

Spatiotemporal behaviour of stochastic and continuum models for biological signalling on stationary and growing domains



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To my parents, Sandra and Thomas Woolley.

You guided me well.

I never knew you were doing it until it was gone.

To my niece Miley Jane Edwards.

Whatever you do in your life, make sure it is something you love.

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Abstract

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One of the main challenges in developmental biology is elucidating the mechanisms underlying the formation and maintenance of complex spatial and temporal structures. This thesis aims to explore two different and yet complementary approaches to address this challenge.

Firstly, we investigate a number of specific developmental systems in order to suggest mechanisms underlying their observed biological behaviour. Critically, we show that, without modifications, standard mathematical formulations are unable to capture certain details. In particular, it is demonstrated that accurate descriptions of domain growth need to be included in order to construct mathematical models that compare favourably with experimental systems. In conjunction with understanding biological development better, we also analytically investigate the existence of spot patterns in a paradigm patterning model, resulting in the use of numerical methods to compare theory with simulation.

The second approach used to investigate spatial-temporal complexity is more theoretical; we aim to characterise the effects of stochastic perturbations and growth on pattern generation. To achieve this we derive a stochastic description of biochemical reactions, diffusion and domain growth that is consistent with the deterministic description in the thermodynamic limit. This is then used to determine conditions under which robust patterns form.

By exploring developmental complexity through these two different approaches of creating broad analytical methods and investigating specific biological models, we not only gain a deeper insight into the creation of complexity in specific cases but also a wider appreciation of how noise can affect such systems.

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Chapter 1

Introduction

“I resolved from the beginning of my quest that I would not be misled by sentiment and desire into beliefs for which there was no good evidence.”

Bertrand Russell, *The Pursuit of Truth*, 1994.

How spatial and temporal complexity emerges from near homogeneity is one of the fundamental questions in developmental biology that remains poorly understood. In particular, the mechanisms by which biological systems maintain robustness, despite being subject to numerous sources of noise, are shrouded in mystery. Not only is producing plausible theoretical models of biological heterogeneity difficult, it is further complicated by the problem of generating robustness (Goodwin *et al.*, 1993; Maini & Othmer, 2001), *i.e.* once we can generate a pattern, how do we ensure that this pattern is consistently reproducible in the face of perturbation (Bunow *et al.*, 1980; Guptasarma, 1995; Harrison *et al.*, 1988; Maini *et al.*, 1997)?

Although molecular genetic studies have led to many advances in identifying the active species involved in patterning, simply identifying genes alone does not help our understanding of the mechanisms by which structures form. This is where the strengths of mathematical techniques lie. Not only are they able to complement experimental results by testing hypothetical relationships, they are also able to predict mechanisms by which populations interact, thus suggesting further experiments (Tomlin & Axelrod, 2007).

The first aim of this thesis is, thus, to apply paradigm pattern formation models to specific developmental systems in order to suggest mechanisms behind the observed phe-

nomena. The applied models will be constructed from deterministic systems of differential equations, as the biological systems are thought to behave as continua on the level at which we observe them. Since, in general, the deterministic framework is well understood (Murray, 2003a,b) our intention is not to further analytical understanding of these equations but, rather, to generate insights into how realistic biological complexity is produced. Perhaps, more importantly, we also highlight the phenomena which the mathematical models fail to capture, as it is only through understanding where a model is deficient that we are able to suggest additional features needed to give rise to the missing observations. This, in turn, leads to experimental predictions, which can be used to test the extended system. Through this continuous cycle of model extension and testing we build up a catalogue of necessary features that a mathematical system must have in order to produce biologically realistic results.

The creation of a mathematical model that reproduces biologically accurate results and testable predictions is only one side of the story. The model must also be able to consistently recreate observed phenomena in the face of realistic levels of noise (Wiesenfeld & Moss, 1995). Hence, the second focus of this thesis is on the robustness problem, of maintaining reproducibility in the face of perturbation. In this thesis the perturbations will arise from differences in initial condition, as well as the inclusion of intrinsic noise in later chapters. This will be a more theoretical problem, focussed on constructing a stochastic framework that is consistent with the deterministic description in the thermodynamic limit. However, its implications on the ability of a mathematical model to recapitulate experimental results are just as important as the investigations into the additional features needed to produce realistic simulations.

The production and maintenance of biological heterogeneity, known as morphogenesis, is an incredibly large and complex theme, which cannot be completely covered in a single thesis. Hence, we restrict ourselves to questioning the effects of growth and stochasticity on morphogenesis. By considering specific biological systems, alongside developing a general stochastic framework for creating and analysing pattern formation, we aim to improve current mathematical modelling approaches and advance understanding of the development of robust complexity.

1.1 Deterministic pattern formation

Many biological patterns, from the distribution of human populations to animal coat markings, can be thought of as heterogeneous distributions of agents. The most widely used framework of describing the evolution of such systems is with partial differential equations (PDEs). Due to their versatility, adaptability and general ease of simulation, systems of PDEs have been used to model numerous biological phenomena, including cell migration (Simpson *et al.*, 2007), heterogeneous cell fate decisions (Solnica-Krezel, 2003), and the formation of pigmentation patterns (Murray, 1981). In many cases, the PDEs are amenable to analytical techniques, which allow us to characterise and summarise the solution's fate and parameter dependencies without the need of simulation (Murray, 2003a,b).

A crucial process underlying morphogenesis is the transport of the agents. In this thesis we identify our agents with biochemical species and, as such, we mainly focus on diffusive mechanisms of pattern formation. However, there are other classes of pattern forming PDEs which contain convective terms, such as 'mechano-chemical' or 'chemotaxis' models that describe the attraction (or repulsion) of one species with respect to another. These usually model living cells, such as *Dictyostelium discoideum*, which are able to sense their surroundings and are thus able to react and move in response to chemical gradients or mechanical forces (Murray, 2003a,b). Haptotaxis (movement due to adhesive gradients) is a related mechanism in which the signalling species does not diffuse, rather it is bound to the domain through which the cells travel (Carter, 1967). Furthermore, the cells are not restricted to sensing chemical gradients; mathematical models can also be used to describe directed movement due to gradients in many other phenomena, such as electric fields (galvanotaxis) (Mycielska & Djamgoz, 2004) or light (phototaxis) (Drescher *et al.*, 2010). Discussion of such models is outside the scope of this thesis, although many of the techniques developed here are applicable to such systems (Murray, 2003a,b; Segel, 1984). We will also not consider the case of cross diffusion or diffusion which is variable in time or space.

The patterns we are considering are thought to arise as the consequence of an observable population, *e.g.* skin cells, responding to diffusing signalling populations, known

as morphogens, *e.g.* proteins. Through diffusion and interactions with other diffusing populations, non-uniform morphogen prepatterns can emerge. The observable population is then thought to undergo concentration-dependent differentiation based on this heterogeneous morphogen distribution, thereby producing a corresponding heterogeneous result in the observable population (Wolpert, 1969, 1989). The deterministic formalism of the PDE framework, which is constructed in Chapter 2, will be used repeatedly throughout this thesis to describe the formation of such morphogen prepatterns.

The French flag model (see Figure 1.1) (Wolpert, 1968, 1969) is perhaps the simplest mechanism of enhancing spatial heterogeneity that can be described with a PDE system, an extended version of which we will meet in Chapter 3. The French flag model postulates that, due to a source of diffusing reagent somewhere in the domain, a concentration gradient is maintained. Cells then detect the local concentration thresholds and differentiate accordingly.

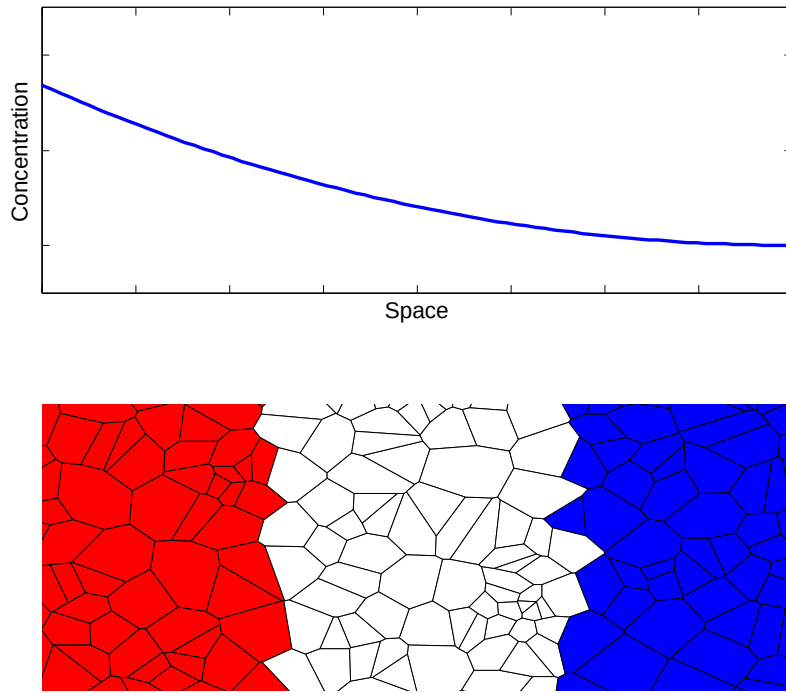


Figure 1.1: French flag model pattern producible by a source of diffusible chemical assumed to lie along the left-hand boundary. The source produces an exponentially decreasing profile similar to that shown in the top image. The different levels of concentration sensed by the polygons (denoting cells) are visualised by their given colour in the bottom image. The cells are red if they can sense a high concentration, white for medium concentrations and blue for low concentrations.

Another model of morphogenesis that we will consider is that of droplet pattern for-

mation (Woolley *et al.*, 2010) (Chapter 4). Here, a coupled system of two morphogens reacts and diffuses in order to produce large circular patterns of high concentration of morphogen and it is suggested that these circular structures could form the pigmentation prepatter of several South American species of fish (Barrio *et al.*, 2009).

Although we use the previously mentioned paradigms to create biologically applicable mathematical models, our main consideration will be with the Turing mechanism of diffusion-driven instability (Turing, 1952). Diffusion is well known for its homogenising properties; however, Turing postulated that when coupled with certain reactions, diffusion could lead to a persistent inhomogeneous state, created through a spontaneous symmetry-breaking of initial near-homogeneous populations. Although we consider a three species system in Chapter 3 we mostly restrict ourselves to two species systems as the basic mathematical properties of the diffusion-driven instability are well understood in this case, and recapitulated in Section 2.2.

Critically, even though the theory behind such systems is well documented, biological realisations of Turing patterns remain theoretical. However, nearly 40 years after their existence was theorised, chemically constructed Turing patterns in the CIMA (chlorite-iodide-malonic acid) (De Kepper *et al.*, 1991) and CDIMA (chlorite dioxide-iodide-malonic acid) (Míguez *et al.*, 2006) reactions were shown to exist (De Kepper *et al.*, 1991, 1982; Rudovics *et al.*, 1996) and later mathematically modelled by Lengyel and Epstein (1991). It is impossible to overstate the importance of the CIMA and CDIMA reactions, as, for the first time, they showed that Turing patterns were not simply theoretical. Indeed, this work has spurred on many other chemists to find alternative reactions, for instance Turing-like patterns have been observed in polyacrylamide-methylene blue-sulfide-oxygen (PA-MBO) reactions (Watzl & Münster, 1995) and also in hexacyanoferrate(II)-iodate-sulfite (FIS) reactions (Lee *et al.*, 1993). Recent developments in theoretical studies of pattern formation have also been aided by experimentalists turning to synthetic systems in order to gain an understanding of how biological systems work (Basu *et al.*, 2005).

Although the number of actual chemical reactions which have been used to produce these patterns is small, the idea of diffusion-driven instability has produced numerous avenues of further investigation. In particular, Gierer and Meinhardt (1972) extended the concept of the Turing mechanism to the paradigm-shifting patterning principle of short-

range-activation-long-range-inhibition and this still stimulates extensive biological research in pattern formation. Indeed, many biochemical gene products have been suggested to be Turing morphogens. For example, in the formation of limb buds there is strong evidence to suggest that signalling molecules of the TGF- β growth factor family act as Turing activators (Entchev *et al.*, 2000; Green & Smith, 1990; Gurdon *et al.*, 1994; Ingham & Fietz, 1995). However, their complementary inhibitors have yet to be identified. Similarly, the regular patterns produced by hair follicles have also highlighted the potential presence of Turing's mechanism (Mooney & Nagorcka, 1985; Mou *et al.*, 2006; Nagorcka & Mooney, 1992). In particular, by comparing simulations with experiments, Sick *et al.* (2006) provide circumstantial evidence that the proteins WNT and DKK can act as Turing morphogens.

The use of Turing systems in describing vascular mesenchymal cell self-organisation during embryogenesis has also led to predictive success (Garfinkel *et al.*, 2004; Sage *et al.*, 2009; Yochelis *et al.*, 2008). Here bone morphogenetic protein 2 (BMP2), which is a known morphogen (Boström *et al.*, 1993), is inhibited by matrix carboxyglutamic acid protein (MGP) (Zebboudj *et al.*, 2002). Critically, MGP is an unusually small particle (Garfinkel *et al.*, 2004) and, thus, is likely to diffuse more rapidly than BMP2. This large difference in the diffusion coefficients of the morphogens is a general property of Turing patterning systems, which we discuss later. Finally, Nakamura *et al.* (2006) have provided compelling evidence that the proteins Nodal and Lefty can interact through a diffusion-driven instability. These reactions are postulated to amplify the initial symmetry breaking that is hypothesised to occur due to embryonic cilia chirality, which, in turn, aids development of the left-right axis of the mouse embryo. However, although these, and other, potential Turing systems have been suggested, the hunt for the first conclusive biological evidence of a Turing structure at the molecular level continues.

Of course, we should also explain why the Turing instability is such an attractive mechanism with which to describe aspects of morphogenesis. Importantly, it is simple; the production of the patterns relies on the natural tendency of molecules to diffuse and react. Also, the mechanism has an inbuilt wavelength. Thus, once the reacting populations have been created and are being released into the system, no external input is needed to specify the spacing of density peaks and troughs as it is self-regulating. This means that Turing patterns can be highly regular over large distances without external regulation.

An obvious step in critiquing Turing’s theory is the identification of morphogens. However, examining the mathematical properties of a model to determine how they compare to biological reality is equally important. For example, one criticism of Turing patterns is that they often have small parameter regions of existence (Murray, 1982) and, in particular, the ratio of activator to inhibitor diffusion coefficients must often be much less than unity (Richter *et al.*, 1981; Sick *et al.*, 2006). However, it is possible to construct Turing models to counteract these generalisations through using three species systems (Klika *et al.*, 2011), or fine-tuning the kinetics and parameters (Baker *et al.*, 2008; Murray, 1982), although the biological plausibility of this fine-tuning is questionable.

Another criticism that Turing models receive is that the produced patterns suffer from a lack of robustness in their evolution (Maini *et al.*, 1997). Minor perturbations in the initial condition, domain geometry or boundary conditions can greatly influence the system (Bard & Lauder, 1974; Crampin *et al.*, 1999). Although the effects of certain perturbations can be ameliorated, domain geometry in dimensions higher than one plays a fundamental role in pattern selection (Murray, 1981).

In Figure 1.2 we illustrate the possible available outcomes of simulating Turing patterns on a two-dimensional domain. For a very thin domain, Figure 1.2(a), the system is essentially one-dimensional and so when Turing patterns are possible stripes are produced. As the domain width is increased we reach a critical length at which the homogeneous state destabilises in the y direction, thus half spots can be generated, Figure 1.2(b). As the domain size increases further more spots are able to fit within the domain as shown in figures 1.2(c) and 1.2(d). It is also worth noting that in Figure 1.2(d) the spots that are away from the boundary form a hexagonal planform. Spots can be made to be arranged in squares, diamond, or even rhombic structures; however, it is this hexagonal arrangement that is generally favoured by experimental systems (Dulos *et al.*, 1996; Rudovics *et al.*, 1996; Yang *et al.*, 2006).

This dependence of the patterns on domain size offers an important insight into the biological possibility of the diffusion-driven instability producing pigmentation patterns. Since spot patterns are formed on large domains, Figure 1.2(d), whilst stripes are preferred on thin domains, Figure 1.2(a), then for tapered domains a transition between spots and stripes can occur, as shown in Figure 1.2(e). This provides us with an immediate check

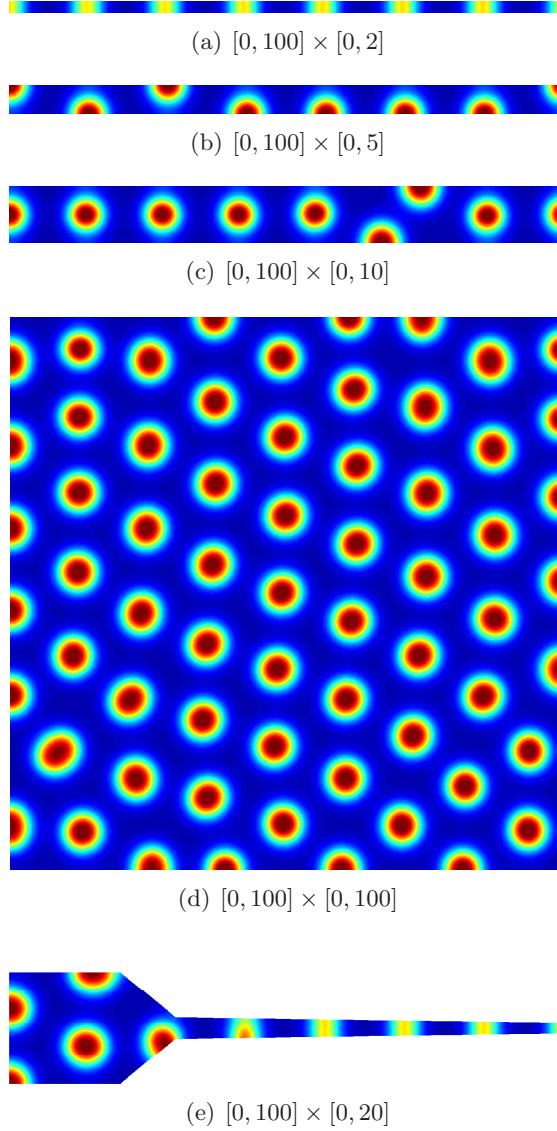


Figure 1.2: Two-dimensional Turing patterns on domains of different sizes. These images are to illustrate the effect of domain size on the patterns. The kinetic system demonstrated is the nondimensionalised Schnakenberg system, equations (2.61) and (2.62), with $\alpha = 0.1$, $\beta = 0.9$ and $D = 40$ (See Appendix A). The solution domain is given under each image except (e) where we have solved the simulated the system on the tapered domain shown and its extreme lengths and widths are given by $[0, 100] \times [0, 20]$. The colour axis goes from 0 (blue) to 4 (red), (see Figure C.1 in Appendix C for the full colour bar mapping). The full details of the kinetic system are discussed later in Section 2.4.

to see if a Turing mechanism is not present, at least in its simplest form, on a thinning geometry. Consider similar tapered domains on animals, such as the body to long thin tails or limbs. In such cases the theory suggests that if a transition occurs then Turing patterns are likely to change from spots on the body to stripes on the thinner section. This form of pattern is indeed observed on the skins of numerous animals, such as those shown

in Figure 1.3(a)-(c). However, the natural world is also able to throw up exceptions, such as the Tapir, Figure 1.3(d), where the main body is striped and it is the smaller regions of the legs and snout which have a spotted pigmentation. Thus, although Turing mechanisms are possible for the animals in figures 1.3(a)-1.3(c), we can be sure that a simple Turing system would be unable to generate the Tapir's complex coat markings (Murray, 1981).

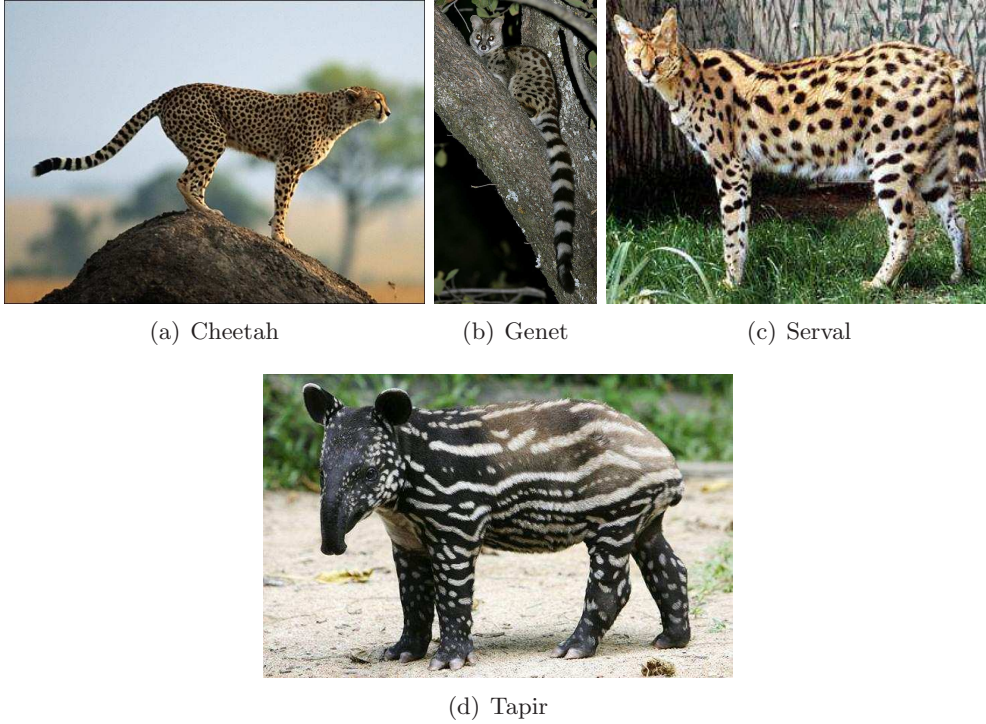


Figure 1.3: Examples of pigmentation patterns.

Due to the importance of domain size during pattern development we are interested in extending mathematical models to encapsulate domain growth into their descriptions. This will increase the realism of such models as animals do not start out with their adult form, rather, they have to grow to their final size. Further, since growth often occurs during early development (Gicquel & Le Bouc, 2006), we are interested in modelling the effects of growth on patterning systems. In particular, in Chapter 2 we demonstrate how growth can robustly support pattern doubling sequences (Crampin *et al.*, 1999).

1.2 Domain growth

Growth is a readily observed process in the biological world and, as such, makes a natural addition to many mathematical models. Not only is growth essential for healthy devel-

opment (Gicquel & Le Bouc, 2006) it has been identified as an important factor in the production of spatial heterogeneity, as it can fundamentally change the observed dynamics of patterning mechanisms (Kulesa *et al.*, 1996a,b; Maini *et al.*, 2003). In particular, Kondo & Asai (1995) observed that, as the size of marine angelfish *Pomocanthus* doubled, new stripes along the skin would develop between the old ones, thereby producing a near constant wavelength. This constant wavelength in fish pigmentation patterns is one of the defining features of a Turing pattern and is very suggestive of a Turing-like mechanism being responsible for the development of the skin pigmentation.

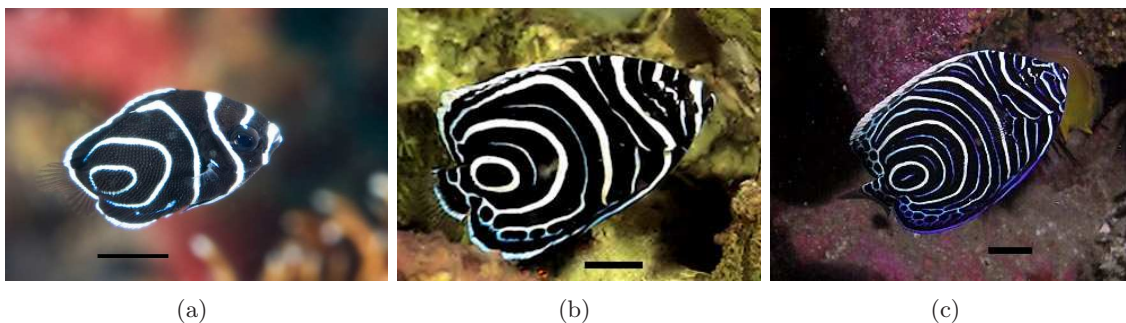


Figure 1.4: Development of stripes on the marine angelfish *Pomocanthus*. (a) Baby. Scale bar 1 cm (b) Juvenile. Scale bar 1 cm. (c) Mature. Scale bar 2 cm.

Apical growth, where tissue growth is restricted to a leading edge, is of particular importance when considering limb buds and root growth (Gandar, 1983; Sinnott & Bloch, 1939). Míguez *et al.* (2006) used the added complexity of photosensitivity in the CDIMA reactions (Dolnik *et al.*, 2001; Sanz-Anchelergues *et al.*, 2001) to study patterns evolving on apically growing, one- and two-dimensional domains, which was then complemented with analogous computational simulations (see Figure 1.5). Interestingly, they discovered that the observed patterns and wavelengths depend on growth velocity, illustrating the importance of the growth time scale in determining the final configuration of chemical and biological patterns. Also, unlike uniform growth, which causes expansion over the whole domain, once the pattern is created behind the leading edge of the apically growing domain it stabilises without reconfiguring as the space gets larger. Note that the boundary conditions at the aperture front are non-standard as chemicals can diffuse between the shaded and unshaded region. Hence the experiments are not immediately open to interpretation using other general studies, which often use very simple boundary conditions (discussed later in Section 2.1).

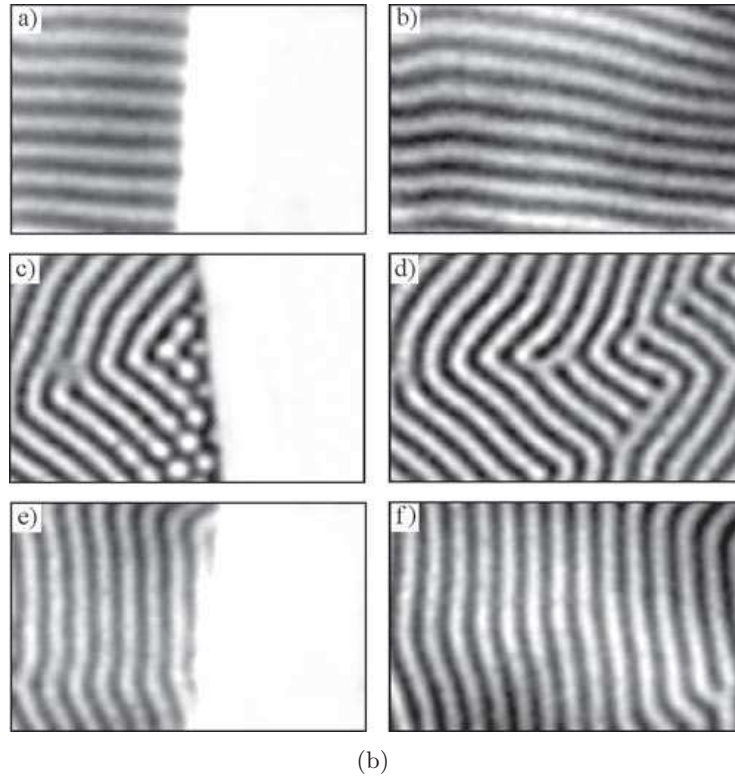
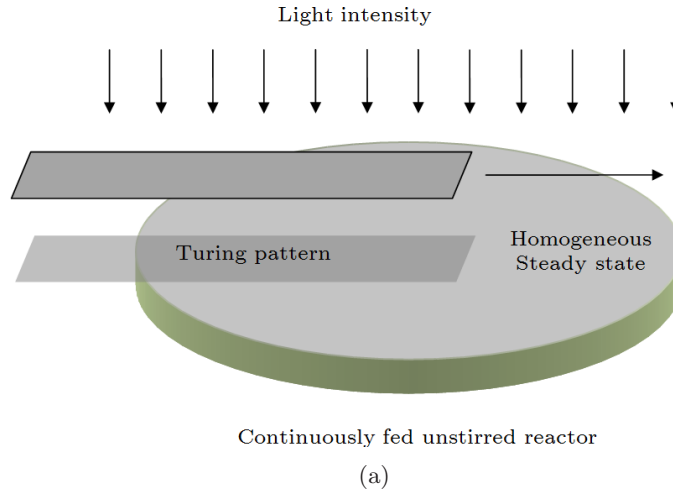


Figure 1.5: (a) Experimental apparatus for viewing Turing structures on an apically growing domain. Adapted from Míguez *et al.* (2006). The chemicals are allowed to diffuse in the continuously fed unstirred reactor (CFUR). However, they only react once they are shaded from the light source. The aperture travels across the CFUR creating an apically growing domain in which Turing patterns can form. (b) Turing pattern formation in a two-dimensional system with moving boundary. Time increases from the first column to the second column. The aperture moves left to right with each row signifying a different aperture speed (a), (b) 0.21 mm/h; (c), (d) 0.43 mm/h; and (e), (f) 1.26 mm/h. Observe that once the pattern has stabilised behind the moving front it hardly changes, despite the increased domain size. Reproduced, with premission, from D. G. Míguez, M. Dolnik, A. P. Muñuzuri and L. Kramer. *Phys. Rev. Lett.*, 96(48304), 2006. “Copyright 2011 by the American Physical Society.”

Growth was first incorporated into reaction-diffusion PDEs in an *ad hoc* manner. Arcuri & Murray (1986) used a domain length with an explicit time dependence which treats domain growth as a reduction in strength of the diffusion rate. Their numerical simulations show a tendency to generate inconsistent pattern sequences. Due to this apparent failure of the mechanism to generate reliable pattern sequences with domain growth they concluded that robust biological patterns must form sequentially as any mechanism that acts over the whole domain is subject to too many sources of error to be capable of robust pattern formation. Through rigorous derivation from first principles (recapitulated in Section 2.3), Crampin *et al.* (1999) showed that certain forms of growth can robustly generate certain wave forms under a persistent pattern doubling mechanism. However, since this analysis relies on the symmetries present in deterministic systems, we are interested in revisiting this problem with the addition of stochastic effects.

1.3 Applications

In Chapter 3 we move away from demonstrating the generic features and mathematical properties of PDE systems to consider two biological applications. Both of these systems are modelled deterministically using the framework set out in Chapter 2 and, although the models share many components, the mechanisms by which heterogeneity is thought to arise are quite different.

The first application we consider is mouse tooth development, where it has been shown experimentally that a genetic feedback loop controls the production of molar domains. In collaboration with experimentalists we demonstrate that the experimentally derived network is consistent with a three species Turing system (Cho *et al.*, 2011). Through modifying the standard Turing mechanism the model is shown to replicate the molar patterns of wild type mice teeth, as well as certain mutant mice. These additional assumptions are biologically plausible and are predictions of the model which can be experimentally tested.

Next, we model the mechanism behind the spatial specification of chick wing digits. Here, it is suggested that the digit forming cells differentiate based on the local concentration, as in the French flag model (Cooke & Summerbell, 1980; Riddle *et al.*, 1993;

Summerbell, 1981; Wolpert, 1989). Similar to the mouse tooth example, it is found that a simple fixed gradient on a stationary domain model (the standard proposed model) is unable to support the observed phenomena and, thus, we need to suggest a number of model innovations which, once again, lead to a number of testable predictions.

Although both of these models contain features that lack experimental evidence they are still able to prove or disprove the consistency of a theoretical concept with experimental observations. Specifically, multiple mechanisms may be quickly tested for their ability to produce a desired outcome. For example, in both applications it is seen that growth is not simply an empty inclusion that is used to increase the realism of the model. The specification of realistic growth is in fact a key aspect of both models, without which experimentally consistent simulations that recapitulate the correct temporal development cannot be produced using the suggested framework.

In Chapter 4 we introduce a different type of pattern formation mechanism, which emphasises the richness of PDE systems in terms of their ability to produce spatial and temporal complexity. Soliton-like structures called ‘droplets’ are found to exist within a paradigm reaction-diffusion model and they can be used to describe the patterning in a number of biological systems, for example on the skin of various fish species (Barrio *et al.*, 2009; Woolley *et al.*, 2010). Spatially localised structures such as these have been the source of increased study in the field of non-linear optics. Cavity solitons, which can form as one stable solution shrinks due to its embedding in the other, are common due to either the polarisation symmetry or quadratic nature of the nonlinearity of many optical systems. Similarly, systems which show the stable coexistence of homogeneous and spatially modulated solutions can be used in the theory of optical coding and memory applications (Firth & Harkness, 1998; Firth & Scroggie, 1996; Gomila *et al.*, 2004).

These droplets can be analysed in paradigm models that contain two non-zero stable steady states that are symmetric, however the asymmetric case is more challenging. In Chapter 4 the properties of the paradigm system are reviewed and then a recently developed perturbation technique (Gomila *et al.*, 2001) is extended to investigate the weakly asymmetric case. Results of mathematical analysis are compared with numerical simulations and shown to give good agreement in the region where the approximations hold.

1.4 Stochastic pattern formation

In all the cases discussed so far deterministic systems of PDEs have been relied upon to capture the relevant observed dynamics. However, biological systems are frequently subject to noisy environments, inputs and signalling. These stochastic perturbations are important when considering the ability of such models to reproduce results consistently. In particular, due to the Turing mechanism being very sensitive to perturbations, stochasticity is able to affect evolving patterns in ways not seen in deterministic simulations (Biancalani *et al.*, 2010). Chapters 5-7 of this thesis are focused on developing methodologies for stochastic modelling and questioning the reliability of the results gained from the deterministic framework.

Before we consider the connection between stochastic and continuous dynamics, we must first clarify the type of noise we are trying to understand. Biologically, noise can be split into two different types, intrinsic and extrinsic (van Kampen, 2007). Although both are sources of stochasticity, their mathematical treatments are very different (Rudge & Burrage, 2008). Intrinsic noise is generated as an inherent part of the system and is due to random fluctuations in population interactions (Gardiner, 1985). Due to its internal nature, the properties of intrinsic noise need to be extracted from the imposed mathematical framework. Extrinsic noise, in comparison, denotes fluctuations from any other source *e.g.* variations in temperature. Although the action of the external noise source on the populations being modelled maybe unknown, a random variable can be applied to mimic its effects. Thus, in the case of external noise, since the random signal is explicitly added, the properties of the noise can be chosen and controlled (Escudero, 2009).

Extensive work has been done in analysing and simulating the effects of external noise on reaction-diffusion pattern formation systems (Escudero, 2009; García-Ojalvo & Sancho, 1994, 1996, 1999; Sagués *et al.*, 2007; Scarsoglio *et al.*, 2011). Simple forms of intrinsic noise have also been investigated (Flegg *et al.*, 2011; Gadgil *et al.*, 2005; Gardiner, 1985; Gardiner *et al.*, 1976; Jahnke & Huisinga, 2007; van Kampen, 2007). However, until recently (Biancalani *et al.*, 2011; Butler & Goldenfeld, 2009; Lugo & McKane, 2008; McKane *et al.*, 2007; McKane & Newman, 2005), there have been few extensions to the modelling

of biological systems that have spatially varying or nonlinear temporal properties. Due to the inherent difficulties involved in analysing stochastic systems, research has focused on efficient algorithms for simulation (Andrews & Bray, 2004; Cao *et al.*, 2005; Gibson & Bruck, 2000; Gillespie, 1976, 1977b, 2001; Qiao *et al.*, 2006; Tian & Burrage, 2004; van Zon & ten Wolde, 2005), thus producing statistics through ensemble data. However, there has been little analysis to complement these results (Leppänen *et al.*, 2003; Zheng-Ping *et al.*, 2008) and any advances in theory have frequently needed heuristic moment closure arguments in order to allow analytic treatment (Baker *et al.*, 2010; Singh & Hespanha, 2007).

Although we take a view that our active species are simple particle reagents in some biochemical system, where the intrinsic noise arises through random thermodynamic fluctuations of collisions, the developed methodology is much more general and can be applied to any biological system in which it is justified to assume that individuals act like interacting particles (Othmer *et al.*, 1988). The intrinsic noise is then generated through some other underlying process that causes the interactions to have a probability distribution, rather than an exact rate. Further, if a particular population can be thought of as undergoing random movement, then diffusion may also be a good model for motility (Murray *et al.*, 1986; Okubo *et al.*, 1989; Woolley *et al.*, 2012).

In Chapter 5 we recapitulate a stochastic simulation algorithm and develop a framework for modelling stochastic systems based on a chemical master equation (CME) formalism. Weak noise expansions then allow us to derive Fokker-Planck equations that characterise the properties of the noise (van Kampen, 2007). These methods are extended by applying Fourier transforms to the Fokker-Planck equation, and give us insights into the potential spatio-temporal dynamics available in such systems (Biancalani *et al.*, 2010; Butler & Goldenfeld, 2011; Woolley *et al.*, 2011b). Although, by using these methods, we will be considering nonlinear complexity through linear analysis, it has been shown that linear dynamics can often afford a good approximation to the full nonlinear behaviour, particularly near homogeneous steady states and early stage bifurcation dynamics (Hinch, 1991; Murray, 2003a,b).

Because of its great importance in biological development, we are interested in applying descriptions of domain growth to the stochastic system. In Chapter 6, we introduce a novel

treatment of growing domains that allows us to cast our stochastic Turing models onto deterministically and stochastically growing domains in order to consider the possible effects of noise on such transition sequences (Woolley *et al.*, 2011a,b). Since domain growth is able to produce robustness in a deterministic Turing system we are interested in observing if the same is true in the stochastic setting.

Previously, heuristic arguments have been used in linking the macroscopic and microscopic descriptions of growth using a ‘box-splitting’ mechanism, such that when the growth ‘reaction’ occurs a compartment is chosen at random and replaced with two daughter compartments, each of which is the same size as the original compartment, with the original parent population split between them (Baker *et al.*, 2010). However, this description of growth is unsuitable for considering the effects of noise on patterning mechanisms as the operation of box-splitting does not have a weak noise limit. In order to bypass this problem of linking the micro- and macro-scopic scales we introduce a mesoscopic level of description, which is analogous to the deterministic technique of mapping a growing domain to a stationary domain (Woolley *et al.*, 2011a,b). The question of why we would want to do this is quite pertinent. The mapping not only allows us to link meso- and macro-scopic scales without the need of moment closure heuristics but we can also use the developed Fourier transform theory in order to analyse complex spatial dynamics. Further, the stochastic realisations are more efficient due to the coarser description of the domain, although by using the mesoscopic description we lose information on the microscopic scale.

In Chapter 7 the stochastic framework and Fourier transforms developed in Chapter 5 are applied to the paradigm Turing kinetics considered in Chapter 2. Far from being a destructive influence, Turing patterns are enhanced by the application of noise. Inside the deterministically derived parameter domain the noise reinforces the creation of exactly the same patterns as the deterministic model; thus the patterns are able to nucleate quicker. Outside of this domain, the deterministically simulated system exhibits only the homogeneous steady state, whereas stochastic systems are still able to support states that are far removed from this homogeneous state. Thus, stochastic effects are able to enlarge the parameter domain over which patterning is realised.

Using the methods developed in Chapter 6 we extend the stochastic Turing description to encapsulate stochastic and deterministic domain growth. Primarily, we are interested

in analysing the effects of stochasticity and growth on robust pattern formation. Although the pattern doubling mechanism is lost we are still able to predict the most likely observed transition sequences as well as suggest ways to manipulate the growth of the domain to produce robust transitions.

We end, in Chapter 8, by trying to extract general conclusions on the addition of growth and stochasticity to pattern formation mechanisms. Given this chance to reflect we also highlight a number of directions in which future research may lead. Finally, we summarise the contributions of this thesis and evaluate our progress in answering the questions that were raised at the beginning of this chapter.

Chapter 2

Methods of deterministic modelling

“Before turning to those moral and mental aspects of the matter which present the greatest difficulties, let the inquirer begin by mastering more elementary problems”

Sir Arthur Conan Doyle, *A Study in Scarlet*, 1888.

As discussed in Chapter 1, PDEs are ubiquitous in their description of biology and it is through these models that we can begin to understand the possible mechanisms by which complexity arises. We start this chapter, in Section 2.1, by reviewing and justifying the primary assumptions underlying deterministic reaction-diffusion systems, since, it is only by understanding where our assumptions hold that we can be sure that our models yield applicable results.

This is followed by the introduction of the Turing mechanism of pattern formation, discussed in Section 2.2. Here, we derive the conditions that a reaction-diffusion system must satisfy in order to produce a diffusion-driven instability, namely that, in the absence of diffusion, the system should evolve to a linearly stable spatially homogeneous steady state, whilst, in the presence of diffusion, this state should be linearly unstable. In Section 2.3 we enlarge the classes of problems that we can consider by allowing the solution domain to grow and show that certain types of growth can produce robust pattern doubling transitions. These transitions can then be used to inform biological insights on the robustness of developing systems.

Examples of the techniques introduced in sections 2.2 and 2.3 are then illustrated in sections 2.4 and 2.5, respectively. In particular we focus on the Schnakenberg kinetics (Schnakenberg, 1979) as a prototypical Turing system¹. We explore the parameter region over which the linear analysis suggests that Turing patterns exist as well as the effect of a number of different types of growth in order to illustrate the possible dynamics available in such systems. Finally, in Section 2.6 we summarise the chapter’s main results.

2.1 Basic model components

This thesis will be primarily concerned with multiple biochemical populations, *e.g.* U_i , where $i = 1, \dots, n$ and, for notational simplicity, they are collectively denoted by the vector, $\mathbf{U} = (U_1, \dots, U_n)$. Further the populations are identified with chemical concentrations, $\phi = (\phi_1, \dots, \phi_n)$. These populations are able to move in a one-, two- or three-dimensional domain, $\mathcal{V}$, with boundary surface $\partial\mathcal{V}$, as well as react with each other according to particular defined kinetics that are either motivated through biological observations or are of mathematical interest, proposed to reflect general aspects of the underlying biology.

The system could be completely described deterministically by Newton’s laws of motion, treating particles as point masses that can collide and bounce off of one another, and where reactions occur when particles collide with sufficient force. However, due to our ignorance of the initial positions and velocities of the active and solvent particles, as well as being unable to cope computationally with the large number of particles involved (which can easily be of the order 10^7 particles and higher (Fletcher *et al.*, 1999)), we instead choose to assume that the discrete populations, U_i , are large enough to be approximated by the continuous chemical concentration, ϕ_i , which is described using deterministic differential equations. Immediately, we see that this assumption has produced an error in our framework as the populations can only physically take integer values, whereas, once we take the continuum limit, the concentrations can take any continuous value. This is consistent with scaling introduced by van Kampen (2007), where stochastic influences on

¹Although the kinetics were originally derived by Gierer & Meinhardt (1972) they were popularised by Schnakenberg (1979) and so we adhere to the widespread terminology of referring to the kinetics as the Schnakenberg system.

the system scales as the reciprocal of the square root of the system size. Thus, if in a specific biological application the population can be justified to be large then a deterministic description is, in general, valid (Cornell *et al.*, 1991).

Since we are dealing with biochemical populations the populations will not be able to sense their surroundings and, thus, in the absence of some external force producing directionality, *e.g.* an electric field, their movement will be a simple random walk down concentration gradients, deterministically modelled by Fick's Law of Diffusion (Fick, 1855)

$$\mathbf{J}_i = -D_i \nabla \phi_i, \quad (2.1)$$

where $\mathbf{J}_i$ is the diffusive flux and D_i is the diffusivity of ϕ_i over the domain, commonly assumed constant and positive over $\mathcal{V}$. The law postulates that the chemicals move from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the size of the concentration gradient. Although the framework is described in the context of molecular particles, it is in fact more general and can be applied to any system where motility is considered to be governed by an unbiased random walk (Murray *et al.*, 1986; Okubo *et al.*, 1989; Woolley *et al.*, 2012).

Each of the concentrations, ϕ_i , is defined at a position $\mathbf{x} \in \mathcal{V}$ and at time $t \geq 0$, thus, the total amount of chemical species within a sub-volume, $\mathbb{V}$, of $\mathcal{V}$ is

$$\int_{\mathbb{V}} \phi_i(\mathbf{x}, t) \, d\mathbf{x}. \quad (2.2)$$

Let $\mathbb{V}$ be an elemental volume of $\mathcal{V}$ with boundary $\partial\mathbb{V}$. Then the rate of change of the concentration within this elemental volume is equal to the net creation of the chemical species due to reaction kinetics, F_i , plus the net flux of the species through the boundary. Mathematically, this is captured in the form

$$\frac{d}{dt} \int_{\mathbb{V}} \phi_i(\mathbf{x}, t) \, d\mathbf{x} = \int_{\partial\mathbb{V}} -\mathbf{J}_i \cdot d\mathbf{S} + \int_{\mathbb{V}} F_i(\phi_i) \, d\mathbf{x}. \quad (2.3)$$

The domain is fixed in time so we can take the time derivative through the first integral. Further, we can apply the divergence theorem (Kreyszig, 2007) to the second integral to

obtain the following simplified form:

$$0 = \int_{\mathcal{V}} \frac{\partial \phi_i}{\partial t} + \nabla \cdot \mathbf{J}_i - F_i(\phi_i) \, d\mathbf{x}. \quad (2.4)$$

Finally, since the sub-domain was arbitrary and we are assuming Fickian diffusion, equation (2.1), equation (2.4) can be simplified further to

$$\frac{\partial \phi_i}{\partial t} = D_i \nabla^2 \phi_i + F_i(\phi_i). \quad (2.5)$$

This derivation can then be trivially expanded to produce the general coupled PDE system, which involves multiple populations:

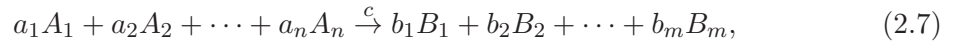
$$\frac{\partial \phi(\mathbf{x}, t)}{\partial t} = \mathbf{D} \nabla^2 \phi(\mathbf{x}, t) + \mathbf{F}(\phi(\mathbf{x}, t)), \quad (2.6)$$

$$\phi(\mathbf{x}, 0) = \phi_0(\mathbf{x}) \quad \forall \mathbf{x} \in \mathcal{V},$$

$$\mathbf{G}(\phi(\mathbf{x}, t)) = 0 \quad \forall \mathbf{x} \in \partial \mathcal{V} \text{ and } t > 0,$$

where $\mathbf{F} = (F_1(\phi), \dots, F_n(\phi))$, $\mathbf{D}$ is a diagonal matrix of diffusivities, which is constant in space and time, and the functional form of $\mathbf{G}$ specifies how the chemicals behave on the boundary (Murray, 2003a).

The form of $\mathbf{F}$ specifies the population's kinetics from the underlying reaction system and is usually derived by using the Law of Mass Action, which is based on collision theory (Guldberg & Waage, 1879). The law states that if a_i molecules of species A_i (corresponding concentration ϕ_i) react to form b_j molecules of product B_j with deterministic rate constant c as



then the reaction rate is proportional to the product of stoichiometrically weighted powers of the reactants,

$$\text{Rate of forward reaction} = c \prod_{p=1}^n \phi_p^{a_p}. \quad (2.8)$$

The deterministic reaction rate parameter, c , is the constant of proportionality for the forward rate of reaction. Physically, c depends on the temperature of the system, however,

we only consider systems in which the temperature does not vary.

Finally, we emphasise that the Law of Mass Action also depends on the large population assumption. For example, if we are dealing with reactions in which multiple particles of the same population are involved, *i.e.* $a_i > 1$ for some i , then in the case of low numbers of particles we would have to account accurately for the possible number of combinations of distinct particles that could produce the reaction (Erban *et al.*, 2007). We delay in-depth discussions of such considerations until Chapter 5 where these issues become important.

In this thesis we will be particularly interested in Neumann, or zero-flux boundary conditions, *i.e.*

$$\mathbf{G} = \frac{\partial \phi}{\partial \mathbf{n}} = \mathbf{0}, \quad (2.9)$$

where $\mathbf{n}$ is the outward pointing normal of $\partial\mathcal{V}$. This simply states that the rate of change of the density through the boundary is zero and, as such, no material may leave the domain. Although our primary interest will be with zero-flux conditions, in Chapter 3, we also use Dirichlet, or fixed density boundary conditions, which, as the name suggests, simply fixes the density of the solution on the boundary,

$$\mathbf{G} = \phi - \mathbf{C} = \mathbf{0}, \quad (2.10)$$

where $\mathbf{C}$ is normally a constant. When we consider chick limb formation in Section 3.2 this boundary condition is further extended to encapsulate a time-dependent form, in order to model an increasing source of the protein Sonic hedgehog on the left-hand side of the domain. In Chapter 4 we also consider periodic boundary conditions on a square domain, $[0, L] \times [0, L]$,

$$\phi(0, y) = \phi(L, y), \quad \phi(x, 0) = \phi(x, L), \quad \forall 0 \leq x, y \leq L. \quad (2.11)$$

This is because our interest lies with understanding the dynamics which the kinetics can produce, rather than with the effects that boundary conditions can have. Other boundary conditions, *e.g.* reactive boundary conditions (Erban & Chapman, 2007), also exist although they are not considered here.

Systems such as equation (2.6) are known as ‘reaction-diffusion’ equations. They can

account for biological patterning through a threshold-mediated response to morphogen concentration gradients (Wolpert, 1969, 1994). It is assumed that the chemically produced or amplified spatial heterogeneity forms a prepattern to which cells respond by differentiating in a concentration-dependent fashion.

As mentioned in Section 1, the French flag model of morphogenesis is perhaps the simplest postulated mechanism of concentration-dependent cell differentiation. The mathematical formalisation of the example illustrated in Figure 1.1 is given by

$$\frac{\partial \phi}{\partial t} = D \frac{\partial^2 \phi}{\partial x^2} - \gamma \phi, \quad (2.12)$$

$$\phi(0, t) = 1 \quad \text{and} \quad \frac{\partial \phi}{\partial x}(L, t) = 0 \quad t > 0, \quad (2.13)$$

where ϕ is a diffusing morphogen, L is the length of the domain, D_i is the diffusion rate and γ is the rate of population decay and suitable initial conditions are prescribed. Although theoretically simple, as it only relies of the production of a stationary gradient from a source, by combining two or more different sources, which are off-set in the domain, we can produce quite complicated patterns (see Figure 2.1). The basic mathematical model of Figure 2.1 will be of a similar form to equation (2.12) in two dimensions, except that it may include more complicated source terms and boundary conditions. The robustness of gradients that arise from such models has been analytically considered elsewhere (Saunders & Howard, 2009; Tostevin *et al.*, 2007) and, thus, we do not reproduce the work here.

Not only can reaction-diffusion models produce stationary gradients but this form of PDE system is able to produce temporal complexity such as travelling waves and moving fronts (Barrio *et al.*, 2009), even without the Turing instability. Thus, unless biologically motivated to add further components to capture relevant dynamics, we concentrate on capturing the maximum amount of complexity through the simplest forms of reaction-diffusion equations.

2.2 Turing instability

Any thermodynamically closed system can only contain transient patterning and must eventually tend to a state of equilibrium, which will be spatially homogeneous. If a chem-

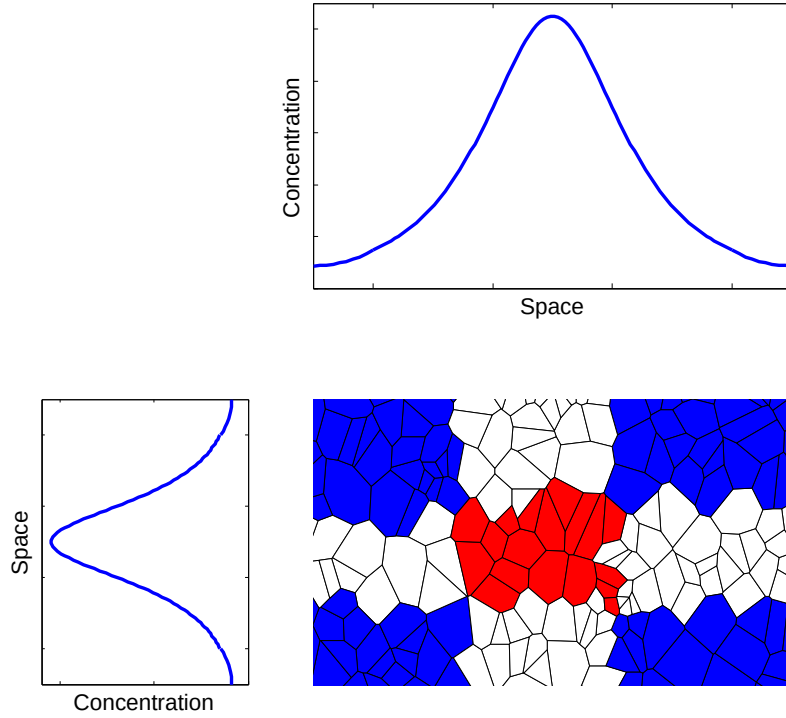


Figure 2.1: The patterning produced by a more complicated system of diffusible chemicals in two dimensions. The source is assumed to form a line along the central horizontal axis of the domain and the strength of the source is spatially modulated by the top Gaussian profile. The transverse concentration profile along the central vertical axis will then be similar to that of the left Gaussian profile. The boundary conditions are zero flux. The action of the sources are visualised in the bottom right image where the polygons (denoting cells) are coloured based on the local concentration. They are red if they can sense a high concentration, white for medium concentrations and blue for low concentrations.

ical system has a non-depleting source of reactant it can be driven away from thermodynamic equilibrium and produce spatially heterogeneous and temporal patterns without violating the laws of thermodynamics (Nicolis & Prigogine, 1977).

A paradigm mechanism postulated to produce heterogeneity is Alan Turing’s diffusion-driven instability (Turing, 1952). Turing conjectured that diffusion, normally known as a homogenising action, could destabilise a spatially homogeneous stable steady state. This counterintuitive idea has been proven to not contradict the basic thermodynamic principles (Prigogine & Glansdorff, 1971) and, as such, it has become the cornerstone of biological morphogenesis (Murray, 2003b). The ideas of diffusion-driven instability have also been applied to such diverse areas as semiconductor physics (Balkarei *et al.*, 1988), hydrodynamics (White, 1988) and even astrophysics (Nozakura & Ikeuchi, 1984).

At its simplest, the instability can be characterised by competition between two dif-

fusing populations, an activator and an inhibitor (Gierer & Meinhardt, 1972; Kapral & Showalter, 1995). Normally, the activator promotes its own production in a positive feedback loop, which, in turn, is controlled by an inhibitor in a negative feedback loop.

If the reaction domain is small enough such that the populations are well-mixed everywhere and diffusion can be discounted, *i.e.* the product of the diffusion rate and reaction time scale is much greater than the domain size squared, the reactions will simply tend to a homogeneous stable steady state of concentration. However, as the domain size increases, diffusion can destabilise the homogeneous state. Explicitly, if the inhibitor diffuses faster than the activator, local growth in the activator is able to occur whilst the inhibitor prevents activator spreading (Borckmans *et al.*, 1995), thus, once the domain is large enough, spatial heterogeneity will arise. This mechanism produces stationary patterns which are forms of ‘dissipative structures’, named as they persist by dissipating the input (Segel & Jackson, 1972) and, so, the system evolution is irreversible.

The application of Turing’s theory to biology has three main criticisms (Maini *et al.*, 1997). Firstly, there is no conclusive proof that Turing patterns actually exist in biology. Candidate proteins have been suggested, but there is currently nothing conclusive at the molecular level (Baker *et al.*, 2008). However, as discussed in Chapter 1, Turing structures have been found in the CIMA and CDIMA chemical reactions (De Kepper *et al.*, 1991; Kærn *et al.*, 2002).

Secondly, the solutions can be very sensitive to small perturbations and thus the model appears unrealistic when applied to many examples in morphogenesis where the patterning process is robust (Keller, 2002). This argument can be countered by research showing that different types of boundary conditions and domain growth can increase the robustness of pattern selection (Arcuri & Murray, 1986; Crampin *et al.*, 1999). It is also the case that in certain biological scenarios, variation is expected and even useful, *e.g.* animal pigmentation.

The third criticism is that generally the ratio of inhibitor diffusivity to activator diffusivity needs to be large for the Turing instability to exist (see equations (2.23) and (2.29)). This criticism can also be ameliorated as it has been experimentally observed that complexes of one reactant can form making the molecule bigger, effectively reducing its diffusivity (De Kepper *et al.*, 1991). Also in specific models it is possible to reduce the

ratio of the diffusion coefficients to almost one (Pearson & Horsthemke, 1989). Although the possibility of such parameter fine-tuning in a biological system is an open question.

Thus, any major criticism of the diffusion-driven instability, so far, can be countered with appropriate extensions and additions to the basic mechanism. The fundamental conditions of existence of the Turing instability are derived in the next section.

2.2.1 Conditions for the Turing instability

For clarity the current formulae are quoted for reaction-diffusion equations of two components, concentrations (ϕ, ψ) , with Neumann (zero flux) boundary conditions in a one-dimensional domain, $[0, L]$. Extensions to higher dimensions and various other boundary conditions are possible (Dillon *et al.*, 1994; Murray, 2003a). In full generality the equations are

$$\phi_t = D_\phi \phi_{xx} + f(\phi, \psi), \quad (2.14)$$

$$\psi_t = D_\psi \psi_{xx} + g(\phi, \psi), \quad (2.15)$$

where the subscripts x and t denote partial derivatives and suitable initial conditions are defined to close the system. Unless otherwise stated, since we assume that in the absence of diffusion there is at least one positive stable homogeneous steady state. The chosen initial conditions will be random fluctuations about such a state.

As just mentioned, the first requirement of a diffusion-driven instability is that there exists a spatially homogeneous linearly stable steady state *i.e.* there exists (ϕ_0, ψ_0) such that $f(\phi_0, \psi_0) = g(\phi_0, \psi_0) = 0$ and all of the eigenvalues of the Jacobian (evaluated at the homogeneous steady state),

$$J(\phi_0, \psi_0) = \begin{pmatrix} \frac{\partial f}{\partial \phi}(\phi_0, \psi_0) & \frac{\partial f}{\partial \psi}(\phi_0, \psi_0) \\ \frac{\partial g}{\partial \phi}(\phi_0, \psi_0) & \frac{\partial g}{\partial \psi}(\phi_0, \psi_0) \end{pmatrix}, \quad (2.16)$$

have negative real part. The second requirement is that the steady state becomes linearly unstable in the presence of diffusion. In order to derive necessary conditions for pattern formation to occur, the steady state is perturbed using functions that also satisfy the boundary conditions. Since we are using zero flux boundary conditions we use a Fourier

cosine expansion of the form $(\phi(x, t), \psi(x, t)) = (\phi_0 + \hat{\phi}(x, t), \psi_0 + \hat{\psi}(x, t))$, where,

$$\begin{pmatrix} \hat{\phi} \\ \hat{\psi} \end{pmatrix} = \sum_{m=1}^{\infty} \begin{pmatrix} a_m \\ b_m \end{pmatrix} e^{\lambda_m t \cos(k_m x)}, \quad (2.17)$$

and $k_m = m\pi/L$, is called the wave mode. The growth rate λ_m informs us as to the stability of the homogeneous steady state with respect to the wave mode, k_m . If the real part of λ_m is negative for all m then any perturbations will tend to decay exponentially quickly. However, in the case that the real part of λ_m is positive for any value of m , our expansion solution suggests that the amplitude of these modes will grow exponentially quickly and so the homogeneous steady state is now linearly unstable. Moreover, in the case where there are multiple growing modes, small alterations in the initial conditions (which are bound to occur, since we are using random initial conditions) can lead to completely different final outcomes. This is the robustness problem and it is discussed in Section 2.4.2.

Substituting equation (2.17) into the linearised form of equations (2.14) and (2.15), we obtain,

$$\mathbf{0} = \begin{pmatrix} \lambda_m + D_\phi k_m^2 - f_\phi & -f_\psi \\ -g_\phi & \lambda_m + D_\psi k_m^2 - g_\psi \end{pmatrix} \begin{pmatrix} a_m \\ b_m \end{pmatrix}, \quad (2.18)$$

where f_ϕ , f_ψ , g_ϕ and g_ψ denote the partial derivative of the function with respect to the subscript variable, evaluated at the steady state. This matrix equation has a non-trivial solution $((a_m, b_m) \neq (0, 0))$ if and only if the determinant is zero,

$$\lambda_m^2 + \lambda_m((D_\phi + D_\psi)k_m^2 - f_\phi - g_\psi) + D_\phi D_\psi k_m^4 - k_m^2(D_\phi g_\psi + D_\psi f_\phi) + f_\phi g_\psi - f_\psi g_\phi = 0. \quad (2.19)$$

Letting $h(k^2) = D_\phi D_\psi k^4 - k^2(D_\phi g_\psi + D_\psi f_\phi) + f_\phi g_\psi - f_\psi g_\phi$, the linear stability of the homogeneous steady state is now given by the signs of the real parts of $\lambda_\pm$ (Murray, 2003b), where

$$\lambda_\pm = \frac{f_\phi + g_\psi - (D_\phi + D_\psi)k_m^2 \pm \sqrt{(f_\phi + g_\psi - (D_\phi + D_\psi)k_m^2)^2 - 4h(k_m^2)}}{2}. \quad (2.20)$$

First, we consider the linear stability in the case where there is no diffusion, $D_\phi = D_\psi = 0$.

For the homogeneous steady state to be linearly stable, the real parts of both eigenvalues need to be negative. Thus

$$\Re(\lambda_{\pm}) = \Re\left(\frac{f_{\phi} + g_{\psi} \pm \sqrt{(f_{\phi} + g_{\psi})^2 - 4h(0)}}{2}\right) < 0, \quad (2.21)$$

$$\implies f_{\phi} + g_{\psi} < 0, \quad (2.22)$$

and

$$h(0) = f_{\phi}g_{\psi} - f_{\psi}g_{\phi} > 0. \quad (2.23)$$

Now, for the Turing instability to occur, once diffusion is included ($k_m \neq 0$) at least one of the eigenvalues must have positive real part. Since $f_{\phi} + g_{\psi} < 0$ by equation (2.22), it follows that $f_{\phi} + g_{\psi} - (D_{\phi} + D_{\psi})k_m^2 < 0$, thus the real part of λ_{-} is always negative. The only way to obtain an instability is if the real part of λ_{+} is positive. From (2.20), this occurs if $h(k_m^2) < 0$. Explicitly

$$D_{\phi}D_{\psi}k_m^4 - k_m^2(D_{\phi}g_{\psi} + D_{\psi}f_{\phi}) + f_{\phi}g_{\psi} - f_{\psi}g_{\phi} < 0, \quad (2.24)$$

$$\implies k_{-}^2 < k_m^2 < k_{+}^2, \quad (2.25)$$

where

$$2D_{\phi}D_{\psi}k_{\pm}^2 = D_{\phi}g_{\psi} + D_{\psi}f_{\phi} \pm \sqrt{(D_{\phi}g_{\psi} + D_{\psi}f_{\phi})^2 - 4D_{\phi}D_{\psi}(f_{\phi}g_{\psi} - f_{\psi}g_{\phi})}. \quad (2.26)$$

For inequality (2.25) to be realised k_{+}^2 needs to be real and positive, implying

$$D_{\phi}g_{\psi} + D_{\psi}f_{\phi} > 0, \quad (2.27)$$

$$(D_{\phi}g_{\psi} + D_{\psi}f_{\phi})^2 - 4D_{\phi}D_{\psi}(f_{\phi}g_{\psi} - f_{\psi}g_{\phi}) > 0. \quad (2.28)$$

Since $f_{\phi}g_{\psi} - f_{\psi}g_{\phi} > 0$, from inequality (2.23), these two inequalities yield one condition,

$$D_{\phi}g_{\psi} + D_{\psi}f_{\phi} > 2\sqrt{D_{\phi}D_{\psi}}\sqrt{(f_{\phi}g_{\psi} - f_{\psi}g_{\phi})} > 0. \quad (2.29)$$

Thus inequalities (2.22), (2.23), (2.25) and (2.29) form the conditions needed for a Turing

instability in a reaction-diffusion system.

$$\begin{aligned}
& f_\phi + g_\psi < 0, \\
& f_\phi g_\psi - f_\psi g_\phi > 0, \\
& D_\phi g_\psi + D_\psi f_\phi > 2\sqrt{D_\phi D_\psi} \sqrt{(f_\phi g_\psi - f_\psi g_\phi)} > 0, \\
& k_-^2 < \left(\frac{m\pi}{L}\right)^2 < k_+^2.
\end{aligned}$$

Inequalities (2.22) and (2.29), together with the fact that D_ψ and D_ϕ are both positive, imply that, not only are f_ϕ and g_ψ of opposite signs, but also that D_ψ/D_ϕ is either larger or smaller than one. Explicitly, when $g_\psi > 0$,

$$\frac{D_\phi}{D_\psi} > -\frac{f_\phi}{g_\psi} > 1. \quad (2.30)$$

Similarly, when $g_\psi < 0$,

$$\frac{D_\phi}{D_\psi} < -\frac{f_\phi}{g_\psi} < 1. \quad (2.31)$$

Further, from inequality (2.23), we deduce that if f_ϕ and g_ψ are of opposite sign then f_ψ and g_ϕ must also be of opposite sign. Thus, if a set of kinetics is to exhibit a Turing instability it must have a Jacobian of the form (including the symmetries when $+ \leftrightarrow -$)

$$J_c = \begin{pmatrix} + & + \\ - & - \end{pmatrix}, \quad J_p = \begin{pmatrix} + & - \\ + & - \end{pmatrix}. \quad (2.32)$$

Definition 1. A kinetic mechanism for which the Jacobian is of type J_c (type J_p) is said to be a cross (pure) activator-inhibitor mechanism (Dillon *et al.*, 1994).

In physical terms pure kinetics comprise a short-range, self-activator which is inhibited by a long-range self-inhibitor, hence the activator and inhibitor are in phase. The cross kinetic form causes a self-activator to deplete the self-inhibitor. This, in turn, feeds production of the activator, causing the spatial oscillations to be in antiphase (Crampin *et al.*, 1999).

Due to the Turing instability producing a pitchfork bifurcation structure (Bode & Purwins, 1995), any pattern based on wave mode m may take either of two polarities, $\pm \cos(m\pi x/L)$. The polarity adopted by the solution depends on the initial conditions, as does the pattern mode.

When $m = 1$, k_m takes its minimum value, namely $k_1 = \pi/L$. By rearranging inequality (2.25), we see that

$$\frac{\pi}{k_+} < L, \quad (2.33)$$

and so there is a lower bound on L , below which no pattern can form. By rearranging inequality (2.25), once again, to obtain a bound on m ,

$$\frac{k_-L}{\pi} < m < \frac{k_+L}{\pi}, \quad (2.34)$$

we notice that by increasing L not only do the limits of inequality (2.34) increase but the range between these limits also grows and, so, as L increases, increasing numbers of higher modes are able to destabilise the homogeneous state, leading to the problem of robustness. Specifically, unless the initial condition is identical in each simulation, small initial perturbations can lead to different wave modes and, thus, vastly different final patterns. This problem of robustness can be successfully dealt with, at least in the deterministic setting, by allowing the system to undergo domain growth (Crampin *et al.*, 1999), which is considered in Section 2.3.

2.2.1.1 Higher order analysis

In the previous section we derived the analytical conditions which must be satisfied in order for a reaction diffusion system to produce Turing patterns. In Section 2.4 we use a prototypical set of Turing kinetics to demonstrate the power of the analysis and, in particular, we illustrate the utility of the wave mode inequality, equation (2.25), in predicting the final pattern.

However, we must remember that linear analysis assumes that the perturbations to the homogeneous steady state are growing exponentially without bound. It is in fact the action of the nonlinear terms that bound the solution and therefore the standard linear analysis does not pick up these details. In order to calculate the amplitudes of the Turing

structures it is possible to use weakly nonlinear perturbative methods to consider the primary branches bifurcating from the homogeneous steady state (Auchmuty & Nicolis, 1975; Grindrod, 1996; Nicolis, 1995). The method is known as weakly perturbative as it is only valid near the bifurcation point.

The method proceeds by expanding the PDE system in terms of a small parameter ϵ . One of the system parameters, *e.g.* the inhibitor's diffusion coefficient, D , is chosen as the bifurcation parameter. The critical point, $D = D_c$, at which Turing patterns occur is noted and we perturb the coefficient around this critical point, *e.g.* $D = D_c + \epsilon^2 D_2$. Observe that the perturbation is positive since for $D < D_c$ the homogeneous steady state is stable. Further, we make the observation that the pattern formation actually occurs on three time scales. For $t \approx \mathcal{O}(1)$ after the system has been initiated we are in a regime where the amplitude is small and the linear analysis is valid. For $t \approx \mathcal{O}(1/\epsilon^2)$ the nonlinearities begin to interact with the linear kinetics and, finally, as $t \rightarrow \infty$ the system achieves its steady state. Hence, for nonlinear analysis, we are interested in the $\mathcal{O}(1/\epsilon^2)$ time scale, and, so, we adapt the time parameter, t , to include a long time scale, $t = t + \tau$, where $\tau = \epsilon^2 t$ and t and τ are assumed to act like independent variables (Grindrod, 1996). Finally, by expanding the amplitude

$$a_n = a_{n0} + \sum_{m=1}^{\infty} \epsilon^m a_{nm}(x, t, \tau), \quad (2.35)$$

and b_n similarly, we can collect the different orders of ϵ and thereby derive ODEs for the amplitudes. These, in turn, can then be used to predict the amplitude of the Turing peaks.

Since this method is limited in its validity and we are simply interested in the observed wave modes rather than pattern amplitude we bypass this analytical technique and refer the interested reader instead to a number of helpful textbooks (Drazin, 1992; Grindrod, 1996; Hinch, 1991; Nicolis, 1995).

2.2.1.2 Higher dimension considerations

As mentioned in Section 2.2.1, the Turing stability analysis can be carried out in higher dimensions with little change to the method, except the expansion needs to encapsulate perturbations in the extra dimensions. For instance, in two dimensions, on a rectangular

domain, with zero flux boundary conditions, the perturbation is now of the form,

$$\begin{pmatrix} \hat{\phi} \\ \hat{\psi} \end{pmatrix} = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \begin{pmatrix} a_{mn} \\ b_{mn} \end{pmatrix} e^{\lambda_{mn}t} \cos(k_{xm}x) \cos(k_{yn}y), \quad (2.36)$$

where k_{xm} and k_{yn} are the wave modes in the x and y directions respectively and there is no necessary reason why they should be the same. This leads to the problem of solution degeneracy.

In one dimension, there exists a degeneracy in the choice of polarity as, for any pattern mode that is based on a $\cos(kx)$ form, $-\cos(kx)$ is also a possible solution with opposite polarity. In spatial dimensions higher than one, the system has another kind of solution degeneracy. Not only is it possible to have multiple wave modes available but also the wave mode is now a vector, not a scalar. In the spatially unbounded problem, infinitely many Fourier modes will grow, as the bifurcation parameter is pushed passed its critical value. This leads to a degeneracy since any wave vector $\mathbf{k}$, which is associated with a critical wave number $|\mathbf{k}| = k_c$, such that $\lambda_m(k_c) > 0$, defines a growing mode. This means that in two dimensions, $|\mathbf{k}| = \sqrt{k_{xm}^2 + k_{yn}^2} = k_c$ is a circle of possible growing Fourier modes, in three dimension the analogue is a spherical shell, *etc.* However, in the spatially bounded problem only a finite number of Fourier modes can have wave vectors that lie on the critical circle in two dimensions, or critical sphere in three dimensions (Judd & Silber, 2000).

In two dimensions rectangular, hexagonal, rhombal patterns are possible. In three dimensions lamellae, prisms, and various other cubic structures all exist, making prediction even more difficult (Callahan & Knobloch, 1999). Weakly nonlinear theory and equivariant bifurcation theory (Borckmans *et al.*, 1995; Callahan & Knobloch, 1996, 1997, 1999; Leppänen, 2004) can be used to produce amplitude equations near a critical bifurcation point that separates the homogeneous and patterned stationary stable states. However, even though it is possible to use non-linear techniques to obtain the final amplitude of a chosen mode, we are still unable to predict exactly which mode will be chosen from random initial conditions. The best indicator we have for this task is to say that the ‘quickest’ growing mode is most likely to be chosen, although even this is of limited use

once large numbers of linearly growing modes become available. In the next section we introduce the growing domain formalism and show how this is able to generate robustness in certain cases.

2.3 PDEs on a growing domain

Growth inevitably occurs during development (Dassule *et al.*, 2000; Serrano & O’Farrell, 1997; Summerbell, 1981). This raises the issue of time scale. Does growth occur slowly enough that it may be considered to decouple from the patterning mechanism or does it form an integral part of the developmental process? The most prominent biological evidence we have to suggest that Turing patterns and growth interact is in the species of fish *Pomocanthus*. It was noticed that the patterns on *Pomocanthus* skin show a certain amount of scale invariance; as the fish grows it gains more stripes, but the pattern wavelength remains approximately constant (see Figure 1.4). This is highly indicative of a Turing type pattern.

Another example is in the development of the lower jaw of the alligator *Alligator mississippiensis*. The alligator’s teeth do not develop in numerical order from front to back (Kulesa *et al.*, 1996a). Instead, the teeth form front to back in the order fourth, first, fifth, second, sixth, third, seventh, as shown in Figure 2.2. In this application it was observed that the mechanisms of growth and Turing patterning were not sufficient to produce the correct evolution, thus a gradient in one of the model parameters was also postulated (Kulesa *et al.*, 1996a,b). Through this extra assumption the authors were able to produce a successful qualitative comparison between their simulations and the development of alligator tooth primordia. These additional assumptions foreshadow our own experiences when working with standard models of morphogenesis, which are discussed in Chapter 3, as we discover that certain additional properties of the system must be assumed before we are able to correctly generate both the final pattern, as well as the correct evolution of the system to that pattern.

