1. Introduction

Structural and functional ideas in developmental biology have been somewhat eclipsed by the recent advent of developmental genetics. The role of genetic information in determining the development of organised and differentiated structures from a single cell is commonly deduced by studying the effects of gene mutations, determining that a certain gene is *necessary* for the correct development of a particular structure. The revolution in molecular genetics has led to unprecedented discoveries and advances, however, there is a danger that something has been lost in the thrust of this research. While nobody would dispute that genes and gene products act in concert, our understanding of the mechanisms by which differentiated structures emerge from the interaction of genes and their products is at risk of being overlooked in the drive to identify (and label) the genetic components involved in the process. The implicit assumption that all growth and form of an organism can be explained in terms of gene instructions, that organisms are 'genetically programmed', is opposed by the alternative view that spatial organisation, illustrated by the adaptive and regulatory properties of developing organisms, must be explained otherwise.

The study of physical aspects of growth and form has a long and distinguished history. D'Arcy Thompson's celebrated book [125] draws parallels between biological forms and structures arising in physical systems. That physical laws constrain what is possible (in terms of morphology or pattern) is indisputable. However, to understand how morphology is determined and regulated it is necessary to postulate physical mechanisms that may plausibly coordinate the spatio-temporal emergence of structure.

This thesis is concerned with one such class of mechanism, based on simple physical principles, which can generate spatial pattern from initial homogeneity. In the context of morphogenesis, Alan Turing [126] proposed that a set of chemicals which react and diffuse within a substrate could lead to the spontaneous symmetry breaking of an initially homogeneous distribution of the chemical concentrations, and the generation of spatial patterns. Before discussing this model in more detail, we consider pattern formation in biological systems, and various alternative modelling approaches.

1.1 Models for Biological Pattern Formation

Any instance of a heterogeneous distribution of gene product or differentiated tissue constitutes a pattern, and any such scenario may be interrogated as to the mechanisms of organisation and regulation of the pattern. A multitude of different morphologies in many different areas of biology have been the subject of mathematical modelling. Several biological systems have attained status of paradigm in theoretical work in this field, including the segmentation of the insect embryo [58, 118], limb development [79, 26], the formation of animal coat markings [88] and the arrangement of hair follicles and feather primordia in skin [90, 89]. Certain unicellular organisms have also

been studied experimentally and theoretically in the context of pattern formation, for example the generation of whorls in the marine alga *Acetabularia* [44] and the branched and star-shaped morphologies of *Micrasterias* [66, 48, 50]. For these species (where each organism has only one nucleus) it is most apparent that structure must develop through spatially distributed physical processes occurring within the cell. At the other end of the scale, patterns in population density (often called 'patchiness') are studied in ecological settings [121, 83, 96, 80].

These examples raise an important theoretical consideration. For the patterning of animal skins, a greater or lesser degree of variation is often displayed between members of the same species and even between closely (genetically) related animals. However, the mechanisms regulating segmentation and limb development, for example, must be able to reliably generate the same number of pattern elements despite normal biological variation, for example in the size or geometry of the region in which the pattern develops. Models whose purpose it is to describe the mechanisms of spatial organisation must be able to account for whichever of these alternative features is observed in the biological system under scrutiny. We return to this crucial issue in the discussion of reaction-diffusion models below.

Two general categories for models of pattern formation may be described, which encompass most theoretical research to date, namely chemical prepattern and cell motility (or mechano-chemical) models. The latter consider the aggregation of cell populations subject to chemical signals and mechanical forces, where it is supposed that cell differentiation occurs in response to increased cell density (see the book by Murray [88] and references therein). In this thesis we are primarily concerned with models of the chemical prepattern type. Here it is argued that a pattern is first established in the concentration of certain chemicals (termed *morphogens*), and subsequent differentiation into different tissue types occurs according to whether or not the concentration exceeds some threshold locally. Thus it is implicitly assumed that the chemical pattern is established on a faster timescale than the response of the cellular machinery, so that the formation and interpretation of the pattern decouple.

The idea of threshold-mediated response to morphogen concentration gradients is developed in Wolpert's notion of positional information [134, 135]. Much of this work considers cellular response to (possibly multiple) simple gradients. Crick [20] established that gradients could form on realistic timescales over distances of a millimetre or less under the mechanism of passive or facilitated diffusion of morphogen from a localised source.

