Mechanical aspects of mesenchymal morphogenesis

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SUMMARY

Many embryonic cells generate substantial contractile forces as they spread and crawl. These forces mechanically deform each cell's local environment, and the resulting distortions can alter subsequent cell movements by convection and the mechanisms of contact guidance and haptotaxis. Here we develop a model for the cumulative effects of these cell-generated forces and show how they can lead to the formation of regular large-scale patterns in cell populations. This model leads to several predictions concerning the effects of cellular and matrix properties on the resulting patterns. We apply the model to two widely studied morphogenetic processes: (a) patterns of skin-organ primordia, especially feather germ formation, and (b) the condensation of cartilagenous skeletal rudiments in the developing vertebrate limb.

INTRODUCTION

Many morphogenetic processes involve coordinated movements of populations of mesenchymal cells. The patterns of skin organ dermal papillae and the condensation of chondrocytes in cartilage formation are two examples. A number of factors known to affect cell motion have been proposed which could help orchestrate these cellular movements, including chemotactic morphogens, contact guidance by the extracellular matrix (ECM) (Weiss, 1929; Abercrombie, 1970; Bell, Ivarsson & Merrill, 1979), haptotaxis – motion up an adhesive gradient (Carter, 1967; Harris, 1973), contact inhibition and simple forward biasing of cell motion (Trinkaus, 1982; Englander & Davies, 1979; Gail & Boone, 1970; Gould, Selwood, Day & Wolpert, 1974).

While all of these mechanisms probably act at some point in development, it is not clear how they conspire to generate organized spatial aggregations of cells. In this paper we will describe a simple mechanism by which patterned aggregates of motile cells can come about. The mechanism we shall describe is speculative in the sense that we have not demonstrated unequivocally that embryonic patterns do arise this way. Nevertheless, since the process we shall describe is an

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inevitable consequence of known cellular properties, it is difficult to imagine that it does not contribute to cellular morphogenesis. To buttress our scenario we shall describe how our model explains a number of in vitro observations, and propose a partial explanation for the formation of skin organ primordia and cartilage anlagen in chondrogenesis.

The role mechanical forces can play in driving morphogenetic movements of epithelial sheets has been discussed by Odell, Oster, Burnside & Alberch (1981), where they showed that mechanics on the scale of cells and embryonic tissues had a peculiar and counterintuitive character: forces generated by cell shape changes at one position in the embryo were equilibrated over the entire embryo virtually instantly. Thus entire tissues could be regarded as being in mechanical equilibrium. Consequently, mechanical forces could coordinate the shape changes of large cell sheets, without the intervention of long-range chemical signalling.

Our model is based on two properties of mesenchymal cells in vivo: (i) cells spread and migrate within a substratum consisting of fibrous extracellular matrix (ECM) and other cells (Hay, 1981). (ii) motile cells can generate large traction forces on the ECM (Harris, Stopak & Wild, 1981). From this, we will show that the mechanical interaction between the motile cells and their elastic substratum affect the cells' motion in such a way as to organize large scale spatial patterns.

We shall base our arguments on very simple and well-established assertions concerning cell motions and ECM properties. Nevertheless, we make use of physical and mathematical arguments that may be unfamiliar to some readers. Therefore, we have organized the paper as follows. In Section II we describe how to model various types of cell motions. Our treatment here is heuristic and we place the mathematical formulation in the Appendix. Even so, this section is necessarily somewhat technical, and can be skipped on first reading – analogous to a materials & methods section in an experimental paper. Section III discusses the morphogenetic consequences of cell traction in the simplest one-dimensional setting. Section IV applies the model to the specific case of dermal papillae formation during development of feather germs. Section V shows how mechanical forces can contribute to chondrogenic condensations during limb morphogenesis. Section VI discusses other settings where traction-induced patterns may contribute to morphogenesis. Finally, Section VII summarizes our findings about the possible role of cell tractions in development.

II. DESCRIPTION OF THE MODEL

In this section we shall give a heuristic description of our model for mesenchymal morphogenesis. We have provided a word equation and/or diagrams to accompany the mathematical expressions (which are elaborated in the Appendix), so that the model can be understood qualitatively from the text alone. We commence our discussion by describing certain characteristics of cell motion, and how they can be described quantitatively.
Cell movements and a classification of guidance mechanisms

Mesenchymal cells within embryos nearly all have a capacity for autonomous locomotion which is analogous to that of amoebae, but is perhaps best described as crawling. The individual cells move by exerting forces upon their surroundings, which generally consist of a fibrous extracellular matrix and the surfaces of other cells. These tractions require that the moving cell has established anchor points to its substratum. The traction forces are exerted by cellular extensions, often flattened, which Abercrombie termed ‘leading lamellae’ (Harris, 1983). Most cells have several of these protrusions extending in opposing directions. Since the traction exerted by each is directed centripetally, the result is a tug-of-war with net cell displacement occurring at the direction of the lamellae with the strongest tension, and/or the strongest adhesions to the substratum.

The speed and direction of cell movements can be influenced by several kinds of extracellular factors. In order to discuss the various mechanisms of cell guidance it is useful to adopt the following generally accepted terminology for cell motions (c.f. Lackie & Wilkinson, 1981).

1. Kineses

A generalized increase in motile activity without any directional component is called ‘kinesis’. In this mode cells execute a strictly random walk within an isotropic substratum, their motion being analogous to a randomly diffusing particle. Note, however, that even a randomly diffusing cell will move statistically down a concentration gradient; however, it is convenient to distinguish this kind of ‘directed’ movement from substrate-directed movement.

2. Taxes

Directed motion up (or down) a gradient in the cells’ environment is called ‘taxis’. For example, motion toward a source of chemoattractant is positive chemotaxis. An important taxis for our considerations is ‘haptotaxis’, which is the directed movement of cells in response to adhesion gradients in their substratum (Carter, 1965; Harris, 1973). A taxis can appear as a biased random walk (i.e. biased diffusion/kinesis); this distinction, however, is only important when we model the various components of cell motion mathematically.

3. Guidance

Cues in the substratum such as strains or curvature can induce directed motion; Weiss (1929) called this ‘contact guidance’ when referring to the orientation of cell extension and displacement parallel to tracts of extracellular matrix fibres. Guidance differs from taxes by its property of ‘bidirectionality’. That is, cells following a groove or fibre cannot distinguish directions: the fibre provides an orientation cue, but not a preferred direction.
Lackie & Wilkinson (1981) call kinesis, taxis and guidance 'scalar', 'vector' and 'tensor' cues, respectively, since the first is non-directional, the second is directional, and the third is bidirectional (see also Keller et al. 1977). This terminology is in accord with the kinds of terms required to model cell motions mathematically.

**Random cell motions**

Random, nondirected cell movement is analogous to diffusion of particles in a gas or liquid, and the same mathematical treatment can be used to describe both situations. This remains true even when cells migrating in a medium, with no directional cues exhibit an appreciable tendency to keep moving for long periods in roughly the same direction (Trinkaus, 1982). Random motions are conventionally modelled as a diffusion process akin to Fick's Law governing diffusing molecules (Lackie & Wilkinson, 1981; Okubo, 1980; Peterson & Noble, 1972). We denote by $J$ the flux of cells; that is, the number of cells passing through a cm$^2$ window per unit time. Fick's law states that this flux is proportional to the gradient in cell density, which we denote by $n$ (the number of cells per cubic centimeter):

$$J = \text{Flux of cells (number of cells crossing 1 cm}^2/\text{sec})$$

$$\propto \text{gradient in cell density}$$

$$= -D \nabla n$$

$$= -D \frac{dn}{dx} \text{ (in one spatial dimension)} \quad (1)$$

where $D$ [cm$^2$/sec] is the diffusion constant, and $\nabla n$ denotes the gradient in cell density. The minus sign in Fick's law ensures that cells disperse away from regions of high concentration (i.e. down the gradient in cell density).

It turns out that Fick's law (1) is not sufficient to model random cell motion *in vivo*. Since it is based on the assumption of a very dilute system, so that interactions between cells are weak and limited at most to nearest neighbours. Typical cell densities encountered in mesenchymal cell populations are quite high, and cells interact strongly with their neighbours, either adhering to or avoiding them. Moreover, cells frequently interact with other cells beyond their nearest neighbours via filopodia and other cell protuberances (see, for example, Plates 8 & 11 in Hinchliffe & Johnson, 1980). In order to model this type of interaction we must add to Fick's law a long-range interaction term. In Appendix A.1 we show how to model this effect, which amounts to adding an additional 'diffusion' term to Fick's law (this term is called 'biharmonic diffusion' in the literature, for reasons discussed in Appendix A.1). Thus the expression for random cell motions becomes

$$J = \text{short-range random motion (Fick's Law) +}$$

$$\text{long-range random motion (biharmonic diffusion)} \quad (2)$$
Directed cell motions

The diffusive processes described in the preceding section would, by themselves, cause a cell population to approach a homogeneous spatial distribution. Countering these dispersive forces are several effects which tend to organize cell populations by allowing cells to move into regions where the cell density is already higher than the surrounding area. Several such antidiffusive effects can be deduced from known phenomena of cell behaviour in tissue culture; we will discuss some of these effects in this section.

Cells can be dragged passively: convective motion

Cells can be passively dragged along by the contractions of its immediate neighbours, or ride on the substratum which is being dragged by the contractions of distant cells. For cells in vivo, the substratum is the extracellular matrix and/or other cells. The expression for convective cell motions has the form

\[ [\text{convective cell flux}] = [\text{cell density}] [\text{mean cell velocity}] \]  

The velocity here is the local mean velocity of the matrix the cells are sitting on.

Cells will follow an adhesive gradient: haptotaxis

Motile cells will move from less adhesive to more adhesive regions of their substrata (Gustafson & Kinnander, 1960; Gustafson & Wolpert, 1967; Carter, 1965, 1967; Harris, 1973; Pouyssegur & Pasten, 1979). The directionality of this movement results from competition between opposite sides of individual motile cells: each side of a cell forms adhesions in the substratum and engages in a tug-of-war with net displacement occurring in direction of that side with the strongest pull and the firmest attachments to the substratum. Thus a cell’s otherwise random motion will be biased up the adhesive gradient. We can model this effect by writing the cell flux due to haptotaxis as

\[ \mathbf{J} = [\text{cell density}][\text{average cell velocity up the gradient}] \]

The simplest way to relate the net cell velocity up the adhesive gradient to the steepness of the gradient is to assume that the mean velocity of migration is proportional to the steepness of the gradient. That is,

\[ \mathbf{J} \sim n \text{ [gradient in adhesiveness]} \]

The local density of adhesive sites [No. sites/cm^3] is proportional to the density of the matrix material. Therefore, we can write the above equation as

\[ \mathbf{J} = \alpha n \text{[gradient in ECM density]} \]  

where the parameter \( \alpha \) is the ‘haptotactic coefficient’, which measures a cell’s tendency to move up an adhesive gradient – analogous to the diffusion coefficient, which measures the tendency of a cell to move down a cell-density
gradient. The effect of (4) is similar to that for chemotaxis: the gradient in adhesive-site density is replaced by the gradient in chemoattractant. We shall ignore chemotactic effects in our subsequent discussion since evidence is lacking that they contribute to metazoan morphogenesis.