The theory needed to include growth in reaction-diffusion PDEs is reviewed in the next section and, as we shall see, growth is not simply an effective increase in the distance between any two points in the system, which, when mapped back to the stationary domain,

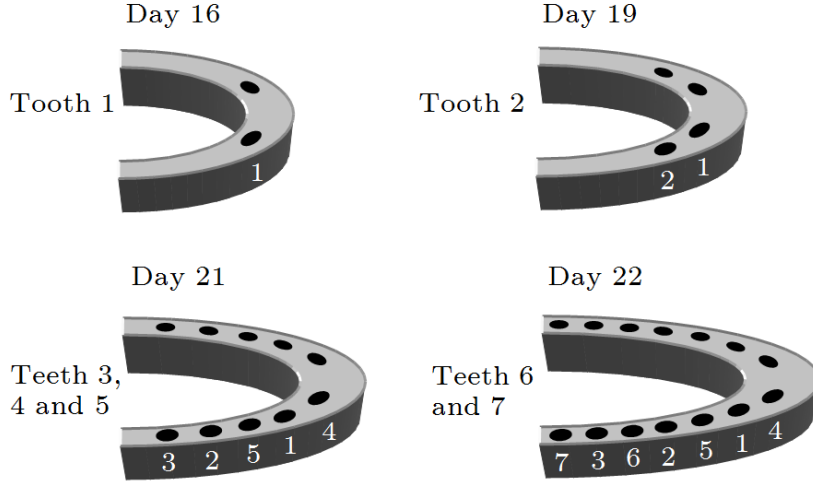


Figure 2.2: Spatial pattern of the first seven tooth primordia in the lower right half jaw of *A. mississippiensis*. Adapted from Westergaard and Ferguson (1986).

would lead to an alteration in the diffusion rate; growth also gives rise to dilution which affects the system kinetics. Furthermore, Crampin *et al.* (1999) showed that certain forms of growth could robustly generate certain wave modes under a persistent peak splitting mechanism.

2.3.1 Domain growth

Let $\phi(\mathbf{x}, t)$ be the concentration of a chemical in which we are interested at position $\mathbf{x}$, at time t . As in Section 2.1, consider an elemental volume $\mathbb{V}(t)$ in the growing domain $\mathcal{V}(t)$, which moves with the flow caused by domain growth, and let $\mathbf{a}$ be the velocity field of the flow generated by the growth of the domain,

$$\frac{d\mathbf{x}}{dt} = \mathbf{a}(\mathbf{x}, t). \quad (2.37)$$

Observe that, due to the growth, the elemental volume is now time-dependent, which contrasts with the time-independent elemental volume used in Section 2.1.

Generally, $\mathbf{a}$ may not have a closed form as it will be determined by some extended system of constitutive equations (Crampin, 2000; Neville *et al.*, 2006). However, to make progress, we restrict our view to simple analytical forms, which are still able to characterise a large class of different types of growth.

The conservation form of this system, equating the rate of change of concentration

with the flux of chemical in and out of the volume, is given by:

$$\frac{d}{dt} \int_{\mathbb{V}(t)} \phi \, d\mathbf{x} = \int_{\mathbb{V}(t)} -\nabla \cdot \mathbf{J} + F(\phi) \, d\mathbf{x}, \quad (2.38)$$

where the arguments of ϕ , (x, t) , have been omitted. Since the elemental volume is time-dependent we apply Reynolds' Transport Theorem (Acheson, 1990) to the left-hand side. Thus,

$$\frac{d}{dt} \int_{\mathbb{V}(t)} \phi \, d\mathbf{x} = \int_{\mathbb{V}(t)} \frac{\partial \phi}{\partial t} + \nabla \cdot (\mathbf{a}\phi) \, d\mathbf{x}. \quad (2.39)$$

Finally, using Fick's law, equation (2.1), and noting that the elemental volume is arbitrary, we obtain:

$$\frac{\partial \phi}{\partial t} + \nabla \cdot (\mathbf{a}\phi) = \mathbf{D}\nabla^2 \phi + F(\phi), \quad \mathbf{x} \in \mathcal{V}(t) \text{ and } t \in [0, \infty), \quad (2.40)$$

(for details see Crampin, 2000; Crampin *et al.*, 1999, 2002a; Crampin & Maini, 2001). As $\nabla \cdot (\mathbf{a}\phi) = \phi \nabla \cdot \mathbf{a} + \mathbf{a} \cdot \nabla \phi$, the term $\mathbf{a} \cdot \nabla \phi$ is interpreted as the transport of material around the domain, and $\phi \nabla \cdot \mathbf{a}$ as the dilution term, arising due to local volume increase.

Given appropriate initial and boundary conditions these equations completely define the system. However, it is problematic to numerically solve the equations on a growing domain, since the algorithm needs to encapsulate moving grid points. Instead, we use a Lagrangian description of the domain which maps each point in the growing system back along its trajectory to a fixed domain. This mapping is a well-defined bijection as we assume that no two distinct elements may be in the same spatial location at the same time and, thus, due to the assumed continuity of the mapping and the domain being connected, no trajectory may cross another. Hence, if $\mathbf{X} = (X_1, X_2, X_3)$ is the initial location of an element and $\mathbf{x}(t) = (x_1(t), x_2(t), x_3(t))$ is the location of the element at time t then the function $\Gamma(\mathbf{X}, t)$ is defined by $\mathbf{x}(t) = \Gamma(\mathbf{X}, t)$. Obviously, $\Gamma(\mathbf{X}, 0) = \mathbf{X}$. Due to Γ being a well-defined bijection we can construct the inverse map, $\mathbf{X} = \mathbf{\Lambda}(\mathbf{x}, t)$. Finally, without loss of generality, we ignore simple translations and rotations and define the origin to be a fixed point of the domain *e.g.* $\Gamma(\mathbf{0}, t) = \mathbf{0}$, for all $t \geq 0$.

The velocity vector field is defined by

$$\mathbf{a} = \frac{\partial \mathbf{\Gamma}}{\partial t}(\mathbf{X}, t). \quad (2.41)$$

Using the chain rule we can expand (2.41) (Crampin, 2000) to show

$$\frac{\partial^2 \Gamma_i}{\partial t \partial X_k} = \sum_{j=1}^3 \frac{\partial a_i}{\partial x_j} \frac{\partial \Gamma_j}{\partial X_k}, \quad (2.42)$$

where Γ_i and a_i are the i^{th} components of $\mathbf{\Gamma}$ and $\mathbf{a}$, respectively. Equation (2.42) then allows us to determine the trajectories of each element using their initial location and local expansion rate.

2.3.2 Growth in one dimension

We now look at the case where the velocity field is fully specified by $a(x, t) = dx/dt = \partial \Gamma / \partial t$. Assuming that the domain growth is isotropic allows us to decompose the trajectory function, $\Gamma(X, t)$, into a spatial function and a temporal function multiplied together. In particular, we specify a new function $l(t)$ via $x(t) = \Gamma(X, t) = Xl(t)$. We now transform equation (2.40) from the growing domain variables (x, t) to the stationary domain variables (X, t) using

$$\left. \frac{\partial}{\partial t} \right|_x = \left. \frac{\partial}{\partial t} \right|_X - X \frac{\dot{l}(t)}{l(t)} \frac{\partial}{\partial X} \text{ and } \frac{\partial}{\partial x} = \frac{1}{l(t)} \frac{\partial}{\partial X}, \quad (2.43)$$

where $\dot{l}(t) = dl(t)/dt$. Thus, equation (2.40) becomes

$$\frac{\partial \phi}{\partial t} - X \frac{\dot{l}(t)}{l(t)} \frac{\partial \phi}{\partial X} + \frac{1}{l(t)} \frac{\partial}{\partial X} \left(l(t) X \phi \right) = \frac{D}{l(t)^2} \frac{\partial^2 \phi}{\partial X^2} + F(\phi), \quad (2.44)$$

$$\implies \frac{\partial \phi}{\partial t} + \frac{\dot{l}(t)}{l(t)} \phi = \frac{D}{l(t)^2} \frac{\partial^2 \phi}{\partial X^2} + F(\phi). \quad (2.45)$$

For simplicity, from now on, since we are always going to be dealing with the Lagrangian formalism, we replace the X variable with the x variable.

All that is left to do is specify the form of growth, $l(t)$. For example, if the domain

grows exponentially with rate r then $l(t) = \exp(rt)$, and

$$\frac{\partial \phi}{\partial t} = \frac{D}{l^2} \frac{\partial^2 \phi}{\partial x^2} + F(\phi) - r\phi, \quad (2.46)$$

is the equation for the concentration, $\phi(x, t)$, undergoing reaction kinetics defined by the functional form F on such a domain. Similarly,

$$\frac{\partial \phi}{\partial t} = \frac{D}{l^2} \frac{\partial^2 \phi}{\partial x^2} + F(\phi) - \frac{r}{1 + rt} \phi, \quad (2.47)$$

is the equation for the concentration, $\phi(x, t)$, undergoing reaction kinetics defined by F on a linearly growing domain, $l(t) = 1 + rt$. Hence, we see that the growth modifies the reaction kinetics due to dilution effects. Often, when r is small, the dilution term is often neglected (Crampin & Maini, 2001). However, Neville *et al.* (2006) expanded this derivation further to include density-dependent growth and showed that relatively quick growth can influence the observed dynamics. Since the addition of dilution effects does not increase the complexity of simulation we always retain this term.

2.3.3 Persistent mode doubling

As observed in the previous section, growth can affect the diffusion rate and kinetics in a time-dependent manner. Thus, it should be unsurprising that the temporal evolution of a reaction-diffusion system can be altered by the inclusion of growth. In Figure 2.8 we illustrate the effects of simulating a Turing system on an exponentially growing domain and note that within a certain range of growth rates the Turing peaks are able to undergo a consistent peak doubling through a splitting mechanism, such that the wave mode expressed on a domain of length $2L$ will be twice that of a domain of length L . This self-similarity on exponentially growing domains was justified analytically by Crampin *et al.* (1999) and is briefly recapitulated here for completeness.

Suppose that the domain growth is monotonically increasing. Using the evolution equation (2.45), eliminate t in favour of $\gamma = l^2(t)$. This yields

$$\frac{d\gamma}{dt} \frac{\partial \phi}{\partial \gamma} + \frac{1}{2\gamma} \frac{d\gamma}{dt} \phi = \frac{D}{\gamma} \frac{\partial^2 \phi}{\partial x^2} + F(\phi). \quad (2.48)$$

A tent map, $p : [0, L] \mapsto [0, L]$, is now constructed to effectively double the spatial frequency, the effect of which can be seen in Figure 2.3. This is defined as

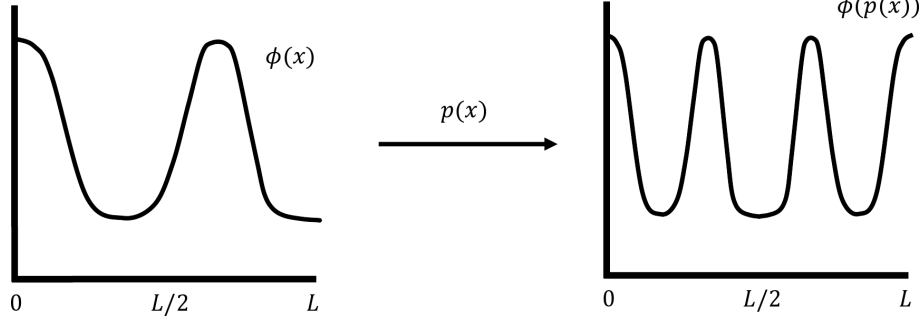


Figure 2.3: Schematic diagram of the effect of the tent map on a spatial solution.

$$p(x) = \begin{cases} 2x & 0 \leq x < \frac{L}{2}, \\ 2(L - x) & \frac{L}{2} \leq x < L, \end{cases} \quad (2.49)$$

with first derivative defined as

$$\frac{dp(x)}{dx} = \begin{cases} 2 & 0 \leq x < \frac{L}{2}, \\ -2 & \frac{L}{2} \leq x < L, \end{cases} \quad (2.50)$$

where the derivative at $x = L/2$ is taken to be the appropriate one-sided limit. The second derivative is then

$$\frac{d^2p(x)}{dx^2} = 0 \text{ for } x \in [0, L]. \quad (2.51)$$

We now consider $\bar{\phi}(x) = \phi(p(x), \gamma)$. Firstly, we check that the function has continuous second order derivatives in x . Specifically, it is enough to show that the derivatives of the function are continuous at $x = L/2$. The first derivative is,

$$\frac{\partial \bar{\phi}}{\partial x} = \frac{dp}{dx} \frac{\partial \phi}{\partial p}(p, \gamma), \quad (2.52)$$

and due to the zero-flux boundary conditions,

$$\lim_{x \uparrow L/2} \frac{\partial \bar{\phi}}{\partial x} = 2 \frac{\partial \phi}{\partial p}(L, \gamma) = 0 = -2 \frac{\partial \phi}{\partial p}(L, \gamma) = \lim_{x \downarrow L/2} \frac{\partial \bar{\phi}}{\partial x}. \quad (2.53)$$

Similarly, the second derivative is

$$\frac{\partial^2 \bar{\phi}}{\partial x^2} = \left(\frac{dp}{dx} \right)^2 \frac{\partial^2 \phi}{\partial p^2}(p, \gamma) + \underbrace{\frac{d^2 p}{dx^2}}_{=0} \frac{\partial \phi}{\partial p}(p, \gamma), \quad (2.54)$$

implying that,

$$\lim_{x \uparrow L/2} \frac{\partial^2 \bar{\phi}}{\partial x^2} = 2^2 \frac{\partial^2 \phi}{\partial p^2}(L, \gamma) = (-2)^2 \frac{\partial^2 \phi}{\partial p^2}(L, \gamma) = \lim_{x \downarrow L/2} \frac{\partial^2 \bar{\phi}}{\partial x^2}. \quad (2.55)$$

Hence, continuity of up to and including the second derivative is assured.

If $\phi(x, \gamma)$ is any solution to the original equation, (2.48), then $\bar{\phi} = \phi(p(x), \gamma)$ satisfies

$$h(\gamma) \frac{\partial \bar{\phi}}{\partial \gamma} + \frac{1}{2\gamma} h(\gamma) \bar{\phi} = \frac{D}{4\gamma} \frac{\partial^2 \bar{\phi}}{\partial x^2} + F(\bar{\phi}), \quad (2.56)$$

where $h(\gamma) = d\gamma/dt$. Finally, since $\phi(x, 4\gamma)$ satisfies

$$\frac{h(4\gamma)}{4} \frac{\partial \phi}{\partial \gamma} + \frac{1}{2\gamma} h(\gamma) \phi = \frac{D}{4\gamma} \frac{\partial^2 \phi}{\partial x^2} + F(\phi), \quad (2.57)$$

then it is observed that equations (2.56) and (2.57) are identical when

$$h(4\gamma) = 4h(\gamma). \quad (2.58)$$

This implies that h has the form of an Eulerian homogeneous function and thus (Di Cera, 1995),

$$\frac{d\gamma}{dt} = h(\gamma) \propto \gamma, \quad (2.59)$$

and, so, γ is an exponential function of t . We can specify the form completely by noting that the growth rate is assumed to be r and that $\gamma(0) = l(0)^2 = 1$. Hence, $l(t) = \exp(rt)$ and $\gamma = \exp(2rt)$.

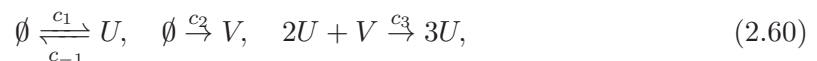
Finally, since we are only interested in bounded solutions to the reaction diffusion problems they can be shown to be unique (Rothe, 1982) and, so, if for any γ' , $\phi(p(x), \gamma') = \phi(x, 4\gamma')$ for all x , then the solutions are equal for all $\gamma \geq \gamma'$. Thus, exponential domain growth is necessary for persistent peak splitting. Using this idea of self-similarity, the window of interest may be reduced to the interval $[\gamma, 4\gamma]$, for if a pattern mode doubles

within this interval then the pattern will double in the interval $[4\gamma, 16\gamma]$ and in the interval $[16\gamma, 64\gamma]$, etc. It should be noted that when simulating this system, due to imperfections in the numerical method, $\phi(p(x), \gamma') - \phi(x, 4\gamma')$ will be small, but non-zero. However, stability of the solution is implicitly assumed, in which case, the value of $\phi(p(x), \gamma') - \phi(x, 4\gamma')$ will always remain small.

2.4 Schnakenberg example

Now that we have briefly taken an overview of the deterministic framework and theoretical methods that we can use to analyse the PDE systems, we demonstrate these principles on a specific choice of reaction kinetics, known as the Schnakenberg kinetics (Schnakenberg, 1979). The Schnakenberg kinetics were popularised in 1979, where they were derived as one of eleven sets of kinetics that demonstrated the necessary properties for limit cycle behaviour, these being that the system needs at least three reactions with at least one of those being autocatalytic (Schnakenberg, 1979). Since then much analysis has been carried out on the Schnakenberg kinetics (DeVillie *et al.*, 2006; Iron *et al.*, 2004; Ward & Wei, 2002), which can be thought of as a simplified version of the Brusselator model (Adomian, 1995). Here, we illustrate the various dynamics the kinetics can exhibit by investigating various regions within its parameter space.

For populations U and V the Schnakenberg reactions can be written as



where the c_n are deterministic reaction rates and $\emptyset$ represents substances which are not explicitly modelled. By letting ϕ and ψ be the concentrations of U and V , respectively, and allowing the reactants to diffuse, after nondimensionalisation (shown in Appendix A), the PDE system becomes,

$$\phi_t = \nabla^2 \phi + \alpha - \phi + \phi^2 \psi, \quad (2.61)$$

$$\psi_t = D \nabla^2 \psi + \beta - \phi^2 \psi, \quad (2.62)$$

$$t > 0, \quad \mathbf{x} \in \mathcal{V},$$

where α , β and D are nondimensionalised constants and the system is supplemented with appropriate initial and boundary conditions.

2.4.1 Turing stability

Using the conditions set out in Section 2.2.1 a homogeneous steady state, (ϕ_0, ψ_0) , is driven to instability by diffusion when

$$\frac{(\phi_0^2 + 1)}{2\phi_0} > \psi_0 > \frac{(\phi_0 + \sqrt{D})^2}{2D\phi_0}, \quad (2.63)$$

is satisfied (see Appendix A for details). This information is collected in Figure 2.4. The parameter region of diffusion-driven instability, Region D of Figure 2.4(b), appears to be unbounded. However, in order for the system to be realistic, the deterministic rates of reaction, c_i , are all positive. This means that we only consider the region where α and β are non-negative, since $\alpha = c_1/c_{-1}\sqrt{c_3/c_{-1}}$ and $\beta = c_2/c_{-1}\sqrt{c_3/c_{-1}}$ (see Appendix A). Thus, the criticism of a small Turing unstable parameter region is quite pertinent in this case. Furthermore, observe that, not only do Turing structures exist, but, due to the Schnakenberg system's unusual property of all physical solutions being bounded (proven in Appendix B), the oscillatory and Turing mechanisms are able to interact, adding temporal complexity to the available patterns.

Figure 2.4 is based on the linear analysis of the reaction kinetics and, thus, the transition from one type of dynamics to the next may not be as sharp as suggested. There may even be dynamics which are not seen through this approximation. This is illustrated in Figure 2.5 where we explore the (α, β) parameter space to show the various available dynamics in one dimension.

Region A According to Figure 2.4(a), in the absence of diffusion, the critical points are unstable nodes and, thus, phase plane trajectories tend to a periodic orbit (see Appendix B). Through numerical simulations shown in Figure 2.5(a), diffusion quickly homogenises the region and the system simply oscillates temporally as though it were an uncoupled system of ODEs. The period of the oscillations can then be derived through non-linear analysis (Hinch, 1991).

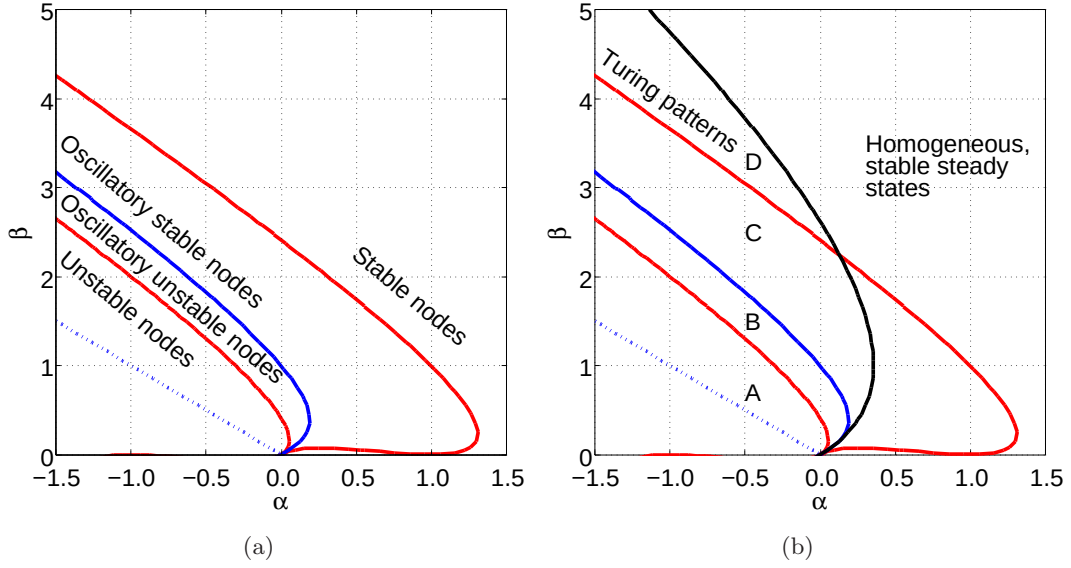


Figure 2.4: (a) Stability diagram of the Schnakenberg kinetics, equations (2.61) and (2.62). The unique spatially homogeneous steady state of this system is $\phi_0 = \alpha + \beta$ and $\psi_0 = \beta/(\alpha + \beta)^2$. The equations for the lines have been derived in terms of (ϕ_0, ψ_0) and have been converted to the (α, β) parameter space using the aforementioned equalities. The blue line, $2\phi_0\psi_0 - 1 - \phi_0^2 < 0$, divides the parameter space according to whether the stationary state is linearly stable or unstable. The red lines delineate the bifurcation between real and complex eigenvalues. Between the red lines, $(\phi_0 - 1)^2/2\phi_0 < \psi_0 < (\phi_0 + 1)^2/2\phi_0$, the eigenvalues have a non-zero imaginary part. The boundary of the region of positive critical states is shown as the dotted line. (b) Turing stability diagram of the Schnakenberg kinetics. The lines are the same as in (a) except for the addition of the black solid line $2D\beta = (\alpha + \beta)(\alpha + \beta + \sqrt{D})^2$, which indicates the border of Turing unstable states. See Appendix A for more details. Parameter $D = 40$.

Region B Due to the interaction of the Turing and oscillatory mechanisms, Turing-Hopf patterns exist, as shown in Figure 2.5(b). The transition from simple oscillatory to Turing-Hopf patterns is not sharp. Near the A, B border the system will tend to an oscillating solution, similar to that seen in Figure 2.5(a). Near the B, C border, which separates the stable and unstable, oscillatory, steady states, the oscillations are damped and produce stationary heterogeneous states.

Region C Here, the temporal oscillations are damped, as in the inset of Figure 2.5(c), resulting in stationary Turing structures, which appear to be stable. It should be noted that the oscillations that exist within regions B and C are not predicted by Turing's linear theory as the eigenvalues of the unstable modes are always real (see equation (2.20)).

Region D In this region, non-oscillating, Turing patterns appear to be stable, as shown in Figure 2.5(d). The difference between these Turing patterns and those in Region C, apart from the initial damped oscillations, is that these appear to be more robust in their pattern selection, as will be shown in Section 2.4.2.

2.4.2 The robustness problem

Setting $D = 40$, $\alpha = 0.1$ and $\beta = 0.9$, places the system in Region C, thus Turing structures appear from damped oscillations, as seen in Figure 2.5(c). By inserting the chosen values into equation (2.24), it is possible to derive the growing modes k ,

$$\frac{31 - \sqrt{31^2 - 4 \times 40}}{2 \times 40} < k^2 < \frac{31 + \sqrt{31^2 - 4 \times 40}}{2 \times 40},$$

$$\implies k_- = 0.1836 \lesssim k \lesssim 0.8610 = k_+. \quad (2.64)$$

Not all of these modes are growing at the same rate, since the eigenvalue, λ , varies with k^2 , as can be seen in Figure 2.6(a). The mode with highest linear growth rate, k_c , corresponds to the largest positive eigenvalue given by equation (2.20). In order to calculate k_c we differentiate the positive root with respect to k_m to find the point at which $\lambda_+(k_m)$ attains a maximum. In the general case we find that,

$$k_c^2 = \frac{D_\phi D_\psi (f_\phi - g_\psi) \pm \sqrt{-f_\psi g_\phi D_\phi D_\psi (D_\phi + D_\psi)^2}}{D_\phi D_\psi (D_\phi - D_\psi)}, \quad (2.65)$$

where the chosen root depends on the signs and sizes of the partial derivatives and the diffusion coefficients, which, in turn, depend on the kinetics.

In the current case of the nondimensionalised Schnakenberg kinetics, equation (2.65) can be simplified to

$$k_c = \sqrt{\frac{D((\alpha + \beta)^3 + \beta - \alpha) - \sqrt{2}\sqrt{D\beta(1 + D)^2(\alpha + \beta)^3}}{D(1 - D)(\alpha + \beta)}}. \quad (2.66)$$

As discussed, due to the assumed boundary conditions and form of perturbation used, $k_m = m\pi/L$, where m is an integer. However, generally, $k_c L/\pi$ will not be an integer, thus, the fastest growing wave number, m_c , will be the closest integer to this that is still

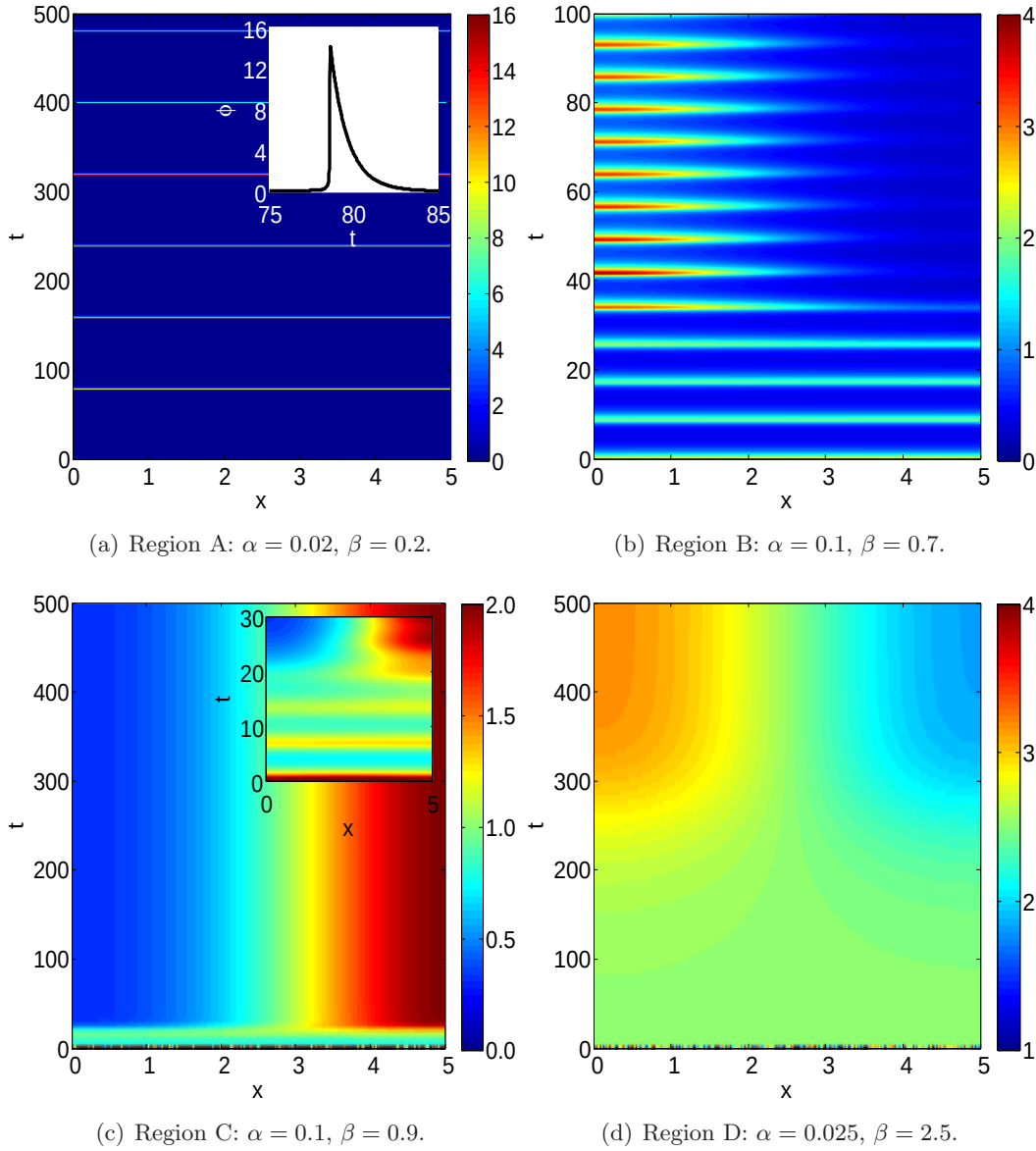


Figure 2.5: One-dimensional simulations of the various dynamics produced by the Schnakenberg kinetics, equations (2.61) and (2.62). Note that, in each case, only ϕ is shown and that ψ is of a similar shape but out of phase, spatially and temporally. (a) In Region A temporal oscillations dominate, producing a spatially homogeneous non-steady pattern. The inset shows the profile of one of the peak solutions. (b) In Region B Turing patterns and oscillations interact producing non-steady Turing patterns. (c) Region C supports stationary Turing patterns, although they are preceded by oscillations, which are seen in the inset. (d) A typical example of the stationary Turing patterns formed in Region D. Initial conditions were randomly generated and in all simulations $D = 40$ and $L = 5$.

inside the Turing unstable interval. This wave mode corresponds to the fastest growing exponential term in the expansion solution, equation (2.17), and, as such, we expect it to be the dominant wave mode seen in the simulations. For the current parameters, the

growing wave modes for various values of L are shown in Table 2.1, alongside the fastest growing mode, m_c .

L	Growing wave modes	Fastest growing mode, m_c
20	$2 \leq m \leq 5$	3
40	$3 \leq m \leq 10$	5
60	$4 \leq m \leq 16$	8

Table 2.1: The range of wave modes to which the homogeneous steady state is linearly unstable, computed for the Schnakenberg kinetics, equations (2.61) and (2.62), for the given L . Parameters are $\alpha = 0.1$, $\beta = 0.9$ and $D = 40$.

L	Growing wave modes	Fastest growing mode, m_c
20	$m = 4$	4
40	$8 \leq m \leq 9$	8
60	$11 \leq m \leq 13$	12
80	$15 \leq m \leq 18$	16

Table 2.2: The range of wave modes to which the homogeneous steady state is linearly unstable, computed for the Schnakenberg kinetics, equations (2.61) and (2.62), for the given L . Parameters are $\alpha = 0.025$, $\beta = 2.5$, $D = 40$.

In Figure 2.6(b), these data are plotted for all lengths, $L \in [0, 100]$. The blue horizontal lines indicate the ranges of L over which each wave number is growing. Observe that the range of existence for each mode becomes larger as m increases, also the amount of overlap between modes increases. This overlap leads to the robustness problem; it is difficult to generate a mode consistently with random initial conditions, especially for higher modes where the overlap region is particularly large. Although there will be a fastest growing wave number, which is indicated by the red solid line, this may not always be the wave number chosen. Indeed, by choosing specific initial conditions, it would be possible to make the system evolve into any of the available modes, but such exactness is unlikely to occur in biological systems (Ozbudak *et al.*, 2002; Rao *et al.*, 2002).

To illustrate the robustness problem, the system was simulated with different initial conditions one hundred times for $L = 20, 40$ and 60 , with $\alpha = 0.1$ and $\beta = 0.9$. In each case the initial condition was the steady state with the addition of a random perturbation taken from a normal distribution with mean equal to zero and standard deviation equal to one (the absolute value was taken if the resulting number was negative). We chose a normal distribution as all wave modes are excited equally by definition. Hence the results should not be biased by the initial conditions. The frequency of the pattern modes which

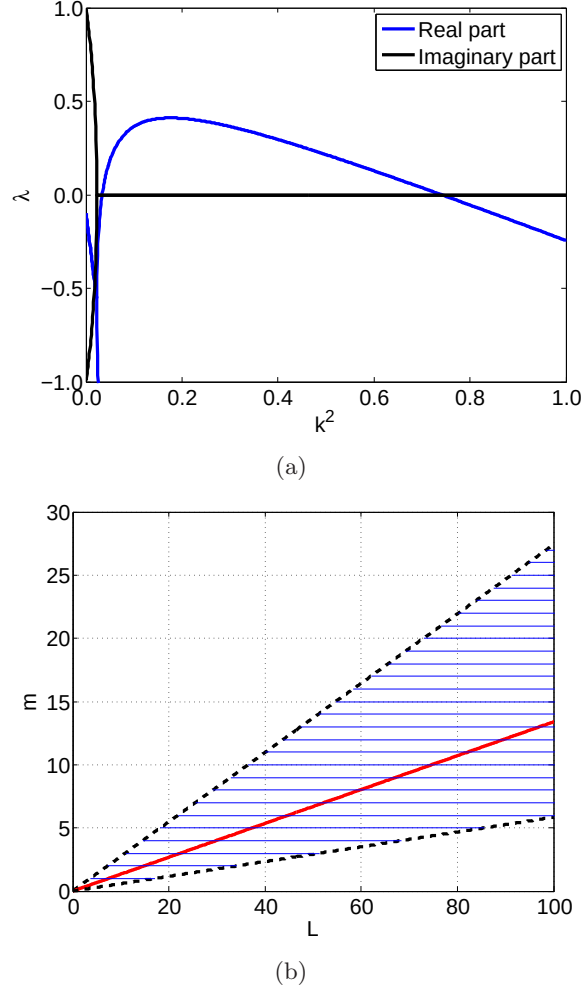


Figure 2.6: Visualising the unstable modes of the Schnakenberg kinetics, equations (2.61) and (2.62). (a) Plot of equation (2.20), which predicts the growth rate of each mode. (b) Bounds on the wave numbers of possible growing modes. The dashed lines are $m = Lk_+/\pi, m = Lk_-/\pi$, respectively, and they are calculated from their definition in equation (2.26). The thick solid red line shows the most unstable mode, $m_c = Lk_c/\pi$, where k_c is given by equation (2.65). The thin solid blue lines are the possible integer modes. Parameters are $\alpha = 0.1$, $\beta = 0.9$ and $D = 40$.

arose was then recorded and is shown in Figure 2.7(a) and can be compared with the range of wave numbers and critical wave numbers for each L in Table 2.1. Figure 2.7(a) illustrates the robustness problem well as it shows how varied the final pattern mode can be. Figure 2.7(b) clearly shows that a much more robust system is produced with parameters $\alpha = 0.025$ and $\beta = 2.5$. The corresponding wave mode ranges are shown in Table 2.2. For $L = 20, 40$ and 60 we obtain exactly the critical mode over all of the one hundred simulations. Even when L is increased further to 80 , it loses robustness only slightly as only 14 simulations out of 100 evolved to the lower wave number $m = 15$. This

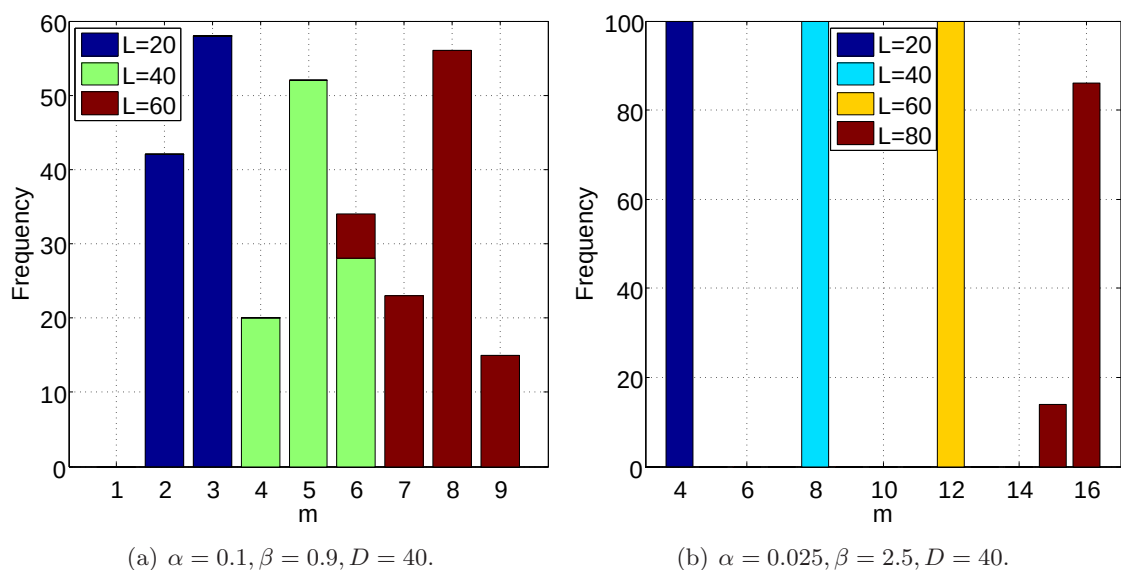


Figure 2.7: Frequency of each wave number over 100 simulations of the Schnakenberg reaction kinetics, equations (2.61) and (2.62) for the values of L shown. The system was simulated using the pdepe algorithm in Matlab 7.12. Each simulation was run for 10^4 time units to ensure that the system was at steady state and each domain was discretised into 500 spatial compartments. Once the simulations had been run, the dominant wave mode observed at the final time point was recorded.

contrasts with the $(\alpha, \beta) = (0.1, 0.9)$ case, where even $L = 20$ shows a large variability with nearly half of the simulations evolving to the lower wave number of $m = 2$. One of the reasons for this increased robustness is that the interval of possible wave modes is shorter in the $(\alpha, \beta) = (0.025, 2.5)$ case, compared to $(\alpha, \beta) = (0.1, 0.9)$. For example, when $L = 20$, $(\alpha, \beta) = (0.025, 2.5)$ guarantees a wave number of four. However, this cannot be the only reason, since the $(\alpha, \beta) = (0.025, 2.5)$ case shows a greater robustness than $(0.1, 0.9)$, even when the interval increases to include multiple wave modes. Thus, we conjecture that the added complexity of temporal oscillations due to the complex eigenvalues can increase the variability of the final chosen pattern mode.

To assume that the reaction rates are such that the system lies within Region D we would need considerable fine-tuning of the parameter values due to the small size of the parameter domain. Hence, the system needs some adaptation in order to produce a robust evolution. In the next section we use growth as a means of providing exactly this behaviour.

2.5 Schnakenberg kinetics on a growing domain

Having considered the kinetics on stationary domains, the evolution of the system has been seen to be extremely variable due to the homogeneous state being unstable to multiple wave modes. In order to prevent this, we use the theory developed in Section 2.3 to cast the Schnakenberg system on a growing domain. As time increases the domain size increases and, as discussed in the previous section, this means that more, higher wave modes are able to grow, whereas lower wave modes begin to decay (see equation (2.25) and Figure 2.6(b)). Through this mechanism transition sequences of pattern modes can be produced.

To be able to compare the deterministic to the stochastic results later in the thesis, the dimensional form of the kinetics are used. From equation (2.45) the Schnakenberg kinetics on an exponentially increasing domain are explicitly

$$\frac{\partial \phi}{\partial t} + c_4 \phi = D_\phi e^{-2c_4 t} \frac{\partial^2 \phi}{\partial x^2} + c_1 - c_{-1} \phi + c_3 \phi^2 \psi, \quad (2.67)$$

$$\frac{\partial \psi}{\partial t} + c_4 \psi = D_\psi e^{-2c_4 t} \frac{\partial^2 \psi}{\partial x^2} + c_2 - c_3 \phi^2 \psi, \quad (2.68)$$

where the c_i parameters are the deterministic rates of the respective reactions. In particular, c_4 , is the rate of exponential growth. As before, the initial conditions are randomly generated and the boundary conditions are Neumann. The initial bifurcation from the homogeneous steady state is through the diffusion-driven instability, as derived in Section 2.4.1. In the case of slow growth, once the pattern has formed growth causes the solution to evolve in two dynamical regimes. The amplitude of the pattern is modulated in a quasi-steady state as the domain length increases with time. Figure 2.8(b) shows that this pattern persists until, at some point, the solution undergoes a transition to a new quasi-steady pattern; undergoing a fast, dynamic reorganisation from a mode m to a mode $2m$ pattern. Subsequently, a pattern of higher spatial frequency is established which persists, with slow amplitude modulation, until the solution undergoes further transitions (Crampin, 2000; Crampin *et al.*, 1999, 2002a).

It has been shown that these transitions can fail if the growth is either too fast or too slow (Barrass *et al.*, 2006; Neville *et al.*, 2006). During the jump from one stable mode to another the trajectory moves through a window where wave modes k , $m < k < 2m$, may

also be stable (Barrass *et al.*, 2006), thus, if the growth is too slow, period doubling may break down due to the accumulated computational errors which lead to asymmetric peak splitting producing a period doubling failure (Figure 2.8(a)). Alternatively, if growth is too fast, (Figure 2.8(c)), period doubling breaks down because the system does not reside for sufficiently long in a specific range of stable wave modes to allow the intended pattern to become established.

Although break down at lower growth rates is an artefact of the solution technique, it is not biologically irrelevant. Biological processes will often have to operate under exposure to noise, as well as many spatial inhomogeneities. Due to the sensitivity of this transition mechanism, in Chapter 7 we will be interested in stochastically simulating Turing systems on growing domains in order to consider the effects of noise on the wave mode evolution.

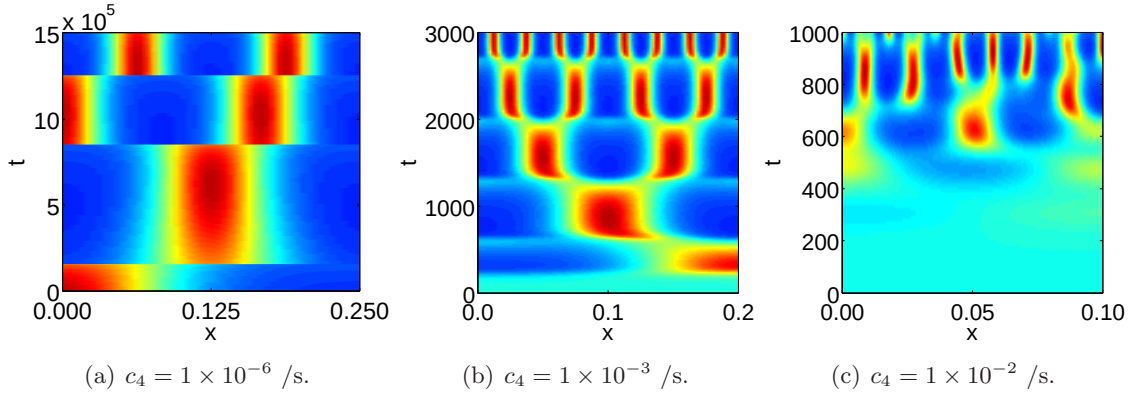


Figure 2.8: Solutions of equations (2.67) and (2.68) where $D_\phi = 10^{-4} \text{ mm}^2/\text{s}$, $D_\psi = 10^{-2} \text{ mm}^2/\text{s}$, $c_1 = 0.1 \text{ mol}/(\text{mm s})$, $c_{-1} = 0.2 /s$, $c_2 = 0.3 \text{ mol}/(\text{mm s})$, $c_3 = 0.1 \text{ mm}^2/(\text{mol}^2 \text{ s})$ and in each case the domain was growing exponentially with rate c_4 as noted beneath each image. The spatial axis is in millimetres and the temporal axis is in seconds. The initial conditions were random perturbations around the steady state, $(u, v) = (2, 7.5)$. The perturbations were generated from a normal distribution with mean equal to zero and standard deviation equal to one. The simulations have been plotted on the fixed Lagrangian domain as it is easier to see the transition sequences. The colour axis in each image runs from 0 (blue) to 5 (red) mol/mm.

2.5.1 Other types of growth

Having shown that exponential domain growth is necessary for period doubling transitions to occur for all time, we now consider the effect of different types of domain growth.

2.5.1.1 Linear growth

Here, $l(t) = 1 + c_4 t$ and, as can be seen in Figure 2.9, prolonged frequency doubling behaviour is not supported by uniform linear growth, although the first few doubling transitions, modes $1 \mapsto 2 \mapsto 4 \mapsto 8$, do occur. Despite being unable to predict what will happen after the doubling transition sequence breaks down, we can at least predict when the breakdown will occur. Crampin *et al.* (1999) noted that the critical growth rate for mode doubling failure on the exponentially growing domain was a useful indicator as to when failure would occur for sub-exponential growth.

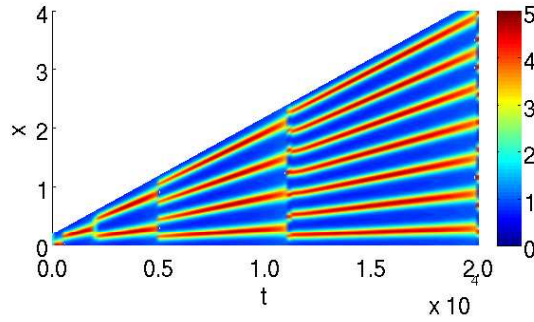


Figure 2.9: Solution of equations (2.67) and (2.68) where $D_\phi = 10^{-4}$ mm²/s, $D_\psi = 10^{-2}$ mm²/s, $c_1 = 0.1$ mol/(mm s), $c_{-1} = 0.2$ /s, $c_2 = 0.3$ mol/(mm s), $c_3 = 0.1$ mm²/(mol² s) and the domain is growing linearly with rate $c_4 = 1 \times 10^{-3}$ /s. The spatial axis is in millimetres and the temporal axis is in seconds. The initial conditions were random perturbations around the steady state, $(u, v) = (2, 0.75)$ mol.

2.5.1.2 Logistic growth

So far we have only considered unbounded forms of growth. Of course this is not physical and should only be used in regimes of initial growth where the domain can be thought to be increasing quickly. However, if the pattern formation is thought to be generated over a time scale on which the growth rate slows and the domain size saturates then we should look towards more appropriate descriptions of growth, such as logistic growth. The form of the growth function is then

$$l(t) = \frac{l_\infty}{1 + (l_\infty - 1)e^{-c_4 l_\infty t}}, \quad (2.69)$$

where l_∞ is the scaling of the initial to the saturated length. Thus, if the initial length is L_0 then the length of the domain tends to a final length of $L_\infty = L_0 l_\infty$.

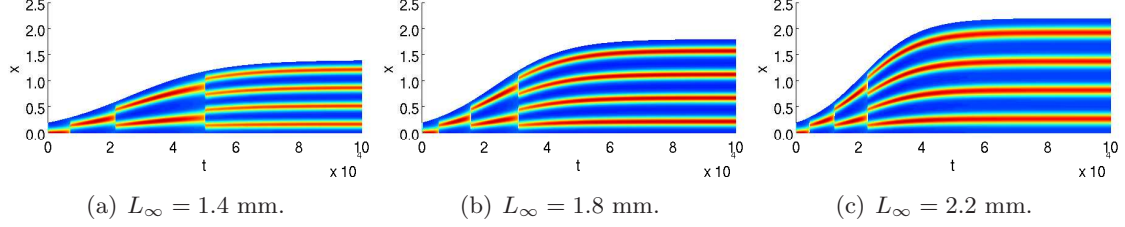


Figure 2.10: Solutions of equations (2.67) and (2.68) where $D_\phi = 10^{-4}$ mm²/s, $D_\psi = 10^{-2}$ mm²/s, $c_1 = 0.1$ mol/(mm s), $c_{-1} = 0.2$ /s, $c_2 = 0.3$ mol/(mm s), $c_3 = 0.1$ mm²/(mol² s) and the domain was growing logistically with rate $c_4 = 1 \times 10^{-5}$ /s. The spatial axis is in millimetres and the temporal axis is in seconds. The initial conditions were random perturbations around the steady state, $(u, v) = (2, 0.75)$ mol. The simulations have been plotted on the growing domain. Each simulation had an initial length $L = 0.2$ and final length, L_∞ , is noted beneath each figure. The colour axis in each image runs from 0 (blue) to 5 (red) mol/mm.

As we would expect, Figure 2.10 shows that, once the domain size begins to saturate, the pattern transitions stop. We are able to perturb the final size of the domain whilst maintaining the same final mode. Thus the transitions have a certain degree of insensitivity to the final length, $L_\infty = L_0 l_\infty$. This is unlike a general stationary domain simulation, where small changes to the domain size can lead to different modes being chosen (Crampin *et al.*, 1999).

2.5.1.3 Apical growth

Here, we relax the condition that growth is isotropic and consider growth that is restricted to the tip of the domain. We model this by assuming that the dilation rate, $\mathcal{D}$, is zero everywhere except in the proliferating region, which is maintained at one edge and has width δ ,

$$\frac{\partial a}{\partial x} = \mathcal{D} = \begin{cases} 0, & 0 \leq x \leq L(t) - \delta \\ S_{tip}(t), & L(t) - \delta \leq x \leq L(t) \end{cases}, \quad (2.70)$$

and so $l(t) = 1 + \delta \int_0^t S_{tip}(t') dt'$.

Setting $S_{tip} = c_4/\delta$, we can take the limit $\delta \rightarrow 0$, *i.e.* considering the proliferating region to be much smaller than the remainder of the domain. Thus, the system can be reduced to an Eulerian moving boundary problem (Crampin *et al.*, 2002b). The scaled

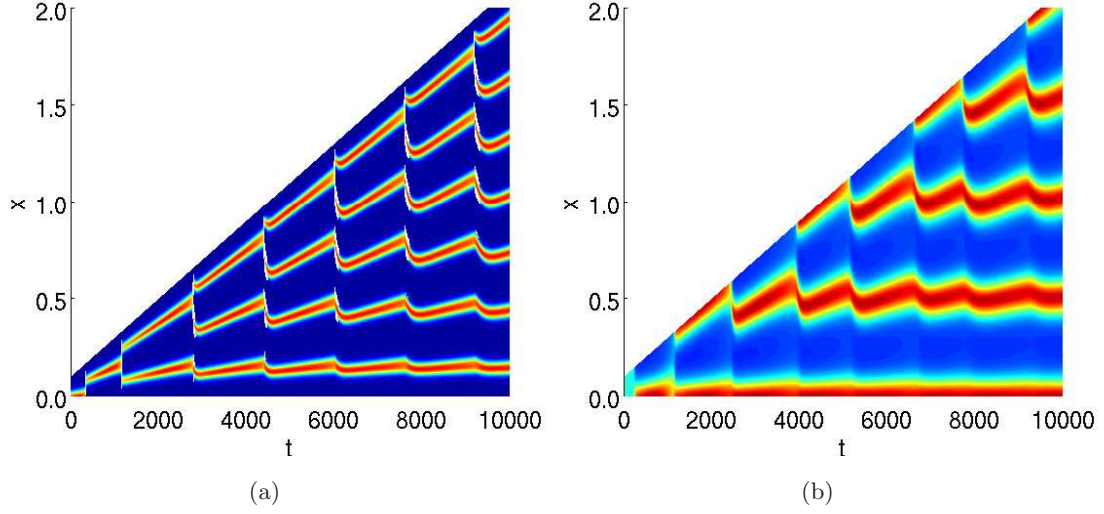


Figure 2.11: Solutions of equations (2.67) and (2.68) where $D_\phi = 10^{-4}$ mm²/s, $D_\psi = 10^{-2}$ mm²/s and the domain was growing apically at a linear rate, $c_4 = 1 \times 10^{-3}$ /s. The spatial axis is in millimetres and the temporal axis is in seconds. The rest of the parameters are (a) $c_1 = 0.1$ mol/(mm s), $c_{-1} = 1$ /s, $c_2 = 0.9$ mol/(mm s), $c_3 = 1$ mm²/(mol² s) and (b) $c_1 = 0.1$ mol/(mm s), $c_{-1} = 0.2$ /s, $c_2 = 0.3$ mol/(mm s), $c_3 = 0.1$ mm²/(mol² s). The initial conditions were random perturbations around the, respective, steady states. The colour axis in each image runs from 0 (blue) to 5 (red) mol/mm.

reaction-diffusion system, in the Lagrangian coordinate system, is

$$\frac{\partial \phi}{\partial t} = \frac{D}{l^2} \frac{\partial^2 \phi}{\partial X^2} + X \frac{\dot{l}}{l} \frac{\partial \phi}{\partial X} + F(\phi). \quad (2.71)$$

Figure 2.11 shows the numerical solution of the Schnakenberg system on an apically growing domain for two different sets of parameters. We consider these two different regimes in order to illustrate an important difference between isotropic and apical growth. Isotropic growth generates robustness through a period doubling transition, whereas Figure 2.11 shows that apical growth is able to produce robust sequences that either only visit even modes, as in Figure 2.11(a), where the wave mode transitions are $2 \mapsto 4 \mapsto 6 \mapsto 8$, or visits every wave mode, as in Figure 2.11(b), where the wave mode transitions are $2 \mapsto 3 \mapsto 4 \mapsto 5$. Other sequences, *e.g.* only odd modes, may also be available. Thus, from this one simple mechanism of growth we are able to produce multiple robust transition sequences.

2.6 Discussion

This chapter has recapitulated the development of the deterministic framework of PDEs and their extension to growing domains, which will be used throughout this thesis to model biological pattern formation. By deriving the equations from the underlying conservation form, we were able to clarify the assumptions upon which the framework rests. In particular, we make use of the large population assumption, Fick's Law of Diffusion and the Law of Mass Action. These assumptions may not always hold, suggesting that a system of continuous reaction-diffusion equations may not always lead to the correct description of the available dynamics (Erban *et al.*, 2007). Through developing connections between deterministic systems and their stochastic analogues, we will understand which properties of continuum analysis, developed in this section, can be transferred and used to describe the more complex dynamics of the stochastic model.

We began by firstly considering the French flag model of morphogenesis, which was followed by the Turing instability. Although this demonstrates that we are able to amplify and produce spatial heterogeneity we are still left with the problem of lack of robustness, which was illustrated in the particular context of the Schnakenberg kinetics. Although the Schnakenberg kinetics have the desirable property that the solutions never suffer from blow up when $\alpha > 0$ (Appendix B) this causes the system to have oscillatory dynamics which compound this lack of robustness by making the final pattern more variable. This wave mode variability increases the importance of understanding the effect of noise on such systems as stochastic simulations are able to produce different final patterns from the same initial conditions (see Section 7.4). Hence, ways of generating robustness need to be investigated.

In this chapter we have seen that such a simple addition as growth has enabled us to produce much richer dynamics than would otherwise be possible. In particular, we can generate a frequency doubling sequence of patterns, providing the speed of growth is not too fast, nor too slow. Importantly, this sequence is not sensitive to the initial conditions, contrary to the results of Saunders & Ho (1995) which are based on the numerical analyses of a model by Arcuri & Murray (1986). However, this contradiction is understandable since Arcuri & Murray (1986) do not derive a model from first principles, instead they

introduce a time-dependent scaling parameter, which distorts the relationship between the time scales for pattern growth and domain growth. Consequently, their model fails to produce robust transitions.

Although only one spatial dimension has been discussed in this chapter, the ideas are generalisable to higher dimensions (Painter *et al.*, 1999) and different geometries (Gjorgjieva & Jacobsen, 2007). However, with increasing dimension the number of possible patterns and arrangements of these patterns also increases. For example, in two dimensions we can produce either spots or stripes (Ermentrout, 1991), with the spots having the ability to be arranged in square, diamond, rhombic or hexagonal patterns (Leppänen, 2004). In three dimensions lamellae, prisms, and various other cubic structures all exist making prediction even more difficult (Callahan & Knobloch, 1996, 1999). Equally, we must ensure that the domain discretisation is fine enough not to interfere with the patterning mechanism (Madzvamuse, 2006; Madzvamuse *et al.*, 2003).

The recapitulation of Crampin’s self-similarity argument analytically demonstrates how robustness can be generated through the use of exponential growth (Crampin *et al.*, 1999). Primarily, the proof depends on the Neumann boundary conditions, so as to preserve the derivative across the doubled pattern. Small perturbations to these boundary conditions, as well as periodic boundary conditions, have also been computationally shown to allow period doubling (Crampin, 2000). However, even this mechanism is not without its problems; Figure 2.8 shows that robust doubling is only realised within a specific (but large) region of growth rates. Outside of this region the transition sequences once again break down. Under linear growth the sequence is also found to break down after some number of doubling events. By comparing the linear growth rate with the slowest exponential growth rate that can support robust pattern doubling sequences it is possible to predict the time at which the doubling will break down.

For logistic growth, not only is the system able to reliably tend to a single mode, but it does so even with perturbations to the final domain length. This is particularly significant as it allows regulation without the need for parameter fine-tuning. This insensitivity, whereby a specific number of pattern elements is laid down despite significant variation in the domain size, is desirable in many biological systems. Although scale invariance has been considered by previous authors (Hunding & Sørensen, 1988; Othmer & Pate,

1980) they concluded that some form of feedback from the domain size into the kinetic parameters would be necessary. Here it is seen that semi-scale invariance arises as a natural consequence of the sequence formation mechanism. This is important in the context of biological pattern formation, as not only do patterns need to form, but, also, in many cases, it is imperative that the transition sequences be reliably producible.

Finally, apical growth was considered and was equally able to produce robust transition sequences, although not the normal period doubling bifurcations. Instead, within different parameter regimes, we are able to produce sequences which increase consecutively or only produce even modes. This ability to produce different robust sequences is a beneficial facet of growth as it shows that the same mechanism is able to account for a number of different phenomena.

In the next chapter we apply this PDE framework to two different biological applications, limb formation in chickens and molar primordia signalling in mice. In both cases robust development of heterogeneity is observed. In collaboration with experimentalists we have derived mathematical models, based on the PDE framework developed in this chapter, in order to suggest the mechanisms by which similar cells can evolve to different fates. Importantly, the inclusion of growth is seen as not only an addition to the realism of the model but is actually crucial to generating the observed results.

Chapter 3

Biological applications of growth

“Childbirth is a miracle. No, it’s not... It’s a chemical reaction, that’s all.”

Bill Hicks, Revelations, 1993.

In the previous chapter we derived the deterministic formalism in which we will construct reaction-diffusion PDE systems. Here, we apply these techniques to two biologically motivated problems. In both cases we observe that the addition of growth is needed to reproduce the experimental phenomena.

We first consider the development of mice teeth by investigating the production of spatial heterogeneity in the dental lamina which forms the tooth precursors. It is well known that the gene pathways of Wnt, Sonic hedgehog (Shh) and Sostdc1 form an important signalling network (Laurikkala *et al.*, 2003; Munne *et al.*, 2009; Sarkar & Sharpe, 1999) and, recently, it has been shown experimentally that their gene products are able to interact through a feedback mechanism (see Figure 3.2(b)) (Cho *et al.*, 2011). We construct a three-component PDE model based upon this feedback network to account for the concentrations of Wnt, Shh and Sostdc1 as activator, mediator and inhibitor, respectively. Crucially, we show that this system is able to support a Turing instability, allowing us to interpret the production of molar domains through high and low concentrations of the activator. Moreover, through the application of growth to the domain, we are able to account for consecutive molar production. However, we observe that, although we are able to produce patterns, in order to create the spatial sequence observed experimentally the model predicts that mixed boundary conditions need to be used.

The second biological system to which we apply the deterministic framework is chick wing digit formation. Chick wing digits were one of the first biological systems in which it was suggested that cells are able to sense a long-range morphogen gradient, as in the French flag model (Cooke & Summerbell, 1980; Riddle *et al.*, 1993; Summerbell, 1981; Wolpert, 1969, 1989). Experiments have led to the discovery of a signalling region at the posterior margin of the chick wing bud (the polarizing region), which is suggested to excrete Shh as a diffusible morphogen that sets up a gradient across the wing bud, so that cells at different positions are exposed to different concentrations (Sanz-Ezquerro & Tickle, 2000; Tickle *et al.*, 1975; Towers *et al.*, 2011). Here, we model the Shh as a diffusing system with a source on the boundary to describe the effects of the polarising region. The identities of the digits are then specified by thresholds which the concentrations breach. Similar to the mouse tooth model, we find that certain assumptions are needed in order to achieve not only the correct concentration levels, but the correct time evolution of the gradient. This gradient model is then extended to include the polarising region in order to see if the same biological mechanism applies to chick leg digit production.

3.1 Mouse tooth spatial patterning

This section is based on work published in Cho *et al.* (2011). The mathematical model was developed by Thomas E. Woolley, Ruth E. Baker and Philip K. Maini and based on experiments designed and carried out by Sung-Won Cho, Sungwook Kwak, Min-Jung Lee, Eun-Jung Kim, Hee-Jin Kim, Jeon-Soo Shin and Han-Sung Jung, with additional insights provided by Cheryl Tickle. Wild-type mice have one incisor and three molars in one quadrant of the jaw. These develop at precise locations and at different times (see Figure 3.1). The development of multiple teeth in the jaw of vertebrates has been extensively studied (Ahn *et al.*, 2010; Cai *et al.*, 2007; Dassule *et al.*, 2000; Dassule & McMahon, 1998), but the genetic mechanism governing the spatial patterning of teeth still remains to be elucidated.

Shh has been identified as a key signalling molecule; it is involved in the spatial patterning of many organs, *e.g.* teeth, hair follicles and feathers (Laurikkala *et al.*, 2003; Närhi *et al.*, 2008; Nohno *et al.*, 1995; St-Jacques *et al.*, 1998), which develop in the outer

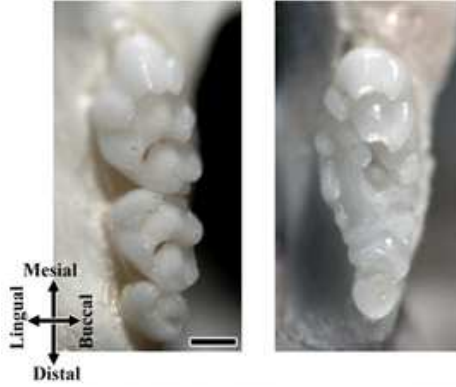


Figure 3.1: Wild-type (left) and Teeth in a mouse with reduced Shh expression (right). In the wild-type mouse three distinct molars can be produced whereas the reduced Shh expression causes the molars to form one long tooth. The scale bar is $500\ \mu\text{m}$ and is the same in both pictures. Reproduced from Cho *et al.* (2011), with permission.

layer of the early embryo, or ectoderm. *Sostdc1* (also known as USAG-1, ectodin and Wise) is an established secreted inhibitor of Wnt pathways and also regulates the spatial patterning of teeth (Kassai *et al.*, 2005; Laurikkala *et al.*, 2003; Munne *et al.*, 2009). We propose a new reaction-diffusion model, in which Wnt, Shh and *Sostdc1* act as the activator, mediator and inhibitor, respectively, and confirm that such interactions can generate the tooth pattern of wild-type mice, as well as suggest reasons behind the patterning breakdown in mutant mice.

Successful computer models of tooth development already exist (Järvinen *et al.*, 2006; Salazar-Ciudad & Jernvall, 2002, 2010). However, due to the high spatial resolution of single teeth produced by these models, they are computationally unable to accommodate molar domain splitting and development of multiple structures (Järvinen *et al.*, 2006). Thus, they are unable to simulate the development of the entire mouse jaw. Here we derive, simulate and adapt a new reaction-diffusion mechanism for spatial patterning of the teeth with the aim of hypothesizing how the sequence of tooth primordia positions could be set by a system of interacting chemicals. Our intention is to reproduce experimental results concerning the spatial distribution of the developing molars. Thus, our method complements that of Järvinen *et al.* (2006), as, once we know the global distribution of Wnt, Shh and *Sostdc1*, we would be able to use their highly detailed numerical methods to model evolving molars.

Due to the lack of experimentally derived parameters, this model should be used to

elucidate qualitative behaviour only. Indeed, it is our intention to characterise the possible underlying mechanics that could produce the observed patterning rather than quantify them. Thus, the parameter values used are primarily found through computational experimentation and illustrate a concept. They should not be taken as indicative values for the biological system.

3.1.1 A new reaction-diffusion model for spatial patterning of teeth

In mice that have sustained activity of the Wnt pathway in the epithelium it has been shown that multiple extra teeth grow (Järvinen *et al.*, 2006; Kuraguchi *et al.*, 2006; Liu *et al.*, 2008). It is also known that sustained epithelial Wnt signalling in the hair and teeth of mice upregulates Shh and Sostdc1 (Liu *et al.*, 2008; Närhi *et al.*, 2008). Here it is assumed that Wnt does not activate Sostdc1 directly but it is by the increase in Shh Cho *et al.* (2011). Deletion of Wnt10b severely decreases expression of Shh in tongue fungiform papillae (Iwatsuki *et al.*, 2007). Conversely, Shh is able to suppress Wnt10b in early developing mandibles (Dassule & McMahon, 1998), and inhibition of Shh signalling by 5E1 (an antibody against Shh protein) increases the expression of Wnt signalling in fungiform papillae (Iwatsuki *et al.*, 2007). It is assumed that the action of the Shh on Wnt does not occur directly, but through the mediator Sostdc1 Cho *et al.* (2011). Furthermore, Wnt signalling in tooth germs of reduced Sostdc1 and Shh mice is significantly elevated compared with that in mice with only reduced Sostdc1 (Ahn *et al.*, 2010). Finally, Cho *et al.* (2011) found that there was no detectable expression of Sostdc1 expression in dental mesenchyme from mice treated with the Shh blocking antibody at E14 and conversely that Sostdc1 expression can be induced in the dental mesenchyme at E14 with Shh protein. They also notice that certain areas which are inhabited by Wnt are free from Sostdc1, leading them to postulate that Wnt directly inhibits the production of Sostdc1. These interactions lead us to propose a Wnt-Shh-Sostdc1 negative feedback loop, which can be modelled as consisting of an activator, mediator and inhibitor, respectively. The key features of this model, shown in Figure 3.2(b), are:

1. Wnt activates Shh, which in turn activates Sostdc1;
2. Wnt is inhibited by Shh through its activation of Sostdc1;

3. Wnt inhibits Sostdc1 production.

Additionally, if we assume that

4. Wnt is a self-activator,

then we are able to model this network of gene interactions as a reaction-diffusion system, based on a three population Turing system (Gierer & Meinhardt, 1972). Unlike features 1-3, property 4 is a mathematically motivated assumption and should be seen as a prediction of the model. However, it is not totally without evidence as Wingless (Wg), a gene product similar to Wnt in the fruit fly *Drosophila melanogaster*, has been shown to be a self-activator (Julius *et al.*, 2009; Tegner *et al.*, 2003).

Our aim is to show that the Gierer-Meinhardt system can reproduce the observations that there are three distinct regions of activator, mediator and inhibitor concentration and that these three regions do not form simultaneously, but instead occur consecutively. These three distinct sites will then be interpreted as the prepatter of the molar placement. Further, we want the model to be able to recreate the result that if Shh expression is reduced then the molar prepatterns become fused together, as shown in Figure 3.1 and illustrated in Figure 3.2(a).

In order to create the reaction-diffusion system we initially consider the effective relationship between only Wnt and Shh. From the biological justification above we know that the self activator Wnt activates Shh, whilst Shh inhibits Wnt (through the action of Sostdc1). We also know that the populations are in phase and, so, are dealing with a “pure” kinetic type system (see Chapter 2). Once we have fixed the type of kinetics it is often hypothesised that their actual forms are unimportant (as verified numerically for two species deterministic systems) (Murray, 2003b). Hence, we simply choose the two population Gierer-Meinhardt system (Gierer & Meinhardt, 1972), on which to base our Turing system. Identifying Wnt to be the standard activator and Shh to be the standard inhibitor we use the following set of non-dimensional equations:

$$\frac{\partial A}{\partial t} = D_a \nabla^2 A + \frac{A^2}{M} - \beta A + \alpha, \quad (3.1)$$

$$\frac{\partial M}{\partial t} = D_m \nabla^2 M + \mu(A^2 - M), \quad (3.2)$$

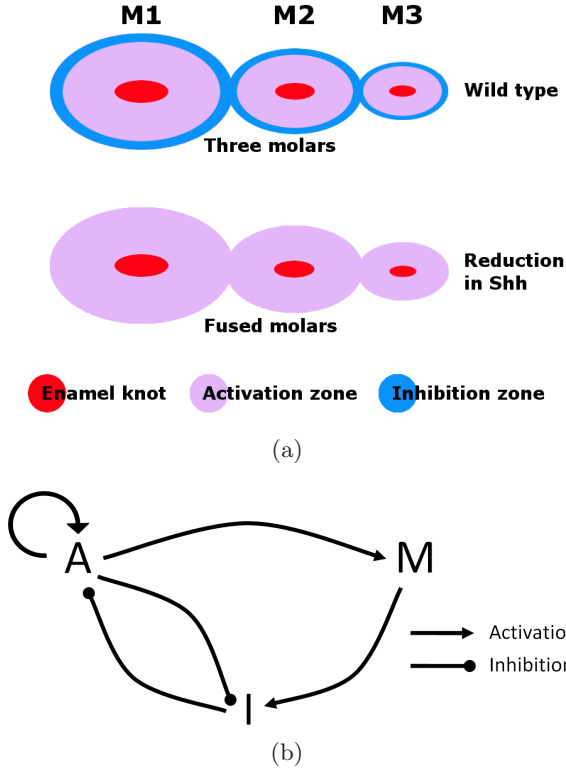


Figure 3.2: (a) Experimentally developed caricature of the molar domain, adapted from Cho *et al.* (2011). (b) Reaction network derived from the work of Cho *et al.* (2011) and others, see text for details. Here, A is the activator, Wnt, M is the mediator, Shh and I is the inhibitor, Sostdc1.

where A , M are Wnt and Shh concentrations, respectively, and D_a , D_m , μ and α are positive constants. This system of equations is then expanded to allow Sostdc1 to take the place of the inhibitor in (3.1). Furthermore, we know that Sostdc1 is activated by the mediator and inhibited by the activator. Using this information the system becomes

$$\frac{\partial A}{\partial t} = D_a \nabla^2 A + \frac{A}{I} - \beta A + \alpha, \quad (3.3)$$

$$\frac{\partial M}{\partial t} = D_m \nabla^2 M + \mu(A^2 - M), \quad (3.4)$$

$$\frac{\partial I}{\partial t} = D_i \nabla^2 I + \delta(M - AI), \quad (3.5)$$

where Shh is now known as the mediator and I is the Sostdc1 concentration. D_i and δ are two further non-dimensional positive constants. In all of the following simulations, the initial conditions are perturbations about the homogeneous steady state using pseudo-random numbers that range from negative one to one. Numerical simulations are produced

using the finite element simulation software COMSOL 4.2.0.150. The domain is a fixed, two-dimensional space that has been discretised into 3264 elements.

Initially, we simulate the system on a nondimensional rectangular domain of size $[0, 14] \times [0, 3]$, which is large enough to support the formation of a three-spot pattern, using the parameter values in Figure 3.3. In the previous chapter, we simply considered Neumann boundary conditions. However, these conditions allow half peaks to form against boundaries, as seen in Figure 2.5(c). Indeed, if we simulate equations (3.3)-(3.5) with zero-flux boundaries,

$$\frac{\partial A}{\partial \mathbf{n}} = \frac{\partial M}{\partial \mathbf{n}} = \frac{\partial I}{\partial \mathbf{n}} = 0, \quad (3.6)$$

(Figure 3.3(a)) then the Turing spots can similarly form on the side of the domain, which is unphysical. In order to prevent this from occurring we alter the boundary conditions to Dirichlet conditions and fix the concentrations to zero on the boundary. It is found through simulation that by applying Dirichlet boundary conditions to the variable M the numerical scheme breaks down. This instability is caused by population blow up of A , which arises due to setting $M = 0$ on the boundary, which can be seen by considering system (3.1)-(3.2), on which system (3.3)-(3.5) is based. Similarly, applying the condition just to I leads to problems because of the term A/I in equation (3.3). Hence, we apply the zero boundary condition to A .

As can be seen in Figure 3.3(b), spots are now robustly generated along the central axis of the domain. However, the value of the inhibitor has increased dramatically near the boundary. In order to compensate for this behaviour we simulate both variables A and I with zero Dirichlet boundary conditions, leading to a system with mixed boundary conditions. Note that this does not cause problem with the A/I term in equation (3.3) as A tends to zero faster than I . Although both sets of boundary conditions are biologically feasible, as zero Dirichlet conditions model chemical sinks, whilst Neumann conditions model impermeable boundaries, it remains a prediction of the system that the boundary conditions are mixed in this way. Although we have chosen boundary conditions to stop the formation of molar prepatterns on the boundary and to keep the order of concentration approximately equal, there are a number of other ways which we could have achieved these. For instance there has been a recent suggestion that there may be sources of inhibitor along

the boundary (Sharpe, *pers. comms.*), which would stop boundary spots from forming. Equally, we could have used a saturating form of the Gierer-Meinhardt kinetics to stop the large spike of Inhibitor concentration near the boundary. Thus, we observe that it is not the specific form of the boundary conditions that is important, but, rather, their influence on the final solution that is crucial.