Theoretical approaches may also be divided into discrete (cellular) and continuum descriptions. Turing's original discussion of the reaction-diffusion mechanism considers both possibilities, however, the analysis is restricted to the case of diffusive coupling between cells (or through the tissue), which is by no means the only mechanism of cellular communication demonstrated in biology. Much is known at the molecular level about intercellular signalling. Cells can demonstrate active regulation of signals

and passage of substances, which are ignored in diffusion-based models. In some instances signalling molecules are held in the cell membrane and bind to receptors on adjacent cells only, a process known as juxtacrine signalling. Lateral inhibition, for which ligand binding down-regulates ligand and receptor expression, generates fine-grained patterns where the wavelength is two cell diameters (high and low expression levels of the ligand are found on alternate cells). This mechanism is observed in the *Delta-Notch* signalling pathway [17], and typically selects a subset of cells from an initially equivalent field which adopt a different cell fate. A recent model due to Owen *et al.* [104, 105] considers lateral induction, where signalling results in up-regulation of ligand and receptor expression (positive feedback), and has demonstrated that longer wavelength patterns may be generated in a mechanism with only nearest-neighbour cell communication. In two dimensions spot and stripe patterns may be generated by this model.

Before going on to discuss the reaction-diffusion mechanism in more detail, we briefly discuss the justification for such modelling, as well as some of the pitfalls. In this context it is useful to distinguish between *modelling* and *simulation* of a natural phenomenon. It would be a tall order (if not impossible) to describe all of the detailed processes involved in any single biological pattern forming event. However, such an all-encompassing mathematical description (or some approximation to it) would constitute a simulation of the system. This is not the intention of the sorts of models we have described above; rather the aim is to discover whether some smaller set of processes may in themselves be sufficient to account for the phenomenon. The model is built to encapsulate only those mechanisms of interest, and is analysed to determine qualitatively whether the phenomenon may be attributed to the interaction of these mechanisms. In this sense we are constructing and testing *theories* of pattern formation. Furthermore, a simple model, such as a reaction-diffusion system, may be considered to represent a caricature of some more complicated (and unidentified) system, which captures the dynamics of the higher dimensional system. In this sense different classes of model may be studied as paradigms for pattern formation, representing some level of mathematical abstraction from the physical reality. Clearly this is only satisfactory from the biological point of view if connections can be made from the mathematical analysis to physically measurable quantities. Of course a successful comparison between model behaviour and the natural phenomenon does not guarantee that the model constitutes the correct explanation. It may be the case that several models with different underlying assumptions generate similar behaviour. This is found to be the case for reaction-diffusion models and certain mechano-chemical models for pattern formation, where the underlying mathematical structure of the pattern forming bifurcation are similar, both mechanisms generating an intrinsic pattern wavelength. Hence it may be difficult to distinguish between the predictions of models describing very different mechanisms purely in terms of phenomenology.

1.2 Reaction-Diffusion Theory

In 1952, Turing proposed that pattern formation during morphogenesis might come about through an instability in systems of reacting chemicals, driven by diffusion. The resulting chemical prepatterns, states the hypothesis, are subsequently interpreted as positional information by competent cells and cell fates are determined via prepattern-dependent differentiation. As will be shown in the following chapter, a set of two or more chemicals is required to interact in a well defined manner in order that heterogeneous patterns may arise in their concentrations. Significantly, the diffusion-driven instability (DDI) requires disparity between the diffusivities of the chemicals.

This mechanism for spatial and spatio-temporal pattern formation is of great theoretical interest as it represents a spontaneous spatial symmetry-breaking phenomenon in a simple physical system. Any thermodynamically closed system, where there is no transfer of matter or heat into or out of the system, must evolve towards thermodynamic equilibrium. Pattern formation in such systems can be only transient. However, Prigogine and Nicolis [94] showed that if nonequilibrium (or far-from-equilibrium) thermodynamic conditions are maintained in an open reactor, e.g. by providing a constant supply of reactant, then heterogeneous patterns may be sustained. In the mechanism described by Turing these patterns have an intrinsic wavelength which does not depend on the physical size of the reactor, and patterns tend to demonstrate periodicity. Turing's theory has found application in fields far removed from developmental biology—see for example the book by Walgraef [130].