Cells can follow cues in the substratum: contact guidance

Numerous experiments have shown that cells will follow geometrical cues in their substratum, such as aligned fibres, grooves, curvature, etc. (Elsdale & Bard, 1972; Dunn & Ebendahl, 1978; O'Hara & Buck, 1979; Belousov, 1979a,b, 1980; Belousov, Dorfman & Cherdantzer, 1975; DeHaan, 1964; Katz & Lasek, 1980). Weiss (1929) called this phenomena 'contact guidance'. The principle difference between cell guidance and taxis is that the latter is directional, the cells moving up or down a gradient, while the former is bidirectional: the cells move along the guidance cue in either of two opposite directions.

A particular type of guidance that can be important occurs when cells are moving within an oriented fibrillar matrix: they will tend to follow the alignment of the matrix (see also Ebendahl, 1976). This alignment can be brought about by mechanical strains produced by the cell tractions (c.f. Lackie & Wilkinson (1981), pp. 17–24). Moreover, cells that align themselves by substratum cues will also tend to exert greater tractions along their long axes, thus further contributing to stress orientation in the guidance direction. We shall call this strain guidance, and it can be modelled easily by allowing the diffusion coefficients in (2) to be functions of the strain, which we denote by $\varepsilon$. ($\varepsilon$ is a second rank tensor, which is defined mathematically in the Appendix.) Other types of cell guidance are modelled the same way; for example, if cells follow local curvature cues, then $\varepsilon$ would be replaced by the local curvature tensor (which is also of second rank).

Finally, if there is appreciable cell division within the volume element, a mitotic source term must be included. In the following discussion we shall assume for simplicity that cell division follows a sigmoidal, or logistic growth curve of the form

$$\text{mitotic rate} = rn(N-n)$$

where $r$ is the mitotic rate and $N$ the maximum cell population.

The equation for cell motion in an extracellular matrix

Now that we have derived expressions for the various kinds of cell fluxes we can add all the effects together in an equation that will describe the net motion of a population of cells migrating through a fibrous extracellular matrix. Consider a small volume element fixed within the ECM. The rate of change of cell density within that volume element is given by the balance between the total cell flux into and out of the volume element:
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\[ \text{rate of change of cell density in a volume element} \]
\[ = [\text{flux into the volume}] - [\text{flux out of the volume}] \]
\[ + [\text{cell division rate}] \quad (6) \]

If we insert the various flux expressions into (6) we arrive at the model equation for cell motion:

\[ \text{rate of change of cell density} = [\text{short range random motion}] \]
\[ + [\text{long range random motion}] \]
\[ + [\text{haptotaxis}] \]
\[ + [\text{convection of cells on ECM}] \]
\[ + [\text{cell division}] \quad (7) \]

The mathematical version of equation (7) is derived in Appendix A.1.

Modelling the extracellular matrix

Equation (7) simply describes how a population of cells would move within a substratum (ECM) with a given adhesive gradient and applied strains. As it stands, no pattern is likely to emerge unless it is imposed by some external agent. In order that the cells evolve spatial patterns spontaneously, without external cues there must be a mechanism for the cells themselves to control their guidance factors.

Cells exert large tractions which deform the substratum

Spreading and migrating cells can exert extremely large traction forces on their substratum. These cell-generated forces can substantially deform the substratum: on artificial silicone-rubber culture media the strains extend for hundreds of cell diameters (Harris et al. 1980). In vivo, it is known that fibroblasts migrating into wound areas produce the disfiguring scars characteristic of wound contraction (e.g. Gabbiani, Masno & Ryan, 1972). The large mechanical tractions that mesenchymal cells can generate provide a unique mechanism for cells to control their own guidance cues and thus to coordinate their morphogenetic movements over distances of at least several centimetres.

Cell tractions create adhesive gradients and guidance cues

A spreading or migrating cell adheres to the matrix material at various points on its surface, and exerts a contractile force, or traction, on these adhesions. If this contractile force is stronger than the adhesions elsewhere on the cell, the cell will pull itself in the direction of the net force vector exerted by these appendages. If the matrix material is deformable, these contracting appendages will also draw the matrix in toward the cell, compressing and aligning it. Thus the cell can be viewed as a local source of contractile force within the ECM – a centre of compression – which bunches up the matrix nearby. Fig. 1 shows how cells explanted on a silicone-rubber substratum produce compression wrinkles under
the cells, and tension wrinkles radiating outward from the contracting cells. These tension wrinkles can extend many hundreds of cell diameters, affecting cells quite distant from the centre of contraction.

Along with the strain lines, the contracting cells will compress the matrix so as to create a gradient in the density of adhesive sites, as shown schematically in Fig. 2. Thus a previously isotropic ECM, when seeded with contractile cells, will not remain isotropic: the cells’ contractions create strain guidance cues and adhesive gradients which guide the surrounding cells inward toward the centre of contraction.

*The ECM is in mechanical equilibrium with the cellular forces*

Mechanics at the cell and tissue level does not follow the laws of Newtonian mechanics, where force = mass × acceleration. The reason is that, for cellular and embryonic processes, the inertial terms in the equations of motion are negligible compared to viscous and elastic forces. That is, there are no inertial effects at work in cellular mechanics: cells obey Archimedian mechanics wherein motion ceases instantly when the applied forces are turned off (Purcell, 1977;
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Odell et al. 1981; Oster, Odell & Alberch, 1980; Oster, 1983). So in describing the mechanical balance of forces between the cells and the ECM the following equilibrium condition holds true at every instant:

\[
\left\{ \begin{array}{l}
\text{Elastic restoring forces} \\
\text{produced by the ECM}
\end{array} \right\} = \left\{ \begin{array}{l}
\text{Traction forces generated} \\
\text{by the cells}
\end{array} \right\}
\] (8)

In words: the cell tractions are in mechanical equilibrium with the elastic forces of the matrix material. In order to realize this word equation mathematically we must call on some elementary results from the theory of elasticity; in keeping with the heuristic nature of our discussion we refer the reader to Appendix A for this discussion. For our purposes we can imagine the ECM as a three-dimensional 'bedspring', as illustrated in Fig. 3.

From the figure we see that if the matrix material is stretched along, say, the x-direction, then not only are the springs parallel to the applied force stretched, but there is a lateral compression as well. Thus deformations in one direction are necessarily accompanied by deformations in the other directions. To account for this effect we need another material constant in addition to the modulus of elasticity, \( E \) (Young's modulus). Poisson's ratio, \( \nu \), accounts for the phenomenon that, when an elastic material is stretched in one direction, it compresses transversally. \( \nu \) measures the magnitude of this lateral compression; it is the Poisson effect that accounts for the tension wrinkles in the elastic substratum in Fig. 3.

Another consequence of the Poisson effect will be important when we discuss feather germ morphogenesis. If the ECM is stretched between two centres of cell traction, then not only will the material between these centres be aligned, but the matrix density will increase as well due to the transverse compression (see Fig. 2). Fibrous materials can have Poisson ratios much higher than ordinary elastic

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**Fig. 2.** In an undeformed, isotropic matrix the adhesive sites are randomly distributed, giving no preferred directional cues. Cell tractions will compress the material and generate a gradient in adhesive sites. The adhesive gradient will be oriented towards the centre of traction, and — via the Poisson effect (c.f. Fig. 3) — towards the centreline joining two compression centres.
Fig. 3. An elastic matrix can be viewed as a three-dimensional 'bedspring' consisting of elastic elements cross linked at various points. If a longitudinal strain is imposed by opposing cell tractions, a transverse compression will be generated. The magnitude of this transverse compression is given by the Poisson ratio: $v = (W - W_0)/(L - L_0)$. For fibrous materials, $v$ can be much larger than 1; Stopak (1983, pers. comm.) has measured values as high as 5 to 8 in collagen gels.

materials; Stopak (1983) has measured the ratios of lateral compression to longitudinal extension between fibroblast explants in collagen gels and found values as high as 5 to 8. Thus the transverse compression effect that accompanies cell traction can be as large as the compression due to the tractions themselves. This transverse compression creates a transverse component of the adhesive gradient. Haptotaxis should therefore cause cells to migrate into the region between traction centres as well as toward the centres of traction.

**Cells tend to align along strain directions**

Several workers have documented the phenomenon of 'strain alignment': within an aligned matrix, such as a collagen gel, cells will configure themselves such that their long axis is in the same direction as the matrix orientation (Harris et al. 1981; Bellows, Melcher & Aubin, 1982). This phenomenon suggests a role for matrix orientation in the development of oriented tissues, such as muscle and tendon (Harris, 1983).

At the cost of considerably complicating the model one can add an 'orientation vector' to the cell description. However, this may not really be necessary, since the displacement field, $u$, of the matrix shown in Fig. 3 provides a good cue to cell polarization. From this figure we see that the Poisson effect will first guide cells into the region between two centres of contraction, where they will tend to align along the axis between the centres. Since increasing fibre density may
eventually impede cell motion, if the Poisson compression between the contraction centres is too great, the cells migrating into this region may not actually be able to migrate far towards the ‘poles’. Thus they will settle down in their axial orientation and proceed with their subsequent differentiation – which, indeed, may be influenced by their state of orientation and packing density.

The extracellular matrix can be modelled as a simple viscoelastic material

The simplest elastic material to model mathematically would be one which was both isotropic (resisting distortions equally in all directions) and linearly elastic (stress directly proportional to strain). The artificial silicone-rubber substrate approximates these ideal properties, at least at small strains. On the other hand, extracellular matrix material, such as collagen and GAG, can certainly be highly anisotropic and nonlinear. For example, collagen gels frequently exhibit ‘plasticity’: deformations are apt to ‘set’ and the material remains deformed even after stresses are removed. However, to keep things manageable we shall assume that the ECM is initially isotropic, offering no cues for orienting cell movement, until the cell tractions generate these cues. There is no difficulty in extending our theory to cover situations in which the matrix is anisotropic or nonlinear, and these generalizations will be dealt with in a subsequent publication.

Modelling the traction forces developed by the cells

We will assume that the magnitude of the contraction forces developed by a population of cells is proportional to the number of cells acting. Moreover, this force should also be proportional to the density of matrix material on which they adhere, since if there were no matrix there could be no traction (recall that we have already assumed that the density of adhesive sites is proportional to the matrix density). That is

\[
\text{Stress developed by the cells} = \tau n
\]

where \(\rho [\text{gm/cm}^3]\) is the matrix density and \(\tau [\text{cm}^7/\text{sec}^2]\) is the proportionality constant that measures the force generated per cell per unit mass of matrix. Strictly speaking, the traction \(\tau\), should be a decreasing function of cell density, \(\tau = \tau(n)\), since there must be an upper limit on the amount of force an aggregation of cells can generate. The form of \(\tau(n)\) is an empirical question, and is unimportant for our discussion so long as we impose an upper limit to the maximum cell traction.

