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Early Biological Morphogenesis and Nonlinear Dynamics

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Biological pattern formation is a process that so far has gone largely unexplained. Mechanisms for cellular differentiation, and pattern forming processes, must both have emerged within the same short time interval. It now seems unlikely that the great diversity of observed patterns can be accounted for by the action of one or a few specific patterning genes, or, in its extreme form, the idea that there might be a single such proto pattern gene. Many seemingly related pattern forming processes, such as segment formation, depend on unrelated genetic mechanisms. Thus another common feature as a proto pattern gene is needed to understand the onset of pattern formation.

Experimental biologists favour mechanisms in which morphogenetic gradients are the main source of positional information. Specific combinations of genes (cues) are activated differentially by thresholds along particular regions on the gradient. Among theoreticians the pattern forming processes are believed to some extent to be dependent upon true symmetry breaking processes, which can occur in most nonlinear control systems, of which the Turing mechanism is an example.

It is argued here that the common fundamental feature of gene control systems is a high degree of cooperativity. Such highly nonlinear systems may originally have been capable of reading subtle differences in the concentration of a controller, but the evolutionary pressure to refine this kind of processes in single cells would eventually have led to gene clusters with steep off-on control, and this is seen experimentally in many such systems. Some nonlinear systems of this type have for some time been known among theoreticians to be prone to multistability (a necessary precondition for cell differentiation), chemical time oscillations and spontaneous spatial pattern formation by Turing's mechanism. Increasing cooperativity in the defining rate laws greatly facilitates the tendency for such phenomena to arise, and we demonstrate this explicitly for Turing pattern formation. We argue that all these phenomena arose simultaneously with the capability of interpreting positional information along simple gradients.

Introduction

Genes containing the homeobox sequence, which are key regulatory genes, are widespread and highly conserved in higher multicellular animals. The evolutionary establishment of some of these classes seems to be at least as ancient as the flatworm. It has been speculated that the homeobox class of genes is somehow connected to the origin of pattern formation in multicellular systems, such as establishment of bilateral symmetry, and later segmentation. A recent overview of the experimental results, which may define the genes essential for defining a common ancestor of metazoa, was given in Shenk & Steele (1993).

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However, the basis of pattern formation emerged well before the Cambrian explosion some 600 million years ago. Many "defining" characteristics for the metazoa are widely distributed among unicellular eukaryotes. Positional information seems to be present already in the ciliates, and fungi and plants arose much earlier than the metazoans, with their own systems of pattern forming processes. Thus the building blocks for some form of pattern formation were present long before their use in multicellular pattern forming processes, and these building blocks must then have originated with different essential roles.

It will be stressed here that the actual pattern forming processes observed in early evolution have a more common feature than a single "pattern

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protogene'' and thus the homeobox genes are not ''pattern'' genes as such, but rather convenient control genes linked to another set of fundamental pattern forming processes. In discussions of homeotic genes and their function, it has been stressed that the function of these genes is especially striking because it is not dedicated to the production of any particular type of pattern (Kenyon, 1994). Thus the gene cluster (the zootype), which has been suggested to define the kingdom Animalia, is a system of positional information and does not necessarily encode any particular pattern (Slack *et al*., 1993). Also homeobox genes emerged much earlier in evolution than the zootype and the common feature in terms of pattern forming capability is not just that these genes encode proteins which control other genes. It will be suggested that the key feature of these genes, from the standpoint of pattern formation, is their ability to create highly cooperative, on–off dynamics. Such dynamics is well known to be capable of generating a rich variety of well controllable patterns.

Homeobox genes are involved in segmentation of *Drosophila* and the control mechanism has been intensively studied experimentally. As this system is one of the most well-known systems genetically, the control mechanism leading to segmentation in *Drosophila* has recently been the subject of intense research, in the hope of gaining an insight into the fundamental pattern forming mechanisms governing early embryonic development (see Wright *et al*., 1989; Riddihough, 1992*a*,*b*; Wilkinson, 1993).

A gene hierarchy seems to control the initial transition from the egg to a segmented embryo in *Drosophila*. In the initial phase of this hierarchy, the maternal gene *bicoid* creates a gradient of its protein along the anterior-posterior axis of the embryo, and this gradient provides positional information for the so-called gap gene *hunchback*, which in turn provides another gradient for other gap genes.

It is experimentally well established that a decrease of these gradients by a factor of two is enough to control the activation of other genes in an on–off manner. This must mean that the control is highly nonlinear with effective Hill number of the order of 8 or higher. This feature is also found in many other gene control systems.

The primary pair-rule genes appear next in the *Drosophila* hierarchy. These genes are each expressed in a series of seven stripes. The mechanism for the formation of these ''zebra'' stripes is not known. Control by a combination of maternal and gap genes above in the hierarchy seems to be involved in the expression of particular stripes (Small *et al*., 1992).

How a number of independent particular stripe generators (cues) could co-operate to form the observed four sets (genes *eve* and *ftz*, and *hairy* and *runt*) of *equally spaced* and *precisely phase shifted* stripes is a much more difficult problem. Theoreticians have pointed out that the ''zebra'' stripes may alternatively be degenerated by a true symmetry-breaking mechanism such as Turing's mechanism; that is, by an autocatalytic reaction-diffusion system which is known to be capable of producing such stripes autonomously. The equal spacing and the phase relation is easily obtained with such a mechanism. The particular pair-rule stripes could then be activated by a combination of maternal, gap *and* Turing pattern interactions. This combined cue–Turing mechanism thus suggests that the pair-rule genes respond to some degree to ''all-stripe'' control, as is found for *ftz* (Hafen *et al*., 1984; Hiromi *et al*., 1985). The main alternative explanation, the pure cue model (individual stripe control), was devised to account for experimental results of the promoter region for *hairy*, which has independent regions for particular stripes, or stripe combinations.

