Theoretical aspects of stripe formation in relation to Drosophila segmentation

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Summary

Many aspects of Drosophila segmentation can be discussed in one-dimensional terms as a linear pattern of repeated elements or cell states. But the initial metameric pattern seen in the expression of pair-rule genes is fully two-dimensional, i.e. a pattern of stripes. Several lines of evidence suggest a kinetic mechanism acting globally during the syncytial blastoderm stage may be responsible for generating this pattern. The requirement that the mechanism should produce stripes, not spots or some other periodic pattern, imposes preconditions on this act, namely (1) sharp anterior and posterior boundaries that delimit the pattern-forming region, and (2) an axial asymmetrizing influence in the form of an anteroposterior gradient. Models for Drosophila segmentation generally rely on the gradient to provide positional information in the form of concentration thresholds that cue downstream elements of a hierarchical control system. This imposes restrictions on how such models cope with experimental disturbances to the gradient. A shallower gradient, for example, means fewer pattern elements. This need not be the case if the gradient acts through a kinetic mechanism like reaction-diffusion that involves the whole system. It is then the overall direction of the gradient that is important rather than specific concentration values. We illustrate this and some related properties of reaction-diffusion models with computations using a specific model, a variant of the Brusselator, and discuss features that appear to be both relevant to Drosophila and of general significance: (1) sharp boundaries and gradients can together orient and stabilize multistripe patterns and (2) boundaries, including fixed boundaries established within a pattern-forming region, may be important for controlling the subdivision of large pattern domains into smaller elements as is observed in the expression of pair-rule genes.

Key words: pattern formation, stripe formation, segmentation, gradients, Drosophila, reaction-diffusion models, Brusselator.

Introduction

The application of molecular techniques to Drosophila has produced a wealth of information on spatial and temporal patterns of gene expression in the early embryo, summarized in several recent reviews (Gergen et al. 1986; Scott & O'Farrell, 1986; Scott & Carroll, 1987; Akam, 1987). The establishment of the basic metameric pattern is most clearly shown in the expression of pair-rule genes like fushi-tarazu (ftz). The pattern was first described by Hafen et al. (1984), and it evolves over time (Weir & Kornberg, 1985). ftz is expressed in the syncytial blastoderm stage first as a broad, uniform band. Then, during cell cycle 14, just prior to cellularization, it is rapidly resolved into a series of seven regularly-spaced circumferential stripes. Other pair-rule genes express essentially the same striped pattern in individually different ways, with stripes partially or fully offset from ftz.

It is now accepted that pattern formation proceeds in a sequential fashion at a progressively decreasing spatial scale (e.g. see Akam, 1987). The embryo begins with maternally derived anterior and posterior determinants that evidently act as sources for a set of anteroposterior gradients (Nüsslein-Volhard et al. 1987). The embryo is then subdivided into a series of large domains by the action of a class of genes known as gap genes, into double-segment repeats by pair-rule genes, and finally into segmental repeats within
which narrow files of cells are specified, for example, by segment-polarity genes (Akam, 1987). Many of these genes were first identified by Nüsslein-Volhard & Wieschaus (1980) as part of a systematic search for genes affecting pattern. Control is hierarchical. Genes expressed early in the sequence act to control later-acting genes, i.e., those located downstream in the control system. In discussing the nature of the control processes involved in segmentation, especially with regard to the hierarchical or combinatorial features of that control, it may be appropriate to treat the problem as essentially one-dimensional as, for example, Scott & O’Farrell (1986) have done. The body plan then becomes simply a linear series of repeating cell states. In dealing with the earlier patterning events, i.e., those leading up to the formation of \textit{ftz} stripes, the full two-dimensional nature of the pattern becomes more important. The stripes are regularly spaced, precisely aligned and show individual reproducible differences (e.g., the last \textit{ftz} stripe is always broader). A complete treatment of segmentation must explain why stripes are formed, rather than some other pattern, as well as explaining detailed features of the final pattern.