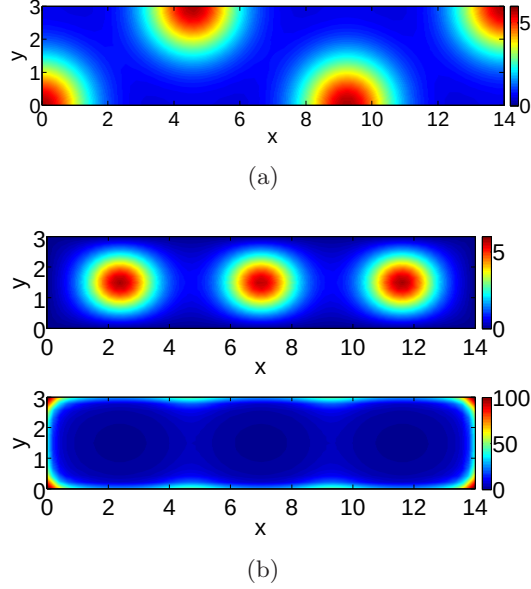


Figure 3.3: Simulation of equations (3.3)-(3.5) on a stationary rectangular domain of size $[0, 14] \times [0, 3]$ with varying boundary conditions. Only the activator is shown in (a) and the activator and inhibitor are shown in (b). (a) All concentrations have zero-flux boundary conditions. (b) I and M have zero-flux boundary conditions, whilst A has Dirichlet boundary conditions. Nondimensional parameters are $D_a = 0.1$, $D_m = 0.9$, $D_i = 0.1$, $\alpha = 0.1$, $\beta = 0.5$, $\mu = 0.4$ and $\delta = 100$.

The next problem that we contend with is producing the three spots sequentially; observations suggest that the teeth form consecutively in the order M1, M2 and, finally, M3 (Cho *et al.*, 2011). In order to accommodate this evolution, we apply the insight gained in Section 2.3 and use the inclusion of dental laminar growth to produce the pattern transitions. Immediately, we are able to discount uniform growth as this causes the M2 spot to appear between the spots M1 and M3 (Figure 3.4), implying that the transition sequence of spots is $M1 \mapsto M1\text{-}M3 \mapsto M1\text{-}M2\text{-}M3$, contradicting the observations that the teeth form consecutively. In order to produce the correct transition evolution we assume the model undergoes apical domain growth, which produces spots consecutively.

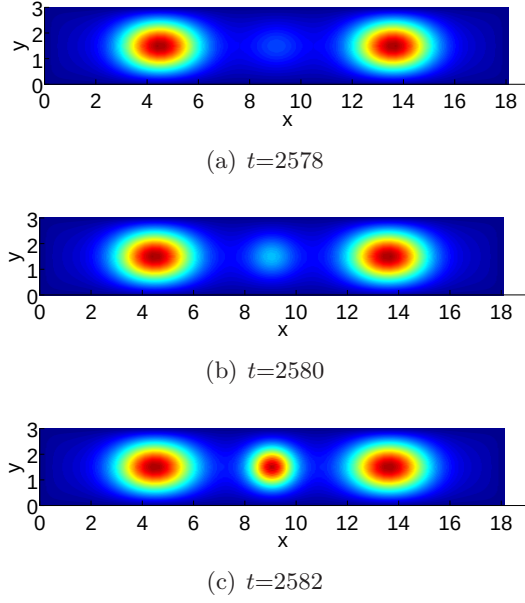


Figure 3.4: Simulation of equations (3.3)-(3.5) on a uniformly logistically growing rectangular domain, which is initially $[0, 3] \times [0, 3]$. Only the concentration of A is visualised. A and I have zero Dirichlet boundary conditions, whilst M has zero-flux boundary condition. Nondimensional parameters are $D_a = 0.1$, $D_m = 0.9$, $D_i = 0.1$, $\alpha = 0.1$, $\beta = 0.5$, $\mu = 0.4$ and $\delta = 100$. The colour axis ranges from 0 (blue) to 6 (red). The time of each simulation is given under each figure.

Further, Figure 3.5 suggests that, from an initial approximately square domain at embryonic day 12 (E12), two molar domains are visible when the dental lamina has grown approximately three to four times its original length along the jaw axis with minimal growth perpendicular to this. The third molar domain then develops when the length of the domain has grown four to five times its original length and the molars stop developing once the domain is approximately six times its original length. Thus, we use the theory developed in Section 2.3 to cast equations (3.3)-(3.5) onto a two-dimensional rectangular domain, which grows apically in the x -direction. The length, L , of the domain is governed by

$$L(t) = 3l(t), \quad (3.7)$$

where

$$l(t) = 1 + 5t/(t + 500). \quad (3.8)$$

Thus, from a square domain, $[0, 3] \times [0, 3]$, the domain will grow to a final rectangular shape, $[0, 18] \times [0, 3]$.

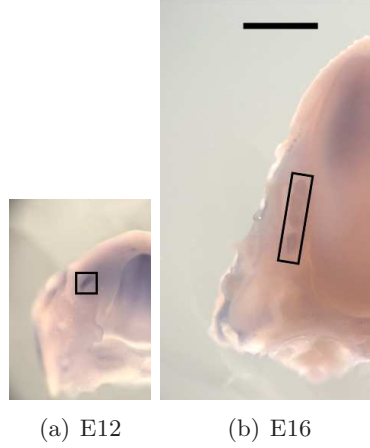


Figure 3.5: Shh expression along the jaw at times E12 and E16. Scale bar 1mm. Both photos are taken at the same magnification. Photos courtesy of Sung-Won Cho, Yonsei University.

As can be seen from Figure 3.6 the model can indeed exhibit the desired observations. Initially, a single Turing spot forms, which then splits at around $t = 300$ to form the patterns of M1 and M2. Note also that the simulations will contain a two spot pattern when the domain is three to four times its original size. Finally, at $t = 1150$, when the domain is four to five times its original size, the M2 spot splits giving the third and final spot. Hence, we are able to produce a consecutively increasing pattern of regions of activator high density along the centre axis.

Finally, it has been observed (Figure 3.1) that, upon experimentally reducing the amount of Shh (mediator) in the system, fused teeth formed instead of three distinct molars. In order to try and capture this change we increase the removal rate of the mediator by replacing equation (3.4) with

$$\frac{\partial M}{\partial t} = \nabla^2 M + \mu(A^2 - M) - \gamma. \quad (3.9)$$

As can be seen in Figure 3.7, the Turing spots are now fused together in a large region of activator, which we would interpret as the prepattern of a fused set of molars. Further, we reproduce the accompanying experimental observation that, upon reducing the levels of Shh, we increase the levels of Wnt and decrease the levels of Sostdc1 (Cho *et al.*, 2011).

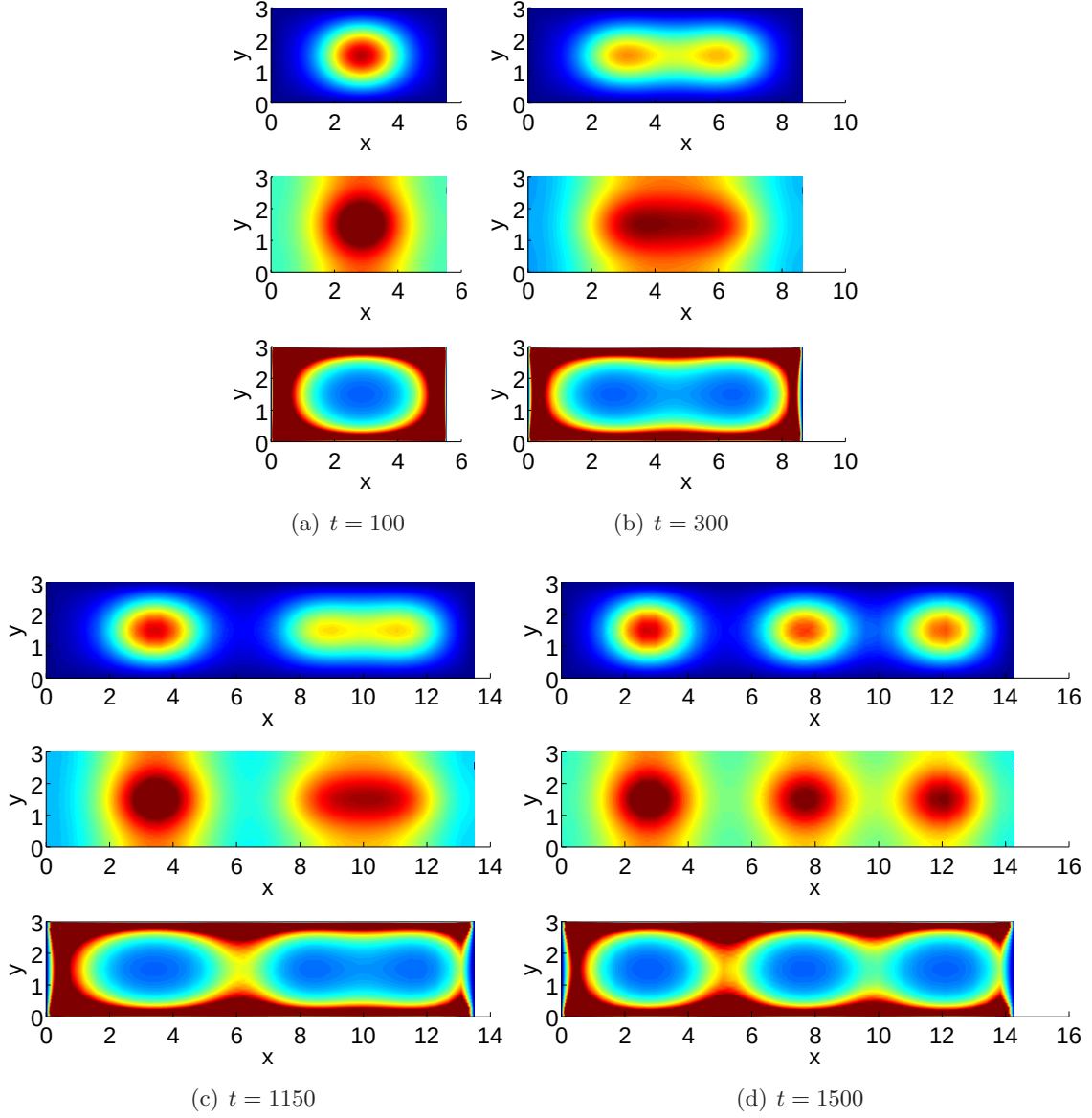


Figure 3.6: Simulation of equations (3.3)-(3.5) on an apically growing domain, at different time points. In each figure the top plot is the activator concentration, A , the middle domain is the mediator concentration, M , and the bottom image is the inhibitor concentration, I . Nondimensional parameters $D_a = 0.1$, $D_m = 0.9$, $D_i = 0.1$, $\alpha = 0.1$, $\beta = 0.5$, $\mu = 0.4$ and $\delta = 100$. The colour axis ranges from 0 (blue) to 6 (red).

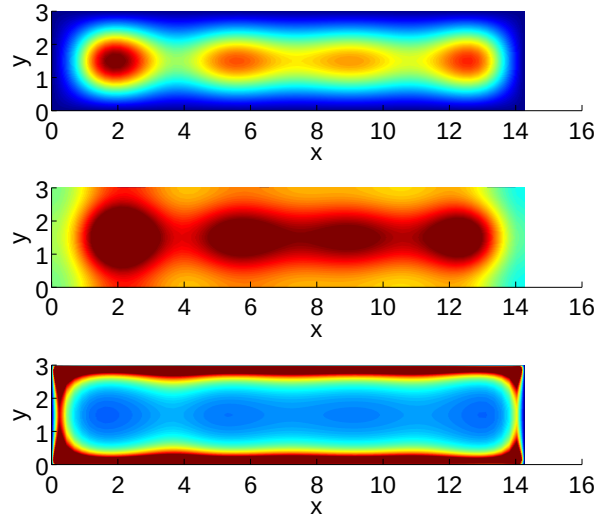


Figure 3.7: Same simulation as in Figure 3.6(d), except that equation (3.4) has been replaced by equation (3.9) and $\gamma = 1$.

3.1.2 Limitations

An observed property of the experiments that we have thus far been unable to encapsulate is illustrated in Figure 3.8; in the case of sustained Wnt activity, molars are produced with irregular sizes and spacing. Similar irregular prepatterns have been shown to exist in Turing systems through the use of: Robin boundary conditions (Myerscough *et al.*, 1998); spatially varying diffusion coefficients (Benson *et al.*, 1998); or extremely small diffusion ratios (Ward & Wei, 2002). Further, due to the conditions needed to produce such a pattern (sustained Wnt activity), observations suggest that temporal effects are important, as Wnt is still activated even when the molars begin to form. Hence, the available space into which the gene products can diffuse is altered by the molar development, producing an extremely complicated time-dependent geometry. Thus, although possible, it is a non-trivial task to generate the observed irregular spot structures with a simple Turing model. The generation of such irregular evolving patterns is thus suggested as an avenue of future work.

Finally, it should be noted that the three molar domains vary greatly in size, with the left-most molar being much larger than the right. This appears to indicate that the system has a spatial inhomogeneity. We have been unable to satisfactorily produce the variations in molar size through perturbing the domain geometry and so we resort to using a spatial variation in the parameter $\mu \mapsto \mu \exp(xl(t)/32)$ as an *ad hoc* method of altering

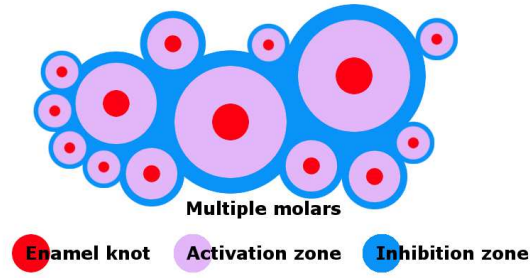


Figure 3.8: Caricature of observed molars in mice with sustained Wnt activity, adapted from Cho *et al.* (2011).

the heights of the activator and inhibitor peaks. The idea being that a larger activator peak is the prepattern for a larger molar domain. This can be seen in Figure 3.6(d) where the right-hand peak is smaller than the left. However, as with many facets of this model, this spatial variance is a prediction that needs to be tested.

3.1.3 Summary

Through the application of a three species coupled PDE system we have been able to show that the feedback mechanism, experimentally discovered by Cho *et al.* (2011), is able to support a modified Turing system. This preliminary research supports the idea that the molar domains are obtained by the creation of a three-spot Turing pattern and, further, that growth plays a fundamental role in the development of the structures.

Although the model is intended to give a purely qualitative view of the tooth formation mechanism it can be used to give predictions of how the mouse tooth pattern will change if the conditions are perturbed in different ways. Here we have only considered the actions observed when Shh is reduced, but similar investigations could be made into the effects of reducing the other two populations. Equally, by observing the change in pattern which occurs when domain growth is increased or decreased, we can obtain experimentally testable predictions. Finally, by understanding the mechanism behind the creation of the molar domains we are able to understand the evolution of gene expression distribution. These reaction-diffusion models can then be used in conjunction with previously developed models that construct the molar shapes in order to create a multiscale system linking the gene expression to the final molar form (Salazar-Ciudad & Jernvall, 2010).

3.2 Chick limb development

This section is based on work completed in collaboration with Matthew Towers and Cheryl Tickle from the University of Bath, who did all of the experimental work and provided biological insights. We have then constructed a mathematical model to test the hypothesis that the experimental observations of chick limb temporal development can be explained using a simple gradient model.

Chick wing digits were one of the first biological systems where it was suggested that cells were able to sense a long-range morphogen gradient, as in the French flag model (Cooke & Summerbell, 1980; Riddle *et al.*, 1993; Summerbell, 1981; Wolpert, 1989). Explicitly, as shown in Figure 3.9, the chick wing digits are known as (posterior to anterior) 4, 3 and 2 and it has been recently found that the fate of each digit is prescribed by the local level of morphogen which it senses (Towers *et al.*, 2008; Yang *et al.*, 1997). Digits in the chick leg are also seen to form under similar conditions. The digits in the leg are known by the Roman numerals I-IV, where the chick limb digits 2, 3 and 4 are similar to chick leg digits I, II and III. Digit IV in the leg has no analogue in the chick wing.



(a)

Figure 3.9: Wild-type digit development in the chick wing (top) and chick leg (bottom). Adapted by permission from Macmillan Publishers Ltd: Nature Communications, Towers *et al.* (2011) copyright 2011.

Embryological experiments have led to the discovery of a region of specialized cells at the posterior margin of the chick wing bud, called the polarizing region (Smith & Wolpert, 1981). Subsequently, it was revealed that the polarizing region expresses Sonic hedgehog

(Shh) (Riddle *et al.*, 1993) which encodes a protein that displays all the characteristics of a polarizing region signal (Yang *et al.*, 1997) as well as also inducing the growth of the digit-forming field, required for specification of antero-posterior positional values (Towers *et al.*, 2008, 2011).

3.2.1 Timing of digit specification by promotion in the chick wing and chick leg

In order to examine the effects of Shh signalling on the development of the digits, experiments adding cyclopamine (which is an inhibitor of Shh) at different times were completed (Towers *et al.*, 2011). This reduction in Shh leads to the inhibition of development of posterior digits. In addition, at embryonic stage 19, the normal polarising region was replaced with a polarising region that expressed green fluorescent protein (Gfp). This allowed the experimentalists to follow the fate of the polarizing region of cells.

Inhibition of Shh signalling in wing buds at embryonic stage 19 (4 hours after the onset of Shh transcription) resulted in wings that occasionally had a single digit 2 (image A of Figure 3.10). At stage 20, 8 hours after the onset of Shh, wings with digit pattern 3-2 (posterior to anterior) were observed (image B in Figure 3.10) and at stage 21, 12 hours after the onset of Shh, wings with a 4-3-2 pattern (posterior to anterior) were produced (top of Figure 3.9). These fate maps show that cells adjacent to the polarizing region in the chick wing are promoted through a series of anterior to posterior digit identities over 12 hours.

When Shh signalling is inhibited in the chick leg at different developmental stages posterior digits are also lost (Summerbell, 1981; Towers *et al.*, 2011) and the digit patterns obtained are I-I (posterior to anterior) at stage 19 (middle image of Figure 3.10), II-II-I (posterior to anterior) at stage 20 (image C in Figure 3.10), III-III-II-I (posterior to anterior) at stage 21 (image D in Figure 3.10) and a IV-III-II-I (posterior to anterior) pattern after stage 22, or 16 hours after the onset of Shh (bottom of Figure 3.9). These digit patterns are much more difficult to interpret than those of cyclopamine-treated wings and do not indicate a simple stepwise anterior to posterior change in digit identity. The observations on the development of digits in chick wings and legs have been summarised in Table 3.1.

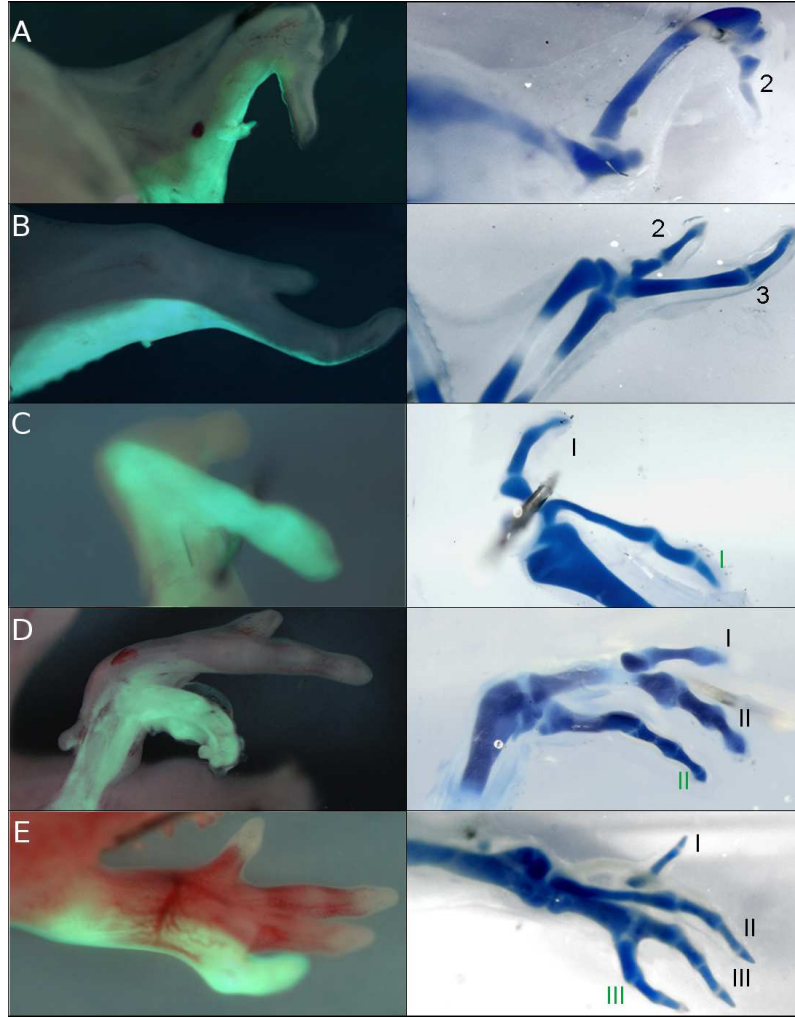


Figure 3.10: Observed inhibited development after the addition of cyclopamine at different times. A: 4 hours after Shh signalling begins in the wing. B: 8 hours after Shh signalling begins in the wing. C: 4 hours after Shh signalling begins in the leg. D: 8 hours after Shh signalling begins in the leg. E: 12 hours after Shh signalling begins in the leg. Adapted by permission from Macmillan Publishers Ltd: Nature Communications, Towers *et al.* (2011) copyright 2011.

Embryonic stage	Number of hours after onset of Shh expression	Digit pattern observed in the chick wing (posterior to anterior)	Digit pattern observed in the chick leg (posterior to anterior)
19	4	2	I-I
20	8	3-2	II-II-I
21	12	4-3-2	III-III-II-I
22	16	4-3-2	IV-III-II-I

Table 3.1: Experimentally observed digit development in chick limbs and chick wings.

3.2.2 Mathematical modelling of chick wing digit patterning

Taking together the detailed data about how Shh specifies the three digits of the chick wing, we construct a mathematical model to see if this is consistent with a gradient-based

mechanism. We then explore whether the same model is applicable to the chick leg with four digits. Several parameters are needed in order to model chick wing digit specification by a Shh gradient:

Size of the initial digit-forming field. Fate maps show that the initial size of the field is $300\mu\text{m}$ (Towers *et al.*, 2008).

Growth rate of the field. The digit-forming field then undergoes a 50% uniform increase in size over the observed 12 hours (Towers *et al.*, 2008).

Time taken for digit specification. The digits take 12 hours to specify in the wing and 16 hours in the leg. This comes from cyclopamine experiments (Towers *et al.*, 2011).

Digit specification Shh thresholds. Through evidence based on defined amounts of Shh being released from microbeads and the numbers of grafted Shh expressing cells, it is estimated that twice the amount of Shh is required to specify each digit in an anterior-posterior sequence. Explicitly, a digit 2 is specified by a Shh concentration of 2 nM, thus digits 3 and 4 are specified by concentrations of 4 and 8 nM, respectively. In the case of the chick leg digit specification, digits I, II and III are identified with wing digits 2, 3 and 4, respectively, leaving digit IV to be specified by a concentration of 16 nM (Towers, *pers. comms.*).

Degradation and diffusion rates of Shh. The degradation and diffusion rates of Shh protein are taken to be $\gamma = 1/60^2$ /s and $D = 13 \mu\text{m}^2/\text{s}$, respectively (Dillon *et al.*, 2003; Saha & Schaffer, 2006).

As mentioned in Section 3.2, the chick wing digits have been the subject of many previous models. However, these previous models tend to focus on reproducing the spatial distribution of the digits (Hentschel *et al.*, 2004; Izaguirre *et al.*, 2004; Newman *et al.*, 2008; Newman & Frisch, 1979; Wilby & Ede, 1975), often using Turing and related mechanisms in order to produce the heterogeneity. Very few have dealt with the temporal development of the digits (Hentschel *et al.* (2004) being an exception). Thus, we return to chick digit development in order to create a new gradient model based on the new

experimental evidence of sequential digit promotion. The intention being that, due to their complementary nature, it would be possible to couple the spatial patterning systems through their dependence on Shh to the temporal evolution of the gradient signal in order to produce a more realistic system. In addition, to the author's knowledge, there have been no previous attempts to simulate patterns of more than three digits, similar to that seen in the chick leg digit pattern, using the French flag model.

Our model consists of a one-dimensional representation of the chick limb bud which is approximately $600\text{ }\mu\text{m}$ in size. This limb bud domain can then be split up into three sections of interest (see Figure 3.11). The first $100\text{ }\mu\text{m}$ is occupied by the polarising region cells, which produce the Shh that diffuses into the rest of the domain. The next $300\text{ }\mu\text{m}$ of cells form the digit forming field. These cells are able to differentiate to form the chick digits. The last $200\text{ }\mu\text{m}$ is the anterior of the limb bud. Although the cells in this region do not transform into chick digits we model them in order that we may use the right-hand side of the limb bud as an insulating boundary, at which we prescribe a Neumann boundary condition.

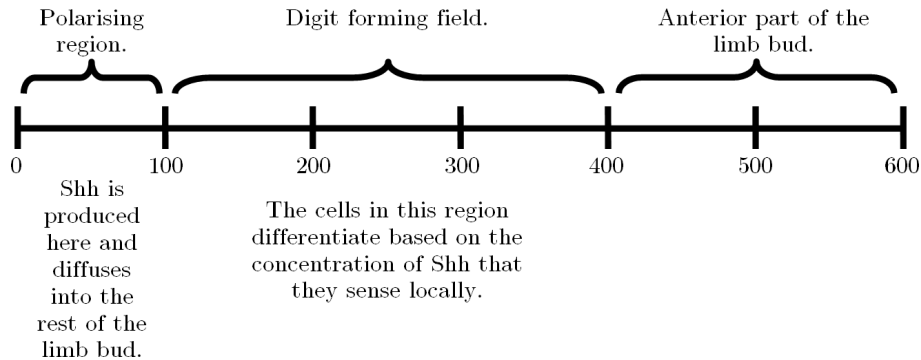


Figure 3.11: Illustration of the chick limb bud domain before growth. The lengths shown are in micrometers. In the chick wing three digits form in the digit forming field. In the chick leg an extra digit forms within the polarising region as well as the three digits that form within the digit forming field.

Since, in the chick wing, the polarising region acts only as a source of Shh we do not model the polarising region and instead replace it with a boundary source term. The form of this source is then varied throughout this investigation. As we are ignoring the first $100\text{ }\mu\text{m}$ of the limb bud we only need to contend with an initial domain of $500\text{ }\mu\text{m}$. Over the next 12 hours we assume that the domain grows uniformly linearly (Hentschel *et al.*, 2004) and its size increases by 50% to $750\text{ }\mu\text{m}$, where the digit forming field makes up the

first 450 μm and the last 300 μm form the limb bud anterior.

Finally, we comment on what we are trying to achieve through use of the gradient model. Experimental evidence suggests that each digit is specified by a doubled concentration of Shh, starting with 2 μM defining a digit 2, 4 μM defining a digit 3 and 8 μM defining a digit 4. We assume this to be an average value for the cells to sense and, thus, we aim to produce a Shh concentration that is greater than these thresholds at the centre of each digit, which are equally spaced along the domain. For example, for three digits to form on a domain of final size 450 μm then each digit has a final width of 150 μm to form within. The centres of these digits are at $x = 75, 225$ and 375 μm , respectively. Thus, for these digits to have been promoted to a 4-3-2 pattern we need the concentration of Shh at $x = 75$ μm to be greater than 8 μM , the concentration of Shh at $x = 225$ μm to be greater than 4 μM and the concentration of Shh at $x = 375$ μm to be greater than 2 μM . Before we consider the growing domain case, we first examine the stationary domain case.

3.2.2.1 Fixed boundary conditions and non-growing domain

Initially, we test whether a simple model of Shh diffusion is sufficient to produce the relative concentration thresholds required to specify three digits in 12 hours over a non-growing domain. Not only does this allow us to visualise the generic model results but it also demonstrates where the model is deficient. These deficiencies enable us to suggest ways in which to adapt the system in order to recapitulate the experimental data.

We solve the diffusion equation with linear decay on a non-growing domain of length 500 μm . The left-hand boundary condition is a fixed source, which models the effects of the polarising region, and a zero-flux condition is applied to the right-hand boundary. The concentration of Shh, u , diffusivity, D , and degradation rate, γ , have units $\text{nM}/\mu\text{m}$, $\mu\text{m}^2/\text{s}$ and $1/\text{s}$, respectively. This leads to the following set of equations:

$$\frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} - \gamma u, \quad x \in [0, 500], \quad (3.10)$$

$$u(0, t) = s, \quad \frac{\partial u}{\partial x}(500, t) = 0, \quad u(x, 0) = 0. \quad (3.11)$$

The simulations of this system are shown in Figure 3.12.

Since we want a final patterned state of 4-3-2 on a stationary digit forming field of

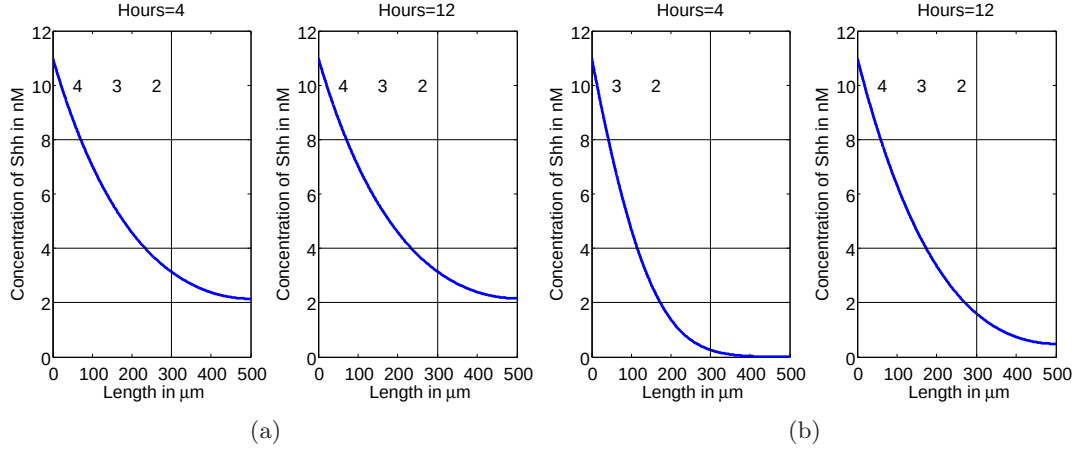


Figure 3.12: Numerical solution of equation (3.10) with boundary conditions (3.11). The horizontal lines at 2, 4 and 8nM show the thresholds beyond which digits are specified as digits 2, 3 and 4 respectively. The vertical line indicates the end of the digit forming field. The three numbers equally spaced along the digit forming field specify the form of digit that would develop at that time point. Simulation time is given above each figure. (a) Parameters: $s = 11 \text{ nM}/\mu\text{m}$, $D = 13 \mu\text{m}^2/\text{s}$ and $\gamma = 1/60^2 \text{ /s}$. (b) Parameters: $s = 11 \text{ nM}/\mu\text{m}$, $D = 0.05 \times 13 \mu\text{m}^2/\text{s}$ and $\gamma = 0.05 \times 1/60^2 \text{ /s}$.

length $300 \mu\text{m}$, we know that the concentration at $x = 50, 150$ and $250 \mu\text{m}$ must be greater than 8, 4 and $2 \mu\text{M}$, respectively. As can be seen in Figure 3.12(a), this certainly can be achieved, using parameter values that are the same order as those in the literature (Dillon *et al.*, 2003; Saha & Schaffer, 2006). However, the time scale on which the thresholds are breached is much too fast.

In order to try and fix this, we alter the time scale $t = Tt'$, where T is a dimensionless scale to be decided. Substituting this time scale in equation (3.10) the diffusion and decay rate then become $D \mapsto TD$ and $\gamma \mapsto T\gamma$, respectively. From Figure 3.12(b) we see that when $T = 0.05$ the system does not immediately go to steady state, although the first two digits still develop too early. Thus, for the system to produce the correct evolution over the 12 hours we would need $T < 0.05$. However, this then means that the diffusion and decay parameters are much smaller than the literature suggests. Thus, we conclude that, for the parameter values that we are using, a stationary domain with a fixed source boundary condition cannot reproduce the correct development of digit promotion.

3.2.2.2 Domain growth and non-linear decay

In the previous section we demonstrated that a stationary domain system with a fixed source boundary condition at $x = 0 \mu\text{m}$ fails to produce a gradient that breaches the appropriate thresholds over the desired spatial and temporal scales. However, we neglected the critical observation that the domain grows by 50% over the 12hr period we are considering. Hence, in this section we simulate the same diffusion and decay dynamics, but on a domain which grows uniformly linearly with rate $r = 1/(2 \times 60^2 \times 12) \text{ /s}$, although we keep the same boundary conditions as before. By using the theory derived in Section 2.3 we map this growing domain back to the stationary domain, giving:

$$\frac{\partial u}{\partial t} = \frac{D}{(1+rt)^2} \frac{\partial^2 u}{\partial x^2} - \gamma u - \frac{r}{1+rt} u, \quad x \in [0, 500], \quad (3.12)$$

$$u(0, t) = s, \quad \frac{\partial u}{\partial x}(500, t) = 0, \quad u(x, 0) = 0. \quad (3.13)$$

In Figure 3.13 we see that growth does help in slowing down the development of the gradient, as the time scale of the system only needs to be reduced by a factor $T = 1/9$, instead of $T = 1/20$, before we see a gradient that develops over the 12hr period. However, although growth helps, this system's evolution is still too quick.

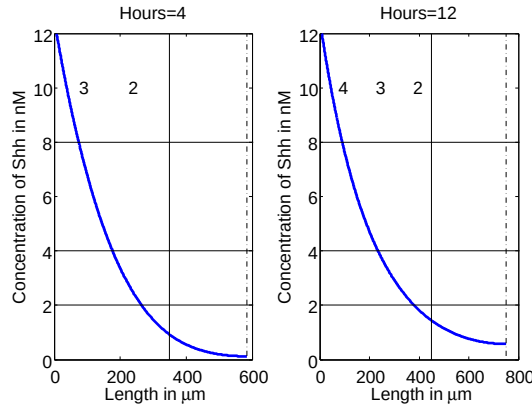


Figure 3.13: Evolution of a gradient on a growing domain with fixed boundary conditions. Solution of equation (3.12) with boundary condition equation (3.14). The dot-dashed vertical line represents the end of the limb bud domain. All other lines and numbers are explained in Figure 3.12. Parameters $s = 12 \text{ nM}/\mu\text{m}$, $D = 13/9 \mu\text{m}^2/\text{s}$ and $\gamma = 1/(9 \times 60^2) \text{ /s}$.

Simultaneously, we also considered different decay forms other than linear (not shown). For higher decay nonlinearities the descent from the left boundary becomes steeper. Al-

though this would aid the system in spanning the range of concentration needed, this comes at the price of making the right-hand side of the gradient shallower. Thus, it would be harder to detect changes in gradient as we move across the field. Also, the similarity of linear, quadratic and cubic kinetics means there is probably not enough variability to distinguish between them experimentally. Due to these factors we use linear decay to give the best compromise of decay over distance.

3.2.2.3 Increasing boundary condition and growing domain

In order to slow down development of the gradient even further we explore the possibility that the polarising region source does not saturate immediately by allowing Shh levels in the polarizing region to accumulate over time. Thus, we adapt the system in the previous section to include an increasing boundary source term of the form,

$$u(0, t) = a \left(1 + \tanh \left(\frac{t - b}{c} \right) \right). \quad (3.14)$$

In this simulation (Figure 3.14), not only does the system have the correct time scale with digits forming every 4 hours and parameters of the order given in the literature, but also the results span the range needed to reproduce the observed concentration doubling over the correct spatial scale. Furthermore, we can also recapitulate results that occur on domains with inhibited growth.

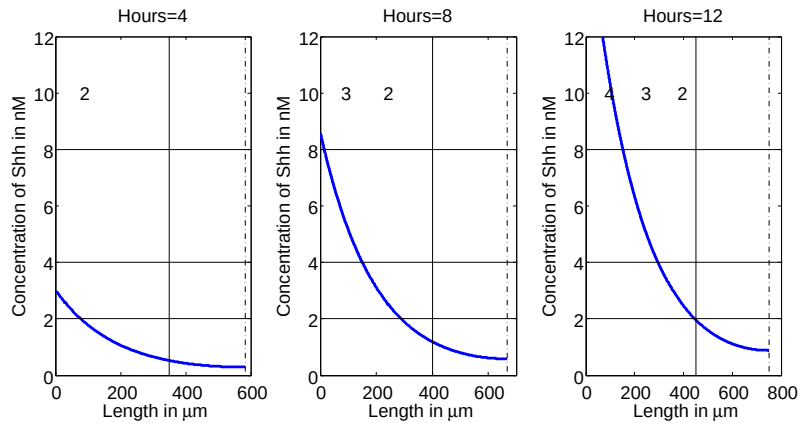


Figure 3.14: Solution of equation (3.10) with boundary condition equation (3.14) on a uniformly linearly growing domain. All other lines and numbers are explained in figures 3.12 and 3.13. Parameters $D = 13 \mu\text{m}^2/\text{s}$, $\gamma = 1/60^2 \text{ /s}$, $a = 9\sqrt{2} \text{ nM}/\mu\text{m}$, $b = 10 \times 60^2 \text{ s}$ and $c = 6 \times 60^2 \text{ s}$.

It is experimentally seen that by applying trichostatin A (TSA) to the developing limb bud growth is inhibited. This then leads to the abnormal development of only a 4-3 digit pattern instead of the full 4-3-2 digit pattern (Towers *et al.*, 2011). By reducing the domain growth to a 25% increase, instead of 50% increase, we are able to recapitulate these observations, as seen in Figure 3.15. Finally, if growth is restricted even further by the addition of cyclopamine only a digit 4 is produced (Towers *et al.*, 2011). By removing growth from the domain altogether, this outcome is also produced as a result of our model (Figure 3.16).

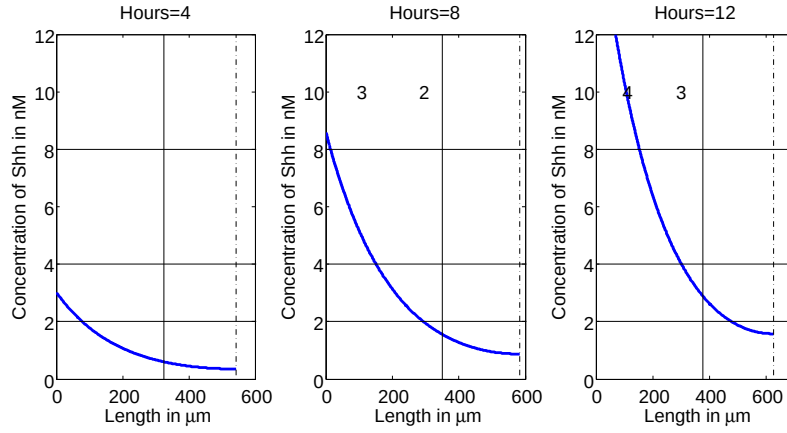


Figure 3.15: Solution of equation (3.10) with boundary condition equation (3.14) on a uniformly linearly growing domain with rate $r = 1/(4 \times 12 \times 60^2)$ /s. All other lines and numbers are explained in figures 3.12 and 3.13. Parameters $D = 13 \mu\text{m}^2/\text{s}$, $\gamma = 1/60^2$ /s, $a = 9\sqrt{2} \text{ nM}/\mu\text{m}$, $b = 10 \times 60^2 \text{ s}$ and $c = 6 \times 60^2 \text{ s}$.

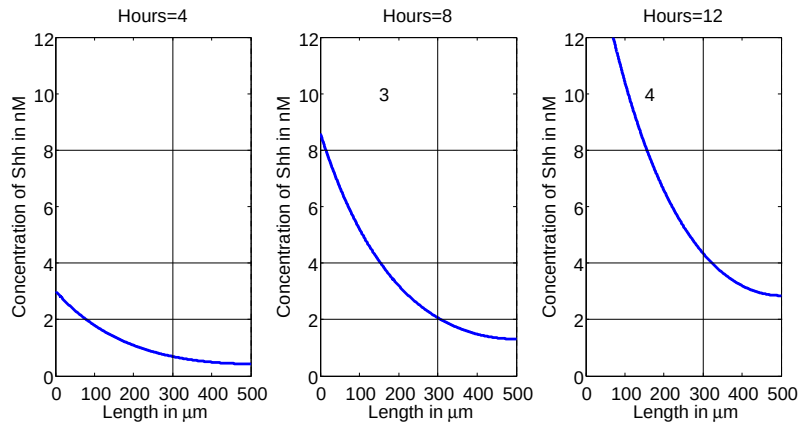


Figure 3.16: Solution of equation (3.10) with boundary condition equation (3.14) on a stationary domain, *i.e.* $r = 0$ /s. All other lines and numbers are explained in figures 3.12 and 3.13. Parameters $D = 13 \mu\text{m}^2/\text{s}$, $\gamma = 1/60^2$ /s, $a = 9\sqrt{2} \text{ nM}/\mu\text{m}$, $b = 10 \times 60^2 \text{ s}$ and $c = 6 \times 60^2 \text{ s}$.

3.2.3 Adaptation to a chick leg model

Having mathematically modelled chick wing digit specification, we ask whether a similar concentration gradient could specify the four digits of the chick leg over 16 hours, assuming that the concentration thresholds for digits I, II and III are the same as in the wing digits 2, 3 and 4, respectively, and that a digit IV can be specified in the polarizing region with a concentration level of $16 \mu\text{m}$. The observed developmental timings are shown in Table 3.1.

To encompass the modelling of the polarising region we extend the domain to the entire limb bud and note that this polarising region is also able to grow (see Figure 3.11). Since we have previously used a saturating boundary source term with a tanh form we model this source region with a logistically growing concentration, to which the homogeneous solution would be a tanh function. Note that, in the polarizing region, the degradation term is nonlinear, although it is linear in the digit forming field. If we were to use a simple constant creation/linear degradation model then the system saturates too quickly. Logistic growth, on the other hand, allows the Shh concentration to undergo slow growth at both the beginning and end of the simulation. However, this change from linear to nonlinear degradation is currently unexplained experimentally and can be thought of as modelling effects present in the polarising region, that are currently unknown. Finally, since the polarising region is able to grow, we map the growing domain back to the initial stationary region, giving equations of the form:

$$\frac{\partial u}{\partial t} = \frac{D}{(1+rt)^2} \frac{\partial^2 u}{\partial x^2} + \alpha u(\beta - u) - \frac{r}{1+rt} u \quad x \in [0, 100], \quad (3.15)$$

$$\frac{\partial u}{\partial t} = \frac{D}{(1+rt)^2} \frac{\partial^2 u}{\partial x^2} - \gamma u - \frac{r}{1+rt} u, \quad x \in [100, 600], \quad (3.16)$$

$$\frac{\partial u}{\partial x}(0, t) = \frac{\partial u}{\partial x}(600, t) = 0, \quad u(x, 0) = 0. \quad (3.17)$$

Note that, since we are working on the entire limb bud, both boundary conditions are zero Neumann, which models the impermeable tissue.

Figure 3.17 shows the simulation of system (3.15) and (3.16) when the growth is allowed to continue after the 12 hours at a constant rate of $r = 1/(2 \times 60^2 \times 12)$ /s. We see that the resulting evolution of the gradient is encouraging, but not quite as desired.

Although we can reproduce the experimentally observed evolution of the digits in the digit forming field, *i.e.* I at hour 4, II-I at hour 8 and III-II-I at hour 12 and beyond, we find that the digit within the polarising region develops in the sequence II $\rightarrow$ III $\rightarrow$ III $\rightarrow$ IV. This contrasts with the experimental observation that the polarising region digit is promoted consecutively from I to IV every four hours (see Table 3.1). Thus, the mechanism behind chick leg digit specification is a possible subject for future investigation.

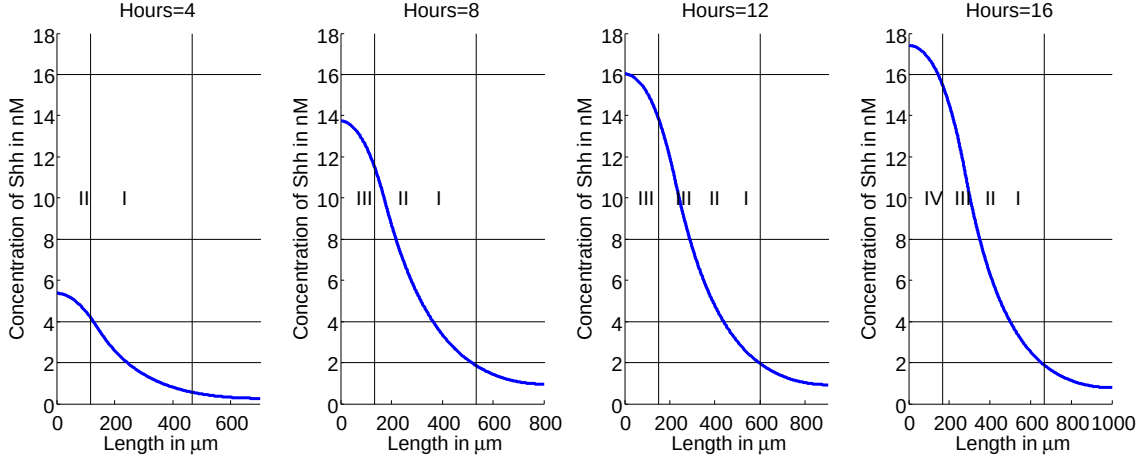


Figure 3.17: Solution of system (3.15) and (3.16) with boundary conditions (3.17) on a uniformly linearly growing domain. The left-hand black vertical line denotes the end of the polarising region. The right-hand black vertical line denotes the end of the digit forming field. All other lines and numbers are explained in figures 3.12 and 3.13. Parameters $D = 13 \mu\text{m}^2/\text{s}$, $\gamma = 1/60^2 \text{ /s}$, $\alpha = 2.2/(15 \times 60^2) \text{ /s}$ and $\beta = 20 \text{ nM}/\mu\text{m}$.

3.3 Discussion and conclusion

In this chapter we have considered two different developing biological systems in which it has been suggested that morphogenesis occurs due to diffusing gene products. Both of these systems were modelled deterministically, using the framework as set out in Chapter 2. An important aspect linking both developing systems is the application of growth, without which the observed experimental phenomena could not have been reproduced.

In the case of the activator-mediator-inhibitor feedback loop (Section 3.1.1) we saw that, at least in principle, it was possible to use a system of three diffusing chemicals to produce precursor tooth patterns, which can then be linked to the spacing of the tooth positions of the mouse. Although the spatial heterogeneity could be produced by simply relying on a Turing instability, the sequential creation of molars was shown to

be lacking, as, without some other mechanism controlling the appearance of the spots, the Turing instability would occur throughout the domain due to exponential growth of perturbations.

Thus, apical growth was used in order to produce the sequential appearance of the spot prepatterns. Initially, the domain is only large enough to contain a single spot, but, as the domain becomes larger, higher wave modes grow and, thus, extra spots are produced. Note that if uniform growth is used instead of apical growth, the third spot develops in the middle of the domain, implying that molar domain development occurs in the order $M1 \mapsto M1-M3 \mapsto M1-M2-M3$. This contradicts the experimental observations that the transitions actually occur in the order $M1 \mapsto M1-M2 \mapsto M1-M2-M3$.

Similarly, in the case of chick limb formation, without growth, a simple gradient model is unable to produce the levels of Shh required to specify the observed digit state. However, growth was not enough to specify temporal evolution of the digits exactly. As shown in Section 3.2.2.1, if we assume that the polarising region is able to produce a constant source of diffusing Shh, then the system equilibrates far too quickly in comparison with experiment. Thus, on the boundary, we applied an increasing source of Shh that saturates at the desired level. Using these two mechanisms of growth and increasing sources we were able to specify the chick wing digits completely on domains that model wild-type behaviour, as well as pathological cases where domain growth was reduced.

This model was then extended to encompass the polarising region, allowing us to test whether the same gradient model was also suitable for describing chick leg development. The difference between the two systems being that, in the leg, a digit also forms inside the polarising region. To model dynamics within the polarising region we chose a form of Shh creation and degradation in the polarising region consistent with the saturating boundary source used in the chick wing model. Once again, growth was needed in order to fully specify the evolution of digit specification as observed in the digit forming field. However, digit specification within the polarising region does not follow the experimental results leading to the prediction that extra factors are needed within the leg polarising region in order to promote the digit as observed.

As discussed in Section 3.1.2, many components of these models lack evidence of their existence, and these difficulties are further compounded by a distinct lack of experimentally

derived parameters. Thus, we must consider the utility of such models. The benefit of modelling such systems is in the ability to prove or disprove the concept of a theory. If it is impossible to realise the observed phenomena through using simple mechanisms that are based on the experimental reasoning, *e.g.* Turing patterns, which rely on the experimentally detected feedback loop, and growth, which is seen during development, then we would immediately be able to state that there must also be some other fundamental mechanism involved. Mathematical models may be able to suggest what that mechanism is, leading to directed experimental design.

For instance, in the mice teeth development, the three molars that are produced are of different sizes. The growth and feedback loop mechanisms were unable to produce this observation, so we modified the model in a number of different ways to account for this. One particular idea was to alter the domain geometry. Thus, we tried a tapered domain, which was larger on the left-hand side than on the right-hand side. Although, verbally, this appears to be a good idea as the smaller right-hand edge would cause the spots to be smaller, physically the difference between the two sides of the domain was not large enough to support this outcome and the mathematical simulations (not shown) showed little difference in actual size. The only addition to the model that was seen to give a consistent result with observation is that of having a spatial gradient.

Although we have placed this gradient in the parameter μ , which controls the strength of the coupling between the activator and the mediator, there are, no doubt, other parameters which this gradient could have been applied to, in order to produce the observed alterations in spot size just as well. The important issue is not that any single one of the coefficients has a spatial gradient, but the fact that a gradient is needed. Hence, we have theoretically shown that a spatial gradient affects the system more than altering the geometry, an important observation that could not have been deduced from a verbal model.

Similarly, in the case of the chick wing digits, Shh is known to be secreted in the polarising region and then diffuse into the rest of the limb bud. We have shown that it must take time for this source in the polarising region to reach its maximum value, otherwise the domain would reach its steady state quicker than is observed. Again, this increasing source term is a facet of the system that could not have been realised through

experimental insights alone. Furthermore, on application of the extended chick wing model to the chick leg, we saw that the correct developmental transitions of the digit in the polarising region could not be realised as the concentration breached a higher threshold than expected. This suggests that control of digit development in the polarising region is different to that suggested in the digit forming field. Specifically, the promotion of digits inside the polarising region cannot be completely dependent on concentration. In support of this idea there is circumstantial evidence which implies that the cells in the polarising region differentiate to their final fates based on the time over which they are subject to Shh signalling, as well as the concentration that they sense (Towers, *pers. comms.*).

In this chapter we have developed mathematical models for specific applications that have been motivated by experimental results. In contrast, in the next chapter, we investigate a mathematically motivated system that is able to demonstrate a completely different set of diffusive patterns called droplets. These bear a remarkable similarity to pigmentation patterns seen on stingrays and South American catfish. Since these patterns appear to be unique to individual stingrays we do not need to worry about robustness issues. Further, since we have no information on the temporal evolution of the patterns we cannot say how growth affects the patterns. Thus, growth is not considered in this context.

Chapter 4

Stable droplets

*“Your manuscript is both good and original. But the part that is good is not original, and
the part that is original is not good.”*

Attributed to Dr Samuel Johnson, 1709 – 1784.

This chapter is based on work published in Woolley *et al.* (2010)

In the previous chapter we considered two distinct examples of morphogenesis and used the insights gained from Chapter 2 to suggest how basic PDE systems could be extended to encapsulate the additional intricacies of evolving systems. Thus, our primary intention was not to produce detailed exploration into the dynamics available in such systems but, rather, to focus on demonstrating that certain mathematical mechanisms could provide the observed experimental results, through appropriate interpretation of the simulated populations. Similarly, in this chapter, we use a mathematically motivated system of PDEs to model the pigmentation patterns of certain types of South American fish. However, we do not have the corresponding biological insight that accompanied the models in the previous chapter. Thus, instead of specifying particular aspects of the model, which can then be tested against data, we approach the system from a theoretical point of view and try to understand its general features.

In Section 4.1 we introduce the paradigm reaction-diffusion system that we will consider and illustrate its various properties. Due to the general structure of the model it contains a number of different pattern formation mechanisms within its dynamics. We demonstrate these possible patterns and then focus on a specific type of structure known

as droplets. These droplets have been shown to have an analytically tractable structure in similar systems where there are two non-zero stable steady states that are symmetric. Here, we extend this recently developed perturbation technique (Gomila *et al.*, 2004) to investigate the weakly asymmetric case. Specifically, our goal is to derive an approximate equation for the radius of the asymmetric droplets, and this is completed in Section 4.2. At the end of Section 4.2 we turn to numerical methods to validate our analysis and comparisons are made in Section 4.3; agreement is shown to be good in the region where the simplifying assumptions hold. Finally, in Section 4.4 we present conclusions and, in particular, question whether droplets are able to develop on a biologically time scale.

4.1 The model

The system we study is a mathematical caricature derived to encompass the general features of a Turing system (Barrio *et al.*, 1999) and we shall hereafter refer to it as the BVAM model. Specifically, not only was it designed to contain the basic Turing instability, as derived in Section 2.2, but it also incorporates the observation that the Turing patterning mechanism depends heavily on the competition between any quadratic and cubic kinetic terms, namely the absence of quadratic nonlinearities favours stripe patterns, whilst the presence of quadratic terms tends to produce spots (Ermentrout, 1991; Nagorcka & Mooney, 1992).

Since any system containing a Turing instability must have a stable steady state in the absence of diffusion, which can be taken to be the origin without loss of generality, Barrio *et al.* (1999) used a generic set of reaction-diffusion equations, which had been Taylor expanded about the zero steady state to inspire the BVAM model. Critically, they kept both quadratic and cubic nonlinearities, whilst ignoring higher order terms. For a pair of deterministic concentrations, (u, v) , the specific form of the BVAM equations are

$$u_t = D\delta\nabla^2 u + \alpha u(1 - r_1 v^2) + v(\tau - r_2 u), \quad (4.1)$$

$$v_t = \delta\nabla^2 v + v(\beta + \alpha r_1 v u) + u(\gamma - r_2 v), \quad (4.2)$$

where D , α , β , δ , τ , r_1 and r_2 are constants. We note that it is easier to work with

the nondimensional form of the equations and so we let $u = U\hat{u}$, $v = V\hat{v}$, $t = T\hat{t}$ and $x = L\hat{x}$, where the capital letters are the dimensional scales and the hatted variables are nondimensional. After rearrangement we obtain

$$a = \frac{\tau}{\alpha}, b = \frac{\beta}{\alpha}, h = \frac{\gamma}{\alpha}, C = \frac{r_2}{\alpha\sqrt{r_1}}, \eta = \frac{L^2\alpha}{\delta}, T = \frac{L^2}{\delta}, \quad (4.3)$$

and

$$\frac{\partial u}{\partial t} = D\nabla^2 u + \eta(u + av - Cuv - uv^2), \quad (4.4)$$

$$\frac{\partial v}{\partial t} = \nabla^2 v + \eta(bv + hu + Cuv + uv^2), \quad (4.5)$$

where the hats have been dropped for simplicity and C , D , a , b , h and η are nondimensionalised constant parameters, of which D and η are positive and the others can take either sign. C and h are particularly important parameters of this system. The term C adjusts the relative strength of the quadratic and cubic nonlinearities; this causes the system to alter its preference in terms of spotted or striped patterns. C is also the symmetry breaking parameter, in the sense that when $C = 0$ the spatially uniform steady states are symmetric about zero and asymmetric otherwise. The parameter h is important as it controls the number of spatially uniform steady states. Specifically, if $h = -1$ then only the trivial, stable steady state, $(u_0, v_0) = (0, 0)$, exists. In order to minimise boundary effects we shall use periodic boundary conditions throughout this chapter.

System (4.4)-(4.5) can be written in the more compact form,

$$\frac{\partial \mathbf{u}}{\partial t} = \mathbf{D}\nabla^2 \mathbf{u} + \mathbf{W}(\mathbf{u}, h) + C\mathbf{S}(\mathbf{u}), \quad (4.6)$$

where

$$\mathbf{u} = \begin{pmatrix} u \\ v \end{pmatrix}, \mathbf{D} = \begin{pmatrix} D & 0 \\ 0 & 1 \end{pmatrix}, \mathbf{W}(\mathbf{u}, h) = \eta \begin{pmatrix} u + av - uv^2 \\ bv + hu + uv^2 \end{pmatrix},$$

and

$$\mathbf{S}(\mathbf{u}) = uv \begin{pmatrix} -1 \\ 1 \end{pmatrix}.$$

If $C = 0$ and $h \neq -1$, there are three spatially uniform steady states, $(u_0, v_0) = (0, 0)$ and

$(u_0, v_0)_\pm = v_\pm(-g, 1)$, where

$$v_\pm = \pm \sqrt{\frac{b}{g} - h}, \quad g = \frac{a+b}{1+h}, \quad (4.7)$$

and the other parameters are chosen to make $v_\pm$ real. The stability of these points can be seen by considering the signs of the real parts of the eigenvalues of the Jacobian of the system (Jordan & Smith, 2002),

$$\mathbf{J} = \begin{pmatrix} \eta(1-v^2) & \eta(a-2uv) \\ \eta(h+v^2) & \eta(b+2uv) \end{pmatrix}. \quad (4.8)$$

If one fixes the value of h , the complex eigenvalues, $\lambda = \sigma + i\tau$, can be examined in the plane (g, f) , where $f = b/g - h = v_\pm^2$. The (g, f) space can be divided into five regions, each with different dynamics, as shown in Figure 4.1. Detailed analyses of the model in these regions have been published elsewhere (Barrio *et al.*, 2009; Leppänen, 2004; Varea *et al.*, 2007) so we omit it here.

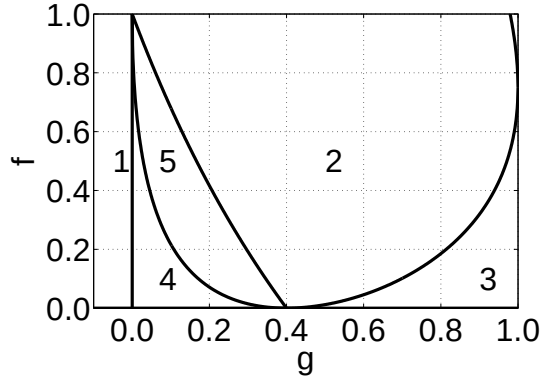


Figure 4.1: Phase plane, in the absence of diffusion, of equations (4.4) and (4.5). Parameters are $D = 0$, $C = 0$, and $h = -2.5$. The lines separating the regions are derived from the dispersion relation $\lambda^2 - \lambda \text{Tr}(\mathbf{J}) + \text{Det}(\mathbf{J}) = 0$ where $\lambda = \sigma_j + i\tau_j$, $j = 1$ and 2 . In region 1, $\sigma_1 < 0$, $\sigma_2 > 0$ and $\tau = 0$ (saddle points). In region 2, $\sigma_{1,2} < 0$ and $\tau \neq 0$ (oscillating stable points). In region 3, $\sigma_{1,2} < 0$, and $\tau = 0$ (stable points). In region 4, $\sigma_{1,2} > 0$ and $\tau = 0$ (unstable points). In region 5, $\sigma_{1,2} > 0$ and $\tau \neq 0$ (oscillating unstable points).

These equations present a rich bifurcation structure and a variety of patterns which makes the model a very versatile system for modelling biological phenomena (Aragon *et al.*, 1998; Barrio *et al.*, 1999). In a wide region of parameter space the one-dimensional

simulations produce travelling wave solutions (also known as fronts) that move with a constant velocity that depends on the asymmetry of the shape of the profile. From random initial conditions the number of fronts produced is such that a definite spatial scale appears. If too many fronts are created, so that their separation is less than the spatial scale, the excess are annihilated when the fronts collide, leaving free to move only those that have separation larger than the distance given by the scale (Varea *et al.*, 2007).

The picture in two dimensions is more revealing; from random initial conditions the two phases immediately separate and the fronts start moving with a velocity that now depends also on the local curvature. The points where the curvature changes sign are then stationary and spiral movement of rotating stripes of constant width is observed (Figure 4.2(d)). In a different region of parameter space the velocity of the fronts becomes zero and a stationary labyrinthine pattern is formed (Figure 4.2(b)). Finally, when one changes the parameters around this region, the fronts start curling and closing the regions occupied by the minority phase, droplets are formed and some of the small ones disappear, while a number of the large ones shrink to a given radius that depends on the parameters of the model (see figures 4.2(a) and 4.4). In all other situations, fronts connecting two nonequivalent homogeneous states move in such a way that the stable state associated with the eigenvalue with the most negative real part annihilates the other (Michaelis *et al.*, 2001).

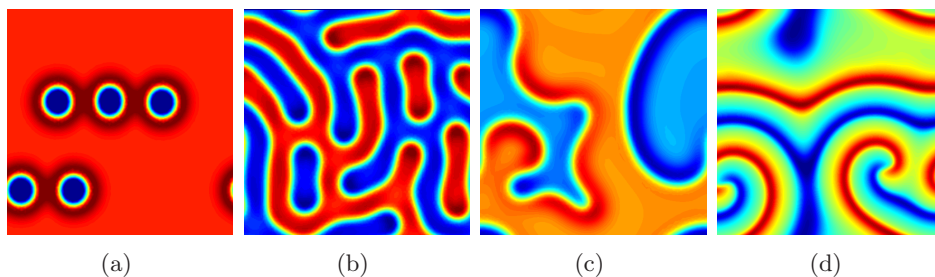


Figure 4.2: Patterns obtained from equations (4.4) and (4.5) when $h \neq -1$ simulated using COMSOL 4.2.0.150. (a) and (b) are stationary solutions and found in region 2 of Figure 4.1. (c) and (d) Travelling wave fronts and oscillating spiral patterns found near the border between regions 2 and 5 of Figure 4.1. In all cases, periodic boundary conditions are used. All parameters, domain sizes and range of colour axes can be found in Appendix C.

As a result of the interactions between various bifurcation structures the BVAM model can give qualitative agreement with the complicated patterns found on the skin of the

freshwater catfish *Pseudoplatystoma fasciatum* (Surubim pintado), *P. tigrinum* (Surubim atigrado) and *P. coruscans* (Surubim manchado), by a simple change of one parameter (see figures 4.3(a) and 4.3(b)).

Another application of the model has been to the pigmentation patterning in freshwater stingrays of the family *Potamotrygonidae*. The patterns of the potamotrygonid species are mostly established by birth, or shortly thereafter. They have highly intricate dorsal colour patterns, which do not look like simple Turing patterns. These patterns include variously shaped spots (de Carvalho *et al.*, 2003). By weakly coupling two BVAM systems together not only can we obtain a good qualitative comparison between the stingray *Potamotrygon motoro* and the simulations (see figures 4.3(c) and 4.3(d)) but we can also account for the differences between genders and the change in patterning that occurs as the stingray matures (Barrio *et al.*, 2009).

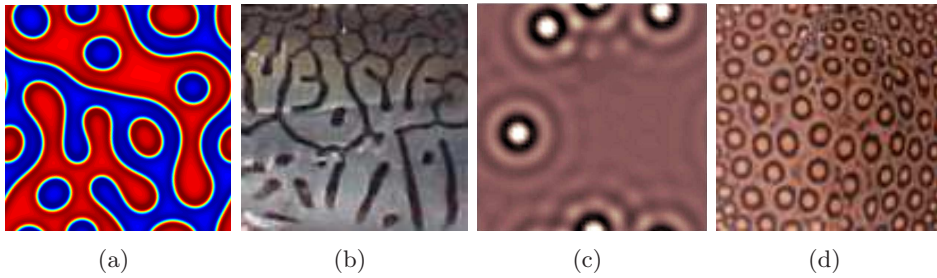


Figure 4.3: (a) An example of the pattern obtained from a single BVAM system with parameter values $D = 0.516$, $\eta = 0.35$, $C = 0.005$, $a = 0.05775$, $b = -0.30525$ and $h = -1.32$. (b) The skin of the catfish *Surubim Tigrinum*. (c) An example simulation of pattern produced when two BVAM models are coupled together. The domain is square with side length 120. Reproduced from R. A. Barrio, R. E. Baker, B. Vaughan Jr, K. Tribuzy, M. R. de Carvalho, R. Bassanezi, and P. K. Maini. Phys. Rev. E, 79(3), 2009. “Copyright 2009 by the American Physical Society.” (d) The skin pattern of the stingray *Potamotrygon motoro*.

A spotted pattern similar to that observed on the skin of stingrays is found within the bistable region of the BVAM model, and it is this situation which is of interest in this chapter. The spots are actually large, stable domain walls separating the two stable homogeneous solutions, presenting a fixed radius (see Figure 4.4). Spatially localised structures such as these have been the source of increased study in the field of non-linear optics. Cavity solitons, which can form as one stable solution shrinks due to its embedding in the other, are common due to either the polarisation symmetry or quadratic nature of the nonlinearity of many optical systems. Similarly, systems which show the stable

coexistence of homogeneous and spatially modulated solutions can be used in the theory of optical coding and memory applications (Firth & Harkness, 1998; Firth & Scroggie, 1996; Gomila *et al.*, 2004).

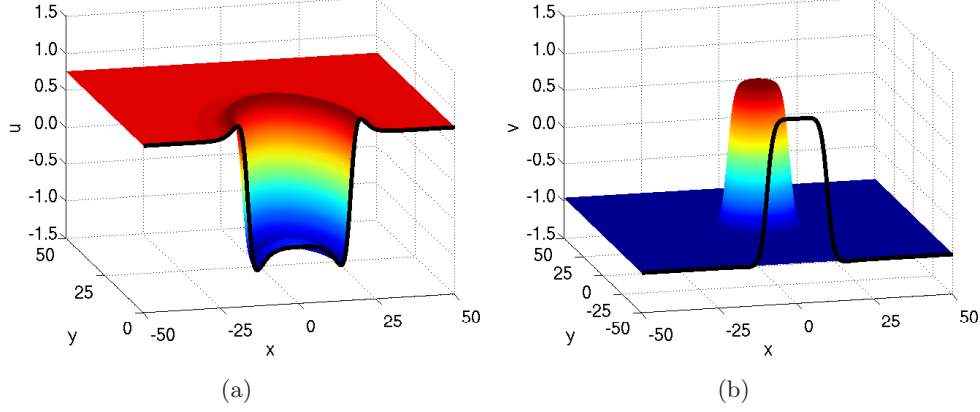


Figure 4.4: (a) Cut away concentration profile of the stationary, stable droplet, u , with the central profile emphasised in black. (b) Concentration of the stationary, stable droplet, v , with central profile in black. u and v were found by solving equations (4.4) and (4.5) using COMSOL 4.2.0.150. Parameters: $C = 0.005$, $D = 0.516$, $\eta = 0.35$, $h = -1.32$, $a = 0.05775$ and $b = -0.30525$.

Droplets are related to solitons and other local structures such as nuclei, although their structure is fundamentally different. Nuclei are never stable. Starting from a cylindrical initial condition, like that seen Figure 4.4(a), nuclei occur when the inner phase, which is surrounded by the domain walls, is favoured. Thus, a structure starting with a large radius will grow to infinity (Gunton *et al.*, 1983). The stability of dark ring cavity solitons comes from the interacting oscillatory tails of the solution front profiles (Gomila *et al.*, 2004; Oppo *et al.*, 1999). In the symmetric kinetic system, when $C = 0$, the stability of the droplet is dependent on the curvature. We will show that, in the more general asymmetric case ($C \neq 0$), the existence and stability of the droplets also depends crucially on the size of the asymmetry, C . Due to its existence depending on curvature, the state associated with stable droplets cannot exist in one dimension.

We believe that, although we must turn to numerical simulation in the end, the analysis gives us insights into the structure, stability and reality of these patterns. To analyse the dynamics we extend the methods developed by Gomila *et al.* (Gomila, 2003; Gomila *et al.*, 2001) whilst examining the evolution of domain walls in non-linear optical systems with a symmetric complex Ginzburg-Landau model. There it was shown quite generally that if:

- (i) a system is invariant under translation and the spatial parity transformation, $\mathcal{P}$, *e.g.* for any solution of the system $\mathbf{u}(x) = (u(x), v(x))^T$ $\mathcal{P}\mathbf{u}(x) = \mathbf{u}(-x)$ is also a solution;
- (ii) there exists a discrete symmetry, $\mathcal{Z}$, which allows the existence of two and only two, non-zero stable homogeneous solutions and the symmetry maps one solution into the other;
- (iii) in one dimension there exist Ising fronts that connect these solutions;

then one is able to derive a formula for the radius of the droplets. This analysis can then be extended to encompass the case when the steady states are asymmetric (Gomila *et al.*, 2004).

Definition 2. A front $u(x)$ is called an Ising wall (or front) if it is invariant with respect to the compound transformation, $\mathcal{S} = \mathcal{Z}\mathcal{P}$ (Michaelis *et al.*, 2001).

We now note that since the Laplacian is invariant under translation and the transformation, $\mathcal{P}$, the whole system will be invariant also. Secondly, in the case $C = 0$, the form of the kinetics are such that they cause the steady states to be equivalent under the discrete symmetry $v_+ \rightarrow v_-$. Finally, as Ising fronts form between the two homogeneous solutions (Figure 4.5) we can invoke the results of Gomila *et al.* (2001). The interfaces can be seen to have a shape very similar to

$$u = -gv_- \tanh(\alpha_1 x) + \alpha_2 \sin(\alpha_3 x) e^{-\alpha_4 |x|}, \quad (4.9)$$

where $\{\alpha_i\}_{i=1}^4$ are constants which can be determined (Coullet *et al.*, 1987; Varea *et al.*, 2007): $\alpha_4 = \sigma$ and $\alpha_3 = \tau$ are the real and imaginary parts, respectively, of the eigenvalues of the linearised system about one of the non-zero critical points; α_1 and α_2 can be fitted numerically.

If $C \neq 0$ and $h \neq -1$ then

$$v_{\pm} = \frac{-C \pm \sqrt{C^2 + 4 \left(\frac{b}{g} - h \right)}}{2}, \quad (4.10)$$

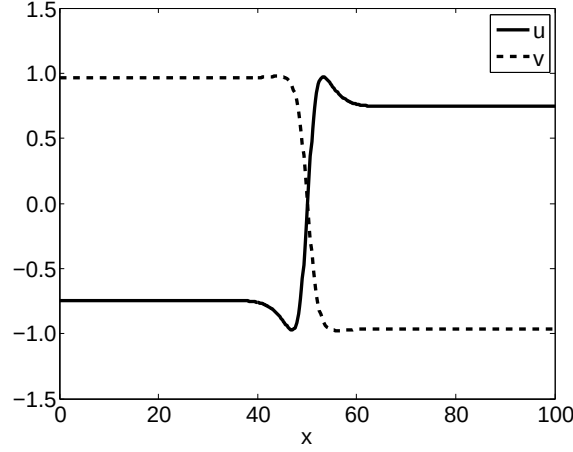


Figure 4.5: Stationary, symmetric structure profiles of u (solid line) and v (dashed line), obtained by solving equations (4.4) and (4.5) in one dimension with parameters $C = 0$, $D = 0.516$, $\eta = 0.35$, $h = -1.32$, $a = 0.05775$ and $b = -0.30525$.

where, once again, the other parameters are assumed to be chosen to ensure that $v_{\pm}$ are real. In this case the non-zero steady states are no longer equivalent and, so, the fronts can move as the asymmetry causes the system to favour one of the states. It is this case that we now analyse further.