Less is known about the structure of the promoter of the remaining pair-rule genes, however. For *eve*, stripe 2 and 3 seem to be controlled analogously to *hairy*. Almost nothing is known about the promoter region of *runt*. The possibility of an ''all-stripe'' control component has recently been revived by studies of *runt* (Klingler & Gergen, 1993; Kagoshima *et al*., 1993), and a revision of the *hairy* control, as *hairy* stripes 3 and 4 are activated in a concerted mode in a broad region, which is then resolved by action of *runt* (Hartmann *et al*., 1994). The role of gene *runt* is currently under substantial revision, as it seems to be more complex than suggested by the above hierarchical model. Thus *runt* has recently been shown to be involved in the regulation of the gap genes (Tsai & Gergen, 1994).

We argue here that the feature that makes possible reliable positional information read-out along a gradient also makes the control system prone to yield pattern formation by a number of other symmetry-breaking mechanisms. Thus gradient control, multi-stability, oscillations, waves and Turing-like pattern forming mechanisms are likely to have evolved in a concerted manner, rather than in succession.

Transcription factors in general are reviewed in Pabo & Sauer (1992). Recent reviews on *Drosophila* have appeared in Ingham (1988), Pankratz & Jäckle (1990) and Nüsslein-Volhard (1991). Recent models of the gap level appear in Struhl *et al*. (1992), and in Jäckle *et al*. (1992). The idea of a common gene complex defining the kingdom Animalia appears in Slack *et al*. (1993). Pattern formation in reaction-diffusion systems were originally discussed by Turing (1952) and

references to Turing-type models may be found in Hunding *et al*. (1990) and Hunding (1993). For information on properties of nonlinear control systems in general, as related to biology, see Nicolis & Prigogine (1977), Murray (1989) and Maini *et al*. (1993).

Early Insect Morphogenesis

Drosophila has a rapid establishment of body plan, in comparison with many other, especially evolutionary primitive, insects. In recent experiments on morphogenesis in less advanced insects it appears that the cues are absent, at least in the form they have in *Drosophila*. Thus these cues, and perhaps cues in general, are not as important for pattern generation as may be inferred from the processes studied in *Drosophila*. This again opens the question as to what is the fundamental pattern (stripe) forming mechanism (see French, 1993).

It has become increasingly clear that the cues in *Drosophila* are not the actual fundamental system for general segment formation. One may speculate that the cues are evolutionary late additions to a fundamental segmentation mechanism, which has been obscured by the addition of gap and pair-rule genes. Neither of these seem to take part in generating one segment after another in less evolutionary advanced insects such as the grasshopper (Patel *et al*., 1992). An intermediate control system with some gap and pair-rule genes seems to be present in beetles (Sommer & Tautz, 1993; French, 1993). The homeotic gene complex in the beetle *Tribolium castaneum* is reviewed in Beeman *et al*. (1993). In *Triboleum*, a pair of *hairy* stripes form and vanish, but a subsequent pair appear in a growth zone posteriorly. So far (D. Tautz, personal communication), it is not clear whether this posterior pair comes on and off in a cyclical way, or how the *hairy* gene expression is related to the subsequent expression of gene *engrailed* in the cells emerging from the growth zone. *en* appears in the regions where *hairy* has faded away, to yield a growing number of *en* stripes.

However, this process does not take place in a syncytium, and thus stripe control by combinations of diffusing large molecules as proteins (cues) seems unlikely. This recent discovery has reopened the debate regarding the evolutionary significance of the cue control system found in *Drosophila*. However, studies of the pair-rule gene *eve* in *Tribolium* indicate that it controls *en* expression much as is seen in *Drosophila* (Patel *et al*., 1994), and Susan J. Brown reports unpublished data to the effect that *runt* and *ftz* are present as well in *Tribolium* (Brown *et al*., 1994). Insect segmentation as a model for evolutionary change is discussed in Patel (1994).

Other interpretations connected to autocatalytic reactions, and reaction-diffusion systems, are possible as well. The much faster overall development rate required in the life cycle of insects such as *Drosophila* may have favoured the observed simultaneous triggering of all seven zebra stripes. The addition of *bicoid* and the gap genes may then be seen as stabilizers of this process, and the subsequent cues as an intricate system to make sure that each generated stripe lines up properly with the local gap genes. Another role for the gap genes during evolution may have been to map out some coarse regions within which simultaneous triggering of stripes could occur, such as an *hb* region first, then perhaps addition of a *Kr* region, something which may actually play a role in the observed beetle morphogenesis. Sequential formation of *en* expression, as observed in the grasshopper, is occasionally seen to involve more than one stripe, which evolve simultaneously (Patel *et al*., 1989).

The results found in the morphogenesis of the beetle may be tentatively taken in support of this view. One may envisage a comprehensive model for early, medium and late evolutionary segment formation based upon a robust global Turing stripe generator in which one stripe after another is activated, while an inhibitor gradually vanishes posteriorly (grasshopper), then an intermediate form in which the global Turing pattern is activated in a zone comprising a few stripes and this progress zone moves posteriorly, while an inhibitor gradually vanishes (beetle), and finally the rapid all at once stripe activation in *Drosophila*, in which the global Turing stripe generator is activated over a large region in several somewhat overlapping subregions provided by the gap genes (Fig. 1). In this model the essential stripe generator is the global Turing pattern, which ensures a prepattern of equally spaced stripes that is then exploited in a progressively more and more complex manner in different species as these move up the evolutionary ladder. Indeed successive segment formation may not be a reliable indicator of a fundamentally different mechanism from that in *Drosophila*. In the tobacco hawkmoth *Manduca sexta* the blastoderm expression pattern of genes *hb*, *Kr* and *runt* are much the same as in *Drosophila*, but successive segment formation is present (Kraft & Jäckle, 1994).