The conservation equation for matrix material

To complete our model for cell motion we must keep track of the matrix material. Where cell tractions bunch up the ECM its density increases, and where it is stretched out its density decreases. Thus we can write a mass balance on the matrix density as
\[
\begin{aligned}
\left\{ \text{rate of change in matrix density} \right\} &= \text{[flux in]} - \text{[flux out]} \\
\text{in a small volume element} & \quad (10)
\end{aligned}
\]

By adding a term to the right-hand side of (10) we could also account for matrix secretion by the cells. However, we shall assume for simplicity that, on the time scale of cell movements we are interested in, matrix secretion is negligible.

Equations (7), (8) and (10) comprise a complete model for cell motion in an elastic ECM. While the mathematical structure of the corresponding equations derived in Appendix A appears complicated (it is), the physical interpretation of the model is straightforward (see Fig. 4). Equation (7) is simply a balance equation describing how local cell density changes due to cell division and the various types of cell motion: kinesis, haptotaxis and strain guidance. Equation (8) expresses the fact that cell tractions must be counterbalanced by the elastic forces of the ECM. Equation (10) is a mass balance on the matrix material. We emphasize that these equations simply embody the experimentally well-documented properties of cell motility; we have not invented any novel or hypothetical behaviours for cells. The mechanical equation coupling the cells to the ECM is the simplest possible model: more complicated assumptions would only enhance the model's pattern-forming predictions.

*The parameters control the model's behaviour in dimensionless groupings*

The mathematical model formulated in Appendix A contains 10 parameters, which in principle are measurable: we list them here:

\[
\{D_1(\epsilon), D_2(\epsilon), \alpha, r, \tau, N, E, v, \mu_1, \mu_2\} \quad (11)
\]

![Diagram](image_url)

**Fig. 4.** The causal chain contained in the model equations. Cell tractions, necessary for cell motion and spreading, also deform the extracellular matrix material. These deformations move cells attached to the matrix convectively, and produce guidance cues which influence cell motions via contact guidance and haptotaxis. In turn, these directional cues tend to cause cells to aggregate into patterned clusters.
Each parameter controls a different physical effect. Nondirectional cell migration – cell kinesis – is controlled by the magnitude of the diffusion parameters, $D_1$ & $D_2$, while the cells’ sensitivity to contact guidance is incorporated into their dependence on the strain, $\varepsilon$. $\alpha$ controls the haptotactic response, and $\tau$ governs the amount of traction the cell exerts on the ECM. $r$ and $N$ control the mitotic rate and the maximum cell density, respectively. $E$ and $\nu$ characterize the elastic properties of the ECM, and $\mu_1$ and $\mu_2$ are two viscosity coefficients that describe its viscous properties (see Appendix A). In addition, it will turn out that the size and shape of the domain will be important characteristics in determining the spatial patterns. This will add geometrical parameters to the above list.

In the Appendix we show that these parameters affect the model’s behaviour not singly, but in dimensionless groups. For example, when discussing the effect of cell traction it is not the parameter $\tau$ which is important, but rather the dimensionless traction parameter

$$
\tau^* = \tau \varphi_0 N (1+\nu) / E
$$

(12)

where $\varphi_0$ is some initial density of the ECM.

After nondimensionalizing the model there remains but seven composite parameter groupings that govern the model’s behaviour. We want to emphasize the point that variations in one parameter can be compensated for by variations in another. This interdependence is important for understanding how anatomical patterns are regulated, and in particular how the system can compensate for imposed or experimental alterations. In equation (12), for example, a reduction in cell traction can be compensated by increases in cell density, or changes in elastic properties of the matrix material.

The parameters are experimentally measurable

The model parameters listed in (11) can be divided into three groups: (1) cell properties ($\{D_1, D_2, \alpha, \tau, r, N\}$; (2) matrix properties $\{E, \nu, \mu_1, \mu_2\}$; (3) geometrical parameters (e.g. length, $W$, and width, $B$, of the domain, and shape = $B/W$). The viscoelastic properties of the ECM are easily accessible to measurement by standard physical techniques, as are the geometrical parameters (c.f. Wainwright, Biggs, Currey & Gosline, 1976). The cell properties $D_1$, $D_2$ and $\alpha$ can be measured by the same techniques discussed by Lackie & Wilkinson (1981): i.e. by measuring mean free paths and cell trajectories. The division rate parameters, $r$ & $N$ are also measurable, in principle, although in vivo estimates may have to be based on estimates of mitotic index from autoradiographic and other techniques. Finally, the crucial traction parameter, $\tau$, can be estimated in vitro by measuring the amount of deformation cells can produce in a calibrated silicone-rubber substratum (Harris, Wild & Stopak, 1980).
III. HOW THE PATTERNING MECHANISM WORKS

One-dimensional spatial patterns

Consider the simplest possible geometric arrangement: a strip of elastic ECM in which cells are free to migrate only along the x-axis, as shown in Fig. 5. Suppose that the cells are initially distributed uniformly in the region between \( x = 0 \) and \( x = L \) with cell density \( N \), and further assume that the cell traction, \( \tau \), is quite low. In this situation the cells will distribute themselves uniformly over the strip, and no spatial pattern will form.

The uniform steady state can become unstable

Now let cell traction gradually increase. (Note that equation (12) shows that increasing \( \tau \) has the same effect as increasing the ECM density, \( \rho_0 \), the cell density, \( N \), or the Poisson ratio, \( \nu \), since they all enter in the same way into the dimensionless traction parameter, \( \tau^* \).) At first, the cell and matrix densities will continue to remain uniform everywhere. However, there is a critical value of \( \tau = \tau_c \) whereupon the uniform cell distribution commences to break up into local

![Diagram showing cell density distribution](image)

Fig. 5. A strip of ECM where the initial cell traction is below the critical level, \( \tau_c \), will not be able to support any aggregation centres. If \( \tau \) rises above the bifurcation value, \( \tau_c \), a single aggregation centre will arise. If \( \tau \) rises still further, a second bifurcation threshold is reached wherein the stable cell distribution exhibits two aggregation centres. Successive bifurcations will yield cell distributions with larger numbers of periodic aggregation centres, whose separation can be calculated approximately from equation (13). The same pattern can be generated by varying other parameters than the cell traction \( \tau \).
cell concentrations. In technical terms, this is a bifurcation point, where the previously stable uniform distribution becomes unstable, and a new stable solution to the equations emerges. This new solution is characterized by islands of high cell density alternating with regions of low cell density. How this comes about in the model is described in Appendix B; intuitively the reason is as follows.

Below the critical traction, $\tau_c$, the aggregate cell tractions deform the ECM and create local strain guidance cues and adhesive gradients as described above. However, these organizing effects are too weak to overcome the dispersive influence of the random movements. When the critical traction is reached the organizing forces start to overwhelm the dispersive action of diffusion. When a local concentration of cells forms by a chance fluctuation the contractions of these cells create a local focus of adhesiveness as well as a radial strain guidance field. Together these cues focus the movement of neighbouring cells in toward the established centre of contraction. At first glance, the only structure that could arise is an aggregation at a single focus. However, here the mathematics predicts the surprising result that multiple cell condensations can occur. This happens because the contractile forces generated in the growing focus of contraction are resisted by the elasticity of the matrix material. That is, the aggregate passive elastic resistance of the ECM far away from the condensation region eventually equilibrates with the contractile action of the cells within the condensation, thus effectively limiting the range of influence of a cell aggregation.

Here we have used the cell traction as the bifurcation parameter. We could equally well have used any of the other parameters.

In some ways this process is analogous to what happens in diffusion-reaction instabilities: at short ranges the contraction of a cell aggregation recruits the neighbouring cells, but this attraction is attenuated as one moves away from the centre. Thus contraction is analogous to short-range activation, and the elastic attenuation by the ECM plays the role of a long-range inhibitor (Meinhardt, 1982; Murray, 1981a,b).

From the analysis outlined in the Appendix the model predicts that when patterns first appear the spacing between the cell aggregations is given approximately by the expression

$$\text{Spacing} \approx 2\pi[D_2/rN]^{1/4}$$  \hspace{1cm} (13)

However, the precise pattern that eventually evolves can only be determined by numerical simulation of the model equations. These simulations will be presented in a separate publication; here we shall restrict ourselves to a qualitative exposition of the phenomenon of mechanical pattern formation. Note that the expression in equation (13) covers the case when cell division is appreciable ($r > 0$); patterns can still form in the absence of cell division ($r = 0$), but another formula must be used to estimate the spacing.
Spatial patterns can arise without cell motility

In our discussion so far we have focused on cell aggregation brought about by haptotactic steering of cell motions. However, it turns out that the model predicts that cell aggregation patterns can arise even if cells are not motile, so that the only mechanism of cell motion is by being passively dragged toward a contraction focus. The reason for this phenomenon is less intuitively obvious than patterns that arise from cell migration; we discuss this effect in the Appendix: it turns out that it can only happen in dense cell cultures wherein cell filopodia extend over several cell diameters. This is the case in chondrogenic condensations, which we shall discuss below, and possibly in other situations as well.

Spatial patterns may arise from a combination of effects

Fig. 6 illustrates an important feature of this morphogenetic mechanism that we mentioned above: combinations of parameters may have equivalent effects. The bifurcation to spatial patterns as \( \tau \) increases occurs along path B, as shown. There are other ways of moving into the domain of spatial structure. For

\[
\frac{\sqrt{\tau D_2}}{\alpha \phi_0} = \frac{\sqrt{\tau N D_2}}{\alpha \phi_0}
\]

\[
\frac{\tau \phi_0 N (1 + v)(1 - 2v)}{E (1 - v)}
\]

Fig. 6. The bifurcation diagram showing the domain in dimensionless parameter space wherein spatial structures will arise. (The bracketed expressions are the equivalent dimensional expressions.) This diagram shows that the same pattern can arise from various combinations of effects, since the crucial quantities are the dimensionless parameter collections. Thus a system initially at P can cross the bifurcation threshold along path B if the cell traction, \( \tau \), increases, along path D if the haptotaxis, \( \alpha \), increases and/or the random motion, \( D_2 \), decreases; increasing the matrix density, \( \phi_0 \), will cause the system to traverse path C, since \( \phi_0 \) appears in both the dimensionless quantities; similarly, increasing cell density, \( N \), will generate spatial patterns along the path A.
example, a path like A can be traversed by increasing the cell density, N, or path D by increasing the haptotaxis parameter, α, and path C by increasing the matrix density, ϕ₀. By varying the matrix properties, E and ν we can move along some other path. Indeed, it is clear that variations in combinations of these can also initiate bifurcations, so that the emergence of spatial pattern may not be attributable to a single cell property, but may result from a composite of factors affecting both cell behaviour and matrix properties.

*Patterns of increasing complexity arise as domain size increases*

Successive bifurcation boundaries can also be traversed by increasing the domain size. This mechanism is analysed in detail in Appendices A.3 and A.4. Both domain size and cell/matrix properties can cause spatial structures to emerge. This is an especially important process since tissue size and shape are being constantly altered during embryonic growth.

*Simultaneous versus sequential pattern formation*

In the discussion so far we have assumed that the system parameters which initiate the bifurcation to spatial patterns change simultaneously over the entire tissue domain. That is, all cells pass through the bifurcation boundary at the same time. This is probably rare in embryogenesis, for tissues grow in size by cell division and recruitment, and so there are frequently substantial gradients in tissue 'age' – and therefore cell properties – by the time morphogenesis commences.