4.2 Analysis of droplets in the BVAM model

To analyse the droplets we must first simplify system (4.6), using assumptions that allow us to keep only the crucial dynamics of the droplets and thus remove the dark ring cavity solitons from the system. We construct a coordinate system, (w, s) , which moves with the front. w is the normal coordinate to the front and s is the arc-length along the front (see Figure 4.6). The change of coordinates is then defined as

$$\mathbf{x}(w, s, t) = \mathbf{X}(s, t) + w\hat{\mathbf{n}}(s, t), \quad (4.11)$$

where $\mathbf{x}$ is the position vector in the reference frame and $\mathbf{X} = (X, Y)$ is the position vector of the line front in the reference frame. The vector $\hat{\mathbf{n}}$ is the unit normal vector to the line front in the reference frame and if we let $\hat{\mathbf{x}}$ and $\hat{\mathbf{y}}$ be the normal unitary vectors along the x and y axis, respectively, then $\hat{\mathbf{n}} = Y_s\hat{\mathbf{x}} - X_s\hat{\mathbf{y}}$, where the subscript s denotes the partial derivative along the curve. Note that, for simplicity, the normalising factor,

$\sqrt{X_s^2 + Y_s^2}$, has been encapsulated into the terms X_s and Y_s . The Jacobian matrix of this transformation is:

$$\mathcal{T}(w, s, t) \equiv \frac{\partial \mathbf{x}(w, s, t)}{\partial(w, s)} = \begin{pmatrix} Y_s & X_s + wY_{ss} \\ -X_s & Y_s - wX_{ss} \end{pmatrix}, \quad (4.12)$$

from which we can derive the change of coordinate metric,

$$\mathcal{G}(r, s, t) \equiv \mathcal{T}^T \mathcal{T} = \begin{pmatrix} 1 & 0 \\ 0 & (1 + w\kappa)^2 \end{pmatrix}, \quad (4.13)$$

where we have made use of the fact that

$$X_s^2 + Y_s^2 = 1, \quad (4.14)$$

and κ is defined to be the local curvature of the front line, which satisfies the identities (Stoker, 1989; Tutu, 1997):

$$\kappa(s, t) \equiv \nabla \cdot \hat{\mathbf{n}} = X_s Y_{ss} - Y_s X_{ss}, \quad (4.15)$$

and

$$\kappa(s, t) \equiv \sqrt{X_{ss}^2 + Y_{ss}^2}. \quad (4.16)$$

From this metric we can derive the Laplacian in the new coordinates (w, s) using the formula (Gomila, 2003; Stone & Goldbart, 2009),

$$\nabla_{w,s}^2 = \frac{1}{\sqrt{\mathcal{G}_{11}\mathcal{G}_{22}}} \left(\frac{\partial}{\partial w} \left(\sqrt{\frac{\mathcal{G}_{22}}{\mathcal{G}_{11}}} \frac{\partial}{\partial w} \right) + \frac{\partial}{\partial s} \left(\sqrt{\frac{\mathcal{G}_{11}}{\mathcal{G}_{22}}} \frac{\partial}{\partial s} \right) \right), \quad (4.17)$$

$$= \frac{\kappa}{1 + w\kappa} \frac{\partial}{\partial w} + \frac{\partial^2}{\partial w^2} - \frac{w\kappa}{(1 + w\kappa)^3} \frac{\partial}{\partial s} + \frac{1}{(1 + w\kappa)^2} \frac{\partial^2}{\partial s^2}. \quad (4.18)$$

The temporal transform is derived through the chain rule,

$$\frac{\partial}{\partial t} = \frac{\partial}{\partial w} \frac{\partial w}{\partial t} + \frac{\partial}{\partial s} \frac{\partial s}{\partial t} + \frac{\partial}{\partial t'}, \quad (4.19)$$

where $t = t'$. The term,

$$\hat{n}_v = -\frac{\partial w}{\partial t}, \quad (4.20)$$

is the normal front velocity and the term $\partial s / \partial t$ is the tangential velocity, which is neglected as we are primarily interested in the breakdown of rotationally invariant cylindrical structures and so the front velocity is more important. Under this coordinate transformation,

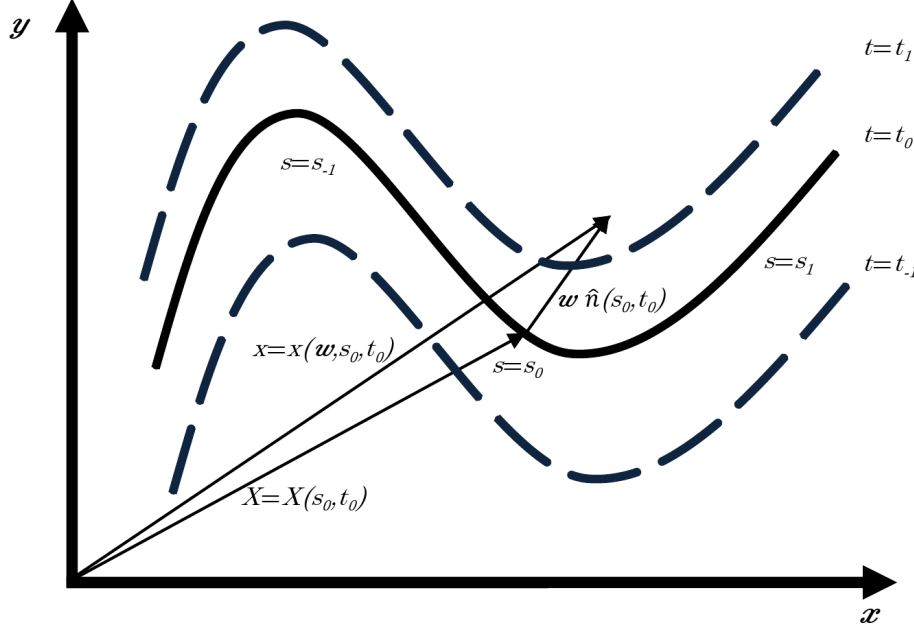


Figure 4.6: Illustration of the new coordinate variables given by equation (4.11). $\mathbf{x}$ is the position vector in the reference frame and $\mathbf{X}$ is the position vector of the line front. The vector $\hat{\mathbf{n}}$ is the unit normal vector to the line front, w is the normal coordinate to the front and s is the arc-length along the front. The various s_i are illustrative points along the front. Similarly, the t_i denote the evolution of the system at different time points.

equation (4.6) can be written in the form

$$\frac{\partial \mathbf{u}}{\partial t} = \mathbf{D} \frac{\partial^2 \mathbf{u}}{\partial w^2} + \left(\hat{n}_v \mathbf{I} + \frac{\kappa \mathbf{D}}{1 + w\kappa} \right) \frac{\partial \mathbf{u}}{\partial w} + \frac{\kappa^2 \mathbf{D}}{(1 + w\kappa)^2} \frac{\partial^2 \mathbf{u}}{\partial \theta^2} + \frac{w\kappa^2 \mathbf{D}}{(1 + w\kappa)^2} \frac{\partial \mathbf{u}}{\partial \theta} + \mathbf{W}(\mathbf{u}, h) + C\mathbf{S}(\mathbf{u}), \quad (4.21)$$

where $\theta = \kappa s$ is the azimuthal angle. Since we are looking for a cylindrical droplet structure with radius R , these can be translated to $\hat{n}_v = \dot{R}$, where the dot denotes the time derivative, and $\kappa = 1/R$.

To enable us to analyse the equation effectively we make the following assumptions which will be justified later:

1. in the frame of reference of the fronts, the droplet structure is stationary;

2. the stable droplet has no θ (azimuthal angle) dependence;
3. the radius of the stable droplet, R , is large, $R \gg 1$;
4. the asymmetry is small ($C \approx 0$) but different from zero;
5. we are able to perturb around the stationary profile of the one-dimensional symmetric system, $\mathbf{u}_0$, using a slightly curved front of the form $\mathbf{u} = \mathbf{u}_0(w) + \mathbf{u}_1(w, s, t)$, where $|\mathbf{u}_1| \ll |\mathbf{u}_0|$ and the solutions are only weakly dependent on t .

Assumption 1 is justified as we are looking for stationary structures, so we can equate the time derivative to zero. By considering Figure 4.7 we see the differences between the soliton structure and droplet profiles. The stable droplets have a larger radius and are more cylindrical in structure than the soliton structures. Assumptions 2 and 3 keep these important properties in the simplified model and are justified by considering Figure 4.4. As noted in Section 4.1, when $C = 0$ the linearised system is symmetric and an analytical estimate for the radius of the droplets can be found (Gomila *et al.*, 2004). Here, we are dealing with the asymmetric case, $C \neq 0$. However, since we are extending the symmetric method our analytically derived radius will only approximate the simulated radius results well when this asymmetry is small, thus justifying Assumption 4. Finally, Assumption 5 is critical in enabling us to reduce the dimension from two to one, by allowing us to treat the domain walls as essentially one-dimensional with the curvature as a small perturbation around this.

By considering equation (4.21) and applying assumptions 1 to 4 we see that the leading order term, $\mathbf{u}_0(w)$, will be the solution of

$$F = \frac{d^2}{dw^2} \begin{pmatrix} Du \\ v \end{pmatrix} + \eta \begin{pmatrix} u + av - uv^2 \\ bv + hu + uv^2 \end{pmatrix} \equiv 0. \quad (4.22)$$

Further, after use of the assumptions and Taylor's theorem, the correction term, $\mathbf{u}_1$, will satisfy

$$0 = \mathbf{D} \frac{\partial^2 \mathbf{u}_1}{\partial w^2} + \left(\dot{R}I + \frac{1}{R} \mathbf{D} \right) \frac{\partial \mathbf{u}_0}{\partial w} + \mathbf{J}(\mathbf{u}_0, h) \mathbf{u}_1 + C \mathbf{S}(\mathbf{u}_0). \quad (4.23)$$

We let

$$M = \mathbf{D} \frac{\partial^2}{\partial w^2} + \mathbf{J}(\mathbf{u}_0, h), \quad (4.24)$$

$$\Rightarrow M\mathbf{u}_1 = -C\mathbf{S} - \left(\dot{R}I + \frac{1}{R}\mathbf{D} \right) \frac{\partial \mathbf{u}_0}{\partial w}, \quad (4.25)$$

where $\mathbf{J}$ is the standard Jacobian of the kinetic terms, *i.e.* equation (4.8) evaluated at $(\mathbf{u}_0, h)$, and $\mathbf{S} = \mathbf{S}(\mathbf{u}_0)$ is a constant. We note that M is singular as $\mathbf{u}_0$ is a solution of equation (4.22). Hence, there exists a null vector, $\mathbf{e}_0 = d\mathbf{u}_0/dw$, such that $M\mathbf{e}_0 = \mathbf{0}$. We can now use the Fredholm alternative (Keener, 2000) to derive a solvability criterion. We define $\mathbf{a}_0$ to be a null mode of the adjoint operator M^T *e.g.* $M^T\mathbf{a}_0 = \mathbf{0}$ and since $\mathbf{a}_0 \rightarrow \mathbf{0}$ as $w \rightarrow \pm\infty$ we define the inner product to be $\langle \mathbf{a}, \mathbf{b} \rangle = \int_{-\infty}^{\infty} \mathbf{a} \cdot \mathbf{b} dw$. Thus, equation (4.25) has a solution if and only if

$$0 = \left\langle \mathbf{a}_0, -C\mathbf{S} - \left(\dot{R}I + \frac{1}{R}\mathbf{D} \right) \frac{\partial \mathbf{u}_0}{\partial w} \right\rangle, \quad (4.26)$$

$$\Rightarrow \dot{R} = -C \frac{\langle \mathbf{a}_0, \mathbf{S} \rangle}{\langle \mathbf{a}_0, \mathbf{e}_0 \rangle} - \frac{1}{R} \frac{\langle \mathbf{a}_0, \mathbf{D}\mathbf{e}_0 \rangle}{\langle \mathbf{a}_0, \mathbf{e}_0 \rangle}. \quad (4.27)$$

Next define

$$\nu_0(h) = -\frac{\langle \mathbf{a}_0, \mathbf{S} \rangle}{\langle \mathbf{a}_0, \mathbf{e}_0 \rangle}, \quad \gamma(h) = \frac{\langle \mathbf{a}_0, \mathbf{D}\mathbf{e}_0 \rangle}{\langle \mathbf{a}_0, \mathbf{e}_0 \rangle}. \quad (4.28)$$

Depending on the signs of the various terms, the equilibrium radius, R_0 , is given by

$$R_0 = \frac{\gamma(h)}{\nu_0(h)} = -\frac{1}{C} \frac{\langle \mathbf{a}_0, \mathbf{D}\mathbf{e}_0 \rangle}{\langle \mathbf{a}_0, \mathbf{S} \rangle} = -\frac{1}{C} \frac{\int_{-\infty}^{\infty} \mathbf{a}_0 \cdot \mathbf{D}\mathbf{e}_0 dw}{\int_{-\infty}^{\infty} \mathbf{a}_0 \cdot \mathbf{S} dw}. \quad (4.29)$$

We will be interested in the parameter $\gamma(h)$ later, as the value $h = h_c$, at which $\gamma(h_c) = 0$, marks the stability bifurcation point in the symmetric case ($C = 0$), as can be seen from equation (4.27). Namely, when $\gamma(h) < 0$ equation (4.27) implies that the radius grows without bound. Conversely, in the case $\gamma(h) > 0$, equation (4.27) implies that the radius will shrink to zero. However, what is actually observed is that, as the radius shrinks, curvature effects become important and a stable non-zero radius can be obtained. The analysis behind this case was completed by Gomila *et al.* (2001). Note also that when $\langle \mathbf{a}_0, \mathbf{e}_0 \rangle = 0$, equation (4.27) is ill-defined. This occurs at an Ising-Bloch transition (Skryabin *et al.*, 2001) so we consider only parameter regions far away from this bifurcation

point. As can be seen from Figure 4.7 the profile changes from the positive solution to

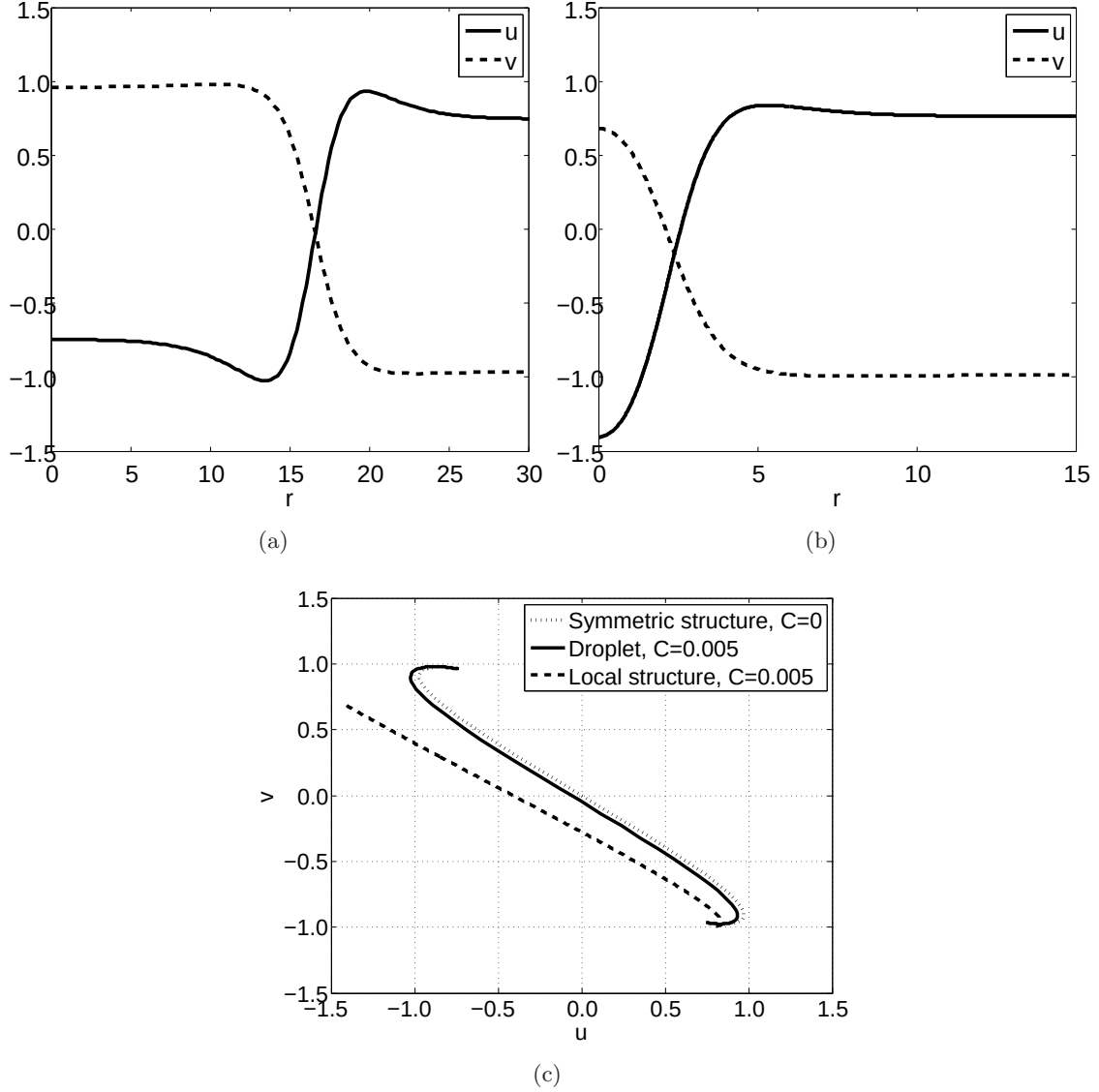


Figure 4.7: (a) Radial profiles of droplet structures u (solid line) and v (dashed line). (b) Radial profiles of soliton structures u (solid line) and v (dashed line). (c) Phase plane plot. The dotted trajectory is the solution to equation (4.22). The solid trajectory is the droplet profile. The dashed trajectory is the soliton profile. The latter two solutions were obtained by solving equation (4.32) with different initial conditions. In (a) and (b) $C = 0.005$, the values of C in (c) are noted in the figure. Other parameters are $D = 0.516$, $\eta = 0.35$, $h = -1.32$, $a = 0.05775$ and $b = -0.30525$.

the negative solution over a short interval so we define the radius to be the point where the profile of v crosses zero.

Now that we have derived a formula for the theoretical radius we must turn to computation in order to evaluate $\mathbf{a}_0$ and $\mathbf{e}_0$. Firstly, we compute $\mathbf{u}_0$; the solution of the

one-dimensional, stationary, symmetric equation (4.22). In order to compute the solution, we apply a Newton-Raphson method (Süli & Mayers, 2003) that uses (4.22) as well as its linearised form,

$$M = \mathbf{D} \frac{d^2}{dw^2} + \eta \begin{pmatrix} 1 - v^2 & a - 2uv \\ h + v^2 & b + 2uv \end{pmatrix}. \quad (4.30)$$

We discretise F and M using a second order finite difference spatial operator to approximate the derivatives. This creates systems containing banded matrices that can be solved efficiently (McSloy, 2002). If the system is initialised with a solution that is close enough to the droplet wall form the iterates will tend to converge to a solution of equation (4.22) which is $\mathbf{u}_0 = (u_0, v_0)$. Due to the walls having the explicit form shown in equation (4.9), a tanh function was used as the initial seed. Finally, $\mathbf{e}_0$ is calculated using the definition

$$\mathbf{e}_0 = \frac{d\mathbf{u}_0}{dw}, \quad (4.31)$$

and $\mathbf{a}_0$ is calculated by finding the null vector of M^T , which can be done using any number of numerical techniques (Moler & Stewart, 1973; Van Loan, 1975).

We could have similarly calculated $\mathbf{e}_0$ by finding the null vector of M . However, algorithms used for computing eigenvectors return the normalised version of $\mathbf{e}_0$ which would have led to a scaling error as it appears only in the numerator of R_0 . The scaling of $\mathbf{a}_0$ does not matter as it appears in both the numerator and denominator of $\gamma(h)$.

To compare the approximation with the actual computed radius of the droplets we use the Newton-Raphson method on the radial, angular independent, stationary, asymmetric equation

$$\hat{F} = \left(\frac{d^2}{dr^2} + \frac{1}{r} \frac{d}{dr} \right) \begin{pmatrix} Du \\ v \end{pmatrix} + \eta \begin{pmatrix} u + av - uv^2 \\ bv + hu + uv^2 \end{pmatrix} + \eta Cuv \begin{pmatrix} -1 \\ 1 \end{pmatrix} \equiv 0, \quad (4.32)$$

which uses the linearised system,

$$\widehat{M} = \mathbf{D} \left(\frac{d^2}{dr^2} + \frac{1}{r} \frac{d}{dr} \right) + \eta \begin{pmatrix} 1 - v^2 & a - 2uv \\ h + v^2 & b + 2uv \end{pmatrix} + \eta C \begin{pmatrix} -v & -u \\ v & u \end{pmatrix}. \quad (4.33)$$

By solving the stationary equation (4.32), we are able to find both stable and unstable structures. The radial stability of the structure is found naturally by considering the eigenvalues of $\widehat{M}$, which is available quite readily due to its involvement in the Newton-Raphson method (Süli & Mayers, 2003).

4.3 Results

Observe in Figure 4.8 that when the radius is large there is good agreement between the computed one-dimensional radius from equation (4.32) and the analytically derived radius gained from the perturbation analysis in equation (4.29). However, the approximation breaks down for $h > -1.32$. The reason for this breakdown is due to Assumption 4, the assumption of shallow curvature, or large radius, becoming invalid for $h \approx -1.32$.

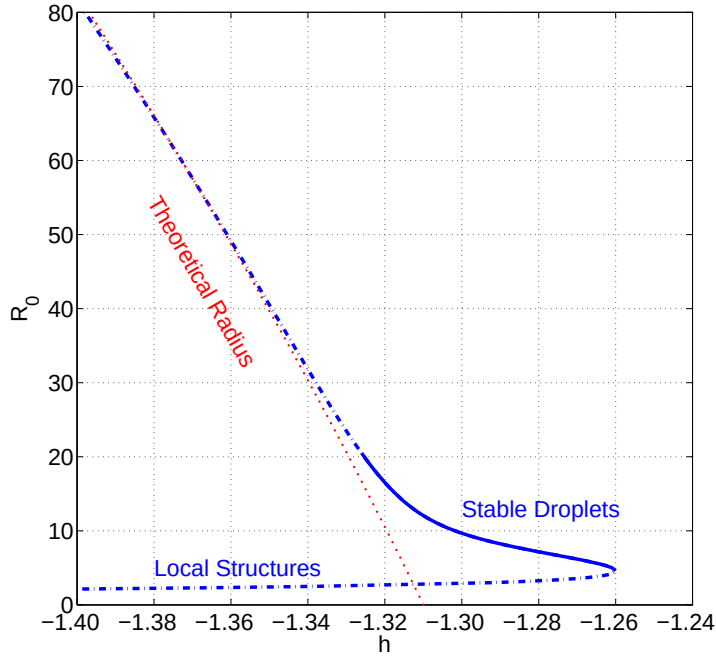


Figure 4.8: Comparison of the computed steady state radius of the perturbed system with the analytically derived radius attained from the one-dimensional equations (4.22) and (4.32). The dashed line indicates an instability to either radial or azimuthal perturbations. The solid line indicates a radius stable to radial and azimuthal perturbations. The dotted line is the theoretical prediction from equation (4.29) with $C = 0.005$ and all other parameters as in Figure 4.7. The stability of the equations (4.4) and (4.5) with a two-dimensional Laplacian was found by using COMSOL 4.2.0.150 to compute finite element solutions on a mesh of 9440 elements. Through a bisection parameter search the stability disappears at $h_d = -1.326$, correct to 3 decimal places.

For h_c defined as the point of modulational instability, as in Section 4.2 ($\gamma(h_c) = 0$), then to three decimal places h_c can be calculated by a bisection search to be $h_c = -1.310$. Thus, in the symmetric case, if $h < h_c$ then $\gamma(h) < 0$ (data not shown) and so equation (4.27) predicts that the radii are unstable and will grow without bound. This is corroborated in Figure 4.9. In this situation either a labyrinthine pattern will result or the most abundant phase will wipe the other out leaving a homogeneous steady state. For $h > h_c$ a finite radius is seen to exist, however, we have not considered the stability of these droplet structures, only their existence.

Although the symmetric system cannot have stable droplets for $h < h_c$, when $C > 0$ we see from equation (4.27) that the asymmetry is able to stabilise the droplets for a larger parameter region so, as Figure 4.8 clearly shows, stable droplets exist for $h < h_c$. For $h < h_c$ ($\gamma < 0$), both droplet and local structure (dark ring cavity soliton) profiles exist in the radial stationary equation (4.32). Further, the one-dimensional analysis predicts that all droplet profiles should be stable to radial perturbations as the simulations suggest that $\nu_0 < 0$ throughout this parameter region. This is corroborated by the eigenvalues of the Jacobian, $\hat{M}$, having negative real part.

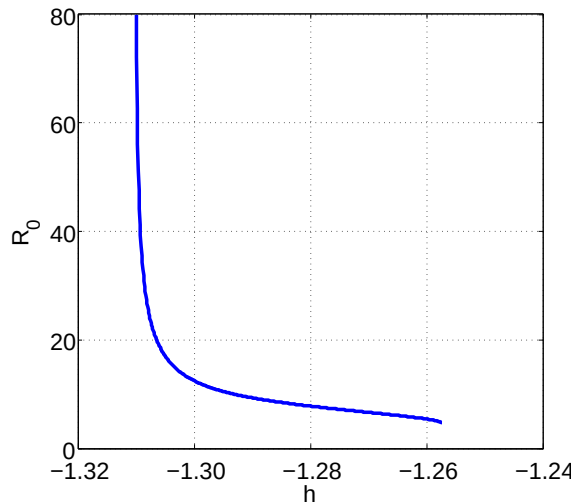


Figure 4.9: Computed radius of droplet structures from the one-dimensional system (4.32) with $C = 0$ and all other parameters as in Figure 4.7. The stability of these structures has not been considered.

It should be noted that, although all the droplet profiles are stable to radial perturbations, modulational instabilities of their circular wall in the azimuthal direction can occur. In two-dimensional simulations (Figure 4.10) the stability of the droplets disap-

appears somewhere between $h = -1.33$ and -1.32 . To simulate the two-dimensional equations we used COMSOL 4.2.0.150 to compute finite element solutions on a mesh of 9440 elements and through a bisection parameter search we found the boundary of stability to be $h = -1.326 = h_d$ correct to three decimal places. For $h > h_d$ the droplets were able to stabilise (Figure 4.10(g)), for $h < h_d$ the droplets succumbed to azimuthal instabilities and broke down (Figure 4.10(d)). The local structures were never seen to be stable, corroborating the stability information gained from the asymmetric radial equation.

Within the region $h_d < h < -1.26$ the droplets are not only stable but they also appear to be very persistent. Figure 4.11 shows that, from pseudo-random initial conditions ($u(x, y, 0) = \sin(90000x + 9000y) + \cos(239845xy)$, $v(x, y, 0) = \sin(45678x + 4659y) - \cos(379586xy) - \sin(20000x + 88000y)$), the system exhibits stable droplets. The only other pattern observed to be viable in this region from pseudo-random initial conditions is stripes (results not shown). These stripes are not the labyrinthine patterns we would normally observe. The stripes are able to form due to the periodic boundary conditions as they form a continuous band through the domain producing a structure with no end. If the stripes are cut they shrink to droplets as they are unable to support unjoined ends, unlike the curved heads seen in figures 4.10(f) and 4.10(h) which labyrinthine patterns produce. Altering the boundary conditions have the potential to stop such stripes forming, leaving stable droplets as the only pattern available.

4.4 Conclusion

The BVAM model examined here presents Turing, Hopf and Turing-Hopf bifurcations when $h \neq -1$. This richness of behaviour allows us to gain insight into the mechanisms of pattern formation and selection in a wide variety of problems. In particular, in the bistable regime it is possible to obtain stable, cylindrical structures, with large radius. An important fact underlying this phenomenon is that $\nu_0 < 0$ throughout the chosen parameter region. If instead $\nu_0 > 0$, the structures would not have been stable droplets, they would have been unstable nuclei.

Observe in figures 4.10 and 4.11 that the stable structures evolve into their stationary forms much quicker than the unstable structures. The stable structures form on a time

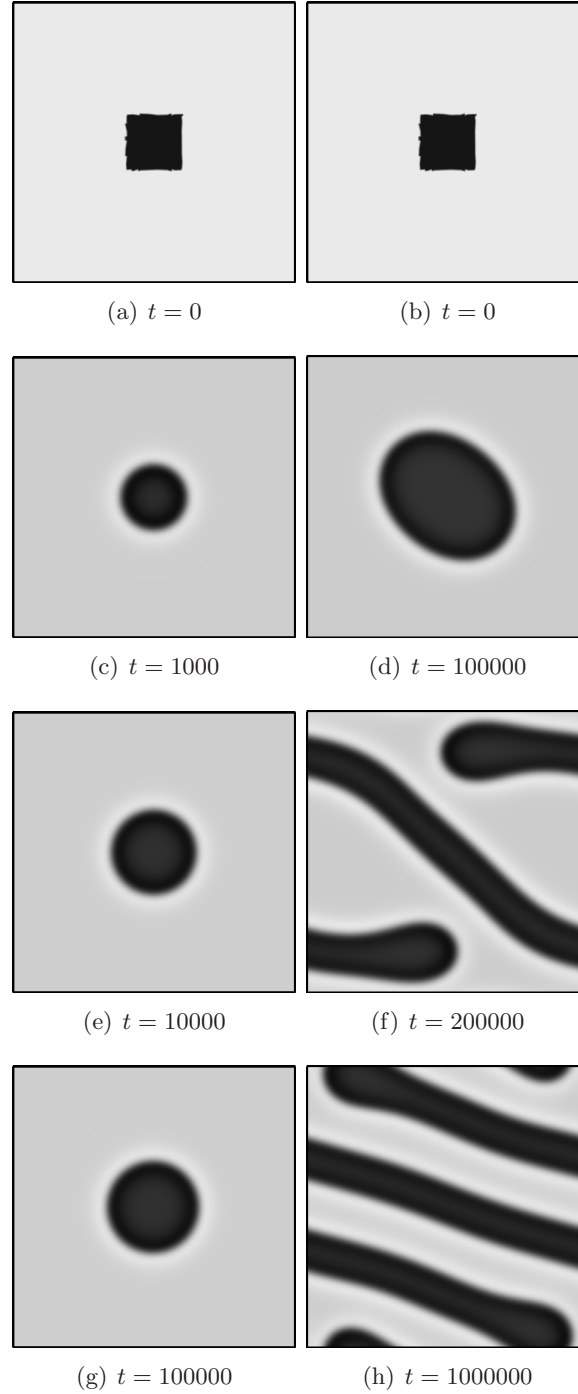


Figure 4.10: Time evolution of equations (4.4) and (4.5) with square initial conditions. Solved using COMSOL 4.2.0.150 on a discretised domain of 9440 elements. The left column has $h = -1.32$ and so is in the stable region, thus the system reaches a final droplet steady state. The right column has $h = -1.33$ and so is in the unstable region, thus it produces a labyrinthine steady state. Other parameters are $D = 0.516$, $\eta = 0.35$, $C = 0.005$, $a = 0.05775$ and $b = -0.30525$. The grey scale is from -1.2 (darkest) to 1.2 (lightest) and the domain is $[-50, 50] \times [-50, 50]$.

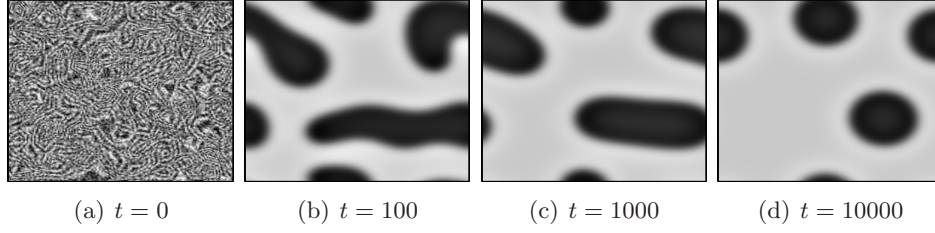


Figure 4.11: Time evolution of pseudo-random initial conditions to stable droplets. Solved using COMSOL 4.2.0.150 on a discretised domain of 9440 elements. Parameters $D = 0.516$, $\eta = 0.35$, $C = 0.005$, $h = -1.3$, $a = 0.05775$ and $b = -0.30525$. The grey scale is from -1.4 (darkest) to 1.4 (lightest) and the domain is $[-50, 50] \times [-50, 50]$.

scale of order 10^4 (nondimensional units) whereas the unstable structures form on a longer time scale of order 10^6 . As the patterns take such a long time to form in the nondimensional system, we must determine if these time scales are consistent with the notion of the BVAM mechanism as a potential candidate for biological patterning. To answer this we first must return to the original dimensional form of the BVAM model, equations (4.1) and (4.2). In particular we are interested in the scalings that were derived in equation (4.3). At birth, stingrays range from 10-100 mm in length (Thorson *et al.*, 1983). Since $\hat{x} = 100$, we use $L = 10^{-2}$ cm. For the diffusivity, $\delta = 10^{-6} - 10^{-7}$ cm²/sec are biologically consistent values (Dawes, 1956; Dillon *et al.*, 2003), giving $T = 10^2 - 10^3$ seconds, which implies $t = 10^6 - 10^7$ seconds $\approx 12 - 115$ days. Since the gestation period of the stingrays varies between 3 and 9 months (Charvet-Almeida *et al.*, 2005), stable droplet formation through a bistable system is therefore a viable mechanism of pattern formation.

We have studied the effect of a symmetry-breaking parameter on the existence and stability of stable droplets and local structures. The strength of this technique lies in the fact that, although the droplets can only exist in two dimensions, we can still use a one-dimensional analysis to derive an asymptotic approximation for the radius. From equation (4.29) we can see that the form of the asymmetry, S , the size of asymmetry, C , and the nondimensionalised diffusion coefficient, D , are all critical factors when it comes to determining the size and stability of droplets.

We have shown that droplet and local structure profiles are both solutions to the stationary radial equations given by (4.32). Further, the droplet profiles are always stable to radial perturbations whereas the local structures are always unstable to radial perturbations. In two dimensions the parameter range of existence is much smaller due to

the destabilising effects of azimuthal perturbations on the droplet structure. However, in the region of existence, not only are the droplets stable but they also appear to be quite persistent.

The first order perturbation analysis gives a good approximation for $-1.4 < h < -1.32$ when $C = 0.005$. As $h \rightarrow -1.32$, the assumption of the droplets having large radius begins to break down, thus the reduction of the model based on gently curved fronts is no longer valid. Due to the reduction of the model from two dimensions to one, the theory is currently unable to predict when curvature effects become important and this extension is suggested as future work. Note that the parameters used in this chapter have not been finely tuned in any sense and for higher values of C the region of stability may actually increase, although the theoretical approximation will become worse.

Although the BVAM system is able to produce Turing patterns the droplet structures we consider exist due to the models exhibited bistability and the interaction of the curvature of the wave fronts, thus, they are not simply dependent on diffusion for their existence, as in a standard Turing pattern. Another critical difference between the BVAM system and a standard Turing mechanism is that a Turing mechanism cannot generate spots and stripes at the same time. In the case of the BVAM model, these patterns are easily generated, as illustrated in Figure 4.3(a).

Since the asymmetry that causes the existence of droplet forms is very small ($C = 0.005$) and the parameter region over which droplets exist is also quite small we must consider what effects the noise might have on these structures *e.g.* noise may possibly cause the existence space to become larger by pushing the asymmetry further, or potentially counteract their existence and thus cause stable droplets to be unattainable in the BVAM system. The existence, or non-existence, of these structures in a noisy setting has serious implications on droplets being biologically realisable. In the next chapter, techniques of stochastic analysis and simulation are developed to deal with exactly the reaction-diffusion systems that we are interested in. In particular, noise is shown to affect systems near bifurcation points in ways that are not seen deterministically, thus greatly altering their evolution.

Chapter 5

Methods of stochastic modelling

“Iacta alea est”

Julius Caesar, 49 BC.

This chapter is based on work published in Woolley *et al.* (2011b).

Up until now, we have considered the construction of complexity through the production of biochemical prepatterns, which have been described by systems of deterministic equations. Although the PDE formulation allows us to see the main effect of many types of kinetics, stochastic effects are also important. This is particularly true in cases where only small numbers of reactive individuals exist, *e.g.* ‘avalanche’ style reactions such as those applied to blood clotting mechanisms (Macfarlane, 1964). Here, a single blood clotting factor is created, which reacts to form a second. This product is then an enzyme for another reaction, which itself is also an enzymatic reaction. These reactions cascade down until thrombin, a coagulation protein, is produced in the bloodstream (Kaminski & McDonagh, 1983). It is highly important that we are able to model this kind of system accurately as the expected waiting time for clots to start forming needs to be carefully considered by medical practitioners since clots must not be allowed to build up and invade parts of the vascular system (Macfarlane, 1964). However, the deterministic framework rests upon the assumption that our population is large enough to treat it as a continuum. This is certainly not true in the case of this avalanche reaction. The creation and reactions that these clotting factors undergo will be a much more probabilistic process.

Stochasticity has also been incorporated into genetic networks involving gene expres-

sion and regulation, since increasing experimental evidence implies that fluctuations play a significant role in these processes (El Samad *et al.*, 2005; Gibson & Mjolsness, 2004). Concepts such as genetic switches rely upon stochasticity to push them between two steady states, whereas deterministically these systems would simply relax to one of the stable steady states, depending on the initial conditions (Loinger *et al.*, 2007; Tian & Burrage, 2006).

As discussed in Chapter 2, if a reaction system contains a large number of individuals that are well-mixed then the Law of Mass Action is valid. However, in order to be rigorous in our application we must be able to define the term ‘large’. Further, in the case that we can use a continuum description, it must be understood that the deterministic equations are only approximations to the true population values, as the populations are discrete, integer quantities rather than continuous valued variables.

The system could be described deterministically through Newton’s laws of motion if all positions and velocities of the individuals were known at some initial time (ignoring quantum effects), but it is certainly not deterministic in the low dimensional space of the PDE description. Since knowing the exact state of every individual is an impossibility (except for very small numbers of particles), we instead turn to a probabilistic description of the system. This allows us to clearly define and derive the probability of being in a specific state.

In this chapter we focus on a stochastic description of diffusion, as it is a fundamental mechanism of transport that underpins a large number of biological processes (Dillon *et al.*, 2003; Koch, 1990; Lawrence *et al.*, 1994) and the extension to reactive systems is simple. Diffusion of chemical constituents is modelled as a space-jump process (Baker *et al.*, 2010; Othmer *et al.*, 1988). The one-dimensional domain is partitioned into compartments of equal size and each compartment contains an indexed population. The individual particles of the chemical populations are then allowed to undergo unbiased random walks by jumping from one compartment to the next *i.e.* each particle has an equal probability per unit time, or transition rate, of jumping to one of its neighbouring boxes.

In Section 5.1 we approach the probabilistic description of a diffusive system through a chemical master equation (CME) formalism and use white noise expansions to derive Fokker-Planck equations that allow us to characterise the properties of the noise (van

Kampen, 2007). In Section 5.2 we are then able to take advantage of a recently developed method that applies Fourier transform techniques to the Fokker-Planck equation. These techniques give us insights into the potential spatio-temporal dynamics which are available in such systems. Although we use linear analysis to understand nonlinear complexity, it has been shown that linear dynamics can often give us useful insights about the full nonlinear system (Hinch, 1991; Murray, 2003a,b). Finally, in Section 5.3, we apply this technique to the specific case of diffusion and discuss the results in Section 5.4.

5.1 Stochastic framework

The CME provides an exact description of the evolution of the probabilities of the active chemical species being in a certain state. By deriving the CME, the system is reduced to specifying only the populations and interactions between them, whilst ignoring their specific positions and velocities. Note that, although the exact position of each particle is ignored they are still constrained to inhabit a given domain. Later, when we discuss the spatially extended system, this will simply mean that the particles can be said to inhabit a certain interval, but their positions within this interval cannot be specified. The justification for this lies in the conditions needed for the system to be considered ‘well mixed’. Fundamentally, an assumption is made that the majority of molecular collisions are non-reactive. The net effect of these non-reactive collisions is to cause the positions of each particle to be uniformly random in the system volume. Thus, ignoring nonreactive collisions, only changes in populations due to defined reactions are considered (Gillespie, 2007).

Although the general CME is derived below, in most cases it has high dimension and is nonlinear, thus, exact solutions exist only in rare cases. However, it can be solved in the sense of using Stochastic Simulation Algorithms (SSAs) to create statistically exact realisations of the system, where averaging over many simulations is equivalent to solving the CME (Gillespie, 1977b) (see Section 5.1.1).

Suppose in the system there are K chemical populations, $\{S_1, \dots, S_K\}$, undergoing J reactions, $\{R_1, \dots, R_J\}$. Since we will be primarily concerned with spatially extended systems the subscript, $i = 1, \dots, K$, indexes over both the chemical population and its

spatial location. As in Chapter 2, let $U_i(t)$ be the integer random variable of individuals of species S_i and for simplicity define $\mathbf{U}(t) = (U_1(t), \dots, U_K(t))$ to be the state vector of the system at time t . Each reaction, R_j , is specified by its stoichiometric vector $\boldsymbol{\nu}_j = (\nu_{1j}, \dots, \nu_{Kj})$ and the propensity function $a_j(\mathbf{u})$, where $\mathbf{u}$ is a single realisation of $\mathbf{U}$ (Gillespie, 1992).

Definition 3. *The stoichiometric vector $\boldsymbol{\nu}_j = (\nu_{1j}, \dots, \nu_{Kj})$ is the state change vector of reaction R_j . Thus, if the system is in state $\mathbf{u}$, and reaction R_j occurs, then the state becomes $\mathbf{u} + \boldsymbol{\nu}_j$ (Gillespie, 2007).*

Definition 4. Fundamental premise of stochastic chemical kinetics

The propensity function $a_j(\mathbf{u})$ is defined to be such that:

$$a_j(\mathbf{u})dt = \text{the probability, given } \mathbf{U}(t) = \mathbf{u}, \text{ that one } R_j \text{ reaction will occur in} \quad (5.1)$$

the next infinitesimal time interval $[t, t + dt)$.

Initially, Definition 4 was regarded with some contempt, for in bimolecular reactions the reaction only occurs when two appropriate molecules collide. However, Definition 4 seems to suggest that any two appropriate elements are able to react despite their separation, thus two molecules which are far apart have the same probability of reacting as two that are very close. Gillespie showed that these misgivings, although plausible, were unjustified (Gillespie, 1977a) by showing that the results were equivalent whether or not the probabilities were conditioned.

The propensity function has the empirical mathematical form (Gillespie, 1976)

$$a_j(\mathbf{u}) = \kappa_j h_j(\mathbf{u}), \quad (5.2)$$

where the function $h_j(\mathbf{u})$ is the number of distinct combinations of R_j reactants available in state $\mathbf{u}$ and its form depends on the order of the reaction. This will be discussed later in Section 5.1.2.

Definition 5. *The coefficient κ_j is the specific probability rate constant and is defined to*

be such that:

$$\begin{aligned} \kappa_j dt &= \text{the probability that a set of } R_j \text{ reactant molecules will undergo} \\ &\text{the } R_j^{\text{th}} \text{ reaction in the next infinitesimal time interval } [t, t + dt). \end{aligned} \quad (5.3)$$

The stoichiometric matrix,

$$\boldsymbol{\nu} = \{\nu_{ij}\}_{i=1, \dots, K}^{j=1, \dots, J}, \quad (5.4)$$

and the propensity vector, $\mathbf{a}^T = (a_1, \dots, a_J)$, follow from these definitions. The rest of the theory follows from these definitions and the Laws of Probability (Grimmett & Stirzaker, 2001).

For a mathematically rigorous derivation of the CME we need a final theorem from Gillespie (1992):

Theorem 5.1.1. *The probability of more than one reaction occurring in the system in the interval $[t, t + dt)$ is $o(dt)$.*

Thus, we only need to consider states that are one reaction removed from the state we are interested in. By defining $P(\mathbf{u}, t + dt | \mathbf{u}_0, t_0)$ to be the probability of being in a state $\mathbf{u}$ at a time t , given initial condition $\mathbf{u}_0$ and t_0 , then the rate of change of P over time is the net probability flux into the state $\mathbf{u}$, or, in other words, the probability of being in a state $\mathbf{u}$ is equal to the probability of being in state $\mathbf{u}$ and no reaction happening, plus that of being in a state one reaction, R_j , away from $\mathbf{u}$ and the R_j reaction occurring in the time dt :

$$\begin{aligned} P(\mathbf{u}, t + dt | \mathbf{u}_0, t_0) &= P(\mathbf{u}, t | \mathbf{u}_0, t_0) \left(1 - \sum_{j=1}^J a_j(\mathbf{u}) dt \right) \\ &\quad + \sum_{j=1}^J P(\mathbf{u} - \boldsymbol{\nu}_j, t | \mathbf{u}_0, t_0) a_n(\mathbf{u} - \boldsymbol{\nu}_j) dt + o(dt). \end{aligned} \quad (5.5)$$

Upon rearranging and taking the limit as $dt \rightarrow 0$ equation (5.5) becomes

$$\begin{aligned} \frac{\partial}{\partial t} P(\mathbf{u}, t | \mathbf{u}_0, t_0) &= \sum_{j=1}^J [P(\mathbf{u} - \boldsymbol{\nu}_j, t | \mathbf{u}_0, t_0) a_j(\mathbf{u} - \boldsymbol{\nu}_j) - P(\mathbf{u}, t | \mathbf{u}, t_0) a_j(\mathbf{u})], \\ &\stackrel{\text{def}}{=} \sum_{j=1}^J (R_j - 1) [P(\mathbf{u}, t | \mathbf{u}_0, t_0) a_j(\mathbf{u})]. \end{aligned} \quad (5.6)$$

In the case of zeroth and first order reactions, *i.e.* reactions in which zero or one active species, respectively, reacts to form another, the CME is linear in $\mathbf{u}$ and can be solved directly (Jahnke & Huisinga, 2007) or by using probability generating functions, which lead to first order partial differential equations (PDEs) for the moments of the probability distribution (McQuarrie, 1967). Similarly, second order reactions lead to second order PDEs for the generating function. Hence, except for very special cases, second and higher order CMEs are not completely, analytically, solvable, due to the nonlinearities in the system.

5.1.1 The stochastic simulation algorithm

Due to the inherent complications in working with the CME, Gillespie (1976, 1977b) developed the SSA to enable the production of simulated trials, which, when averaged, solve the CME. Specifically, the principle theoretical construct upon which the numerical procedure is based is not the system probability function, P , nor any of its derived quantities, but rather an entity called the reaction probability density function.

Definition 6. *Given a system such as that described in Section 5.1, the probability density function, $p(\tau, j | \mathbf{u}, t)$, is the joint probability density function of two random variables, τ and j , where τ is the time to the next reaction and j is the index of the next reaction, given that a system is in state $\mathbf{u}$, at time t . p is defined to be such that:*

$$\begin{aligned} p(\tau, j | \mathbf{u}, t) dt &\equiv \text{the probability, given } \mathbf{U}(t) = \mathbf{u}, \text{ that the next reaction in the} \\ &\text{system will occur in the infinitesimal time interval} \\ &[t + \tau, t + \tau + dt), \text{ and will be an } R_j \text{ reaction.} \end{aligned}$$

This can be written in the form

$$p(\tau, j)d\tau = p_0(\tau)a_j(\mathbf{x})d\tau, \quad (5.7)$$

where $p_0(\tau)$ is the probability that no reaction occurs in the time interval $[t, t + \tau)$. Using the above definitions,

$$p_0(\tau + d\tau) = p_0(\tau) \left(1 - \sum_{j=1}^J a_j(\mathbf{u})d\tau \right). \quad (5.8)$$

Upon rearranging and letting $d\tau \rightarrow 0$, we derive:

$$p_0(\tau) = \exp \left(- \sum_{j=1}^J a_j(\mathbf{u})\tau \right). \quad (5.9)$$

Inserting equation (5.9) into equation (5.7) it is seen that

$$p(\tau, j) = a_j \exp(-a_0\tau), \quad (5.10)$$

where

$$a_0(\mathbf{u}) = \sum_{j=1}^J a_j(\mathbf{u}). \quad (5.11)$$

Equation (5.10) shows that τ is a Poisson random variable with mean and standard deviation $1/a_0$ which is statistically independent of j . One way to simulate τ and j is to generate two uniformly random numbers¹, r_1 and r_2 , from the interval $[0,1]$ and calculate,

$$\tau = \frac{1}{a_0(\mathbf{u})} \ln \frac{1}{r_1}, \quad (5.12)$$

$$j = \text{smallest integer satisfying } \sum_{n=1}^j a_n(\mathbf{u}) > r_2 a_0(\mathbf{u}). \quad (5.13)$$

These steps can be combined to form Gillespie's standard SSA:

1. initialise the time $t = t_0$ and the system state $\mathbf{u} = \mathbf{u}_0$;

¹More accurately the numbers calculated using any computational library are pseudorandom in that the numbers form a cycle based on an initial seed. The generator used in the following simulations is based on the Mersenne twister algorithm which has a cycle length of $2^{19937} - 1$ which is approximately 4.3×10^{6001} ; this is many orders of magnitude larger than the estimated number of particles in the observable universe, which is 10^{87} , and is sufficient for the purposes of this thesis.

2. evaluate each $a_j(\mathbf{u})$ and the sum $a_0(\mathbf{u})$ (equations (5.2) and (5.11));
3. generate τ and j using equations (5.12) and (5.13);
4. update $t := t + \tau$, $\mathbf{u} := \mathbf{u} + \boldsymbol{\nu}_j$;
5. return to step 2 until t exceeds a predetermined limit.

Since the SSA treats each particle separately, for systems with relatively large population sizes, this method will often be much slower than an algorithm solving the analogous deterministic system (Morton & Mayers, 2005). Particularly, when there is a mixture of fast and slow dynamics, the fast reaction channels will fire more, often causing most of the simulation time to be spent simulating reactions which may approach a steady state very quickly, thus, an adiabatic approximation may be appropriate.

A number of adaptations have been made to the basic Gillespie algorithm in order to speed up the simulations or to encompass the generalisation to spatial populations (Cao *et al.*, 2005, 2006; Engblom *et al.*, 2009; Gibson & Bruck, 2000; Gillespie, 2001; Gillespie & Petzold, 2003; Hattne *et al.*, 2005; Tian & Burrage, 2004). Presently, we use a basic SSA with a modified search to simulate all the data in this thesis. This is done, firstly, to maintain accuracy. In τ -leaping methods (Cao *et al.*, 2005, 2006; Cotter *et al.*, 2011; Gillespie, 2001; Gillespie & Petzold, 2003; Tian & Burrage, 2004), accuracy is reduced as we no longer simulate each individual reaction, but, instead, the system is updated with a stochastic estimate of how many reactions would have fired during a time step of length τ . Secondly, the standard SSA is much easier to code than the binary tree search method needed for the efficient algorithm presented by Gibson & Bruck (2000). Since our simulations run on the order of a few seconds to a few hours our needs are satiated with the simple yet accurate method of the standard SSA outlined above.

5.1.2 Weak noise expansion

Although we may not be able to solve the CME exactly, except in certain, special, circumstances, we are able to systematically produce an approximation of it by expanding the equations in terms of a suitable system parameter, denoted Ω , and assuming that, as this parameter is increased, the stochastic fluctuations become lower order. This allows

separation of mean-field and stochastic parts by yielding deterministic and probabilistic equations, respectively. By deriving equations for the mean-field dynamics and the effects of the noise about these, we are able to compare analytic results to ensemble data gained from SSA simulations.

Motivated by the central limit theorem (Grimmett & Stirzaker, 2001), we define a new random variable, η_i (which is of order one as $\Omega \rightarrow \infty$), through the relation,

$$U_i = \phi_i \Omega + \eta_i \sqrt{\Omega}. \quad (5.14)$$

Here, we only consider one spatially extended population and, thus, we fix $\Omega = U_i(0) = u(0)$, if the initial condition is homogeneous and Ω is the mean value of the $U_i(0)$, $i = 1, \dots, K$, otherwise. Thus, Ω does not have a spatial dependence. In the case of multiple spatially extended populations we define Ω to be the order of magnitude of the smallest homogeneous steady state. In the case of no steady state (spatially uniform, or otherwise) we once again use the smallest spatial average of the initial conditions. Thus, by making Ω large we ensure that all populations satisfy the weak noise assumption.

ϕ_i is the expected ratio of population at time t to the initial population and is a dimensionless ‘macroscopic’ variable. Since it is macroscopic we assume it is of order one compared to Ω , thus the various orders of the model can be separated. The statistics of U_i and η_i are linked through the identification (van Kampen, 2007)

$$P(\mathbf{U}, t) = \Pi(\boldsymbol{\eta}, t). \quad (5.15)$$

Thus, in the limit,

$$\lim_{\Omega \rightarrow \infty} \frac{\mathbf{U}}{\Omega} = \boldsymbol{\phi} = (\phi_1, \dots, \phi_K). \quad (5.16)$$

This is consistent with the ‘rule of thumb’ argument, identified by van Kampen (2007), that noise scales with the inverse square root of the population size. Also, it encapsulates the intuitive idea that, as the population grows, individual interactions become less important and we can treat the population as a continuum.

Using Taylor series we are now able to expand the operator $R_j P(\mathbf{u}, t)$ (shown in equation (5.6)) about this new function Π and the microscopic propensity function, $a_j(\mathbf{u} -$

ν_j), about the macroscopic rate, $\mathbf{a}_j(\phi)$, in terms of Ω .

Fixing the number of molecules and differentiating equation (5.15) with respect to time gives (Elf & Ehrenberg, 2003)

$$\frac{\partial P}{\partial t} = \frac{\partial \Pi(\boldsymbol{\eta}, t)}{\partial t} + \sum_{i=1}^K \frac{d\eta_i}{dt} \frac{\partial \Pi(\boldsymbol{\eta}, t)}{\partial \eta_i} = \frac{\partial \Pi}{\partial t} - \sqrt{\Omega} \sum_{i=1}^K \frac{d\phi_i}{dt} \frac{\partial \Pi}{\partial \eta_i}. \quad (5.17)$$

To expand the right-hand side of equation (5.6), we consider the effect of a general reaction operator, R_j , on both the transition rates, a_j , and the probability density, Π .

First, we consider the relation between the microscopic propensity function, $a_j(\mathbf{u} - \nu_j)$, and macroscopic rate, $\mathbf{a}_j(\phi)$, which is used to define the macroscopic variables, ϕ_i , as solutions of

$$\frac{d\phi_i}{dt} = \sum_{j=1}^J \mathbf{a}_j(\phi) \nu_{ij}. \quad (5.18)$$

For instance, for a general zeroth order reaction, where $\emptyset$ is a non-modelled species, *e.g.*



the microscopic propensity rate is simply $a_j(\mathbf{u}) = \kappa$. Upon using a mass balance argument we find that this relates to the macroscopic propensity rate by a factor of Ω , namely $\mathbf{a}_j(\phi) = \kappa/\Omega$. For first order reactions a scaling is not needed, but, in general, for a p^{th} order reaction,

$$S_{j_1} + \dots + S_{j_p} \xrightarrow{\kappa} S_i, \quad (5.20)$$

where the indices $j_1, \dots, j_p$ are all different, the microscopic propensity is then proportional to the product of all the populations, $a_j(\mathbf{u}) = \kappa u_{j_1} u_{j_2} \dots u_{j_p}$, whereas $\mathbf{a}_j(\phi) = \kappa \Omega^{p-1} \phi_{j_1} \phi_{j_2} \dots \phi_{j_p}$. In the case of repeated indices we take into account that the reactions must occur between distinct particles. For instance suppose

$$S_{j_1} + \dots + n S_{j_q} + \dots + S_{j_p} \xrightarrow{\kappa} S_i, \quad (5.21)$$

the microscopic propensity is then

$$a_j(\mathbf{u}) = \kappa u_{j_1} u_{j_2} \dots u_{j_q} (u_{j_q} - 1) \dots (u_{j_q} - (n - 1)) \dots u_{j_p}, \quad (5.22)$$

whereas $\mathbf{a}_j(\phi) = \kappa \Omega^{p-1} \phi_{j_1} \phi_{j_2} \dots \phi_{j_q}^n \dots \phi_{j_p}$.

Furthermore, because of the relationship between a and $\mathbf{a}$, we can expand the microscopic propensity function $a_j(\mathbf{u} - \boldsymbol{\nu}_j)$ about the macroscopic rate $\mathbf{a}_j$, as

$$\begin{aligned} a_j(\mathbf{u} - \boldsymbol{\nu}_j) &= a_j \left(\phi \Omega + \boldsymbol{\eta} \sqrt{\Omega} - \boldsymbol{\nu}_j \right), \\ &= \Omega \mathbf{a}_j \left(\phi + \frac{\boldsymbol{\eta}}{\sqrt{\Omega}} - \frac{\boldsymbol{\nu}_j}{\Omega} \right) + \mathcal{O} \left(\frac{1}{\Omega} \right), \\ &= \Omega \left(\mathbf{a}_j(\phi) + \frac{1}{\sqrt{\Omega}} \sum_{i=1}^K \frac{\partial \mathbf{a}_j(\phi)}{\partial \phi_i} \eta_i + \mathcal{O} \left(\frac{1}{\Omega} \right) \right). \end{aligned} \quad (5.23)$$

Similarly,

$$\begin{aligned} R_j P(\mathbf{u}, t) &= P(\mathbf{u} - \boldsymbol{\nu}_j, t) = \Pi \left(\boldsymbol{\eta} - \frac{\boldsymbol{\nu}_j}{\sqrt{\Omega}}, t \right), \\ &= \left(1 - \frac{1}{\sqrt{\Omega}} \sum_{i=1}^K \nu_{ij} \frac{\partial}{\partial \eta_i} + \frac{1}{2\Omega} \sum_{i=1}^K \sum_{l=1}^K \nu_{ij} \nu_{lj} \frac{\partial^2}{\partial \eta_i \partial \eta_l} + \mathcal{O} \left(\frac{1}{\Omega^{\frac{3}{2}}} \right) \right) \Pi(\boldsymbol{\eta}, t). \end{aligned} \quad (5.24)$$

Upon inserting equations (5.17), (5.23) and (5.24) into equation (5.6) and ignoring terms of order $1/\sqrt{\Omega}$, we obtain

$$\begin{aligned} \frac{\partial \Pi}{\partial t} - \sqrt{\Omega} \sum_{i=1}^K \frac{d\phi_i}{dt} \frac{\partial \Pi}{\partial \eta_i} &= \\ \sum_{j=1}^J \left(-\sqrt{\Omega} \sum_{i=1}^K \nu_{ij} \frac{\partial}{\partial \eta_i} + \frac{1}{2} \sum_{i=1}^K \sum_{l=1}^K \nu_{ij} \nu_{lj} \frac{\partial^2}{\partial \eta_i \partial \eta_l} \right) &\left(\mathbf{a}_j(\phi) + \frac{1}{\sqrt{\Omega}} \sum_{i=1}^K \frac{\partial \mathbf{a}_j(\phi)}{\partial \phi_i} \eta_i \right) \Pi(\boldsymbol{\eta}). \end{aligned} \quad (5.25)$$

Collecting terms of order $\sqrt{\Omega}$ leads to the macroscopic equation,

$$\sum_{i=1}^K \frac{d\phi_i}{dt} \frac{\partial \Pi}{\partial \eta_i} = \sum_{i=1}^K \sum_{j=1}^J \mathbf{a}_j(\phi) \nu_{ij} \frac{\partial \Pi}{\partial \eta_i}, \quad (5.26)$$

which is satisfied by the definition of $\mathbf{a}_j(\phi)$, equation (5.18).

Collecting terms of order one gives rise to a Fokker-Planck equation (Risken, 1989)

which defines the dynamics of the probability density,

$$\begin{aligned}\frac{\partial \Pi}{\partial t} &= \sum_{j=1}^J \left(- \sum_{i,l=1}^K \frac{\partial \mathbf{a}_j}{\partial \phi_l} \nu_{ij} \frac{\partial (\eta_i \Pi)}{\partial \eta_l} + \frac{1}{2} \mathbf{a}_j \sum_{i,l=1}^K \nu_{ij} \nu_{lj} \frac{\partial^2 \Pi}{\partial \eta_i \partial \eta_l} \right), \\ &= - \sum_{i,l=1}^K A_{il} \frac{\partial (\eta_l \Pi)}{\partial \eta_i} + \frac{1}{2} \sum_{i,l=1}^K B_{il} \frac{\partial^2 \Pi}{\partial \eta_i \partial \eta_l},\end{aligned}\tag{5.27}$$

where (Elf & Ehrenberg, 2003)

$$\mathbf{A} = \{A_{il}\} = \{\partial[\boldsymbol{\nu} \mathbf{a}]_i / \partial \phi_l\},\tag{5.28}$$

$$\mathbf{B} = \{B_{il}\} = \boldsymbol{\nu} \text{diag}(\mathbf{a}) \boldsymbol{\nu}^T,\tag{5.29}$$

and $\mathbf{a}^T = (\mathbf{a}_1, \dots, \mathbf{a}_J)$.

5.1.3 Spatially extended systems

In order to proceed generally we must consider how the reactions affect the structure of the matrices $\mathbf{A}$ and $\mathbf{B}$. Since we are considering reaction-diffusion equations, it is important that we define diffusion on the micro- and macro-scopic scales. Although other descriptions are possible (Andrews & Bray, 2004), here, we model diffusion as a space-jump process (Baker *et al.*, 2010; Othmer *et al.*, 1988) (see Figure 5.1).

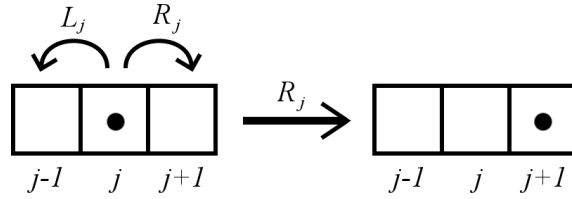


Figure 5.1: Diagram illustrating space-jump description of diffusion. Each particle has equal probability per time unit of moving left as of moving right. If, for example, the right diffusion reaction, R_j , occurs one particle from box j moves to box $j + 1$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

A domain of length L is partitioned into K one-dimensional compartments of size $\Delta_E = L/K$ and each compartment contains an indexed population. Higher dimensional domains can also be considered by replacing the one-dimensional compartments with appropriate analogues (Erban *et al.*, 2007). For K compartments, in a one-dimensional domain, with

Neumann, zero-flux boundary conditions, each containing a species S_i , with population $U_i = \Omega\phi_i + \sqrt{\Omega}\eta_i$ and microscopic diffusion coefficient d_E , the reactions are:



Since these are first order equations, the equations governing their mean values are equivalent to the deterministic reactions (McQuarrie, 1967),

$$\frac{d\phi_1}{dt} = d_E (\phi_2 - \phi_1), \quad (5.31)$$

$$\frac{d\phi_i}{dt} = d_E (\phi_{i-1} - 2\phi_i + \phi_{i+1}) \quad i = 2, \dots, K-1, \quad (5.32)$$

$$\frac{d\phi_K}{dt} = d_E (\phi_{K-1} - \phi_K). \quad (5.33)$$

Comparing these equations with the second order finite difference approximation of the one-dimensional Laplacian with zero-flux boundary conditions, which is derived through Taylor expansions of the continuous formulation (Morton & Mayers, 2005), shows that their descriptions are equivalent if $d_E = D/\Delta_E^2$, where D is the macroscopic rate of diffusion (Gardiner *et al.*, 1976). Thus, because the reactions are linear, the expected value of the populations undergoing a stochastic description of diffusion approaches the deterministic diffusion equation as Δ_E tends to zero.

Similar to simulating a discretised reaction-diffusion PDE, if the discretised domain is too coarse the simulations will be inaccurate. Thus, there is a restriction on the compartment size being too large. However, unlike PDEs, the compartment size may be restricted from being too small as the specific probability rate constants may depend on the size of the compartment, thus the limit $\Delta_E \rightarrow 0$ is not necessarily well-defined (Erban & Chapman, 2009; Twomey, 2007). Such problematic cases are not dealt with in the present context.

Using the matrix terminology developed in Section 5.1.2, the stoichiometric matrix,

$\boldsymbol{\nu}$, and the macroscopic transition rate, $\mathbf{a}$, are given by:

$$\boldsymbol{\nu} = \begin{pmatrix} -1 & 0 & \cdots & 0 & 0 & 0 & 1 & 0 & \cdots & 0 \\ 1 & \ddots & \ddots & \vdots & \vdots & \vdots & -1 & \ddots & \ddots & \vdots \\ 0 & \ddots & \ddots & 0 & \vdots & \vdots & 0 & \ddots & \ddots & 0 \\ \vdots & \ddots & \ddots & -1 & \vdots & \vdots & \vdots & \ddots & \ddots & 1 \\ 0 & \cdots & 0 & 1 & 0 & 0 & 0 & \cdots & 0 & -1 \end{pmatrix}, \quad \mathbf{a} = \begin{pmatrix} d_E \phi_1 \\ \vdots \\ d_E \phi_K \\ d_E \phi_1 \\ \vdots \\ d_E \phi_K \end{pmatrix}, \quad (5.34)$$

where $\boldsymbol{\nu}$ is a $K \times 2K$ matrix. The first $K \times K$ elements are the population change vectors of diffusion to the right *e.g.* the j^{th} column ($j < K$) is the population transition of the reaction

$$S_j \rightarrow S_{j+1}. \quad (5.35)$$

Similarly, for $j > K + 1$, the j^{th} column is the population transition of

$$S_j \rightarrow S_{j-1}. \quad (5.36)$$

The two zero columns in the centre show that no particles may leave the domain, which corresponds to Neumann boundary conditions. The j^{th} element of $\mathbf{a}$ is the corresponding weighting probability of the j^{th} reaction.

Using definitions (5.28) and (5.29) we have,

$$\mathbf{A} = \begin{pmatrix} -d_E & d_E & 0 & \cdots & 0 \\ d_E & -2d_E & d_E & \ddots & \vdots \\ 0 & \ddots & \ddots & \ddots & 0 \\ \vdots & \ddots & d_E & -2d_E & d_E \\ 0 & \cdots & 0 & d_E & -d_E \end{pmatrix}, \quad (5.37)$$

$$\mathbf{B} = \begin{pmatrix} 2d_E\phi & -2d_E\phi & 0 & \dots & 0 \\ -2d_E\phi & 4d_E\phi & -2d_E\phi & \ddots & \vdots \\ 0 & \ddots & \ddots & \ddots & 0 \\ \vdots & \ddots & -2d\phi & 4d_E\phi & -2d_E\phi \\ 0 & \dots & 0 & -2d_E\phi & 2d_E\phi \end{pmatrix}, \quad (5.38)$$

where ϕ is the homogeneous steady state solution of the system (5.31)-(5.33). The symmetric, tridiagonal forms of $\mathbf{A}$ and $\mathbf{B}$ are the key features used when deriving their spatio-temporal Fourier transforms. Any other reactions that are added into the one population system are assumed to act within each compartment, thus reagents in box i can only react with other reagents in box i . This means that any additional reactions simply add extra terms to the diagonals of $\mathbf{A}$ and $\mathbf{B}$, thus, we only need consider a symmetric, tridiagonal matrix for the following analysis to be general.

5.2 Fourier transforms

The Fokker-Planck equation (5.27) can be converted to its corresponding Langevin equation, which has the general form (Elf & Ehrenberg, 2003; Gillespie, 1996),

$$\frac{d\boldsymbol{\zeta}}{dt} = \mathbf{A}\boldsymbol{\zeta} + \boldsymbol{\lambda}(t), \quad (5.39)$$

where $\boldsymbol{\zeta} = (\eta_1, \dots, \eta_K)$ and $\boldsymbol{\lambda} = (\lambda_1, \dots, \lambda_K)$. The λ_i are Gaussian random variables with zero mean and covariance specified by,

$$\langle \lambda_i(t) \lambda_j(t') \rangle = B_{ij} \delta(t - t'), \quad (5.40)$$

where $\langle x \rangle$ is the mean value of x over a number of simulations. Note that, since the λ_i are only uniquely defined up to their covariances, the Langevin equation is non-unique (Gillespie, 1996). However, since we only work with the covariances, this is not a problem that will concern us.

Temporal and spatial Fourier transform theory can be applied to Langevin equation (5.39) in order to discern inhomogeneous behaviour (Biancalani *et al.*, 2010; Black &

McKane, 2010; Butler & Goldenfeld, 2009; Lugo & McKane, 2008; McKane *et al.*, 2007; McKane & Newman, 2005). In this section we recapitulate this theory, paying particular attention to the form of expansion used and the effects of boundary conditions, whilst extending the theory to non-steady state kinetics.

5.2.1 Temporal Fourier transform

In this thesis we will be primarily interested in the spatial transform, as the biological patterns we study are normally stationary. Since the temporal transform has applications in systems with no stable steady state *e.g.* limit cycles, it is covered for completeness (Reichenbach *et al.*, 2006). Further, we extend current analytical results to non-stationary kinetics, highlighting the importance of carefully handling the boundary conditions.

As we will see later (Section 5.2.2), the spatial Fourier transform reduces the set of coupled ordinary differential equations (ODEs) in equation (5.39) to a single ODE. Hence, in this section we consider only a single species, S , with population U and neglect diffusion.

5.2.1.1 Stationary kinetics

To illustrate the difference between stationary and non-stationary kinetics, we first consider the simple system of logistic growth,

$$S \xrightleftharpoons[\beta/\Omega]{\alpha} 2S. \quad (5.41)$$

Using the theory set out in Section 5.1.2, the deterministic equation describing the mean-field dynamics of U is

$$\dot{\phi} = \alpha\phi - \beta\phi^2, \quad (5.42)$$

with stable steady state $\phi_s = \alpha/\beta$. Further, we can easily find that $\mathbf{A} = A = \alpha - 2\beta\phi_s = -\alpha$ and $\mathbf{B} = B = \alpha\phi_s + \beta\phi_s^2 = 2\alpha^2/\beta$, which allows the calculation of the Langevin equation,

$$\dot{\eta} = -\alpha\eta + \lambda, \quad (5.43)$$

where λ is given by equation (5.40). At this point we would like to apply the discrete temporal Fourier transform (Briggs & Henson, 1995),

$$\tilde{f}(\omega) = \Delta_t \sum_{i=0}^{M-1} \exp(I\omega\Delta_t i) f(\Delta_t i), \quad (5.44)$$

where f is a function that has been sampled M times over an interval $[0, T]$ such that $\Delta_t = T/M$ and $I = \sqrt{-1}$ is the imaginary unit.

The Fourier transform of η is simply $\tilde{\eta}$ and, previously, when taking the temporal Fourier transform of the time derivative, the approximation (Biancalani *et al.*, 2010; McKane & Newman, 2005)

$$\dot{\tilde{\eta}} = -I\omega\tilde{\eta}, \quad (5.45)$$

has been used. However, this approximation does not encompass any contribution from the initial and final values, as they are assumed to be small. Here, we use this approximation successfully, which is justified later. In Section 5.2.1.2, the approximation fails when it is applied to kinetics which do not tend to a stable steady state.

Applying the discrete Fourier transform to equation (5.43) and using approximation (5.45), we obtain

$$-I\omega\tilde{\eta} = -\alpha\tilde{\eta} + \tilde{\lambda}, \quad (5.46)$$

$$\implies \tilde{\eta} = \frac{\tilde{\lambda}}{\alpha - \omega I}. \quad (5.47)$$

Further,

$$\begin{aligned} \langle \tilde{\lambda}(\omega) \tilde{\lambda}(\omega') \rangle &= \Delta_t^2 \sum_{i=0}^{M-1} \sum_{j=0}^{M-1} \exp(I\omega\Delta_t i) \exp(I\omega'\Delta_t j) \langle \lambda(\Delta_t i), \lambda(\Delta_t j) \rangle, \\ &= \Delta_t^2 \sum_{i=0}^{M-1} \sum_{j=0}^{M-1} \exp(I\omega\Delta_t i) \exp(I\omega'\Delta_t j) B\delta(\Delta_t(i-j)), \\ &= \Delta_t B \sum_{i=0}^{M-1} \exp(I(\omega + \omega')\Delta_t i), \end{aligned} \quad (5.48)$$

implying

$$\langle \tilde{\lambda}(\omega) \tilde{\lambda}(-\omega) \rangle = \Delta_t B M. \quad (5.49)$$

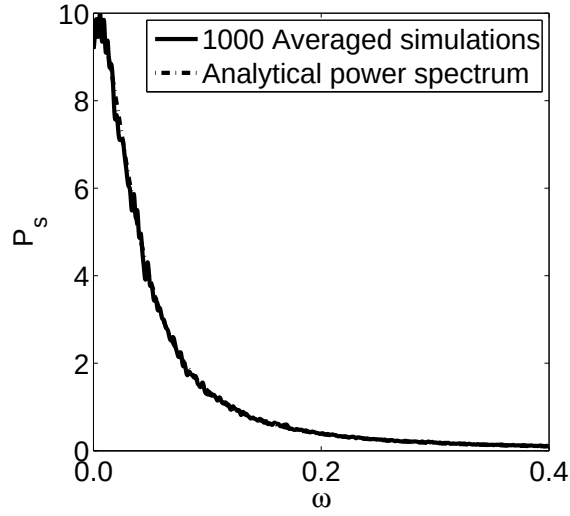


Figure 5.2: Comparison of the power spectrum given by equation (5.50) (dashed line) and 1000 averaged replicates of the stochastic system (solid line) each scaled by a factor of $T = 1000$. Parameters are $\Omega = 100$, $\alpha = 0.1$ and $\beta = 0.2$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

Thus we are able to calculate the power spectrum of the noise,

$$P_s(\omega) = \frac{\langle |\tilde{\eta}|^2 \rangle}{\Delta_t M} = \frac{2\alpha^2}{\beta(\alpha^2 + \omega^2)}. \quad (5.50)$$

In Figure 5.2 we compare the power spectrum, equation (5.50), with the averaged ensemble of 1000 Fourier-transformed stochastic simulations, scaled by $\Delta_t M = T$. The figure clearly shows an extremely good fit between theory and simulation.

5.2.1.2 Non-stationary kinetics

Let us now consider kinetics that do not tend to a stationary steady state, *e.g.* linear growth,

$$\emptyset \xrightarrow{r} S. \quad (5.51)$$

Here, we obtain

$$P_s(\omega) = \frac{\langle |\tilde{\eta}|^2 \rangle}{T} = \frac{r}{\Omega \omega^2}. \quad (5.52)$$

Qualitatively the curves in Figure 5.2 are the same but quantitatively the analytic solution is quite different. Thus, in order to obtain a better theoretical comparison to the ensemble power spectrum, we ignore approximation (5.45) and return to the Langevin equation.

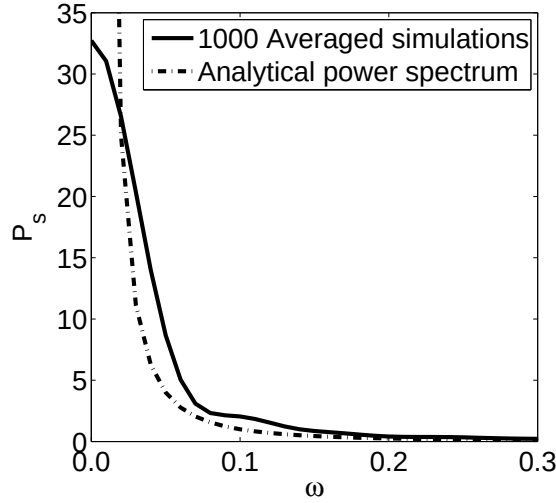


Figure 5.3: Comparison of the linear growth power spectrum, equation (5.52) (dashed line) and 1000 averaged replicates of the stochastic system (solid line). Each result has been scaled by a factor of $T = 100$. Parameters are $\Omega = 1000$ and $r = 10$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. *Phys. Rev. E*, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

5.2.1.3 Corrected Fourier transform of the time derivative

The Langevin equation arising from the weak noise expansion method is always linear. Thus, following van Kampen (2007), instead of taking the Fourier transform of the time derivative, we simply solve the linear ODE and take the Fourier transform of the resulting covariances.

