PATTERN FORMATION IN TISSUE INTERACTION MODELS

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1. INTRODUCTION

Embryogenesis depends on a series of processes which generate specific patterns at each stage of development. For example, gastrulation, chondrogenesis, formation of scale, feather and hair primordia all involve major symmetry breaking. These ubiquitous spatial pattern formation requirements depend on specific pattern generation mechanisms which are still unknown. They are the subject of much research both theoretical and experimental. In the case of integumental patterns, for example, we do not in general even know when in development the pattern is actually formed. This was the key question studied by Murray *et al.* (1990) in a recent theoretical and experimental paper on alligator (*Alligator missippiensis*) stripes.

Here we shall concentrate on some of the patterns formed in the skin during embryogenesis. In many of these situations highly specific control of the pattern is not crucial – the distribution of spots on an animal's coat or scales and pigment patterns on some snakes exhibit considerable diversity. However, in several other pattern formation processes, such as those involved in chondrogenesis, control is very important. Furthermore, with any spatial pattern generator, one must address the problem of robustness, dependence on initial conditions, mode of pattern initiation, parameter dependence and so on.

Various mathematical models have been proposed to account for the formation of such patterns. For example, Murray (1981a,b; 1989) showed that reaction diffusion models exhibit spatial patterns consistent with many observed animal coat markings, while Bard (1981) and Cocho *et al.* (1987) obtained similar types of patterns in cellular automata models. Recently, Murray & Myerscough (1991) examined a cell-chemotaxis model for snake skin patterning and demonstrated that many of the observed patterns are similar to the bifurcating spatially heterogeneous solutions of their model equations (Maini *et al.*, 1991). Although skin patterning in reptiles poses several interesting developmental problems (see, for example, Maderson, 1985 and Ferguson, 1985), little work has been done specifically on this problem. The above paper by Murray *et al.* (1990) on alligator stripe patterns is one example.

The ability of reaction diffusion and mechanochemical models to generate regular patterns such as stripes and spots which are arranged in a rhombic or hexagonal array

is well known. Furthermore, that these patterns also appear in chemical systems has recently been demonstrated experimentally by De Kepper *et al.* (1991) and Ouyang & Swinney (1991).

Vertebrate skin forms many specialized structures, such as, hair, scales, feathers and glands, which are distributed over the skin in a highly ordered fashion. Despite a vast amount of experimental investigation, the underlying mechanisms involved in the formation and distribution of these appendages are still not well understood. However, it is clear from interspecific transplants that interaction between different tissue types plays a crucial role in these patterning processes.

To date, most models for pattern formation have ignored tissue interaction (see Murray, 1989, 1992 for reviews). Recently, however, a number of models have been proposed which take such interaction into account. All of these comprise individual sub-models for the epidermal and dermal layers of skin, which are then coupled to represent the interaction. Typically, each sub-model is of reaction-diffusion or of mechanochemical type, and is capable, separately, of generating spatial pattern. Shaw & Murray (1990) showed how mechanism coupling could induce spatial pattern formation when neither of the sub-models was individually able to do so. In this paper we shall focus on the tissue interaction model of Cruywagen & Murray (1992) which is based on sound experimental observations. We shall consider the model in the context of feather germ patterning on the chick embryo.

In Section 2 we briefly discuss the biology of tissue interaction, concentrating specifically on the epidermal-dermal interaction in chick skin. In Section 3 we review some previous models for tissue interaction and in Section 4 we outline the model of Cruywagen & Murray. In Section 5 we show that this model exhibits sequential spatial patterns in two-dimensions.

2. BIOLOGY OF TISSUE INTERACTION

Vertebrate skin is composed of two layers: an ectodermal epithelium, the epidermis, consisting of columnar cells, overlies a mesodermal mesenchyme, the dermis. The layers are separated by a fibrous basal lamina. Epithelial cells array themselves into sheets and may present a regular *paving stone* appearance. The dermal cells are much more loosely packed and move around in a jelly-like extracellular matrix (ECM). During skin development sweat glands, hair follicles and other skin structures project down from the epidermis into the dermis.

Feather bud development in the chick has been widely studied experimentally (Davidson, 1983a,b; Chuong & Edelman, 1985a,b) and the various stages of feather formation are described by Sengel (1976). The first feather rudiments on the chick back become visible six days after egg fertilization. A feather primordium, or feather germ, consists of an epidermal thickening, or placode, overlying a dermal cell condensation, or papilla. Initially a row of equally spaced feather primordia appears along the dorsal midline. Lateral rows of feather buds then appear sequentially from the dorsal row outwards to form a regular rhombic array of primordia. There is no general agreement on the sequence of events in the formation of papillae and placodes. However, there *is*

strong evidence for dermal-epidermal coupling (Chuong & Edelman, 1985a; Nagorcka & Mooney, 1982, 1985).

