

# THE SCALE OF PATTERN FORMATION

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## INTRODUCTION

There are many kinds of patterns in biology and a number of quite different mechanisms for generating them. For example, patterns due to pigment in some cases depend largely on variations in the movement of melanocytes, as in the case of chicken feathers. Other patterns are likely to be produced, at least in part, by lineage mechanisms. This is probably true in the formation of bristles in insects. In *Rhodnius*, for example, the single bristle mother-cell divides twice, thus producing a small group of four cells, which become the trichogen, the tormogen, the nerve cell and the neurilemma cell (Wigglesworth, 1953). In this case, the relative movement of the cells also appears to play some part in forming the spatial pattern of the group.

A pattern may exist even when it is not immediately obvious by visual inspection. Such a case is the retina of the amphibian eye, studied by Gaze and Jacobson (see the review by Gaze, 1967), in which some mechanism fairly early in the development of the retina instructs the cells where they lie in the tissue, so that they know to which part of the optic tectum to send their axons. It is this latter type of pattern which I shall be particularly concerned with here. Wolpert (1969) has suggested the use of the term 'positional information' to describe such cases. It is, of course, not limited to cases where the pattern is invisible.

Most of the examples of such systems which have been studied show regulation. Moreover, as far as can be determined, the relative movement of cells does not seem to occur to any appreciable extent. Thus the pattern is unlikely to be due entirely to lineage. There must be a mechanism, involving some sort of communication between cells, which enables each cell to discover its location in the tissue. This paper is concerned with the general nature of such communication. The very important topic of the junctions between cells in a tissue is not dealt with here, except by implication.

## TWO BASIC MECHANISMS

One possibility, which has a long history, is that the tissue in some way sets up a gradient of concentration of some chemical (or chemicals), here called a morphogen, and that each cell recognizes the local concentration of the morphogen. Another quite different mechanism has been suggested by Goodwin & Cohen (1969). (See also their papers in this Symposium.) It is thus of some value to contrast these two hypothetical mechanisms. It turns out that this can be done in rather general terms, since each is an example of two distinct types.

*Random walk mechanisms*

The first type might be called random walk mechanisms. They can loosely be described as follows. Consider a line of cells labelled *A*, *B*, *C*, etc. In such cases cell *A* affects cell *B* in some way. *B* then affects both cell *C* and cell *A*, and so on. In short, each cell always affects all its neighbours. The effect produced by each cell on its neighbours must be small enough to avoid producing an explosion. In most cases, such as simple diffusion, there is a conservation law which says that the total amount of the 'effect' (for example, the amount of material diffusing) remains constant, except where there are sources or sinks. The *maximum* distance the effect has travelled after a certain time depends critically upon how small an effect can be detected. It is therefore more convenient to describe the *mean* distance,  $\bar{x}$ , the effect has travelled in time *t*. In the simplest cases this is given by

$$\frac{\bar{x}^2}{t} = \text{constant.}$$

This shows clearly that the velocity with which the 'effect' travels falls off with distance. Small distances are influenced very rapidly, large distances very slowly. A similar formula also applies in two or three dimensions.

*Signalling mechanisms*

The second type I shall call signalling mechanisms. In such cases cell *A* affects cell *B*, cell *B* affects cell *C* (but not *A*), cell *C* affects cell *D* (but not cell *B*), and so on. This can happen for several reasons. In physical cases it may be due, for example, to momentum. In biological cases it could be because the line of cells has an intrinsic polarity. However, this feature is not essential. An alternative is to invoke a refractory period. That is, cell *C* attempts to affect both cell *B* and cell *D*, but the former has become temporarily refractory and thus only the latter is affected.

The signal travels, in simple cases, with a constant velocity, so that

$$x/t = \text{constant.}$$

Thus, although small distances will be influenced sooner than big distances the contrast between the times involved is not so great as in the random walk process. Again the formula is not restricted to one-dimensional cases. Signalling mechanisms can broadly be divided into two classes. In the first, which might be called amplitude decay mechanisms, the influence of each cell on the next is such that the amplitude of the signal gets less and less at each successive step. This could be done if the output of each cell were proportional to the input. The constant of proportionality must be fixed so that the signal decays slowly rather than increasing explosively. A refractory period is necessary to avoid a random walk. If the amplitude at the source is fixed, any cell can find where it is in the tissue by measuring the amplitude of the signal which reaches it. Such a system will not regulate unless complicated further.