\textbf{Gradients rule, or do they?}

There is much experimental support for the idea that gradients are a widespread feature of insect epithelia involved in morphogenetic control, their role being to provide positional cues to cells (Lawrence, 1973). Nüsslein-Volhard et al. (1987) summarizes the evidence for the existence of at least two primary gradients in the early embryo, generated from the anterior and posterior pole of the embryo, and dependent on \textit{bicoid} and the \textit{oskar} group of genes, respectively. Further, anteroposterior gradients in distribution of the products of two other genes, \textit{caudal} and \textit{hunchback}, can be directly visualized (Mlodzik et al. 1985; Macdonald & Struhl, 1986; Tautz et al. 1987).

A gradient is usually assumed to work as follows: a stable, monotonic concentration gradient of some substance is established, cells determine their position with reference to the concentration they encounter locally and then respond in a position-specific way. Gergen et al. (1986) discuss several ways this might be done. Specifying structures in detail in such a fashion would require that cells reading the gradient discriminate very small concentration differences. Even in principle there must be limits, and it seems doubtful that all the details of anteroposterior patterning in \textit{Drosophila} could be coded in a single gradient. Nor is this idea supported by the evidence. It is easier to accept the idea that only the initial subdivision of the body, e.g., into relatively coarse cardinal domains as proposed by Meinhardt (1986), is controlled directly in this way. The gap genes, in Meinhardt’s model, interact in various ways reminiscent of reaction-diffusion models, which sharpens the domains they form. The location of the domains along the embryonic axis is determined by concentration-dependent input from the gradient. Stripes arise, as they would in any positionally cued model, because the gradient is assumed to have cylindrical symmetry about the anteroposterior axis: the gradient acts as a ruler whose markings are in register around the whole circumference of the body. Cells at a given level along the axis read the same value regardless of circumferential position. The effect of experimental alterations to the gradient is readily predictable. Steepling it should compress pattern. In contrast, a shallower gradient means fewer positional values, so fewer pattern elements will be expressed. This is desirable for explaining some experimental effects but not others. It may be that segment number is reduced in the mutant \textit{bicaudal} because the gradient is shallower than normal and half normal length, due to duplication, as suggested by Meinhardt (1986). But with \textit{caudal}, which is required for normal \textit{ftz} expression and segmental pattern, an abnormally shallow gradient has minimal effect on these (Macdonald & Struhl, 1986). There is therefore a need to examine how gradients might affect striped patterns, perhaps control them, but in a way that decouples control from specific concentration values. Kinetic theories for pattern formation, of which reaction-diffusion models are a major subclass, have this capability. The main purpose of this paper is to illustrate this and related features of such models to show how an anteroposterior gradient can order pattern elements oriented perpendicular to it.

\textbf{Reaction-diffusion and morphogenetic control in two dimensions}

Kinetic theories for pattern formation are numerous and diverse. Theoreticians are giving increasing attention to the task of classifying these with respect to their overall capabilities and limitations (Gierer, 1981; Murray, 1982; Harrison, 1982, 1987), which is important, since a broad perspective is needed to avoid misleading conclusions based on the peculiarities of any one theory or model. The diversity lies in the dynamics of chemical interactions, described theoretically by differential equations with space and time variables. Such systems of equations are notoriously sensitive to the initial and boundary conditions employed. These can be as important as the dynamical diversity within the
equations in governing pattern. There has in the past been a tendency to use initial and boundary conditions chosen for mathematical convenience. There is now a trend to use conditions more relevant to specific real biological situations set up, for example, by previous stages of patterning. This opens the way to studies of how pattern-forming mechanisms might be coupled hierarchically, in a time sequence (see Harrison & Hillier, 1985, for an example from algal morphogenesis).

Given that we are interested here in the effect of an input to a model on the behaviour of that model, how much does it matter which model is being used? Sensitivity to input is a very general property of kinetic theories and, of these, two categories have been fairly extensively studied: reaction-diffusion and mechanochemical theory, the latter involving the viscoelastic properties of cytoplasm and the cytoskeleton (Oster & Odell, 1984; Goodwin & Trainor, 1985). In general, mechanochemical models are promising for pattern formation involving the egg cell cortex (Cheer et al. 1987), but stripe patterns are not affected by anti-cytoskeletal agents (Edgar et al. 1987). This makes diffusion-based models more appealing for the particular case of Drosophila segmentation.