There are many ways in which sets of cells can influence the behaviour of other nearby cell populations. Here we focus on epithelial and mesenchymal cell communication during skin morphogenesis which mainly involves *action-at-close-range*, or so called *proximate* interactions (see, for example, Gilbert, 1988). There are two types of proximate interactions: *instructive* and *permissive*. In instructive interactions specific instructions are given by one group of cells to another. For example, if one places the optic vesicle of the embryonic eye adjacent to a part of the head ectoderm, which in the normal course of development would have formed skin, then specific information is passed to that region of the ectoderm so that a lens rather than skin develops (McKeehan, 1951).

On the other hand, in permissive interactions no specific instructions are passed, but development proceeds only in the presence of another tissue. Epithelial cell mitosis, for instance, usually occurs only in the presence of adjacent embryonic mesenchyme (Gilbert, 1988).

Several authors, such as Rawles (1963), Dhouailly (1973, 1975), Sengel (1976), Wessels (1977) and Dhouailly & Maderson (1984), have demonstrated the importance of instructive interaction between the epithelial and mesenchymal layers during embryonic skin pattern formation. Dhouailly (1973, 1975) studied the interaction by combining interspecific epidermal and dermal tissues from three different classes of animal — reptiles (lizards), birds (chicks) and mammals (mice). The results of her recombination experiments strongly suggest that messages originate from the dermis to influence the patterns formed in the epidermis. For example, chick dermis explanted with any type of epidermis forms the type of appendage specific to the epidermis, but the typical shape, size and distribution are similar to that seen in feather bud formation.

Gallin *et al.* (1986) found that disrupting the balance of epidermal neural cell adhesion molecules (N-CAMs) in chick skin leads to dramatic changes in the patterning of feather germs. This shows that the epidermis, in turn, can influence patterns in the dermis. Furthermore, their results appear to implicate cell adhesion molecules (CAMs) in the signalling process.

There are effectively two possible ways in which instructions can be transmitted between the mesenchyme and the epithelium (Saxén *et al.* 1976): via chemical signals, for example, paracrine signalling; or through the mechanical interaction of epithelial and mesenchymal cells, which are in direct contact with each other. To date, it is not known which mechanism, if not both, is involved in mediating interaction, and signalling molecules have yet to be isolated.

3. MODELS FOR TISSUE INTERACTION – A BRIEF REVIEW

Nagorcka (1986) proposed a tissue interaction mechanism to account for the initiation of skin organ primordia. The model consists of a system of reacting and diffusing chemicals (termed morphogens) in the epidermis controlled by a chemical switch mechanism in the dermis. The spatial pre-pattern in morphogen concentration set up in the epidermis then serves to provide positional information (Wolpert, 1981) for epidermal cell patterning and induces dermal cell condensation. Variations of this model have also been used; for example, see Nagorcka & Mooney (1982, 1985), Nagorcka (1984) and Mooney & Nagorcka (1985).

A mechanochemical tissue interaction model was proposed by Nagorcka *et al.* (1987) in which a reaction-diffusion system in the epidermal layer is coupled to a mechanical system in the dermis. In this model, the morphogen concentration in the epidermis controls certain mechanical properties in the dermis. In turn, dermal cells produce a factor which causes morphogen production in the epidermis. They demonstrated numerically that the model can generate regular complex spatial patterns consistent with scale patterns observed in certain reptiles. This was confirmed by a detailed analytical study of a similar system by Shaw & Murray (1990). In these composite models, each sub-model is capable of generating spatial pattern of any desired wavelength, hence, if coupled appropriately, the full model can exhibit a superposition of patterns with two distinct wavelengths. The resultant patterns are similar to those observed in scale patterns on reptiles (Figure 1). Recently, Bentil & Murray (personal communication, 1991) have shown that even rather simple models can produce the complex spatial patterns exhibited by these composite models.

A different interaction mechanism for feather germ formation was proposed by Chuong & Edelman (1985a). They proposed that a specific factor produced by the L-CAM positive dermal cells, maybe a hormone or peptide, triggers the formation of dermal condensations. This factor may act as a chemotactic agent and stimulate N-CAM expression to induce N-CAM linked papillae. This agrees with the experimental results of Gallin *et al.* (1986). Furthermore, the recombination experiments of Dhouailly (1973, 1975) suggest that a dermally produced signal is involved in epidermal patterning. Chuong & Edelman (1985a) therefore proposed that epidermal placode formation is induced by a factor produced by the developing dermal condensations. When feather germ formation is completed, the inductive factors are modified so as to halt dermal aggregation. Since these factors can still be active in neighbouring tissue, periodic feather germ patterns could thus be formed in a self propagating manner.