One has an uncomfortable feeling that such a mechanism might be difficult for a cell to achieve. I have not been able to produce a really convincing reason against it, although the proportionality constant would have to be fixed very accurately. The time behaviour of the cell would need to be largely independent of the amplitude of the signal, but I do not see why this should present an insuperable difficulty.

The second class of signalling mechanism might be called the phase difference mechanism. In this, the signal is regenerated at each step to a constant amplitude, so that one cannot use the amplitude alone to give positional information. To overcome this difficulty Goodwin & Cohen (1969) suggested that two signals, synchronized at the source, are sent out which travel with different velocities. A cell receiving the signals then obtains its position by measuring the phase difference between them. In order for the system to regulate a further elaboration is necessary.

Strictly the pair of signals need only be sent once, but it is obviously an advantage to send them many times, so that the cell can integrate its response in some way to obtain a more efficient and reliable indication of position.

*Examples*

It is easy to give examples of the two main kinds of mechanism from physics or biology. The diffusion of a solute in a stationary liquid is the classic case of a random walk process. However, it is not essential for an actual material substance to move an appreciable distance, as the example of the diffusion of heat shows rather clearly. In the biological context an influence of one cell on its neighbours, such that they then influence all their

neighbours, is strictly all that is required. In practice, one would not be surprised to find an actual molecule diffusing.

Physical examples of signalling mechanisms are plentiful, since any wave has this character, though not usually because of a refractory period. The classic example in biology is the transmission of the action potential down a nerve axon. Here, as in the mechanism suggested by Goodwin and Cohen, it is necessary to postulate a refractory period.

At the moment no decision can be made between the two sorts of model. The Goodwin-Cohen type of interaction appears very plausible as an explanation of the aggregation of certain slime moulds and possibly the locomotion of their slugs, but it has yet to be shown that it conveys positional information, as opposed to polarity. The random walk model, in the form of a steady gradient of concentration, has often been postulated but never clearly established. At least nobody has decisively isolated a morphogen and proved that it acts in this way. Nevertheless, this mechanism is so simple compared with the rather elaborate biochemical apparatus required by the Goodwin-Cohen phase difference mechanism, especially if the latter has to regulate, that I thought it was worth exploring further.

#### CONCENTRATION GRADIENT MECHANISMS

The obvious model (which has often been suggested before) is that one cell, or set of cells, becomes a source, producing a morphogen and holding its concentration there to a fixed value. Another cell, or set of cells, becomes a sink, which destroys the morphogen, maintaining its concentration at the sink at a fixed low level, probably close to zero. The morphogen diffuses through the tissue from source to sink and after a time a steady concentration gradient is set up. Any cell in the tissue can thus find one co-ordinate of its position by measuring the local concentration of the morphogen. Such a system is easily capable of regulation, provided the position of the source and sink can be imposed by other considerations. For example, it might be arranged that they always formed at the two edges of a piece of tissue.

It turns out that if such a concentration gradient mechanism is indeed operating there is a very severe restriction on the distance over which the gradient can be set up in a limited time. I have already set out the arguments in detail elsewhere (Crick, 1970). In brief, even if the morphogen is a rather small organic molecule there is a clear upper limit to the rate at which it can diffuse in a watery solution. Moreover, to move reasonably rapidly in a tissue there will probably have to be a fairly fast process of facilitated diffusion between cells. Whatever the exact details of the process

— for example, whether the morphogen moves in the intracellular space, or, alternatively, only within cells by means of special cell-to-cell junctions — it is impossible to set up a concentration gradient faster than a certain rate, the value of which can be estimated approximately. The actual figures are given in the paper quoted. In broad terms they show that if there is only a few hours available in development to set up a gradient system, it cannot be much larger than a millimetre or two. Shorter times would imply smaller distances.

#### *The basic postulate*

One can thus propose a basic postulate which states: 'when a pattern is first set up the size of the tissue embodying it is always small'.

'Pattern' is restricted to cases of positional information; 'small' is defined numerically in terms of the time available. A very rough and ready rule would be

$$x \leq \sqrt{t}$$

where  $x$  is in millimetres and  $t$  is in hours. Here  $x$  is the distance between the source and the sink, and  $t$  is the time available to set up the gradient. It should be stressed that it is the time needed for setting up which is limiting. After a gradient has been established it is possible to conceive mechanisms by which, during the growth of the tissue, it could be extended to cover quite considerable distances.