Turing's ideas have been applied to a wide variety of pattern formation problems in biology. However, the theory has received important criticism on several fronts. Firstly, although many molecules have been identified which appear to act as diffusive signals, some of which may act as morphogens in the Wolpertian sense, no set of chemicals has been demonstrated to operate in the manner that Turing described in a biological system. In fact, it is only relatively recently that Turing patterns have been demonstrated conclusively under controlled conditions in artificial chemical systems, discussed in the following section.

We have already hinted at the second major source of criticism of Turing's theory of pattern formation in biology. In many situations the number of pattern elements (for example the number of wavelengths generated in one dimension) is crucial. Turing patterns have been shown to display strong sensitivity to the size and geometry of the solution domain. This criticism, which has come to be known as the *robustness* problem, was first brought to light concerning the segmentation of *Drosophila*. Kauffman [59] suggested that periodic gene expression patterns observed during the early development of insects could be explained by a reaction-diffusion model, which appeared to give qualitatively similar patterns on regular and rather symmetric domains. Subsequent work [10] showed that patterns which do not resemble those occurring naturally are obtained for minor perturbations of the size and shape of the domain. In

fact it was subsequently discovered that the segmentation of the *Drosophila* embryo is achieved in a manner much closer to that described by Wolpert, where each individual element of the apparently periodic pattern is separately controlled and regulated, and the pattern is generated in a cascade of gene switching [2].

The root of this problem is in the fact that for domains of anything but very small aspect ratio (the ratio of domain size to intrinsic pattern wavelength) there are many different patterned solutions which may be generated, the number increasing as the domain size is increased, and the selection between these different patterns depends sensitively on initial data and domain geometry. Bard and Lauder [6] draw the same conclusion, finding in a series of numerical experiments that patterns in discrete cellular simulations are sensitive to the number of cells, concluding that only unpredictable *mosaic* patterns are possible. More recently Saunders and Ho [118] have considered segmentation of growing systems, concluding once again that reaction-diffusion does not constitute a reliable pattern generation mechanism. Dillon *et al.* [26] have shown that the multiplicity of solutions may be reduced by varying the boundary conditions. We will demonstrate that the consideration of domain growth during pattern formation may have important consequences for the robustness issue.

An alternative way of viewing the robustness issue is to argue that the reaction-diffusion mechanism fails to demonstrate the regulatory properties that we described earlier. While for given initial conditions it may be possible to select the desired pattern by judicious choice of domain size, in general biological systems are subject to natural variation in such parameters and reliable pattern generation requires a certain degree of scale invariance. To achieve this regulatory property, various modifications to the theory have been proposed, requiring some form of feedback from the domain size to the parameters in the function describing the reaction rates [100, 52]. We will discuss scale invariance later, in light of results we will present for pattern formation on growing domains. Before turning to consider domain growth we discuss the realisation of chemical patterns in laboratory experiments.

1.3 Chemical Pattern Formation

Travelling waves in chemical systems have been known for some time in the Belousov-Zhabotinsky reaction, however, reactions demonstrating the stationary patterns predicted by Turing have only been discovered within the last decade. General reviews of spatio-temporal phenomena in chemistry can be found in Epstein and Showalter [36] and Johnson and Scott [56] and, for spatial patterns, Maini *et al.* [78].

The experimental realisation of Turing patterns was precipitated by the development of gel reactors where reactants undergo diffusive transport through an aqueous gel, which serves to suppress any convective motion. First introduced by De Kepper and Boissonade in Bordeaux, the Gel Strip reactor has two reservoirs containing chemically inert sets of reactants which are allowed to diffuse into a thin rectangular ribbon of gel from opposite sides. In the middle of the ribbon both sets of chemicals are

present and may react. The concentrations in the two reservoirs can be held constant to maintain nonequilibrium conditions. The first unambiguous experimental observation of Turing patterns was reported by this group in the CIMA reaction[1] [13, 23]. Here the gel was loaded with starch primarily to aid visualisation. Starch, a large molecule with low mobility in the gel matrix, forms a complex with iodide, one of the reacting species, effectively reducing its diffusion coefficient to provide the necessary conditions for Turing patterns to form. Subsequent observations were reported by Ouyang and Swinney [103] using a variation on the design, the Gel Disk reactor, where patterns form in the plane perpendicular to the concentration gradients so that larger patterned domains can be observed. A vast amount of theoretical work has been done to develop analytical models of these complicated reactions, the aim being to reproduce the phenomena and to calculate phase and bifurcation diagrams describing the chemical systems.