Note in the following, general, derivation we consider the finite time continuous transform as we expect the continuous and discrete transforms to correspond as $\Delta_t \rightarrow 0$ (Briggs & Henson, 1995). Let

$$\dot{\eta} = c\eta + \lambda, \quad (5.53)$$

where $c \neq 0$ constant² and

$$\langle \lambda(t')\lambda(t'') \rangle \stackrel{def}{=} g(t', t'')\delta(t' - t''). \quad (5.54)$$

Since equation (5.53) is linear we can integrate the system to obtain

$$\eta(t) = e^{ct} \int_0^t \lambda(t')e^{-ct'} dt', \quad (5.55)$$

²We treat the singular case of linear growth separately.

and so

$$\begin{aligned}\langle \eta(t_1)\eta(t_2) \rangle &= e^{c(t_1+t_2)} \int_0^{t_1} \int_0^{t_2} g(t', t'') e^{-c(t'+t'')} \delta(t' - t'') dt' dt'', \\ &= e^{c(t_1+t_2)} \int_0^{\min(t_1, t_2)} g(t', t') e^{-2ct'} dt'.\end{aligned}\quad (5.56)$$

Simplifying the notation of $g(t', t')$ to $g(t')$ and applying the temporal transform gives

$$\begin{aligned}\langle |\tilde{\eta}(\omega)|^2 \rangle &= \int_0^T \int_0^T e^{(I\omega(t_1-t_2))} e^{c(t_1+t_2)} \int_0^{\min(t_1, t_2)} g(t') e^{-2ct'} dt' dt_1 dt_2, \\ &= \int_0^T \int_0^{t_2} e^{(I\omega(t_1-t_2))} e^{c(t_1+t_2)} \int_0^{t_1} g(t') e^{-2ct'} dt' dt_1 dt_2 \\ &\quad + \int_0^T \int_{t_2}^T e^{(I\omega(t_1-t_2))} e^{c(t_1+t_2)} \int_0^{t_2} g(t') e^{-2ct'} dt' dt_1 dt_2, \\ &= 2 \int_0^T \int_0^{t_2} \cos(\omega(t_1 - t_2)) e^{c(t_1+t_2)} \int_0^{t_1} g(t') e^{-2ct'} dt' dt_1 dt_2.\end{aligned}\quad (5.57)$$

To make further progress we assume a form for g . If the kinetics have a stable steady state then g will be a constant,

$$g(t') = g. \quad (5.58)$$

Whence,

$$\begin{aligned}\langle |\tilde{\eta}(\omega)|^2 \rangle &= \frac{g(c^3 + c\omega^2)T}{(c^2 + \omega^2)^2 c} - \frac{g(4c^2 \cos(\omega T) + 4\omega \sin(\omega T)c)e^{cT}}{2(c^2 + \omega^2)^2 c} \\ &\quad + \frac{g(c^2 + \omega^2)e^{2cT}}{2(c^2 + \omega^2)^2 c} - \frac{g(-3c^2 + \omega^2)}{2(c^2 + \omega^2)^2 c}.\end{aligned}\quad (5.59)$$

Further, from the derivation of $\mathbf{A}$ in Section 5.1.2, c will be the eigenvalue of the homogeneous system thus, since we are assuming stable kinetics, c will be negative and the exponentials will decay. Considering only the long time solution leads to

$$\langle |\tilde{\eta}(\omega)|^2 \rangle = \frac{gT}{(\omega^2 + c^2)}. \quad (5.60)$$

Letting $c = \alpha$ and $g = 2\alpha^2/\beta$, we can compare this with the power spectrum of the logistic kinetics derived in Section 5.2.1.1. We see that additional terms associated with the temporal boundary values are of lower order, thus in the long time limit they can,

legitimately, be ignored.

Now suppose the kinetics are not stationary and thus $c > 0$. This will then give a time-dependent $g = g(t')$. In general, equation (5.57) no longer has a closed form solution, thus, we must consider solutions on a case by case basis.

For exponential kinetics, we see that $c = r$ and $g(t) = r \exp(rt)$. Thus,

$$\langle |\tilde{\eta}(\omega)|^2 \rangle = \frac{-2re^{rT} \sin(\omega T) + \omega (e^{2rT} - 1)}{\omega (\omega^2 + r^2)}. \quad (5.61)$$

Figure 5.4(a) convincingly shows that equation (5.61) is a good approximation to the numerically simulated power spectrum. However, the power spectrum of exponential growth does not tend to a limit when scaled by $1/T$ and so the power spectrum is dependent on the final time value of the observed domain.

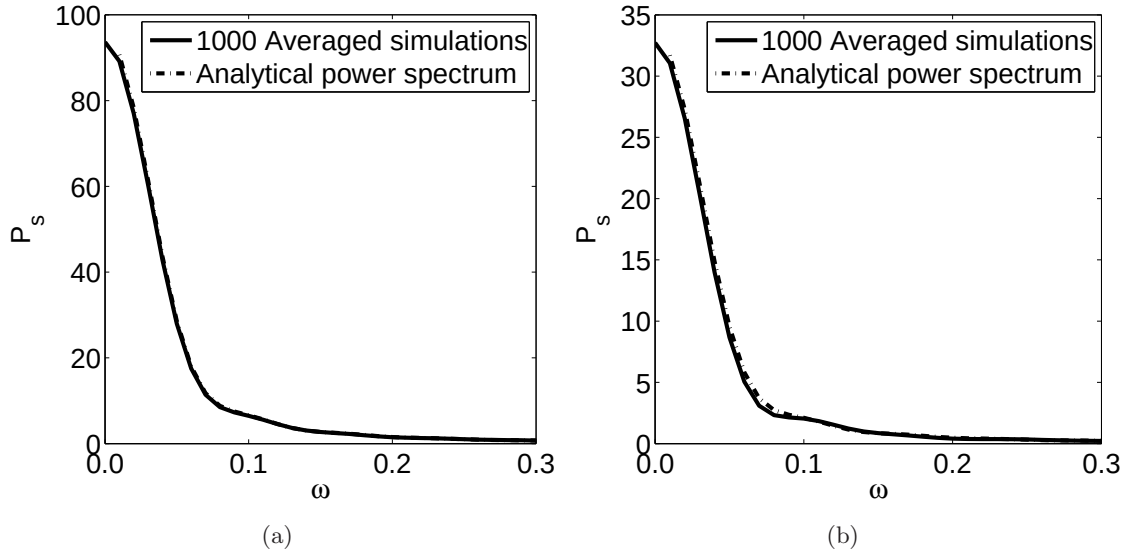


Figure 5.4: Comparison of the corrected power spectrum (dashed line) and 1000 averaged replicates of the stochastic system (solid line). Each result has been scaled by a factor of $T = 100$. (a) Exponential growth. Analytic power spectrum given by equation (5.61). (b) Linear growth. Analytic power spectrum given by equation (5.62). Parameters are as in Figure 5.3. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. *Phys. Rev. E*, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

Finally, for the singular case of linear growth, the same procedure can be followed from equation (5.53) but this time $c = 0$. This leads to

$$\langle |\tilde{\eta}(\omega)|^2 \rangle = 2 \frac{rT}{\omega^2 \Omega} - 2 \frac{r \sin(\omega T)}{\omega^3 \Omega}. \quad (5.62)$$

Comparing this with the originally derived estimate, equation (5.52), we find a factor of two that was originally missing, as well a term of the form $r \sin(\omega T)$. From Figure 5.4(b) we, once again, see excellent correspondence between theory and simulation.

5.2.2 Spatial Fourier transform

We now consider the discrete spatial Fourier transform of a spatially extended system with a population diffusing over the domain, see Section 5.1.3. In previous work, the utilised transform has been analogous to the temporal transform (Biancalani *et al.*, 2010; Lugo & McKane, 2008),

$$\hat{f}(k) = \Delta_x \sum_{j=1}^K \exp(-Ik\Delta_x(j-1))f(x_j), \quad (5.63)$$

where f is a function defined over a space which has been discretised into K uniformly spaced points with spacing Δ_x , such that $x_j = j\Delta_x$.

Since our simulations employ Neumann boundary conditions, which do not disappear on using the above transform, we choose, instead, to use the discrete Fourier cosine expansion (Briggs & Henson, 1995),

$$\hat{f}(k) = \Delta_x \sum_{j=1}^K \cos(k\Delta_x(j-1))f(x_j). \quad (5.64)$$

Note that the correction $(j-1)$ in the cosine function is simply to account for the fact that, spatially, we have defined our populations to start with an index one instead of zero. In contrast, the temporal initial condition is defined to occur at time $t = 0$ thus the index for the temporal transformation starts at zero.

Taking the discrete spatial transform of the discrete Laplacian of η , with $\Delta_x = \Delta_E$,

gives

$$\begin{aligned}
& \Delta_E \cos(k\Delta_E 0) d_E[-\eta_1 + \eta_2] + \Delta_E \cos(k\Delta_E(K-1)) d_E[\eta_{K-1} - \eta_K] \\
& + \Delta_E \sum_{j=2}^{K-1} \cos(k\Delta_E(j-1)) d_E[\eta_{j-1} - 2\eta_j + \eta_{j+1}], \\
& = -2d_E \hat{\eta}_k + \Delta_E \cos(k\Delta_E 0) d_E \eta_1 + \Delta_E \cos(k\Delta_E(K-1)) d_E \eta_K \\
& + \Delta_E \sum_{j=1}^{K-1} \cos(k\Delta_E j) d_E \eta_j + \Delta_E \sum_{j=2}^K \cos(k\Delta_E(j-2)) d_E \eta_j, \\
& = -2d_E \hat{\eta}_k + \Delta_E \cos(k\Delta_E 0) d_E \eta_1 + \Delta_E \cos(k\Delta_E(K-1)) d_E \eta_K \\
& + \Delta_E \sum_{j=1}^{K-1} [\cos(k\Delta_E(j-1)) \cos(k\Delta_E) - \sin(k\Delta_E(j-1)) \sin(k\Delta_E)] d_E \eta_j \\
& + \Delta_E \sum_{j=2}^K [\cos(k\Delta_E(j-1)) \cos(k\Delta_E) + \sin(k\Delta_E(j-1)) \sin(k\Delta_E)] d_E \eta_j, \\
& = 2d_E \hat{\eta}_k (\cos(k\Delta_E) - 1) - k\Delta_E^2 \frac{(\cos(k\Delta_E 1) - \cos(k\Delta_E 0))}{k\Delta_E} d_E \eta_1 \\
& + k\Delta_E^2 \frac{(\cos(k\Delta_E(K-1)) - \cos(k\Delta_E K))}{-k\Delta_E} d_E \eta_K. \tag{5.65}
\end{aligned}$$

Taking the limit $\Delta_E \rightarrow 0+$, whilst keeping $\Delta_E K = L$ and k fixed, gives

$$\lim_{\Delta_E \rightarrow 0+} \frac{[\cos(k\Delta_E 1) - \cos(k\Delta_E 0)]}{k\Delta_E} = \sin(0) = 0, \tag{5.66}$$

$$\lim_{\Delta_E \rightarrow 0+} \frac{[\cos(k\Delta_E(K-1)) - \cos(k\Delta_E K)]}{-k\Delta_E} = \sin(kL). \tag{5.67}$$

Hence, by choosing $k = m\pi/L$, $m = 0, 1, \dots, K-1$, the boundary terms in equation (5.65) are encapsulated within the form of expansion and they leave only terms that are of lower order than the leading order term, $2d_E \hat{\eta}_k (\cos(k\Delta_E) - 1)$. Further, by expanding the cosine function and remembering that, as $\Delta_E \rightarrow 0$, $d_E \Delta_E^2 = D$ is finite,

$$\lim_{\Delta_E \rightarrow 0} 2d_E \hat{\eta}_k (\cos(k\Delta_E) - 1) = -k^2 D \hat{\eta}_k, \tag{5.68}$$

which is exactly the solution given by the continuous transform of the Laplacian (Lugo & McKane, 2008).

Thus, the spatial Fourier cosine transform of the Langevin equation, generated by the

space-jump description of diffusion (equation (5.39), where $\mathbf{A}$ and $\mathbf{B}$ are given by equation (5.38)), is given by

$$\frac{d\hat{\eta}_k}{dt} = 2(\cos(k\Delta_E) - 1)d_E\hat{\eta}_k + \hat{\lambda}_k. \quad (5.69)$$

Notice that the spatial cosine transform has reduced a set of K coupled ODEs in equation (5.39) to a single ODE, equation (5.69), which can be solved for all $k = m\pi/L$, $m = 0, \dots, K-1$.

The explicit form of $\langle \hat{\lambda}_k(t) \hat{\lambda}_{k'}(t') \rangle$ can be found similarly. Since $\mathbf{B}$ is tridiagonal and symmetric we can completely specify its elements by using Kronecker delta functions,

$$B_{ij} = b_0\delta_{i,j} + b_1\delta_{|i-j|,1}, \quad (5.70)$$

where the b_0 are the diagonal elements and the b_1 are the elements on the super- and sub-diagonals. Once again we use a discrete cosine transform, which, as shown above, automatically incorporates the boundary conditions, thus,

$$\langle \hat{\lambda}_k(t) \hat{\lambda}_{k'}(t') \rangle = \Delta_E^2 \sum_{i,j=1}^K \cos(k\Delta_E(i-1)) \cos(k'\Delta_E(j-1)) (b_0\delta_{i,j} + b_1\delta_{|i-j|,1}) \delta(t-t'), \quad (5.71)$$

can be then simplified to

$$\langle \hat{\lambda}_k(t) \hat{\lambda}_k(t') \rangle = \frac{\Delta_E^2 K}{2} (b_0 + 2 \cos(k\Delta_E) b_1) \delta(t-t'). \quad (5.72)$$

We have been able to apply the spatial Fourier cosine transform analytically due to the spatial homogeneity of the coefficients. If the coefficients were also constant in time, the temporal transform would cause no trouble and this case has been dealt with successfully (Alonso *et al.*, 2007; Biancalani *et al.*, 2010; Butler & Goldenfeld, 2009; McKane & Newman, 2004, 2005; Reichenbach *et al.*, 2006). For completeness, and as an example of using both transforms together, in the next section we apply both techniques to our stochastic description of diffusion.

5.3 Temporal and spatial Fourier cosine transform of diffusion

In the previous sections, we demonstrated the temporal transform on both stable and unstable systems. Further, we derived the spatial Fourier cosine transform of the space-jump description of diffusion. Here, we demonstrate both of the transforms simultaneously on the mechanism of diffusion, in order to compare the stochastic space-jump description with its deterministic analogue. Although the system tends to a stationary steady state, meaning we could use the approximation given by equation (5.45), we derive the equation fully using the same method as in Section 5.2.1.3 and apply the temporal Fourier transform to equation (5.69).

Let $C(k) = 2d_E(1 - \cos(k\Delta_E))$, then

$$\hat{\eta}_k = \exp(-C(k)t) \int_0^t \hat{\lambda}_k \exp(C(k)t') dt'. \quad (5.73)$$

This implies

$$\langle \hat{\eta}_k(t_1) \hat{\eta}_k(t_2) \rangle = e^{-C(k)(t_1+t_2)} \int_0^{t_1} \int_0^{t_2} \langle \hat{\lambda}_k \hat{\lambda}_k \rangle e^{C(k)(t'_1+t'_2)} dt'_1 dt'_2. \quad (5.74)$$

By considering the matrices in equation (5.38) and substituting the correct values into equation (5.72) we obtain

$$\begin{aligned} \langle \hat{\lambda}_k(t) \hat{\lambda}_k(t') \rangle &= 2\Delta_E^2 K d_E \phi (1 - \cos(k\Delta_E)) \delta(t - t'), \\ &= \Delta_E^2 K \phi C(k) \delta(t - t'). \end{aligned} \quad (5.75)$$

Thus,

$$\langle \hat{\eta}_k(t) \hat{\eta}_k(t) \rangle = \frac{\Delta_E^2 K \phi}{2} \left(1 - e^{(-2C(k)t)} \right). \quad (5.76)$$

Since $C(k) \geq 0$ the long time covariance is time independent,

$$\lim_{t \rightarrow \infty} \langle \hat{\eta}_k(t) \hat{\eta}_k(t) \rangle = \begin{cases} 0 & \text{if } k = 0, \\ \frac{\Delta_E^2 K \phi}{2} & \text{if } k \neq 0. \end{cases} \quad (5.77)$$

As this is constant for all spatial frequencies, $k \neq 0$, then, as we would expect, the noise tends not to favour any one frequency over another. Thus stochastic effects in the diffusion equation do not produce a patterned state.

The temporal Fourier transform is derived by considering

$$\langle \tilde{\eta}_k(\omega) \tilde{\eta}_k(-\omega) \rangle = \int_0^T \int_0^T \exp(i\omega(t_1 - t_2)) \langle \hat{\eta}_k(t_1) \hat{\eta}_k(t_2) \rangle dt_1 dt_2. \quad (5.78)$$

The system is completely integrable and so, by suppressing the argument k in $C(k)$, we obtain

$$\begin{aligned} \langle |\tilde{\eta}_k(\omega)|^2 \rangle &= \frac{\Delta_E^2 K \phi}{2(\omega^2 + C^2)^2} \left[-(\omega^2 + C^2) (\cosh(2CT) - \sinh(2CT)) \right. \\ &\quad \left. + (-4\omega C \sin(\omega T) + 4C^2 \cos(\omega T)) (\cosh(CT) - \sinh(CT)) \right. \\ &\quad \left. \omega^2 - 3C^2 + 2CT\omega^2 + 2C^3T \right]. \end{aligned} \quad (5.79)$$

The long time form simplifies to

$$\begin{aligned} \langle |\tilde{\eta}_k(\omega)|^2 \rangle &= \frac{C \Delta_E^2 K \phi T}{(\omega^2 + C^2)}, \\ &= \frac{2d_E(1 - \cos(k\Delta_E)) \Delta_E \phi L T}{(\omega^2 + 4d_E(1 - \cos(k\Delta_E))^2)}, \end{aligned} \quad (5.80)$$

where T is the total time of simulation and $L = K\Delta_E$ is the length of domain.

Figure 5.5 shows that simulation, once again, compares favourably with theory. Note that $(k, \omega) = (0, 0)$ is a singular point of equation (5.80) and, as such, the analytic solution tends to infinity near this point. Since the white noise process is uncorrelated in time and space this is the expected behaviour of the spectrum. However, the average of a finite number of discrete simulations is necessarily finite (Reichenbach *et al.*, 2006) and so we would expect that the approximation of the ensemble solution to the analytical solution to become worse near this point. This is clearly seen in Figure 5.5(c).

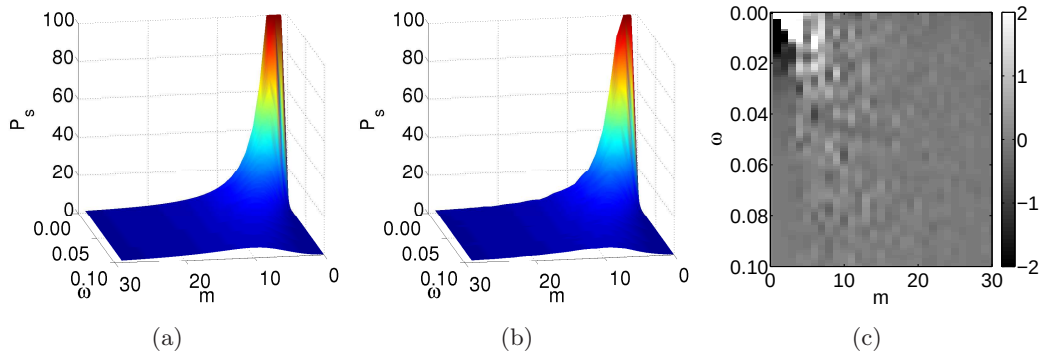


Figure 5.5: Left: power spectra of 1000 averaged simulations. Middle: analytically derived power spectra; equation (5.80) scaled by $\Delta_E L T$. Right: analytical value minus the ensemble values. Note that $(k, \omega) = (0, 0)$ is a singular point of equation (5.80) thus the analytic solution tends to infinity, whereas averaged simulations will always be finite. This is why the difference between the two spectra is close to zero everywhere, except near the origin. Note that, in order to see the variability around zero, we have fixed the colour axis to be in the interval $[-2, 2]$. Thus, although the difference does become infinite near the origin, any value above two is coloured white. Parameters used: $\phi = 1$, $d_E = 1$, $\Delta_E = 1/100$, $T = 1000$ and $L = 1$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. *Phys. Rev. E*, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

5.4 Discussion

The intention of this chapter has been to introduce a number of analytical and computational methods for understanding stochastic systems. We based our approach on a CME description of the stochastic system, which we are only able to solve exactly in the sense that the average of a large number of simulations will tend to a solution. In order to obtain an analytical hold on this equation we then approximated the system using a weak-noise expansion, allowing us to derive deterministic equations for the mean-field dynamics and a Fokker-Planck equation, which describes the stochastic effects.

Akin to the CME, the explicit form of the Fokker-Planck equation is not particularly useful. Thus we discussed and clarified the recent use of Fourier transform techniques in studying stochastic reaction-diffusion systems (McKane & Newman, 2004). We illustrated the importance of understanding the dependence of the power spectra on the initial and final values and using the correct discrete expansion to compensate for the spatial boundary conditions. Using these ideas we were able to obtain a general formula for the temporal power spectrum of a one population system, which either evolves under steady or non-steady state dynamics, equation (5.57). This result can easily be generalised to

higher numbers of populations, although it may not have a closed form. These techniques were then further extended to the case of spatial systems and the application of the spatial Fourier cosine transform to a tridiagonal matrix, which introduced factors of $\cos(k\Delta_E)$.

Focusing on diffusion as the simplest example to which to apply the framework, we see that the analytically derived spatio-temporal power spectrum gives a good comparison to the averaged stochastic spectra as well as giving us insights into the expected results. The main aspect of note in this spectrum is the infinite power spike at $(0, 0)$. This is consistent with the intuitive approach that a system of diffusing particles are not correlated in time *i.e.* the system is memoryless. The spatial power spectrum of diffusion, equation (5.77), further confirms that the diffusing particles act as desired, showing that, in the long time limit, all wave modes are independent of time and that (except for the zero mode which has power, $P = 0$, since the mean-field solution is subtracted) all wave modes tend to have the exact same power. This flat frequency profile implies that no mode is dominant in the diffusing system and, as such, no stable patterns exist.

Having used the stochastic framework and Fourier transforms to confirm the properties of the diffusion equation, in the next chapter we extend these methods to encompass domain growth. Although this has been done before (Baker *et al.*, 2010), it has been found that the current framework does not have a well-defined thermodynamic limit. Thus, we intend to mimic the idea used in Chapter 2 and project the growing domain back onto a fixed domain. Although this does clear the problem of the limiting process, it does produce new complications. In particular, diffusion no longer acts as expected.

Chapter 6

Growth in stochastic systems

“What does chance ever do for us? In the human body, for instance, chance, i.e. the operation of causes without design, may produce a wen, a wart, a mole, a pimple, but never an eye”

William Paley, Natural Theology, 1809.

As discussed in Chapter 1, growth is of fundamental importance in early stages of development. As such, there has recently been interest in deriving a stochastic description of growth in the space-jump framework (Baker *et al.*, 2010) which is consistent with continuum theory (Crampin *et al.*, 1999). In the previous chapter we developed and clarified the Fourier transform and CME framework that was recently proposed (Lugo & McKane, 2008), which allowed us to analyse the spatio-temporal patterning capabilities of stochastic systems. Here, we aim to use the exact same techniques in order to characterise the effects of growth on diffusing systems. This chapter is based on work in Woolley *et al.* (2011a,b).

Growth has previously been defined through a box-splitting operation on the populations (Baker *et al.*, 2010). We denote this an Eulerian form of growth. Initially, if an interval $[0, L]$ is split into K boxes of length L/K , and the growth ‘reaction’ occurs in the j^{th} box, a new box of size L/K is created next to the j^{th} box, as in Figure 6.1, and the

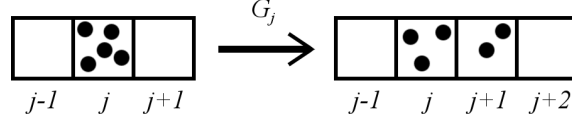


Figure 6.1: Diagram illustrating domain growth. If the j^{th} box divides then a new box is created next to the j^{th} box. The population in the j^{th} box is split as evenly as possible between the j^{th} and $j+1^{th}$ boxes and the rest of the populations are updated accordingly. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

populations are updated according to the rules:

$$\begin{aligned}
 U_K &\mapsto U_{K+1}, \\
 U_{K-1} &\mapsto U_K, \\
 &\vdots \\
 U_{j+1} &\mapsto U_{j+2}, \\
 U_j &\mapsto \lceil U/2 \rceil_j + \lfloor U/2 \rfloor_{j+1},
 \end{aligned} \tag{6.1}$$

where $\lfloor x \rfloor$ is the largest integer less than x and $\lceil x \rceil$ is the smallest integer greater than x . Note that various other splitting rules have been used, including splitting the population uniformly randomly over the two boxes, however, these have been seen to have little effect on the simulations (details not shown).

However, although microscopically this is a description of growth, the resulting CME that defines the operation does not have a weak noise limit. Because of this, we use representations analogous to those of Crampin *et al.* (1999) and map the growing Eulerian domain to a stationary Lagrangian domain where a weak noise limit can be prescribed.

Let x_E be the time-dependent Eulerian coordinate on a uniformly expanding, one-dimensional domain, $[0, L(t)]$. From Section 2.3.1 the mean-field diffusion equation then takes the form (Crampin, 2000; Crampin *et al.*, 1999; Crampin & Maini, 2001)

$$\frac{\partial \phi}{\partial t} + \frac{\partial}{\partial x_E} (a(x_E, t)\phi) = D \frac{\partial^2 \phi}{\partial x_E^2} \text{ on } [0, L(t)], \tag{6.2}$$

where $a(x_E, t)$ is the flow generated by the growth. Since growth is uniform we assume there exists a growth function, $n(t)$, such that $L(t) = L_0 n(t)$ and $n(0) = 1$. Thus we can

relate the Eulerian coordinates to a time-independent Lagrangian coordinate, x_L , through $x_E = x_L n(t)$. This then determines the local flow through

$$a(x_E, t) = \frac{\partial x_E}{\partial t}. \quad (6.3)$$

Converting to Lagrangian coordinates and dropping the subscript L ,

$$\frac{\partial \phi}{\partial t} + \frac{\dot{n}}{n} \phi = \frac{D}{n^2} \frac{\partial^2 \phi}{\partial x^2} \text{ on } [0, L_0]. \quad (6.4)$$

Finally, by rescaling $\phi_L = \phi n$ and, again, dropping the subscript L , we remove the dilution terms to obtain

$$\frac{\partial \phi}{\partial t} = \frac{D}{n^2} \frac{\partial^2 \phi}{\partial x^2} \text{ on } [0, L_0]. \quad (6.5)$$

Thus, in the mean-field, we simply reproduce the diffusion equation with a time-dependent diffusion coefficient, $D/n(t)^2$. We use this knowledge of the deterministic system to inform the transition rates, or ‘the rate of jumping between the discretised boxes’.

6.1 Lagrangian coordinates

So far we have been able to formulate expressions for the power spectra of stochastically diffusing populations using both spatial and temporal transforms. However, with the introduction of domain growth, the diffusion coefficient gains a time dependence, as shown in the previous section. This dependence does not take a simple form akin to those studied in Section 5.2.1.2 and, thus, temporal Fourier transforms have to be calculated numerically. However, since we are primarily considering stationary structures as models of inhomogeneous patterning we concentrate purely on spatial Fourier transform methods.

In order to model a time-dependent Eulerian domain we construct a fixed Lagrangian domain $[0, L]$ split into K boxes of length $\Delta_L = L/K$ (see Figure 6.2). Box i is then able to contain a number of further subdivisions, N_i , identified to be the number of Eulerian boxes in Lagrangian box i . As the domain evolves with stochastic fluctuations in the spatial variable the Lagrangian domain remains fixed by definition, with domain growth represented by alterations in the number of Eulerian boxes in each Lagrangian box. This

has the immediate interpretation of each Eulerian box corresponding to a biological cell (or group of cells), with the number of cells varying in time within a given Lagrangian element and thus inducing domain growth.

This microscopic variable, $N_i(t)$, is also controlled stochastically and is identified, as in Section 5.1.2, as a linear combination of a deterministic part, n_i , which defines the average ratio of the total size of the corresponding Eulerian sub-boxes, $N_i(t)\Delta_E$, at a time t to Lagrangian box size, Δ_L , and a stochastic part, $\epsilon_i(t)$, through

$$N_i = n_i\theta + \epsilon_i\sqrt{\theta}, \quad (6.6)$$

where

$$\theta \stackrel{def}{=} \frac{\Delta_L}{\Delta_E}, \quad (6.7)$$

is the scaling of Eulerian box length to Lagrangian box length. Initially, the $N_i(0)$ are taken to be equal for all i thus,

$$\Delta_L n_i(0) = \theta \Delta_E = N_i(0) \Delta_E, \quad (6.8)$$

(see Figure 6.2) and hence $n_i(0) = 1$ for all i . Note in the case that the domain growth can be assumed to be deterministic, we simply drop the ϵ_i variable or, more rigorously, let $\theta \rightarrow \infty$.

Similar to the Eulerian description in Section 5.1.2, we link the random variables $\mathbf{N} = (N_1, \dots, N_K)$ and $\mathbf{U} = (U_1, \dots, U_K)$ to $\boldsymbol{\epsilon} = (\epsilon_1, \dots, \epsilon_K)$ and $\boldsymbol{\eta} = (\eta_1, \dots, \eta_K)$ through the identification

$$P(\mathbf{U}, \mathbf{N}, t) = \Pi(\boldsymbol{\eta}, \boldsymbol{\epsilon}, t), \quad (6.9)$$

where $P(\mathbf{U}, \mathbf{N}, t)$ is, once again, the probability of being in state $(\mathbf{U}, \mathbf{N})$ at time t .

In the deterministic setting, we have shown that the effect of mapping a growing domain to a static one is simply realised as a scaling of the diffusion coefficient (Crampin *et al.*, 1999; Woolley *et al.*, 2011b). On expanding in the weak noise limit the leading order term is the deterministic equation; hence we require a stochastic domain growth generalisation of the jump rates, which in the deterministic growth scenario are given by

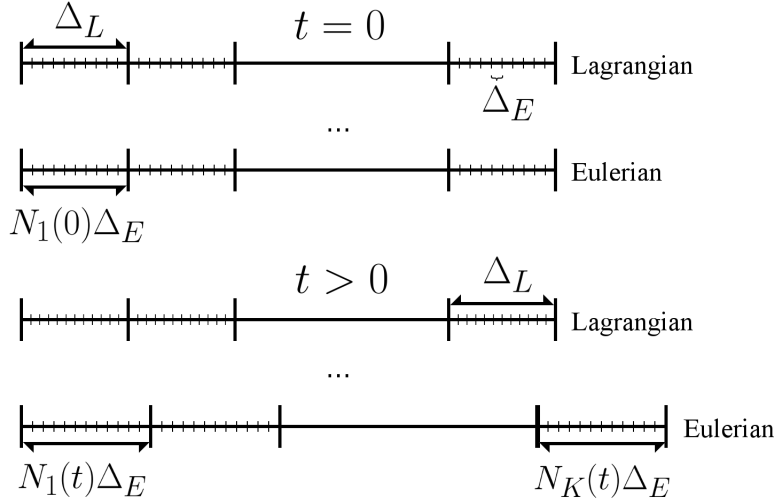


Figure 6.2: Illustrating the connection between the Lagrangian description of the domain and the corresponding Eulerian description. See text for details. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

$d_L = D/\Delta_L^2$ (see equation (6.5)).

The stochastic effects of the domain are then added, noting that

$$\lim_{\theta \rightarrow \infty} \frac{N_i(t)}{\theta} = n_i(t), \quad (6.10)$$

and substituting $n_i(t)$ for N_i/θ . However, since growth is assumed to be uniform, all of the Eulerian subdivisions, N_i , of a Lagrangian box i are controlled identically and so, in the mean-field, the $n_i(t)$ will be equal for all i . Thus, many forms of dependence of the diffusion coefficient on the spatial variables will give rise to equivalent forms of mean-field growth. Two particular examples are considered in Section 6.3.

Presently, we continue with a general diffusion coefficient, $d_{i,j}$, which is the transition rate from Lagrangian box i to Lagrangian box j such that $d_{i,j} \rightarrow d_L/n(t)^2$ as $\theta \rightarrow \infty$. Although $d_{i,j}$ is the transition rate between boxes i and j we do not explicitly define its dependence on the Eulerian populations. In particular, we do not know if the description is symmetric, *i.e.* $d_{i,j}$ may not be equal to $d_{j,i}$.

By suppressing the unchanged variables, the CME describing diffusion in the La-

grangian framework, on a stochastically growing domain, has the general form

$$\begin{aligned}
\frac{\partial P}{\partial t} = & \sum_{i=1}^{K-1} d_{i,i+1} ((U_i + 1)P(\dots, U_i + 1, U_{i+1} - 1, \dots) - U_i P) \\
& + \sum_{i=2}^K d_{i,i-1} ((U_i + 1)P(\dots, U_{i-1} - 1, U_i + 1, \dots) - U_i P) \\
& + \sum_{i=1}^K G_i(\mathbf{N} - \boldsymbol{\nu}_i)P(\mathbf{N} - \boldsymbol{\nu}_i) - G_i(\mathbf{N})P.
\end{aligned} \tag{6.11}$$

The first two lines of the summation describe right and left jumps, respectively. The last line defines growth through the increase in the number of subdivisions of each box. This feeds back to the diffusion terms through the dependence of the $d_{i,j}$ on N_i . In equation (6.11) the form of growth is left general through the use of the operator G_i . We now specify the growth to be uniformly linear with rate r *i.e.* the rate of increase in subdivisions, N_i , of Lagrangian box i is constant over time, hence, $G_i(\mathbf{N}) = r/\theta$. This is easily modified *mutatis mutandis*.

Approaching the CME through the formalism of Van Kampen's linear noise expansion method (van Kampen, 2007), gives

$$\begin{aligned}
\frac{\partial \Pi}{\partial t} - \sqrt{\Omega} \sum_{i=1}^K \frac{\partial \Pi}{\partial \eta_i} \frac{d\phi_i}{dt} - \sqrt{\theta} \sum_{i=1}^K \frac{\partial \Pi}{\partial \epsilon_i} \frac{dn_i}{dt} = & \sum_{i=1}^{K-1} d_{i,i+1} \frac{1}{\sqrt{\Omega}} \left(\frac{\partial}{\partial \eta_i} - \frac{\partial}{\partial \eta_{i+1}} \right) (U_i \Pi) \\
& + \frac{1}{2\Omega} \sum_{i=1}^{K-1} d_{i,i+1} \left(\frac{\partial}{\partial \eta_i} - \frac{\partial}{\partial \eta_{i+1}} \right)^2 (U_i \Pi) + \sum_{i=1}^{K-1} d_{i+1,i} \frac{1}{\sqrt{\Omega}} \left(-\frac{\partial}{\partial \eta_i} + \frac{\partial}{\partial \eta_{i+1}} \right) (U_{i+1} \Pi) \\
& + \frac{1}{2\Omega} \sum_{i=1}^{K-1} d_{i+1,i} \left(-\frac{\partial}{\partial \eta_i} + \frac{\partial}{\partial \eta_{i+1}} \right)^2 (U_{i+1} \Pi) - \sum_{i=1}^K \frac{1}{\sqrt{\theta}} \frac{r}{\theta} \frac{\partial \Pi}{\partial \epsilon_i} + \frac{1}{2\theta} \frac{r}{\theta} \sum_{i=1}^K \frac{\partial^2 \Pi}{\partial \epsilon_i^2}.
\end{aligned} \tag{6.12}$$

Since

$$\frac{N_i}{\theta} = n_i + \frac{\epsilon_i}{\sqrt{\theta}} \rightarrow n_i \text{ as } \theta \rightarrow \infty, \tag{6.13}$$

we use the approximation

$$\frac{1}{n_i(t) + \frac{\epsilon_i(t)}{\sqrt{\theta}}} \approx \frac{1}{n_i(t)} - \frac{\epsilon_i}{\sqrt{\theta} n_i(t)^2} + \frac{\epsilon_i^2}{\theta n_i(t)^3}. \tag{6.14}$$

This approximation allows us to explicitly consider the various orders of θ .

It should be noted that equation (6.14) holds only when $|\epsilon_i(t)| \ll \sqrt{\theta} n_i(t)$. Since we

are using a weak noise expansion, motivated by the central limit theorem, we assume that, at leading order, the noise will be Gaussian and able to take values of arbitrary size (van Kampen, 2007). However, $\langle \epsilon_i \rangle = 0$ and initially $\langle \epsilon_i^2 \rangle = 0$, since the starting conditions are assumed to be known. Through the Fokker-Planck equation, generated from the linear noise expansion, we are able to derive expressions for the evolution of the variance. Thus, we can put probabilistic bounds on how likely equation (6.14) is to hold at a certain time. This is done for the examples considered in Section 6.4.

Derivatives with respect to ϵ_i decouple from equation (6.12) and, thus, to first order in $\sqrt{\theta}$,

$$\dot{n}_i = \frac{r}{\theta}, \text{ for } i = 1, \dots, K. \quad (6.15)$$

Thus, $n_i(t) = n(t) = 1 + rt/\theta$, using the initial condition $n_i(0) = 1$. Similarly, by using equation (6.14), we can equate leading orders of $\sqrt{\Omega}$ to obtain

$$\frac{\partial \phi_1}{\partial t} = \frac{d_L}{n^2} (\phi_2 - \phi_1), \quad (6.16)$$

$$\frac{\partial \phi_i}{\partial t} = \frac{d_L}{n^2} (\phi_{i+1} - 2\phi_i + \phi_{i-1}), \text{ for } i = 2, \dots, K-1, \quad (6.17)$$

$$\frac{\partial \phi_K}{\partial t} = \frac{d_L}{n^2} (\phi_{K-1} - \phi_K). \quad (6.18)$$

Remembering that $n(t) = 1 + rt/\theta$ and defining $d_L = D/\Delta_L^2$, where D is the macroscopic diffusivity, we can see that on taking $K \rightarrow \infty$ or, equivalently, $\Delta_L \rightarrow 0$, we retrieve the discretised diffusion PDEs mapped from the growing Eulerian domain onto a stationary Lagrangian domain.

Having removed the leading order equations of Ω and θ we obtain terms of order $\sqrt{\Omega/\theta}$ which are exactly the terms that would arise from using Elf and Ehrenberg's matrix method (Elf & Ehrenberg, 2003), discussed in Section 5.1.2. The only generalisation being that the scaling factors, which normally cancel, remain. Thus the general Fokker-Planck equation for a diffusing species on a stochastically growing domain is

$$\frac{\partial \Pi}{\partial t} = - \sum_{i,j=1}^{2K} A_{ij} \frac{\partial}{\partial \zeta_i} (\zeta_j \Pi) \sqrt{\frac{\Theta_j}{\Theta_i}} + \frac{1}{2} \sum_{i,j=1}^{2K} B_{ij} \frac{\partial^2 \Pi}{\partial \zeta_i \partial \zeta_j} \frac{\Theta_j}{\Theta_i}, \quad (6.19)$$

where Θ_i is the scaling for the variable ζ_i . In the current case $\zeta_i = \eta_i$ and $\Theta_i = \Omega$ for

$i = 1, \dots, K$, whilst $\zeta_i = \epsilon_i$ and $\Theta_i = \theta$ for $i = K + 1, \dots, 2K$.

If $\theta \gg \Omega$ growth may be modelled deterministically. This case is considered in Section 6.2. Conversely, if $\theta \ll \Omega$ the noise generated through growth is far more important than that generated through population fluctuations. This case is not considered, since, biologically, we expect to be dealing with low population copy numbers and slow growth relative to diffusion. For simplicity, when stochastic growth is considered, we fix this ratio of nondimensionalised parameters to be one. In this case, stochastic effects from domain growth and the diffusing populations are of the same order and are, thus, important.

Currently, we are unable to link the Lagrangian density to the Eulerian scale since we assume that the particle population within a Lagrangian box is uniformly distributed. However, although we cannot directly link populations to a specific Eulerian box, we can link them to the large groups of Eulerian boxes which make up the Lagrangian boxes. Ultimately, because we can only rigorously link the meso- and macro-scopic scale our interpretation of the patterns must remain on this scale.

As mentioned, we use a basic SSA to simulate both the Eulerian and Lagrangian systems. Although we do not change the algorithm, our transformation from the Eulerian to the Lagrangian domain has the added bonus of producing quicker simulations. The Lagrangian population is generated by summing over an appropriate number of Eulerian populations. Thus, since we reduce the number of simulated populations, we decrease the time spent searching for the activated reaction. Further, by increasing the spatial scale we reduce the diffusion propensity function, which increases the possible simulated time steps. However, we do lose the microscopic detail of being able to link the particles to their Eulerian subdivisions of the Lagrangian domain. Hence, if a mesoscopic description of a considered system is appropriate, the benefits of the Lagrangian mapping are worth considering.

6.2 Spectra of diffusion on deterministically growing domains

In the case that the domain growth is deterministic the analysis still holds, the only specification being that all the ϵ_i are set to zero, thus, the Fokker-Planck equation (6.19)

reduces to a much simpler form as we do not need to consider the interaction of two different population scales. Further, by using the Lagrangian formulation of growth we are able to invoke results from Section 5.2, with the identifications that $\Delta_E \mapsto \Delta_L$ and $d_E \mapsto d_L/n(t)^2$. Thus, we can analytically derive the spatial Fourier cosine transformation of diffusion with the generalisation that the diffusion coefficient is now time-dependent,

$$\begin{aligned} \langle \hat{\eta}_k(t_1) \hat{\eta}_{k'}(t_2) \rangle &= \exp \left(- \left(C(k) \int_0^{t_1} \frac{1}{n(t')^2} dt' \right) - \left(C(k') \int_0^{t_2} \frac{1}{n(t')^2} dt' \right) \right) \\ &\times \frac{\Delta_L^2 K \phi}{2} \left(\exp \left(2C(k) \int_0^{\min(t_1, t_2)} \frac{1}{n(t')^2} dt' \right) - 1 \right) \delta_{k-k', 0}. \end{aligned} \quad (6.20)$$

This expression could be used to calculate the full power spectrum by the temporal Fourier transform, equation (5.44). However, the resulting double integral would have to be evaluated numerically. Since we are primarily interested in systems that tend to a temporal steady state, but may have inhomogeneous spatial behaviour, our main concern lies with the spatial Fourier cosine transform which produces a complete, closed solution.

By varying the growth law we are able to derive the spatial power spectra of exponential growth, $n = e^{rt}$,

$$P_s(k, t) \stackrel{def}{=} \frac{\langle |\hat{\eta}_k(t)|^2 \rangle}{\Delta_L^2 K \phi} = \frac{1}{2} \left(1 - \exp \left(\frac{2d_L}{r} (1 - \exp(-2rt)) (\cos(k\Delta_L) - 1) \right) \right), \quad (6.21)$$

$$P_s(k) = \lim_{t \rightarrow \infty} P_s(k, t) = \frac{1}{2} \left(1 - \exp \left(\frac{2d_L}{r} (\cos(k\Delta_L) - 1) \right) \right), \quad (6.22)$$

linear growth, $n = 1 + tr/\theta$,

$$P_s(k, t) = \frac{1}{2} \left(1 - \exp \left(\frac{4d_L\theta}{r} \left(1 - \left(1 + \frac{r}{\theta}t \right)^{-1} \right) (\cos(k\Delta_L) - 1) \right) \right), \quad (6.23)$$

$$P_s(k) = \lim_{t \rightarrow \infty} P_s(k, t) = \frac{1}{2} \left(1 - \exp \left(\frac{4d_L\theta}{r} (\cos(k\Delta_L) - 1) \right) \right), \quad (6.24)$$

and no growth, $n = 1$,

$$P_s(k) = \lim_{t \rightarrow \infty} P_s(k, t) = \frac{1}{2} (1 - \exp(4d_L t (\cos(k\Delta_L) - 1))), \quad (6.25)$$

$$\lim_{t \rightarrow \infty} P_s(k) = \begin{cases} 0 & \text{if } k = 0, \\ \frac{1}{2} & \text{if } k \neq 0, \end{cases} \quad (6.26)$$

which recapitulates the results in Chapter 5. From these expressions we note that, by reducing the growth rate, r , we reduce the difference between the growth and static domain spectra, as expected.

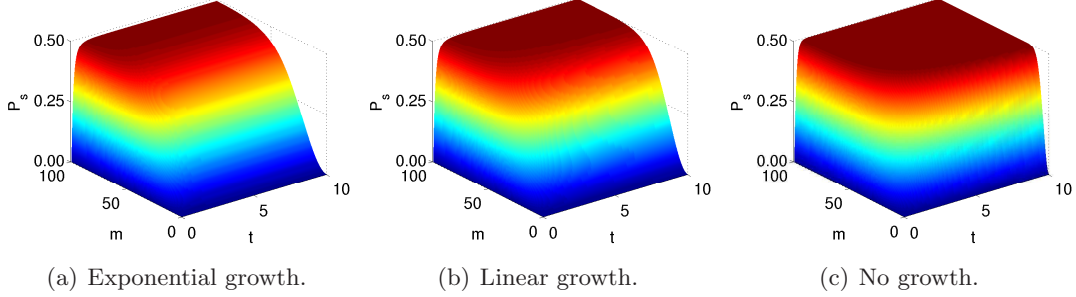


Figure 6.3: Spatial power spectra of diffusion on exponentially and linearly growing domains, compared to that of a static domain. For exponential growth $r = 1$, for linear growth $r/\theta = 1$ and for all simulations $\Delta_L = 1/100$ and $d_L = 1$. Note that $k = m\pi/L$ and $L = 1$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. *Phys. Rev. E*, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

From Figure 6.3, we see that growth affects the active spatial frequencies of diffusion. In the static domain case, all non-zero wavelengths will eventually have the same strength, as can be seen from equation (6.26), thus no spatial frequency can dominate. However, with linear and exponential growth, the lower spatial frequencies will have greatly reduced power for all time. Thus, we expect to see consistent spatial heterogeneity occurring where growth is relatively fast.

Individual stochastic realisations of diffusion on domains undergoing deterministic linear growth with different rates of growth are shown in Figure 6.4. The simulations show many transient inhomogeneous states but, by considering Figure 6.4(e), we clearly see consistently high population values in certain Lagrangian boxes lasting approximately 200 or more simulated time units which are not seen in the static domain case. This is corroborated by Figure 6.4(f), which shows that certain spatial frequencies, $m = 2, 3$ and 4, are being excited for long periods of time.

This implies that, for single realisations of the system, certain wavelengths will be excited. However, these wavelengths are chosen randomly amongst all the possible excited frequencies since we expect an averaged ensemble to smooth out individual peaks. This idea is best seen when the system undergoes extremely fast growth, or equivalently, for large values of r . In Figure 6.5(a) $r = 100$ and we see that, once a heterogeneous state is

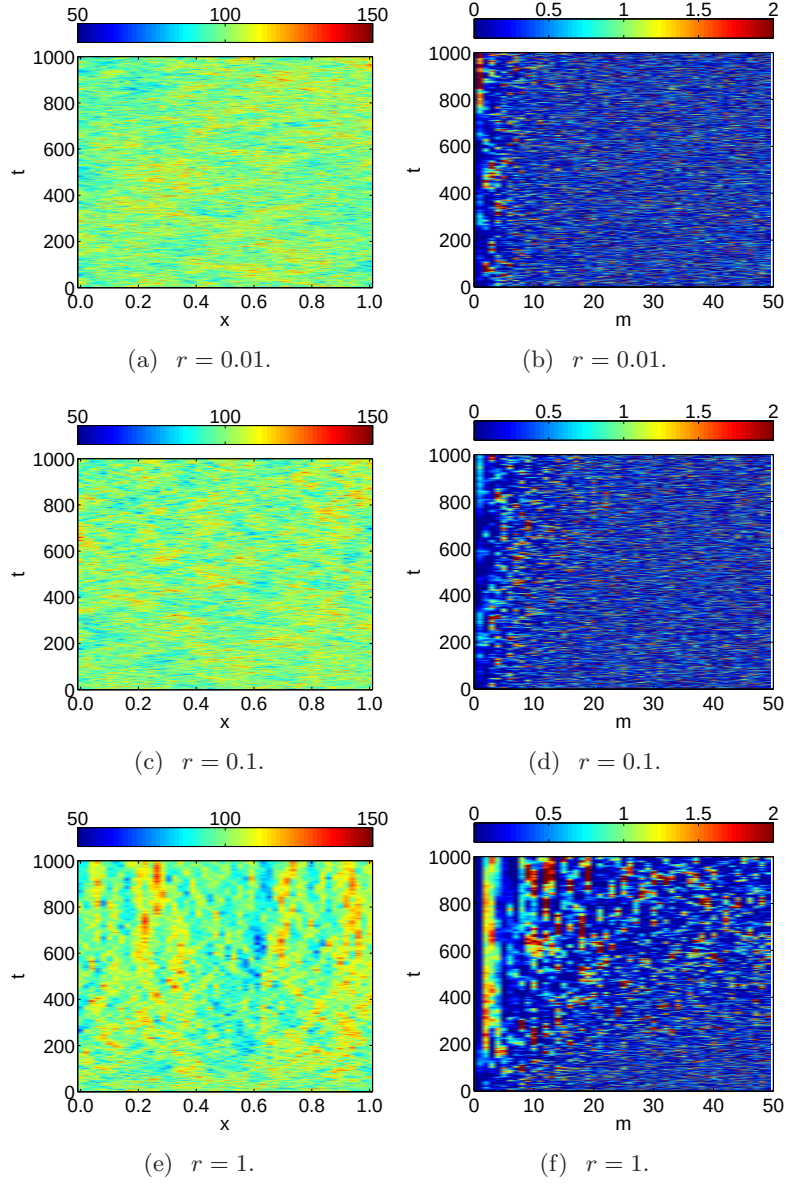


Figure 6.4: Left column: single realisations of a diffusion process on a linearly growing domain (mapped on to the Lagrangian domain) for different rates of growth. Initially there were 50 Lagrangian boxes, each containing 100 simulated Eulerian boxes and each Lagrangian box contained 100 particles. The growth rate r is noted beneath each figure, $\Delta_L = 1/50$ and $d_L = 1$. Right column: Spatial Fourier cosine transformation of the realisations in the left column. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

reached, it lasts for all remaining simulation time. Thus, although for a single simulation, as in Figure 6.5(a), definite wavelengths set in (see Figure 6.5(b)), when an ensemble of solutions are averaged, as in Figure 6.5(d), they produce a power spectrum that matches the analytical solution, Figure 6.5(c), as expected.

The reason behind this persistent heterogeneity is understandable; initially the domain is quite small, so diffusion is able to carry the particles between the Lagrangian boxes and, due to the stochasticity of the simulated diffusion process, it is highly likely that the system will evolve to an inhomogeneous state. If the system is not growing then this state is transient. However, in the case of fast growth, the system grows quicker than diffusion can transport particles. Thus, each Lagrangian box effectively becomes decoupled from the rest of the system, causing any effects of spatial noise to be fixed for the rest of the simulation.

Specifically, we can capture this reasoning, in the linear growth case, by using the non-dimensional parameter grouping $d_L\theta/r$. By considering equation (6.24) and by inspection of Figure 6.4 we see that the persistent peaks set in for $r > 0.1$. Thus, for

$$\frac{d_L\theta}{r} < 1000, \quad (6.27)$$

we would expect non-transient heterogeneities to form. Otherwise diffusion will lead to homogeneity. Thus, the stochastic structures depend on the balance of diffusion and growth, as expected. Due to this ratio being based on observation, equation (6.27) should only be used as an approximate value. Other growth forms will yield similar non-dimensional parameter groupings.

To see that equation (6.27) is consistent with our physical intuition we consider approximate orders of realistic physical quantities. Using $D = 10^{-6} \text{ cm}^2/\text{s}$ (Dillon *et al.*, 2003) and taking the view that each Lagrangian box, $\Delta_L=1\text{mm}$, is made up of a number of Eulerian subdivisions, which can be identified with the size of a cell, $\Delta_E = 10\mu\text{m}$ (Alberts *et al.*, 2008), we obtain

$$\frac{D\theta}{1000\Delta_L^2} = \frac{10^{-10} \times 10^2}{10^3 \times 10^{-6}} = 10^{-5}/s < r. \quad (6.28)$$

Since 10^5 seconds is approximately one day this suggests that if a substance is diffusing in a one-dimensional tissue which is uniformly growing faster than one cell per day then we would expect inhomogeneities to form.

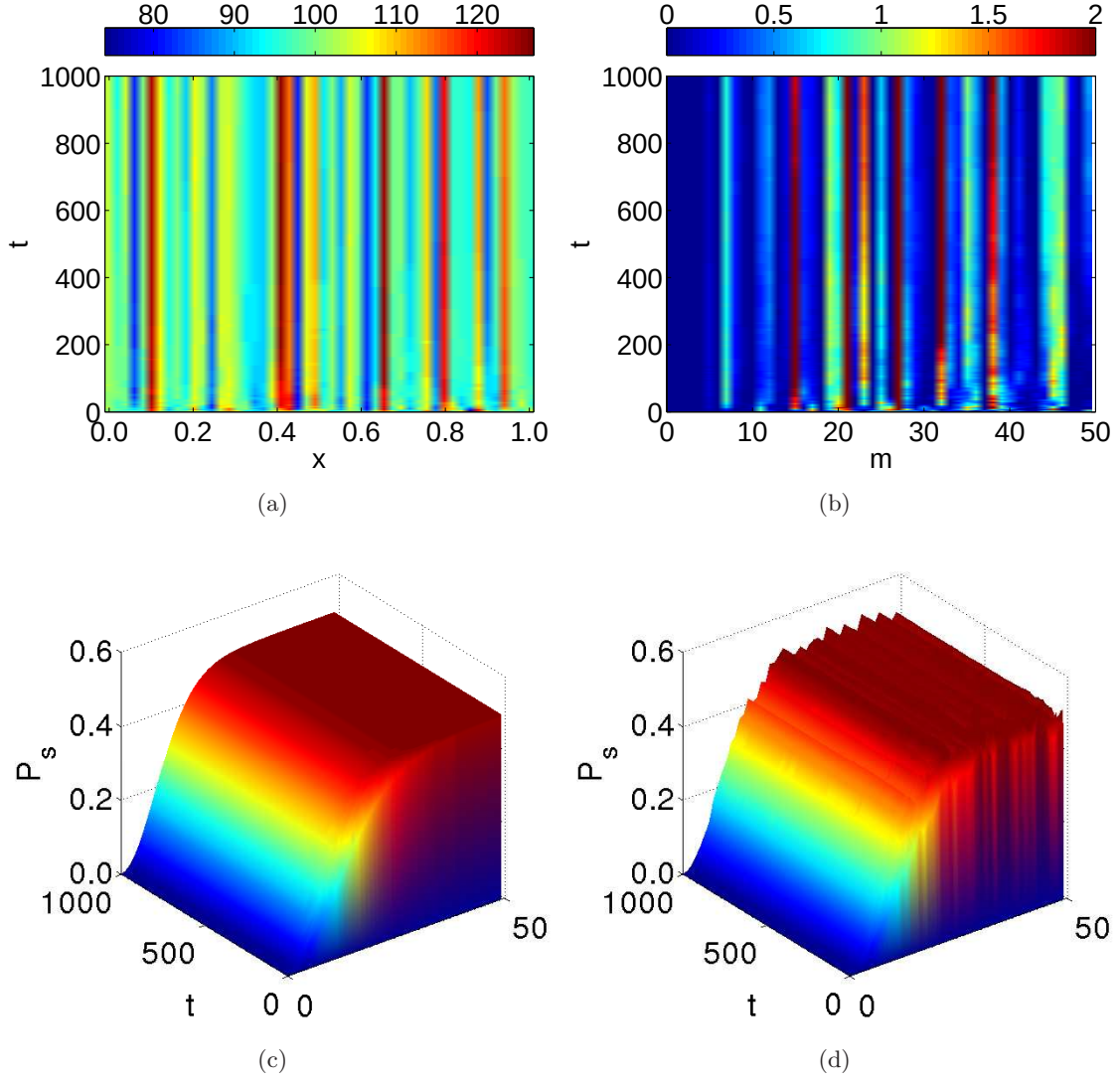


Figure 6.5: (a) A single realisation of a diffusion process on a fast linearly growing domain. The fixed pattern is indicative of the heterogeneities formed. (b) Power spectrum of the simulation in (a). Note that certain spatial frequencies are more excited than others. (c) Analytic power spectrum of diffusion on a linearly growing domain, equation (6.24). (d) 1000 averaged power spectra derived from stochastic simulations of diffusion on a linearly growing domain. Parameters are $d_L = 1$, $\Delta_L = 1/50$, $r = 100$, $k = m\pi/L$, $L = 1$ and $\theta = 100$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 79(2), 2011. “Copyright 2011 by the American Physical Society.”

6.3 Different growing domain descriptions

Growth has previously been defined through a box-splitting operation on the populations (Baker *et al.*, 2010). Initially, if an interval $[0, L]$ is split into K boxes of length L/K , and the growth ‘reaction’ occurs in the j^{th} box, a new box of size L/K is created next to the j^{th} box and the parent population is split between these two new daughter boxes. Various splitting rules have been used, including splitting the population uniformly randomly over the two boxes, however, these have been seen to have little effect on the stochastic simulations.

However, although microscopically this is a description of growth, the resulting CME that defines the operation does not have a weak noise limit, which is why we have constructed the Lagrangian domain. Due to this lack of a link between the scales, we cannot derive the spatial dependence of the Lagrangian transition rates from the Eulerian diffusion transition rates. In Section 6.2, the specific dependence of the space-jump process on the discretisation of the domain did not matter as the growth was deterministic and, thus, the Lagrangian boxes were identical. In this section we will show that the dependence of the diffusion coefficient on the spatial variables can have important consequences for the outcome of the simulations, which can be characterised by the different shapes of their power spectra.

6.3.1 Calculation of transition rates

Since there is currently a non-uniqueness in the transition rate description we will consider the following two types of Lagrangian diffusion (see Figure 6.6 for a schematic diagram of the two types):

1. Jumping from the midpoint of a Lagrangian box to the midpoint of an adjoining Lagrangian box. We call this midpoint to midpoint diffusion (MMD). The justification for this description of diffusion is that we expect the particles to be uniformly distributed, due to the well-mixed assumption. Thus, we suppose that we can model diffusion as jumping between the Lagrangian centroids, due to the symmetry of density about this point.
2. Jumping a distance equal to the current Lagrangian box length from the midpoint

of the Lagrangian box to an adjoining Lagrangian box. We call this approximate midpoint to midpoint diffusion (AMMD). The justification of this is that, since growth is uniform on average, the N_i should all be approximately equal, given a homogeneous initial condition. Thus moving a distance equal to the Lagrangian box from the centre of the box would move the particle to approximately the centre of the adjoining Lagrangian box.

Note that we are not saying that AMMD is a better description of diffusion than MMD, which is a natural description to use. We use AMMD simply because it is a different form of transition rate dependence that produces contrasting effects at the population level, even though it should approximate the action of MMD.

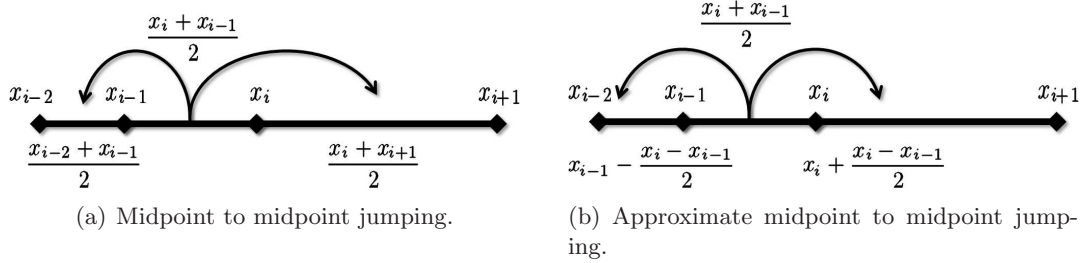


Figure 6.6: Illustration of different jumping paradigms. Note that initially each Lagrangian box will be the same size, but, due to stochastic growth, it is highly likely that the boxes will be perturbed away from the average length. Thus, the small, medium and large sizes are for illustrative purposes only. See text for details. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 84(4), 2011. “Copyright 2011 by the American Physical Society.”

The transition rate is the conditional probability of jumping to a particular adjoining box per unit time. Thus, one possible way of deriving the transition rates is to first calculate the expected first exit time of a particle in a domain and then condition it based on the probability of either a left or right transition.

Although the first exit time will have a distribution, we appeal to the central limit theorem (Grimmett & Stirzaker, 2001) and assume that we are using sufficiently large populations of particles to justify the claim that the first exit time will be close to the mean and have small variance. This assumption also needs to be fulfilled in order to use the linear noise expansion in Section 5.1, where we need Ω to be large enough to justify the separation of various orders.

Although other approaches are possible (Kinateder, 2001; Redner, 2001; van Kampen, 2007), we use Dynkin's formula applied to the case of diffusion (Øksendal, 2003) to derive the exit time. The expected first exit time, $\langle \tau \rangle$, of a random walk with diffusion coefficient D , from the interval $[-R, R]$ starting at a point $a \in [-R, R]$ is given by

$$2D\langle \tau \rangle = R^2 - a^2. \quad (6.29)$$

Hence, for the case of MMD, we start at $(x_i + x_{i-1})/2$ and we want to know when we first leave the region $[(x_{i-2} + x_{i-1})/2, (x_i + x_{i+1})/2]$. Translating these points to be around zero gives

$$R = \frac{1}{2} \left(\frac{x_i + x_{i+1}}{2} - \frac{x_{i-2} + x_{i-1}}{2} \right) = \frac{x_i + x_{i+1} - x_{i-2} - x_{i-1}}{4}, \quad (6.30)$$

$$a = \frac{x_i + x_{i-1}}{2} - \frac{1}{2} \left(\frac{x_i + x_{i+1}}{2} + \frac{x_{i-2} + x_{i-1}}{2} \right) = \frac{x_i + x_{i-1} - x_{i-2} - x_{i+1}}{4}. \quad (6.31)$$

Thus,

$$2D\langle \tau \rangle = \frac{(x_{i+1} - x_{i-1})(x_i - x_{i-2})}{4}. \quad (6.32)$$

Hence, $1/\langle \tau \rangle$ is the expected rate of leaving the interval. However, this rate has not been conditioned on to which adjoining Lagrangian box the particle diffuses.

By considering a simple random walk process the probability of hitting the left-hand boundary first, when starting at a , is (Grimmett & Stirzaker, 2001)

$$p_l = \frac{R - a}{2R} = \frac{x_{i+1} - x_{i-1}}{x_i + x_{i+1} - x_{i-2} - x_{i-1}}. \quad (6.33)$$

Similarly, the probability of hitting the right-hand boundary first is

$$p_r = \frac{R + a}{2R} = \frac{x_i - x_{i-2}}{x_i + x_{i+1} - x_{i-2} - x_{i-1}}. \quad (6.34)$$

Combining these probabilities with the transition rates gives the conditional transition rates of jumping left, l_i , and jumping right, r_i , from the midpoint of a Lagrangian box to

the midpoint of the adjoining Lagrangian box:

$$l_i = \frac{p_l}{\langle \tau \rangle} = \frac{8D}{(x_i - x_{i-2})(x_i + x_{i+1} - x_{i-2} - x_{i-1})}, \quad (6.35)$$

$$r_i = \frac{p_r}{\langle \tau \rangle} = \frac{8D}{(x_{i+1} - x_{i-1})(x_i + x_{i+1} - x_{i-2} - x_{i-1})}. \quad (6.36)$$

The case of AMMD is treated similarly:

$$R = x_i - x_{i+1}, \quad (6.37)$$

$$a = 0, \quad (6.38)$$

$$2D\langle \tau \rangle = (x_i - x_{i+1})^2, \quad (6.39)$$

$$p_l = \frac{1}{2}, \quad (6.40)$$

$$p_r = \frac{1}{2}. \quad (6.41)$$

We then extend these descriptions to the case of a stochastically growing domain and use $(x_i - x_{i-1}) = \Delta_E N_i$. Remembering that $\theta = \Delta_L/\Delta_E$ and $D = d_L \Delta_L^2$ we can derive the following expressions for the dependency of stochastic diffusion on growth.

MMD transition rates from Lagrangian box i :

$$l_i = \frac{8d_L}{\frac{1}{\theta^2}(N_i + N_{i-1})(N_{i-1} + 2N_i + N_{i+1})}, \quad (6.42)$$

$$r_i = \frac{8d_L}{\frac{1}{\theta^2}(N_i + N_{i+1})(N_{i-1} + 2N_i + N_{i+1})}. \quad (6.43)$$

AMMD transition rates from Lagrangian box i :

$$l_i = \frac{d_L}{\frac{1}{\theta^2}N_i^2}, \quad (6.44)$$

$$r_i = \frac{d_L}{\frac{1}{\theta^2}N_i^2}. \quad (6.45)$$

6.3.2 Comparison of different transition rates

Since $N_i = \theta n_i + \sqrt{\theta} \epsilon_i$, we see that all of the transition rates derived in the last section will give rise to the same mean-field description of diffusion on a growing domain as $\theta \rightarrow \infty$. Although these two descriptions are equivalent in the limit of large θ , Figure 6.7 shows

that AMMD leads to more consistent local maxima and minima than MMD, *i.e.* stripes, which are not apparent in figures 6.7(a) and 6.7(c), are visible in figures 6.7(b) and 6.7(d).

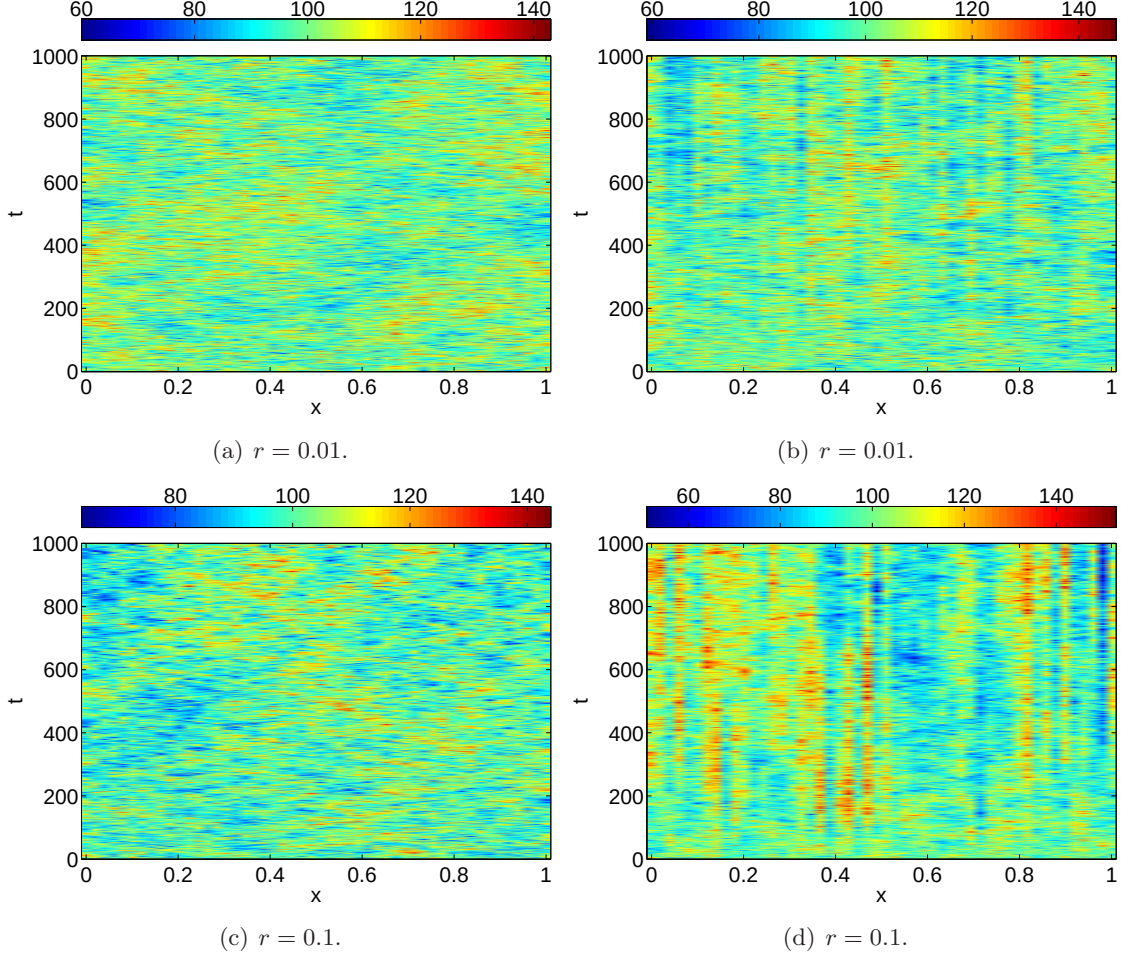


Figure 6.7: Single realisations, comparing different forms of Lagrangian diffusion on linearly growing domains. Left column: MMD, the time dependence of the transition rate is defined by equations (6.42) and (6.43). Right column: AMMD, the time dependence of the transition rate is defined by equations (6.44) and (6.45). In each case the domain is growing linearly with rate r given under each figure. Other parameters are $d_L = 1$, $K = 50$ and $\theta = 100$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 84(4), 2011. “Copyright 2011 by the American Physical Society.”

These extrema form under equations (6.44) and (6.45) and not equations (6.42) and (6.43) due to the different dependencies on the spatial variables. Notice in MMD that the effect of having a local extremum of Eulerian subdivisions, N_i , is reduced. This is due to the local number of subdivisions being averaged against the number of Eulerian subdivisions in the adjacent boxes, N_{i-1} and N_{i+1} . However, now consider AMMD and

suppose a particular Lagrangian box, i , contains a local maximum of Eulerian subdivisions, N_i . By assumption, the transition rate out of this box will be smaller than the transition rates into the box, *i.e.*

$$\frac{d_L}{N_i(t)^2/\theta^2} < \frac{d_L}{N_{i+1}(t)^2/\theta^2} \text{ and } \frac{d_L}{N_i(t)^2/\theta^2} < \frac{d_L}{N_{i-1}(t)^2/\theta^2}. \quad (6.46)$$

Hence, more particles flow into the box producing a local maximum of the population, U_i . Further, this maximum will last as long as the box contains a local maximum of Eulerian boxes. Conversely, if the Eulerian population is a local minimum, the transition rates out of the box will be higher than the transition rates into the box. Thus, the box will tend to a population minimum.

As a final comment we emphasise that this heterogeneity is not simply due to the description of diffusion we are using. The stochasticity is critical in the nucleation of the stripes, as it is the varying number of subdivisions that creates the local extrema. This, in turn, reinforces the asymmetry of the descriptions, as shown in equation (6.46). In using the weak noise expansion we assume that the number of Eulerian boxes per Lagrangian box is large enough such that the stochastic effects are of lower order and, thus, the size of the Lagrangian boxes should differ only by a small amount. Hence, we are illustrating that these small differences on the Eulerian scale can have large effects on the Lagrangian population.

When we were considering the basic case of our stochastic description of diffusion on a static or deterministically growing domain the matrices $\mathbf{A}$ and $\mathbf{B}$ were $K \times K$ tridiagonal. Now, we have the additional population of Lagrangian subdivisions, N_i , thus, the matrices will be $2K \times 2K$ in size. Generally, they will be $pK \times pK$ in size where p is the number of distinct, spatially extended, reacting species. This $2K \times 2K$ (more generally $pK \times pK$) matrix can be split up into $K \times K$ sub-matrices, which can be linked to the covariances of each of the species, with each sub-matrix having strong diagonal features. These diagonal features are discussed in the following section.

6.4 Fourier analysis of additional reactions

In this section we methodically apply the developed Fourier techniques to the case of MMD on a linearly growing domain, with rate of growth r . The case of AMMD is similar and discussed later. First, we define the stoichiometric matrix and the macroscopic propensity functions:

$$\nu = \left(\begin{array}{ccccc|ccccc|cccc} \text{Right jump} & & & & & \text{Left jump} & & & & & \text{Growth} & & & & \\ \hline -1 & 0 & \cdots & 0 & 0 & 0 & 1 & 0 & \cdots & 0 & 0 & \cdots & \cdots & 0 & \text{Effect on} \\ 1 & \ddots & \ddots & \vdots & \vdots & \vdots & -1 & \ddots & \ddots & \vdots & \vdots & \ddots & \ddots & \vdots & \text{diffusing} \\ 0 & \ddots & \ddots & 0 & \vdots & \vdots & 0 & \ddots & \ddots & 0 & \vdots & \ddots & \ddots & \vdots & \text{population} \\ \vdots & \ddots & \ddots & -1 & \vdots & \vdots & \vdots & \ddots & \ddots & 1 & \vdots & \ddots & \ddots & \vdots & \\ 0 & \cdots & 0 & 1 & 0 & 0 & 0 & \cdots & 0 & -1 & 0 & \cdots & \cdots & 0 & \\ \hline 0 & \cdots & \cdots & \cdots & 0 & 0 & \cdots & \cdots & \cdots & 0 & 1 & 0 & \cdots & 0 & \text{Effect on} \\ \vdots & \cdots & \cdots & \cdots & \vdots & \vdots & \cdots & \cdots & \cdots & \vdots & 0 & \ddots & \ddots & \vdots & \text{Eulerian} \\ \vdots & \cdots & \cdots & \cdots & \vdots & \vdots & \cdots & \cdots & \cdots & \vdots & \vdots & \ddots & \ddots & 0 & \text{boxes} \\ 0 & \cdots & \cdots & \cdots & 0 & 0 & \cdots & \cdots & \cdots & 0 & 0 & \cdots & 0 & 1 & \end{array} \right), \quad (6.47)$$

$$\left(\begin{array}{c} \frac{4d_L\phi_1}{(n_1+n_2)^2}, \quad \frac{8d_L\phi_2}{(n_2+n_3)(n_1+2n_2+n_3)}, \quad \cdots, \quad \frac{8d_L\phi_{K-1}}{(n_{K-1}+n_K)(n_{K-2}+2n_{K-1}+n_K)}, \quad \frac{4d_L\phi_K}{(n_K+n_{K-1})^2}, \\ \frac{4d_L\phi_1}{(n_1+n_2)^2}, \quad \frac{8d_L\phi_2}{(n_2+n_1)(n_1+2n_2+n_3)}, \quad \cdots, \quad \frac{8d_L\phi_{K-1}}{(n_{K-1}+n_{K-2})(n_{K-2}+2n_{K-1}+n_K)}, \quad \frac{4d_L\phi_K}{(n_K+n_{K-1})^2}, \\ \frac{r}{\theta}, \quad \cdots, \quad \frac{r}{\theta} \end{array} \right) = \mathbf{a}^T. \quad (6.48)$$

The stoichiometric matrix has been split into six sub-matrices. Each sub-matrix is $K \times K$ in size and three of these are non-zero. The first $K \times K$ elements are the population change vectors of transitions to the right. Similarly the second sub-matrix is comprised of the transition vectors of jumps to the left. The last non-zero sub-matrix controls the change of Eulerian subdivisions in each Lagrangian box. In this case we are dealing with linear growth of Eulerian populations with rate r . The zeros in the rest of the sub-matrices show that diffusion does not alter the number of Eulerian subdivisions and, similarly, the Eulerian subdivisions do not affect the diffusing populations except through the transition

rate dependencies.