It has been pointed out by Wolpert (Wolpert, 1969), independent of the above argument, that in fact the experimental data do suggest that a rule of this sort may be true. He notes that 'most embryonic fields seem to involve distances of less than 100 cells, and often less than 50'. His examples (his Table 1) are all taken from animals. A quick glance at botany would suggest that the same statement may be true there, but I speak without expert knowledge.

Even if it turns out that this basic postulate is (almost) always correct, it would still not decisively favour the gradient hypothesis over the phase mechanism of Goodwin and Cohen. It is certainly true that signalling mechanisms are ideal for sending information over large distances. That is why they are used in the nerve axon. However, in order to convey positional information, as opposed to merely sending a sequence of pulses, two distinct signals must be sent. Each cell then obtains its position by comparing the phases of the two signals. It could be argued that if there is a limit to the precision with which this comparison can be made, there will be a limit to the distance (or number of cells) which can usefully be covered before the pattern starts to repeat again. If so, the phase difference mechanism might not work efficiently over big distances, at least if position had to be specified rather precisely.

On the other hand, the demonstration of an example of positional information which grossly violated the distance rule suggested above would certainly imply that, in that case at least, the mechanism is unlikely to be based on simple diffusion.

### FLUID FLOW MECHANISMS

There is a third possible general type of mechanism which could give positional information but it can only occur in certain cases. This is one based on the flow of a fluid. If a chemical is produced at one point in a stream so that there is a steady concentration at that point and for one reason or another its concentration decays as it travels down the stream (either because it is unstable, or destroyed by an enzyme, or absorbed along the way) then clearly its concentration will act as an indicator of 'position' to cells bordering the fluid channel.

The extreme case of this, in which the positional information is negligible, is when the concentration of the chemical changes hardly at all as it is carried down the stream. In such instances the chemical would normally be classed as a hormone.

Gradient effects of this sort may well occur in the vascular system of animals and plants. However, I think it would be better not to use the term positional information, in Wolpert's sense, in such a context.

### SOME GENERAL REMARKS ABOUT CONCENTRATION LEVELS

The simple theory of concentration gradients has two basic assumptions. The first is that a source or sink cell can *maintain* its internal concentration of the morphogen at (approximately) a fixed level. The second is that any cell in the gradient can *recognize* the concentration of morphogen within it or around it.

There does not appear to be any real difficulty about the first requirement. A cell can easily arrange to hold the concentration of a morphogen more or less constant, in spite of small variations in the size of the cell, or in the rate at which the morphogen is lost from the cell. Moreover, this level can be genetically determined in a relatively simple way. The reason for this springs from the very nature of enzymes. To make things easy, let us consider a simple enzyme whose steady rate follows classical Michaelis-Menten kinetics. Such an enzyme has two basic parameters, which depend on the precise stereochemical structure of the enzyme. This is mainly, if

not entirely, derived from the amino acid sequence of the enzyme and is thus to a large extent genetically determined. The two parameters are:

- (1) the maximum rate of action of the enzyme, which happens at high substrate concentrations. Note that *for a cell* this also depends on the *number* of enzyme molecules in the cell. Though a cell will certainly control this number in some way, it is not clear how accurately it will control it. For example, if the volume of the cell changes with time, will the number of enzyme molecules tend to remain the same, or their concentration?
- (2) The Michaelis constant of the enzyme. This is the concentration of the substrate at which the enzyme works at half its maximum rate. Note that, as opposed to the maximum rate, this parameter is independent of the number of enzyme molecules in the cell. There is thus a very real sense in which an enzyme molecule embodies an absolute value of the concentration of its substrate.

Naturally an actual enzyme is likely to be more complicated. The two parameters will probably change with general factors such as pH and temperature but under normal physiological conditions such factors are likely to be fairly effectively controlled by the cell. The parameters may be alterable by certain rather specific molecules (activators, inhibitors) but this merely implies that the enzyme can recognize, albeit in a somewhat complicated way, the absolute concentration of molecules other than its substrate. Finally, enzymes often consist of several subunits which interact, and do not follow normal Michaelis-Menten kinetics. However, the curve of rate-of-action versus substrate concentration is then often steeper at the half-maximum rate value, and thus may in practice define a substrate concentration more precisely than in the simple case.

### A PRECIPITATION MECHANISM

A quite different scheme for keeping a fixed concentration of a morphogen in a cell is to choose a chemical which will precipitate (or perhaps co-precipitate) at the concentration required. This mechanism has the advantage that the control is very sharp, and that there will be a reserve of the morphogen available in the precipitate. One might speculate that protein might be involved in two subsidiary roles. One protein might be used to facilitate nucleation of the precipitate and thus avoid super-saturation. A second one might be used to interact allosterically with the precipitate (but not with the free morphogen) and thus form part of a control mechanism to reduce the metabolic supply of the morphogen as the precipitate accumulates. More elaborate schemes, involving storage in vesicles, or the precipitation of a morphogen precursor, are obviously possible.