To see the effect of such age gradients on the pattern we reconsider the one-dimensional tissue in Fig. 5, but now suppose that the tissue has developed by growing from the left (call this the anterior end). Thus by the time the entire domain has formed there will be an anterior–posterior age gradient down the tissue. Now, further suppose that as each cell arises by division its properties (e.g. its traction parameter, τ) commences to increase. Thus there will be established a monotonic 'maturity' gradient along the tissue in concert with the age gradient.

At some point the anterior-most cells will commence to pass through the bifurcation boundary in Fig. 6, and morphogenesis will be initiated amongst these cells. Cells further down the line will not yet be competent to participate in pattern formation, their parameters being still too 'immature'. It is clear that if the age gradient is monotonically decreasing along the tissue, a 'maturation wave' will sweep from anterior to posterior, leaving behind it a field of activated cells, competent to commence aggregation patterns. This wave is purely kinematic: there need be no 'signal' passed from cell to cell, the wave front being merely the locus where the cells are ageing across the bifurcation boundary. (Intercellular signalling by chemical diffusion or gap junction coupling is certainly possible, and can easily be included in the model; however, to keep the
discussion simple we shall neglect this possibility here. The role of intercellular signalling will be discussed in a subsequent publication.

The pattern which arises in this situation depends not only on the cell and matrix properties, as we discussed above, but also on the speed with which the maturation wave passes down the tissue. If the velocity of the maturation wave front is much faster than the rate of cell aggregation, then the pattern that arises will be the same as in the situation discussed above where the parameters were increased simultaneously across the entire tissue. (This is the case covered by equation 13). However, if the wave speed is much smaller than the rate of morphogenesis, then the pattern that arises will reflect the effective size of the domain during condensation. In the intermediate case, when the maturation wave speed is comparable to the rate of cell aggregation, calculation of the foci spacing is more complicated, but the resulting pattern is qualitatively similar. This interaction between maturation waves and local aggregation potential provides a rich palette of pattern-formation possibilities, which we shall explore in a subsequent publication.

**Domain shape also affects pattern formation: higher dimensional patterns**

During embryonic growth, not only does the size of tissues change, but their shape as well. To appreciate the effect of domain shape on the pattern of cell aggregation we must examine two- and three-dimensional domains. For simplicity, consider a simple rectangular domain of length W and width B, as shown in Fig. 7. There are now two geometrical parameters: the domain size, S = BW, and the domain shape, s = B/W, which can be varied independently. In the Appendix we show that as the shape parameter, s, is varied, a two-dimensional sequence of patterns emerges consisting of isolated peaks in cell density. The mechanism operates exactly as in the one-dimensional case discussed above: aggregation domains become too large to hold together, and give way to smaller aggregations. The important new feature here is that tissue geometry enters in an essential way to determine the morphogenetic pattern. This same influence of geometry on pattern was studied by Murray (1981a,b) in the setting of morphogen-based models of patterns on animal coats and lepidopteran wings.

In the next two sections we shall discuss how this morphogenetic mechanism can contribute to embryogenesis in some specific settings.

**IV. SKIN ORGAN MORPHOGENESIS: FEATHER GERMS**

The earliest stages in the development of feather germs and scales begin the same way: an epidermal layer underlain by a layer of dermal (mesenchymal) cells forms a regular spatial pattern of primordia. Each primordium consists of one or more layers of columnar epidermal cells (placodes) beneath which are aggregations of dermal cells (papillae) (Sengel, 1976). We shall focus our attention on
the most well studied of these systems: the feather germs in fowl (Holmes, 1935; Linsenmayer, 1972; Dhouailly, 1975; Dhouailly et al. 1980; Davidson, 1983a,b).

Numerous transplant experiments indicate that the spatial pattern of feather germs is dictated by the dermis, although subsequent development is a coordinated phenomenon involving both dermal and epidermal layers (c.f. Section 6) (Wessells, 1977; Sengel, 1971, 1976; Sengel & Rusauoen, 1968; Cairns & Saunders, 1954). Moreover, dermal papillae appear to form in vivo more from immigration (Stuart et al. 1972) than from local cell proliferation (Wessells, 1965), while in vitro significant mitoses are observed during condensation (Davidson, 1983a,b). As we shall see, either or both can give rise to the pattern.

Consider a large patch of dermal tissue, which we model as an area of ECM seeded with an initially low population of dermal cells. Referring to Fig. 6, we can see that an increase in cell density, either by proliferation at a local initiation site or by immigration, and/or a change in the other cell/matrix properties contained in the dimensionless groupings, will commence a sequence of bifurcations leading to a spatial array of cell aggregations. In the case where the pattern develops simultaneously over the whole region, only a nonlinear analysis of the model equations will reveal the exact form of these patterns, although the linear analysis developed in the Appendix gives a good idea of what to expect. However, recent experiments by Davidson (1983a,b) on cultured chick skin indicate that the primordia form sequentially rather than simultaneously (see also Linsenmayer, 1972). This is in accord with our discussion above on sequential pattern formation; we need only suppose that there is an age or tension gradient in the tissue prior to condensation. In this case, the pattern of condensations is easier to derive from the model.

The initial dermal aggregation in the dorsal ptya are appears as a columnar condensation of dermal cells situated directly over the midline, as shown in Fig. 5 (c.f. Holmes, 1935; Wessells and Evans, 1968, 1977; Stuart & Moscona, 1967). This column then subdivides into isolated clumps forming the papillae. As the condensations increase in size, tension lines develop joining the aggregation centres;
this is in exact accord with the predictions of the model wherein cell tractions align the ECM between condensation centres. The condensations spread posteriorly and laterally as a ‘wave’.

Consider a cross section of the presumptive feather tract along the midline – corresponding to Fig. 5. Aggregation can be initiated at the anterior end when any increase in cell density (by proliferation or lateral immigration) or traction pushes the system over the bifurcation boundary. The uniform aggregation will then become locally unstable as discussed in the previous section, and break up into isolated condensations whose local spacing can be computed from the cell and matrix properties (e.g. equation 13). The pattern will propagate posteriorly and laterally along the age gradient as we discussed above.

If the ECM is isotropic then the aggregation wave will spread radially from the initiation site (or perpendicularly to the age-contours). If, however, there is a pre-stress in the tissue then the papillae will form faster in the direction of the tension lines. Thus the temporal development of the model papillae can be made to imitate the in vivo situation wherein feather primordia form first along the dorsal axis from anterior to posterior, then secondary rows spreading laterally to form a ‘chevron-shaped’ array. A full numerical simulation of the two-dimensional pattern will be presented in a subsequent publication; here our analysis indicates that the model can indeed generate dermal condensations in the manner illustrated in Fig. 8.

![Diagram](image)

**Fig. 8.** The model predicts the sequence of bifurcations shown, leading from a uniform distribution to a columnar array, which then breaks up into isolated aggregations. Subsequent aggregations spread laterally to form a periodic array. Depending on the anisotropic character of the substratum (e.g. prestresses), the final periodic array can exhibit a variety of geometries ranging from hexagonal to square or rhombic patterns.
Which comes first: placodes or papillae?

Skin organ primordia consist not only of papillae, as we have described above, but of placodes – columnar aggregates of epithelial cells. These placodes lie in such precise geometrical relationships with the papillae that it seems almost certain that there is some coordinating mechanism between the two (c.f. Section VI). It has been well established by transplantation experiments that the pattern of primordia is largely controlled by the dermal layer. However, the epidermis is also capable of forming patterns in the absence of the dermis, as exemplified by lens epithelium.

While it is clear that the dermis controls the pattern of feather germs, there are conflicting reports about whether the dermal condensations commence before or after the epidermal thickenings.

In a separate study Oster & Odell (1983) (see also Oster & Alberch, 1982) developed a mechanical model for epithelial foldings; they found that an epithelial layer can indeed form spontaneous patterns analogous to placodes. If a prestress was imposed – such as that generated by the condensing dermal cells – then the placode pattern echoed the papillae. A complete analysis of the mechanical equilibria between dermal and epidermal layers is in preparation. Our purpose here is only to call attention to the possibility that one tissue type may affect another by imposing a stress pattern on it. Thus mechanical interactions between differential cell types may be important in certain kinds of morphogenetic processes.

Here we have taken the opposite view: that dermal cells can form periodic condensations independently of the epidermis – in accordance with the in vitro experiments of Stopak (1983), Stopak & Harris (1983). Moreover, it is known from in vivo studies that epidermis which does not normally form placodes will do so when transplanted over a papillae-forming dermis. From the viewpoint of the mechanical theory both tissues are capable of pattern formation, and which one – dermis or epidermis – initiates the process is an empirical question beyond the scope of the model. However, since the traction forces generated by the dermal cells can be quite large, the model supports the view that the dermis controls the final pattern, if not its initiation.

V. CHONDROGENESIS

Formation of bone anlagen by condensation of chondrocytes has been one of the most studied of morphogenetic phenomena (Hall, 1978; Hinchliffe & Johnson, 1980; Thorogood, 1983). The exact mechanism that initiates the condensations remains undecided, however, as does the mechanism that determines the pattern. There is no shortage of theories that address these issues, and most of them involve gradients of diffusible morphogens. In this section we will show that some aspects of limb morphogenesis and pattern formation can be understood
Fig. 9. Chondrogenesis, as predicted by the cell-traction model. A cylindrical limb bud growing from one end (the progress zone) will – at a critical cell density, for example – bifurcate from a uniform distribution of cells to form an axial condensation, as shown in (A). As the cells contract and migrate toward the central condensation their tractions will deform the limb into increasingly elliptical cross sections. At a critical ellipticity, a secondary bifurcation will occur leading to two condensations, as shown in (B). Between the two stable structures there is a small amorphous region wherein the uniform cell distribution is briefly stable, suggestive of a 'joint'. While the bifurcation here is geometry-induced, variations in other cell and/or matrix properties as the cell emerge from the progress zone can trigger similar bifurcations. The bifurcation diagram in Fig. 6 shows how the various combinations of cell and matrix properties conspire with geometry to produce the pattern of aggregations.

using the simple physical model we have formulated here. (In fact, in the context of our model there is no conceptual distinction between pattern formation and morphogenesis – a distinction that seems to have grown out of the various morphogen-based models.)

Consider the situation sketched in Fig. 9: we model the growing limb bud as a cylinder with cell proliferation occurring at the distal surface (e.g. the progress zone in birds). Now consider successive cross sections of the limb as shown in the figure; a given cross section experiences a gradual increase in cell density due to proliferation and immigration.

As the system approaches the bifurcation value, two effects come into play. First, the cells begin to migrate centripetally to form a central condensation. At
the same time, their tractions commence to deform the limb cross section, which is initially almost circular, into an elliptical shape. Thus, as the initial condensation forms the limb shape is flattened, as shown in the figure:

Recalling our discussion of the effect of domain shape, we see that a critical ellipticity is eventually reached wherein the single central condensation is no longer stable. The system then bifurcates to form two condensations, as shown.

A possibly important prediction of the model is that the primary and secondary bifurcations are separated by a short region wherein the uniform cell distribution is stable. That is, there is a small transition gap between the first and second condensations which is suggestive of a 'joint' region. Note also that the cell tractions which flatten the limb will eventually produce a cross section which is not convex, making the production of phalanges inevitable (c.f. Fig. 10).