Finally the posterior growth zone in the beetle may generate stripes, not by a reaction-diffusion mechanism, but by a closely related set of reactions, which yield chemical oscillations. If a certain activator concentration rises and vanishes repeatedly over time, the cells being in the zone when the activator is on may

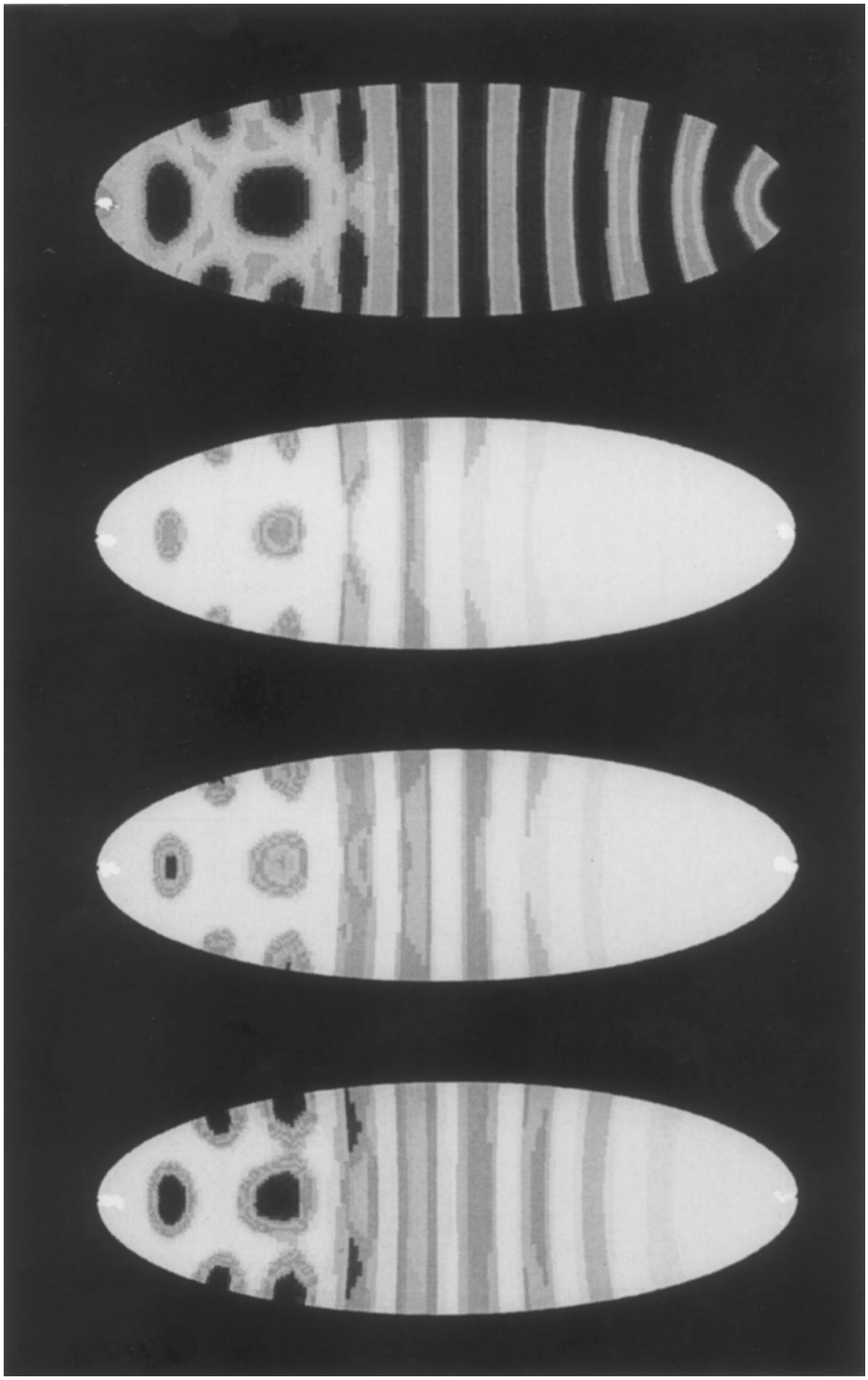

FIG. 1. Computer simulation of stripe prepattern in a three-dimensional region resembling an early insect embryo. Spontaneous pattern formation occurs in reaction-diffusion systems. In the model shown here, a gradient is imposed posteriorly (right) and the resulting space dependent rate constant in the RD system creates a Turing pattern (a) which yields stripes posteriorly, but anteriorly the stripes break up to yield a highly symmetrical pattern which may play a role in head formation. Once established, this RD prepattern governs the read out of genes related to segment formation in the embryo. If an inhibitor is present with high concentration posteriorly, such genes are prohibited from being activated posteriorly and no segments form. When the inhibitor gradually vanish, stripes are activated one after another (b–d). If the inhibitor gradient triggers another activator gene in a zone along the inhibitor gradient, this progress zone would activate a few stripes simultaneously along the Turing pattern, and eventually all stripes become activated from left to right. Finally, several such distinct activator regions may form, as realized with the gap genes in *Drosophila*, to yield activation of all the stripes along the Turing pattern simultaneously.

subsequently express another gene, say *en*, and when the cells leave the growth zone, the result is a growing number of *en* stripes as the embryo grows.

The point here is that *all* such mechanisms are potential segment generators, and it may thus not be a particular gene, which is the common ancestral pattern generator. Rather, the class of highly nonlinear autocatalytic chemical reactions may be a more likely candidate underlying repetitive processes in early evolution. Such systems may easily yield chemical time-oscillations, which may be used to generate segments sequentially as described above, but the very same chemical processes are prone to yield Turing-type patterns, as we will discuss below.

Such autocatalytic systems may also be present in the systems involved in cell-to-cell communication. Even Turing's mechanism may be generalized to this situation, with cell-to-cell communication rates replacing simple diffusion. The essence of a Turing pattern generator is the balance between growth from an autocatalytic process and dissipation by another mechanism, of which diffusion is only one member of the class.

There may thus be several chemical control systems around that have generated repetitive patterns in early evolution, and it may be that various segmentation processes are genetically related only superficially (Riddihough, 1992*a*,*b*).