Within the reaction-diffusion category, there are still many models from which to choose. Some are better than others at producing a well-regulated pattern of stripes. Meinhardt & Gierer (1980) have devised one that is particularly stripy, based on long-range activation of mutually exclusive states. Meinhardt (1986) includes it as part of his comprehensive model for segmentation and cell specification within segments. He invokes its striping capabilities, however, mainly as a way of sustaining narrow files of cells in individual segments, not for establishing the initial striped domains. As discussed above, these are essentially being marked off against the gradient. The Meinhardt & Gierer model is nevertheless potentially applicable to all steps of the patterning sequence, from the establishment of gap domains to stabilizing files of cells within segments. It also has other interesting features. As a minimum, reaction-diffusion models require two interacting substances, styled variously X and Y, or A and I for activator and inhibitor. The Meinhardt & Gierer model requires two matched, interacting pairs of such substances, i.e. four substances in all (see Gierer, 1981, for details and examples). These are produced in a mutually exclusive fashion. Cells become switched on for production of one or the other of these pairs, but not both. The dynamics thus have a satisfying resemblance to the way genetic switches are supposed to operate in development with the further implication that regulatory genes involved in pattern should operate in pairs. Applied to the pair-rule genes, this means control of double segment periodicity would be expected to involve, at its core, at least a pair of genes with complementary control functions and fully offset striped patterns of expression.

Making stripes: a role for gradients and boundaries

The Meinhardt & Gierer (1980) model has not yet been subjected to detailed theoretical study and is somewhat complex for illustrating the general effects on reaction-diffusion models of boundaries and gradients. A simpler model is better for this purpose. We use here a variant of the Brusselator (Fig. 1), a model that has been extensively studied in relation both to spatial pattern (e.g. Nicolis & Prigogine, 1974; Kubiček et al. 1978; Murray, 1982) and time oscillations (Tyson & Light, 1973; Schnakenberg, 1979). It is representative of a large reaction-diffusion class called depletion models. One of the two substances required, X, is a self-enhancing (autocatalytic) activator. The other, Y, is used up in the production of X. This makes it a de facto inhibitor of X, because X production slows down as Y is depleted.

Numerous analyses have shown that simple depletion models are not particularly good at producing stripes (Gierer, 1981; Harrison, 1982). Arrays of spots are more typical, but a sufficiently strong asymmetrizing influence can cause stripes to form instead of spots, as shown in Figs 2A–C. In a square domain, with no-flux boundaries all around and no asymmetrizing influences, the Brusselator gives spots in a roughly hexagonal array. The mildest asymmetry is a change in boundary conditions that effectively wraps the square into a vertical cylinder; no flux boundaries at top and bottom, periodic boundary conditions along the sides. This is used in the computations shown in Fig. 2. The result, in Fig. 2A, is the same as would be obtained with an open square.

![Diagram](image)

**Fig. 1.** The model reaction scheme used for Figs 2 and 3, a variant of the Brusselator. The model has two morphogens, X and Y, and rate constants a–e. Y is converted to X via two routes, at a basic rate governed by first-order rate constant b, and by autocatalysis at rate constant c times the square of X concentration. Diffusivities, $D_X$ and $D_Y$, must be such that X, the autocatalytic morphogen, is slower diffusing than Y by a large factor, i.e. at least 25 times.
Greater sensitivity to the boundary can be obtained by adjusting the values of parameters, i.e. of rate constants a–e in Fig. 1. The values we have used in the computations are given in each figure legend, with further analytical treatment of their meaning deferred for a more theoretical account. This is sufficient for our present purpose. The algebraic conditions needed for choosing values are discussed systematically by Lacalli & Harrison (1979). Specifically, for stripe formation, values must satisfy conditions for region (d) behaviour in fig. 2 of that paper. In practice, this means increasing the decay constant for Y (rate constant e). Y, the depleted substance or quasi-inhibitor, is the faster-diffusing of the two substances and carries the main burden of maintaining communication throughout the morphogenetic domain. Results like those shown here in Fig. 2B are then obtained: there is an incipient tendency to form stripes.