It is a requirement for such a mechanism that the solubility of the morphogen is tolerably constant under normal physiological conditions. This is probably not too difficult to achieve.

#### GENERAL REMARKS

Thus it is not difficult to devise schemes for a cell to maintain, at the source of the gradient, a pre-set concentration of the morphogen which is relatively independent of variations in cell size, etc. It is particularly easy to make a sink, if the sink holds the concentrations of the morphogen near zero, since then all that is required is an enzyme in the sink cells to destroy the morphogen very rapidly, even at very low concentrations.

I can see no strong reason why double gradients should be an advantage. A double gradient system is one in which the concentration of one morphogen increases from right to left, another increases from left to right and the cells respond to the *ratio* of the concentration of the two morphogens. This may, of course, turn out to be useful in special cases. The transmission of information by chemicals travelling down a long nerve axon might be such a case.

While the problem of setting up the gradient of a morphogen seems peculiarly simple, that of responding to and registering a morphogen level is not quite so straightforward, and the molecular mechanisms are likely to be more complex. I do not think there is any insuperable difficulty but I shall postpone a discussion of this until another occasion.

#### POLARITY, GRADIENTS AND CONTOURS

In this last section I make a few simple comments on these three concepts.

The word 'gradient' is customarily used in embryology in a rather loose sense. It implies that there is an underlying field of a scalar quantity (usually, a concentration) which varies smoothly over the tissue, usually with a fairly steady slope, and that each cell in the tissue can recognize and respond to the value of the scalar (that is, the concentration) at that point. The mathematical sense of the word gradient corresponds, however, to the actual slope of the scalar field at a particular point. Mathematically one can always derive a vector field ( $\text{grad } \phi$ ) from the scalar field ( $\phi$ ), so that mathematically a 'gradient', in the biological sense, implies a polarity, that is the maximum slope ( $\text{grad } \phi$ ) of the scalar field.

A system of polarities, on the other hand, necessarily implies a vector field. Whereas one can always derive a vector field from a scalar one, the converse is not always true. The mathematical criterion that this can be

done is that  $\text{curl } \mathbf{V}$  is everywhere zero. However, it is not unreasonable to hope that biological vector fields may often arise as the slope of an underlying scalar field.

The fact that these operations can be done mathematically does not necessarily imply that the tissue (or cells) can do them. One can imagine a situation in which there was a gradient of concentration in a tissue but the cells did not respond to the space derivative of the concentration (i.e. to the polarity), but did respond to the average concentration within each cell. The polarity would then be only formal. Similarly, one can imagine a tissue having a polarity, the cells of which could not recognize the scalar field which one could derive from it.

The cuticles of insects in general (and *Rhodnius* and *Oncopeltus* in particular) have a polarity, as shown, for example, by the direction of the bristles or hairs. The classic work of Locke (1959, 1960) on *Rhodnius* and later work of Lawrence on both *Rhodnius* and *Oncopeltus* (see the article by Lawrence in this Symposium) show clearly that in these cases there is also a gradient of some sort. Moreover, the bristles and hairs appear to direct their polarity according to the slope of the scalar field, at least to a first approximation.

There are a number of cases in biology (for example, the lines on the cuticle of *Rhodnius*, or our own fingerprints) in which a system of lines looks rather like a set of contours. However, true contours have rather special properties. A contour is, strictly speaking, a line joining points having the same value in a scalar field. To avoid unnecessary complications we shall assume that this field is reasonably smooth, well-behaved and not perfectly flat.

True contours cannot come to a dead end, or branch, except in very special cases. These special cases occur when a bit of the contour is, by accident, exactly on a minimum or maximum of the scalar field. However, a slightly different choice of contour level will remove the dead end or branch point. A more useful criterion might be that these special points should only occur occasionally and as fairly close pairs. This assumes that stretches of contour lying exactly on a maximum or a minimum will only happen by chance, and for short distances at a time.

In general, if a system of lines (like, say, fingerprints), looking roughly like a set of contours, is found to have fairly frequent branch points and dead ends, it is unlikely to be a set of true contours. Examination of one's fingerprints show that they have just this character. Of course, this does not mean that the lines, although not true contours, do not follow approximately some underlying scalar field.

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