Nonlinear Dynamics is Triggered by Gene Clusters

In this section, we demonstrate for particular models that control systems, which gradually approach off–on control, greatly facilitate pattern formation by Turing's mechanism. Usually, effective Hill numbers in such studies have been taken to be less than ~ 3 as this conforms to the actual values measured for enzyme regulation in the cytoplasm. In the *genetic* control systems discussed above, however, it is common to have substantially larger Hill numbers, often apparently in excess of 8. In the following we will explore this feature.

A Turing system of the first kind is defined as

$$\partial c/\partial t = F(c) + D\Delta c. \tag{1}$$

Simple linear stability theory has been treated in many sources (see for example Murray, 1989). For a reaction-diffusion system of the form

$$\frac{\partial x}{\partial t} = f(x, y) + D_1 \Delta x \tag{2}$$

$$\frac{\partial y}{\partial t} = g(x, y) + D_2 \Delta y, \tag{3}$$

one defines the Jacobian matrix

$$J = \begin{pmatrix} \partial f/\partial x & \partial f/\partial y \\ \partial g/\partial x & \partial g/\partial y \end{pmatrix} = \begin{pmatrix} f_x & f_y \\ g_x & g_y \end{pmatrix} = \begin{pmatrix} a & b \\ c & d \end{pmatrix}, \tag{4}$$

where the derivatives are evaluated at the stationary concentrations where $f = g = 0$.

There are only two classes (I) and (II) of such Turing systems defined by the following two Jacobians

$$J = \begin{pmatrix} + & - \\ + & - \end{pmatrix} \text{(I)} \quad J = \begin{pmatrix} + & + \\ - & - \end{pmatrix} \text{(II)}. \tag{5}$$

Two further Jacobians may be obtained from the trivial operation of interchanging x and y with the result

$$J = \begin{pmatrix} - & + \\ - & + \end{pmatrix} \text{(I}') \quad J = \begin{pmatrix} - & - \\ + & + \end{pmatrix} \text{(II}'). \tag{6}$$

The first class (I) is known as an activator-inhibitor system. The second class (II) has no obvious classification of activators or inhibitors. Both x and y enhance the first rate, and both decrease the second rate. One may define the self-activator to be the substance which has a positive element ($+$ in the above matrices) in the diagonal, i.e. the component which enhances its own formation. The other component generally is self-inhibitory (minus sign in the diagonal) and it is well established that it must diffuse faster than the self-activator.

In class (I) (the standard activation-inhibition system) the two substances x and y are *coincident* with respect to maxima in the emerging patterns: x is high where y is *high* and vice versa, contrary to conventional wisdom. The second class (II) has high x where y is low, but this is not an activator-inhibitor system.

The eigenvalues to J are found from

$$\lambda^2 - \lambda(a+b) + (ad - bc) = 0, \tag{7}$$

i.e.

$$\lambda^2 - \lambda Tr + \det = 0 \tag{8}$$

for short. Both eigenvalues have negative real part iff

$$Tr < 0 \tag{9}$$

$$\det > 0. \tag{10}$$

Note that inequality (9) means that the self-inhibitor has a stronger effect upon itself than the auto activation of the activator, a point which is usually not incorporated in some popular qualitative "explanations" of the onset of Turing instabilities.

Such popularizations have been criticized in Cross & Hohenberg (1993), where it is argued that Turing pattern formation explained by simple local activation, lateral inhibition considerations may be intuitively appealing but somewhat restrictive.

When diffusion is added, the diagonal elements of J change to $(a-k^2D_1)$ and $(d-k^2D_2)$ and the eigenvalues are found from

$$\lambda^2-\lambda[Tr-k^2(D_1+D_2)]+\det -k^2(aD_2+dD_1)+k^4D_1D_2=0. \quad (11)$$

This may generate eigenvalues with positive real part (see Fig. 2).