In Fig. 2C, a gradient, from high at bottom to low at top, is imposed on the values of two of the rate constants, c and e. The gradient in c, the autocatalytic rate constant, is the more important. Overall top-to-bottom change in c is by a factor of 2, and the increased stripe-forming power of the mechanism is quite striking. The pattern-forming mechanism remains fully interactive at all times throughout the morphogenetic domain. The pattern develops somewhat sequentially, the stripes forming first at the high end of the gradient.

Since Drosophila makes seven stripes, it is useful to show that the model can do the same. This is shown in Fig. 3, for a system roughly 60% longer than in Fig. 2. The gradient is also stretched, so it is shallower, but more stripes are produced. Stripe number depends on spacing, which is little influenced by the magnitude of c. Ratios of the other parameters are more important determinants of spacing, and these are not much changed by the gradient. Fig. 3B is included to show that the pattern does not depend on width. Stripes can develop simply because a system is so narrow as to behave in a pseudo-one-dimensional fashion. This is discussed by Murray (1981), in relation to the striped tails of various mammals.

Based on our computations, patterns with large numbers of stripes as in Fig. 3, are relatively difficult to sustain, i.e. they require a relatively careful adjustment of parameter values. Patterns of three and four stripes or less are much more robust. This may be one reason why Drosophila segmentation occurs in steps with the formation first of coarse subdivisions, essentially broad stripes, and then finer stripes within these. Reaction-diffusion could be responsible for either or both of these processes. Small numbers of stripes are also very robust to changes in the gradient. We have obtained identical three- and four-striped patterns in computations in which the slope of the gradient in c differs by over an order of magnitude, with all conditions otherwise the same.

The above effects depend on boundaries and gradients, both of which provide a bias to the computation on a large, whole-system scale. The way this works may be understood in a very general way, as follows: the unpatterned system can be thought of as initially homogeneous, but subject to random disturbances that contain, in effect, the rudiments of many different sets of equally spaced stripes, with different spacings. The dynamics of the mechanism allows a limited number of these disturbances to grow to expression while others are suppressed. The reaction-diffusion mechanism acts, in a sense, as a chemical amplifier. We can show this graphically by plotting growth rate for pattern rudiments of different spacing versus spacing, as shown in Fig. 4. Such plots are relevant to all reaction-diffusion models, not just the Brusselator, though not all models will be as sensitive to their effects. For stable pattern, the curve in Fig. 4 must pass through a maximum and then go negative at larger spacings. The pattern established will have a characteristic spacing (λ, the pattern 'wavelength') corresponding as closely to that with maximum growth rate as boundary conditions permit. If the curve did not go negative past this point, very-large-scale disturbances (i.e. uniformity) would grow and wipe out the pattern. All large-scale biases in the system, including straight boundaries and linear

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**Fig. 2.** Stages in the generation of three different two-dimensional patterns, time given at the lower right-hand corner of each plot. Each series shows the emerging pattern of distribution on a square grid for morphogen X. The method for generating the pattern is a standard one in modelling: starting with a uniform but unstable steady state, random disturbances are introduced and pattern grows from these. Concentration maxima are dark, minima are light. Boundary conditions are: no-flux at top and bottom, wrap-around (periodic) along the sides, which gives a vertical cylinder, representing the segmentation zone of the Drosophila blastoderm.

(A) The basic tendency of the model is to make spots.

Parameters: a = 0.01, b = 0.001, c = 0.035, d = 0.01, e = 0.003, Dx = 0.004, Dx = 0.18, with no gradients.

(B) Parameter values are adjusted to improve amplification of large-scale features of the system, the top and bottom boundaries in this case, since there is no gradient. Stripe-like elements persist.

Parameters: a = 0.0175, b = 0.001, c = 0.03, d = 0.01, e = 0.02, and otherwise as in A. (C) As in B but with a gradient (bottom to top) in the magnitude of c (0.04 to 0.02) and of e (0.025 to 0.015). The stripes are stable indefinitely. Choosing a square computational grid as a first approximation of reality gives stripes with straighter edges than would be the case with, for example, a more realistic hexagonal grid.
Metameric pattern in Drosophila

A

B

C

3000 4000 6000 9000 40000

1000 2000 4000 9000 40000

800 1600 2000 4000 40000
Fig. 3. (A) Stripes formed in sequence on a longer system. Parameters, gradients and boundary conditions are the same as in Fig. 2C. The pattern is indefinitely stable, even on a wider system (B).