Gallin *et al.* (1986) constructed a model to simulate this mechanism in which cells were modelled as discrete units responding stochastically to chemical signals. In their model a signal E_s, produced by the L-CAM linked epidermal cells, increases the mitotic rate, aggregation and N-CAM expression of the mesenchyme. A dermal signal D_s, produced by the N-CAM positive condensations, in turn induces placode formation in the epithelium. The dermal signal also downregulates the production of E_s which then halts the formation of papillae and placodes. Although the signals E_s and D_s are treated as diffusible morphogens, acting as intercellular chemical messengers, the model is also

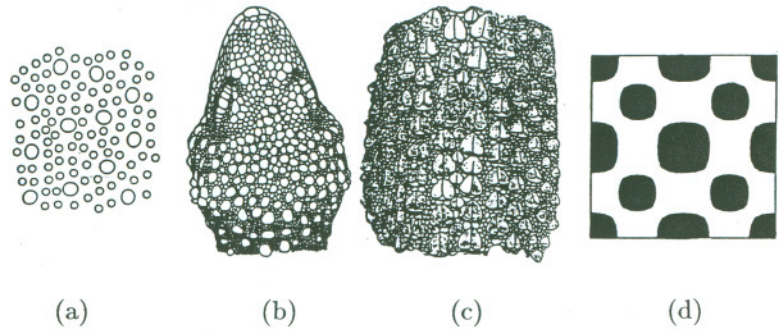

Figure 1: Complex spatial skin patterns. (a) Small and large diameter feather follicles, in the skin area under the beak after 12 days of incubation, in the common coot *Fulica (genus) atra L.* (redrawn from Gerber, 1939). Small and large scales in the dorsal head region (b) and on the back (c) of the lizard *Cyrtodactylus (genus) fedtschenkoi* (Leviton & Anderson, 1984). (d) Morphogen profile from a numerical solution of a reaction-diffusion-mechanochemical tissue interaction model (Shaw, 1989). Regions in which the morphogen concentration exceeds a set threshold are shaded.

consistent with direct cell-cell signalling, whether chemical or mechanical. Their results were in good agreement with the experimental observations.

The model of Cruywagen & Murray (1992) is a continuum tissue interaction model based on the discrete model of Gallin *et al.* (1986). We briefly describe the model in the next section and refer the reader to the original paper for full details.

4. MODEL EQUATIONS

The model of Cruywagen & Murray (1992) involves seven field variables in space and time. The epidermal variables are

$N(\mathbf{x}, t)$ = the epidermal cell density at position $\mathbf{x}$ and time t;

$u(\mathbf{x}, t)$ = the displacement at time t of a material point in the epidermis which was initially at $\mathbf{x}$;

$\hat{e}(\mathbf{x}, t)$ = the epidermal concentration of a signal morphogen, produced in the epidermis, at position $\mathbf{x}$ and time t;

$\hat{s}(\mathbf{x}, t)$ = the epidermal concentration of a signal morphogen, received from the dermis, at position $\mathbf{x}$ and time t.

Similarly, the variables for the dermis are

$n(\mathbf{x}, t)$ = the dermal cell density at position $\mathbf{x}$ and time t;

$s(\mathbf{x}, t)$ = the dermal concentration of a signal morphogen produced in the dermis, at position $\mathbf{x}$ and time t;

$e(\mathbf{x}, t)$ = the dermal concentration of a signal morphogen, received from the epidermis, at position $\mathbf{x}$ and time t.

(Morphogen variables and related constants specific to the epidermal layer are distinguished from those of the dermal layer by using the hat symbol.)

The epithelial sheet is modelled as a two-dimensional, visco-elastic continuum in equilibrium and it is assumed that epidermal cells move only by convection. The chemical $\hat{e}(\mathbf{x}, t)$, secreted by epidermal cells, is assumed to diffuse from a high concentration in the epidermis, across the basal lamina, to a lower concentration in the dermis. There the morphogen, represented by $e(\mathbf{x}, t)$, acts as a chemoattractant for dermal cells thus inducing papilla formation. Similarly the morphogen $s(\mathbf{x}, t)$ is the signal produced by the dermal cells which then diffuses through the basal lamina into the epidermal layer. There the morphogen, represented by $\hat{s}(\mathbf{x}, t)$, increases cell traction thus causing cell aggregation which leads eventually to placode formation. The scenario is sketched in Figure 2.