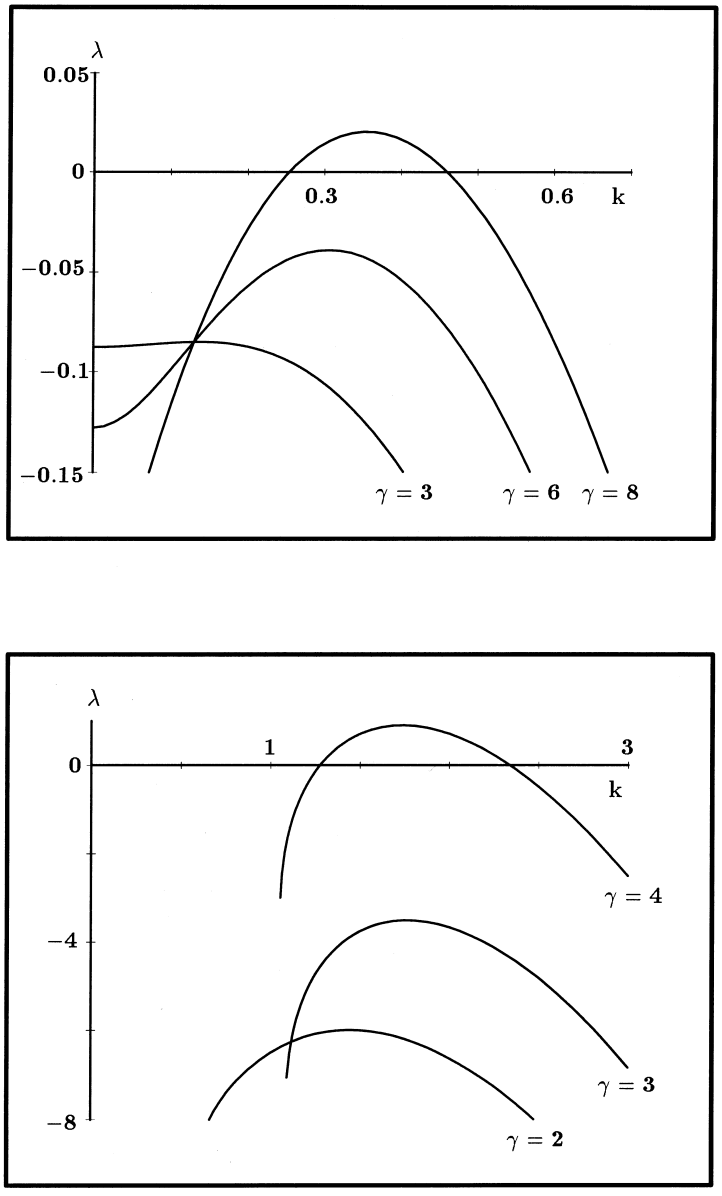

FIG. 2. Pattern formation by Turing's mechanism is facilitated by increasing cooperativity and thus high effective Hill constants γ in the defining rates. Such an increase in nonlinearity has occurred during evolution in gene control systems, for example where the promoter of the gene has developed several binding sites for gene controlling proteins. Cooperativity with effective γ in excess of 8 has been recorded experimentally for several different gene control systems. Such high Hill constants may have developed under evolutionary pressure, as they are necessary for accurate control in other contexts, even in single cells, but such control systems also become increasingly prone to create spontaneous pattern formation by Turing's mechanism. We have plotted the dispersion relation [eqn (11) in the text] for two mechanisms: eqns (27) and (28) (upper) with $\alpha=0.068$ and $D_-/D_+=5.0$, for $\gamma=3$, 6, 8 respectively, and eqns (61) and (62) (lower) with $A=8$, $B=42$, $C=4$, $D_-/D_+=5.0$, for $\gamma=2$, 3, 4 respectively.

As $Tr<0$ we have $D_1 \neq D_2$, otherwise we cannot have

$$aD_2 + dD_1 > 0, \tag{12}$$

which is an immediate necessary condition for eigenvalues with positive real part. The system is unstable if the last term is negative:

$$P(k^2) = \det - k^2(aD_2 + dD_1) + k^4 D_1 D_2 < 0. \tag{13}$$

The minimum of this polynomial obtains for

$$k^2 = \xi = \frac{aD_2 + dD_1}{2D_1 D_2} \tag{14}$$

and $P(\xi)<0$ and $\det > 0$ evaluate to

$$0 < \det < \frac{(aD_2 + dD_2)^2}{4D_1 D_2}. \tag{15}$$

This is the standard inequality derived in many sources. Note though that we may obtain an explicit inequality for $x = D_1/D_2$. Rearranging eqn (15) we get

$$d^2x^2 - (2ad - 4bc)x + a^2 > 0. \tag{16}$$

This has two real roots x_1, x_2:

$$x = \frac{(\det - bc) \pm 2\sqrt{-bc\ \det}}{d^2}. \tag{17}$$

Only one of these applies though: From eqn (12) we have

$$dx > -a \tag{18}$$

but from eqn (17)

$$dx = \frac{(\det - bc) \pm 2\sqrt{-bc\ \det}}{d}. \tag{19}$$

For the root x_- we get

$$dx + a = \frac{(\det - bc) - 2\sqrt{-bc\ \det}}{d} + a \tag{20}$$

$$= \frac{\det}{2}\,\frac{\sqrt{\det} - \sqrt{-bc}}{d}. \tag{21}$$

Since $ad<0$ for the Turing matrices with d positive this implies $ad - bc < -bc$ and thus

$$\frac{\sqrt{\det} - \sqrt{-bc}}{d} < 0. \tag{22}$$

However, this root does not fulfil inequality (18) and thus x_- does not apply. The other root yields

$$x \equiv \frac{D_-}{D_+} > \frac{(\det - bc) + 2\sqrt{-bc\ \det}}{d^2}, \tag{23}$$

which holds for the Turing matrices with positive d, (I′) and (II′) in eqn (6). We have stressed that the inequality contains the diffusion constant for the self-inhibitor D_- divided by the diffusion coefficient for the self-activator.

The remaining Turing matrices obtain by simple interchange of system 1 with system 2, and thus a negative d is interchanged with a positive a. Thus for Turing matrices of the form (I) and (II) [eqn (5)], we have

$$\frac{D_-}{D_+} > \frac{(\det - bc) + 2\sqrt{-bc\ \det}}{a^2}. \tag{24}$$

Related expressions have been derived elsewhere but we will use these particular inequalities for the ratio of diffusion coefficients to show that the diffusion constants may become almost equal in magnitude when the cooperativity of the defining kinetics becomes as high as is seen in genetic control systems. This has not been shown before.

We shall demonstrate this for a number of particular mechanisms.

(A) THE SEL'KOV MODEL

$$\frac{\partial x}{\partial t} = v - \frac{k_1 x y^\gamma}{1 + K y^\gamma} + D_1 \Delta x \tag{25}$$

$$\frac{\partial y}{\partial t} = \frac{k_1 x y^\gamma}{1 + K y^\gamma} - k_2 y + D_2 \Delta y. \tag{26}$$

Here component one is generated by a constant uniform rate v and transformed into component two by Hill-type kinetics. Component two is created by the same rate and decomposed by first-order kinetics. Usually the denominator is neglected and the equations renormalized to the form

$$\frac{\partial x}{\partial t} = 1 - x y^\gamma + D_1 \Delta x \tag{27}$$

$$\frac{\partial y}{\partial t} = \alpha x y^\gamma - \alpha y + D_2 \Delta y \tag{28}$$

which has the stationary solution $x^* = y^* = 1$ and the Jacobian elements evaluate to $a = -1$, $b = -\gamma$, $c = \alpha$ and $d = \alpha(\gamma - 1)$.