As the growing limb extends and continues to flatten due to the cell tractions, subsequent bifurcations produce more complicated condensation patterns. A detailed comparison with actual cartilage anlagen must be performed numerically - a study presently underway. However, from the linear analysis we have performed in the Appendix we can deduce some qualitative features of the pattern. For example, the description above assumed that the growing limb bud is simply a cylinder; however, if we take into account the actual shape of the limb bud - a flattened paddle, thicker at the anterior - then we can draw some general conclusions about the pattern of condensations.

There are three features that strongly influence the shape and sequence of condensations in the model: limb geometry, including (i) the cross-sectional shape of the limb, (ii) the limb profile (i.e. the 'paddle' shape), and (iii) the tissue age (i.e. cell property) profile down the limb. As the limb grows, cell tractions flatten its cross-sectional profile as discussed above, so that the limb becomes progressively planar towards its distal tip. If the limb bud is initially not perfectly elliptical in cross section, but rather more like an aerofoil - thicker on, say, the posterior side - then as the limb grows, the cell tractions will accentuate this, and affect the size and sequence of the bifurcations so that they don't occur symmetrically with respect to the anterior–posterior axis. Moreover, if the constant tissue-age contours are not planes normal to the cylinder axis, then the bifurcation boundaries will also not be planes normal to the limb axis. Thus the size and shape of the condensations will be affected by the curved geometry of the 'critical' age contour (i.e. the contour corresponding to the bifurcation boundary). Finally, since nearby condensations compete in recruiting cells - both from one another, and from the as yet uncondensed distal regions - it is difficult to say just what the shape and sequence of condensations will be without detailed numerical simulations of actual situations. Fig. 10 gives a hypothetical scenario illustrating these points.

We emphasize that the traction model addresses only one aspect of chondrogenesis: the physical mechanism of cell condensation and limb flattening. Other important aspects of the process, such as the shape and pattern of the cartilage
cell orientations, are not contained in the model. (However, one can show that the condensations will generally not be perfectly cylindrical, but will be thicker at the ends than in the middle – a pattern typical of limb bones.) Nor do we address the problem of cell differentiation, although we suspect that the increased cell packing and contact generated during condensation may play an important role in cueing subsequent differentiations. Finally, we should mention that since cells should orient themselves along the strain lines (i.e. in the direction of the displacement vector \( u \), in the Appendix), then it would appear that the model predicts that condensing cells will be oriented radially, pointing inward toward the axis of the condensation. In fact, cells are aligned circumferentially around the condensation. However, we must realize that the model only addresses the initial stages of condensation, wherein the cell orientations must be radial in order for them to migrate to the centre (see, however, the discussion below of condensations without motility). Later on during the process of chondrogenesis other phenomena commence to dominate the process. For example, chondrocytes secrete hyaluronic acid, a powerful osmotic agent, which
Mechanical aspects of mesenchymal morphogenesis could induce fluid influx to the condensation, swelling the matrix and producing circumferential 'hoop' stresses. Cells would then be aligned along these circumferential strains, in accordance with observations.

The above scenario for the formation of bone patterns depends only on the ability of chondroblasts to exert tractions on their neighbours, either directly or via the extracellular matrix. As we have discussed above, we can assume that as cells leave the progress zone and 'age', one of the cell parameters — perhaps cell traction or diffusivity — changes slowly. Our discussion of dimensionless parameters shows that there are several equivalent variations; only experiment can distinguish between them. Indeed, the model suggests a number of experiments which can directly test the theory, and which combination of factors is most important in determining the pattern. For example, substances which affect the traction properties of mesenchymal cells can alter the patterns of cellular condensation: in Fig. 6 we see that if it is reduced sufficiently, no pattern can obtain.

Finally we point out in the Appendix that the approach we have taken is consistent with the notion of positional information (Wolpert, 1971) in the interpretation of experimental data.

Condensations can occur in the absence of cell motility

In the previous analyses cell condensations arose because of the effect of traction-induced anisotropy on cell motility. However, even if cells are not

---

Fig. 10. (A) The type of axial condensation is influenced by the limb cross-sectional shape. (1) A cylinder will produce a single cylindrical condensation (e.g. a humerus or femur). (2) An aerofoil-shaped domain will produce unequal axial condensations (radius/ulna, tibia/fibula). (3) In a long, thin cylinder, the axial condensations will be broken up into segments by axial bifurcations (phalanges).

(B) A hypothetical scenario showing a possible pattern of bifurcations according to the model. Condensations will commence to appear when cells have aged through the critical age contour. Actually, the age contour is really a contour of dimensionless parameters (c.f. Fig. 6); however, we shall assume that the bifurcation value is monotonically increasing with age, so that we can ignore the difference here.

(1) A 'humerus' has condensed along the axis of the model limb bud as the critical age contour. a*, progresses distally down the limb (a type 1 bifurcation as described in (a)). (2) When a* reaches the critical cross-sectional ellipticity, a bifurcation to two axial condensations ('radius' & 'ulna') occurs (a type-2 bifurcation). (3) By now the growing limb has deformed the cross section and the a*—contour such that the next (type 2) bifurcation occurs only on the 'radius', commencing the 'carpals'/tarsals'.

(4) Because the cell proliferation is greater at the distal midline tip, the a*—contour is much elongated along the midline. Thus the next bifurcation is also binary, and the 'metacarpals' are of unequal length. (5) By now, the limb cross section has been so flattened by the tractions of the condensing cells that the cross section can no longer maintain its convexity. Thus a radial buckling of the cross-section commences. This effectively isolates the condensations from one another, and the geometric domain reverts to an effective cylinder. The only kind of bifurcations that can occur in a long, thin cylinder are type 3. Thus the eventual termination of the limb in phalanges is an inevitable consequence of the flattening of the limb. How many phalanges appear will depend on the limb geometry (cross section and profile) and on the a*—contour at the time when the radial buckling which isolates the phalanges occurs.
motile, (i.e. no random motion or haptotaxis), it is possible to produce cell condensations by passive convection (i.e. being dragged by the contractions of nearby cells). It is intuitively clear that a uniform distribution of cells is not stable even without cell motility: a local centre of contraction grows stronger by dragging in nearby cells, so that the formation of at least a single aggregation focus is inevitable once traction commences. However, analysis of the model equations reveals the surprising possibility that cell traction alone can produce spatial patterning beyond a single aggregation focus. The conditions under which this can take place are that the cells are quite densely packed to begin with, so that contractile filopodia can extend beyond nearest neighbours and attach to more distant cells. Mesenchymal populations in the limb bud appear to fulfill this condition, raising the possibility that the chondrogenic condensations discussed above may arise with very little cell motility. The extent to which cell migration contributes to aggregation is an experimental question; here we can only demonstrate the theoretical possibilities.

VI. MESENCHYMAI AND EPITHEIAL CELLS INTERACT MECHANICALLY

Migrating cells frequently use as their substratum the basal lamina which covers the basal surface of an epithelial layer. Examples include neural crest cells, invaginating mesoderm in amphibian gastrulas and pioneer neurons in insect CNS. In such situations it is natural to suspect that the migrating cells obtain some of their guidance cues from the alignment of the fibres that constitute the basal lamina or, if the lamina is thin or absent, from the orientation of the grooves between the epithelial cells themselves.

A number of investigators have suggested that the shape changes which epithelial layers undergo during morphogenesis might provide guidance cues for subsurface migratory cells by aligning the ECM close to their basal surface. Conversely, knowing the strong tractions mesenchymal cells are capable of exerting on their substratum, it is likely that there is a reciprocal mechanical dialogue between epithelia and mesoderm which can create patterns of cell conformation and movement that could not be realized by a unilateral interaction.

There are numerous examples of situations where a morphogenetic process depends on coordination between epithelial and mesenchymal cell populations (c.f. Wessells, 1977). For example, invagination and branching of lung and gland epithelia appear to depend on a dynamic equilibrium between contracting epithelial cells which secrete a basal lamina, and motile mesenchymal cells which degrade the lamina (Bernfield, Cohn & Banerjee, 1973).

VII. DISCUSSION

Observations on the motile behaviour of cells in vivo and in vitro suggest
Mechanical aspects of mesenchymal morphogenesis

several mechanisms that may contribute to mesenchymal cell morphogenesis. These include contact inhibition, forward bias, chemotaxis, haptotaxis and various guidance cues such as local curvatures, fibrous tracks and strain lines. However, it is not obvious how these phenomena combine to organize the behaviour of cell populations into spatial patterns.

Each of the above mechanisms can be cast in quantitative terms and combined into a mathematical model that describes how cells migrate within a fibrous extracellular matrix (ECM). The model is built around the assumption that motile cells develop large traction forces that can deform the ECM within which they move. These deformations create strain guidance and haptotactic cues which influence the cells’ motion. The surprising result of our analysis is that these well-documented properties of motile cells can conspire to generate a variety of spatial patterns.

The way in which these patterns arise is easy to understand. The tractions generated by the cells compress the ECM and create a density and adhesive gradient which orients motile cells towards the centres of contraction. Indeed, if cells are densely enough packed, it turns out that condensations can occur by traction alone, without appreciable cell motility.

At the same time the cells’ contractions are countered by the elastic properties of the extracellular matrix and by other cells. These parallel forces act to limit the range of influence of a contractile centre. Thus a population of contractile cells can condense into one or many islands of aggregation; the specific pattern depends not only on the traction forces of the cells, but on the viscoelastic properties of the substratum and on the geometry of the tissue as well. The purpose of the model is to show how these various factors conspire to create the spatial patterns.

We have used the cell traction model to examine the initiation of feather germ patterns in fowl dermis and the formation of cartilage condensations during early limb morphogenesis. In both cases we find that the model can reproduce the essential features of the cell aggregation patterns.

We do not mean to suggest by our analysis that mechanical tractions are the universal mechanism by which mesenchymal cells generate spatial patterns. However, it is gratifying that simple physics can reproduce morphogenetic phenomena that heretofore have been modelled by sensitively tuned diffusion-reaction schemes, involving hypothetical morphogens. This unexpected property of mesenchymal cells suggests lines of experimental inquiry different from those inspired by chemical gradient models.

By examining the model in nondimensional terms, we have shown how various combinations of cell and matrix properties (e.g. cell division, traction, motility, haptotaxis, matrix elasticity) can, individually and in concert, produce equivalent patterns. For example, a decrease in cell traction can be compensated by an increase in ECM density or adhesiveness, or by increased cell division, to arrive at the same spatial structure. This introduces the notion of equivalent and
compensating effects in morphogenesis, and suggests that many patterns are not
the result of a single process, but rather emerge as the consensus of a number of
interacting phenomena. Beyond demonstrating that this theoretical possibility
exists, the model shows how the component processes combine, and conversely,
how they may be disentangled and studied separately.

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MATHEMATICAL APPENDICES

In these appendices we outline the mathematical aspects of the model and justify the statements made heuristically in the main body of the paper. In section A.1 we discuss more fully the derivation of the model equations. In section A.2 we nondimensionalize the equations and discuss the significance of the relevant dimensionless parameters. In A.3 we perform a linear analysis of the model equations so as to isolate the parameter ranges which can generate spatial patterns, and in A.4 we investigate the bifurcation behaviour of the model in a few geometrical settings relevant to our discussions in the text. This will demonstrate the importance of a full non-linear analysis, as well as numerical investigations which will be presented elsewhere.