The propensity vector has also been split into the three types of reactions in order to aid visualisation. The first and second lines are the macroscopic propensities of jumps right and left. The final line contains the propensity functions describing linear growth. Note the scaling of θ is needed because linear growth is a zeroth order reaction.

We can now calculate the matrices $\mathbf{A}$ and $\mathbf{B}$ using their definitions, equations (5.28) and (5.29). The matrices will have the following forms

$$\mathbf{A} = \begin{pmatrix} \mathbf{a} & \mathbf{b} \\ \mathbf{c} & \mathbf{d} \end{pmatrix}, \quad \mathbf{B} = \begin{pmatrix} \boldsymbol{\alpha} & \boldsymbol{\beta} \\ \boldsymbol{\beta} & \boldsymbol{\gamma} \end{pmatrix}, \quad (6.49)$$

where all the sub-matrices are symmetric. Further, $\mathbf{c} = \mathbf{d} = \boldsymbol{\beta} = \mathbf{0}$, $\boldsymbol{\gamma} = (r/\theta)\mathbf{I}$, where $\mathbf{I}$ is the $K \times K$ identity matrix and $\mathbf{a}$ and $\boldsymbol{\alpha}$ are tridiagonal matrices with diagonal entries $a_0 = -2d_L/n^2$, $\alpha_0 = 4d_L\phi/n^2$ and off-diagonal entries $a_1 = d_L/n^2$, $\alpha_1 = -2d_L\phi/n^2$, respectively, where $n = 1 + rt/\theta$.

The form of $\mathbf{b}$ is pentagonal. To see this, consider the i^{th} ($2 < i < K - 1$) population, U_i . U_i will depend on the transition of particles out of the i^{th} box, l_i and r_i , as well as the transition of particles from the $(i - 1)^{th}$ and $(i + 1)^{th}$ boxes into the i^{th} box, namely r_{i-1} and l_{i+1} . By considering equations (6.42) and (6.43), we see that l_{i+1} , l_i , r_i and r_{i-1} will depend on the variables n_{i-2} , n_{i-1} , n_i , n_{i+1} and n_{i+2} . Thus, when the derivative is taken with respect to each variable to form $\mathbf{A}$, there will be at most five non-zero terms, all symmetric about the diagonal. Fortunately, the sub- and super-diagonals of $\mathbf{b}$ reduce to zero. Hence, the only non-zero terms of $\mathbf{b}$ are the diagonal, second-sub- and

second-super-diagonal. The explicit form of $\mathbf{b}$ is

$$\mathbf{b} = \begin{pmatrix} \frac{d_L \phi}{4n^3} & 0 & -\frac{d_L \phi}{4n^3} & 0 & \dots & \dots & 0 \\ 0 & \frac{d_L \phi}{4n^3} & 0 & -\frac{d_L \phi}{4n^3} & 0 & \dots & 0 \\ -\frac{d_L \phi}{4n^3} & 0 & \frac{d_L \phi}{2n^3} & 0 & -\frac{d_L \phi}{4n^3} & \ddots & \vdots \\ 0 & \ddots & \ddots & \ddots & \ddots & \ddots & 0 \\ \vdots & \ddots & -\frac{d_L \phi}{4n^3} & 0 & \frac{d_L \phi}{2n^3} & 0 & -\frac{d_L \phi}{4n^3} \\ 0 & \dots & 0 & -\frac{d_L \phi}{4n^3} & 0 & \frac{d_L \phi}{4n^3} & 0 \\ 0 & \dots & \dots & 0 & -\frac{d_L \phi}{4n^3} & 0 & \frac{d_L \phi}{4n^3} \end{pmatrix}. \quad (6.50)$$

Using these forms of $\mathbf{A}$ and $\mathbf{B}$ we are able to derive equations for the corresponding general Fokker-Planck equation for the variables η_i, ϵ_i , where

$$U_i = \Omega \phi_i + \sqrt{\Omega} \eta_i, \quad (6.51)$$

$$N_i = \theta n_i + \sqrt{\theta} \epsilon_i. \quad (6.52)$$

Remember that, in order to simplify the algebra, we set $\theta = \Omega$. The general Fokker-Planck equation is thus,

$$\begin{aligned} \frac{\partial \Pi}{\partial t} = & - \sum_{i,j} \mathbf{a}_{ij} \frac{\partial}{\partial \eta_i} (\eta_j \Pi) - \sum_{i,j} \mathbf{b}_{ij} \frac{\partial}{\partial \eta_i} (\epsilon_j \Pi) - \sum_{i,j} \mathbf{c}_{ij} \frac{\partial}{\partial \epsilon_i} (\eta_j \Pi) - \sum_{i,j} \mathbf{d}_{ij} \frac{\partial}{\partial \epsilon_i} (\epsilon_j \Pi) \\ & + \frac{1}{2} \sum_{i,j} \alpha_{ij} \frac{\partial^2 \Pi}{\partial \eta_i \partial \eta_j} + \sum_{i,j} \beta_{ij} \frac{\partial^2 \Pi}{\partial \eta_i \partial \epsilon_j} + \frac{1}{2} \sum_{i,j} \gamma_{ij} \frac{\partial^2 \Pi}{\partial \epsilon_i \partial \epsilon_j}. \end{aligned} \quad (6.53)$$

From equation (6.53) we are able to derive the covariances (van Kampen, 2007),

$$\langle \dot{\eta}_l \eta_m \rangle = \sum_j \mathbf{a}_{lj} \langle \eta_j \eta_m \rangle + \sum_j \mathbf{a}_{mj} \langle \eta_l \eta_j \rangle + \sum_j \mathbf{b}_{lj} \langle \epsilon_j \eta_m \rangle + \sum_j \mathbf{b}_{mj} \langle \eta_l \epsilon_j \rangle + \alpha_{lm}, \quad (6.54)$$

$$\langle \dot{\eta}_l \epsilon_m \rangle = \sum_j \mathbf{a}_{lj} \langle \eta_j \epsilon_m \rangle + \sum_j \mathbf{b}_{lj} \langle \epsilon_j \epsilon_m \rangle + \sum_j \mathbf{c}_{mj} \langle \eta_l \eta_j \rangle + \sum_j \mathbf{d}_{mj} \langle \eta_l \epsilon_j \rangle + \beta_{lm}, \quad (6.55)$$

$$\langle \dot{\epsilon}_l \epsilon_m \rangle = \sum_j \mathbf{c}_{lj} \langle \eta_j \epsilon_m \rangle + \sum_j \mathbf{c}_{mj} \langle \epsilon_l \eta_j \rangle + \sum_j \mathbf{d}_{lj} \langle \epsilon_j \epsilon_m \rangle + \sum_j \mathbf{d}_{mj} \langle \epsilon_l \epsilon_j \rangle + \gamma_{lm}, \quad (6.56)$$

where $\dot{} = \partial/\partial t$. Due to linearity and the highly-banded, symmetric nature of matrices $\mathbf{A}$

and $\mathbf{B}$ we are immediately able to take the Fourier cosine transform of these equations (see Woolley *et al.* (2011b) for detailed derivations). Note that, although we have not considered the case of a pentagonal matrix explicitly, it is a simple extension to show that the resulting Fourier transform can be generalised to

$$b_0 + 2 \cos(k\Delta_E)b_1 + 2 \cos(2k\Delta_E)b_2, \quad (6.57)$$

where b_0 are the diagonal elements, b_1 are the super- and sub-diagonal elements and b_2 are the second-sub- and second-super-diagonal elements.

Using the forms of the various sub-matrices discussed above yields

$$\begin{aligned} \langle \hat{\eta}_k \dot{\hat{\eta}}_k \rangle &= (4a_1 \cos(k\Delta_L) + 2a_0) \langle \hat{\eta}_k \hat{\eta}_k \rangle + (4b_2 \cos(2k\Delta_L) + 2b_0) \langle \hat{\eta}_k \hat{\epsilon}_k \rangle \\ &\quad + \frac{\Delta_L^2 K}{2} (\alpha_0 + 2 \cos(k\Delta_L) \alpha_1), \end{aligned} \quad (6.58)$$

$$\langle \hat{\eta}_k \dot{\hat{\epsilon}}_k \rangle = (2a_1 \cos(k\Delta_L) + a_0) \langle \hat{\eta}_k \hat{\epsilon}_k \rangle + (2b_2 \cos(2k\Delta_L) + b_0) \langle \hat{\epsilon}_k \hat{\epsilon}_k \rangle, \quad (6.59)$$

$$\langle \hat{\epsilon}_k \dot{\hat{\epsilon}}_k \rangle = \frac{\Delta_L^2 K}{2} \gamma_0. \quad (6.60)$$

The case of AMMD can be treated similarly. The benefit of this different description of diffusion is that the transition probabilities only depend on n_{i-1} , n_i and n_{i+1} , so the submatrix $\mathbf{b}$ is at most tridiagonal. Thus, it has an even simpler form than MMD.

6.4.1 Power spectra comparison

We now compare the derived descriptions of diffusion. In all cases the domain is growing linearly with rate r , thus $n = 1 + rt/\theta$.

Figures 6.8 and 6.9 compare the simulated and analytic spectra of the two descriptions. Notice that the simulations have been allowed to proceed until $t = 1 \times 10^5$. Although the theory and simulation are still comparable we should pay attention to the cautionary note in Section 6.1 on how long equation (6.14) stays valid. From equation (6.53) we see that the variance of linear growth is $\langle \epsilon_i(t)^2 \rangle = rt/\theta$. As approximately 99.7% of the Gaussian distribution is between three standard deviations either side of the mean, then to have 99.7% of the data simulated correctly, we need to satisfy $3\sqrt{\langle \epsilon_i(t)^2 \rangle} \ll \sqrt{\theta}n(t)$. Expanding

	Midpoint to midpoint	Approximate midpoint to midpoint
a_0	$\frac{-2d_L}{n^2}$	$\frac{-2d_L}{n^2}$
a_1	$\frac{d_L}{n^2}$	$\frac{d_L}{n^2}$
b_0	$\frac{d_L\phi}{2n^3}$	$\frac{4d_L\phi}{n^3}$
b_1	0	$\frac{-2d_L\phi}{n^3}$
b_2	$\frac{-d_L\phi}{4n^3}$	0
α_0	$\frac{4d_L\phi}{n^2}$	$\frac{4d_L\phi}{n^2}$
α_1	$\frac{-2d_L\phi}{n^2}$	$\frac{-2d_L\phi}{n^2}$
γ_0	$\frac{r}{\theta}$	$\frac{r}{\theta}$

Table 6.1: Explicit forms of the Fokker-Planck coefficients.

this implies

$$\frac{rt/\theta}{(1 + rt/\theta)^2} \ll \frac{\theta}{9}. \quad (6.61)$$

Since θ is chosen to be large in order to satisfy the assumptions of the linear noise approximation, it follows that $\theta/9 \gg 1$. Further, it can easily be shown that $u/(1+u)^2 \leq 1/4$ for all $u \geq 0$, hence, the inequality holds for all $t \geq 0$. Thus, we expect the analysis to always be a good match for the simulation. However, although the analysis and simulations should always match, the physical interpretation of the domain discretisation will eventually break down as it will become too coarse to accurately portray the spatial dynamics.

Not only do figures 6.8 and 6.9 show a good comparison between simulation and theory, but we also see differences between the two descriptions of diffusion. Both descriptions lead to an initial increase of power, above $P_s = 0.5$. However, MMD only excites comparatively low wave modes, $m < 30$, whereas AMMD excites all wave modes. In both simulations all wave modes, except $m = 1$, relax to a steady state of $P_s = 0.5$. For $m = 1$ it should be noticed that AMMD has a higher value of power than MMD, however, overall, the stationary power spectra are very similar.

We can also compare these two types of stochastic growth with deterministic growth, Figure 6.3(c), which was considered in Section 6.2. In the case of deterministic linear growth, the power spectrum is a monotonically increasing function of $k = \pi m/L$, hence we would never see the excited wave modes that occur in figures 6.8 and 6.9. Equally, as a function of time, the power spectrum is monotonic and thus the initial increase past

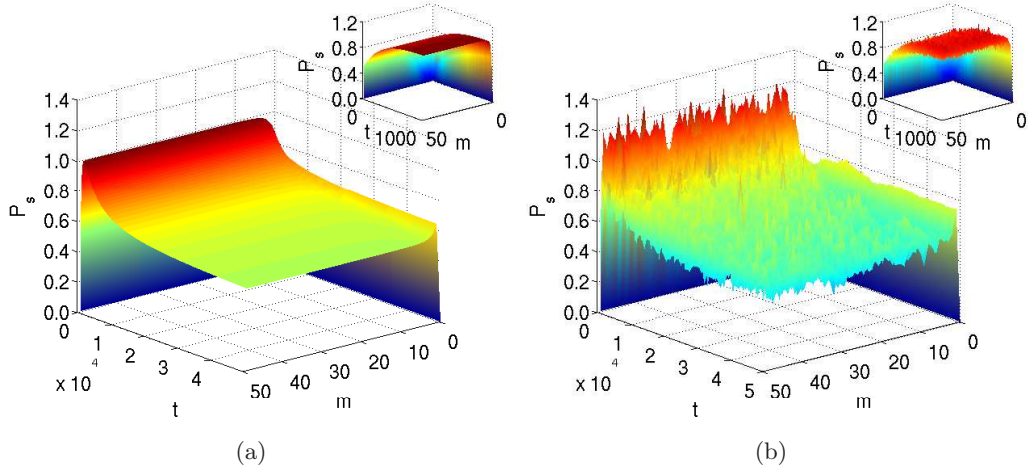


Figure 6.8: Comparison of the theoretical power spectra, (a), and the averaged power spectra of 200 simulations of MMD, (b). Parameters are $r = 0.1$, $\Delta_L = 1/50$, $\Omega = \theta = 100$, $k = m\pi/L$, $L = 1$ and $d_L = 1$. The inset in each figure shows a close up of the first 1000 time units and the stochastic spectrum is averaged over 1000 simulations. In each case the spectrum has been scaled by a factor of $\Delta_L^2 K\phi$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 84(4), 2011. “Copyright 2011 by the American Physical Society.”

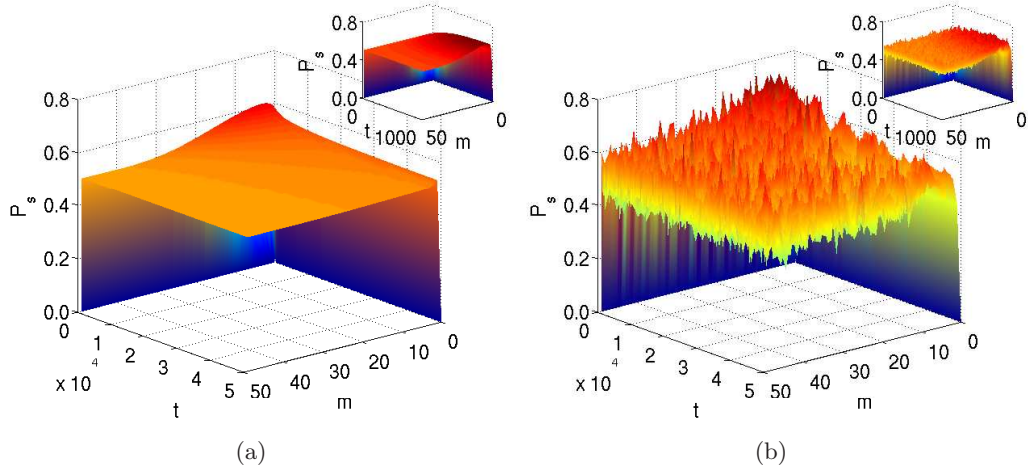


Figure 6.9: Comparison of the theoretical power spectra, (a), and the averaged power spectra of 200 simulations of AMMD (b). Parameters are $r = 0.1$, $\Delta_L = 1/50$, $\Omega = \theta = 100$, $k = m\pi/L$, $L = 1$ and $d_L = 1$. The inset in each figure shows a close up of the first 1000 time units and the stochastic spectrum is averaged over 1000 simulations. In each case the spectrum has been scaled by a factor of $\Delta_L^2 K\phi$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 84(4), 2011. “Copyright 2011 by the American Physical Society.”

$P_s = 0.5$ in the stochastic descriptions is never seen.

As noted, in the limit $r \rightarrow 0$ we regain the static domain equations. Similarly, in the limit $\theta \gg \Omega$, the differences between the stochastic and deterministic formalisms

disappear. However, within the region that the magnitudes of θ and Ω are comparable, we gain further evidence that noise can fundamentally affect systems in ways that are not considered through a purely deterministic description. Further, we conclude that although MMD and AMMD may be equivalent in the mean-field, the internal noise generated by the particular descriptions of diffusion affects the simulations in different ways. Hence, in order to simulate a system using a Lagrangian description correctly, we must be sure of the underlying stochastic description of movement. Finally, we comment on the issue that the time scale for the power spectra to reach their steady states is extremely long, approximately 2×10^4 time units. Mathematically this causes no problems, however, experimentally, the noise generated by growth on short time scales may be important since it is these initial effects that will determine the final inhomogeneities that arise. Thus, unless the growth of a diffusing system is adiabatically slow, noise cannot be ignored.

6.5 Logistically growing domain

As a final example, we consider the application of AMMD on a logistically growing domain,

$$N_i \stackrel{a}{\rightleftharpoons} N_i + 1. \quad (6.62)$$

The number of subdivisions, $N_i = \theta n_i + \sqrt{\theta} \epsilon_i$, in a Lagrangian box is controlled by the mean-field equation,

$$\dot{n} = an - b\theta n^2, \quad (6.63)$$

and K Langevin equations. These decouple and consist of K identical equations of the form

$$\dot{\epsilon} = (a - 2b\theta n_s)\epsilon + \lambda, \quad (6.64)$$

where $\langle \lambda \lambda \rangle = an_s + b\theta n_s^2$. Since $n_s = a/(b\theta)$, the variance of the random variable ϵ is

$$\langle \epsilon(t) \epsilon(t) \rangle = (1 - e^{-2at})n_s. \quad (6.65)$$

We now consider two sets of parameter values. These are $a = 1$, $b = 10^{-2}$ and $a = 10^{-3}$, $b = 10^{-5}$. Since the value of θ is unchanged, the steady state, n_s , is unchanged

and thus the long time steady state of the variance is the same. The difference between the two sets of coordinates is simply a matter of time scale. To transform the first set to the second set we use the transformation $t \mapsto t/1000$. Based on this transformation, the first set of parameters is denoted fast and the second set is denoted slow. The reason for this differentiation of the two scales can be seen by comparing Figure 6.10(h) and Figure 6.10(i). Although the (long time, steady state) variances of the two systems will be the same, the fast parameters cause perturbations about the mean to be faster, whereas the slow parameters produce a much weaker restorative force and thus the stochastic trajectory is able to undertake extended periods away from the mean.

These two different scales are able to give very different forms of growth. Since we start the domain length at the mean-field steady state of equation (6.63) we expect the domain size not to alter very much, thus in Figure 6.10 no domain growth (left column) is compared with quickly fluctuating logistic domain growth (middle column) and slowly fluctuating domain growth (right column). By following the routine set out in Section 6.4 we can construct the theoretical power spectrum for the logistically growing domain. Specifically, we are only interested in the steady state, which, for $k \neq 0$, is

$$P_s(k) = \frac{((2ad_Lb^2\theta^2 + 8d_L\phi b^3\theta^3)(\cos(k\Delta_L) - 1) - a^4)}{2a(2d_Lb^2\Omega^2(\cos(k\Delta_L) - 1) - a^3)}. \quad (6.66)$$

Note that $P_s(0) = 0$ since we subtract the expected value and thus the constant mode, $k = 0$, always has zero power. Again, we obtain excellent comparison between theory and simulation.

On comparing the power spectrum of diffusion on a slow logistically growing domain, Figure 6.10(c), with diffusion on a static domain, Figure 6.10(a), apart from the increase in power they appear very similar. However, when we compare actual diffusion simulations, fast logistic domain growth, Figure 6.10(e), appears to be more similar to the stationary domain case, Figure 6.10(d), than the highly patterned slow logistic growth, Figure 6.10(f). Since the time scale of the deviations in the slow logistic growth case is 1000 times slower than that for fast logistic growth, the slow reacting domain is able to undergo longer periods away from the mean value whereas the faster time scale of the middle column causes the system to rapidly correct itself whenever it overshoots the mean (compare

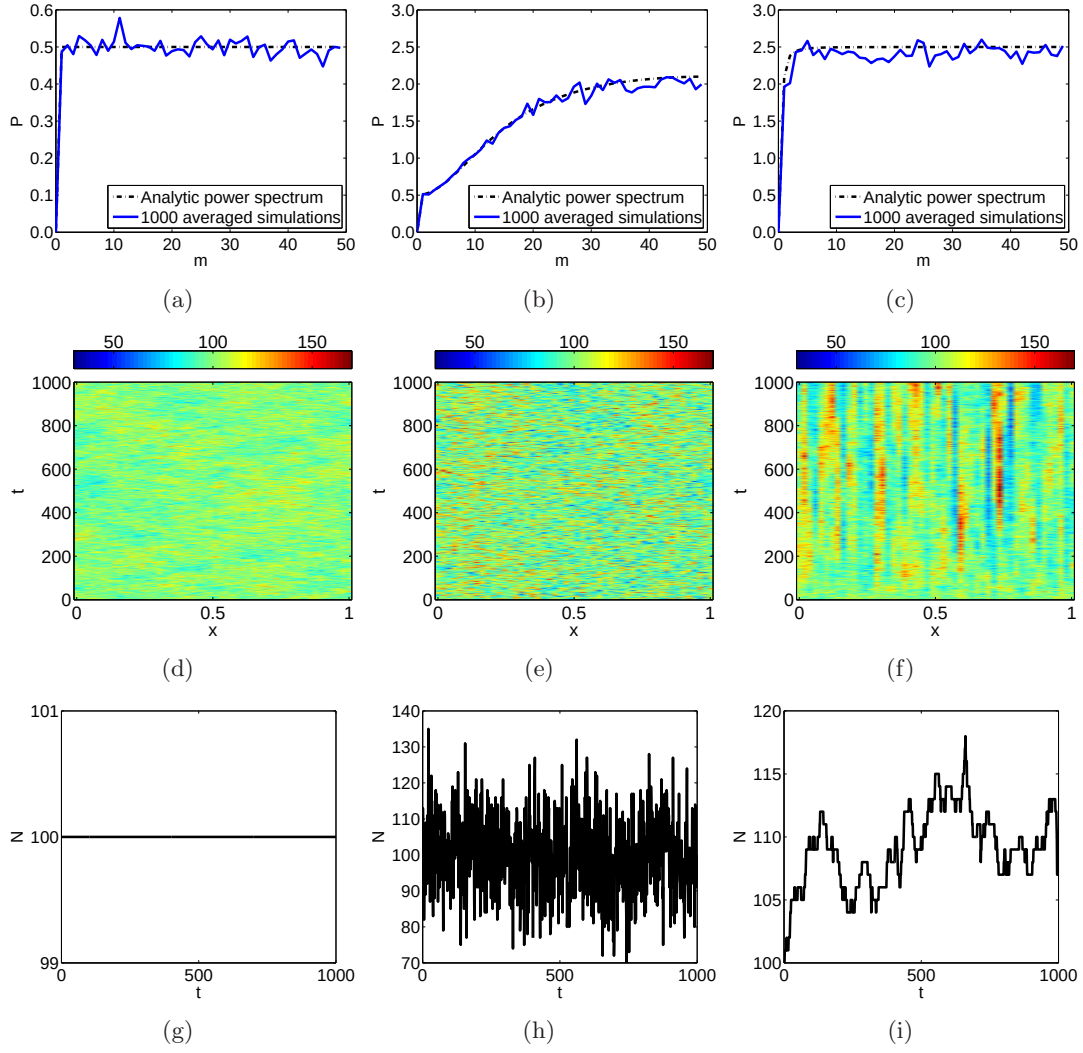


Figure 6.10: Comparison of diffusion on a static domain compared with domains undergoing logistic growth with two different time scales. Left column: no growth. Middle column: logistically growing domain with parameters $a = 1$ and $b = 1/100$. Right column: logistically growing domain with parameters $a = 10^{-3}$ and $b = 10^{-5}$. Top row: steady state power spectrum of stochastic system averaged over 1000 trials, each scaled by $\Delta_L^2 K \phi$ and compared with the theoretical steady state power spectrum, equation (6.66), similarly scaled. Middle row: Single realisations of the simulations. Bottom row: single realisations of the number of subdivisions in the first Lagrangian box of the simulations in the middle row. Parameters are: $d_L = 1$, $\Omega = \theta = 100$, $\Delta_L = 1/50$, $L = 1$, $k = m\pi/L$ and $\phi = 1$. Reproduced from T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. Phys. Rev. E, 84(4), 2011. “Copyright 2011 by the American Physical Society.”

figures 6.10(h) and 6.10(i)). These extended meanderings enable the slower system to enter a patterned state; wherever the number of subdivisions in a Lagrangian box is higher than those surrounding it, the particles will diffuse slower out of the box than into it. Thus it is likely that this box will become a local maximum. Similarly, if a Lagrangian

box has a lower number of subdivisions than those surrounding it, the diffusion out of this box will be faster than the diffusion into it, causing the box to become a local minimum. Thus, the longer the number of subdivisions of a Lagrangian box is perturbed away from the mean value, the higher the probability that the box will contain a local extremum of population. This then produces the patterned state that we see.

Hence, we have shown that, although we expect a homogeneous particle distribution on the logistically growing domain, growth is able to cause patterning, which is not possible in the deterministically growing case. Furthermore, the same form of growth can give rise to different effects depending on the time scale of the stochastic fluctuations.

6.6 Summary and conclusion

In this chapter we extended the methods discussed in Chapter 5 to encompass the possibility of domain growth. Clearly, we have shown, analytically and computationally, that stochastic effects are critical when considering diffusing populations on a growing domain. The Fourier techniques have allowed us to highlight in general that stochastic systems are able to exhibit much richer dynamics than their deterministic counterparts. Specifically, domain growth fundamentally changes the power spectrum of diffusion and, further, relatively quick growth supports the nucleation of states that are far removed from the deterministic mean-field steady state.

We then extended the analysis once again to consider the case of stochastic domain growth, which led to the discovery that the Lagrangian framework causes the description of diffusion to be non-unique. In order to specify an actual description of diffusion under Lagrangian growth, simulations were run using a variety of descriptions that are equivalent in the mean-field, such as those given in equations (6.42) and (6.43) and equations (6.44) and (6.45). These were then compared with Eulerian box-splitting simulations which had been mapped back to the Lagrangian domain (data not shown). Although the steady state power spectra were similar, there were fundamental differences in the evolution to the steady state. This is not surprising as currently there is no rigorous limit linking these two scales. Although they are both descriptions of growth, only the Lagrangian formulation can be treated rigorously in a thermodynamic limit. However, this is not a

big problem for the Lagrangian framework. It simply implies that if we are to use the spatial mapping we must be clear about the underlying dependence of the motion on the domain.

Due to the non-uniqueness of the description of diffusion, we considered two justified descriptions of diffusion on a Lagrangian growing domain: midpoint to midpoint diffusion (MMD) and approximate midpoint to midpoint diffusion (AMMD). Through simulations we have shown that the analysis compares favourably with Fourier transforming ensemble data. Further, at steady state, the power spectra are very similar. However, their respective evolutions to the steady state are completely different with AMMD exciting all wave modes to a higher power than MMD, which only transiently excites comparably low wave modes.

Physically, it should be noted that the Lagrangian description of growth becomes less valid as time increases; the domain is growing but the discretisation remains fixed. If we were considering systems with spatial dynamics, we would have to take account of this by only allowing the system to evolve up until the point at which the Lagrangian discretisation is too coarse to capture the relevant structures. However, although the physicality of the description becomes less valid, we have shown that we never need worry about the analytical approximations breaking down as we are able to simulate the data as accurately as we need, simply by increasing the parameter θ (see equation (6.61)).

These ideas were further extended to the example of diffusion on a logistically growing domain. Importantly, we saw the surprising result that the same type of growth can affect diffusion in different ways depending on the speed of correction of the stochastic perturbations. We concluded that there is a huge difference between a stationary domain and one which is stationary on average. Further, by inspection, the simulated results of diffusion on a stationary domain were much closer to the case of fast logistic kinetics, than slow logistic kinetics (see figures 6.10(d)-(f)). Although explainable, this is not necessarily an intuitive result as the slow logistic power spectrum qualitatively resembles the stationary domain case (see figures 6.10(a)-(c)).

We have shown that stochastic growth effects can play an important role when considering diffusing populations on a growing domain. Deterministically, the system will simply relax to a homogeneous steady state, whereas stochastic fluctuations in the underlying do-

main are able to cause non-transient structures to form, which have a wavelength that arises from a well-defined power spectrum. Importantly, we have demonstrated that this domain growth need not be monotonic; although deterministically logistic growth does produce a monotonic growth to a final steady state, the intrinsic noise of the simulations causes the stochastic domain to oscillate about this mean-field with the interpretation that Eulerian subdivisions are being destroyed as well as created. We thus conclude that it is very important to clarify the dominant sources of noise, whether they be the population dynamics, growth or both, as the relationship between the respective noise scales, Ω and θ , is able to produce large differences in the resulting power spectra.

In the next chapter we return to biological patterning paradigm models and apply this developed stochastic framework. We are interested in explaining the effects of stochasticity on such systems as noise has already been shown to extend the parameter regions over which Turing patterns are able to exist (Biancalani *et al.*, 2010). Previously, this small parameter region of existence has been seen to be one of the criticisms of using Turing systems as biological paradigms of pigmentation patterning (Murray, 1982). We will further this re-examination of Turing systems by considering the effects of noise on the robustness of pattern generation (Woolley *et al.*, 2011c).

Chapter 7

Stochastic Turing patterns

“Noise is the source of all evil.”

Philip K. Maini, 6th November 2010.

This chapter is based on work in Woolley *et al.* (2011c).

As discussed in Chapter 1, mathematical modelling is of fundamental importance in developmental biology due to its ability to suggest and test mechanisms by which complex biological patterns can arise (Maini *et al.*, 2006). Although many mechanisms have been proposed (Barrio *et al.*, 2009; Owen *et al.*, 2000; Satnoianu & Menzinger, 2000; Woolley *et al.*, 2010; Zeng *et al.*, 2004), we focus on the Turing system as a model of morphogenesis (Kondo & Asai, 1995; Liaw *et al.*, 2001; Meinhardt *et al.*, 2003) as it has been postulated to be the key mechanism behind feather bud, limb bud and hair follicle development (Cho *et al.*, 2011; Lin *et al.*, 2009; Maini *et al.*, 2006; Sick *et al.*, 2006; Turing, 1952). In most cases these models have been constructed in a deterministic framework and the effects of intrinsic noise on pattern formation have only recently been considered (Biancalani *et al.*, 2010). Indeed, due to the sensitivity of the Turing model to perturbations in parameter values, domain geometry, *etc.*, it has been seen that stochastic effects are able to influence the evolving patterns in ways not seen through deterministic simulations (Biancalani *et al.*, 2010).

Previous research in the area of stochastic reaction-diffusion systems has focused on analysing and simulating the effects of external noise (Escudero, 2009; García-Ojalvo & Sancho, 1994, 1996, 1999; Sagués *et al.*, 2007). External noise is modelled as an additional

effect that is controlled by the user, thus its properties are known *a priori*. Here, we focus on the role of intrinsic stochasticity by modelling diffusion of the chemical constituents as a space-jump process (Baker *et al.*, 2010; Othmer *et al.*, 1988), *i.e.*, the individual particles of the chemical populations undergo unbiased random walks on a domain which has been discretised into boxes. Reactions between constituent populations are assumed to act within each box, thus reagents in box i can only react with other reagents in box i . For each stochastic system a CME can be generated as an exact description of the evolution of the system (Gardiner, 1985). Linear noise expansions then allow derivation of Fokker-Planck equations that characterise the properties of the noise, which can then be converted into ODEs that define the covariances (van Kampen, 2007). Recently developed spatial Fourier transform techniques (Butler & Goldenfeld, 2009; Lugo & McKane, 2008; McKane *et al.*, 2007; McKane & Newman, 2005; Woolley *et al.*, 2011a,b) can then be applied in order to gain an insight into the potential spatial dynamics that are possible in such systems.

Primarily, we can explore which wave modes are stochastically excited inside and outside of the deterministic Turing domain. Within the parameter region that allows deterministic systems to realise Turing patterns we analytically demonstrate that the stochastically excited wave modes correspond exactly to their deterministic analogues. Linear analysis predicts that these stochastically excited wave modes grow exponentially with time and, since the noise perturbs the populations away from the uniform steady state, patterning is able to form much earlier in a stochastic system than its deterministic counterpart. Outside of this region deterministic systems are unable to sustain viable patterns, whereas the noise inherent in a stochastic system is able to produce a state which is consistently far removed from the homogeneous steady state, a result that was previously noted by Biancalani *et al.* (2010).

Having considered the stationary domain case we use the growth mapping that was developed in Chapter 6 to explore stochastic reaction-diffusion systems on growing domains. We are interested in the effects of stochasticity in such systems, as, in the deterministic setting, it has been shown that domain growth is able to support robust pattern doubling sequences (Crampin *et al.*, 1999). Since robustness in a deterministic Turing system is achieved through domain growth we may naïvely suspect that robustness will exist in

the stochastic formulation, since the excited wave modes are identical in both systems. However, it is quickly realised that stochasticity destroys any sense of pattern doubling robustness. Even in the limit of large populations stochasticity is able to cause a breakdown in the doubling eventually. Although we lose period doubling as a mechanism of robustness, stochastic systems appear to excite consecutive modes and thus we investigate the robustness of this mechanism through simulation.

In order to examine Turing systems we need to consider at least two spatially extended populations that interact through nonlinear reactions. Thus, in this section, we briefly recapitulate the weak noise expansion applied to a system of two diffusing populations, $U(x, t)$ and $V(x, t)$, that are coupled through non-delayed reactions, on a one-dimensional domain. The domain is partitioned into one-dimensional boxes of size Δ_E where each box, i , contains the indexed populations U_i and V_i . Thus, for two particle types that have been discretised into K boxes in a one-dimensional domain of length L , with zero Neumann boundary conditions and stochastic diffusion coefficients d_u and d_v , respectively, the diffusion reactions are

$$U_1 \xrightleftharpoons[d_u]{d_u} U_2 \xrightleftharpoons[d_u]{d_u} \dots \xrightleftharpoons[d_u]{d_u} U_K, \quad (7.1)$$

$$V_1 \xrightleftharpoons[d_v]{d_v} V_2 \xrightleftharpoons[d_v]{d_v} \dots \xrightleftharpoons[d_v]{d_v} V_K. \quad (7.2)$$

We use U_i and V_i to stand for both respective species and populations and this abuse of notation should not cause confusion. We define new random variables, η_{ui} and η_{vi} (which are of order one as $\Omega \rightarrow \infty$), for $i = 1, \dots, K$, through the relations

$$U_i = \phi_i \Omega + \eta_{ui} \sqrt{\Omega}, \quad (7.3)$$

$$V_i = \psi_i \Omega + \eta_{vi} \sqrt{\Omega}, \quad (7.4)$$

where Ω is the magnitude of the smallest homogeneous steady state of the populations U or V . ϕ_i and ψ_i are the expected ratios of the populations at time t to the order of magnitude, Ω , and are dimensionless ‘macroscopic’ variables. The statistics of U_i and V_i

are linked to η_{ui} and η_{vi} , respectively, through the identification (van Kampen, 2007)

$$P(\mathbf{U}, \mathbf{V}, t) = \Pi(\boldsymbol{\eta}_u, \boldsymbol{\eta}_v, t). \quad (7.5)$$

Through using the relationships between P , Π , a_j and $\mathbf{a}_j$, we are able to expand and separate equation (5.6) in terms of decreasing orders of Ω (Woolley *et al.*, 2011a,b). The leading order terms are of size $\mathcal{O}(\sqrt{\Omega})$ and define the deterministic dynamics of the macroscopic equations,

$$\sum_{i=1}^K \left(\frac{d\phi_i}{dt} \frac{\partial \Pi}{\partial \eta_{ui}} + \frac{d\psi_i}{dt} \frac{\partial \Pi}{\partial \eta_{vi}} \right) = \sum_{j=1}^J \left(\sum_{i=1}^K \mathbf{a}_j(\phi, \psi) \nu_{ij} \frac{\partial \Pi}{\partial \eta_{ui}} + \sum_{i=1}^K \mathbf{a}_j(\phi, \psi) \nu_{K+ij} \frac{\partial \Pi}{\partial \eta_{vi}} \right). \quad (7.6)$$

This is satisfied by the definitions of ϕ_i and ψ_i .

The second order term in the expansion is of size $\mathcal{O}(1)$ and gives rise to a Fokker-Planck equation (Risken, 1989) which defines the dynamics of the probability density,

$$\frac{\partial \Pi}{\partial t} = - \sum_{i,l=1}^{2K} A_{il} \frac{\partial (\eta_l \Pi)}{\partial \eta_i} + \frac{1}{2} \sum_{i,l=1}^{2K} B_{il} \frac{\partial^2 \Pi}{\partial \eta_i \partial \eta_l}, \quad (7.7)$$

where $\eta_i = \eta_{ui}$ for $i = 1, \dots, K$ and $\eta_i = \eta_{vi-K}$ for $i = K + 1, \dots, 2K$. The coefficients A_{il} and B_{il} are defined through the matrix equations (Elf & Ehrenberg, 2003) given in Section 5.1.

7.1 General form

Further, in the case of stochastic growth, we need to consider the additional population $N_i = \theta n_i + \sqrt{\theta} \epsilon_i$, as in Section 6.1. Thus, we have the extra scaling, θ , to account for and the general Fokker-Planck equation for stochastic reactions on a stochastically growing domain is

$$\frac{\partial \Pi}{\partial t} = - \sum_{i,j=1}^{3K} A_{ij} \frac{\partial}{\partial \zeta_i} (\zeta_j \Pi) \sqrt{\frac{\Theta_j}{\Theta_i}} + \frac{1}{2} \sum_{i,j=1}^{3K} B_{ij} \frac{\partial^2 \Pi}{\partial \zeta_i \partial \zeta_j} \frac{\Theta_j}{\Theta_i}, \quad (7.8)$$

where Θ_i is the scaling for the variable ζ_i . In the current case $\zeta_i = \eta_{ui}$ for $i = 1, \dots, K$, $\zeta_i = \eta_{vi-K}$ for $i = K + 1, \dots, 2K$, $\zeta_i = \epsilon_{i-2K}$ for $i = 2K + 1, \dots, 3K$, $\Theta_i = \Omega$ for $i = 1, \dots, 2K$ and $\Theta_i = \theta$ for $i = 2K + 1, \dots, 3K$. Note that, for simplicity, whenever we

consider stochastic growth we fix the scaling parameters to be equal, *i.e.* $\theta = \Omega$. Later, once we have compared deterministic and stochastic growth in Section 7.4.3 and shown that, not only is the noise generated from growth much smaller than that generated from reactions, we also see that the pattern evolution of a Turing system on a stochastically growing domain is sufficiently similar to one undergoing deterministic growth so that it is appropriate to investigate only deterministic growth. Finally, we fix the form of Lagrangian diffusion to be AMMD (see Section 6.3). This defines the transition rates left and right to be equal to

$$\frac{d_L}{\frac{1}{\theta^2} N_i^2}, \quad (7.9)$$

where $d_L = d_u$ or d_v depending on the population (Øksendal, 2003; Woolley *et al.*, 2011a).

In general, a system of non-delayed, coupled reaction-diffusion equations on a deterministically growing domain can be rescaled to remove dilution and projected on to the stationary domain thus giving the form (see Section 2.3) (Crampin, 2000; Crampin *et al.*, 1999; Crampin & Maini, 2001):

$$\frac{\partial \phi}{\partial t}(x, t) = \frac{D_u}{n(t)^2} \nabla^2 \phi(x, t) + f(\phi(x, t), \psi(x, t), t), \quad (7.10)$$

$$\frac{\partial \psi}{\partial t}(x, t) = \frac{D_v}{n(t)^2} \nabla^2 \psi(x, t) + g(\phi(x, t), \psi(x, t), t), \quad (7.11)$$

where $\phi(t)$, $\psi(t)$ and $n(t)$ are the spatially continuous forms of the discretised populations $\phi_i(t)$, $\psi_i(t)$ and $n_i(t)$. Also, due to the rescaling of the reaction kinetics using $n(t)$ (see Section 7.3), the kinetic functions of the reactions, f and g , will normally be time-dependent. Thus, except for the diffusion term (which uses a three-point stencil in one dimension), the discretised system will contain only terms evaluated at the same spatial and temporal points. Further, since the diffusive operator only includes two extra additional spatial terms, the matrices $\mathbf{A}$ and $\mathbf{B}$, defined in equations (5.28) and (5.29), are symmetric with all of the sub- and super-diagonal entries being identical. This allows the equations to be spatially Fourier transformed analytically, even in the case of time-dependent coefficients. Although, by considering different stochastic descriptions of diffusion, the number of non-zero super- and sub-diagonals may change, the matrices will always be symmetric and so

spatial Fourier transforms will always be applicable.

For stochastic growth, the matrices $\mathbf{A}$ and $\mathbf{B}$ will be $3K \times 3K$ in size, as we have three distinct, spatially discretised populations, $\mathbf{U}$, $\mathbf{V}$ and $\mathbf{N} = (N_1, N_2, \dots, N_K)$. In the case that the growth rate is independent of the chemical concentrations, $\mathbf{A}$ and $\mathbf{B}$ will have sparse forms. These matrices can be split up into nine $K \times K$ sub-matrices of the form

$$\mathbf{A} = \begin{bmatrix} \mathbf{a} & \mathbf{b} & \mathbf{e} \\ \mathbf{c} & \mathbf{d} & \mathbf{f} \\ \mathbf{0} & \mathbf{0} & \mathbf{g} \end{bmatrix}, \quad \mathbf{B} = \begin{bmatrix} \boldsymbol{\alpha} & \boldsymbol{\beta} & \mathbf{0} \\ \boldsymbol{\beta} & \boldsymbol{\gamma} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \boldsymbol{\delta} \end{bmatrix}, \quad (7.12)$$

where $\mathbf{b}$ and $\mathbf{c}$ are diagonal and the zeros exist because a change in the number of Eulerian subdivisions, N_i , only affects the chemical species, U_i and V_i , through the diffusion coefficient. Since we are considering linear growth $\mathbf{g} = \mathbf{0}$ as well, however, this is not generally true. Since we would like to consider stochastic growth the sub-matrices $\mathbf{a}$, $\mathbf{d}$, $\mathbf{e}$ and $\mathbf{f}$ have non-unique forms due to different possible spatial dependencies in the definition of diffusion on a Lagrangian domain (Woolley *et al.*, 2011a). However, as mentioned, in the present case we fix the definition of diffusion to be AMMD, so that the matrices $\mathbf{a}$, $\mathbf{d}$, $\mathbf{e}$ and $\mathbf{f}$ will be tridiagonal and symmetric.

For the matrix $\mathbf{B}$, we first note that, by definition, it is symmetric and the sub-matrices $\boldsymbol{\alpha}$, $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$ are of the same structure as the corresponding sub-matrices of $\mathbf{A}$. The sub-matrix $\boldsymbol{\delta}$ is diagonal since each growth ‘reaction’ only affects a single Lagrangian box. For a specific worked example of the derivation of $\mathbf{A}$ and $\mathbf{B}$ see Section 7.3.

Using matrices $\mathbf{A}$ and $\mathbf{B}$ we are able to derive the corresponding general Fokker-Planck equation for the variables η_{ui} , η_{vi} and ϵ_i :

$$\begin{aligned} \frac{\partial \Pi}{\partial t} = & - \sum_{i,j} \mathbf{a}_{ij} \frac{\partial}{\partial \eta_{ui}} (\eta_{uj} \Pi) - \sum_{i,j} \mathbf{b}_{ij} \frac{\partial}{\partial \eta_{ui}} (\eta_{vj} \Pi) - \sum_{i,j} \mathbf{e}_{ij} \frac{\partial}{\partial \eta_{ui}} (\epsilon_j \Pi) \\ & - \sum_{i,j} \mathbf{c}_{ij} \frac{\partial}{\partial \eta_{vi}} (\eta_{uj} \Pi) - \sum_{i,j} \mathbf{d}_{ij} \frac{\partial}{\partial \eta_{vi}} (\eta_{vj} \Pi) - \sum_{i,j} \mathbf{f}_{ij} \frac{\partial}{\partial \eta_{vi}} (\epsilon_j \Pi) \\ & + \frac{1}{2} \sum_{i,j} \boldsymbol{\alpha}_{ij} \frac{\partial^2 \Pi}{\partial \eta_{ui} \partial \eta_{uj}} + \sum_{i,j} \boldsymbol{\beta}_{ij} \frac{\partial^2 \Pi}{\partial \eta_{ui} \partial \eta_{vj}} + \frac{1}{2} \sum_{i,j} \boldsymbol{\gamma}_{ij} \frac{\partial^2 \Pi}{\partial \eta_{vi} \partial \eta_{vj}} + \frac{1}{2} \sum_{i,j} \boldsymbol{\delta}_{ij} \frac{\partial^2 \Pi}{\partial \epsilon_i \partial \epsilon_j}. \end{aligned} \quad (7.13)$$

From this we can immediately derive the covariances as (van Kampen, 2007),

$$\begin{aligned}\langle \dot{\eta}_{ul} \eta_{um} \rangle &= \sum_j \mathbf{a}_{lj} \langle \eta_{uj} \eta_{um} \rangle + \sum_j \mathbf{a}_{mj} \langle \eta_{ul} \eta_{uj} \rangle + \sum_j \mathbf{b}_{lj} \langle \eta_{vj} \eta_{um} \rangle + \sum_j \mathbf{b}_{mj} \langle \eta_{ul} \eta_{vj} \rangle \\ &+ \sum_j \mathbf{e}_{lj} \langle \epsilon_j \eta_{um} \rangle + \sum_j \mathbf{e}_{mj} \langle \eta_{ul} \epsilon_j \rangle + \boldsymbol{\alpha}_{lm},\end{aligned}\quad (7.14)$$

$$\begin{aligned}\langle \dot{\eta}_{ul} \eta_{vm} \rangle &= \sum_j \mathbf{a}_{lj} \langle \eta_{uj} \eta_{vm} \rangle + \sum_j \mathbf{b}_{lj} \langle \eta_{vj} \eta_{vm} \rangle + \sum_j \mathbf{c}_{mj} \langle \eta_{ul} \eta_{uj} \rangle + \sum_j \mathbf{d}_{mj} \langle \eta_{ul} \eta_{vj} \rangle \\ &+ \sum_j \mathbf{e}_{lj} \langle \epsilon_j \eta_{vm} \rangle + \sum_j \mathbf{f}_{mj} \langle \eta_{ul} \epsilon_j \rangle + \boldsymbol{\beta}_{lm},\end{aligned}\quad (7.15)$$

$$\begin{aligned}\langle \dot{\eta}_{vl} \eta_{vm} \rangle &= \sum_j \mathbf{c}_{lj} \langle \eta_{uj} \eta_{vm} \rangle + \sum_j \mathbf{c}_{mj} \langle \eta_{vl} \eta_{uj} \rangle + \sum_j \mathbf{d}_{lj} \langle \eta_{vj} \eta_{vm} \rangle + \sum_j \mathbf{d}_{mj} \langle \eta_{vl} \eta_{vj} \rangle \\ &+ \sum_j \mathbf{f}_{lj} \langle \epsilon_j \eta_{vm} \rangle + \sum_j \mathbf{f}_{mj} \langle \eta_{vl} \epsilon_j \rangle + \boldsymbol{\gamma}_{lm},\end{aligned}\quad (7.16)$$

where $\dot{} = \partial/\partial t$. Partial differential equations governing the covariances of $\langle \eta_{ul} \epsilon_m \rangle$, $\langle \epsilon_l \eta_{vm} \rangle$ and $\langle \epsilon_l \epsilon_m \rangle$ can be found similarly.

7.2 Spatial transform

We now use the discrete spatial Fourier transform, equation (5.64). Hence, the differential equations governing the spatial power spectra of the system are specified by:

$$\begin{aligned}\langle \dot{\widehat{\eta}}_{uk} \widehat{\eta}_{uk} \rangle &= (4a_1 \cos(k\Delta_L) + 2a_0) \langle \widehat{\eta}_{uk} \widehat{\eta}_{uk} \rangle + 2b_0 \langle \widehat{\eta}_{vk} \widehat{\eta}_{uk} \rangle \\ &+ (4e_1 \cos(k\Delta_L) + 2e_0) \langle \widehat{\eta}_{uk} \widehat{\epsilon}_k \rangle + \frac{\Delta_L^2 K}{2} (\alpha_0 + 2 \cos(k\Delta_L) \alpha_1),\end{aligned}\quad (7.17)$$

$$\begin{aligned}\langle \dot{\widehat{\eta}}_{uk} \widehat{\eta}_{vk} \rangle &= (2(a_1 + d_1) \cos(k\Delta_L) + a_0 + d_0) \langle \widehat{\eta}_{uk} \widehat{\eta}_{vk} \rangle + b_0 \langle \widehat{\eta}_{vk} \widehat{\eta}_{vk} \rangle + c_0 \langle \widehat{\eta}_{uk} \widehat{\eta}_{uk} \rangle \\ &+ (2e_1 \cos(k\Delta_L) + e_0) \langle \widehat{\epsilon}_k \widehat{\eta}_{vk} \rangle + (2f_1 \cos(k\Delta_L) + f_0) \langle \widehat{\eta}_{uk} \widehat{\epsilon}_k \rangle + \frac{\Delta_L^2 K}{2} \beta_0,\end{aligned}\quad (7.18)$$

$$\begin{aligned}\langle \dot{\widehat{\eta}}_{vk} \widehat{\eta}_{vk} \rangle &= (4d_1 \cos(k\Delta_L) + 2d_0) \langle \widehat{\eta}_{vk} \widehat{\eta}_{vk} \rangle + 2c_0 \langle \widehat{\eta}_{uk} \widehat{\eta}_{vk} \rangle \\ &+ (4f_1 \cos(k\Delta_L) + 2f_0) \langle \widehat{\epsilon}_k \widehat{\eta}_{vk} \rangle + \frac{\Delta_L^2 K}{2} (\gamma_0 + 2 \cos(k\Delta_L) \gamma_1),\end{aligned}\quad (7.19)$$

where, for $w \in \{a, b, c, d, e, f, \alpha, \beta, \gamma, \delta\}$, w_0 and w_1 are the diagonal and off-diagonal elements of the sub-matrix $\mathbf{w}$, respectively. ODEs governing the Fourier transforms of the covariances for $\langle \widehat{\eta}_{uk} \widehat{\epsilon}_k \rangle$, $\langle \widehat{\epsilon}_k \widehat{\eta}_{vk} \rangle$ and $\langle \widehat{\epsilon}_k \widehat{\epsilon}_k \rangle$ can be found similarly. Hence, we have reduced

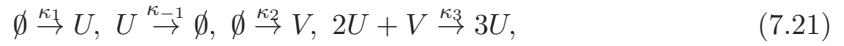
the system from $6K \times 6K$ to six ODEs, which define the dynamics of the spatial power spectra of the stochastic variables. These equations tell us which spatial wave modes are activated and, thus, give us insights into the patterns we should expect. However, it should be noted that we have lost information concerning the cross correlations. This not a problem since we are only concerned with the activated wave modes predicted by the power spectra.

7.3 Derivation of stochastic Schnakenberg reaction rates

In this section we use the specific example of Schnakenberg reaction kinetics to illustrate the effect of this spatial mapping. Since the mean-field equations of the CME are the deterministic equations in the system equations (7.26) and (7.27) we use the deterministic reaction rates to produce stochastic analogues by substituting $n_i(t)$ for N_i/θ and noting that

$$\lim_{\theta \rightarrow \infty} \frac{N_i(t)}{\theta} = n_i(t). \quad (7.20)$$

Letting κ_i be the stochastic rate of reaction, the Schnakenberg system (Schnakenberg, 1979) consists of the chemical reactions,



plus diffusion of the two reacting species. We consider the Schnakenberg system on a uniformly growing domain, which causes the Eulerian coordinates, x_E , to behave like $x_E = x_L n(t)$, where x_L is the fixed Lagrangian coordinate and $n(t)$ encapsulates the definition of growth (noting $n(0) = 1$). The mean-field equations take the form (Crampin, 2000; Crampin *et al.*, 1999; Crampin & Maini, 2001),

$$\frac{\partial \phi}{\partial t} + (a\phi)_{x_E} = D_u \frac{\partial^2 \phi}{\partial x_E^2} + \frac{\kappa_1}{\Omega} - \phi \kappa_{-1} + \phi^2 \psi \kappa_3 \Omega^2, \quad (7.22)$$

$$\frac{\partial \psi}{\partial t} + (a\psi)_{x_E} = D_v \frac{\partial^2 \psi}{\partial x_E^2} + \frac{\kappa_2}{\Omega} - \phi^2 \psi \kappa_3 \Omega^2. \quad (7.23)$$

Converting to Lagrangian coordinates and dropping the L yields

$$\frac{\partial \phi}{\partial t} + \frac{\dot{n}}{n} \phi = \frac{D_u}{n^2} \frac{\partial^2 \phi}{\partial x^2} + \frac{\kappa_1}{\Omega} - \phi \kappa_{-1} + \phi^2 \psi \kappa_3 \Omega^2, \quad (7.24)$$

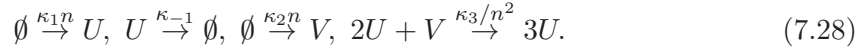
$$\frac{\partial \psi}{\partial t} + \frac{\dot{n}}{n} \psi = \frac{D_v}{n^2} \frac{\partial^2 \psi}{\partial x^2} + \frac{\kappa_2}{\Omega} - \phi^2 \psi \kappa_3 \Omega^2. \quad (7.25)$$

Finally, by rescaling $\phi_L = \phi n$ and $\psi_L = \psi n$, we remove the dilution terms and upon ignoring the subscript, L , again, we obtain

$$\frac{\partial \phi}{\partial t} = \frac{D_u}{n^2} \frac{\partial \phi}{\partial x} + \frac{\kappa_1 n}{\Omega} - \phi \kappa_{-1} + \frac{\phi^2 \psi \kappa_3 \Omega^2}{n^2}, \quad (7.26)$$

$$\frac{\partial \psi}{\partial t} = \frac{D_v}{n^2} \frac{\partial \psi}{\partial x} + \frac{\kappa_2 n}{\Omega} - \frac{\phi^2 \psi \kappa_3 \Omega^2}{n^2}. \quad (7.27)$$

Using this as a template we redefine the stochastic rates of reaction so as to simulate the reactions on a Lagrangian domain,



Thus, the mean-field equations of these reactions (plus diffusion scaled similarly) are exactly equations (7.26) and (7.27).

Assuming deterministic growth, the stoichiometric matrix is

$$\nu = \begin{bmatrix} \mathbf{R} & \mathbf{L} & \mathbf{0} & \mathbf{0} & \mathbf{I} & -\mathbf{I} & \mathbf{0} & \mathbf{I} \\ \mathbf{0} & \mathbf{0} & \mathbf{R} & \mathbf{L} & \mathbf{0} & \mathbf{0} & \mathbf{I} & -\mathbf{I} \end{bmatrix}, \quad (7.29)$$

where $\mathbf{L}$ and $\mathbf{R}$ are the $K \times K$ stoichiometric matrices for jumps left and right, respectively (see equations (7.1) and (7.2)), and $\mathbf{I}$ is the $K \times K$ identity matrix. The corresponding vector of macroscopic transition rates, $\mathbf{a}$, is

$$\mathbf{a}^T = \left(\frac{d_1 \phi_1}{n^2}, \dots, \frac{d_1 \phi_K}{n^2}, \frac{d_2 \psi_1}{n^2}, \dots, \frac{d_2 \psi_K}{n^2}, \frac{n}{\Omega} \kappa_1, \dots, \frac{n}{\Omega} \kappa_1, \phi_1 \kappa_{-1}, \dots \right. \\ \left. \dots, \phi_N \kappa_{-1}, \frac{n}{\Omega} \kappa_2, \dots, \frac{n}{\Omega} \kappa_2, \phi_1^2 \psi_1 \kappa_3 \frac{\Omega^2}{n^2}, \dots, \phi_n^2 \psi_n \kappa_3 \frac{\Omega^2}{n^2} \right). \quad (7.30)$$

These then determine the desired matrices $\mathbf{A}$ and $\mathbf{B}$ which have the forms shown in equations (5.28) and (5.29). Noting that we are interested in the homogeneous state,

$\phi_i = \phi^*$ and $\psi_i = \psi^*$ for all i , we can write down the elements of the matrices $\mathbf{A}$ and $\mathbf{B}$ as

$$\begin{aligned}
a_1 &= \frac{d_1}{n^2}, \quad a_0 = \frac{-2d_1}{n^2} - \kappa_{-1} + 2\phi^*\psi^*\kappa_3\frac{\Omega^2}{n^2}, \\
b_0 &= \frac{\phi^{*2}\kappa_3\Omega^2}{n^2}, \\
c_0 &= \frac{-2\phi^*\psi^*\kappa_3\Omega^2}{n^2}, \\
d_1 &= \frac{d_2}{n^2}, \quad d_0 = \frac{-2d_2}{n^2} - \phi^{*2}\kappa_3\frac{\Omega^2}{n^2}, \\
\alpha_1 &= \frac{-2d_1\phi^*}{n^2}, \quad \alpha_0 = \frac{4d_1\phi^*}{n^2} + \frac{n\kappa_1}{\Omega} + \kappa_{-1}\phi^* + \phi^{*2}\psi^*\kappa_3\frac{\Omega^2}{n^2}, \\
\beta_0 &= \frac{-\phi^{*2}\kappa_3\psi^*\Omega^2}{n^2}, \\
\gamma_1 &= \frac{-2d_2\psi^*}{n^2}, \quad \gamma_0 = \frac{4d_2\psi^*}{n^2} + \frac{n\kappa_2}{\Omega} + \phi^{*2}\psi^*\kappa_3\frac{\Omega^2}{n^2},
\end{aligned}$$

where, for $w \in \{a, b, c, d, e, f, \alpha, \beta, \gamma, \delta\}$, w_0 and w_1 are the diagonal elements and off-diagonal elements of the sub-matrix $\mathbf{w}$, respectively.

In the case that growth is stochastic the stoichiometric matrix and the macroscopic transition rate vector in equations (7.29) and (7.30), respectively, will need to be extended in the obvious way. From these we can then calculate the final elements needed to completely define the matrices (7.12),

$$\begin{aligned}
e_1 &= -2\frac{d_u\phi^*}{n^3}, \quad e_0 = \frac{4d_u\phi^*}{n^3} + \frac{\kappa_1}{\Omega} - 2\phi^{*2}\psi^*\kappa_3\frac{\Omega^2}{n^3}, \\
f_1 &= -2\frac{d_v\psi^*}{n^3}, \quad f_0 = \frac{4d_v\psi^*}{n^3} + \frac{\kappa_2}{\Omega} + 2\phi^{*2}\psi^*\kappa_3\frac{\Omega^2}{n^3}, \\
\delta_0 &= \frac{r}{\theta}.
\end{aligned} \tag{7.31}$$

7.4 Simulation comparison

We now face the problem of comparing theory with simulation. The derived theory is only applicable at the spatially homogeneous steady state. However, in the Turing unstable parameter region the system will try to evolve into a patterned state, making the conclusions drawn from the theory invalid. Thus, we are in exactly the same situation as with deterministic Turing linear analysis; we can only predict to which modes a spatially uniform steady state is unstable and not which mode (or supposition of modes) will be

finally displayed at steady state. Thus, the theory has two applications. Firstly, it can be used to suggest which wave modes are being initially excited and question whether they correspond to the modes available in the deterministic system. Secondly, we can explore the parameter region outside the Turing unstable regime. In the deterministic case, we would obtain no patterns. However, it has been shown that, in this regime, the stochastic description of the problem may be able to support structures which have been referred to as ‘stochastic Turing patterns’ (Biancalani *et al.*, 2010; Zheng-Ping *et al.*, 2008).

7.4.1 Stationary domain

As an initial illustration we simulate a deterministic system and compare it to its stochastic analogue. To complement this we investigate the stochastic and deterministic wave modes to which the uniform steady state is linearly unstable, both inside and outside of the deterministic Turing patterning parameter domain.

7.4.1.1 Inside the Turing parameter domain

Turing systems on stationary domains normally do not exhibit pattern selection robustness because the homogeneous steady state is unstable to multiple wave modes, as illustrated in Figure 7.1. This figure shows that, as the length of the domain, L , increases, the number of possible final patterns also increases. For example, when the length of the domain is $L = 0.4$, the spatially uniform steady state is unstable to wave modes two and three. Thus, from random initial conditions, we cannot be certain as to which final wave mode the system will evolve. However, similar to the case of deterministic Turing patterns, for small numbers of possible linearly growing wave modes we can expect that the fastest growing mode will generally dictate the solution of the system. Figures 7.2(a) and 7.2(b) show that modes two and three are realisable in both the stochastic and deterministic simulations.

By considering the spatial power spectra in figures 7.2(c) and 7.2(d) we can now compare the stochastically excited modes with the actual evolution of the system. Figure 7.2(c) clearly shows that both modes two and three are excited in the stochastic spectrum. The fact that wave mode two grows quicker than wave mode three offers a reason behind the difficulty in generating a mode three wave pattern; out of 100 simulations only seven

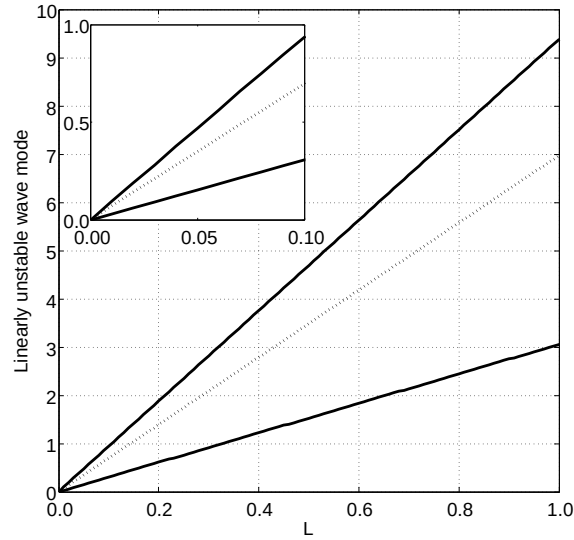


Figure 7.1: Linearly growing wave modes of the Schnakenberg kinetics for varying domain lengths. The two solid lines delineate the region of possible linearly growing wave modes. The dotted line denotes the maximum eigenvalue of the dispersion relation *i.e.* the fastest growing Fourier mode in the continuous system. The inset magnifies the region $[0,0.1]$. Parameters used: $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$ and $\kappa_3 = 1 \times 10^{-5}$.

displayed a stable mode three pattern, similar to that shown in Figure 7.2(b).

On comparing figures 7.2(c) and 7.2(d) we see that the theoretical power spectrum grows without limit, whilst the spectrum of the single stochastic realisation tends to a finite power value. This shows the limitation of the theory discussed at the start of Section 7.4, *i.e.* since we are using a linearised version of the Fokker-Planck equation we can only predict the excited wave modes rather than their amplitude. This is comparable to the standard Turing analysis (Murray, 2003a,b; Turing, 1952).

7.4.1.2 Outside the Turing parameter domain

To illustrate the second use of our theory, we now extend our investigation to parameter regions which do not allow deterministic systems to pattern. For $L = 0.1$ the inset of Figure 7.1 suggests that no deterministic Turing patterns exist, which is confirmed by deterministic simulation (not shown). Contrarily, Figure 7.3(a) clearly shows that the inclusion of noise promotes patterning. It has equally been observed that intrinsic noise can excite temporal dynamics (Erban *et al.*, 2009). However, although stochastic Turing patterns are able to exist outside of the deterministic parameter domain, they are unable

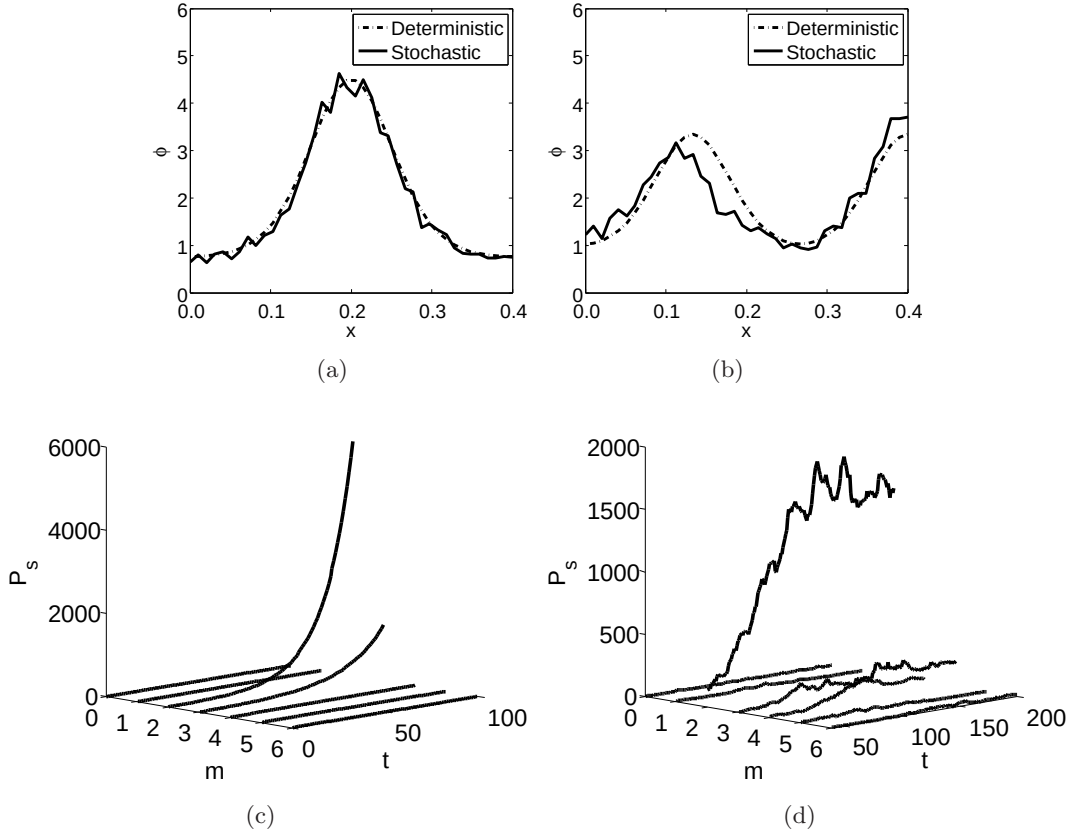


Figure 7.2: (a)-(b) For the given parameter values, both wave mode two and three patterns are possible using the Schnakenberg kinetics. Here we compare the stochastic and deterministic systems for each mode, respectively. (c) Evolution of the theoretical spatial power spectrum, equation (7.17), using the Schnakenberg kinetics. (d) Power spectrum of the single stochastic simulation shown in (a). Parameters used: $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$, $\kappa_3 = 1 \times 10^{-5}$ and $\Omega = 100$, on a stationary domain of length $L = 0.4$ discretised into $K = 40$ boxes.

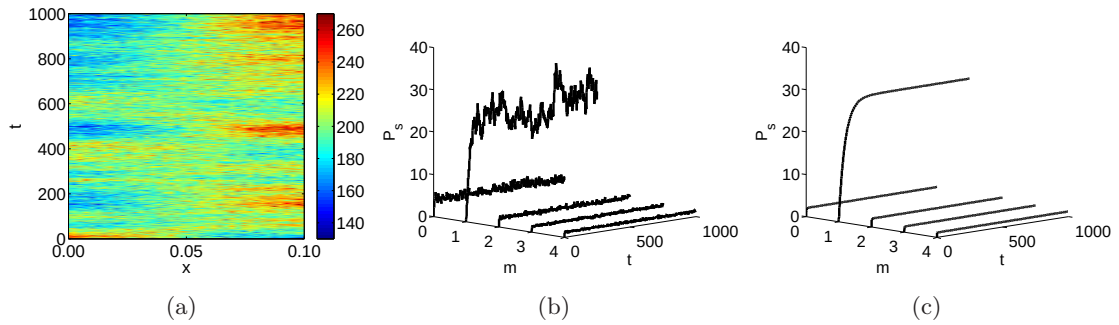


Figure 7.3: Left: evolution of the population U during a single stochastic simulation with Schnakenberg kinetics. Middle: averaged power spectrum of 100 simulations. Right: evolution of the theoretical spatial power spectrum, equation (7.17), with Schnakenberg kinetics. Parameters used: $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$, $\kappa_3 = 1 \times 10^{-5}$ and $\Omega = 100$ on a stationary domain of length $L = 0.1$ discretised into $K = 40$ boxes. For the given parameters the steady state is $U = 200$.

to stabilise over the entire simulation time. Due to the instability of these ‘stochastic Turing patterns’, the structures are able to undergo polarity switching. Note that polarity switching is also possible within the normal Turing parameter region if noise levels are sufficiently high. However, it is realised more easily outside this region because of the competing effects that are occurring; the noise tends to pattern the domain, whereas the mean-field dynamics tend to homogenise the system.

In Figure 7.3(c) we see that, since we are not inside the Turing unstable parameter region, the linearised power spectrum predicts that stochastically excited wave modes no longer continuously grow without bound and instead tend to a constant value of power. This compares well with the averaged power spectrum of the simulated data shown in Figure 7.3(b). Here, we see the theory works far better than we would expect it to. As mentioned previously, we only expect a good comparison when the population is near its steady state value, yet Figure 7.3 clearly shows that the theory may hold even in situations where the stochastic kinetics are such that a spatial mode is being activated to move the system away from the steady state. Currently, there is debate as to how the noise is able to act constructively over such long time scales. However, by considering equations (7.17) and (7.19), we can be sure that the wave modes are excited due to the second-order moments of our Gaussian approximation to the noise.

7.4.2 Sensitivity to initial conditions

Throughout simulating the stochastic and deterministic system we have noticed that the time taken between system initiation and pattern realisation can be influenced by the form of initial conditions. In figures 7.4(a)-7.4(c) the same deterministic system is simulated three times with three different initial conditions. In each case a normally distributed random variable with mean zero and standard deviation σ was added to each spatial point of the homogeneous steady state. If, upon the addition of this random number, the initial condition was negative, the absolute value of the number was taken. Figures 7.4(a)-7.4(c) show the effect of decreasing the standard deviation over the three simulations. Clearly, we can see that the size of stochastic perturbation affects the resulting time between simulation initiation and pattern formation.