Stability towards homogeneous oscillations are provided by inequalities (9) and (10), that is,

$$Tr = \alpha(\gamma - 1) - 1 < 0 \tag{29}$$

and $\det = \alpha$. Thus an increasing cooperativity, measured by Hill constant γ, make the system more prone to autonomous oscillations, a well-known result. For $\gamma > 1$ the Jacobian becomes a Turing matrix of the form (II′) in eqn (6). The ratio between diffusion constants, inequality (23), evaluates to

$$\frac{D_-}{D_+} > \frac{1 + \gamma + 2\sqrt{\gamma}}{\alpha(\gamma - 1)^2} > \frac{1 + \gamma + 2\sqrt{\gamma}}{\gamma - 1}, \tag{30}$$

where we have used inequality (29). Inequality (30) may be rewritten

$$\frac{D_-}{D_+} > \frac{\sqrt{\gamma}+1}{\sqrt{\gamma}-1}. \tag{31}$$

For increasing cooperativity γ the ratio between diffusion constants approaches one.

In the following we shall demonstrate that the same may be shown for a number of other mechanisms. Whenever the effective Hill constant increases the critical ratio of diffusion constants approaches one. Thus Turing instabilities are greatly facilitated by high nonlinearity in the kinetics.

(B) AN EXTENSION OF THE BRUSSELATOR SCHEME

$$\frac{\partial x}{\partial t} = Ax^\gamma y - (B+1)x + 1 \tag{32}$$

$$\frac{\partial y}{\partial t} = -Ax^\gamma y + Bx. \tag{33}$$

Usually γ is set to 2. Here we consider higher values. The stationary solutions are $x^* = 1$, $y^* = B/A$. With $w = B(\gamma - 1)$, the Jacobian elements evaluate to $a = w - 1$, $b = A$, $c = -w$ and $d = -A$. Thus to have a Turing matrix [of the form II in eqn (5)] we must have $w - 1 > 0$. The inequality (9) evaluates to

$$(w-1) - A < 0 \tag{34}$$

and $\det = A$. Thus the ratio of diffusion constants must satisfy inequality (24) which evaluates to

$$\frac{D_-}{D_+} > \frac{A(1+w+2\sqrt{w})}{(w-1)^2} \tag{35}$$

$$> \frac{\sqrt{w}+1}{\sqrt{w}-1}, \tag{36}$$

where we again have used the condition for the trace, inequality (34). Since $w = B(\gamma - 1)$, increasing cooperativity again results in a ratio of diffusion constants approaching one.

Note, however, that now an increase in γ may be compensated by a lower value of the effective rate constant B. However, if the other kinetics is unchanged, but the system has its cooperativity increased, then the system becomes increasingly a Turing system.

(C) THE GENERALIZED SCHNACKENBERG MODEL

$$\frac{\partial x}{\partial t} = A - x + x^\gamma y \tag{37}$$

$$\frac{\partial y}{\partial t} = -x^\gamma y + B. \tag{38}$$

With the temporary abbreviation

$$w_1 = A + B \tag{39}$$

the stationary state evaluates to

$$x^* = w_1 \tag{40}$$

$$y^* = \frac{B}{(w_1)\gamma} \tag{41}$$

and the Jacobian becomes

$$J = \begin{pmatrix} \frac{B\gamma}{w_1} - 1 & w_1^\gamma \\ -\frac{B\gamma}{w_1} & -w_1^\gamma \end{pmatrix}. \tag{42}$$

This is a Turing matrix of type II [eqn (5)] if matrix element $a > 0$, that is

$$\frac{B\gamma}{w_1} - 1 > 0. \tag{43}$$

The determinant is w_1^γ and the trace inequality (9) is

$$Tr = \frac{B\gamma}{w_1} - 1 - w_1^\gamma < 0. \tag{44}$$

The ratio of the diffusion constants, inequality (24), evaluates to

$$\frac{D_-}{D_+} > (w_1)^\gamma \frac{1 + \frac{B\gamma}{w_1} + 2\sqrt{\frac{B\gamma}{w_1}}}{\left(\frac{B\gamma}{w_1} - 1\right)^2}. \tag{45}$$

From inequality (44)

$$(w_1)^\gamma > \frac{B\gamma}{w_1} - 1 \tag{46}$$

and thus (45) becomes,

$$\frac{D_-}{D_+} > \frac{w-1}{(\sqrt{w}-1)^2} = \frac{\sqrt{w}+1}{\sqrt{w}-1}, \tag{47}$$

with

$$w = \frac{B\gamma}{w_1} = \frac{B\gamma}{A+B}. \tag{48}$$

Again the ratio of diffusion constants approaches one when γ increases as w is proportional to the Hill constant γ.

(D) THE GENERALIZED GIERER AND MEINHARDT MODEL

$$\frac{\partial x}{\partial t}=A-Bx+\frac{x^\gamma}{y} \tag{49}$$

$$\frac{\partial y}{\partial t}=x^\gamma-y. \tag{50}$$

Observe that the term with high nonlinearity is not taken to be the same in the two rates, contrary to our earlier models. With

$$w_1=\frac{B}{A+1}, \tag{51}$$

the stationary concentrations are

$$x^*=\frac{1}{w_1} \tag{52}$$

$$y^*=\frac{1}{w_1^\gamma}. \tag{53}$$

The Jacobian elements evaluate to $a=w_1\gamma-B$, $b=-w_1^\gamma$, $c=\gamma w_1^{-\gamma+1}$ and $d=-1$ respectively. This is a Turing matrix of type I in eqn (5) provided $w_1\gamma-B>0$. The trace and determinant evaluate to

$$Tr=w_1\gamma-B-1<0 \tag{54}$$

$$\det=B>0. \tag{55}$$

The ratio of diffusion constants is evaluated from inequality (24) to yield

$$\frac{D_-}{D_+}>\frac{B+\gamma w_1+2\sqrt{\gamma B w_1}}{(w_1\gamma-B)^2}. \tag{56}$$

It is convenient to change to variable

$$w_2=\frac{\gamma}{A+1}, \tag{57}$$

and thus $\gamma w_1=Bw_2$. Using the trace inequality (54), $B(w_2-1)<1$, inequality (56) becomes

$$\frac{D_-}{D_+}>\frac{1+w_2+2\sqrt{w_2}}{B(w_2-1)^2} \tag{58}$$

$$>\frac{1+w_2+2\sqrt{w_2}}{w_2-1} \tag{59}$$

$$=\frac{\sqrt{w_2}+1}{\sqrt{w_2}-1}. \tag{60}$$

As w_2 is proportional to the Hill coefficient γ, we again find that, for increasing cooperativity, the ratio between diffusion coefficients tends to 1.