Fig. 4. A plot of growth rate for incipient striped patterns of different spacing versus spacing for a reaction-diffusion model. Patterns of finite wavelength $\lambda$ are fastest growing. This is a simplified version of the exponential growth rate curve for the initial (linear) phase of pattern formation characteristic of reaction-diffusion models generally, adjusted for the choice of parameter values used in Figs 2B, 2C and 3. From Lacalli & Harrison (1979, fig. 2). Similar plots will be found in other theoretical papers.

gradients, act as continuous sources of such disturbances. The effects of these disturbances are made to decay by the patterning mechanism, but their continual presence has an effect on the final outcome: patterns with some large-scale character (stripes) are favoured over those with none (spots). Figs 2B, 2C and 3, to which the curve in Fig. 4 would apply, show this. Fig. 2A gives spots, but its parameters yield a version of Fig. 4 in which the curve first goes negative, then imaginary. This is because it derives from the solution of a quadratic equation containing a negative square root. This generally means some type of oscillatory behaviour is to be expected. In this case, it means that large-scale biases are prevented from having a sustained effect.

A final difficulty arises concerning the robustness of this and related models against change in the numerical value of the parameters. This problem has several aspects. First, robustness of this type might seem to be desirable in a biological control system, but is by no means necessary. In contrast to strictly physical phenomena, living systems are products of long evolution, which implies the possibility of stringent selection, e.g. of enzyme-controlled reaction rates, and little need for quantitative robustness. Second, the kinetic parameters in reaction-diffusion models can themselves vary widely in value so long as certain ratios between them remain within a restricted range. This has implications for how genetic control of such a system might operate, as an obvious advantage is to be gained by having the genes responsible controlled as a group so their products are always present in fixed ratios. Finally, as is true to an extent of many
models, it may be necessary to sacrifice robustness in some aspects to gain it elsewhere. Here we are looking for a particular type of robustness, i.e. constancy in number of pattern repeats against change in gradient steepness, which we have obtained for changes in slope by factors of up to an order of magnitude.

**Subdividing the initial pair-rule pattern: an exercise in scale reduction**

The above computations show stripes with constant spacing being generated roughly in sequence from one end of the system to the other. The real sequence, in the case of the pair-rule pattern, is somewhat different. *ftz*, for example, is first expressed in a broad band which is progressively subdivided to two, then four, and finally seven stripes (Weir & Kornberg, 1985; Macdonald et al. 1986). *even-skipped* and *hairy* do much the same, though with characteristic individual differences (Ingham et al. 1985; Macdonald et al. 1986; Frasch et al. 1987), while only *paired* shows obvious trace of an antero-posterior sequence (Kilchherrefa/. 1986). Periodicity is clearly being generated after the initial activation of the pair-rule genes, and the initial pattern is split up in what appears to be an orderly, spatially coordinated fashion. This can be taken as circumstantial evidence for the involvement of a kinetic mechanism of some type, e.g. reaction-diffusion. This is discussed, for example, by Akam (1987). Ho et al. (1987) reach a similar conclusion from ether shock experiments, which alter segment number but produce, nevertheless, many examples of ordered, evenly spaced pattern.

Pattern subdivision is a common feature of reaction-diffusion models. Typical behaviour for the Brusselator is for concentration peaks of the autocatalytic morphogen to broaden and then split in the centre (Fig. 5A–C). The peaks move as a new concentration minimum is established. If pattern spacing is fixed, such splitting will occur as the system increases in size, so more pattern repeats can be accommodated. If size is fixed, splitting can occur due to changing parameter values, which reduces the effective wavelength of the patterning mechanism. Typically, wavelength will be long when precursors are in poor supply and short as they become more concentrated (Harrison & Tan, 1988). If the precursor supply is at first building up from a low value, the pattern might be expected to start out with a small number of large parts, and later split up into smaller elements. Incorporating a parameter change of this type into the model used here is one way of converting its sequential behaviour to something closer to what is observed in reality. The detailed shape of the curve (e.g. in Fig. 5) is less important in this regard than the relative positions and movements of concentration maxima and minima. Peaks can be broadened and flattened by adjusting the model in various ways, e.g. through saturation effects or by using fixed-concentration boundary conditions as suggested by Arcuri & Murray (1986), without altering the basic behaviour.