A.1 Derivation of the model equations

As we discussed in the body of the paper the model consists of motile cells migrating within an elastic medium, the extracellular matrix (ECM). The ECM is deformed by the traction forces of the cells, and in turn this induced anisotropy affects the cell movements. The mathematical model consists of equations governing (1) the cell density (equation 7); (2) the mechanical balance of forces between the cell tractions and the matrix (equation 8); and (3) the balance law governing the matrix material (equation 10).

(1) Cell equation

Let \( n(t, x) \) be the cell density function (= the number of cells at time \( t \) per unit volume at position \( x \)). We write a conservation law which equates the time rate of change of the cell density at \( x \) to the various terms which affect cell motion. This has the general form

\[
\frac{\partial n}{\partial t} = -\nabla \cdot \mathbf{J} + M
\]

where \( \mathbf{J} \) is the flux of cells per unit area and \( M \) is the mitotic rate. The specific form of the constitutive relations \( \mathbf{J} \) and \( M \) we employ are

\[
\frac{\partial n}{\partial t} = -\nabla \cdot \left( [-D_1 \nabla n + D_2 \nabla (\nabla^2 n)] - [\alpha n \nabla \phi] - [n \delta u / \delta t] \right) + r n (N - n)
\]

(A.1)

rate of random dispersal \quad hapto-taxis \quad convection \quad mitosis
change of density by matrix motion

where \( D_1, D_2, \alpha, r \) and \( N \) are all positive. Here \( u(t, x) \) is the displacement vector of the matrix; that is, a material point in the matrix which is initially at position \( x \) undergoes a displacement to \( x + u \). We motivate each of the terms in (A.1) in turn.
(i) Random dispersal. The random dispersal term has two components. If cells simply diffused in a homogeneous, isotropic matrix according to Fick's law, then the flux \( J = -D \nabla n \), where \( D \) is the diffusion coefficient of the cells in the medium. When \( D \) is constant then this term in the conservation equation has the usual form \( D \nabla^2 n \) where \( \nabla^2 \) is the Laplacian. We call this 'harmonic diffusion' (since \( \nabla^2 \) is the harmonic operator); it represents local, or short range, random motion.

The Laplacian operator simply averages the neighbouring cell densities, as can be seen by writing out its finite difference equivalent. Alternatively, one can show that the Laplacian is given by the following expression:

\[
\nabla^2 n \propto [n(x, t)_{av} - n(x, t)]/R^2
\]

where \( n(x, t)_{av} \) is the average cell concentration in a sphere of radius \( R \) about \( x \):

\[
n(x, t)_{av} = \frac{3}{4\pi R^3} \int_V n(x+r, t)dr
\]

where \( V \) is a sphere of radius \( R \).

In vivo, however, mesenchymal cell are quite densely packed; moreover, they typically possess long filopodia which extend beyond their nearest neighbours (see for example Plates 8 & 11 in Hinchliffe & Johnson, 1980). Thus they can sense and respond to conditions beyond their immediate neighbourhood. A cell which can sample the environment in its neighbourhood will respond not only to the local value of the concentration gradient, but to the average value, \( n_{av} \), in that neighbourhood. To the extent that this 'non-local' influence is important to their behaviour we must augment their harmonic diffusion by an additional term. There are several ways of arriving at a model for long range interactions. Cohen & Murray (1981) modelled a population system using a Landau-Ginzberg approach wherein spatial gradient contributions to the interaction energy were included which resulted in the biharmonic diffusion term in equation A.1. A development more in line with the cellular interpretation was provided to us by H. Othmer (Othmer, personal communication). He shows that the flux expression appropriate to diffusing objects which also respond to the local average of the concentration field is given by

\[
J = -D_1 \nabla n + D_2 \nabla (\nabla^2 n)
\]

(A.2)

where the biharmonic diffusion coefficient, \( D_2 \), has dimensions of \([\text{length}]^4/\text{[time]}\).

Both harmonic and biharmonic diffusion terms are stabilizing. To see this immediately consider the higher order diffusion equation

\[
\frac{\partial n}{\partial t} = -\nabla \cdot J = -D_2 \nabla^4 n + D_1 \nabla^2 n
\]

(A.3)

and look for solutions of the form \( n(t, x) \sim e^{\sigma t + ikx} \) where \( k \) is any wave number. Substitution into the equation determines a characteristic equation of the form

\[
\sigma = -D_2 k^4 - D_1 k^2 < 0;
\]

thus all such solutions \( n \to 0 \) as \( t \to \infty \), which implies stability of \( n = 0 \).
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If the matrix material within which the cells are migrating is itself under strain then the matrix is no longer isotropic, and, because of the phenomenon of contact guidance, cells will be biased in their random walks. We can model contact guidance by weighting the diffusion coefficients \( D_1 \) and \( D_2 \) by the strains in the ECM. That is, \( D_1(\varepsilon) \) and \( D_2(\varepsilon) \) are functions of the strain \( \varepsilon = 1/2[\nabla u + \nabla u^T] \), where the superscript denotes the transpose. However, since we will only be performing a linear analysis here we shall choose \( D_1 \) and \( D_2 \) to be positive constants.

(ii) Haptotaxis. As the cells migrate their tractions deform the ECM and thus generate gradients in the matrix density, \( \varrho(t,x) \). As discussed in the text, these density gradients produce gradients in the density of adhesive sites for cell attachment. The simplest model for haptotactic transport is to assume that the cell flux is proportional to this matrix gradient and to the density of cells whose tractions are deforming the matrix. The cell traction coefficient, \( \tau_c \), can be viewed as the compressive stress exerted per cell on a unit mass of matrix.

(iii) Mitosis. We represent this term by a logistic growth model with growth rate, \( \tau \), and maximum density, \( N \). The detailed form of this term is not critical as long as it is qualitatively similar.

(iv) Convection. This term represents the passive movement of cells riding on the matrix, which is itself being towed by distant cell tractions. It is simply the product of cell density and the local matrix velocity, \( \partial u / \partial t \). Note that in this term, and throughout the following treatment, we shall employ the small strain approximation to the matrix deformation.

(2) Cell-matrix interaction

The time scale of embryonic motions is very long (h), and the spatial scale is very small (usually < mm). Therefore, we are in the regime of very low Reynolds numbers (c.f. Purcell, 1977; Odell et al. 1981) and we can assume that the tractions developed by the cells are in equilibrium with the elastic restoring forces developed by the strained matrix material (equation 8). We shall write the equilibrium equations by considering the composite material [cells+matrix] and modifying the usual expression for the viscoelastic stress tensor (Landau & Lifshitz, 1970).

\[
\sigma = \left[ \mu_1 \partial \varepsilon / \partial t + \mu_2 \partial \theta / \partial t \mathbf{I} \right] + \left[ \frac{E}{1 + \nu} \left( \varepsilon + \frac{\nu}{1 - 2\nu} \theta \mathbf{I} \right) \right]
\]

(A.4a)

where:

\( \theta = \nabla \cdot \mathbf{u} \) = the dilatation of the matrix material

\( \mu_1 \) = shear viscosity

\( \mu_2 \) = bulk viscosity

\( E \) = Young's modulus

\( \nu \) = Poisson ratio

\( \mathbf{I} \) = the unit tensor
The modifications we make in A.4a are to account for (a) the phenomena of strain alignment, which increases the elasticity as the ECM is dilated and the fibres align, (b) the contribution of the cell traction to the elastic properties; (c) the effects of external elastic forces on the system.

Physically, the effects of fibre alignment and cross-bridge enhancement appear macroscopically as a nonlinear elastic modulus: \( E(\theta) \). The precise nature of this curve can be determined experimentally, but for our purposes here it is not necessary to assume any more than that \( E(\theta) \) is a monotonically increasing function of \( \theta \).

The contribution of the cell tractions to the stress tensor can be modelled most simply by assuming that the force generated per cell per unit mass of matrix is a saturating function of cell density:

\[
\sigma_{\text{cell}} = \frac{\tau \rho}{(1 + \lambda n)} I \tag{A.4b}
\]

where \( \tau \) [dyne-cm/gm] is characteristic of the cell type and \( \lambda > 0 \) measures the rate of saturation of the traction force.

As we discussed in the previous section, cells exert traction forces on cells beyond their nearest neighbours. Therefore, we must include a nonlocal contribution to the cell traction term just as we did for cell motility. We do this in the simplest possible way, by adding a term to (A.4b)

\[
\sigma_{\text{cell}} = \left[ \frac{\tau \rho}{(1 + \lambda n)} \right] [n + \beta \nabla^2 n] I \tag{A.4c}
\]

where \( \beta > 0 \) measures the magnitude of the nonlocal effect.

With these modifications the equilibrium equations are

\[
\nabla \cdot \sigma + \rho F = 0 \tag{A.4c}
\]

where the composite stress tensor \( \sigma \) is the sum of (A.4a) and (A.4b), and the term \( \rho F \) accounts for body forces. For example, if the cell/matrix material is attached elastically to an external substratum – for example, the subdermal layer in the feather tract system – then this appears as a body force proportional to the displacement of the material from its unstrained position:

\[
F = su
\]

where \( s \) is the elastic constant characterizing the substrate to which the ECM is attached.

Thus our model equations for the ECM, A.4c, is just the classical Stokes equations of linear elasticity with the cell tractions acting as a distributed body force (Landau & Lifshitz, 1970; Lin & Segel, 1974).

(3) \textit{Matrix density equation}

The conservation equation for the matrix material, \( \varrho \), is

\[
\frac{\partial \varrho}{\partial t} = -\nabla \cdot (\varrho \partial u / \partial t) + S(n,u,\varrho) \tag{A.5}
\]
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Here S is the secretion rate of matrix material by the cells. Henceforth, we shall assume that secretion is negligible on the time scale of cell motion and take \( S = 0 \).

Equations (A.1) – (A.5) constitute our mathematical model for cell-matrix interactions; they are three equations for the three quantities \( n, u \) and \( \varphi \).

A.2 Dimensionless parameters controlling the model behaviour

The model equations contain 12 positive parameters:

\[
\{ D_1, D_2, \alpha, \tau, \beta, r, N, \mu_1, \mu_2, E, \nu, \lambda \}
\]

Two additional parameters will appear in the nondimensionalization procedure to follow. (a) The uniform matrix density in the undeformed state, \( \varphi_0 \). (b) Since we are generally interested in finite domains a typical length scale, \( L \), will be associated with the dimensions of the tissue under consideration. Thus there are 14 parameters in all. As usual, rendering the equations nondimensional will both reduce the number of relevant parameters considerably, and highlight the combinations of parameter changes that affect the model behaviour in equivalent ways as we saw in the text.