Stationary patterns have also been recorded in the FIS reaction[2] when initiated with sufficiently large perturbation away from equilibrium. Here pattern formation is achieved by propagating chemical (redox) fronts which halt when they approach each other. In this case labyrinthine patterns [69] have been observed as well as self-replicating phenomena [70], where a localised spot grows, divides and separates, repeating to fill domain. Other phenomena include breathing spot patterns, where the spot radius oscillates [47]. Recently, similar structures have also been reported in the CIMA reaction [22]. However, these patterns are not of Turing type in the strict sense, as we shall see in the following chapter.

1.4 Domain Growth

The motivation for consideration of domain growth in developmental systems is apparent. Most pattern formation takes place during the growth of the organism. However, what may not be immediately clear is whether underlying growth, which is expected to take place over much longer timescales than the generation of pattern via reaction and diffusion, may be considered to decouple from the reaction-diffusion mechanism, as are other cellular processes. Furthermore, it is unclear how the incorporation of domain growth might influence pattern selection. The following is a quote from Cross and Hohenberg's comprehensive review of pattern formation in nonequilibrium systems [21, p.1052]:

'A natural procedure for biological systems is to consider the spatial domain Ω to be a function of time $\Omega(t)$. Then the dynamics of the [reaction-diffusion] equations will be supplemented by the stretching of the domain. In the simplest case one might assume that the timescale for variation of $\Omega(t)$ is slow, but never the less the final pattern obtained might be very different

[1] Chlorine-Iodide-Malonic Acid
[2] Ferrocyanide-Iodide-Sulfite

from the one which would be produced by specifying an initial condition on the fully grown domain $\Omega(t_{final})$.'

It is our intention in this thesis to present a systematic study of the influence of domain growth on pattern formation in reaction-diffusion systems.

Domain growth has previously been considered in reaction-diffusion models for the sequence of emergence of tooth primordia in the developing jaw [65] and for the branching morphology of growing *Micrasterias* [66]. New impetus was recently provided by Kondo and Asai [64], who suggested that a reaction-diffusion mechanism could be responsible for the dynamic changes in pigmentation patterns of the marine angelfish *Pomacanthus*. Unlike mammalian coat markings, the pattern in the skin of these fish changes dynamically during growth of the animal, rather than simply enlarging in proportion to the body size. Juvenile *P. imperator* display concentric stripes and *P. semicirculatus* have a regular array of vertical stripes which increase in number during growth. Juvenile *P. semicirculatus* of less than 2cm in length display three vertical stripes which separate until the length of the fish is approximately 4cm, at which point new stripes appear between the original ones. Similarly at around 8-9cm in length new stripes again appear between the existing ones. In this manner the pattern changes by insertion of new stripes as the animal roughly doubles in length, to preserve the wavelength of the pattern. In *P. imperator* this behaviour is maintained in the adult fish, where horizontal stripes maintain an average spacing. This dynamic regulation of the pattern is quite unlike the static pattern selection we have previously discussed.

In the following chapter we present a detailed discussion of the diffusion-driven instability, including further discussion of the robustness problem, and some mathematical properties of solutions to the model pertinent to pattern formation on growing domains. In Chapter 3 we derive the governing equations for reaction and diffusion processes on a growing domain as a problem in kinematics. Chapter 4 considers a simplified scenario in one spatial dimension where the domain growth is uniform in space. We investigate the effects on pattern formation of the rate at which the domain is growing, and on the reaction kinetics. This latter problem is taken up in Chapter 5 where we examine the dynamical transitions between patterns as the domain grows for different functional forms for the reaction term. Chapter 6 considers nonuniform domain growth and pattern formation in two spatial dimensions. Finally, in Chapter 7 we conclude the thesis with further discussion of reaction-diffusion pattern formation on growing domains.