Since the normal distribution produces white noise all spatial frequencies have the

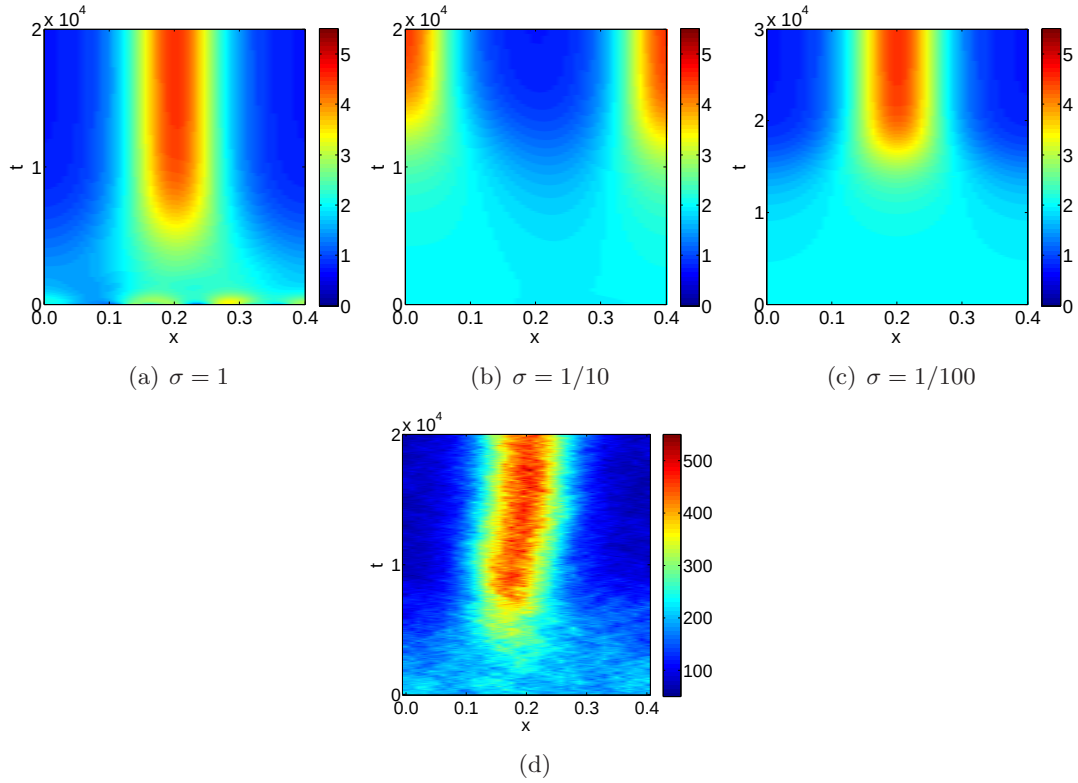


Figure 7.4: (a)-(c) Deterministic pattern formation from homogeneous steady state initial conditions with normally distributed noise (mean zero and standard deviation, σ , given under each figure) added to the deterministic initial conditions. (d) Stochastic pattern formation from homogeneous steady state initial conditions exactly. The illustrated system is the Schnakenberg kinetics, equations (2.67) and (2.68). The deterministic system was simulated using an explicit Euler stepping method and the stochastic system was simulated using a standard Gillespie algorithm. Parameters are $D_u = 10^{-6}$ mm²/s, $D_v = 10^{-4}$ mm²/s, $c_1 = 10^{-3}$ mol/s, $c_{-1} = 2 \times 10^{-3}$ /s, $c_2 = 3 \times 10^{-3}$ mol/s, $c_3 = 10^{-3}$ mol⁻²/s and $c_4 = 0$ /s. Boundary conditions are zero-flux.

same power. We also tested uniformly distributed random initial conditions and pseudo-random initial conditions in order to see if these affected the pattern initiation time in any particular way (results not shown). Although these different initial conditions may excite all wave modes, they will not all have the same power, thus, certain frequencies may dominate. However, it is observed that altering the initial condition in such a way has little effect on the time taken to form patterns, whilst altering the amplitude of the noise produces similar results to those seen in Figure 7.4.

The dependence of the time taken to pattern on the initial condition is easy to understand; if the deterministic system is initialised at exactly the homogeneous steady state then (ignoring machine error) the system will remain at the steady state. However, any

perturbation, no matter how small, will destabilise this state. The small perturbation is assumed to grow exponentially during the beginning of the simulation, but by decreasing σ we decrease the variance of the noise about the homogeneous steady state and, hence, the size of this perturbation. Thus, although the wave mode increases exponentially, it has to increase from a state that is very close to homogeneity when $\sigma = 1/100$ and, so, the time taken for patterns to appear is increased. It is worth noting that, since the wave modes grow exponentially, we would expect a logarithmic relationship between the size of the perturbation and the increase in time.

The deterministic simulations, figures 7.4(a)-(c), can then be compared with the analogous stochastic simulation in Figure 7.4(d). Here, the initial condition was the uniform steady state exactly and it is the intrinsic noise which destabilises the homogeneous state. By considering Figure 7.4 we observe that the patterning time scale of the stochastic simulation is most similar to the deterministic simulation which had the initial condition with perturbations with largest standard deviation. Critically, it is seen that by adding noise to the stochastic system's initial condition the time scale of pattern formation is not affected (results not shown). Thus, not only does the stochastic system display reduced sensitivity to initial conditions, but it is also able to realise a patterned state much more quickly than the deterministic systems, particularly when the initial condition is very close to the homogeneous steady state. This is an important observation, since the initial stochasticity that has been added to initialise each of the deterministic simulations has been generated on an *ad hoc* basis. Here, we see evidence to suggest that characterising the initial conditions correctly can have dramatic effects on the patterning time scale of the deterministic simulations.

Another assumption that we need to address is that nearly all simulations produced thus far have been initiated from the homogeneous steady state. We should question whether these initial conditions are realistic in a biological sense. Certainly, *in vitro* morphogens could be added at the beginning of an experiment to reproduce steady state conditions (Míguez *et al.*, 2006). However, *in vivo*, it will take time for morphogen concentrations to build up to sufficient levels before patterning can occur (Towers *et al.*, 2011). In Figure 7.5 we simulate the exact same system as in Figure 7.4, except that the initial conditions are set to zero in the stochastic case and zero with normally distributed noise

in the deterministic case (the absolute value is taken if the random number is negative). Immediately, we see that the standard deviation has much more influence on the patterning time scale than the change in the initial condition. We should also note that the deterministic formulation of the system is actually invalid when we start from the zero state as we are violating the assumption that there are enough reactant particles to treat the morphogens as continuous quantities, rather than discrete particles.

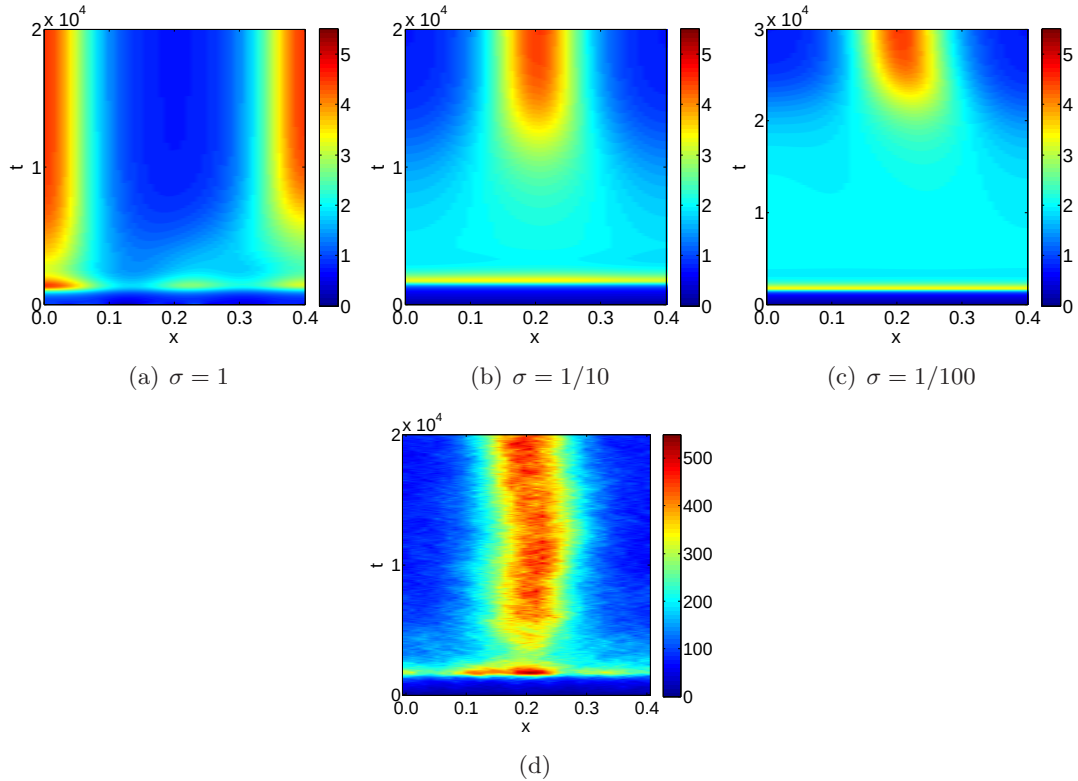


Figure 7.5: (a)-(c) Deterministic pattern formation from zero state initial conditions with normally distributed noise (mean zero and standard deviation, σ , given under each figure) added to the deterministic initial conditions. (d) Stochastic pattern formation from zero steady state initial conditions exactly. Kinetics, parameters, boundary conditions and solution methods are the same as in Figure 7.4.

7.4.3 Comparing deterministic and stochastic growth

Having seen that the intrinsic noise of reactions can affect the patterning properties of a Turing system on a stationary domain, we now include growth to see how this influences mode selection.

In Figure 7.6 we compare not only the deterministic evolution of the Schnakenberg kinetics to their stochastic analogues but we also illustrate the difference between stochastic

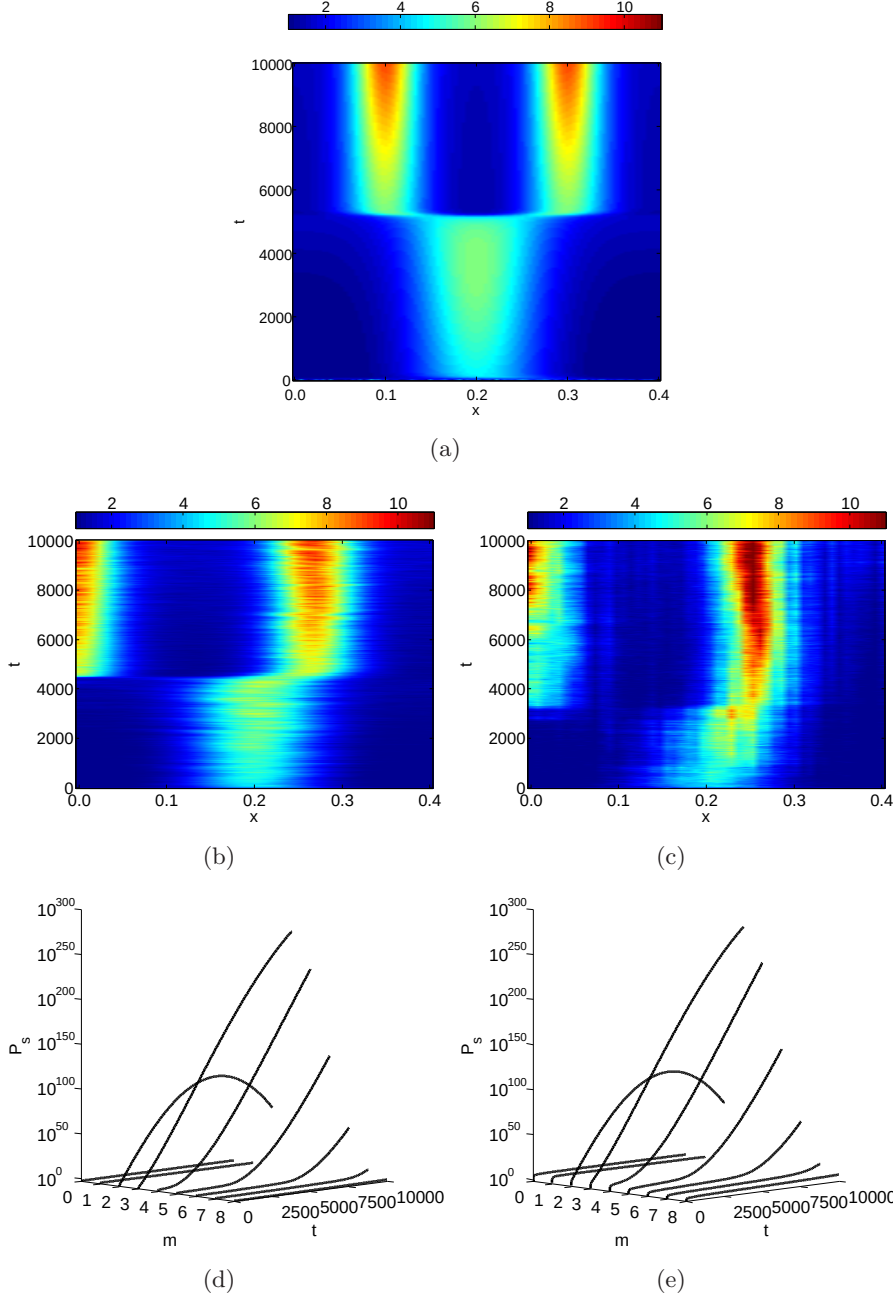


Figure 7.6: (a) Deterministic evolution of the Schnakenberg equations on a linearly growing domain. (b) Stochastic Schnakenberg kinetics on a deterministically, linearly growing domain. (c) Stochastic Schnakenberg kinetics on a stochastically, linearly growing domain. (d)-(e) Evolution of the corresponding theoretical power spectra, $\langle \hat{\eta}_{uk} \hat{\eta}_{uk} \rangle$, on deterministically and stochastically growing domains, respectively. Note that the plots have a log scale on the vertical axis. Parameters used: $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$, $\kappa_3 = 1 \times 10^{-5}$, $\Omega = 100$, $\theta = 100$ and $r/\theta = 10^{-4}$. The initial length of domain is $L = 0.4$ and there are $K = 50$ boxes.

and deterministic descriptions of growth. Starting from an initial length of $L = 0.4$ the domain was allowed to grow uniformly linearly as $n = 1 + rt/\theta$ at a rate of $r/\theta = 1 \times 10^{-4}$. Thus after 10^4 time steps the domain doubles in size, to $L = 0.8$. By considering Figure 7.1 we can see that, for a domain of length $L = 0.4$, the system can evolve to a final pattern of wave mode two or three, whereas when the length of the domain is $L = 0.8$, the system can evolve to a final pattern of any integer wave mode between three and seven, inclusive. This evolution of activated modes is captured in figures 7.6(d) and 7.6(e) where, initially, mode two has the highest power but as time increases further wave modes are activated. As the simulation reaches $t = 2220$ the growth of the second mode begins to slow down and eventually reduces, whilst the third mode overtakes it as the most strongly excited mode. This transition can be linked to the breakdown of period doubling that occurs in the transition from deterministic kinetics to stochastic kinetics, as seen in figures 7.6(a)-7.6(c), although the realisation of the transition occurs much later in the simulated system than estimated from the covariance ODEs.

From comparing figures 7.6(b) and 7.6(c) and figures 7.6(d) and 7.6(e) we can conclude that the inherent noise of the reactions far outweighs the noise generated from the stochastic description of growth, since qualitatively the figures are the same. However, it should be noted that the stochastic growth spectrum has a higher power. The only noticeable difference in the simulations being that, as expected, the stochastic growing domain simulation is noisier; the maximum range of the solution is larger than the deterministic growth simulation and the transition from mode two to three occurs earlier. Practically, since our main consideration is the transition sequences, this implies that it is sufficient to consider deterministic growth.

In both the situations of growing and static domains, whenever we are inside the Turing unstable parameter region the noise excites the same modes as the deterministic Turing analysis. Particularly, due to consecutive wave mode excitation, the primary effect of growth is seen to remove peak-splitting as the mechanism of transition and replace it with consecutive increasing of the wave mode of the solution. This correspondence of activated wave modes is proved in Section 7.5.

7.4.4 Mechanisms of robustness

In the previous section we saw that stochasticity caused a break down of robustness via mode doubling. Although this loss of robustness may not be surprising it does lead us to question the sensitivity of the pattern doubling mechanism, *i.e.* will arbitrarily small noise lead to a break down of pattern doubling? Furthermore, is this new mechanism of consecutive mode excitation robust?

Theoretically, as the population scale, Ω , becomes larger the stochastic simulations should approximate their deterministic counter-parts better. The effect of increasing Ω can be seen in Figure 7.7, where, for values of $\Omega \leq 10^4$, the pattern doubling mechanism breaks down as the system undergoes the transition from wave mode two to three. Although $\Omega = 10^4$ is nowhere near the number of molecules in a mole (10^{23}), numerically we are at the limit of our computational power using single particle interactions. Thus, to consider stochastic effects within larger populations we use a Langevin framework (see Appendix D) in which the noise is correctly scaled (Figure 7.8) (Gillespie, 1996, 2000, 2002). Although this is only an approximation of the system under consideration, as we have moved from a discrete case description to a continuous description, it will at least suggest if the transitions are stable against any form of noise, or whether stochasticity singularly perturbs the deterministic system. From Figure 7.8 we see the first signs of robust doubling reappearing, as the transition $2 \mapsto 4$ stabilises for $\Omega \geq 10^6$ and, further, the transition $2 \mapsto 4 \mapsto 8$ is observed in Figure 7.8(b). Thus, we conjecture that as the parameter Ω increases the doubling transitions will become more and more common. However, for any finite value of Ω there will come a time point at which the stochastic transitions diverge from the deterministic transitions. This is because, as the system evolves to higher and higher wave modes, the bifurcation diagram increases in complexity and therefore it is more likely that the simulations will exhibit one of the other possible modes (Barrass *et al.*, 2006). This breakdown of period doubling is also compounded by the fact that we are using linearly growing domains, as it has been shown analytically that only exponentially growing domains can support period doubling in the deterministic case. Hence, for all sub-exponential growth rates, there will come a point at which even the deterministic system is unable to maintain a pattern doubling transition. However, linear growth is

used in preference to exponential growth, since it greatly reduces the computational time, whilst also being able to produce the first few doubling transitions (see figures 7.6(a) and 7.8(c)).

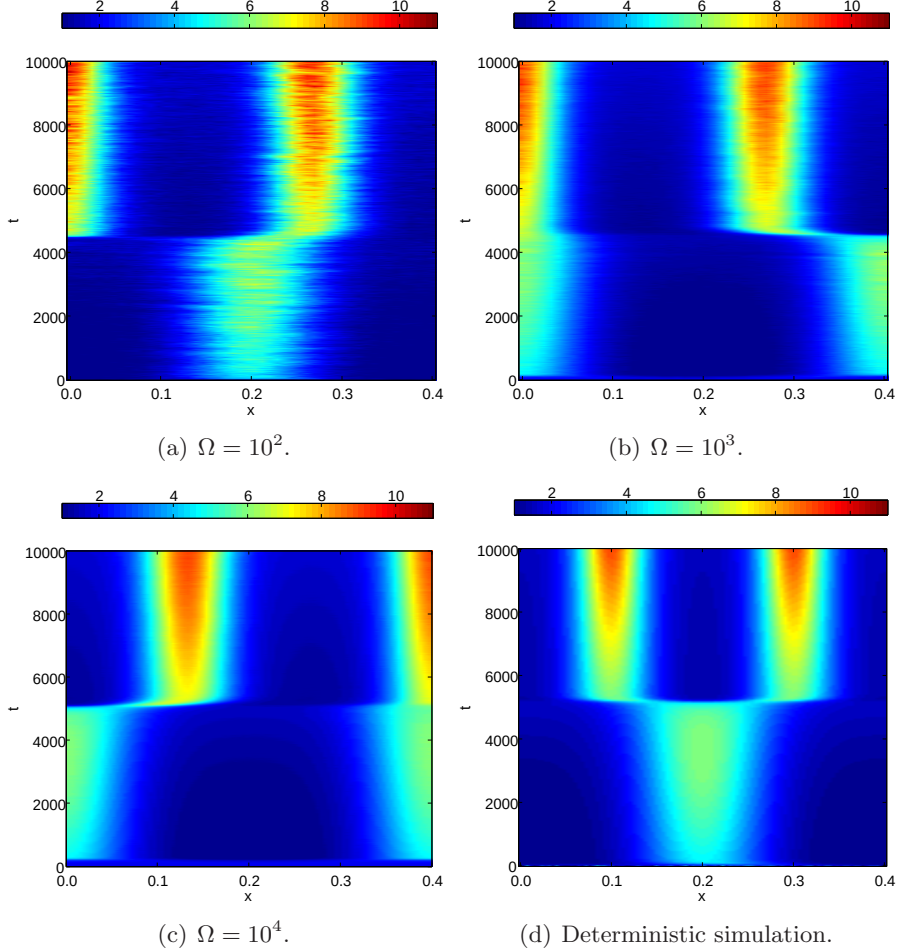


Figure 7.7: Stochastic simulations with increasing value of Ω , noted beneath each figure. Parameters are $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$, $\kappa_3 = 1 \times 10^{-5}$, and $r/\theta = 10^{-4}$. Initially the length of the domain was $L = 0.4$ and the domains in (a) and (b) were discretised into $K = 50$ boxes, whereas the domains in (c) and (d) were discretised into $K = 100$ boxes.

Since our primary concern is with low copy number systems we must return to the hypothesis that, although pattern doubling is not robust, it may be possible to use consecutive wave number increase as a mechanism of robustness, as suggested by Figure 7.6. In Figure 7.9 we show the results of repeated simulation of the stochastic system for cases with $K = 50$ Lagrangian boxes (top row) and $K = 100$ Lagrangian boxes (bottom row) to ensure that the domain discretisation was not affecting the simulations. The simulations show that the phenomenon of sequentially increasing wave numbers is not completely ro-

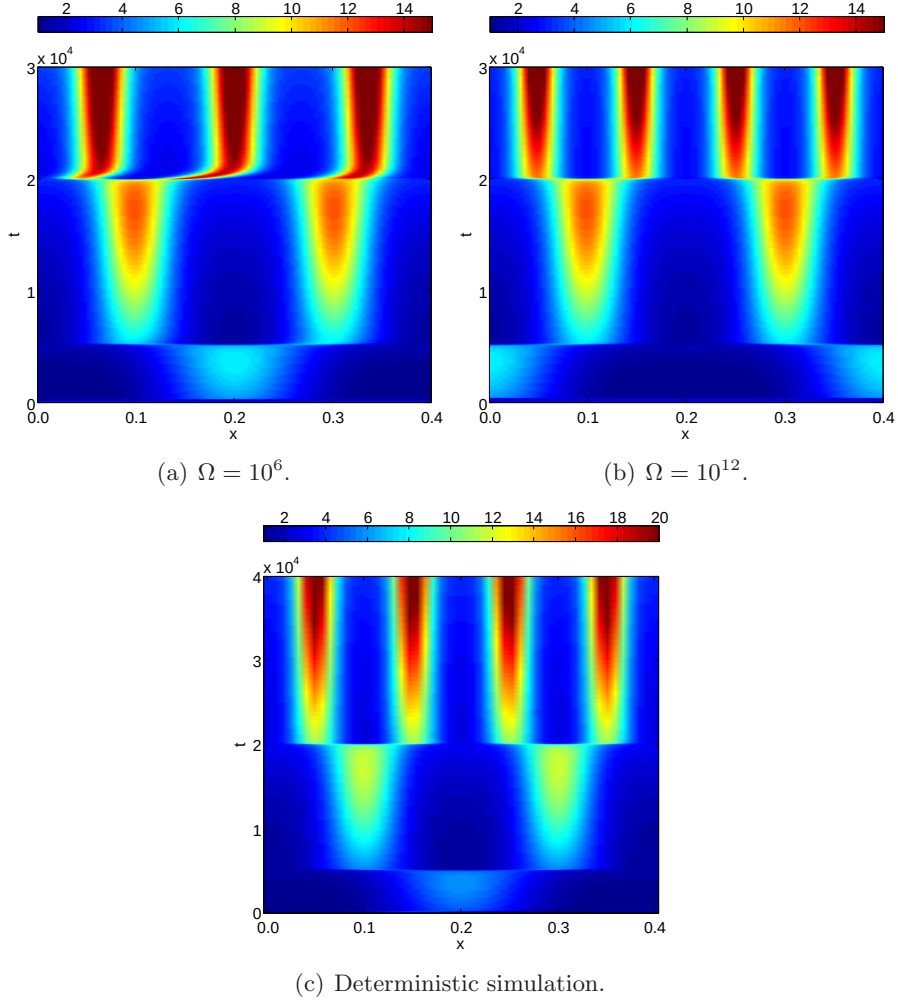


Figure 7.8: Langevin simulations with increasing values of Ω , noted beneath each figure. Parameters are $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$, $\kappa_3 = 1 \times 10^{-5}$, and $r/\theta = 10^{-4}$. Initially the length of the domain was $L = 0.4$ and the domains were discretised into $K = 100$ boxes.

bust as, although the majority of simulations do show transitions $2 \mapsto 3 \mapsto 4$, after this transitions $4 \mapsto 5$ and $4 \mapsto 6$ are both seen and, furthermore, the transition $2 \mapsto 4$ is also possible. Also note that we have no control over the polarity of the solution. Thus, we conclude that, for systems that contain only a small number of active particles, uniformly growing domains may be able to support robust sequences of the lower wave modes. For further evidence of this claim see Figure 7.10. Here, we have simulated the evolution of 100 stochastic systems and calculated the dominant wave mode at each time point. Clearly we see that the most likely transition sequence is a consecutively increasing one since the wave mode with the highest probability increases consecutively. However, this probability reduces with increasing number of active wave modes. For example, the probability that the system will exhibit a wave mode two pattern during the first 5000 time units is 0.98. Similarly, the probability the system will be in a mode three pattern during the next 5000 time units is 0.96. Interestingly, although we can see that the probability of being in a particular state decreases over time, due to more wave modes becoming available, this decrease is not monotonic. Indeed, over the regions where the probability of being in an even mode is highest, this probability is higher than the probability of the preceding odd mode. For example, the probability of being in a mode six pattern during $t \approx 25000 - 30000$ time units is 0.87, whereas the probability of being in a mode five pattern during $t \approx 20000 - 25000$ is 0.81. This can be explained by reasoning that it is an artefact of the mean-field causing the system to exhibit peak splitting. Hence, although small transition sequences of consecutive increasing modes are likely, they are not certain. This was also commented upon by Arcuri & Murray (1986), who studied deterministic Turing systems on growing domains. Due to the inclusion of growth through a simple scaling argument rather than a derivation from first principles, they noticed that their systems would often miss certain wave modes during their evolution. Hence, they concluded that patterns must form sequentially as any mechanism that acts over the whole domain is subject to too many sources of error to be capable of robust pattern formation at high wave modes. Although they did not include a dilution term, which we can now account for if the growth is isotropic, in the stochastic setting we are unable to generate robustness successfully on uniformly growing domains.

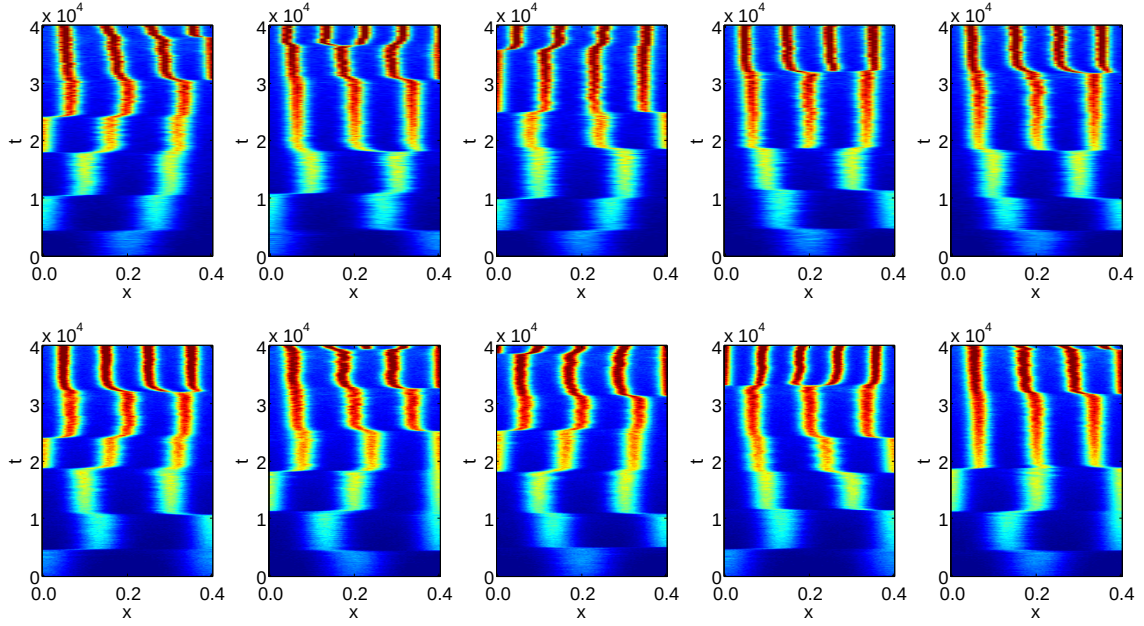


Figure 7.9: Ten stochastic simulations with varying numbers of Lagrangian boxes. Top row: $K = 50$. Bottom row: $K = 100$. Parameters $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$, $\kappa_3 = 1 \times 10^{-5}$, $\Omega = 100$, $\theta = 100$ and $r/\theta = 10^{-4}$. Initial length of domain $L = 0.4$. The colour map is from 0 (blue) to 20 (red).

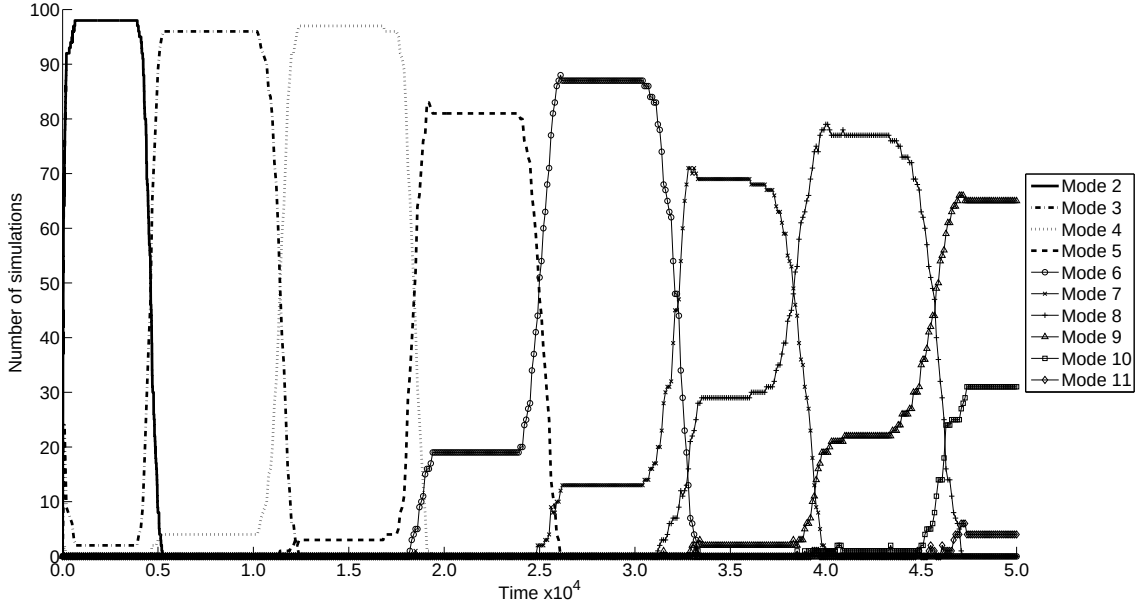


Figure 7.10: Exhibited wave modes of 100 simulations using the same parameters as in Figure 7.9. The domain was discretised into 100 Lagrangian boxes.

7.5 Analysis of the covariance ODEs

Simulations seem to suggest that, inside the parameter region which realises deterministic Turing patterns, the excited wave modes of the stochastic Schnakenberg system correspond to the deterministic analogue, although the transition sequences may not. From considering a general reaction-diffusion system on a deterministic, uniformly growing domain that has been mapped onto a Lagrangian domain,

$$\frac{\partial \phi}{\partial t} = \frac{D_u}{n^2} \frac{\partial^2 \phi}{\partial x^2} + f(\phi, \psi, t), \quad (7.32)$$

$$\frac{\partial \psi}{\partial t} = \frac{D_v}{n^2} \frac{\partial^2 \psi}{\partial x^2} + g(\phi, \psi, t), \quad (7.33)$$

we intend to show that this is true for all Turing systems. As we are assuming slow growth, we use a quasi-steady-state hypothesis and, thus, fix $n = 1$.

From the reaction system that produces the kinetic functional forms f and g we can derive the propensity functions and stoichiometric matrix (see Section 7.3). Then, using the macroscopic steady state values of ϕ and ψ , we can calculate the matrices $\mathbf{A}$ and $\mathbf{B}$ from the definitions in equations (5.28) and (5.29), which allows us to construct the ODE system of Fourier-transformed covariances in (7.17)-(7.19). Rewriting this as a matrix ODE equation we obtain

$$\dot{\mathbf{X}} = \mathbf{M}\mathbf{X} - \mathbf{V}, \quad (7.34)$$

where $\mathbf{X}^T = (\langle \hat{\eta}_{uk} \hat{\eta}_{uk} \rangle, \langle \hat{\eta}_{uk} \hat{\eta}_{vk} \rangle, \langle \hat{\eta}_{vk} \hat{\eta}_{vk} \rangle)$. The explicit form of $\mathbf{M}$ is

$$\mathbf{M} = \begin{bmatrix} 4a_1 \cos(k\Delta_L) + 2a_0 & 2b_0 & 0 \\ c_0 & 2(a_1 + d_1) \cos(k\Delta_L) + a_0 + d_0 & b_0 \\ 0 & 2c_0 & 4d_1 \cos(k\Delta_L) + 2d_0 \end{bmatrix}, \quad (7.35)$$

and $\mathbf{V}$ is defined appropriately from equations (7.17)-(7.19). Since equation (7.34) is linear and autonomous we can solve the ODEs analytically yielding a solution which is the linear

sum of three exponential functions with exponents

$$\lambda_1 = 2a_1 \cos(k\Delta_L) + a_0 + 2d_1 \cos(k\Delta_L) + d_0, \quad (7.36)$$

$$\lambda_{\pm} = 2a_1 \cos(k\Delta_L) + a_0 + 2d_1 \cos(k\Delta_L) + d_0 \pm \left[4(a_1 - d_1)^2 (\cos(k\Delta_L) - 1)^2 + 4b_0c_0 + 4(a_1 - d_1)(2a_1 + a_0 - d_0 - 2d_1) \cos(k\Delta_L) + (a_0 - d_0)^2 - 4(d_1 - a_1)^2 \right]^{\frac{1}{2}}. \quad (7.37)$$

The existence of such a solution also depends on these three values not being zero, which is always satisfied inside the Turing domain, as shown below.

Since we are only interested in the coefficients matrix, $\mathbf{M}$, of the Fourier transformed covariances, we only need to consider the matrix $\mathbf{A}$. This can be split up into four $K \times K$ sub-matrices,

$$\mathbf{A} = \begin{bmatrix} \mathbf{a} & \mathbf{b} \\ \mathbf{c} & \mathbf{d} \end{bmatrix}, \quad (7.38)$$

where $\mathbf{a}$ and $\mathbf{d}$ are symmetric and tridiagonal and $\mathbf{b}$ and $\mathbf{c}$ are diagonal. From their definitions, equation (5.28), we can show that $\mathbf{b} = \partial f / \partial \psi \mathbf{I} = b_0 \mathbf{I}$ and $\mathbf{c} = \partial g / \partial \phi \mathbf{I} = c_0 \mathbf{I}$, where $\mathbf{I}$ is the $K \times K$ identity matrix. The diagonal and off-diagonal values of $\mathbf{a}$ are

$$a_0 = \frac{-2D_u K^2}{L^2} + \frac{\partial f}{\partial \phi}, \quad (7.39)$$

$$a_1 = \frac{D_u K^2}{L^2}, \quad (7.40)$$

respectively, whereas

$$d_0 = \frac{-2D_v K^2}{L^2} + \frac{\partial g}{\partial \psi}, \quad (7.41)$$

$$d_1 = \frac{D_v K^2}{L^2}, \quad (7.42)$$

are the diagonal and off-diagonal elements, respectively, of $\mathbf{d}$. Each term is evaluated at the homogeneous steady state, $\phi_i = \phi^*$ and $\psi_i = \psi^*$, for all values of i . Substituting these

values into equations (7.36) and (7.37) we obtain

$$\lambda_1 = 2 \frac{D_u K^2}{L^2} (\cos(k\Delta_L) - 1) + 2 \frac{D_v K^2}{L^2} (\cos(k\Delta_L) - 1) + f_\phi + g_\psi, \quad (7.43)$$

$$\begin{aligned} \lambda_\pm = \lambda_1 \pm & \left[4 \left(\frac{D_u K^2}{L^2} - \frac{D_v K^2}{L^2} \right)^2 (\cos(k\Delta_L) - 1)^2 + 4(g_\phi f_\psi - f_\phi g_\psi) + (f_\phi + g_\psi)^2 \right. \\ & \left. + 4(f_\phi - g_\psi) \left(\frac{D_u K^2}{L^2} - \frac{D_v K^2}{L^2} \right) (\cos(k\Delta_L) - 1) \right]^{\frac{1}{2}}. \end{aligned} \quad (7.44)$$

Since $(\cos(k\Delta_L) - 1) \leq 0$ and inside the Turing region we know that $f_\phi + g_\psi < 0$ (Murray, 2003b), λ_1 and $\text{Re}(\lambda_-) < 0$. Hence, the only way to obtain an excited mode in the Turing region is if $\text{Re}(\lambda_+) > 0$. In order to satisfy this condition the following inequality for $y = (\cos(k\Delta_L) - 1)$ must be satisfied:

$$\frac{4D_u D_v K^4}{L^4} y^2 + \frac{2K^2}{L^2} (f_\phi D_v + D_u g_\psi) y + f_\phi g_\psi - g_\phi f_\psi < 0. \quad (7.45)$$

Thus, excited modes will exist for

$$-\frac{L^2}{K^2} y_+ < y < -\frac{L^2}{K^2} y_-, \quad (7.46)$$

where

$$y_\pm = \frac{f_\phi D_\psi + g_\psi D_\phi \pm \sqrt{(f_\phi D_\psi + D_\phi g_\psi)^2 - 4D_\phi D_\psi (f_\phi g_\psi - f_\psi g_\phi)}}{4D_\phi D_\psi}. \quad (7.47)$$

Note that inside the Turing unstable domain $y_\pm$ are real and so the steady state is unstable whenever

$$1 - \frac{L^2}{K^2} y_+ < \cos(k\Delta_L) < 1 - \frac{L^2}{K^2} y_-. \quad (7.48)$$

Thus, whenever the two limits of this range are within $[-1, 1]$ we can invert the cosine function to obtain

$$\frac{\arccos(1 - y_- \frac{L^2}{K^2})}{\Delta_L} < k < \frac{\arccos(1 - y_+ \frac{L^2}{K^2})}{\Delta_L}. \quad (7.49)$$

To make sure the domain discretisation does not interfere with the simulations we make the number of boxes, K , large and hence we are able to expand the arccos operator in

terms of large K :

$$\sqrt{2y_-} \left(1 + \frac{1}{12} y_- \left(\frac{L}{K} \right)^2 \right) \lesssim k \lesssim \sqrt{2y_+} \left(1 + \frac{1}{12} y_+ \left(\frac{L}{K} \right)^2 \right). \quad (7.50)$$

Letting $K \rightarrow \infty$, or $\Delta_L \rightarrow 0$, in such a way that $L = K\Delta_L$ remains constant, space becomes a continuum and, thus, using $k = m\pi/L$,

$$\frac{\sqrt{2y_-}}{\pi} L = m_- < m_c < m_+ = \frac{\sqrt{2y_+}}{\pi} L. \quad (7.51)$$

Using equation (7.47), inequality (7.51) can be compared exactly with the activated modes derived from the deterministic Turing analysis (Murray, 2003b). Hence, we can see that inside the deterministically unstable Turing parameter region, the stochastically excited modes are exactly the same as the Turing excited modes.

However, this is only true in the limit $K \rightarrow \infty$ and with slow growth. In a discretised domain of K boxes there can be, at most, K active wave modes and, as the domain grows, higher wave modes also have the possibility of growing. As the activated wave number tends to the number of boxes, the noise excites higher wave modes than otherwise expected. This is illustrated in Figure 7.11, which shows the stochastically excited modes when the domain is discretised into 25 boxes. As the number of modes increases the line begins to distort until its tangent becomes infinite at wave mode 25. In terms of inequality (7.48), the lower root has reduced to below -1, implying that the equation no longer has two, real solutions.

For a given length, L , we are able to use the upper boundary of inequality (7.50) to define a lower bound on K ,

$$\begin{aligned} m &= \frac{kL}{\pi}, \\ &\approx \frac{L}{\pi} \sqrt{2y_+} \left(1 + \frac{1}{12} y_+ \left(\frac{L}{K} \right)^2 \right), \\ &= m_+ + \frac{\sqrt{2}}{12\pi} y_+^{3/2} \left(\frac{L^3}{K^2} \right). \end{aligned} \quad (7.52)$$

The discretisation of the space will become inadequate when, upon spatially Fourier transforming the solution, we observe higher wave modes to be amplified than would be ex-

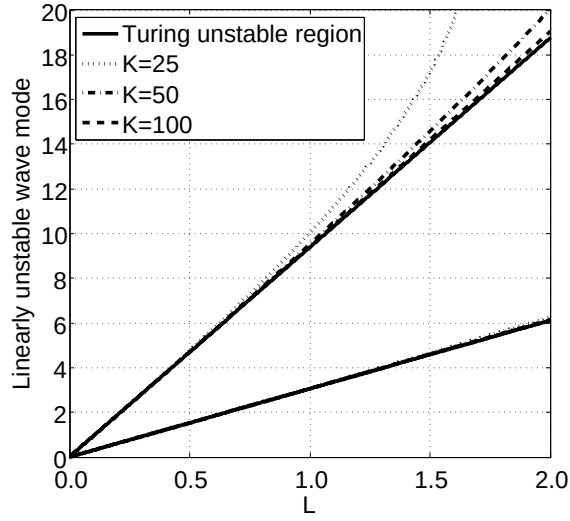


Figure 7.11: Comparison of linearly growing wave modes of the deterministic Turing analysis (black solid lines) and noise excited wave modes for different numbers of boxes, K , using the upper and lower bounds of inequalities (7.48) and (7.51). Parameters are $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$ and $\kappa_3 = 1 \times 10^{-5}$. Note that the lines delineating the lower bounds all lie approximately on top of each other.

pected from the deterministic Turing analysis. From equation (7.52) we see that we need to choose K satisfying

$$\begin{aligned} \frac{\sqrt{2}}{12\pi} y_+^{\frac{3}{2}} \frac{L^3}{K^2} &< 1, \\ \Rightarrow \frac{y_+^{\frac{3}{2}} L^{\frac{3}{2}}}{\sqrt{6\sqrt{2}\pi}} &< K. \end{aligned} \quad (7.53)$$

Inequality (7.53) suggests that the number of boxes in the discretised system needs to grow faster than the length due to the exponent of $3/2$. For the small scale systems we are simulating we can easily choose K large enough not to violate this condition. However, for larger values of L we would need a much larger value of K and thus a much finer discretisation. Since our analysis uses the weak noise limit this would mean including a larger number of simulated particles. This combination of factors implies that as the domain becomes larger the Gillespie SSA will take longer to simulate the system, as each time step becomes smaller due to the propensities increasing with the number of particles.

7.6 Conclusion

In this chapter we have applied previously developed Fourier transform techniques to Turing reaction-diffusion systems on growing domains (see chapters 5 and 6) (Woolley *et al.*, 2011a,b). Our aim was to consider the lack of robustness that is apparent in such paradigm models, when the uniform steady state is linearly unstable to multiple wave modes (Maini *et al.*, 1997). It has been shown in the deterministic case that, upon placing Turing systems on uniformly growing domains, robust peak doubling sequences can be generated (Crampin *et al.*, 1999). However, upon stochastically simulating the systems we have found that this robustness is lost.

Firstly, we considered the specific example of the Schnakenberg kinetics on a stationary domain. Here it was seen that, within the deterministic Turing instability regime, the uniform steady state of the stochastic system is linearly unstable to exactly the same wave modes as the deterministic system. However, although we were able to suggest which wave modes were excited, we were unable to predict which mode the final pattern would adopt. This is similar to the standard Turing analysis, which, due to the linearisation process, suggests that all excited modes tend to grow exponentially and ignores higher order terms that become important as the solution evolves. In the stochastic case, due to our use of a linear noise expansion, we also lose the higher order effects and, thus, our analysis is only valid around the homogeneous steady state.

Although we are unable to predict exactly to which mode the system is going to evolve, if the number of excited modes is small then the power spectrum suggests which mode grows most quickly. This, in turn, suggests to which mode the system will tend. This was demonstrated in Figure 7.2, where the power spectrum suggests that both modes two and three are excited, although wave mode two grows more quickly. This correlates with the fact that, out of 100 stochastic simulations, 93 yielded a stable mode two pattern, whereas only seven exhibited a stable wave mode three pattern. This correlation between highest power mode and realised stable pattern was also seen in the growing domain simulations (Figure 7.6). Here, the power spectrum shows that, although wave mode two is initially the highest power mode, during growth wave mode three surpasses mode two to become the dominant mode. This can then be linked to the exact same transition in the simulated

systems.

Parameters outside the deterministic Turing unstable region were then considered. Although such stochastic Turing patterns have been postulated before (Biancalani *et al.*, 2010), our simulations are, to our knowledge, the first numerical evidence of their actual existence. Further, we have discovered that, due to stochastic effects, polarity switching is possible (Figure 7.3). Due to the system lying outside the deterministic patterning parameter region, the activated modes no longer grow exponentially and we see a good comparison between the theoretical and simulated power spectra. Since the theory only holds near the homogeneous steady state and noise forces the system away from this state, this excellent correspondence is somewhat unexpected.

Having investigated the stationary domain, we then moved on to consider uniformly growing domains. Immediately, it was seen that stochastic effects destroy any hope of robust peak doubling sequences when the population numbers are low. By comparing stochastic and deterministic growth, we see that the noise generated by reactions is more important than the noise generated by stochastic domain growth. Indeed, it is due to the kinetics that activated modes grow exponentially, the effect of stochastic domain growth is simply to increase the power of all modes.

Using this knowledge we focused simply on deterministic growth, which further reduced the size of the system of Fourier covariance ODEs. Using general reaction-diffusion equations we were able to show that the stochastic Turing modes were able to grow exponentially and, further, that these excited modes exactly correspond to their analogous deterministic Turing modes. This has allowed us to suggest that the reason that the peak doubling sequences break down is that the simulations simply switch to the highest power mode, which increases consecutively.

In Section 7.4.4 we investigated this break down further and questioned whether stochasticity was a singular perturbation of the deterministic system. Since the noise scales as $1/\sqrt{\Omega}$, we increased Ω to our computational limit and saw that for large enough populations, $\Omega = 10^6 - 10^{12}$, period doubling can be realised, at least for the first couple of transitions. If we now compare these particle numbers to a physical system it is estimated that the haploid yeast cell contains 10^7 molecules of protein (Futcher *et al.*, 1999). As seen in Figure 7.8(a), by this point the stochastic effects in the system are incredibly

small. Hence, systems of this size and larger could be justified to act as a continuum. However, there are many biological systems which depend on very low copy numbers of active proteins, *e.g.* the genome of the bacterium *Escherichia coli*, where there are only 10-30 molecules of the lac repressor, which is involved in the regulation of gene expression (Guptasarma, 1995). Thus, our focus on small population numbers is fully justified.

Furthermore, in this low copy number regime, even the consecutive inclusion of wave modes is not robust on uniformly growing domains when the homogeneous steady state is unstable to large numbers of wave modes. In this case predicting the evolution of the pattern is incredibly difficult. Thus, our results support those of Arcuri & Murray (1986), who concluded that patterns must form sequentially as any mechanism that acts over the whole domain is subject to too many sources of error to be capable of robust pattern formation at high wave modes. Hence, to generate robustness, in a stochastic environment, we suggest apical growth as a plausible mechanism that can robustly support consecutive wave mode increasing pattern sequences (see Figure 7.12). Thus, if apical domain growth and wave number were connected in some form of feedback loop then, once the desired wave number is reached, growth would stop, leaving a stable pattern of exactly the desired wave mode.

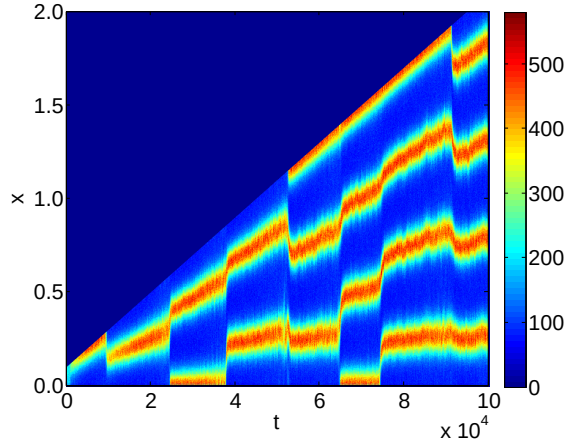


Figure 7.12: Stochastic Schnakenberg kinetics simulated on a deterministic apically growing domain. Parameters are $D_u = 1 \times 10^{-4}$, $D_v = 1 \times 10^{-2}$, $\kappa_1 = 10$, $\kappa_{-1} = 0.2$, $\kappa_2 = 30$, $\kappa_3 = 1 \times 10^{-5}$, $\Omega = 100$ and $r/\theta = 1/5 \times 10^3$. Initially, $L = 0.1$ and the domain was discretised into 25 boxes.

Chapter 8

Discussion and future research

“We can only see a short distance ahead, but we can see plenty there that needs to be done.”

Alan M. Turing, Computing machinery and intelligence, 1950.

Mathematical modelling is now used throughout developmental biology. The strengths of such theoretical methods lie not only in clarifying the underlying mechanisms through which complexity forms but also in their power of prediction, which can be used to inform experimental design. One key component of using mathematical theory to characterise biological systems is understanding how robust their behaviours need to be. There will always be a certain degree of variability between individual living systems. For example, asexual reproduction can result in different phenotypes from the same genotype if individuals are subject to differing environmental conditions. However, even with numerous sources of intrinsic and extrinsic noise, biological processes are able to consistently develop similar results, *e.g.* the same number of digits in chick wings and legs. In this thesis we have addressed this problem of creation and maintenance of patterns by, firstly, gaining a deeper understanding of how complexity is formed in specific cases and, secondly, developing a stochastic formalism enabling us to investigate robustness of the emergent complexity.

In Chapter 2, we derived the deterministic formalism, which was used frequently throughout this thesis to describe biological, or mathematical phenomena. Critically, we demonstrated the power of diffusion as a key mechanism of pattern formation, whether

it be through the construction of a simple gradient, as in a French flag pattern, or through its ability to destabilise a uniform steady state, as in a Turing pattern. These simple systems were then extended to incorporate growing domains. Having recapitulated the deterministic theory and explored the effects of growth on reaction-diffusion systems we were able to contend with our first aim, which was to use current experimental insights to construct realistic developmental models. Critically, we wanted to not only capture the final state of the system but also to recapitulate experimental observations throughout the time evolution of the mathematical simulations.

In Chapter 3 we investigated two distinct cases of molar development in mice and digit development in chick limbs. In both cases growth was shown to be a key assumption necessary for the generation of observed dynamics. In particular, in the case of mice teeth, we needed apical growth to occur in the jaw to ensure consecutive development of molars, whilst growth in the chick wing model was needed to slow down the creation of a gradient solution. Furthermore, in the case of chick digit development, our model has predicted that the source of Shh in the polarising region must increase over time instead of saturating immediately. However, using such a gradient solution we have been unable to recapitulate chick leg digit data accurately, implying that, although cells in the digit forming field can differentiate based on the local concentration of Shh that they sense, the control of digit formation in the polarising region must be more complex than in the rest of the digit forming field.

The second aim of this thesis was to increase our analytical understanding of the theoretical formalisms that we use to model biological phenomena. Chapter 4 encapsulated both aims by allowing us to consider a biologically motivated problem of pigmentation patterns on stingrays, whilst trying to understand the mathematical phenomena known as stable droplets. Analytically, this system was quite tractable and we were able to derive a formula linking the radius of the droplets to the asymmetry and diffusion coefficients of the system. Since we were missing a number of key biological insights, such as potential chemical species that the activator and inhibitor could be identified with, the parameter values were chosen for their ability to demonstrate the droplet phenomenon, rather than their physical interpretation. However, upon using values which were of the correct order of magnitude, it was shown that the droplets can form on biologically realistic time scales

consistent with current observations of stingray gestation periods.

This brings us to chapters 5, 6 and 7, where we extend the formalism derived in Chapter 2 to encompass intrinsic stochastic effects. In particular, in Chapter 5, we start from a CME description and use an assumption of weak noise to derive the mean-field dynamics, as well as the effects of noise upon them. As we are interested in spatial patterning, we then introduce the discrete Fourier cosine transform, which allows us to interrogate the wave modes excited by the noise.

In Chapter 6 we consider the effects of incorporating both deterministic and stochastic domain growth in the derived stochastic framework. This was done by creating a Lagrangian description of growth, akin to that seen in the deterministic case. Critically, this led to the unexpected problem of non-uniqueness when defining a description of diffusion that is consistent with the mean-field thermodynamic limit. Crucially, it was observed that certain combinations of growth and diffusion were able to produce non-transient, non-uniform states without the need of reaction kinetics. Although the inhomogeneity produced by stochastically simulating diffusion on growing domains is not a pattern in the same sense as the Turing mechanism, it does imply that growing tissues that rely on diffusing populations need to be regulated against transient as well as longer lasting stochastic perturbations of the mean-field concentrations.

Upon applying these analytical methods to stochastic Turing systems in Chapter 7, we immediately see that Turing patterns are able to exist and stabilise, even in the face of intrinsic noise. Moreover, noise was shown to have certain beneficial effects, in that stochasticity is able to initiate pattern formation earlier as well as excite wave modes outside of the deterministically derived pattern formation parameter region. However, although noise does provide a number of benefits, it also leads to a breakdown in the pattern doubling mechanism that was used to generate robust transition sequences in Chapter 2. Increasing the population scale and, thus, equivalently, reducing the relative size of stochastic perturbation did enable us to regain the doubling mechanism indicating that, although noise is not a singular perturbation of the deterministic system, it is of paramount importance to understand the level of noise that a system is subject to. Fortunately, robust sequences are still producible for systems with large amounts of noise if the kinetics are simulated on apically growing domains rather than uniformly growing

domains. Through the use of the discrete Fourier cosine transform we are also able to derive an inequality regarding the effect of the discretisation on the stochastically excited wave modes.

8.1 Future work

When applied to systems with zero Neumann boundary conditions the Turing mechanism of diffusion-driven instability is well understood and, as we have demonstrated in Chapter 2, analytical results regarding the doubling of patterns can be derived. However, when the boundary conditions are altered to Dirichlet conditions we find that these analytical results no longer hold, since they depend on the continuity of the derivatives at the boundaries. Furthermore, even the standard Turing analysis is seen not to hold everywhere as fixing the boundary concentrations to be at the homogeneous steady state facilitates patterning outside of the standard Turing domain, as can be seen in Figure 8.1(c). By considering Figure 7.1, which illustrates the wave modes that are able to destabilise the steady state, we can see that, for a length of $L = 0.1$, linear analysis predicts that the uniform steady state should be stable, whereas Figure 8.1(c) demonstrates an inhomogeneous pattern is produced on a domain of length $L = 0.1$. Furthermore, from Figure 8.1, we can see that asymmetric peaks can occur, as well as the breakdown of pattern doubling. Thus, even within this well documented theory, there are many analytical avenues that are still open for exploration.

Although, in Chapter 3, we have achieved some success through the application of deterministic theory to biological examples, there are a number facets of the models that could be improved upon by experimentally testing our assumptions. For instance, the most important assumption in the current model of molar development is that the morphogens satisfy different boundary conditions; the activator and inhibitor have zero Dirichlet boundary conditions, whereas the mediator has zero Neumann boundary conditions. Thus, this simple model has generated a falsifiable prediction, which generates further questions based on whether these mixed boundary conditions are experimentally confirmed or denied. For example, if the morphogens do satisfy different boundary conditions we are led to question how these conditions are produced. Conversely, if the morphogens do not

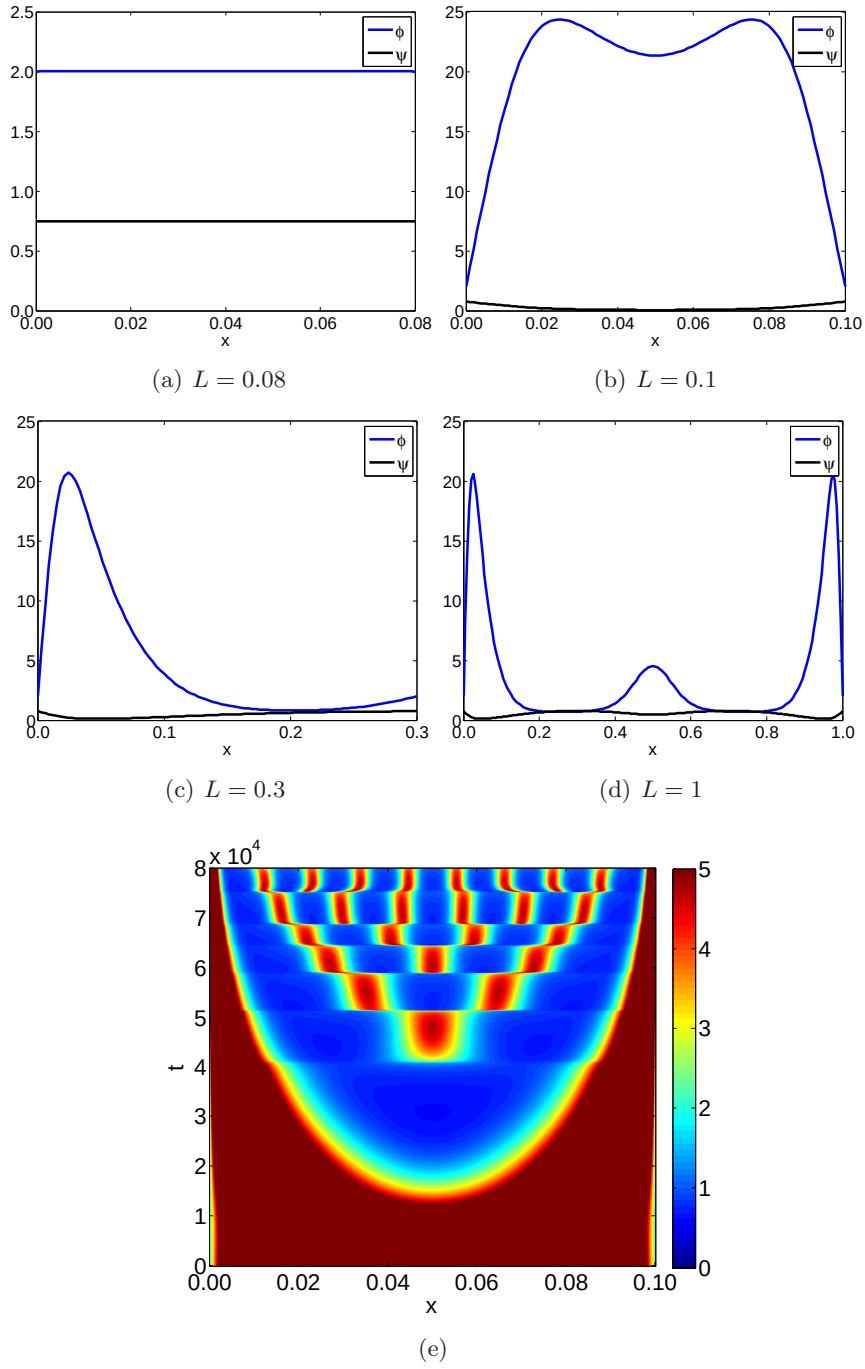


Figure 8.1: Simulation of the Schnakenberg system, equations (2.67) and (2.68), with the boundary concentrations fixed at the uniform steady state. In (a)-(d) the domains are stationary, $c_4 = 0/\text{s}$, with lengths given under each image. The simulations in (a)-(d) have been run to steady state over 10^4 seconds. Note that the lengths used in simulations (a) and (b) are outside of the Turing unstable parameter region (derived through linear perturbation analysis). (e) Domain is undergoing exponential growth with rate $c_4 = 5 \times 10^{-5}/\text{s}$ and initial length $L = 0.1\text{mm}$. The domain is mapped onto the Lagrangian domain for clarity. Other parameters are $D_u = 10^{-4} \text{ mm}^2/\text{s}$, $D_v = 10^{-2} \text{ mm}^2/\text{s}$, $c_1 = 0.1 \text{ mol}/(\text{mm s})$, $c_{-1} = 0.2 / \text{s}$, $c_2 = 0.3 \text{ mol}/(\text{mm s})$ and $c_3 = 0.1 \text{ mm}/(\text{mol}^2 \text{ s})$.

satisfy the predicted boundary conditions, these new experiments concerning biochemical concentrations at the boundary will afford new insights that can be used to modify the model accordingly.

Similarly, there are still open questions concerning the development of chick digits. Although a simple gradient model can specify the temporal effects within the chick wing, the digit specification in the chick leg remains elusive. Currently, circumstantial evidence suggests that cells within the polarising region differentiate based on the duration for which they sense a concentration as well as the concentration level itself. Hence, the chick leg model could be extended to include such a temporal control factor.

Both biological systems have been modelled before and, as such, we have tried to focus on areas that have not been previously considered with the intention of providing new results. However, many of the previously used techniques are complementary to our methods and it is suggested that further work could investigate the coupling of the old and new models together in order to produce multiscale systems. For example, the three species Turing system, which we have used to model morphogen concentrations in the molar domain, could be used in conjunction with the highly detailed computational model developed by Salazar-Ciudad & Jernvall (2002) in order to produce multiple molar shapes. Similarly, in the case of chick digit development, the control of the spatial wavelength is an external input. Thus, our model, which controls the temporal development of Shh concentration, could be linked to one of the existing Turing systems, causing both the temporal and spatial dynamics of the digit promotion to be controlled implicitly.

In Chapter 4, due to a lack of biological data concerning the development of spot patterns in stingrays, we were unable to investigate biological applications of the BVAM model in the depth of detail that was seen in Chapter 3. Clearly, there is much scope for experimental investigations that aim to identify the gene pathways involved in the pigmentation patterning of such species of fish. These results will then provide better insights on which to base our models. Mathematically, there are obvious extensions to growing domains. Although simulations of the BVAM system on a growing square domain have been completed (Barrio, 2008), comparable analytical results are lacking. Furthermore, due to droplet existence depending on a small nonlinearity, we believe that developing stochastic models of these equations will lead to a number of interesting results concerning their

stability in the face of noise.

The stochastic formalism that has been derived in this thesis has multiple opportunities for development. Immediately, we see there is a possibility of extending this analysis to include the temporal transform that was also discussed in Chapter 5. We would then be able to consider the temporal frequencies that the stochastic kinetics excite. Since many patterning mechanisms considered thus far contain the possibility of oscillating dynamics, further investigation is needed to understand how the deterministic and stochastic frequencies interact in order to determine whether noise can destabilise homogeneous steady states to produce oscillations as well as non-uniformity. Equally, the non-uniqueness of mesoscopic jumping rules that was discovered in Chapter 6 clearly warrants further work into analysing the infinite forms of descriptions available in order to see if it is possible to link them back to different assumptions on the microscale jump rules.

Another possible direction of enquiry involves further investigating the role of domain discretisation on the observed patterning dynamics. In Chapter 7 we showed that as the domain becomes coarser higher than expected wave modes are excited, eventually leading to imaginary wave modes. Although physically the coarsity of the domain is invalid, mathematically these imaginary wave modes could perhaps produce oscillating effects within the Turing system that could be analysed with the temporal transform, as discussed above. Therefore, investigations into the possible excited temporal frequencies that are available in such systems have the potential to lead to some novel theoretical discoveries.

The stochastic formalism has many other extensions, including the incorporation of higher dimensions. Using multidimensional Fourier cosine expansions it should be possible to derive power spectra similar to those derived in the one-dimensional case. However, although theoretically viable, simulations in two dimensions will be much slower, as the populations have to be discretised into many more boxes than their one-dimensional counterparts. In Figure 8.2 we demonstrate preliminary evidence indicating that the correspondence between stochastic and deterministic patterns remains good, even in two dimensions. Furthermore, it may be possible to extend the analysis further to encompass higher order terms in the Taylor expansions of the CME in order to characterise the effects which control pattern amplitudes as well as the stability of pattern arrangements.

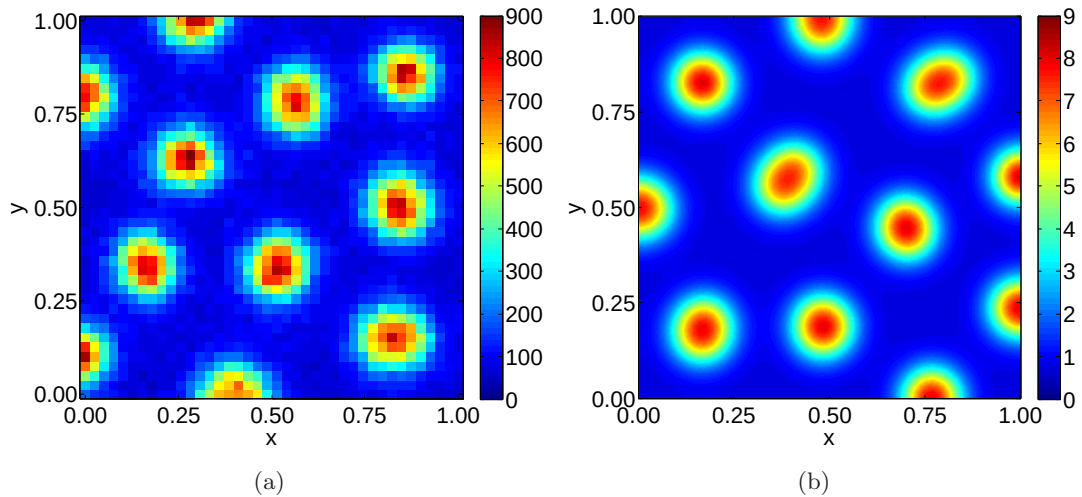


Figure 8.2: Two-dimensional simulations of the deterministic Schnakenberg system, equations (2.67) and (2.68), and its stochastic analogue with zero Neumann boundary conditions. In the stochastic simulation, (a), we illustrate the population U , whereas, in the deterministic case, (b), ϕ is illustrated. These can be linked through the scaling variable, Ω , as approximately $U \approx \Omega\phi$. Parameters are $D_\phi = 10^{-5} \text{ mm}^2/\text{s}$, $D_\psi = 10^{-3} \text{ mm}^2/\text{s}$, $c_1 = 0.01 \text{ mol}/(\text{mm s})$, $c_{-1} = 0.02 / (\text{mm s})$, $c_2 = 0.03 \text{ mol/s}$, $c_3 = 10^{-2} \text{ mm}/(\text{mol}^2 \text{ s})$, $c_4 = 0 / \text{s}$ and $\Omega = 100$. The deterministic simulation was produced using COMSOL 4.2.0.150. The stochastic simulation was produced using a standard SSA.

The main difficulty that we foresee when dealing with higher dimensions is defining descriptions of growth on a mesoscopic scale that are consistent with mean-field descriptions of domain growth, although there are certain simple cases in which the current framework should be immediately applicable. For instance, in the case of a circular domain undergoing axisymmetric growth, we would be able to transform the Cartesian coordinates to their polar forms and then assume no azimuthal angle dependence, allowing us to effectively reduce the system to a one-dimensional problem of controlling the circular domain's radius. However, the creation of a stochastic framework which includes general two-dimensional growing domains is still an open topic of research.

Another direction of future work that we are working on builds upon the insights gained in Section 7.4.2. We seek to apply our stochastic formalism to the case of delayed reaction kinetics, since kinetic delays have been previously shown to cause pathological behaviour in Turing systems (Gaffney & Monk, 2006; Seirin Lee *et al.*, 2010). In particular, it is seen that a small delay in the deterministic reactions can cause a large increase in the patterning time scale. Since noise has the ability to remove the diffusion-driven instability's sensitivity to initial conditions and thereby increase the speed of the patterning mechanism

our aim is to show that noise is able to initiate patterns earlier in delayed systems and, thus, counteract the criticism that Turing patterns are not biologically feasible as they are too sensitive to delays.

Throughout this thesis we have focused on applying the stochastic framework to reaction-diffusion equations. However, there are a great number of similar systems to which these techniques are applicable. Currently, we are investigating cell signalling using stochastic juxtacrine systems. Juxtacrine systems model densely packed cellular structures in the epithelium and assumes that communication between cells is possible using membrane anchored molecules that are able to bind to receptors on the surface of neighbouring cells (Massague, 1990). Similar to reaction-diffusion models, juxtacrine systems can realise morphogen gradients, travelling wave phenomena and spike solutions (Monk, 1998; Monk *et al.*, 2001; Owen & Sherratt, 1998; Owen *et al.*, 1999, 2000; Wearing *et al.*, 2000; Wearing & Sherratt, 2001). In addition, due to the mathematical formulation of the juxtacrine system having the symmetric properties that are similar to the reaction-diffusion mechanism, the stochastic framework and analytical treatment should be immediately applicable.

The juxtacrine model tries to capture the dynamics of reversible binding ligands, which are anchored to cell membranes, by using a discrete set of coupled ODEs. The ODEs consist of three spatially extended populations, such that at each point, $j = 1, 2, \dots, K$, there is a concentration of ligand molecules, $a_j(t)$, unoccupied receptors, $f_j(t)$, and occupied receptors, $b_j(t)$. The juxtacrine dynamics are then modelled by the following equations:

$$\frac{\partial a_j}{\partial t} = \underbrace{-c_1 a_j \langle f_j \rangle}_{\text{binding}} + \underbrace{c_2 \langle b_j \rangle}_{\text{dissociation}} - \underbrace{c_3 a_j}_{\text{decay}} + \underbrace{P_a(b_j)}_{\text{Production}}, \quad (8.1)$$

$$\frac{\partial f_j}{\partial t} = -c_1 f_j \langle a_j \rangle + c_2 b_j - c_4 f_j + P_f(b_j), \quad (8.2)$$

$$\frac{\partial b_j}{\partial t} = c_1 f_j \langle a_j \rangle - c_2 b_j - \underbrace{c_5 b_j}_{\text{Internalisation}}, \quad (8.3)$$

where,

$$\langle g_j \rangle = \frac{g_{j-1} + 2g_j + g_{j+1}}{4}, \quad (8.4)$$

reflects the juxtacrine communication as it indicates that the signal acquired by cell j is an average of those surrounding it. Importantly, note that the factor of $2g_j$ is based on the

assumption that the one-dimensional strip of cells is abstracted from an epithelial sheet of square cells and that each row acts in the same way, meaning that we only need to consider the dynamics of one row. The production rates, P_a and P_f , control the positive feedback through the level of occupied receptors. These concentrations can then be linked to stochastic microscopic populations in exactly the same way as the Turing populations that we have been considering, *e.g.* $A_j = \Omega a_j + \sqrt{\Omega} \eta_{aj}$, where A_j is the stochastic population corresponding to the concentration a_j , Ω is the population scale and η_{aj} is the random variable used to characterise the noise.

Deterministic and equivalent stochastic simulations of system (8.1)-(8.3) can be seen in Figure 8.3. Due to the excellent correspondence of stochastic and deterministic simulations and the fact that the systems are amenable to the discrete Fourier cosine analysis, we are hopeful that analytical approaches will afford us new insights into the juxtacrine system akin to those gained in the reaction-diffusion setting.

Through understanding the impact of such factors as growth and stochasticity on reaction-diffusion systems, this thesis has taken us one step closer to more realistic mathematical models of biological phenomena. Further, we have discovered that many, well understood, regions of mathematics can be given new life simply by considering their stochastic analogues. Critically, the accompanying analytical theory is no more difficult than the deterministic case and, as computers become more powerful, simulations will inevitably run faster. However, it is not enough to constantly travel along the route of mathematical abstraction, for it is only by coupling these mathematical insights with complementary experimental expertise that we can produce a definitive mathematical model that is predictive, as well as descriptive.

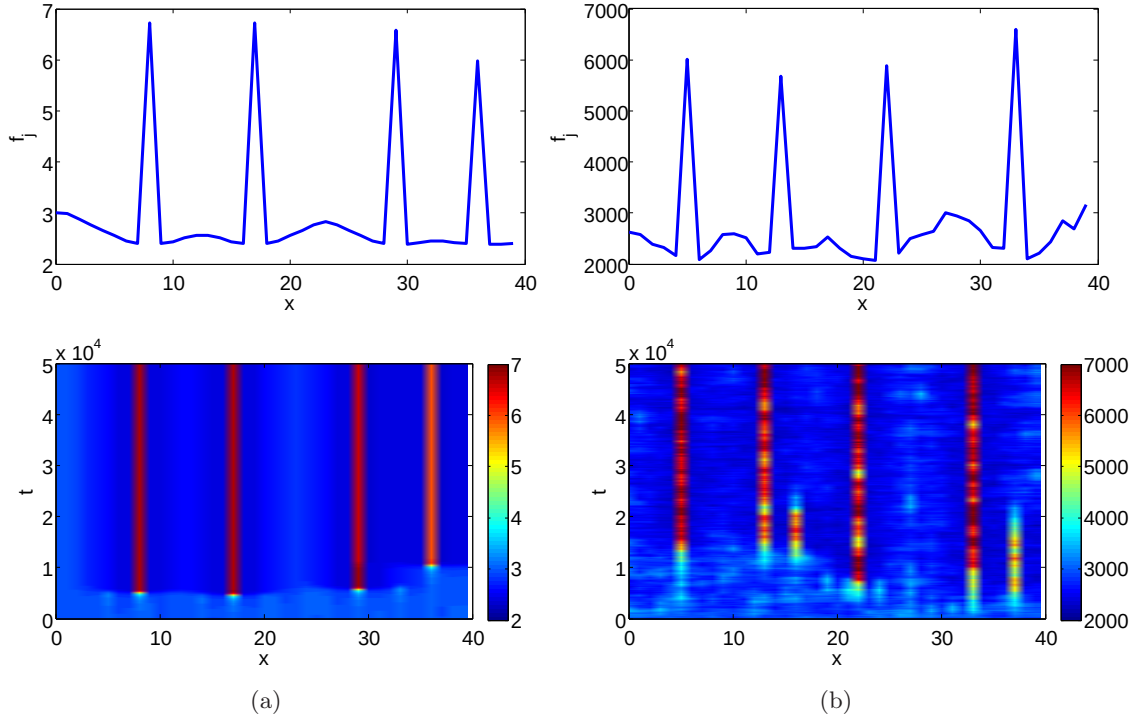
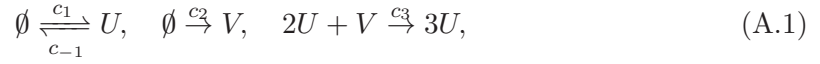


Figure 8.3: Deterministic, (a), and stochastic, (b), simulations of the juxtacrine system, equations (8.1) and (8.3), with zero Neumann boundary conditions. In each case only the f_j population is shown. The top image in each pair shows the final pattern of the system, whilst the bottom illustrates the temporal evolution of the system. The deterministic simulations illustrate the concentration f_j and the stochastic simulations illustrate the corresponding population, F_j . Parameters are $c_1 = 0.3$, $c_2 = 0.12$, $c_3 = 0.006$, $c_4 = 0.03$, $c_5 = 0.019$, $P_a(b) = \alpha_1 b / (\beta_1 + b)$, $P_f(b) = \gamma + \alpha_2 b^3 / (\beta_2 + b^3)$, $\alpha_1 = 0.219$, $\beta_1 = 8$, $\gamma = 0.09$, $\alpha_2 = 0.398$, $\beta_2 = 162$ and $\Omega = 1000$. The deterministic simulation is computed using an explicit Eulerian stepping scheme and a standard SSA is used to calculate the stochastic simulation.