There is no unique way to nondimensionalize the model equations. For example, there are several time scales we could use: \( L^2/D_1 \) and \( L^4/D_2 \) are associated with the cells' random motions in a domain of extent \( L \); the mitotic time scale is \( 1/rN \); the mechanical time scales are associated with \( \mu_1 \) and \( \mu_2 \) and the haptotactic time scale is \( L^2/\alpha \varphi_0 \). The mitotic and mechanical time scales apply equally well in finite and in very large, or infinite, domains. The conditions on the parameters we shall derive for spatial patterning do not depend on which nondimensionalizing scheme we choose. However, the final computed solutions to the model equations do depend on the choice of dimensionless parameters in the sense that they highlight the effect of detailed dimensionless groupings.

To illustrate the procedure we will use the haptotactic time scale \( L^2/\alpha \varphi_0 \). Temporarily, we denote the dimensionless parameters by asterisks, and define the following quantities:

\[
\begin{align*}
    x^* &= x/L, \quad t^* = \alpha \varphi_0 L^2, \quad u^* = u/L, \quad \nabla^* = L \nabla, \quad \theta^* = \nabla^* \cdot u^* = \nabla \cdot u = \theta, \\
    \epsilon^* &= \epsilon, \quad \varphi^* = \varphi/\varphi_0, \quad n^* = n/N, \quad D_1^* = D_1/\alpha \varphi_0, \quad D_2^* = D_2/L^2 \alpha \varphi_0, \\
    \beta^* &= \beta/L^2, \quad \tau^* = \tau \varphi_0 N(1 + v)/E, \quad r^* = rNL^2/\alpha \varphi_0, \quad \mu_1^* = (1 + v) \mu_1 \alpha \varphi_0/EL^2, \\
    \mu_2^* &= (1 + v) \mu_2 \alpha \varphi_0/EL^2, \quad \lambda^* = \lambda N
\end{align*}
\]  

(A.6)

Using these dimensionless variables in equations (A.1) – (A.5) we obtain:

\[
\begin{align*}
    \partial n/\partial t &= D_1 \nabla^2 n - D_2 \nabla^4 n - \nabla \cdot (\nabla \varphi) - \nabla \cdot (n \partial u/\partial t) + rn(1 - n) \quad (A.7) \\
    \nabla \cdot \{ \mu_1 \partial \epsilon/\partial t + \mu_2 \partial \theta/\partial t \mathbf{I} + \epsilon + \varphi \partial \mathbf{I} + [\tau \varphi/(1 + \lambda \nu)][n + \beta \nabla^2 n(\mathbf{I})] - s \varphi u \} &= 0 \quad (A.8) \\
    \partial \varphi/\partial t + \nabla \cdot (\varphi \partial u/\partial t) &= 0 \quad (A.9)
\end{align*}
\]

where for notational convenience we have dropped the asterisk, and defined the parameter \( \hat{\nu} = \nu/(1 - 2\nu) \). The dimensionless parameters now number 9:
Note in (A.6) how variations in different dimensional parameters can have equivalent effects on the dimensionless parameters.

A.3 Linear analysis of the model equations

The uniform equilibrium or steady states of (A.7) – (A.9) are:

\[ \begin{align*}
& u = 0, \quad \varphi = 1, \quad n = 1 \\
& \text{and} \quad u = 0, \quad \varphi = 1, \quad n = 0
\end{align*} \]

Since the \( n = 0 \) state is always unstable, and not of interest here, we consider the stability of the former to small perturbations:

\[ n = 1 + \tilde{n}, \quad u = \tilde{u}, \quad \varphi = l + \varphi \]

where \( \tilde{n}, \tilde{u}, \) and \( \varphi \) are small. Substitution of these into (A.7) – (A.9) and neglecting all but the linear terms results in the linear system of equations:

\[ \begin{align*}
\frac{\partial n}{\partial t} &= D_2 \nabla^4 n - D_1 \nabla^2 n - \nabla^2 \varphi - \nabla \cdot (\partial u / \partial t) + rn \\
\nabla \cdot [\mu_1 \partial \varepsilon / \partial t + \mu_2 \partial \theta / \partial t] + \nabla \cdot [\varepsilon + \varepsilon \theta] + \frac{\tau}{(1 + \lambda)} \nabla n + \frac{\tau}{(1 + \lambda)} \nabla \varphi + \frac{\beta \tau}{(1 + \lambda)} \nabla^3 n - s u \varphi &= 0 \\
\frac{\partial \varphi}{\partial t} + \nabla \cdot (\partial u / \partial t) &= 0
\end{align*} \]  

where for notational convenience we have dropped the tilde.

To determine the stability criteria we look for solutions of the form:

\[ \frac{n}{\varphi} = e^{\alpha + i k \cdot x} \]  

For now, let us set \( \lambda \equiv 0, \beta \equiv 0 \); then substitution into (A.10) – (A.12) gives the dispersion relation which determines \( \sigma \) as a function of the wave vector \( k \):

\[ k^2 \sigma [\mu \sigma^2 + b(k^2) \sigma + c(k^2)] = 0 \]  

where

\[ \begin{align*}
b(k^2) &\equiv \mu D_2 k^4 + \mu D_1 k^2 + (1 + \hat{v} + \mu r - 2 \tau) \\
c(k^2) &\equiv (1 + v - \tau) D_2 k^4 + k^2 [D_1 (1 + v - \tau) - \tau] + r (1 + v - \tau)
\end{align*} \]

Spatially inhomogeneous unstable patterns will appear if for some wave numbers \( k^2 \neq 0 \) the corresponding \( \sigma(k^2) \) from (A.14) has \( \text{Re}(\sigma) > 0 \): such waves will initially grow exponentially. For this to happen \( b(k^2) \) or \( c(k^2) \) must be negative for some \( k^2 \neq 0 \) since, from (A.14), \( \sigma = 0 \) or

\[ 2 \mu \sigma = -b(k^2) \pm [b^2(k^2) - 4 \mu c(k^2)]^{1/2} \]  

(A.17)
Since we require the system to be stable if there is no spatial inhomogeneity in the solutions (A.13), we require the solutions \( \sigma \) in (A.17) to have \( \text{Re}(\sigma) \leq 0 \) for \( k^2 \to 0 \); so we have

\[
\begin{align*}
\text{b}(0) &= 1 + \hat{\nu} + \mu r - 2 \tau > 0, \\
\text{c}(0) &= r(1 + \hat{\nu} - \tau) > 0
\end{align*}
\]

(A.18)

These are satisfied if \( \tau \) is sufficiently small. From (A.6) this can be achieved if \( N \) is sufficiently small. It is the dimensionless grouping in \( \tau^* \) which must satisfy the inequalities (A.18).

We now want the system (A.7) - (A.9) to have unstable spatially inhomogeneous solutions; that is, solutions of the form (A.13) for at least one \( k^2 \neq 0 \), and the corresponding \( \text{Re}(\sigma) > 0 \). From (A.14) - (A.16) and the restrictions (A.18), \( b(k^2) > 0 \) for all \( k^2 \geq 0 \). Thus the only possibility is if \( c(k^2) < 0 \) for some \( k^2 \). From (A.16) this requires the coefficient of \( k^2 \) to be negative, at the least; that is

\[
D_1(1 + \hat{\nu} - \tau) - \tau < 0
\]

A sufficient condition is that

\[
c(k^2) \bigg|_{\text{min}} = r(1 + \hat{\nu} - \tau) - \frac{(\tau - D_1(1 + \hat{\nu} - \tau))^2}{4(1 + \hat{\nu} - \tau)D_2} < 0
\]

So, necessary and sufficient conditions on the dimensionless parameters for spatially unstable modes with \( k^2 \neq 0 \) are (A.18) and, from the last inequality

\[
\beta^2 > 4rD_2, \quad \beta = [\tau/(1 + \hat{\nu} - \tau)] - D_1 > 0
\]

(A.19)

Fig. 11(a) illustrates \( c(k^2) \) for various values of \( D_2 \). With the parameters satisfying (A.18) and (A.19) all \( k \) such that \( c(k^2) < 0 \) are unstable modes. Fig. 11(b) shows the corresponding values of \( \sigma > 0 \). From (A.16) and (A.19) this range of \( k^2 \) values is

\[
[\beta - \{\beta^2 - 4rD_2\}^{1/2}/2D_2 < k^2 < [\beta + \{\beta^2 - 4rD_2\}^{1/2}/2D_2
\]

(A.20)

On the curve \( D_2 = D_c \) in Fig. 11 the critical wave number, \( k_c^2 \), is obtained from (A.16) as the solution of \( c(k^2) = 0 \) and \( \partial c/\partial k^2 = 0 \); the corresponding critical wavelength is

\[
\omega_c = (D_2/r)^{1/4} = 1/k_c
\]

(A.24)

Or, in dimensional variables, since \( \omega^*_c = \omega_c/L_c \), and using (A.6), the critical wavelength for spatial patterning is

\[
\omega_c = (D_2/rN)^{1/4}
\]

(A.25)

For this analysis we see that the instability criterion requires \( \tau \) to be nonzero, and, of course, \( \alpha \), which is implicitly assumed in the nondimensionalization (A.6). This illustrates how crucial is the coupling between the cellular and matrix generated forces in producing spatial patterns. In addition, there must be some
cell proliferation, measured by the parameter $\tau$, and some long-range diffusion so that $D_2 \neq 0$. The viscosities affect only the time scale on which the spatial instability evolves, and so do not enter into the instability criteria or the critical wavelength expression; however, the viscosities do affect the size and structure of the domain of instability (see below).

Thus we see that the parameter space can be divided into domains where spatial patterns can be generated and where they cannot arise. The critical bifurcation surfaces are given by the inequalities in (A.18) – (A.19). The domains for instability are those which give $\text{Re}(\sigma) > 0$ in (A.14). As an illustrative example, setting $D_1 = \mu_1 = \mu_2 = 0$, (A.18) gives

$$2\tau < (1 + \hat{\nu}),$$

and from (A.19)

$$1 > \left[ \frac{1 + \nu - \tau}{\tau} - 1 \right] \cdot 2(rD_2),$$

The domain of instability in the $[rD_2, \tau/(1 + \hat{\nu})]$-plane is shown in Fig. 6 above. If the viscosities $\mu_1$ and $\mu_2$ and the diffusion coefficient, $D_1$ are nonzero, then from (A.18) the domain is given by the (A.18) and (A.19).

As we discussed in the text, spatial patterns can arise even if cell motility is
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negligible ($D_1 = D_2 = \alpha = 0$) providing we include the nonlocal effects of cell traction, $\beta > 0$. This provides a quite different mechanism for cell tractions to form spatial patterns of cell aggregation. There is presently some controversy as to how much cell motility contributes to aggregation phenomena; however, the model does show that both mechanisms, individually or in concert, can generate spatial aggregation patterns.

This analysis shows that certain spatial perturbation modes about the uniform steady state can be made linearly unstable by suitable choices of parameters. These unstable modes will evolve into spatially inhomogeneous solutions, and a linear analysis cannot say what these patterns will be. A numerical study is underway to investigate the nonlinear behaviour of the model.

A.4 Specific geometries and bifurcating patterns

Experience with similar nonlinear systems shows that the existence of linearly unstable modes is generally a good predictor of an ultimate steady state spatial pattern, and for simple structures a reasonable predictor of the final nonlinear pattern. Therefore, it is worthwhile to push the linear analysis a bit further and investigate the role of geometry on the incipient spatial patterns (c.f. Murray, 1981a,b).