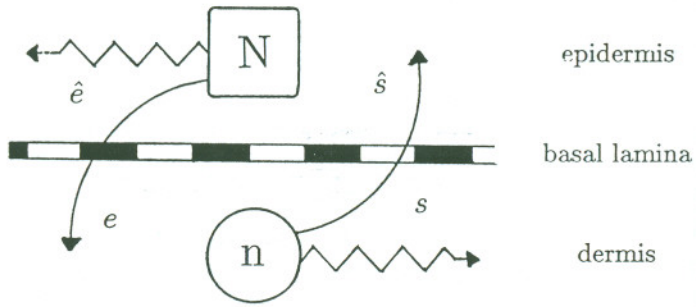

Figure 2: Representation of the tissue interaction mechanism (Cruywagen & Murray, 1992). The dermal cells, n, produce a morphogen, s, which diffuses to the epidermis where it is denoted by $\hat{s}$. In the epidermis $\hat{s}$ increases cell traction which, in turn, causes cell aggregation. Similarly $\hat{e}$, produced by the epidermal cells, N, diffuses to the dermis where it is denoted by $\hat{e}$. In the dermis $\hat{e}$ acts as a chemoattractant for dermal cells, causing cell aggregation.

The model equations are:

$$\frac{\partial N}{\partial t} = \overbrace{-\nabla \cdot N\frac{\partial \mathbf{u}}{\partial t}}^{\text{convection}} \tag{1}$$

$$\nabla \cdot \{\overbrace{\frac{E}{1+\upsilon}[\epsilon - \beta_1\nabla^2\epsilon + \frac{\upsilon}{1-2\upsilon}(\theta - \beta_2\nabla^2\theta)\mathbf{I}]}^{\text{elastic stresses}} + \overbrace{\mu_1\frac{\partial \epsilon}{\partial t} + \mu_2\frac{\partial \theta}{\partial t}\mathbf{I}}^{\text{viscous stresses}} + \overbrace{\tau(\hat{s})\mathbf{I}}^{\text{traction}}\} = \overbrace{\rho\mathbf{u}}^{\text{body forces}} \tag{2}$$

$$\frac{\partial \hat{e}}{\partial t} = \overbrace{\hat{D}_e\nabla^2\hat{e}}^{\text{diffusion}} + \overbrace{f(N,\hat{s})}^{\text{production}} - \overbrace{P_e(\hat{e}-e)}^{\text{dermal signal}} - \overbrace{\hat{\gamma}\hat{e}}^{\text{degradation}} \tag{3}$$

$$\frac{\partial n}{\partial t} = \overbrace{\nabla \cdot D(e)\nabla n}^{\text{diffusion}} - \overbrace{\nabla \cdot n\alpha(e)\nabla e}^{\text{chemotaxis}} + \overbrace{rn(n_0 - n)}^{\text{mitosis}}. \tag{4}$$

$$\frac{\partial e}{\partial t} = \overbrace{D_e\nabla^2 e}^{\text{diffusion}} + P_e(\hat{e}-e) - \overbrace{\gamma n e}^{\text{metabolism}} \tag{5}$$

Equation (1) is the conservation equation for epithelial cell density and equation (2) is the force balance equation for the epithelium, where E is the passive elastic modulus, υ is Poisson's ratio,μ_1 and μ_2 are the shear and bulk viscosities respectively (Landau & Lifshitz 1970), and $\mathbf{I}$ is the unit tensor. The dilation is defined by $\theta = \nabla \cdot \mathbf{u}$ and the strain tensor by $\epsilon = (\nabla\mathbf{u} + \nabla\mathbf{u}^T)/2$, where T indicates the transpose. The positive parameters β_1 and β_2 reflect the strength of the long range elastic forces (see Murray 1989 for a pedagogical discussion). The epidermis is tethered to the basal lamina and the positive parameter ρ reflects the strength of this attachment. Since we are dealing with a system at a very low Reynolds number, inertial terms are ignored in the force balance equation.

The active cell traction is determined by the chemical $\hat{s}$ and is modelled by the function $\tau(\hat{s}) = \tau\hat{s}^2/(1 + c\hat{s}^2)$ where τ and c are positive constants. This specific form is similar to that experimentally observed (Murray & Oster 1984) when the inducing chemical is Ca^{2+}. Strictly speaking, because of the complexity of biological tissue, the elastic modulus E, and both the viscosity coefficients, μ_1 and μ_2, are functions of $\hat{s}$.

Equations (3) and (5) are the conservation equations for the morphogen $\hat{e}$, and similar equations hold for the morphogen s. For simplicity it is assumed that the production of both morphogens is proportional to the respective cell densities N and n, so that, for the case of $\hat{e}$, $f(N,\hat{s}) = k_e N$, where the positive constant, k_e, is the epidermal production rate. During paracrine signalling the chemical molecules are rapidly degraded by enzymes and the positive constant $\hat{\gamma}$ is a measure of the degradation rate. In the dermis the signalling molecules attach to the mesenchyme receptor cells and are metabolized by them. It is assumed that this metabolism is proportional to the receptor cell density, n, and the chemical concentration e. The metabolic rate is denoted by the positive constant γ.