Appendix A

Nondimensionalisation and linearisation of the Schnakenberg kinetics

For populations U and V , the Schnakenberg reactions can be written as



where the c_n are deterministic rates of reaction and $\emptyset$ is a substance not considered. Let ϕ and ψ be the concentrations of U and V , respectively. If in addition the populations are allowed to diffuse, the deterministic equations are

$$\phi_t = D_\phi \nabla^2 \phi + c_1 - c_{-1} \phi + c_3 \phi^2 \psi, \quad (\text{A.2})$$

$$\psi_t = D_\psi \nabla^2 \psi + c_2 - c_3 \phi^2 \psi, \quad (\text{A.3})$$

$$t > 0, \quad x \in \Omega,$$

where D_ϕ and D_ψ are the respective diffusion coefficients and zero flux (Neumann) boundary conditions are assumed.

First, nondimensionalise the system by letting $\phi = \Phi \phi'$, $\psi = \Psi \psi'$, $\mathbf{x} = X \mathbf{x}'$, $t = T t'$, where the primed variables are nondimensional and the capital variables are dimensional reference constants that we can choose. Upon substituting these variables into equations

(A.2) and (A.3) the following equations are obtained:

$$\frac{\Phi}{T}\phi_t = \frac{\Phi}{X^2}D_\phi\nabla^2\phi + c_1 - \Phi c_{-1}\phi + \Phi^2\Psi c_3\phi^2\psi, \quad (\text{A.4})$$

$$\frac{\Psi}{T}\psi_t = \frac{\Psi}{X^2}D_\psi\nabla^2\psi + c_2 - \Phi^2\Psi c_3\phi^2\psi, \quad (\text{A.5})$$

$$t > 0, \quad \mathbf{x} \in \Omega'.$$

We choose to equate the various following parameter groups:

$$\frac{\Phi}{T} = \frac{D_\phi\Phi}{X^2} = c_{-1}\Phi = \Phi^2\Psi c_3, \quad (\text{A.6})$$

$$\frac{\Psi}{T} = c_3\Phi^2\Psi, \quad (\text{A.7})$$

which, upon rearrangement, produces the scales

$$T = \frac{1}{c_{-1}}, \quad X = \sqrt{\frac{D_\phi}{c_{-1}}}, \quad \Psi = \Phi = \sqrt{\frac{c_{-1}}{c_3}}, \quad (\text{A.8})$$

and

$$\phi_t = \nabla^2\phi + \alpha - \phi + \phi^2\psi, \quad (\text{A.9})$$

$$\psi_t = D\nabla^2\psi + \beta - \phi^2\psi, \quad (\text{A.10})$$

$$t > 0, \quad \mathbf{x} \in \Omega',$$

where $\alpha = c_1/c_{-1}\sqrt{c_3/c_{-1}}$, $\beta = c_2/c_{-1}\sqrt{c_3/c_{-1}}$ and $D = D_\psi/D_\phi$. If the domain is one-dimensional, $\mathcal{V} = [0, L]$, then the nondimensionalised form of this is $\mathcal{V}' = [0, L\sqrt{c_{-1}/D_\phi}]$.

A.1 Spatially homogeneous linear stability

Consider the Schnakenberg kinetics without diffusion:

$$\frac{d\phi}{dt} = \alpha - \phi + \phi^2\psi, \quad (\text{A.11})$$

$$\frac{d\psi}{dt} = \beta - \phi^2\psi. \quad (\text{A.12})$$

Using standard analytic techniques (Jordan & Smith, 2002) it is possible to compute the linear stability about the homogeneous steady state¹ $\psi_0 = \alpha + \beta$ and $\phi_0 = \beta/(\alpha + \beta)^2$. The Jacobian evaluated at this critical point is

$$\begin{pmatrix} f_\phi & f_\psi \\ g_\phi & g_\psi \end{pmatrix} = \begin{pmatrix} 2\phi_0\psi_0 - 1 & \phi_0^2 \\ -2\phi_0\psi_0 & -\phi_0^2 \end{pmatrix}, \quad (\text{A.13})$$

$$\Rightarrow \begin{cases} f_u + g_v = 2\phi_0\psi_0 - 1 - \phi_0^2, \\ f_u g_v - f_v g_u = \phi_0^2. \end{cases} \quad (\text{A.14})$$

Substituting these into the conditions derived in Section 2.2.1 yields the eigenvalues of the homogeneous steady state,

$$\sigma_\pm = \frac{2\phi_0\psi_0 - 1 - \phi_0^2 \pm \sqrt{(2\phi_0\psi_0 - 1 - \phi_0^2)^2 - 4\phi_0^2}}{2}. \quad (\text{A.15})$$

For (ϕ_0, ψ_0) to be stable in the absence of diffusion both eigenvalues must have negative real parts, thus, the steady state has to satisfy

$$2\phi_0\psi_0 - 1 - \phi_0^2 < 0, \quad (\text{A.16})$$

$$\phi_0^2 > 0, \quad \text{which is trivially satisfied.} \quad (\text{A.17})$$

Further, by splitting up the stability plane by considering whether $\sigma_\pm$ is real or complex, the possibility of Turing-Hopf bifurcations can be considered. Hence, oscillatory trajectories are possible when $\sigma_\pm$ is complex, which occurs when

$$(2\phi_0\psi_0 - 1 - \phi_0^2)^2 < 4\phi_0^2, \quad (\text{A.18})$$

$$\Rightarrow -2\phi_0 < (2\phi_0\psi_0 - 1 - \phi_0^2) < 2\phi_0, \quad (\text{A.19})$$

$$\Rightarrow \frac{(\phi_0 - 1)^2}{2\phi_0} < \psi_0 < \frac{(\phi_0 + 1)^2}{2\phi_0}. \quad (\text{A.20})$$

¹For physical realisable steady states we must have that α and $\beta > 0$ and, so, $\alpha + \beta \neq 0$

A.2 Diffusion-driven instability

By adding diffusion back into the system, a Turing instability exists when the critical point satisfies equations (A.16), (A.17) and (2.29),

$$2\phi_0\psi_0D - D - \phi_0^2 > 2\sqrt{D}\phi_0 > 0. \quad (\text{A.21})$$

Equations (A.16) and (A.21) can be rewritten as

$$\frac{(\phi_0^2 + 1)}{2\phi_0} > \psi_0 > \frac{(\phi_0 + \sqrt{D})^2}{2D\phi_0}. \quad (\text{A.22})$$

Equations (A.20), (A.21) and (A.22) can then be used to produce the plots in Figure 2.4.

Appendix B

Proof that Schnakenberg trajectories are bounded

Let $(\bar{\phi}, \bar{\psi})$ be any physically realistic initial point¹, *i.e.* $\bar{\phi}$ and $\bar{\psi} > 0$. The construction of a trapping region is split into two cases, $\bar{\phi} < \alpha + \beta$ and $\bar{\phi} \geq \alpha + \beta$. This is due to the dynamics depending on whether the initial condition is to the left or right of the critical point $(\alpha + \beta, \beta/(\alpha + \beta)^2)$.

To begin with, consider the nullclines,

$$(\phi \text{ nullcline}) \quad \psi = \frac{\phi - \alpha}{\phi^2}, \quad (\text{B.1})$$

$$(\psi \text{ nullcline}) \quad \psi = \frac{\beta}{\phi^2}. \quad (\text{B.2})$$

Case 1: $\bar{\phi} < \alpha + \beta$

Pick $0 < x' < \min\{\alpha, \bar{\phi}\}$ and $y' > \max\{1/(4\alpha), \bar{\psi}\}$. This is to make x' less than the root of the ϕ nullcline, α , and to make y' greater than the maximum of the ϕ nullcline. Set $y = \max\{y', \beta/x'^2\}$ and $x = \sqrt{\beta/y}$. Then since the nullcline $\psi = \beta/\phi^2$ is monotonically decreasing we have constructed (x, y) to be on the nullcline, such that $x < \min\{\alpha, \bar{\phi}\}$ and $y > \max\{1/(4\alpha), \bar{\psi}\}$.

¹If $\bar{\phi} = \bar{\psi} = 0$ and $\alpha, \beta > 0$ equations (A.11) and (A.12) show that $\dot{\bar{\phi}}, \dot{\bar{\psi}} > 0$, thus, the trajectories move into the positive quarter plane. Hence, without loss of generality $\bar{\phi}, \bar{\psi} > 0$.

Construct the polygon M such that

$$M = \cup_{i=1}^5 M_i, \quad (\text{B.3})$$

$$M_1 = \{(x, z) : 0 \leq z \leq y\}, \quad (\text{B.4})$$

$$M_2 = \{(z, y) : x \leq z \leq \alpha + \beta\}, \quad (\text{B.5})$$

$$M_3 = \{(z, w(z)) : w(z) = \alpha + \beta + y - z, \quad \alpha + \beta \leq z \leq \phi^*\}, \quad (\text{B.6})$$

$$M_4 = \{(\phi^*, z) : 0 \leq z \leq \psi^*\}, \quad (\text{B.7})$$

$$M_5 = \{(z, 0) : x \leq z \leq \phi^*\}, \quad (\text{B.8})$$

where ϕ^* is the smallest, real root greater than $\alpha + \beta$ of

$$\phi^* - \alpha = \phi^{*2}(\alpha + \beta + y - \phi^*), \quad (\text{B.9})$$

and $\psi^* = \alpha + \beta + y - \phi^*$.

The root, ϕ^* , exists since the point $w(\alpha + \beta) = y$ is above the critical point, $(\alpha + \beta, \beta/(\alpha + \beta))$, due to the construction of $y > 1/(4\alpha)$, which is the maximum of the nullcline. Further,

$$\frac{\phi - \alpha}{\phi^2}, \quad (\text{B.10})$$

is monotonically decreasing, but always positive for $\phi > \alpha$. Finally, $w(\phi) = \alpha + \beta + y - \phi < 0$ for sufficiently large ϕ . Thus, since

$$w(\alpha + \beta) > \max\left(\frac{\phi - \alpha}{\phi^2}\right) > 0 = w(\alpha + \beta + y), \quad (\text{B.11})$$

then, by continuity, there exists $\phi^* \in (\alpha + \beta, \alpha + \beta + y)$ that satisfies equation (B.9).

On M_1 , M_2 , M_4 and M_5 it is trivial to show that the field is directed such that trajectories will move to the inside of M . To show this is true for M_3 , note that $\phi \geq \alpha + \beta \implies \dot{\phi} + \dot{\psi} \leq 0$. Hence, for $\phi \geq \alpha + \beta$, ψ evolves such that $\psi \leq c - \phi$ for some fixed c , which implies that if the trajectory begins below M_3 at $\phi = \alpha + \beta$ it stays below M_3 for all $\alpha + \beta \leq \phi \leq \phi^*$.

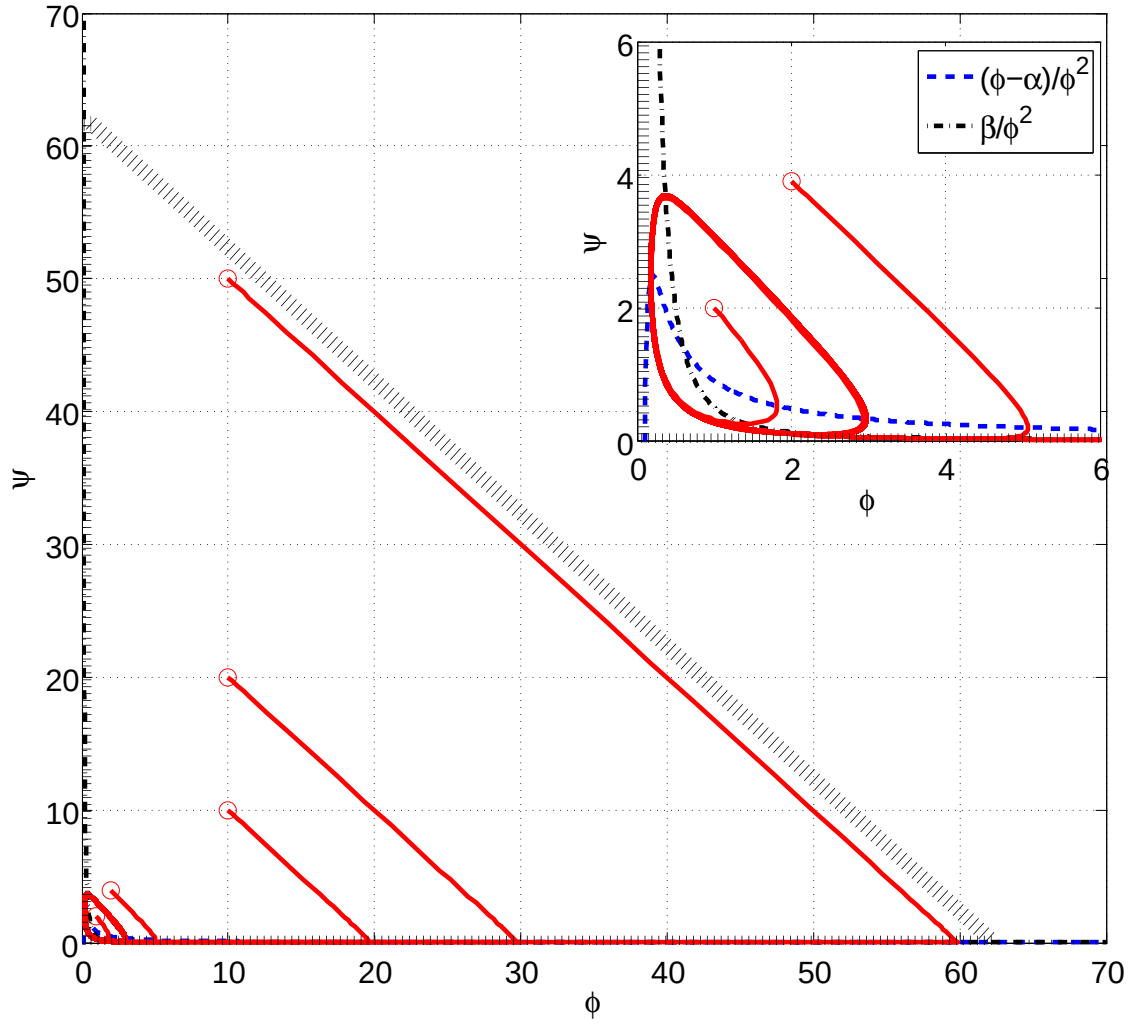


Figure B.1: The trapping region for all points within the dashed polygon M . The solid lines are the trajectories with initial condition marked by a circle. The dashed lines are the nullclines. The inset shows a close-up of the region $[0, 6] \times [0, 6]$. Parameters $\alpha = 0.1$, $\beta = 0.5$, $x = 0.0636$, $y = 61.7284$.

Case 2: $\bar{\phi} \geq \alpha + \beta$

The second case is similar but since the initial point is beyond the intersection of the nullclines, M_3 must be constructed carefully such that it is above the initial point.

First choose $y' > \max\{\bar{\psi}, 1/(4\alpha)\}$. Again, this ensures that y' is above the maximum value of the ϕ nullcline, which occurs at $(2\alpha, 1/(4\alpha))$. Next let $\psi' = \bar{\phi} + y' - \alpha - \beta$. Due to the monotonic decreasing nature of $\psi = \bar{\phi} + y' - \phi$ and $\bar{\phi} \geq \alpha + \beta$, this implies $\psi' \geq y' > \bar{\psi}$. Finally, choose $x < \min\{\alpha, \sqrt{\beta/\psi'}\}$ and $y = \beta/x^2$, constructing M as above.

Thus any initial positive value must evolve along a trajectory which is bounded by

the polygon M . The Poincaré-Bendixson theorem (Bendixson, 1901; Glendinning, 1994; Poincare, 1892) is now stated to complete the proof.

Theorem B.0.1. *Poincaré-Bendixson theorem.*

Given a differential equation $d\phi/dt = F(\phi)$ in the plane, assume $\phi^*(t)$ is a solution curve which stays in a bounded region. Then either $\phi^*(t)$ converges to an equilibrium point where $F(\phi^*) = 0$ as $t \rightarrow \infty$, or it converges to a periodic cycle.

Hence, if α and $\beta > 0$, then all (ϕ, ψ) trajectories are bounded. Note, that this only works in the case of systems with one or two differential equations as in three dimensions, although the trajectories may be bounded, the solution may not tend to a limit cycle, but instead produce a chaotic orbit.

Appendix C

BVAM parameter values

The following table contains that parameter values and axis ranges for each of the images in Figure 4.2:

	(a)	(b)	(c)	(d)
D	0.516	0.8	1	1
η	0.35	0.5	0.5	0.5
a	0.05775	0.04125	0.0570405	0.0817905
b	-0.30525	-0.28875	-0.3045405	-0.3045405
h	-2.5	-2.5	-2.5	-2.5
C	0	0	0	0
Length of side	80	80	80	160
Colour axis, blue to red	[-0.3, 0.3]	[-0.3, 0.3]	[-0.3, 0.3]	[-0.5, 0.5]



Figure C.1: The colour bar that is used in all colour figures through out this thesis. The colour axis is a linear scale from the minimum value (blue) to the maximum value (red).

Appendix D

The Langevin equation

Given a discrete system of chemical populations as described in Chapter 5, we showed that their reaction dynamics can be accurately modelled by a chemical master equation (CME) approach. However, due to the high dimension and nonlinearities usually contained within the CME, it is usually not conducive to simulation. Thus, also in Chapter 5, we introduced Gillespie’s stochastic simulation algorithm (SSA) which can be used to accurately simulate statistically exact stochastic systems

Due to the SSA simulating the reactions of every single particle in the system this algorithm is often computationally expensive. In this appendix we derive the chemical Langevin equation, which allows us to gain the speed of a deterministic simulation whilst approximating the action of the intrinsic noise. Hence, the method of simulating the Langevin equation may be thought of as an intermediate scale between the SSA and deterministic simulation.

Here we derive the Langevin system with respect to a single spatially discretised population, $\mathbf{U}(t) = (U_1(t), U_2(t), \dots, U_K(t))$, but this can easily be expanded to encompass different particle types. The following derivation closely follows that of Gillespie (1996, 2000, 2002). We will also be using notation defined and explained in Chapter 5 without recapitulation.

Suppose the state of the system at a time t is given by $\mathbf{U}(t)$ and for any $\tau > 0$ $K_j(\mathbf{U}(t), \tau)$ is the number of R_j reactions that occur in the interval $[t, t + \tau]$. Since we know the stoichiometric matrix, $\boldsymbol{\nu}$, the population at time $t + \tau$ can be written in the

exact form

$$U_i(t + \tau) = U_i(t) + \sum_{j=1}^J K_j(\mathbf{U}(t), \tau) \nu_{ji}, \quad (\text{D.1})$$

where J is the total number of reactions.

However, computing the actual form of K_j , which is a random variable, for arbitrary $\tau > 0$ is as difficult as solving the CME (Gillespie, 2000). Thus, the power of the Langevin equation is that we approximate K_j with something much more computationally tractable. The simulation of this approximation can be shown to give a good comparison with the SSA when we can satisfy the two following conditions (Gillespie, 2002):

1. τ can be chosen such that the population change over the interval $[t, t + \tau]$ is small;
2. τ can be chosen large enough that each reaction, R_j , is expected to have occurred a large number of times.

Although these two statements appear contradictory, condition (1) suggests that the change in particle numbers is small compared to the entire population, whereas condition (2) implies that the absolute number of reactions should be large. Condition (1) can always be satisfied if the populations are sufficiently large (Gillespie, 2000), which is consistent with the weak noise limit we are considering. Furthermore condition (1) allows us to approximate the update kernel, $K_j(\mathbf{U}(t), \tau)$, by a Poisson random variable. This is achieved through noting that because the populations do not appreciably change their values during the interval $[t, t + \tau]$ all reaction events will be essentially independent of each other. Thus, the update kernel will be the number of times a reaction channel R_j would occur if its propensity function remained constant at the value $a_j(\mathbf{U}(t))$. From the SSA algorithm this can be seen to be a Poisson random variable, $\mathcal{P}_j(a_j(\mathbf{U}(t)), \tau)$, where $\mathcal{P}(a, t)$ is understood to be the number of events that occur in a time interval t given that the probability for an event to occur in any infinitesimal time interval dt is adt . Substituting this into equation (D.1) we deduce that the update rule is approximately

$$U_i(t + \tau) = U_i(t) + \sum_{j=1}^J \mathcal{P}_j(a_j(\mathbf{U}(t)), \tau) \nu_{ji}. \quad (\text{D.2})$$

By assuming that the number of firings for each reaction pathway is large condition (2) then allows us to approximate the Poisson random variable, $\mathcal{P}_j(a_j(\mathbf{U}(t)), \tau)$,

by a normally distributed random variable with mean and variance $a_j(\mathbf{U}(t))\tau$, namely $\mathcal{N}_j(a_j(\mathbf{U}(t))\tau, a_j(\mathbf{U}(t))\tau)$, which is a standard statistical result (Grimmett & Stirzaker, 2001). Thus, we have transformed the original equation (D.1) into

$$U_i(t + \tau) = U_i(t) + \sum_{j=1}^J \mathcal{N}_j(a_j(\mathbf{U}(t))\tau, a_j(\mathbf{U}(t))\tau) \nu_{ji}. \quad (\text{D.3})$$

This normal distribution can then be expanded to yield the standard chemical Langevin equation (CLE) (Gillespie, 2007)

$$U_i(t + \tau) = U_i(t) + \sum_{j=1}^J a_j(\mathbf{U}(t))\tau \nu_{ji} + \sum_{j=1}^J \mathcal{N}_j(0, 1) \sqrt{a_j(\mathbf{U}(t))\tau} \nu_{ji}. \quad (\text{D.4})$$

Thus, in Chapter 7 where the populations are too large to simulate using the standard SSA, we use equation (D.4) to simulate the evolution of the system to give us an approximate idea of how the system changes with the inclusion of intrinsic noise. Notice that if the noise is ignored then the method is just the simple explicit Euler solver of the deterministic equation (Süli & Mayers, 2003; Yang *et al.*, 2005).

Finally, it is important to understand that the derivation above shows that the noise manifests itself from the internal noise through an approximation on the size of the time scale. Many Langevin simulations add a white noise term in an *ad hoc* method of modelling the stochasticity. However, this noise has a much more external form whereas here the derived form of the noise is an inherent part of the discrete Markov process.

Bibliography

- D. J. Acheson. *Elementary Fluid Dynamics*. Oxford University Press, 1990.
- G. Adomian. 1995. The diffusion-Brusselator equation. *Comput. Math. Appl.*, **29**, 1–3.
- Y. Ahn, B. W. Sanderson, O. D. Klein and R. Krumlauf. 2010. Inhibition of Wnt signaling by Wise (Sostdc1) and negative feedback from Shh controls tooth number and patterning. *Development*, **137**, 3221.
- B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter. *Molecular Biology of the Cell*. Garland Science New York, 5th edition, 2008.
- D. Alonso, A. J. McKane and M. Pascual. 2007. Stochastic amplification in epidemics. *J. Roy. Soc. Interface.*, **4**, 575.
- S. S. Andrews and D. Bray. 2004. Stochastic simulation of chemical reactions with spatial resolution and single molecule detail. *Phys. Biol.*, **1**, 137–151.
- J. L. Aragon, C. Varea, R. A. Barrio and P. K. Maini. 1998. Spatial patterning in modified Turing systems: application to pigmentation patterns on marine fish. *Forma*, **13**, 213–221.
- P. Arcuri and J. D. Murray. 1986. Pattern sensitivity to boundary and initial conditions in reaction-diffusion models. *J. Math. Biol.*, **24**, 141–165.
- J. F. G. Auchmuty and G. Nicolis. 1975. Bifurcation analysis of nonlinear reaction-diffusion equations—I. Evolution equations and the steady state solutions. *Bull. Math. Biol.*, **37**, 323–365.

- R. E. Baker, E. A. Gaffney and P. K. Maini. 2008. Partial differential equations for self-organization in cellular and developmental biology. *Nonlinearity*, **21**, R251–R290.
- R. E. Baker, C. A. Yates and R. Erban. 2010. From microscopic to macroscopic descriptions of cell migration on growing domains. *Bull. Math. Biol.*, **72**, 719–762.
- Y. I. Balkarei, A. V. Grigor'yants, Y. A. Rzhanov and M. I. Elinson. 1988. Regenerative oscillations, spatial-temporal single pulses and static inhomogeneous structures in optically bistable semiconductors. *Opt. Commun.*, **66**, 161 – 166.
- J. Bard and I. Lauder. 1974. How well does Turing's theory of morphogenesis work? *J. Theor. Biol.*, **45**, 501–51.
- I. Barrass, E. J. Crampin and P. K. Maini. 2006. Mode transitions in a model reaction-diffusion system driven by domain growth and noise. *Bull. Math. Biol.*, **68**, 981–995.
- R. A. Barrio. *Physics of Emergence and Organization*, chapter Turing systems: A general model for complex patterns in nature, pages 267–296. World Scientific, 2008.
- R. A. Barrio, R. E. Baker, B. Vaughan Jr, K. Tribuzy, M. R. de Carvalho, R. Bassanezi and P. K. Maini. 2009. Modeling the skin pattern of fishes. *Phys. Rev. E*, **79**, 31908.
- R. A. Barrio, C. Varea, J. L. Aragón and P. K. Maini. 1999. A two-dimensional numerical study of spatial pattern formation in interacting Turing systems. *Bull. Math. Biol.*, **61**, 483–505.
- S. Basu, Y. Gerchman, C. H. Collins, F. H. Arnold and R. Weiss. 2005. A synthetic multicellular system for programmed pattern formation. *Nature*, **434**, 1130–1134.
- I. Bendixson. 1901. Sur les courbes définies par des équations différentielles. *Acta. Math.-Djursholm.*, **24**, 1–88.
- D. L. Benson, P. K. Maini and J. A. Sherratt. 1998. Unravelling the Turing bifurcation using spatially varying diffusion coefficients. *J. Math. Biol.*, **37**, 381–417.
- T. Biancalani, D. Fanelli and F. Di Patti. 2010. Stochastic Turing patterns in the Brusselator model. *Phys. Rev. E*, **81**, 046215.

- T Biancalani, T Galla and A. J. McKane. 2011. Stochastic waves in a Brusselator model with nonlocal interaction. *Phys. Rev. E*, **84**, 026201.
- A. J. Black and A. J. McKane. 2010. Stochastic amplification in an epidemic model with seasonal forcing. *J. Theor. Biol.*, **267**, 85–94.
- M. Bode and H. G. Purwins. 1995. Pattern formation in reaction-diffusion systems - dissipative solitons in physical systems. *Physica D*, **86**, 53 – 63.
- P. Borckmans, G. Dewell, A. De wit and D. Walgraef. *Chemical waves and patterns*, chapter 10 Turing Bifurcations and Pattern Selection, pages 325–363. Kluwer Academic Publishers, 1995.
- K. Boström, K. E. Watson, S. Horn, C. Wortham, I. M. Herman and L. L. Demer. 1993. Bone morphogenetic protein expression in human atherosclerotic lesions. *J. Clin. Invest.*, **91**, 1800.
- W. L. Briggs and V. E. Henson. *The DFT: An Owner's Manual for the Discrete Fourier Transform*. SIAM, 1995.
- B. Bunow, J. P. Kernevez, G. Joly and D. Thomas. 1980. Pattern formation by reaction-diffusion instabilities: application to morphogenesis in *Drosophila*. *J. Theor. Biol.*, **84**, 629–649.
- T. Butler and N. Goldenfeld. 2009. Robust ecological pattern formation induced by demographic noise. *Phys. Rev. E*, **80**, 030902.
- T. Butler and N. Goldenfeld. 2011. Fluctuation-driven Turing patterns. *Phys. Rev. E*, **84**, 011112.
- J. Cai, S. W. Cho, J. Y. Kim, M. J. Lee, Y. G. Cha and H. S. Jung. 2007. Patterning the size and number of tooth and its cusps. *Dev. Biol.*, **304**, 499–507.
- T. K. Callahan and E. Knobloch. 1996. Bifurcations on the fcc lattice. *Phys. Rev. E*, **53**, 3559–3562.
- T. K. Callahan and E. Knobloch. 1997. Symmetry-breaking bifurcations on cubic lattices. *Nonlinearity*, **10**, 1179–1216.

- T. K. Callahan and E. Knobloch. 1999. Pattern formation in three-dimensional reaction–diffusion systems. *Physica D*, **132**, 339–362.
- Y. Cao, D. T. Gillespie and L. R. Petzold. 2005. Avoiding negative populations in explicit Poisson tau-leaping. *J. Chem. Phys.*, **123**, 054104.
- Y. Cao, D. T. Gillespie and L. R. Petzold. 2006. Efficient step size selection for the tau-leaping simulation method. *J. Chem. Phys.*, **124**, 044109.
- S. B. Carter. 1967. Haptotaxis and the mechanism of cell motility. *Nature*, **213**, 256–260.
- P. Charvet-Almeida, M. L. G Araújo and M. P. Almeida. 2005. Reproductive aspects of freshwater stingrays (Chondrichthyes: Potamotrygonidae) in the Brazilian Amazon basin. *J. Northw. Atl. Fish. Sci.*, **35**, 165–171.
- S. W. Cho, S. Kwak, T. E. Woolley, M. J. Lee, E. J. Kim, R. E. Baker, H. J. Kim, J. S. Shin, C. Tickle, P. K. Maini and H. S. Jung. 2011. Interactions between Shh, Sostdc1 and Wnt signaling and a new feedback loop for spatial patterning of the teeth. *Development*, **138**, 1807–1816.
- J. Cooke and D. Summerbell. 1980. Cell cycle and experimental pattern duplication in the chick wing during embryonic development. *Nature*, **287**, 697–701.
- S. Cornell, M. Droz and B. Chopard. 1991. Role of fluctuations for inhomogeneous reaction-diffusion phenomena. *Phys. Rev. A*, **44**, 4826–4832.
- S. L. Cotter, K. C. Zygalakis, I. G. Kevrekidis and R. Erban. 2011. A constrained approach to multiscale stochastic simulation of chemically reacting systems. *J. Chem. Phys.*, **135**, 094102.
- P. Coullet, C. Elphick and D. Repaux. 1987. Nature of spatial chaos. *Phys. Rev. Lett.*, **58**, 431–434.
- E. J. Crampin. *Reaction Diffusion Patterns on Growing Domains*. PhD thesis, University of Oxford, 2000.
- E. J. Crampin, E. A. Gaffney and P. K. Maini. 1999. Reaction and diffusion on growing domains: scenarios for robust pattern formation. *Bull. Math. Biol.*, **61**, 1093–1120.

- E. J. Crampin, E. A. Gaffney and P. K. Maini. 2002a. Mode-doubling and tripling in reaction-diffusion patterns on growing domains: a piecewise linear model. *J. Math. Biol.*, **44**, 107–128.
- E. J. Crampin, W. W. Hackborn and P. K. Maini. 2002b. Pattern formation in reaction-diffusion models with nonuniform domain growth. *Bull. Math. Biol.*, **64**, 747–769.
- E. J. Crampin and P. K. Maini. 2001. Modelling biological pattern formation: the role of domain growth. *Comm. Theor. Biol.*, **6**, 229–249.
- H. R. Dassule, P. Lewis, M. Bei, R. Maas and A. P. McMahon. 2000. Sonic hedgehog regulates growth and morphogenesis of the tooth. *Development*, **127**, 4775.
- H. R. Dassule and A. P. McMahon. 1998. Analysis of epithelial-mesenchymal interactions in the initial morphogenesis of the mammalian tooth. *Dev. Biol.*, **202**, 215–227.
- E. A. Dawes. *Quantitative Problems in Biology*. Longman, London, 1956.
- M. R. de Carvalho, N. R. Lovejoy and R. S. Rosa. *Check List of the Freshwater Fishes of South and Central America*. EDIPUCRS, 2003.
- P. De Kepper, V. Castets, E. Dulos and J. Boissonade. 1991. Turing-type chemical patterns in the chlorite-iodide-malonic acid reaction. *Physica D*, **49**, 161–169.
- P. De Kepper, I. R. Epstein, K. Kustin and M. Orban. 1982. Systematic design of chemical oscillators. Part 8. Batch oscillations and spatial wave patterns in chlorite oscillating systems. *J. Phys. Chem.*, **86**, 170–171.
- R. E. L. DeVille, C. B. Muratov and E. Vanden-Eijnden. 2006. Non-meanfield deterministic limits in chemical reaction kinetics. *J. Chem. Phys.*, **124**, 231102.
- E. Di Cera. *Thermodynamic Theory of Site-Specific Binding Processes in Biological Macromolecules*. Cambridge University Press, 1995.
- R. Dillon, C. Gadgil and H. G. Othmer. 2003. Short-and long-range effects of Sonic hedgehog in limb development. *Proc. Nat. Acad. Sci.*, **100**, 10152.
- R. Dillon, P. K. Maini and H. G. Othmer. 1994. Pattern formation in generalized Turing systems. *J. Math. Biol.*, **32**, 345–393.

- M. Dolnik, A. M. Zhabotinsky and I. R. Epstein. 2001. Resonant suppression of Turing patterns by periodic illumination. *Phys. Rev. E*, **63**, 026101.
- P. G. Drazin. *Nonlinear Systems*. Cambridge University Press, 1992.
- K. Drescher, R. E. Goldstein and I. Tuval. 2010. Fidelity of adaptive phototaxis. *Proc. Nat. Acad. Sci.*, **107**, 11171.
- E. Dulos, J. Boissonade, J. J. Perraud, B. Rudovics and P. Kepper. 1996. Chemical morphogenesis: Turing patterns in an experimental chemical system. *Acta Biotheor.*, **44**, 249–261.
- H. El Samad, M. Khammash, L. Petzold and D. T. Gillespie. 2005. Stochastic modelling of gene regulatory networks. *Int. J. Robust. Nonlin.*, **15**, 691–711.
- J. Elf and M. Ehrenberg. 2003. Fast evaluation of fluctuations in biochemical networks with the linear noise approximation. *Genome. Res.*, **13**, 2475–2484.
- S. Engblom, L. Ferm, A. Hellander and P. Lötstedt. 2009. Simulation of stochastic reaction-diffusion processes on unstructured meshes. *SIAM J. Sci. Comput.*, **31**, 1774.
- E. V. Entchev, A. Schwabedissen and M. González-Gaitán. 2000. Gradient formation of the TGF- β homolog Dpp. *Cell*, **103**, 981–992.
- R. Erban and S. J. Chapman. 2007. Reactive boundary conditions for stochastic simulations of reaction–diffusion processes. *Phys. Biol.*, **4**, 16.
- R. Erban and S. J. Chapman. 2009. Stochastic modelling of reaction–diffusion processes: algorithms for bimolecular reactions. *Phys. Biol.*, **6**, 046001.
- R. Erban, S. J. Chapman, I. G. Kevrekidis and T. Vejchodský. 2009. Analysis of a Stochastic Chemical System Close to a SNIPER Bifurcation of Its Mean-Field Model. *SIAM J. Appl. Math.*, **70**, 984.
- R. Erban, S. J. Chapman and P. K. Maini. 2007. A practical guide to stochastic simulations of reaction-diffusion processes. *arXiv:0704.1908v2 [q-bio.SC]*.
- B. Ermentrout. 1991. Stripes or spots? Nonlinear effects in bifurcation of reaction-diffusion equations on the square. *Proc. Math. Phys. Sci.*, **434**, 413–417.

- C. Escudero. 2009. Stochastic growth equations on growing domains. *J. Stat. Mech.*, **2009**, P07020.
- A. Fick. 1855. On liquid diffusion. *Philos. Mag. J. Sci.*, **10**, 31–39.
- W. J. Firth and G. K. Harkness. 1998. Cavity solitons. *Asian J. Phys.*, **7**, 665–677.
- W. J. Firth and A. J. Scroggie. 1996. Optical bullet holes: robust controllable localized states of a nonlinear cavity. *Phys. Rev. Lett.*, **76**, 1623–1626.
- M. B. Flegg, S. J. Chapman and R. Erban. 2011. The two-regime method for optimizing stochastic reaction–diffusion simulations. *J. Roy. Soc. Interface.*
- B. Futcher, GI Latter, P. Monardo, CS McLaughlin and JI Garrels. 1999. A sampling of the yeast proteome. *Mol. Cell. Biol.*, **19**, 7357.
- C. Gadgil, C. H. Lee and H. G. Othmer. 2005. A stochastic analysis of first-order reaction networks. *Bull. Math. Biol.*, **67**, 901–946.
- E. A. Gaffney and N. A. M. Monk. 2006. Gene expression time delays and Turing pattern formation systems. *Bull. Math. Biol.*, **68**, 99–130.
- P. W. Gandar. 1983. Growth in root apices. I. The kinematic description of growth. *Bot. Gaz.*, **144**, 1–10.
- J. García-Ojalvo and J. M. Sancho. 1994. Colored noise in spatially extended systems. *Phys. Rev. E*, **49**, 2769–2778.
- J. García-Ojalvo and J. M. Sancho. 1996. External fluctuations in a pattern-forming instability. *Phys. Rev. E*, **53**, 5680–5689.
- J. García-Ojalvo and J. M. Sancho. *Noise in Spatially Extended Systems*. Springer Verlag, 1999.
- C. W. Gardiner. *Handbook of Stochastic Methods*. Springer Berlin, 1985.
- C. W. Gardiner, K. J. McNeil, D. F. Walls and I. S. Matheson. 1976. Correlations in stochastic theories of chemical reactions. *J. Stat. Phys.*, **14**, 307–331.

- A. Garfinkel, Y. Tintut, D. Petrasek, K. Boström and L. L. Demer. 2004. Pattern formation by vascular mesenchymal cells. *Proc. Nat. Acad. Sci.*, **101**, 9247.
- M. A. Gibson and J. Bruck. 2000. Efficient exact stochastic simulation of chemical systems with many species and many channels. *J. Phys. Chem. A.*, **104**, 1876–1889.
- M. A. Gibson and E. Mjolsness. *Computational Modeling of Genetic and Biochemical Networks*, volume 1, chapter Modeling the activity of single genes, page 48. The MIT Press, 2004.
- C. Gicquel and Y. Le Bouc. 2006. Hormonal regulation of fetal growth. *Horm. Res. Paediatr.*, **65**, 28–33.
- A. Gierer and H. Meinhardt. 1972. A theory of biological pattern formation. *Biol. Cybern.*, **12**, 30–39.
- D. T. Gillespie. 1976. A general method for numerically simulating stochastic time evolution of coupled chemical reactions. *J. Comput. Phys.*, **22**, 403–434.
- D. T. Gillespie. 1977a. Concerning the validity of the stochastic approach to chemical kinetics. *J. Stat. Phys.*, **16**, 311–318.
- D. T. Gillespie. 1977b. Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem.*, **81**, 2340–2361.
- D. T. Gillespie. 1992. A rigorous derivation of the chemical master equation. *Physica A*, **188**, 404–425.
- D. T. Gillespie. 1996. The multivariate Langevin and Fokker–Planck equations. *Am. J. Phys.*, **64**, 1246.
- D. T. Gillespie. 2000. The chemical Langevin equation. *J. Chem. Phys.*, **113**, 297.
- D. T. Gillespie. 2001. Approximate accelerated stochastic simulation of chemically reacting systems. *J. Chem. Phys.*, **115**, 1716.
- D. T. Gillespie. 2002. The chemical Langevin and Fokker–Planck equations for the reversible isomerization reaction. *J. Phys. Chem. A.*, **106**, 5063–5071.

- D. T. Gillespie. 2007. Stochastic simulation of chemical kinetics. *Annu. Rev. Phys. Chem.*, **58**, 35–55.
- D. T. Gillespie and L. R. Petzold. 2003. Improved leap-size selection for accelerated stochastic simulation. *J. Chem. Phys.*, **119**, 8229.
- J. Gjorgjieva and J. Jacobsen. Turing patterns on growing spheres: the exponential case. Dynamical systems and differential equations. In *Proceedings of the 6th AIMS international conference. Discrete and continuous dynamical systems supplement, USA*, pages 436–445, 2007.
- P. Glendinning. *Stability, Instability, and Chaos: An Introduction to the Theory of Nonlinear Differential Equations*. Cambridge University Press, 1994.
- D. Gomila. *Dynamics of Spatial Structures in Nonlinear Optics*. PhD thesis, Universitat de les Illes Balears, 2003.
- D. Gomila, P. Colet, G. L. Oppo and M. S. Miguel. 2001. Stable droplets and growth laws close to the modulational instability of a domain wall. *Phys. Rev. Lett.*, **87**, 194101.
- D. Gomila, P. Colet, G. L. Oppo and M. S. Miguel. 2004. Stable droplets and nucleation in asymmetric bistable nonlinear optical systems. *J. Opt. B: Quantum Semiclass. Opt.*, **6**, 265–270.
- B. C. Goodwin, S. Kauffman and J. D. Murray. 1993. Is morphogenesis an intrinsically robust process? *J. Theor. Biol.*, **163**, 135 – 144.
- J. B. A. Green and J. C. Smith. 1990. Graded changes in dose of a *Xenopus* activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature*, **347**, 391–394.
- G. Grimmett and D. Stirzaker. *Probability and Random Processes*. Oxford University Press, 2001.
- P. Grindrod. *The Theory and Applications of Reaction-Diffusion Equations: Patterns and Waves*. Clarendon Press, 1996.
- C. M. Guldberg and P. Waage. 1879. Concerning chemical affinity. *J. Prakt. Chem.*, **19**, 69–114.

- J. D. Gunton, M. San Miguel and P. S. Sahni. 1983. Phase transitions and critical phenomena. *Academic. London.*, **8**, 267.
- P. Guptasarma. 1995. Does replication-induced transcription regulate synthesis of the myriad low copy number proteins of *Escherichia coli*? *BioEssays*, **17**, 987–97.
- J. B. Gurdon, P. Harger, A. Mitchell and P. Lemaire. 1994. Activin signalling and response to a morphogen gradient. *Nature*, **371**, 487–492.
- L. G. Harrison, K. T. Graham and B. C. Lakowski. 1988. Calcium localization during *Acetabularia* whorl formation: evidence supporting a two-stage hierarchical mechanism. *Development*, **104**, 255.
- J. Hattne, D. Fange and J. Elf. 2005. Stochastic reaction-diffusion simulation with MesoRD. *Bioinformatics*, **21**, 2923.
- H. G. E. Hentschel, T. Glimm, J. A. Glazier and S. A. Newman. 2004. Dynamical mechanisms for skeletal pattern formation in the vertebrate limb. *Proc. R. Soc. Lond. B*, **271**, 1713.
- E. J. Hinch. *Perturbation Methods*. Cambridge University Press, 1991.
- A. Hunding and P. G. Sørensen. 1988. Size adaptation of Turing prepatterns. *J. Math. Biol.*, **26**, 27–39.
- P. W. Ingham and M. J. Fietz. 1995. Quantitative effects of hedgehog and decapentaplegic activity on the patterning of the *Drosophila* wing. *Curr. Biol.*, **5**, 432–440.
- D. Iron, J. Wei and M. Winter. 2004. Stability analysis of Turing patterns generated by the Schnakenberg model. *J. Math. Biol.*, **49**, 358–390.
- K. Iwatsuki, H. X. Liu, A. Grónder, M. A. Singer, T. F. Lane, R. Grosschedl, C. M. Mistretta and R. F. Margolskee. 2007. Wnt signaling interacts with Shh to regulate taste papilla development. *Proc. Nat. Acad. Sci.*, **104**, 2253.
- J. A. Izaguirre, R. Chaturvedi, C. Huang, T. Cickovski, J. Coffland, G. Thomas, G. Forgacs, M. Alber, G. Hentschel, S. A. Newman and J. A. Glazier. 2004. CompuCell, a multi-model framework for simulation of morphogenesis. *Bioinformatics*, **20**, 1129.

- T. Jahnke and W. Huisinga. 2007. Solving the chemical master equation for monomolecular reaction systems analytically. *J. Math. Biol.*, **54**, 1–26.
- E. Järvinen, I. Salazar-Ciudad, W. Birchmeier, M. M. Taketo, J. Jernvall and I. Thesleff. 2006. Continuous tooth generation in mouse is induced by activated epithelial Wnt/ β -catenin signaling. *Proc. Nat. Acad. Sci.*, **103**, 18627.
- D. W. Jordan and P. Smith. *Mathematical Techniques: An Introduction for the Engineering, Physical, and Mathematical Sciences*. Oxford University Press, 2002.
- S. L. Judd and M. Silber. 2000. Simple and superlattice Turing patterns in reaction-diffusion systems: bifurcation, bistability, and parameter collapse. *Physica D*, **136**, 45 – 65.
- A. Julius, M. Zavlanos, S. Boyd and G. J. Pappas. 2009. Genetic network identification using convex programming. *IET Sys. Bio.*, **3**, 155–166.
- M. Kærn, R. Satnoianu, A.P. Muñozuri and M. Menzinger. 2002. Controlled pattern formation in the CDIMA reaction with a moving boundary of illumination. *Phys. Chem. Chem. Phys.*, **4**, 1315–1319.
- M. Kaminski and J. McDonagh. 1983. Studies on the mechanism of thrombin. Interaction with fibrin. *J. Biol. Chem.*, **258**, 10530–10535.
- R. Kapral and K. Showalter. *Chemical Waves and Patterns*. Kluwer Academic Publishers, 1995.
- Y. Kassai, P. Munne, Y. Hotta, E. Penttilä, K. Kavanagh, N. Ohbayashi, S. Takada, I. Thesleff, J. Jernvall and N. Itoh. 2005. Regulation of mammalian tooth cusp patterning by ectodin. *Science*, **309**, 2067.
- J. P. Keener. *Principles of Applied Mathematics*. Westview Press, 2000.
- E. F. Keller. 2002. Developmental robustness. *Ann. Ny. Acad. Sci.*, **981**, 189–201.
- K. K. J. Kinader. 2001. Understanding average Brownian exit time. *Stat. Probabil. Lett.*, **51**, 1–8.

- V. Klika, R. E. Baker, D. Headon and E. A. Gaffney. 2011. The influence of receptor-mediated interactions on reaction-diffusion mechanisms of cellular self-organisation. *In press*.
- A. L. Koch. 1990. Diffusion: the crucial process in many aspects of the biology of bacteria. *Adv. Microb. Ecol.*, **11**, 37–70.
- S. Kondo and R. Asai. 1995. A reaction-diffusion wave on the skin of the marine angelfish *Pomacanthus*. *Nature*, **376**, 765–768.
- E. Kreyszig. *Advanced Engineering Mathematics*. Wiley-India, 8th edition, 2007.
- P. M. Kulesa, G. C. Cruywagen, S. R. Lubkin, M. W. J. Ferguson and J. D. Murray. 1996a. Modelling the spatial patterning of teeth primordia in the alligator. *Acta. Biotheor.*, **44**, 153–164.
- P. M. Kulesa, G. C. Cruywagen, S. R. Lubkin, P. K. Maini, J. Sneyd, M. W. J. Ferguson and J. D. Murray. 1996b. On a model mechanism for the spatial patterning of teeth primordia in the alligator. *J. Theor. Biol.*, **180**, 287–296.
- M. Kuraguchi, X. P. Wang, R. T. Bronson, R. Rothenberg, N. Y. Ohene-Baah, J. J. Lund, M. Kucherlapati, R. L. Maas and R. Kucherlapati. 2006. Adenomatous polyposis coli (APC) is required for normal development of skin and thymus. *PLoS genetics*, **2**, e146.
- J. Laurikkala, Y. Kassai, L. Pakkasjärvi, I. Thesleff and N. Itoh. 2003. Identification of a secreted BMP antagonist, ectodin, integrating BMP, FGF, and SHH signals from the tooth enamel knot. *Dev. Biol.*, **264**, 91–105.
- J. R. Lawrence, G. M. Wolfaardt and D. R. Korber. 1994. Determination of diffusion coefficients in biofilms by confocal laser microscopy. *Appl. Environ. Microb.*, **60**, 1166.
- K. J. Lee, W. D. McCormick, Q. Ouyang and H. L. Swinney. 1993. Pattern formation by interacting chemical fronts. *Science*, **261**, 192–194.
- I. Lengyel and I. R. Epstein. 1991. Modeling of Turing structures in the chlorite-iodide-malonic acid-starch reaction system. *Science*, **251**, 650–652.

- T. Leppänen. *Computational Studies of Pattern Formation in Turing Systems*. PhD thesis, 2004.
- T. Leppänen, M. Karttunen, R. A. Barrio and K. Kaski. 2003. The effect of noise on Turing patterns. *Sup. Prog. Theor. Phys.*, **150**, 367–370.
- S. S. Liaw, C. C. Yang, R. T. Liu and J. T. Hong. 2001. Turing model for the patterns of lady beetles. *Phys. Rev. E*, **64**, 41909.
- C. M. Lin, T. X. Jiang, R. E. Baker, P. K. Maini, R. B. Widelitz and C. M. Chuong. 2009. Spots and stripes: Pleomorphic patterning of stem cells via p-ERK-dependent cell chemotaxis shown by feather morphogenesis and mathematical simulation. *Dev. Biol.*, **334**, 369–382.
- F. Liu, Chu E. Y., B. Watt, Y. Zhang, N. M. Gallant, T. Andl, S. H. Yang, P. Lu, M. M. and Piccolo, R. Schmidt-Ullrich, M. M. Taketo, E. E. Morrissey, R. Atit, A. A. Dlugosz and S. E. Millar. 2008. Wnt/ β -catenin signaling directs multiple stages of tooth morphogenesis. *Dev. Biol.*, **313**, 210 – 224.
- A. Loinger, A. Lipshtat, N.Q. Balaban and O. Biham. 2007. Stochastic simulations of genetic switch systems. *Phys. Rev. E*, **75**, 21904.
- C. A. Lugo and A. J. McKane. 2008. Quasicycles in a spatial predator-prey model. *Phys. Rev. E*, **78**, 51911.
- R. G. Macfarlane. 1964. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature*, **202**, 498–499.
- A. Madzvamuse. 2006. Time-stepping schemes for moving grid finite elements applied to reaction-diffusion systems on fixed and growing domains. *J. Comput. Phys.*, **214**, 239–263.
- A. Madzvamuse, A. J. Wathen and P. K. Maini. 2003. A moving grid finite element method applied to a model biological pattern generator. *J. Comp. Phys.*, **190**, 478–500.
- P. K. Maini, R. E. Baker and C. M. Chuong. 2006. The Turing model comes of molecular age. *Science*, **314**, 1397–1398.

- P. K. Maini, E. J. Crampin, A. Madzvamuse, A. J. Wathen and R. D. K. Thomas. Implications of domain growth in morphogenesis. In *Mathematical modelling & computing in biology and medicine: 5th ESMTB conference 2002*, volume 1, page 67, 2003.
- P. K. Maini and H. G. Othmer, editors. *Mathematical Models for Biological Pattern Formation*. Springer Verlag, 2001.
- P. K. Maini, K. J. Painter and H. N. P. Chau. 1997. Spatial pattern formation in chemical and biological systems. *J. Chem. Soc., Faraday Trans.*, **93**, 3601–3610.
- J. Massague. 1990. The transforming growth factor-beta family. *Annu. Rev. Cell. Biol.*, **6**, 597–641.
- A. J. McKane, J. D. Nagy, T. J. Newman and M. O. Stefanini. 2007. Amplified biochemical oscillations in cellular systems. *J. Stat. Phys.*, **128**, 165–191.
- A. J. McKane and T. J. Newman. 2004. Stochastic models in population biology and their deterministic analogs. *Phys. Rev. E*, **70**, 041902.
- A. J. McKane and T. J. Newman. 2005. Predator-prey cycles from resonant amplification of demographic stochasticity. *Phys. Rev. Lett.*, **94**, 218102.
- D. A. McQuarrie. 1967. Stochastic approach to chemical kinetics. *J. Appl. Probab.*, **4**, 413–478.
- J. M. McSloy. *Computationally Determined Existence and Stability Regimes of Solitonic Phenomena in Nonlinear Optics*. PhD thesis, University of Strathclyde, 2002.
- H. Meinhardt, P. Prusinkiewicz and D. R. Fowler. *The Algorithmic Beauty of Sea Shells*. Springer Verlag, 2003.
- D. Michaelis, U. Peschel, F. Lederer, D. V. Skryabin and W. J. Firth. 2001. Universal criterion and amplitude equation for a nonequilibrium Ising-Bloch transition. *Phys. Rev. E*, **63**, 066602.
- D. G. Míguez, M. Dolnik, A. P. Muñuzuri and L. Kramer. 2006. Effect of axial growth on Turing pattern formation. *Phys. Rev. Lett.*, **96**, 48304.

- C. B. Moler and G. W. Stewart. 1973. An algorithm for generalized matrix eigenvalue problems. *SIAM J. Numer. Anal.*, **10**, 241–256.
- N. A. M. Monk. 1998. Restricted-range gradients and travelling fronts in a model of juxtacrine cell relay. *Bull. Math. Biol.*, **60**, 901–918.
- N. A. M. Monk, J. A. Sherratt and M. R. Owen. *Mathematical Models for Biological Pattern Formation*, chapter Spatiotemporal patterning in models of juxtacrine intercellular signalling with feedback, pages 165–192. Springer Verlag, 2001.
- J. R. Mooney and B. N. Nagorcka. 1985. Spatial patterns produced by a reaction-diffusion system in primary hair follicles. *J. Theor. Biol.*, **115**, 299–317.
- K. W. Morton and D. F. Mayers. *Numerical Solution of Partial Differential Equations: An Introduction*. Cambridge University Press, 2005.
- C. Mou, B. Jackson, P. Schneider, P. A. Overbeek and D. J. Headon. 2006. Generation of the primary hair follicle pattern. *Proc. Nat. Acad. Sci.*, **103**, 9075.
- P. M. Munne, M. Tummers, E. Järvinen, I. Thesleff and J. Jernvall. 2009. Tinkering with the inductive mesenchyme: *Sostdc1* uncovers the role of dental mesenchyme in limiting tooth induction. *Development*, **136**, 393.
- J. D. Murray. 1981. A pre-pattern formation mechanism for animal coat markings. *J. Theor. Biol.*, **88**, 161–199.
- J. D. Murray. 1982. Parameter space for Turing instability in reaction diffusion mechanisms: a comparison of models. *J. Theor. Biol.*, **98**, 143.
- J. D. Murray. *Mathematical Biology I: An Introduction*, volume 1. Springer-Verlag, 3rd edition, 2003a.
- J. D. Murray. *Mathematical Biology II: Spatial Models and Biomedical Applications*, volume 2. Springer-Verlag, 3rd edition, 2003b.
- J. D. Murray, E. A. Stanley and D. L. Brown. 1986. On the spatial spread of rabies among foxes. *P. Roy. Soc. Lond. B. Bio.*, **229**, 111–150.

- M. E. Mycielska and M. Djamgoz. 2004. Cellular mechanisms of direct-current electric field effects: galvanotaxis and metastatic disease. *J. Cell. Sci.*, **117**, 1631.
- M. R. Myerscough, P. K. Maini and K. J. Painter. 1998. Pattern formation in a generalized chemotactic model. *Bull. Math. Biol.*, **60**, 1–26.
- B. N. Nagorcka and J. R. Mooney. 1992. From stripes to spots: prepatterns which can be produced in the skin by a reaction-diffusion system. *Math. Med. Biol.*, **9**, 249.
- T. Nakamura, N. Mine, E. Nakaguchi, A. Mochizuki, M. Yamamoto, K. Yashiro, C. Meno and H. Hamada. 2006. Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. *Dev. Cell.*, **11**, 495–504.
- K. Närhi, E. Järvinen, W. Birchmeier, M. M. Taketo, M. L. Mikkola and I. Liu Thesleff. 2008. Sustained epithelial β -catenin activity induces precocious hair development but disrupts hair follicle down-growth and hair shaft formation. *Development*, **135**, 1019.
- A. A. Neville, P. C. Matthews and H. M. Byrne. 2006. Interactions between pattern formation and domain growth. *Bull. Math. Biol.*, **68**, 1975–2003.
- S. A. Newman, S. Christley, T. Glimm, H. G. E. Hentschel, B. Kazmierczak, Y. T. Zhang, J. Zhu and M. Alber. 2008. Multiscale models for vertebrate limb development. *Curr. Top. Dev. Biol.*, **81**, 311–340.
- S. A. Newman and H. L. Frisch. 1979. Dynamics of skeletal pattern formation in developing chick limb. *Science*, **205**, 662.
- G. Nicolis. *Introduction to Nonlinear Science*. Cambridge University Press, 1995.
- G. Nicolis and I. Prigogine. *Self-Organization in Nonequilibrium Systems*. Wiley, 1977.
- T. Nohno, Y. Kawakami, H. Ohuchi, A. Fujiwara, H. Yoshioka and S. Noji. 1995. Involvement of the Sonic hedgehog gene in chick feather formation. *Biochem. Biophys. Res. Co.*, **206**, 33–39.
- T. Nozakura and S. Ikeuchi. 1984. Formation of dissipative structures in galaxies. *Astrophys. J.*, **279**, 40–52.

- B. K. Øksendal. *Stochastic Differential Equations: An Introduction with Applications*. Springer Verlag, 2003.
- A. Okubo, P. K. Maini, M. H. Williamson and J. D. Murray. 1989. On the spatial spread of the grey squirrel in Britain. *P. Roy. Soc. Lond. B. Biol.*, **238**, 113.
- G. L. Oppo, A. J. Scroggie and W. J. Firth. 1999. From domain walls to localized structures in degenerate optical parametric oscillators. *J. Opt. B: Quantum Semiclass. Opt.* **1**, 1, 133–138.
- H. G. Othmer, S. R. Dunbar and W. Alt. 1988. Models of dispersal in biological systems. *J. Math. Biol.*, **26**, 263–298.
- H. G. Othmer and E. Pate. 1980. Scale-invariance in reaction-diffusion models of spatial pattern formation. *Proc. Nat. Acad. Sci.*, **77**, 4180.
- M. R. Owen and J. A. Sherratt. 1998. Mathematical modelling of juxtacrine cell signalling. *Math. Biosci.*, **153**, 125–150.
- M. R. Owen, J. A. Sherratt and S. R. Myers. 1999. How far can a juxtacrine signal travel? *Proc. R. Soc. Lond. B*, **266**, 579.
- M. R. Owen, J. A. Sherratt and H. J. Wearing. 2000. Lateral induction by juxtacrine signaling is a new mechanism for pattern formation. *Dev. Biol.*, **217**, 54–61.
- E. M. Ozbudak, M. Thattai, I. Kurtser, A. D. Grossman and A. van Oudenaarden. 2002. Regulation of noise in the expression of a single gene. *Nat. Genet.*, **31**, 69–73.
- K. J. Painter, P. K. Maini and H. G. Othmer. 1999. Stripe formation in juvenile *Pomacanthus* explained by a generalized Turing mechanism with chemotaxis. *Proc. Nat. Acad. Sci.*, **96**, 5549.
- J. E. Pearson and W. Horsthemke. 1989. Turing instabilities with nearly equal diffusion coefficients. *J. Chem. Phys.*, **90**, 1588.
- H. Poincare. 1892. Les methodes nouvelles de la mecanique celeste. *Gauthier-Villars*, **1**.
- I. Prigogine and P. Glansdorff. *Thermodynamic Theory of Structure, Stability and Fluctuations*. Wiley-Interscience New York, 1971.

- L. Qiao, R. Erban, C. T. Kelley and I. G. Kevrekidis. 2006. Spatially distributed stochastic systems: Equation-free and equation-assisted preconditioned computations. *J. Chem. Phys.*, **125**, 204108.
- C. V. Rao, D. M. Wolf and A. P. Arkin. 2002. Control, exploitation and tolerance of intracellular noise. *Nature*, **420**, 231–237.
- S. Redner. *A Guide to First-Passage Processes*. Cambridge University Press, 2001.
- T. Reichenbach, M. Mobilia and E. Frey. 2006. Coexistence versus extinction in the stochastic cyclic Lotka-Volterra model. *Phys. Rev. E*, **74**, 51907.
- P. H. Richter, P. Rehms and J. Ross. 1981. Control and dissipation in oscillatory chemical engines. *Prog. Theor. Phys.*, **66**.
- R. D. Riddle, R. L. Johnson, E. D. Laufer and C. Tabin. 1993. Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell*, **75**, 1401–1416.
- H. Risken. *The Fokker-Planck Equation: Methods of Solution and Applications*. Springer, 1989.
- F. Rothe. 1982. Uniform bounds from bounded L_p -functionals in reaction-diffusion equations. *J. Differ. Equations.*, **45**, 207–233.
- T. Rudge and K. Burrage. 2008. Effects of intrinsic and extrinsic noise can accelerate juxtacrine pattern formation. *Bull. Math. Biol.*, **70**, 971–991.
- B. Rudovics, E. Dulos and P. De Kepper. 1996. Standard and nonstandard Turing patterns and waves in the CIMA reaction. *Phys. Scr.*, **T67**, 43–50.
- A. Sage, Y. Tintut, A. Garfinkel and L. Demer. 2009. Systems biology of vascular calcification. *Trends. Cardiovas. Med.*, **19**, 118–123.
- F. Sagués, J. M. Sancho and J. García-Ojalvo. 2007. Spatiotemporal order out of noise. *Rev. Mod. Phys.*, **79**, 829–882.
- K. Saha and D. V. Schaffer. 2006. Signal dynamics in Sonic hedgehog tissue patterning. *Development*, **133**, 889.

- I. Salazar-Ciudad and J. Jernvall. 2002. A gene network model accounting for development and evolution of mammalian teeth. *Proc. Nat. Acad. Sci.*, **99**, 8116–8120.
- I. Salazar-Ciudad and J. Jernvall. 2010. A computational model of teeth and the developmental origins of morphological variation. *Nature*, **464**, 583–586.
- A. Sanz-Anchelergues, A. M. Zhabotinsky, I. R. Epstein and A. P. Munuzuri. 2001. Turing pattern formation induced by spatially correlated noise. *Phys. Rev. E*, **63**, 056124.
- J. J. Sanz-Ezquerro and C. Tickle. 2000. Autoregulation of Shh expression and Shh induction of cell death suggest a mechanism for modulating polarising activity during chick limb development. *Development*, **127**, 4811.
- L. Sarkar and P. T. Sharpe. 1999. Expression of Wnt signalling pathway genes during tooth development. *Mech. Develop.*, **85**, 197–200.
- R. A. Satnoianu and M. Menzinger. 2000. Non-Turing stationary patterns in flow-distributed oscillators with general diffusion and flow rates. *Phys. Rev. E*, **62**, 113–119.
- P. T. Saunders and M. W. Ho. 1995. Reliable segmentation by successive bifurcation. *Bull. Math. Biol.*, **57**, 539–556.
- T. Saunders and M. Howard. 2009. When it pays to rush: interpreting morphogen gradients prior to steady-state. *Phys. Biol.*, **6**, 046020.
- S. Scarsoglio, F. Laio, P. D’Odorico and L. Ridolfi. 2011. Spatial pattern formation induced by Gaussian white noise. *Math. Biosci.*, **229**, 174–184.
- J. Schnakenberg. 1979. Simple chemical reaction systems with limit cycle behaviour. *J. Theor. Biol.*, **81**, 389–400.
- L. A. Segel. *Modeling Dynamic Phenomena in Molecular and Cellular Biology*. Cambridge University Press, 1984.
- L. A. Segel and J. L. Jackson. 1972. Dissipative structure: an explanation and an ecological example. *J. Theor. Biol.*, **37**, 545–559.

- S. Seirin Lee, E. A. Gaffney and N. A. M. Monk. 2010. The influence of gene expression time delays on Gierer–Meinhardt pattern formation systems. *Bull. Math. Biol.*, **72**, 2139–2160.
- N. Serrano and P. H. O’Farrell. 1997. Limb morphogenesis: connections between patterning and growth. *Curr. Biol.*, **7**, R186–R195.
- S. Sick, S. Reinker, J. Timmer and T. Schlake. 2006. WNT and DKK determine hair follicle spacing through a reaction-diffusion mechanism. *Science*, **314**, 1447–1450.
- M. J. Simpson, D. C. Zhang, M. Mariani, K. A. Landman and D. F. Newgreen. 2007. Cell proliferation drives neural crest cell invasion of the intestine. *Dev. Biol.*, **302**, 553–568.
- A. Singh and J. P. Hespanha. Stochastic analysis of gene regulatory networks using moment closure. In *American Control Conference, 2007. ACC’07*, pages 1299–1304, 2007.
- E. W. Sinnott and R. Bloch. 1939. Changes in intercellular relationships during the growth and differentiation of living plant tissues. *Am. J. Bot.*, **26**, 625–634.
- D. V. Skryabin, A. Yulin, D. Michaelis, W. J. Firth, G. L. Oppo, U. Peschel and F. Lederer. 2001. Perturbation theory for domain walls in the parametric Ginzburg-Landau equation. *Phys. Rev. E*, **64**, 56618.
- J. C. Smith and L. Wolpert. 1981. Pattern formation along the anteroposterior axis of the chick wing: the increase in width following a polarizing region graft and the effect of X-irradiation. *J. Embryol. Exp. Morph.*, **63**, 127.
- L. Solnica-Krezel. 2003. Vertebrate development: taming the nodal waves. *Curr. Biol.*, **13**, R7–R9.
- B. St-Jacques, H. R. Dassule, I. Karavanova, V. A. Botchkarev, J. Li, P. S. Danielian, J. A. McMahon, P. M. Lewis, R. Paus and A. P. McMahon. 1998. Sonic hedgehog signaling is essential for hair development. *Curr. Biol.*, **8**, 1058–1069.
- J. J. Stoker. *Differential Geometry*. Wiley-Interscience, 1989.

- M. Stone and P. M. Goldbart. *Mathematics for Physics: A Guided Tour for Graduate Students*. Cambridge University Press, 2009.
- E. Süli and D. F. Mayers. *An Introduction to Numerical Analysis*. Cambridge University Press, 2003.
- D. Summerbell. 1981. The control of growth and the development of pattern across the anteroposterior axis of the chick limb bud. *J. Embryol. Exp. Morph.*, **63**, 161.
- J. Tegner, M. K. Yeung, J. Hasty and J. J. Collins. 2003. Reverse engineering gene networks: integrating genetic perturbations with dynamical modeling. *Proc. Nat. Acad. Sci.*, **100**, 5944.
- T. B. Thorson, J. K. Langhammer and M. I. Oetinger. 1983. Reproduction and development of the South American freshwater stingrays, *Potamotrygon circularis* and *P. motoro*. *Environ. Biol. Fish.*, **9**, 3–24.
- T. Tian and K. Burrage. 2004. Binomial leap methods for simulating stochastic chemical kinetics. *J. Chem. Phys.*, **121**, 10356.
- T. Tian and K. Burrage. 2006. Stochastic models for regulatory networks of the genetic toggle switch. *Proc. Nat. Acad. Sci.*, **103**, 8372.
- C. Tickle, D. Summerbell and L. Wolpert. 1975. Positional signalling and specification of digits in chick limb morphogenesis. *Nature*, **254**, 199–202.
- C. J. Tomlin and J. D. Axelrod. 2007. Biology by numbers: mathematical modelling in developmental biology. *Nat. Rev. Genet.*, **8**, 331–340.
- F. Tostevin, P. R. ten Wolde and M. Howard. 2007. Fundamental limits to position determination by concentration gradients. *PLoS Comput. Biol.*, **3**, e78.
- M. Towers, R. Mahood, Y. Yin and C. Tickle. 2008. Integration of growth and specification in chick wing digit-patterning. *Nature*, **452**, 882–886.
- M. Towers, J. Signolet, A. Sherman, H. Sang and C. Tickle. 2011. Insights into bird wing evolution and digit specification from polarizing region fate maps. *Nat. Commun.*, **2**, 426.

- A. M. Turing. 1952. The chemical basis of morphogenesis. *Phil. Trans. R. Soc. Lond. B*, **237**, 37–72.
- H. Tutu. 1997. Ordering process and Bloch wall dynamics in a two-dimensional anisotropic spin system. *Phys. Rev. E*, **56**, 5036–5043.
- A. Twomey. On the Stochastic Modelling of Reaction-Diffusion Processes. Master’s thesis, University of Oxford, 2007.
- N. G. van Kampen. *Stochastic Processes in Physics and Chemistry*. North Holland, 3rd edition, 2007.
- C. F. Van Loan. 1975. A general matrix eigenvalue algorithm. *SIAM J. Numer. Anal.*, **12**, 819–834.
- J. S. van Zon and P. R. ten Wolde. 2005. Greens-function reaction dynamics: A particle-based approach for simulating biochemical networks in time and space. *J. Chem. Phys.*, **123**, 234910.
- C. Varea, D. Hernández and R. A. Barrio. 2007. Soliton behaviour in a bistable reaction diffusion model. *J. Math. Biol.*, **54**, 797–813.
- M. J. Ward and J. Wei. 2002. The existence and stability of asymmetric spike patterns for the Schnakenberg model. *Stud. App. Math.*, **109**, 229–264.
- M. Watzl and A. F. Münster. 1995. Turing-like spatial patterns in a polyacrylamide-methylene blue-sulfide-oxygen system. *Chem. Phys. Lett.*, **242**, 273–278.
- H. J. Wearing, M. R. Owen and J. A. Sherratt. 2000. Mathematical modelling of juxtacrine patterning. *Bull. Math. Biol.*, **62**, 293–320.
- H. J. Wearing and J. A. Sherratt. 2001. Nonlinear analysis of juxtacrine patterns. *SIAM J. Appl. Math.*, **62**, 283–309.
- B. Westergaard and M. W. J. Ferguson. 1986. Development of the dentition in *Alligator mississippiensis*. Early embryonic development in the lower jaw. *J. Zool.*, **210**, 575–597.
- D. B. White. 1988. The planforms and onset of convection with a temperature-dependent viscosity. *J. Fluid Mech.*, **191**, 247–286.

- K. Wiesenfeld and F. Moss. 1995. Stochastic resonance and the benefits of noise: from ice ages to crayfish and SQUIDS. *Nature*, **373**, 33–36.
- O. K. Wilby and D. A. Ede. 1975. A model generating the pattern of cartilage skeletal elements in the embryonic chick limb. *J. Theor. Biol.*, **52**, 199–217.
- L. Wolpert. *Towards a Theoretical Biology. I. Prolegomena*, volume 1, chapter The French flag problem: a contribution to the discussion on pattern development and regulation, pages 125–133. Edinburgh University Press, 1968.
- L. Wolpert. 1969. Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.*, **25**, 1–47.
- L. Wolpert. 1989. Positional information revisited. *Development*, **107**, 3–12.
- L. Wolpert. 1994. Positional information and pattern formation in development. *Dev. Genet.*, **15**, 485–490.
- T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. 2011a. Influence of stochastic domain growth on pattern nucleation for diffusive systems with internal noise. *Phys. Rev. E*, **84**, 041905.
- T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. 2011b. Power spectra methods for a stochastic description of diffusion on deterministically growing domains. *Phys. Rev. E*, **84**, 021915.
- T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. 2011c. Stochastic reaction and diffusion on growing domains: Understanding the breakdown of robust pattern formation. *Phys. Rev. E*, **84**, 046216.
- T. E. Woolley, R. E. Baker, E. A. Gaffney and P. K. Maini. *Mathematical Modelling of Zombies*, chapter How long can we survive? To appear., 2012.
- T. E. Woolley, R. E. Baker, P. K. Maini, J. L. Aragón and R. A. Barrio. 2010. Analysis of stationary droplets in a generic Turing reaction-diffusion system. *Phys. Rev. E*, **82**, 051929.

- L. Yang, M. Dolnik, A. M. Zhabotinsky and I. R. Epstein. 2006. Turing patterns beyond hexagons and stripes. *Chaos*, **16**, 037114.
- W. Y. Yang, W. Cao, T. S. Chung and J. Morris. *Applied Numerical Methods Using MATLAB*. Wiley-Interscience, 2005.
- Y. Yang, G. Drossopoulou, P. T. Chuang, D. Duprez, E. Marti, D. Bumcrot, N. Vargesson, J. Clarke, L. Niswander, A. McMahon and C. Tickle. 1997. Relationship between dose, distance and time in Sonic Hedgehog-mediated regulation of anteroposterior polarity in the chick limb. *Development*, **124**, 4393.
- A. Yochelis, Y. Tintut, L. L. Demer and A. Garfinkel. 2008. The formation of labyrinths, spots and stripe patterns in a biochemical approach to cardiovascular calcification. *New J. Phys.*, **10**, 055002.
- A. F. Zebboudj, M. Imura and K. Boström. 2002. Matrix GLA protein, a regulatory protein for bone morphogenetic protein-2. *J. Biol. Chem.*, **277**, 4388.
- W. Zeng, G. L. Thomas and J. A. Glazier. 2004. Non-Turing stripes and spots: a novel mechanism for biological cell clustering. *Physica A*, **341**, 482–494.
- F. Zheng-Ping, X. Xin-Hang, W. Hong-Li and O. Qi. 2008. Stochastic simulation of Turing patterns. *Chin. Phys. Lett.*, **25**, 1220–1223.