(a) One-dimensional geometry

For illustrative purposes consider first a one-dimensional domain of length $L$ with zero-flux boundary conditions for the cell and with $\phi = 1$, $u = 0$ at the ends:

$$\phi = 1, \frac{\partial \phi}{\partial x} = 0, u = 0 \text{ on } x = 0, L$$  \hspace{1cm} (A.26)

(In dimensionless terms these conditions are the same, but $x$ varies from 0 to 1.) Solutions of the linear system are of the form

$$\exp(\alpha t) \cos(m \pi x / L)$$
$$\exp(\alpha t) \sin(m \pi x / L), \ m = \text{integer}.$$  

The $m$-modes which are unstable are then given by (A.20) with $k^2 = m^2 \pi^2$ (here $k$ is nondimensionalized with $L$).

In order to see the effect of linear scale on the pattern let $L$ be the unit of length and $l$ the actual domain size; now the boundary conditions hold on $x = 0, l$. Let $\gamma^2 = \rho / L^2$, then in place of (A.20) we have

$$\frac{\gamma^2}{2D_2} [\beta - (\beta^2 - 4rD_2)^{1/2}] < m^2 \pi^2 < \frac{\gamma^2}{2D_2} [\beta + (\beta^2 - 4rD_2)^{1/2}]$$  \hspace{1cm} (A.27)

$$\beta = \frac{\tau}{1 + \tilde{\tau} - \tau} - D_1,$$
$$\gamma = \frac{l}{L}.$$

Now fix all the parameters except $\gamma$, which measures the domain size. As $\gamma$ increases the unstable nodes have an increasing $m$; higher $m$ values correspond
to increasing degrees of spatial structure, as shown in Fig. 12 for the cell density, \(n\) (the matrix density and strain exhibit the same behaviour, although with different phases.)

The relationship between \(m\) and \(\gamma\) is linear (c.f. (A.27)); thus as \(\gamma\) increases it passes through successive bifurcation values, say \(\gamma_1, \gamma_2, \ldots\). At each of these values the largest \(m\) bifurcates to successively higher values. Depending on the particular parameter values, there can be gaps in the \(\gamma\)-space where there are no unstable modes. This phenomenon, which has also been found in a diffusion-reaction instability (Lara & Murray, 1983), turns out to have an interesting biological interpretation in the context of cartilage anlagen.

It is important to note that for a fixed \(\gamma\), successive bifurcations occur as \(D_2\) decreases, although from (A.27) the relationship is more complex than that with \(\gamma\). In fact, bifurcations can occur through variations in all of the parameters: the dimensionless parameter groupings only allow one to identify equivalent parameter effects. This is particularly important in the following discussion of the two-dimensional case.

(b) Two-dimensional geometry

In order to apply the model to biological phenomena such as chondrogenesis and feather germ formation we must consider domains in two and three spatial dimensions. Consider for simplicity a rectangular domain of width \(W\) and breadth \(B\) on the boundaries of which we impose zero flux conditions on \(n\) and \(\varrho\), as well as zero stress. Then solutions to the linearized model equations are of the form

\[
e^{ip_\theta q_\phi + t}[\begin{bmatrix} \sin(p \pi x/W) & \sin(q \pi y/B) \\ \cos(p \pi x/W) & \cos(q \pi y/B) \end{bmatrix}]
\]

where \(p\) and \(q\) are integers, and \(x, y\) are Cartesian spatial coordinates. The condition equivalent to (A.27) is then

\[
\frac{\gamma^2}{2D_2} \left[ \beta - (\beta^2 - 4\tau D_2)^{\frac{1}{4}} \right] < \pi^2 \left[ \frac{p^2}{W^2} + \frac{q^2}{B^2} \right] < \frac{\gamma^2}{2D_2} \left[ \beta + (\beta^2 - 4\tau D_2)^{\frac{1}{4}} \right]
\]

\[
\beta = \frac{\tau}{1 + \hat{\nu} - \tau} - D_1, \quad \gamma = \frac{l}{L}
\]
Here $L$ is some absolute length scale and $l$ is the length associated with the rectangular domain (which is $W$ long and $B$ wide).

From (A.29) we see that the range of $p$ and $q$ corresponding to unstable modes depends not only on all of the parameters plus $\gamma$, but also on the size of the pure numbers $W$ and $B$; that is, the bifurcation depends on the shape of the domain. For example, if $B$ is small enough, any $q \neq 0$ will be such that $\pi^2 q^2 / B$ lies outside the unstable range. This implies that all spatial inhomogeneity will be in the $x$ direction since only $p \neq 0$ can satisfy the inequality (A.29). This influence of domain on shape is similar to that proposed by Murray (1981a, b) for animal coat and lepidopteran wing markings. There he showed that geometry could constrain the type of possible patterns, as it does in the model under discussion here.

If there are nonzero values of $p$ and $q$ which satisfy (A.29) then two-dimensional spatial patterns can arise. If, however, $B$ is too small (that is, the domain is too narrow), then only $p \neq 0$ and the resulting pattern is quasi-one-dimensional. The following example illustrates the dependence of pattern on domain geometry.

The unstable modes for $n$, say, are solutions of the form

$$n(t,x,y) \sim 1 + \sum_{pq} a_{pq} e^{i \omega t} \cos \left( \frac{p \pi x}{W} \right) \cos \left( \frac{q \pi y}{B} \right)$$

(A.30)

where $a_{pq}$ is small and $p,q$ satisfy the inequality (A.29), which we write as

$$\phi(\gamma \beta, r, D_2) < \frac{p^2}{W^2} + \frac{q^2}{B^2} < \Phi(\gamma, \beta, r, D_2)$$

(A.31)

$$\left[ \begin{array}{c} \Phi \\ \phi \end{array} \right] = \frac{\gamma^2}{2\pi^2 D_2} \left[ \beta \pm (\beta^2 - 4rD_2) i \right]$$

with $\beta$ as in (A.27). The growth factor $\alpha_{p,q}$ is the solution (A.19) with $p,q$ from (A.31): these guarantee that $\text{Re} \alpha_{p,q} > 0$.

As an example of this calculation let the parameters be such that $\Phi = 3$, $\phi = 2.7$ and are kept fixed, and $W = 2$ and $B = 1.5$ (that is, the aspect ratio – or domain ‘shape’ – is $B/W = 0.75$). From (A.31) the $p,q$ of interest satisfy

$$2.7 < p^2/4 + q^2/2.25 < 3.0$$

which implies $p = q = 2$. Now, suppose that the aspect ratio changes so that $W = 4$, $B = 1.5$ ($B/W = 0.31$); then

$$2.7 < p^2/16 + q^2/2.25 < 3.0$$

so that $p = 4$, $q = 2$. Fig. 7 shows the patterns that arise from this bifurcation. As the aspect ratio is further reduced more patterns can be generated. This shape-driven bifurcation is discussed in the text in the setting of limb chondrogenesis wherein the increasing distal ellipticity of the developing limb initiates the sequence of patterns from the humerus to the radius and ulna, then to the carpals and finally the digits.
A comparable sequence of patterns can be obtained if the area and aspect ratios are fixed and other parameters in the model vary. For example, from (A.31) it can be shown that Φ increases as D₂ decreases. Thus if we assume that the later mesenchymal cells leave the apical ectodermal ridge the less motile they are (smaller D₂) then more distal patterns will display more spatial structure.

Because of the nonlinear interplay between the parameters and the generated patterns it is possible that, just as in the Turing instability studied by Lara & Murray (1983), there is a gap in the sequence of bifurcations in which there is no spatial pattern. In the setting of chondrogenesis this would correspond to the 'joint' region which separates the bone anlagen.

We emphasize that the results developed here are based on a linear analysis. Even so, certain general features emerge which we are currently investigating in the nonlinear regime by numerical simulation. Moreover, we have considered the pattern of cell concentrations within a domain whose boundaries are fixed. However, the biological problem in the case of chondrogenesis involves boundaries which are free. Thus the forces developed by the cells and matrix will deform the boundary. These changes in domain shape can themselves trigger the bifurcations we have described. That is there is a mechanical coupling between the cell/matrix forces and the domain geometry which can produce spatial patterning. This free boundary problem is formidable, even for numerical simulation.

**The model can be interpreted in terms of positional information**

The notion of positional information (PI) (Wolpert, 1971) is not a 'model' in the usual sense, but rather a way of viewing the phenomenology of development. The basic idea is that cells are assigned a 'positional value' characteristic of their physical position in the embryo. The positional value (PV) is the aggregate of the cell's chemical, physical and genetic properties. In the context of our model, a cell's PV is its parameter values: motility (D₁,₂, α), mitotic rate (r) and traction (τ). Thus, for example, in a limb bud, each cell would have a representative point in 'cell property space': \{D₁, D₂, α, r, τ\}. A cell's behaviour is determined by the model equations once we specify (a) its PV \{D₁, D₂, α, r, τ\} and (b) its environment: the local cell and matrix density (n, φ) and the local ECM properties (E, ν).

The notion of PI per se does not address the issues of how the PV's are assigned, nor how the cell, executes the morphogenetic 'programme' specified by its PV. PI has frequently been interpreted in terms of gradients of diffusible morphogens; a cell's PV is set once it reads out the local concentration of the morphogen. Such models usually do not specify how the cell executes its morphogenetic (cell shape change, protein synthesis, or whatever). In our model, however, we need not address the issue of how the cell parameters are set, but once they are, the equations predict how morphogenesis is executed. Thus the 'chemical prepattern' view of morphogenesis asserts that tissue geometry (i.e. morphogenesis) is a trivial mechanical execution by the cells of a pre-existing chemical pattern:
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chemistry $\rightarrow$ geometry $\rightarrow$ mechanics.

In contrast, the model we have presented here assumes that the tissue geometry is the result of cell mechanics, and need not be specified by a detailed chemical prepattern:

chemistry $\rightarrow$ mechanics $\rightarrow$ geometry.

This view appears to have some advantages over the strictly chemical prepattern scheme. It is true that the mechanical view requires that we specify the cell’s PV (cell property parameters), and in this sense is also a ‘prepattern’. However, this prepattern can be much simpler than a morphogen model would require. For example, in the feather germ model we needed only to specify that a cell’s traction, $\tau$, increased as the cell ‘matured’ – a very simple ‘life history’ prepattern – and the complex geometry of the feather tract was then an automatic and inevitable consequence of cell mechanics. One could produce the same spatial pattern via a morphogen-type model but to do so would require postulating the morphogens, and assuming the correct chemical kinetics and transport properties to generate the spatial pattern of morphogen, and then begging the question of how the cells read out and execute the chemical instructions. We feel that our approach has the advantage of dealing, simultaneously with the pattern-formation and morphogenesis problems, and it does so in terms of cell parameters which are more accessible experimentally than morphogens have proven to be. Moreover, quite complex geometries can arise quite simply from strictly local cellular interactions. Preliminary analysis indicates that our model can generate an even greater range of patterns than are possible with the standard reaction-diffusion models. Moreover the two model types are not mutually exclusive: one can easily include in our model the possibility that cells secrete and respond to chemical cues, which alter the cell parameters, for example, traction, mitosis, etc. We shall investigate this generalization in subsequent publications.