For dermal cell movement, a simple chemotaxis model is proposed, related to the cell-chemotaxis model of Oster & Murray (1989), and based on the *Morphoregulator Hypothesis* (see, for example, Edelman, 1986). This hypothesis states that cell-cell adhesion, mediated by CAMs, controls skin organ morphogenesis and that differences in the effectiveness and concentration of CAMs in the dermis can lead to gradients in cell density which, in turn, lead to spatial patterns. Because chemical modulation can have a marked effect on the binding rates and binding strength of CAMs (Grumet & Edelman 1988), it is assumed that the chemical signal e, is responsible for the CAM expression. This dependence is modelled by the term $\alpha(e)$ in equation (4).

The conservation equation for mesenchymal cell density also incorporates random cell migration, modelled by Fickian diffusion, and cell division, modelled by logistic growth. Here D, the diffusion coefficient, is a function of the chemical e; r and n_0 are positive constants related to cell mitosis.

The model equations (1) – (5) are solved subject to homogeneous Neumann boundary conditions on the morphogen concentrations and the cell densities, and homogeneous Dirichlet conditions on epidermal displacement.

5. PROPAGATING SPATIAL PATTERNS

The majority of models proposed so far only addresses the issue of synchronous pattern formation — it is assumed that as a bifurcation parameter changes, the homogeneous steady state becomes unstable and bifurcates to a spatial pattern which develops simultaneously on the whole domain.

However, in many developmental situations spatial pattern formation occurs sequentially. Regular patterns of repeated units often develop at a frontier of pattern formation (Zeeman 1974) which moves across the prospective area to transform bland tissue into an array of patterned components. As discussed earlier, this is the case for feather pattern formation on the chick skin. Further examples of such waves of pattern formation are in the development of somites (Pearson & Elsdale 1979), reptilian teeth (Edmund 1969), scales (Maderson 1965a,b) and alligator skin patterns (Murray *et al.* 1990).

Such sequential patterning has only been considered in non-tissue interaction models. For example, Myerscough & Murray (1992) considered a chemotaxis model on an one-dimensional domain to describe the propagation of stripes in alligator integument. Nagorcka (1986) considered sequential patterning for reaction diffusion systems on two-dimensional domains.

Here we apply the tissue interaction model described in Section 4 to sequential pattern formation of feather primordia on the chick back, as detailed in Section 2. The full model is a formidable system to solve, so we consider a caricature model (see Cruywagen & Murray, 1992) of the full system which captures the essential features of the full tissue interaction mechanism. The caricature model was solved on a rectangular domain. On such a domain it is possible to choose parameters such that the uniform steady state is linearly unstable at a degenerate bifurcation. In this case more than one type of heterogeneous spatial pattern is possible. Numerical simulations of the model

demonstrate that the spatial pattern which propagates across the domain depends crucially on the pattern that forms initially on the dorsal midline. Figure 3 shows that if spots are initially specified on the dorsal midline, the resultant propagating pattern closely resembles the *chessboard* patterns of feather germs observed on the chick back. On the other hand, if the initial pattern is stripes, then the resultant two-dimensional pattern would be stripes. A detailed discussion of the sequential aspects of pattern formation is presented in Cruywagen *et al.* (1994).

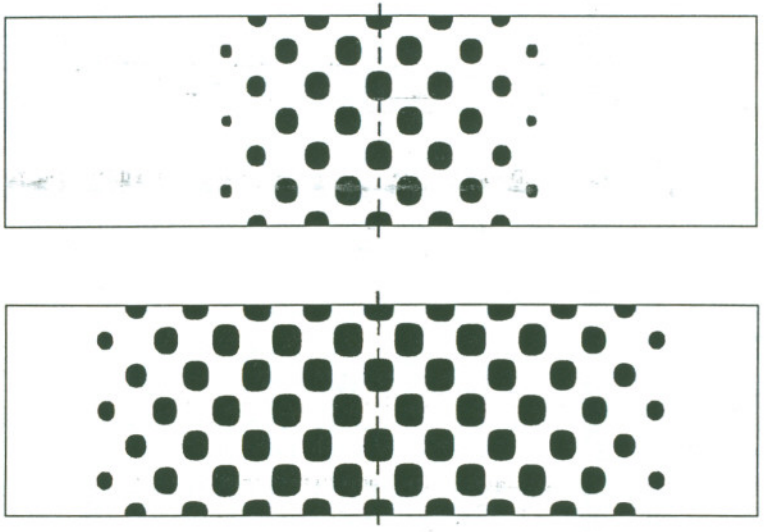

Figure 3: Numerical solution of a caricature model of the full system (1)–(5), see Cruywagen & Murray (1992) for full details. The resultant propagating pattern depends crucially on the initial pattern on the dorsal midline. By specifying an initial pattern of spots in cell density along the dorsal midline, the pattern propagates through the domain to form a chessboard pattern similar to that observed for feather primordia on the chick back. The results are shown at two different times. Areas of high cell density are shaded. The dorsal midline is indicated by the broken line.

