated making use of expression (20) and the recursion formula for Legendre polynomials (18).

Concluding of this second part of the appendix, it is clear that TURING (1952) gave very little introduction to the mathematical methods used for solving the spherical case of reaction-diffusion theory (these notions were only briefly mentioned on the upper half of p. 70). Further on, mathematical techniques are used mostly in analogy with the cylindrical case (see appendix a). This also holds for the interdependence of the constants A_n^m, B_n^m, C_n^m and D_n^m, which follows the matrix formulation for solving the set of differential equations, analogous to the cylindrical case, where LA-functions constitute the co-efficients of the set of differential equations.

Finally, it is not without importance that JEANS (1927, p. 229-230) devoted a paragraph to 'nearly' spherical surfaces. JEANS shows that nearly spherical surfaces can be treated in the same way as spherical surfaces, for the squares of the harmonics describing the small deviations can be neglected. Interestingly, also TURING (1952, p. 71) regards the forms of various 'nearly' spherical structures as closely related to the latter spherical harmonic patterns (see main text).

c) Use of normalized Legendre associated functions

The normalized Legendre associated functions (shortly: normalized LA-functions) are introduced in B. RICHARDS extension of TURING'S posthumously published manuscripts (SAUNDERS, 1992, Part III, pp. 107-118), with a number of references to HOBSON (1931). The rationale is to provide a more exact solution for the morphogenetic equations in the spherical case. As shown at the end of appendix b (**), TURING (1952) also used LA-functions of degree $m = -1$, which refer to the notion of normalized LA-functions. RICHARDS makes extensive use of them in order to describe the reaction-diffusion process in small organisms (see section 2 of main text).

From the differential equation describing the reaction-diffusion process in small organisms, the solution obtained for the concentration function $U(\theta, \phi, t)$ is of the form:

$$U(\theta,\phi,t) = \sum_{m=-n}^{m=n} S_m(t).\overline{P_n^m}(\cos\theta).e^{im\phi} \qquad (22),$$

where U being real and $\overline{P_n^m}(\cos\theta)$ being the normalized LA functions, which are defined by :

$$\overline{P_n^m}(\cos\theta) = A_n^m P_n^m(\cos\theta) \qquad (23),$$

where $P_n^m(\cos\theta)$ represents the usual LA-function, with the condition that $P_n^m(\cos\theta) = P_n^{-m}(\cos\theta)$ and

$$A_n^m = \sqrt{\frac{(2n+1)(n-m)!}{(n+m)!}} \qquad \text{(SAUNDERS, 1992, p. 117).}$$

This property, referring to Hobson (1931, p. 162), is inferred from the theory of conjugate systems of harmonics. A conjugate system of harmonics of degree *n* is defined as a system of *(2n+1)* harmonics, such that for any pair of them the product of these harmonics equals zero, or:

$$\frac{1}{4\pi}\iint \overline{P_n^r}(\cos\theta).\overline{P_n^s}(\cos\theta)dS = \begin{cases} 1 & \text{for } r = s \\ 0 & r \neq s \end{cases} \qquad (24)$$

(SAUNDERS, 1992, p. 108).

The biological relevance of these conjugate systems is obvious, for it enables an important simplification in the number of harmonics describing concentration or potential functions on the sphere. When applied to the differential equation for reaction-diffusion in small organisms (see section 2), it follows that the function $\Phi(\nabla^2)$, which depends on the concentration function U, now can be replaced by a constant I, or:

$$\Phi(\nabla^2)U = IU$$

Accordingly, the general differential equation describing the changes of morphogen concentrations in time is given by:

$$\frac{dU}{dt} = IU + GU^2 - HUV \qquad (25)$$

(SAUNDERS, 1992, p. 108).

According to RICHARDS (see SAUNDERS, 1992, p. 108-111) equation (25) can now be used to derive the unknown solutions $S_m(t)$ in expression (22), following the recursion formula:

$$S_m = \sum_{i=-n}^{i=n}\sum_{j=-n}^{j=n} S_i S_j L_n^{i,j,-m} \qquad (26),$$

where the auxilliary functions $L_n^{p,q,r}$ and $E_n^{p,q,r}$ are defined as follows:

$$L_n^{p,q,r} = \frac{1}{4\pi}\int_0^{2\pi}\int_{-1}^{1} \overline{P_n^p}(\cos\theta)\overline{P_n^q}(\cos\theta)\overline{P_n^r}(\cos\theta).e^{i(p+q+r)\phi}d\cos\theta.d\phi$$

and

$$E_n^{p,q,r} = \frac{1}{2}\int_{-1}^{1} \overline{P_n^p}(\cos\theta)\overline{P_n^q}(\cos\theta)\overline{P_n^r}(\cos\theta).d\cos\theta$$

An important simplification results from application of the following conditions:

$$L_n^{p,q,r} = \begin{cases} E_n^{p,q,r} = E_n^{/p/,/q/,/r/} \\ 0 \end{cases} \quad \text{for} \quad \begin{cases} p+q+r = 0 \\ p+q+r \neq 0 \end{cases}$$

(SAUNDERS, 1992, p. 117).

Numerical examples of these calculations using the recursion formula (26) have been provided by RICHARDS (see SAUNDERS, 1992, pp. 110-114). According to RICHARDS, it is important to remember that the solutions represent deviations from the sphere; a correct balance between the oscillations of the concentration function U and the radius of the initial sphere can be obtained when looking at a suitable biological species (SAUNDERS, 1992, p.111). Examples of such suitable biological species are found in the marine organisms of the class Radiolaria (see Fig. 2.c). These unicellular organisms are surrounded by a skeleton, generally composed of silica, which forms sharp spines that radiate from the outer shell of the skeleton. RICHARDS' calculations for solutions of degree $n = 4$ reveal spheroid bodies with spines at each pole and four around the equator, among others. More complex geometrical forms are obtained by using solutions of higher degree.

** TURING (1952, p. 71) also uses LA-functions with negative integer degree (-1). This results from the use of normalized LA-functions, which notions are explained in appendix c.

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