Both gap and pair-rule genes have a controlling influence over *ftz* pattern, and mutations at both types of loci have the general effect of reducing the total number of stripes, as if the subdivision process were being retarded or arrested. This sometimes affects the whole pattern, as in *hairy* mutants, which have a four-stripe pattern whose elements are normally positioned but incompletely resolved (Howard & Ingham, 1986; Carroll & Scott, 1986). Gap mutants, in contrast, produce mixtures of broad and narrow stripes (Carroll & Scott, 1986) suggesting a degree of autonomy or local control over the subdivision of individual elements of the four-stripe pattern. The differential expression of gap genes along the

![Pattern subdivision](image)
body axis provides a heterogeneity at this stage that could allow for the autonomy observed.

One important feature of the splitting process, judging from Weir & Kornberg (1985), is that ftz minima, once established, remain fixed in position as the peaks between them continue to divide. This is an important observation. In theoretical terms, fixing a particular minimum, which amounts to establishing a new boundary condition within the system, allows for better control of subsequent events. A suggestion of how this might work is shown in Fig. 5. If the process shown in Fig. 5A–C were continued, the two peaks in Fig. 5C would each split again to give four as in Fig. 5E. Suppose some heterogeneity in the system (e.g. due to the gradient or gap gene expression) allowed one peak to get a head start. A pattern of three evenly spaced peaks could take over before the four-peak one. For this to happen, there must be a shift in the position of the central concentration minimum (Fig. 5D). An internal boundary of the fixed-concentration type, established at the site of the original minimum, would prevent this so that only symmetrical modes of subdivision could occur. Four peaks would then form as in Fig. 5E. The gap genes could clearly be involved in some way in this process if either peaks of gap gene expression or boundaries between adjacent cardinal domains could act to define or sustain a particular pair-rule minimum. It is already recognized that the boundaries between cardinal domains may be important in organizing the pair-rule pattern. This is a central component of Meinhardt's 1986 model, for example. The exact positions of such boundaries in relation to the registry of pair-rule stripes is thus of crucial importance in assessing the claims of competing models (Lawrence, 1987). The issue is further complicated, however, if a reaction-diffusion model is involved. We are suggesting that landmarks of an established pattern act to control subsequent patterning events via reaction-diffusion, but without being able to specify which landmarks are most relevant, i.e. whether peaks, troughs or boundaries. How such landmarks act will depend on mechanistic details governing the way output from one model is converted to input for another and there are, in theory, various possibilities. This is one aspect of the theory that requires further study.

Examples of pattern subdivision attributable to reaction-diffusion can be seen elsewhere in biological systems, notably in unicellular algae, in which projecting lobes of the cell wall show a time sequence of branching at progressively decreasing spatial scale (Lacalli & Harrison, 1987; Harrison & Hillier, 1985). Both there and in Drosophila, we are dealing with a process of pattern subdivision in the absence of cellular organization, in giant unicells in the case of the algae and a syncytial array of nuclei in Drosophila. It may not be coincidence that these so far provide the best circumstantial evidence for the involvement of reaction-diffusion in pattern formation. There are a variety of ways cells in an epithelium might interact in order to generate pattern. Local inductions or signalling between neighbours are examples. Models of cell interaction often require cells to interact with specific neighbours on an individual basis, or depend on their ability to determine position or polarity in relation to some fixed frame of reference. Both are facilitated by dividing the system into cells, i.e. discrete units with fixed boundaries able to control events at those boundaries. Nuclei in the syncytial blastoderm will have more limited capabilities and reaction-diffusion may be the best, if not the only, means of pattern formation available to a Drosophila embryo at the syncytial stage.

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