6. DISCUSSION

Although pattern formation in development has been widely studied, the role of tissue interaction and the phenomena of propagating spatial patterns have been comparatively ignored. In this paper we have examined a model for tissue interaction and have shown that it can produce sequential pattern. Perhaps the crucial result of this work is that

the nature of the two-dimensional pattern is determined by the critical pattern which develops along the dorsal midline. That is, a simple quasi-one-dimensional pattern determines the form of the much more complex two-dimensional pattern. This suggests that in development the specification of a one-dimensional pattern may be all that is required to control the propagation of two-dimensional patterns.

Mathematically, propagating patterns pose challenging problems of analytically determining the speed of propagation and the wavelength of spatial patterns. For one-dimensional spatial patterns Myerscough & Murray (1992) have used an asymptotic method to obtain an analytical approximation to these quantities in the case of a cell-chemotaxis model. Cruywagen *et al.* (personal communication, 1992) have used an envelope method to calculate the speed of pattern propagation in the one-dimensional version of the tissue interaction model studied here.

In the tissue interaction models of Nagorcka *et al.* (1987) and Shaw & Murray (1990), for example, two pattern generators were in effect coupled. One of the most interesting aspects of the built-in tissue interaction in these models is that the spectrum of patterned solutions that is obtained is much *smaller* than the sum of the two classes of individual patterns which can be formed by the individual generators. Simulation of the coupled system almost always resulted in a greatly reduced number of excitable modes. There seems to be a strong basin of attraction for a specific and highly restricted subset of the theoretically possible patterns. The *nonlinearity* and the *coupling* appear to enhance the strength of the basin of attraction of specific patterns from the many possible. One interesting exception to the reduction in the number is the case when neither mechanism can produce pattern on its own but coupling them together results in a pattern (Shaw & Murray, 1990).

Spatial pattern locking is an interesting concept which could have far reaching consequences in our understanding of how development actually takes place and could explain the ever present robustness of pattern formation in developing embryos. The concept of basins of attraction in spatial pattern generators was introduced and discussed in more detail by Murray (1992). With spatially homogeneous oscillators, the problem of phase locking is, of course, well known. The mathematical analysis of spatial phase locking is particularly challenging and, analytically, is essentially virgin territory.

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REFERENCES

Bard, J.B.L. (1981). A model for generating aspects of zebra and other mammalian coat patterns. *J. Theor. Biol.* , **93**, 363–385.

Chuong, C.-M., Edelman, G.M. (1985a). Expression of cell adhesion molecules in embryonic induction. I. Morphogenesis of nestling feathers. *J. Cell Biol.*, **101**, 1009–1026.

Chuong, C.-M., Edelman, G.M. (1985b). Expression of cell adhesion molecules in embryonic induction. II. Morphogenesis of adult feathers. *J. Cell Biol.* , **101**, 1027–1043.

Cocho, G., Perez-Pascual, R., Ruis, J.L. (1987). Discrete systems, cell-cell interactions and color pattern of animals. I. Conflicting dynamics and pattern formation. *J. Theor. Biol.*, **125**, 419-435.

Cruywagen, G.C., Maini, P.K., Murray, J.D. (1994). Travelling waves in a tissue interaction model for skin pattern formation *IMA J. Maths. Appl. in Medic. and Biol.* (in press).

Cruywagen, G.C., Murray, J.D. (1992). On a tissue interaction model for skin pattern formation. *J. Nonlinear Sci.*, **2**, 217-240.

Davidson, D. (1983a). The mechanism of feather pattern development in the chick. I. The time determination of feather position. Yevgeny B. Karasik Dept. of Computer Science Tel Aviv Univ. *J. Embryol. exp. Morph.* , **74**, 245-259.

Davidson, D. (1983b). The mechanism of feather pattern development in the chick. II. Control of the sequence of pattern formation. *J. Embryol. exp. Morph.*, **74**, 261-273.

De Kepper, P., Castets, V., Dulos, E., Boissonade, J. (1991). Turing-type chemical patterns in the chlorite-iodide-malonic acid reaction. *Physica D*, **49**, 161-169.

Dhouailly, D. (1973). Dermo-epidermal interactions between birds and mammals: differentiation of cutaneous appendages. *J. Embryol. exp. Morph.* , **30**, 587-603.

Dhouailly, D. (1975). Formation of cutaneous appendages in dermo-epidermal recombination between reptiles, birds and mammals. Wilhelm Roux Arc. *EntwMech. Org.*, **177**, 323-340.

Dhouailly, D., Maderson, P.F.A. (1984). Ultrastructural observations on the embryonic development of the integument of *Lacerta muralis (Lacertilia, Reptilia). J. Morph.*,**179**, 203-228.