(E) THE GENERALIZED LENGYEL–EPSTEIN MODEL

$$\frac{\partial x}{\partial t}=A-x-\frac{Cxy}{1+x^\gamma} \tag{61}$$

$$\frac{\partial y}{\partial t}=B\left(x-\frac{xy}{1+x^\gamma}\right). \tag{62}$$

Here the nonlinearity is only inhibitory, whereas our former examples were cooperative activation. With the abbreviation

$$w_1=\frac{A}{C+1}, \tag{63}$$

the stationary concentrations evaluate to

$$x^*=w_1 \tag{64}$$

$$y^*=1+w_1^\gamma \tag{65}$$

and the Jacobian matrix elements become

$$a=-1-C+\frac{C\gamma w_1^\gamma}{1+w_1^\gamma},\quad b=-\frac{Cw_1}{1+w_1^\gamma} \tag{66}$$

$$c=\frac{B\gamma w_1^\gamma}{1+w_1^\gamma},\quad d=-\frac{Bw_1}{1+w_1^\gamma}. \tag{67}$$

This is a Turing matrix of type I in eqn (5) provided $a>0$, that is

$$-1-C+\frac{C\gamma w_1^\gamma}{1+w_1^\gamma}>0. \tag{68}$$

The condition for the trace evaluates to

$$Tr=-1-C+\frac{C\gamma w_1^\gamma-Bw_1}{1+w_1^\gamma} \tag{69}$$

$$=-(C+1)+\frac{C\gamma(w_2-1)-Bw_1}{w_2}<0, \tag{70}$$

where we have introduced another temporary abbreviation

$$w_2=1+w_1^\gamma. \tag{71}$$

The determinant is

$$\det=(C+1)\frac{Bw_1}{w_2} \tag{72}$$

and the ratio between diffusion constants [inequality (24)] evaluate to

$$\frac{D_-}{D_+} > \frac{Bw_1}{w_2} \times \frac{(C+1)+\dfrac{C\gamma(w_2-1)}{w_2}+2\sqrt{(C+1)C\gamma\dfrac{(w_2-1)}{w_2}}}{\left(\dfrac{C\gamma(w_2-1)}{w_2}-(C+1)\right)^2}. \tag{73}$$

Using inequality (70) in the form

$$\frac{Bw_1}{w_2} > \frac{C\gamma(w_2-1)}{w_2}-(C+1), \tag{74}$$

inequality (73) may be written

$$\frac{D_-}{D_+} > \frac{(C+1)+\dfrac{C\gamma(w_2-1)}{w_2}+2\sqrt{(C+1)C\gamma\dfrac{(w_2-1)}{w_2}}}{\left(\dfrac{C\gamma(w_2-1)}{w_2}-(C+1)\right)} \tag{75}$$

$$= \frac{1+z+2\sqrt{z}}{z-1} \tag{76}$$

$$= \frac{\sqrt{z}+1}{\sqrt{z}-1}, \tag{77}$$

where we have finally introduced variable z by

$$z = \gamma\left(\frac{C}{C+1}\right)\frac{w_2-1}{w_2} = \gamma\left(\frac{C}{C+1}\right)\frac{\left(\dfrac{A}{C+1}\right)^\gamma}{1+\left(\dfrac{A}{C+1}\right)^\gamma}. \tag{78}$$

If

$$\frac{\left(\dfrac{A}{C+1}\right)^\gamma}{1+\left(\dfrac{A}{C+1}\right)^\gamma} \to 1, \tag{79}$$

which obtains for

$$\frac{A}{C+1} \gg 1, \tag{80}$$

variable z becomes proportional to γ and thus again, high cooperativity facilitates Turing structures, as the ratio between diffusion coefficients tend to 1. Condition (80) is the same as stating that the stationary concentration of x fulfils $x^* \gg 1$ which again means that the system operates on the strongly inhibitory side of the term

$$\frac{Cxy}{1+x^\gamma} \tag{81}$$

in the defining rates.

In all the models investigated here, we obtain an expression of the form

$$\frac{D_-}{D_+} > \frac{\sqrt{z}+1}{\sqrt{z}-1} \tag{82}$$

(Table 1), where z is a function of γ, usually a linear function at least for suitable parameter values, and thus z increases with increasing cooperativity γ. It is tempting to suggest that this will always obtain, but even with occasional exemptions to this rule the above results show that a substantial class of models become Turing systems with increasing cooperativity. Thus the usually stated requirement of having effective diffusion coefficients differ by almost an order of magnitude, which is often found in systems with small Hill constants, is relaxed in gene control systems where much larger effective Hill constants have been recorded.

We proceed by noting that the dispersion equation [eqn (11)] yields an increasing Turing region with increasing Hill number. In Fig. 2 we have calculated the eigenvalue λ from eqn (11) as a function of wave number k as is usual, but with the extension of plotting a set of such curves for increasing values of the Hill constant γ.

We conclude that Turing pattern formation may be much more feasible in actual biological systems, if the mechanism is connected to the gene control system with high Hill numbers, than would be expected from studies of inorganic model systems, or experimental realizations of such inorganic systems, as these latter systems rarely have Hill numbers exceeding 2. This discovery thus supplements earlier discussions of the feasibility of a substantial ratio between effective diffusion constants in Turing's mechanism (Hunding & Sørensen, 1988; Pearson & Horsthemke, 1989).