Edelman, G.M. (1986). Cell adhesion molecules in the regulation of animal form and tissue pattern. *Annu. Rev. Cell Biol.* , **2**, 81-116.

Edmund, A.G. (1969). Dentition. In: *Biology of the Reptilia.* (eds. Bellairs, A.d'A. & Parsons, T.S.). Academic Press: London.

Ferguson, M.W.J. (1985). The reproductive biology and embryology of crocodilians. In: *Biology of Reptilia.* Vol. 14 Development A. (eds Gans, C., Billet, F., Maderson, P.F.A.) 329-491. Wiley: New York.

Gallin, W.J., Chuong, C.-M., Finkel, L.H., Edelman, G.M. (1986). Antibodies to liver cell adhesion molecules perturb inductive interactions and alter feather pattern and structure. *Proc. Natl. Acad. Sci. USA.*, **83**, 8235-8239.

Gerber, A. (1939). Die embryonale und postembryonale Pterylose der Alectromorphae. *Rev. Suisse Zool.*, **46**, 161-324.

Gilbert, S.F. (1988) *Development Biology.* 2nd edn. Sinauer Associates, Inc: Sunderland.

Grumet, M., Edelman, G.M. (1988). Neuron-glia cell adhesion molecules interact with neurons and astroglia via different binding mechanisms. *J. Cell Biol.*, **106**, 487-503.

Landau, L. D. , Lifshitz, E. M. (1970). Theory of Elasticity. 2nd ed., Pergamon: New York.

Leviton, A. E. , Anderson, S. C. (1984). Description of a new species of *Cyrtodactylus* from Afghanistan with remarks on the status of *Gymnodactylus longpipes* and *Cytrodactylus fedtschenkoi. J. Herp.*, **18**, 270-276.

Maderson, P.F.A. (1965a). The embryonic development of the squamate integument. *Acta Zool.*, **46**, 275-295.

Maderson, P.F.A. (1965b). The structure and development of the squamate epidermis. In: Biology of the Skin and Hair Growth. (eds. Lyne, A.G. & Short, B.F.) Sydney: Angus and Robertson.

Maderson, P.F.A. (1985). Some developmental problems of the reptilian integument. In: *Biology of Reptilia.* Vol. 14 Development A. (eds Gans, C., Billet, F., Maderson, P.F.A.) 523-598. Wiley: New York.

Maini, P.K., Myerscough, M.R., Murray, J.D., Winters, K.H. (1991). Bifurcating spatially heterogeneous solutions in a chemotaxis model for biological pattern formation. *Bull. Math. Biol.*, **53**, 701-719.

McKeehan, M.S. (1951). Cytological aspects of embryonic lens induction in the chick. *J. exp. Zool.*, **117**, 31-64.

Mooney, J.D., Nagorcka, B.N. (1985). Spatial patterns produced by a reaction-diffusion system in primary hair follicles. *J. Theor. Biol.*, **115**, 229-317.

Murray, J.D. (1981a). A pre-pattern formation mechanism for animal coat markings. *J. Theor. Biol.*, **88**, 161-199.

Murray, J.D. (1981b). On pattern formation mechanisms for Lepidopteran wing patterns and mammalian coat markings. *Phil. Trans. Roy. Soc. Lond.*, **B295**, 473-496.

Murray, J.D. (1989). Mathematical Biology. Springer Verlag: Heidelberg.

Murray, J.D., Deeming, D.C., Ferguson, M.W.J. (1990). Size dependent pigmentation pattern formation in embryos of *Alligator Mississipiensis*: time of initiation of pattern generation mechanism.*Proc. Roy. Soc.*, **B239**, 279-293.

Murray, J.D., Oster, G.F. (1984). Cell traction models for generating pattern and form in morphogenesis. *J. Math. Biol.*, **19**, 265-279.

Murray, J.D., Myerscough, M.R. (1991). Pigmentation pattern formation on snakes. *J. Theor. Biol.*, **149**, 339-360.

Murray, J.D. (1993). Complex pattern formation and tissue interaction. In: *Proceedings 1st European Conference on the Applications of Mathematics to Medicine & Biology* (1990) (eds. Demongeot, J., Capasso, V.) pp. 495-506, Wuerz Publishing: Winnipeg.

Myerscough, M. R., Murray, J.D. (1992). Analysis of propagating pattern in a chemotaxis system. *Bull. Math. Biol.*, **54**, 77-94.

Nagorcka, B. N. (1984). Evidence for a reaction-diffusion system in the formation of hair fibres. *Biosystems*,**16**, 323-332.

Nagorcka, B. N. (1986). The role of a reaction-diffusion system in the initiation of skin organ primordia. I. The first wave of initiation. *J. Theor. Biol.*, **121**, 449-475.