TABLE 1
Ratio of diffusion coefficients D_-/D_+ in a Turing system approaches one when effective Hill constant increases according to eqn (82)

z	D_-/D_+
2	5.83
4	3.00
8	2.09
16	1.67

In eqn (82) z is a function of the effective Hill constant γ and for a number of mechanisms it has been shown here that $z \simeq \gamma$ at least for suitable parameter combinations

Discussion

One role the homeobox genes—or their predecessors, the genes for helix-turn-helix proteins—may have played in this context is to provide a system with very high cooperativity. Initially in evolution such a system may be used simply to read subtle differences of concentrations, quite possible within a single cell. Indeed, several processes need fine tuned control in which the system is required to respond in a nearly off–on manner when concentrations are varied with less than a factor of 2.

Examples are the very central process of control of the cell cycle governing mitosis in which the cell must respond with an entirely new mode when its mass is increased by a factor of 2. Any control system capable of this must meet the additional requirement to adapt to somewhat varying size of the single cell. This necessitates a very precise control, made possible with highly nonlinear off–on kinetics. An example of a model which captures these features may be found in Novak & Tyson (1993).

Second, it may be noted that highly nonlinear off–on control is a central feature of "active gene control". (The experimental basis for dynamic maintenance of multistability in cells, known as active control, as opposed to a once and for all inactivation (passive control), has been reviewed in Blau (1992).) It is experimentally well established that a single gene product may be active in different time intervals of the life of a cell, and thus that the gene may be activated by different combinations of other gene products. In turn, the genes own product may itself be involved in quite different molecular jigzaw puzzles of activating or repressing clusters on the promoter for other genes. In the corresponding theoretical framework, theoretical framework, highly nonlinear off–on control is a central feature of multistability in connected reaction networks. Multistability is a prerequisite for multifunctionality and adaptation, but the step from this level to cell differentiation is short, and models for the emergence of stable reaction cycles rest on such highly nonlinear kinetics (Kauffman, 1993).

Highly nonlinear off–on control is also a prerequisite for interpretation of positional information. It is not enough that a system is able to discern different information: it must also be able to remember it. Thus, later in evolution, when precise size regulation and the basis for multifunctionality and cell differentiation were established, this feature of precise concentration response may then have been explored to read out positional information along a gradient. The cooperativity recorded experimentally is very high, often effective Hill numbers exceeding 8 are inferred. This creates an almost ideal on–off system. Such control could then be used to read position along a gradient and thus to create a local peak of gene expression, or subsequently to yield double stripes on both sides of such a peak, as discussed above.

Such use of positional information is known to be used repeatedly. The initial anterior-posterior gradients in *Drosophila* as well as the dorsal-ventral organization are such examples, and recent studies of gene *hedgehog* point to an important role for such a hierarchy of gradients in wing patterning as well (for references, see O'Farrell, 1994).

However, it is important to realize that any control systems with high Hill numbers will be prone to yield time-oscillations, and this will be true of many Turing systems as well, as remarked above. Moreover, for many model systems, the higher the Hill number, the easier it is to get Turing structures with effective diffusion rates which do not differ much from each other. Thus the usual requirement of approximately an order of magnitude spread between the two effective diffusion rates is relaxed, and a trend towards higher Hill numbers would greatly facilitate the formation of a Turing structure. It should be stressed then, that Turing pattern formation is not a particular special case, but a pattern-forming process, which emerges with the *same* requirements to the control system as pattern formation on the basis of reading positional information along a gradient. In both cases, high Hill numbers are essential.

The main point then is that a gene system which is capable of discriminating small differences in concentration is prone to generate cue control, chemical oscillations and Turing type patterns as well.

The connection between the homeobox genes and the fundamental pattern forming processes may thus not be that of an ancestral pattern gene which has

developed into a class of such genes. Rather, the homeobox genes have the ability to create proteins which bind to the promoter region of another gene. One gene controls the expression of another gene. The protein may during evolution develop several binding sites to the promoter or other proteins. These parts are envisaged to fold up in a sort of three dimensional jigzaw puzzle involving many such binding sites. The result may be highly cooperative control.

In addition, a particular gene may take part in *several* such jigzaw puzzles. Many of the genes already mentioned have this property. An explicit example is *hairy*, which in early evolution seems to be originally connected to the nervous system but later in evolution burrowed to play a role in the initial body plan of *Drosophila*. Thus a particular pattern forming process is not connected to a particular gene, or gene class. Rather, the particular pattern forming process is generated by genes generally capable to be involved in highly cooperative control.

This *property* of high cooperativity in the gene regulatory system then yields a class of control systems, which should be easy to exploit to make particular patterns like sequentially arising stripes, pairs of stripes laid down in succession, or even Turing-type spatial waves, as discussed above.

In summary, the study of nonlinear dynamics, i.e. the properties of nonlinear control systems, are equally important for the understanding of the emergence of patterns in multicellular systems, as are studies of protein structure or the geometry of protein–gene interactions.

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APPENDIX

Numerical Treatment of Pattern Formation

Our efforts to develop efficient software for the calculation of spontaneous pattern formation in biological systems have led to fast codes for vector supercomputers. The numerical method we have used is largely the same as earlier described (Hunding *et al*., 1990). The method of lines was used and thus the system of nonlinear partial differential equations was converted to a large system of ordinary differential equations by discretization of the Laplacian in three curvilinear coordinates. The resulting system is stiff and solved accordingly (modified Gear code).

The Jacobian used in the corrector step is a sparse banded matrix which may be rearranged (chessboard numbering of meshpoints) to yield large blocks within which the solution vector elements may be iterated in parallel (RBSOR method). Implementation on vector computers results in a huge increase in speed: the RBSOR code runs efficiently and close to the top speed of vector machines like the Fujitsu/Amdahl VP1200 (410 MFLOPS sustained speed) and 1320 MFLOPS have been recorded on a two-processor CRAY C90.