Nagorcka, B.N., Mooney, J. D. (1982). The role of a reaction-diffusion system in the formation of hair fibres. *J. Theor. Biol.*, **98**, 575-607.

Nagorcka, B.N., Mooney, J. D. (1985). The role of a reaction-diffusion system in the initiation of primary hair follicles. *J. Theor. Biol.*, **114**, 243-272.

Nagorcka, B.N., Manoranjan, V.S., Murray, J.D. (1987). Complex spatial patterns from tissue interactions – an illustrative model. *J. Theor. Biol.*, **128**, 359-374.

Oster, G.F., Murray, J.D. (1989). Pattern formation models and developmental constraints. *J. Exp. Zool.*, **251**, 186-202.

Ouyang, Q., Swinney, H.L. (1991). Transition from a uniform state to hexagonal striped Turing patterns. *Nature*,**352**, 610-612.

Pearson, M., Elsdale, T. (1979). Somitogenesis in amphibian embryos. I. Experimental evidence for an interaction between two temporal factors in the specification of the somite pattern. *J. Embryol. exp. Morph.* , **51**, 27-50.

Rawles, M. (1963). Tissue interactions in scale and feather development as studied in dermal epidermal recombinations. J. Embryol. exp. Morph. **11**, 765-789.

Saxén, L., Lehtonen, E., Karkinen-Jääskeläinen, M., Nordling, S., Wartiovaara, J. (1976). Are morphogenetic tissue interactions mediated by transmissable signal substances or through cell contacts? *Nature*, **259**, 662-663.

Sengel, P. (1976). Morphogenesis of Skin. Cambridge University Press: Cambridge.

Shaw, L.J., Murray, J.D. (1990). Analysis of a model for complex skin patterns. *SIAM J. Appl. Math.* , **50**, 628-648.

Shaw, L.J. (1989) Tissue Interaction Models for Spatial Pattern and Form. D. Phil thesis, Oxford.

Wessells, N.K. (1977). Tissue Interaction in Development. W. J. Benjamin: Menlo Park.

Wolpert, L. (1981). Positional information and pattern formation. Phil. Trans. Roy. Soc. Lond., **B295**, 441-450.

Zeeman, E.C. (1974). Primary and secondary waves in developmental biology. *Lectures in Mathematics in the Life Sciences* Vol. 4, Rhode Island: American Mathematical Society.

TOWARD ARTIFICIAL COMPETENCE

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This brief essay moves from a personal perspective of research in neurobiology to the identification of some important areas for future research.

My first exposure to neurobiology was in several Gordon Conferences on Theoretical Biology. I didn't find it appealing. All those wiggly voltage graphs. All that incomprehensible electricity. But "cast your bread upon the waters... ." My Gordon Conference background stood me in good stead as a basis for conversation when Hanna Parnas asked me whether I would like to collaborate with her in a study of the control of neurotransmitter release. I had enjoyed working with Hanna when we both were thinking about slime mold development, so that I was receptive when she described the new area of interest that she had picked up on sabbatical. Neurotransmitter release, she argued cogently, was a central phenomenon in neurobiology that deserved careful study. Experiments abounded, and there was much theory – enough of both, and of sufficent quality, to produce a much deserved Nobel Prize for Bernhard Katz, about 20 years ago. In particular, Katz had established that the presence of calcium in the bathing solution was necessary to induce release.

Yet to explain various experiments on facilitation of a second release by a previous stimulus, only the very first theoretical steps had been taken. Hanna argued that what was required was to write and analyze equations for three sub-processes: the entry of calcium into the terminal (induced by an action potential), the subsequent removal of calcium by various mechanisms, and finally the release of neurotransmitter as a function of the intracellular calcium concentration.

We began work in 1979, initiating a collaboration that continues to this day. An early important step occurred when we were pondering experimental results by Cooke, Okamoto, and Quastel (1973). We could see no way to explain some of their findings except to postulate that voltage had further effects, in addition to the accepted one of opening channels for the influx of extracellular calcium. At this point decisive progress was made in a collaborative effort of Josef Dudel (Technological University, Munich) and Yitzhak Parnas (Hebrew University), both experimentalists, together with Hanna. In a series of papers published in *Pflüger's Archiv* (notably Dudel, Parnas, and Parnas, 1983) the so-called "calcium-voltage" hypothesis was developed. Theoretical and experimental work went hand in hand to make a solid case for the hypothesis that the entry of calcium into the nerve terminal was not sufficient for release. A second factor was required. The second factor appeared to be the voltage itself, which not only induced calcium entry but also acted directly to activate a certain molecule or factor that was essential in the promotion of release.

Strong interaction between the theorists and the experimentalists in our group led to the postulation of the following kinetic scheme as the core of the calcium-voltage hypothesis: