

Mathematical models of single and collective cell migration in the cranial neural crest



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A thesis submitted for the degree of
Doctor of Philosophy

Michaelmas 2025

*I dedicate this thesis to my parents, Anne Marie and Kevin.
Your hard work, love, and encouragement throughout my
life have made this work possible; it is as much yours as it
is mine.*

In memoriam

This page is dedicated to those who passed away
before the submission of this thesis.

Lewis Boyd-Hill (1993–2021) and James Boyd-Hill (1997–2023)

My cousins, Lewis and James, were born in Hartlepool, County Durham, and have both recently passed away in an untimely fashion. I will always be grateful for the short amount of time we spent together on this Earth and I hope that, somewhere, they are enjoying each other's company once again. Lewis and James, there are no words that can describe how very deeply you are missed. Your memory will remain with me always.

Mary Boyd (née Skilbeck) (1931–1973) and John Boyd (1934–2017)

My Nana, Mary, and Grandad, John, were born in Hartlepool, County Durham. My Nana passed away at a very young age, and I am very sad that I did not get the chance to meet her. My Grandad lived on long after my Nana's passing and I have enjoyed many visits to Hartlepool throughout my life. I hope that you are both together again, alongside Lewis and James, and that you would both be proud of me.

Eileen Vickers (née Breen) (1935–2025) and William Johnson (1917–1974)

My Granny, Eileen, was born in Mullinahone, County Tipperary, and my Grandad, William, in Ballycumber, County Offaly, Ireland. They met shortly after moving to England. My Grandad passed away long before I was born, but I know lots about him through hearing about the life that he shared with my Granny. I have many fond memories of visiting my Granny after she moved back to Ireland, and I am very sad that both she and my Grandad are not here to see me submit this work.

Paul Kulesa (1962–2025)

Paul was an exceptional scientist who made immeasurable contributions to neural crest biology. He was deeply committed to his work and brought an infectious enthusiasm to research. Paul was also an extraordinarily kind man who always had time to discuss ideas with anyone, regardless of their seniority. Without his contributions to neural crest biology, none of the work presented in this thesis would have been possible. He is deeply missed by everyone in Oxford who had the pleasure of working with him.

May they rest in peace.

Acknowledgements

First and foremost, I would like to thank my supervisors, Professor Ruth Baker and Professor Philip Maini, for their outstanding guidance and support. Over the past four years, I have learned an enormous amount from you both, and my questions have always been met with kindness, patience, and deep insight.

I would also like to thank Professor Mike Giles for his advice regarding the numerical scheme in Chapter 6, Professor Maddy Parsons for biological advice in Chapter 3, and Professor Paul Kulesa (1962–2025) for his innumerable comments and insights that have fundamentally shaped the direction of this thesis. Much of my work has also been influenced by my transfer and confirmation of status assessors, Professor Helen Byrne, Dr Berta Verd, and Professor Mohit Dalwadi, all of whom have provided comments and advice that have greatly improved this thesis. I am also deeply thankful to the IT, cleaning, and services staff of the Andrew Wiles Building, without whom we would have no place to conduct research.

I gratefully acknowledge the Wolfson Centre for Mathematical Biology for providing an excellent environment for doctoral research. In particular, I would like to thank Róisín, Simon, Carles, Sergi, Luke, and Lena for making my office feel like home. I am also grateful to Ian and Niall for our many trips to the Star, to Vedang for our Saturday office sessions, to Jun for our continued political discussions, to Luke for sneaking up on me in the office at least once a day, and to Joe, for his very welcome office visits.

Outside of mathematics, I would like to thank Jack and Jade for their invaluable friendship throughout my doctorate; James, Kenny, and Calum for our (all too infrequent) trips to the pub; my friends from Edinburgh, Peter, Tom, and Dante, for our frequent contact and meet-ups; and my friends from Chester, particularly Dom, Dave, Will, and Mike, for their visits to Oxford, which were always the highlight of my year.

I would also like to thank Mrs Holmes and Mr McManus from the Chester Catholic High School. Their encouragement and passion for mathematics played a formative role in my decision to pursue the subject further. To Mrs Holmes, I think I still owe you many pieces of homework — I hope that this thesis goes some way towards making up for that.

I am grateful to Lady Margaret Hall for offering me a Stipendiary Lectureship over the last three years, and to the amazing students and mathematics tutors there. Thank you to Ian (again), David, Alex, and Fabian for our many lunches together in hall.

I also thank the Biotechnology and Biological Sciences Research Council for offering me a doctoral studentship that allowed me to conduct this research, and the Doctoral Training Centre in Oxford for the wider scientific community it has provided.

Finally, I would like to thank my Mum, Dad, and family for their constant support, and Kat, for believing in me more than I believe in myself. You have helped in more ways than I could possibly list and I do not know what I would do without you.

Abstract

The cranial neural crest is a highly migratory cell population that contributes to the formation of much of the facial skeleton, cranial ganglia, and associated connective tissues during vertebrate embryogenesis. The collective migration of cranial neural crest cells (CNCCs) occurs along well-characterised spatio-temporal pathways and is largely independent of cell proliferation, making the cranial neural crest a paradigm model system for studying the emergence of collective behaviours from both cell–cell and cell–environment interactions. An improved understanding of CNCC migration offers the potential to develop novel therapeutic interventions to counteract the effects of aberrant migration and, more broadly, to provide insight into other biological systems in which collective migration is also of importance, including wound healing and tumour progression.

Accordingly, in this thesis, we develop mathematical models at the single-cell, collective, and continuum scales to interrogate the mechanisms underlying cranial neural crest cell migration. Using the chick embryo as a model system, we construct a hierarchy of mathematical models to study CNCC morphological heterogeneity, phenotypic plasticity, the microenvironmental regulation of CNCC streams, and their emergence via short-range cell–environment and cell–cell interactions.

By formulating a mathematical model resolved at the single-cell scale, we study the variation in the protrusive morphology of CNCCs during migration, before employing a canonical agent-based model to examine how exogenous signalling dynamics give rise to coherent, spatially confined CNCC streams. Following this, we formulate a novel agent-based model to demonstrate the spontaneous emergence of leader and follower phenotypes during collective CNCC migration, and conclude by using continuum models to link microscale cell–cell interactions to emergent collective stream dynamics. In doing so, we provide novel theoretical insight into cranial neural crest migration and generate a series of experimentally testable hypotheses for future *in vivo* and *in vitro* validation.

Statement of originality / Declaration of generative AI use: I confirm that I have read the University and MPLS divisional guidance on generative AI and have complied with it in preparing this thesis.

The mathematical contributions of this thesis, including the formulation of novel mathematical models, the extension of existing mathematical models from the literature, the analysis and interpretation of model outputs, and the conclusions and inferences drawn from them, are my own, except where explicitly stated otherwise. Where ideas have been drawn from the existing literature, the original work has been cited. Where work has been based on a publication I have authored, this has been highlighted at the beginning of a chapter. All projects contained in this thesis were conceptualised without the use of generative AI.

I used the following generative AI tools in the preparation of this thesis: ChatGPT (OpenAI; GPT-4, GPT-4o, and GPT-5); Google Scholar Labs (Google). No confidential or otherwise sensitive research data were entered into these tools.

I used ChatGPT to debug and refactor code that was written in the preparation of this thesis. All debugged and refactored code was then independently checked. ChatGPT was also used to draft code for plotting data, which was subsequently reviewed and edited as necessary. I take full responsibility for all code used in the generation and visualisation of results in this thesis.

I also used ChatGPT to check spelling and grammar in the thesis, advise on the clarity of exposition and phrasing, and to proofread the final version. ChatGPT was also used to check for notational clashes when integrating work from different sources.

I used Google Scholar Labs to search for additional relevant mathematical and biological literature. All literature identified using Google Scholar Labs that was cited in this thesis was independently read and assessed by me.

List of Abbreviations

ABM Agent-based model

ba1–ba4 Branchial arches 1–4

BMP Bone morphogenetic protein

C3a Complement fragment C3a

C12 Colec12

CD253 Cluster of differentiation 253

CFL Courant–Friedrichs–Lewy

CIL Contact inhibition of locomotion

CNCC Cranial neural crest cell

CoA Co-attraction

Colec12 Collectin-12

CXCR4 CXC chemokine receptor 4

Dan Differential screening-selected gene aberrant in neuroblastoma

DTER Distance-to-energy ratio

ECM Extracellular matrix

EMT Epithelial-to-mesenchymal transition

EphB2 Eph receptor B2

FACS Fluorescence-activated cell sorting

Fc Fragment crystallisable region

LCM Laser-capture microdissection

NBL1 Neuroblastoma suppressor of tumorigenicity 1

Npn2 Neuropilin 2

NRG1 Neuregulin 1

ODE Ordinary differential equation

OV Otic vesicle

PCA Principal component analysis

PDE Partial differential equation

PDGF Platelet-derived growth factor

r1–r7 Rhombomeres 1–7

RNA Ribonucleic acid

Sdf1 Stromal cell-derived factor 1

Sema3A Semaphorin 3A

Sema3F Semaphorin 3F

SNCC Sacral neural crest cell

TNCC Trunk neural crest cell

Trail Tumour necrosis factor-related apoptosis-inducing ligand

VEGF Vascular endothelial growth factor

VNCC Vagal neural crest cell

Contents

Introduction and overview	1
1.1 Collective cell migration in biology	3
1.2 Collective cell migration in the neural crest	5
1.3 Mathematical modelling in the study of neural crest cell migration . . .	12
Collective cell migration in the cranial neural crest	19
2.1 Collective cranial neural crest cell migration in <i>Xenopus</i>	20
2.2 The leader–follower hypothesis	21
2.3 Agent-based models of leader–follower cranial neural crest cell migration	22
2.4 Model pseudocode	30
2.5 Model parametrisation	33
Cranial neural crest cell morphology	34
3.1 The biology of eukaryotic actin-driven protrusions	36
3.2 Mathematical models of eukaryotic actin-driven protrusions	38
3.3 Cranial neural crest cell morphology in chick	39
3.4 Mathematical model formulation	40
3.5 Shallow VEGF gradients drive a transition from bet-hedging to speculative phenotypes	50
3.6 Assessment of model predictions in an agent-based model of cranial neural crest cell migration	54
3.7 Discussion	59
Appendix	61
Cranial neural crest cell confinement during collective migration	65
4.1 The biology of cranial neural crest cell confinement	66
4.2 Incorporating Colec12, Trail, and Dan into an agent-based model of cranial neural crest cell migration	71
4.3 Trail and Colec12 confine cranial neural crest cells to the r4–ba2 path- way during collective migration	79
4.4 Cranial neural crest cell-induced degradation of Dan increases stream coherence through differential speed modulation	85
4.5 Trail and Colec12 expression in CNCC-free zones facilitates the ex- change of cranial neural crest cells between adjacent collectives	90
4.6 Discussion	94
Appendix	100

The spontaneous emergence of leaders and followers during cranial neural crest cell migration	103
5.1 Cranial neural crest cell transcriptomic heterogeneity and plasticity <i>in vivo</i>	105
5.2 Cranial neural crest cell phenotypic plasticity in mathematical models .	108
5.3 A spring–polarisation agent-based model of cranial neural crest cell migration	110
5.4 Collective cranial neural crest cell migration requires balanced intercel- lular adhesion, active polarity-driven forces, and cell-induced VEGF degradation	117
5.5 Leader and follower phenotypes emerge spontaneously in a spring– polarisation agent-based model of cranial neural crest cell migration .	121
5.6 A spring–polarisation agent-based model of cranial neural crest cell migration produces migratory behaviour consistent with experimental perturbations in chick and zebrafish	123
5.7 Discussion	127
Appendix	130
Towards a continuum model of collective cranial neural crest cell migration	135
6.1 Modelling background	137
6.2 A minimal model of cranial neural crest cell migration based on cell–cell adhesion	139
6.3 A polarisation-based continuum model of cranial neural crest cell mi- gration	148
6.4 Discussion	153
Appendix	157
Conclusion	165
7.1 Summary	165
7.2 Open questions and future work	169
7.3 Final remarks	171
Bibliography	172

List of Figures

1.1	Schematic of the neural crest and its axial subpopulations	7
1.2	Schematics of the mechanisms of neural crest migration and mathematical frameworks within which to model neural crest migration . .	13
2.1	Schematics of the leader–follower transcriptional analysis in chick and a corresponding agent-based model of r4–ba2 cranial neural crest cell migration	23
3.1	Schematic of the morphological heterogeneity observed within streams of cranial neural crest cells migrating from r4 to ba2 in chick	40
3.2	Schematic of the DTER model	41
3.3	Cell membrane coordinate system for the DTER model	43
3.4	The effect of VEGF gradient steepness on n_p^{opt} and l_p^{opt} in the DTER model	52
3.5	Heatmaps of the DTER in numerical model simulations of the agent-based model outlined in Chapter 2	57
3.6	Parameter sweep of the DTER model for a steep chemical gradient ($d_{\text{crit}} = 0.5$)	62
3.7	Parameter sweep of the DTER model for an intermediate chemical gradient ($d_{\text{crit}} = 3.5$)	63
3.8	Parameter sweep of the DTER model for a shallow chemical gradient ($d_{\text{crit}} = 6.5$)	64
4.1	Schematics of CNCC migration and r4–ba2 stream confinement in chick	67
4.2	Schematic of the expanded ABM geometry	72
4.3	Colec12 and Trail model representations and schematic for zones of confinement	80
4.4	Original Colec12 model representation confinement results	82
4.5	Revised Colec12 and Trail confinement results	83
4.6	The effect of Dan protein in CNCC migration	88
4.7	Colec12 and Trail cell exchange results	92
5.1	Schematic of the full-transcriptome (14,299 gene) single-cell RNA sequencing analysis and subsequent continuous transcriptomic analysis in chick	106
5.2	Schematics of leader–follower, signal integration, and spring–polarisation agent-based models	110
5.3	Visualisation of an example spring–polarisation model simulation and the results of a numerical model parameter sweep over λ , f_0 , and k_{spring}	119

5.4	Three regimes of collective migration for intermediate values of λ . . .	120
5.5	The onset and evolution of VEGF-induced polarisation in numerical spring-polarisation model simulations	122
5.6	The results of perturbations to numerical model simulations based on prior experiments conducted in chick and zebrafish	124
5.7	Mean stream invasion distance (average x -coordinate of stream cen- troids) and maximum VEGF-induced polarisation as a function of the number of leading cells ablated	134
6.1	Schematic of the adhesion-based continuum model of CNCC migration	140
6.2	Example model simulations and the two regimes of follower transport	145
6.3	Heatmaps of M_F/M_0 for $\chi = 10 \mu\text{m}$, $50 \mu\text{m}$, and $100 \mu\text{m}$ for a range of interaction strengths and corresponding scatter plots of M_F/M_0 as a function of μ_{FL} for fixed $\mu_{FF} = 10 \mu\text{m min}^{-1}$	146
6.4	Simulations of the polarity alignment model at $t = 12 \text{ h}$	154
6.5	Example polarity alignment model simulation for $\chi = 10 \mu\text{m}$ and $\lambda = 10 \text{ min}^{-1}$	155

List of Tables

2.1	Default parameter values for the baseline implementation of the leader– follower agent-based model of cranial neural crest cell migration . . .	33
4.1	Default parameter values for the expanded implementation of the leader–follower agent-based model of cranial neural crest cell migration	76
5.1	Default parameter values for the spring–polarisation agent-based model of cranial neural crest cell migration	131

Chapter 1

Introduction and overview

This thesis is concerned with the construction and analysis of mathematical and computational models of both single and collective cell migration in the cranial neural crest. Cranial neural crest cells (CNCCs) are a highly migratory cell population that originates from the anterior regions of the neural tube during the embryonic development of vertebrates (Kulesa et al., 2010). CNCC migration is both collective and highly directed, with streams of CNCCs migrating towards distal targets in the branchial arches. Upon colonisation of the branchial arches, CNCCs subsequently differentiate, underpinning developmental processes including the formation of bone, cartilage, and connective tissue in the head (Cordero et al., 2011; Minoux and Rijli, 2010). A greater understanding of CNCC migration is, therefore, central to elucidating the origins of congenital disorders associated with abnormal craniofacial development (Bolande, 1974; Sato et al., 2019; Vega-Lopez et al., 2018).

Experiments probing migration in the cranial neural crest are widespread. However, many aspects of CNCC migration remain poorly understood. Although it is clear that CNCCs use cell–cell adhesion (McLennan et al., 2012; Taneyhill, 2008), chemotaxis (McLennan et al., 2010, 2015a), co-attraction (CoA) (Carmona-Fontaine et al., 2011), contact inhibition of locomotion (CIL) (Carmona-Fontaine et al., 2008), and interactions with the extracellular matrix (ECM) (McLennan et al., 2020) to move directedly over large distances, a wealth of questions remain regarding the roles of many genes and biological mechanisms implicated in their migration.

In this thesis, we formulate mathematical and computational models of both single and collective CNCC migration to generate testable predictions regarding the role of multiple genes and biological mechanisms identified by our experimental collaborators as crucial in facilitating coherent and directed CNCC migration. The

predictions generated in this context can then be used to guide future experiments that may further elucidate the biological mechanisms underpinning CNCC migration, provide insights into craniofacial anomalies, and in the long term, identify therapeutic opportunities to minimise costly repair surgeries to correct the effects of aberrant migration.

In this chapter, we begin by reviewing collective cell migration in biology, highlighting the neural crest as a paradigmatic model system in which the emergent collective behaviours of cells can be studied. We then review the current mathematical modelling literature in the context of neural crest cell migration, drawing particular attention to the interplay between mathematical modelling and experiments in uncovering the mechanisms through which neural crest cell migration occurs. In Chapter 2, we focus specifically on the cranial neural crest, discussing its utility as a model system within which to study stream-like collective migration in systems where cells are mitotically quiescent. We also discuss, in depth, a canonical *leader-follower* agent-based model (ABM) of CNCC migration from which much of the work in this thesis is derived. In Chapter 3, we employ linear elasticity theory to construct a novel mathematical model of energy optimisation in CNCC chemotaxis and use it to understand the morphological heterogeneity of cells within streams of migrating CNCCs *in vivo*. Chapter 4 then builds upon the leader-follower ABM discussed in Chapter 2, integrating insights from prior experiments to investigate the mechanisms by which CNCCs remain confined to spatially distinct migratory streams during invasion of the second branchial arch. In Chapter 5, we present a novel *spring-polarisation* ABM that incorporates spring-like pairwise intercellular interactions coupled with an ordinary differential equation (ODE) model of cell polarity, which we employ to examine the spontaneous emergence of leader and follower phenotypes during CNCC migration. Finally, Chapter 6 develops a continuum-level representation of CNCC migration, with a particular emphasis on addressing the limitations of existing continuum mathematical models of cell-cell adhesion. In

Chapter 7, we summarise our findings and highlight future avenues of investigation based on the work conducted in this thesis.

Comment on originality. A version of the material in this chapter and Chapter 2 forms part of an invited book chapter in preparation entitled: *Integrating Mathematical Modeling and Experiments in the Study of Collective Neural Crest Cell Migration*, which was written by the author of this thesis and revised alongside Ruth Baker and Philip Maini. The work will appear in the edited volume *Patterns and Forms in Nature: From Organisms to Collective Behaviours in Biological Systems*, which is to be published by Springer Nature.

1.1 Collective cell migration in biology

Collective cell migration underpins many biological processes such as wound healing (Li et al., 2013), tumour progression (Deisboeck and Couzin, 2009), and development (Schumacher, 2019a). In such processes, the migration of cells is governed not only by environmental factors, but also by cell–cell interactions, leading to emergent cell behaviours and migration as coordinated streams, chains, clumps, and other multicellular patterns (Rørth, 2009). These collective behaviours arise in both epithelial and mesenchymal cell populations; however, the underlying biological mechanisms governing migration differ markedly between these contexts (Theveneau and Mayor, 2013).

Epithelial collectives typically maintain stable cell–cell junctions and migrate as cohesive sheets, as observed during wound healing (Poujade et al., 2007). In contrast, mesenchymal collectives are characterised by more transient cell–cell interactions and a greater degree of individual cell autonomy. However, mesenchymal collectives still exhibit coordinated group behaviours (Theveneau and Mayor, 2013). In this thesis, we focus exclusively on collective mesenchymal cell migration, owing

to its central role in vertebrate embryonic development and the many open questions surrounding its underlying mechanisms.

Studies of collective migration date as far back as the late nineteenth and early twentieth centuries (Barfurth, 1891; Eycleshymer, 1907), though historically, its complex and multi-faceted nature means that in-depth experimental investigations have, until recent decades, been precluded by a lack of appropriate technology. However, with recent advances in experimental methods, including spatial transcriptomics (Moses and Pachter, 2022), single-cell RNA sequencing (Saliba et al., 2014), and high-resolution live imaging (Lemon and McDole, 2020), collective migration has emerged as an increasingly prominent focus of cell biology, complementing longstanding studies of single-cell migration.

Clinically, a greater understanding of collective cell migration may inform the development of novel therapeutic strategies and interventions that target coordinated cellular behaviours, with potential applications in limiting collective tumour invasion during cancer progression (Cheung and Ewald, 2016), promoting efficient epithelial migration during wound repair (Friedl and Gilmour, 2009), and preventing aberrant migratory behaviours during development that give rise to congenital defects (Schumacher, 2019a). In this thesis, we focus on the latter, using mathematical modelling to generate experimentally testable hypotheses that advance our understanding of collective cell migration during embryonic development.

To elucidate the principles of collective cell migration during embryonic development, it is imperative to study a system that both robustly exhibits collective behaviour and permits direct experimental interrogation of model-derived hypotheses. The neural crest is a cell population that satisfies both of these criteria (Bronner and Le Douarin, 2012; Le Douarin and Kalcheim, 1999; Mayor and Theveneau, 2013). The migration of neural crest cells has been studied extensively both *in vitro* and *in vivo*. In addition, mathematical modelling has been widely utilised alongside corresponding experiments to generate novel biological hypotheses and to test

existing ones (Giniūnaitė et al., 2020; Szabó and Mayor, 2016). In light of this, this thesis develops mathematical models of both single and collective neural crest cell migration, with a particular focus on the cranial neural crest, to gain biological insights into the mechanisms governing collective cell migration. In the following sections, we introduce the neural crest more broadly, before restricting our focus to the cranial neural crest in Chapter 2.

1.2 Collective cell migration in the neural crest

The neural crest is a transient cell population that contributes to a vast array of developmental processes and structures (Mayor and Theveneau, 2013). In early embryonic development, the ectoderm-derived neural plate is induced as a precursor of the central nervous system. During neurulation, the neural plate bends, folds, and eventually closes to produce the neural tube (Mayanil, 2013) (Figures 1.1(b)i–iii). Prior to and during closure of the neural tube, cells originally at the borders of the neural plate are specified as neural crest cells. These cells then undergo an epithelial-to-mesenchymal transition (EMT) in which they acquire motile capacity, before migrating collectively towards distant target sites throughout the embryo (Figures 1.1(b)iv–v) (Bronner and Le Douarin, 2012; Burns and Thapar, 2006; Szabó and Mayor, 2018). Upon differentiation, neural crest cells contribute to the development of a diverse array of tissues including pigment cells, neurons and glia of the peripheral nervous system, and craniofacial tissues (Donoghue et al., 2008; Martik and Bronner, 2017).

Neural crest cell migration is tightly regulated by coordinated cell–cell interactions, extracellular guidance cues, and the factors expressed within the environment through which migration occurs (Section 1.2.2). Disruption of neural crest specification, migration, or differentiation can lead to a broad spectrum of congenital disorders collectively termed neurocristopathies (Bolande, 1974; Sato et al., 2019;

Vega-Lopez et al., 2018), which has motivated extensive investigation into the mechanisms governing neural crest development and migration.

1.2.1 Axial subpopulations of the neural crest

The neural crest can broadly be divided into four subpopulations according to the region of the embryo from which delamination occurs: cranial (cephalic), vagal, trunk, and sacral (Figure 1.1(a)). Each of these subpopulations gives rise to distinct derivatives that ultimately contribute to the formation of various structures within the embryo.

1.2.1.1 Cranial neural crest

Cranial neural crest cells (CNCCs) emerge from the dorsal neural tube and the hindbrain-derived populations studied in this thesis migrate collectively towards branchial arches 1–4 (ba1–4) (Kulesa et al., 2010). Upon colonisation of the branchial arches, these CNCCs differentiate and contribute to the development of the embryonic facial structure, including cartilage, bone, cranial ganglia, and connective tissues of the head (Knight and Schilling, 2006). Aberrant CNCC migration can also contribute to craniofacial defects such as cleft palate (Siismets and Hatch, 2020). In Chapter 2, we focus in much greater depth on the collective migration of CNCCs, the mechanisms that underpin their migration, and a suite of ABMs used to study their migratory behaviours.

1.2.1.2 Vagal neural crest

Vagal neural crest cells (VNCCs) originate from somite levels 1–7 and are the primary progenitor population of the enteric nervous system (Gershon et al., 1993; Hutchins et al., 2018). After emerging from the neural tube, VNCCs migrate rostro-caudally along the developing gastrointestinal tract in a coordinated wave (Hutchins

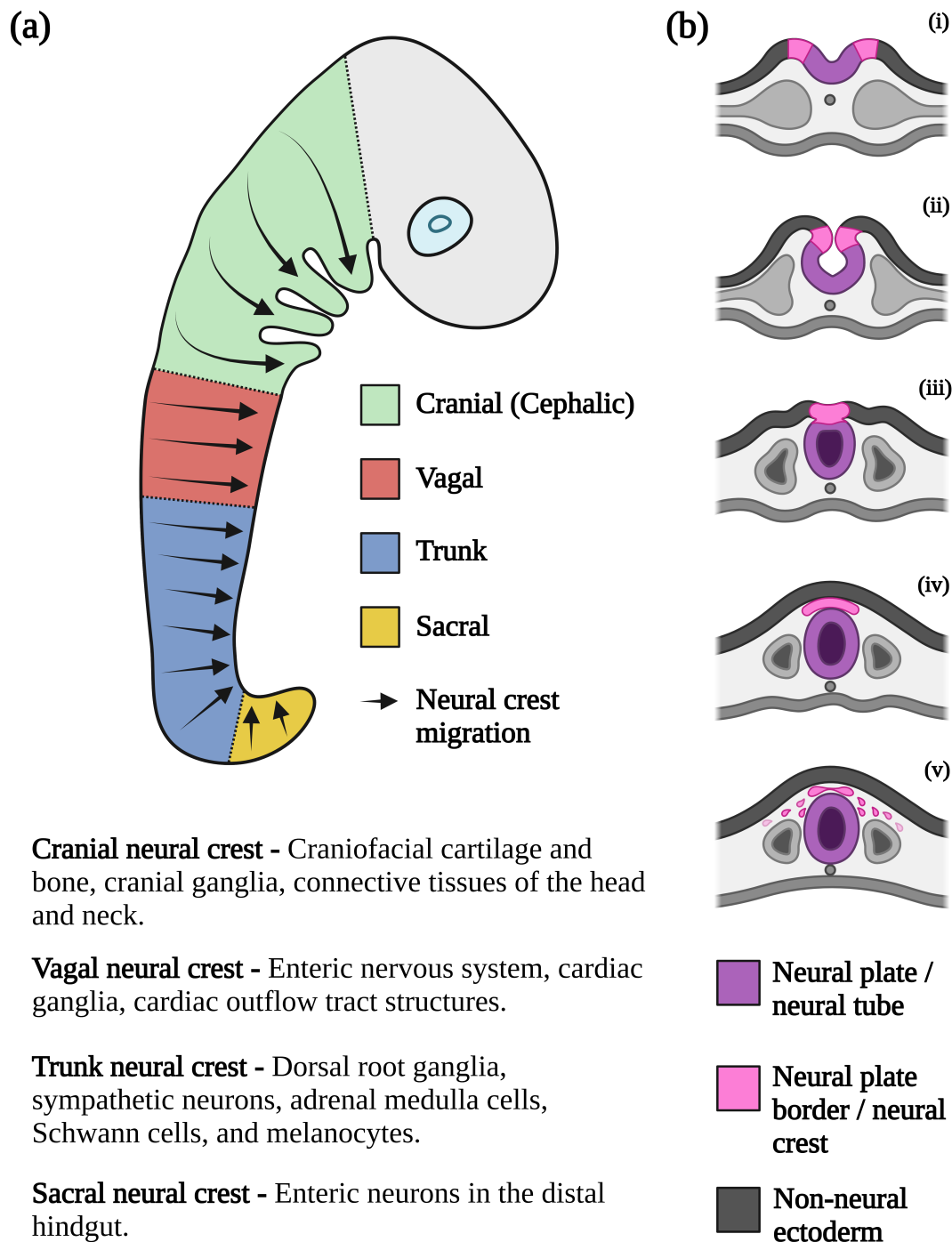


Figure 1.1: **(a)** Schematic of the axial subpopulations of the neural crest and their derivatives. **(b)** Stages of neural crest formation. During stages (i)–(iii), the neural plate (pink/purple) folds to form the neural tube (purple), with neural crest cells specified at the interface between the neural plate and the non-neural ectoderm (dark grey). In stage (iv), neural crest cells are established, undergo EMT, delaminate from the dorsal neural tube, and subsequently migrate along stereotypical pathways to their target tissues in stage (v). Created in BioRender by Samuel Johnson: <https://BioRender.com/rx3t57m>.

et al., 2018; Simpson et al., 2007b). Through continued proliferation and directed migration, VNCCs colonise the gut and subsequently differentiate into neurons and glia that regulate intestinal motility and function (Goyal and Hirano, 1996; Young et al., 2002). Failure of these cells to reach or populate the distal regions of the bowel results in aganglionosis (Butler Tjaden and Trainor, 2013), which most commonly affects the colon. This defect underlies Hirschsprung’s disease, which is a congenital disorder that results in functional intestinal obstruction caused by a lack of enteric ganglia (Montalva et al., 2023). In addition, a spatially restricted subset of the vagal neural crest, termed the cardiac neural crest, colonises the pharyngeal arches and conotruncal region, where it plays essential roles in great vessel patterning and cardiac septation (Kyriakoudi et al., 2024; Stoller and Epstein, 2005).

1.2.1.3 Trunk neural crest

Trunk neural crest cells (TNCCs) arise from the dorsal neural tube between the vagal and sacral regions of the vertebrate embryo (Vega-Lopez et al., 2017). Upon delamination from the neural tube, TNCCs segregate into two principal migratory streams with distinct trajectories and developmental lineages (Gammill and Roffers-Agarwal, 2010). One population migrates dorsolaterally beneath the ectoderm and differentiates into melanocytes that populate the skin. A second population follows a ventral route through the anterior regions of the somites and migrates via the sclerotome to deeper embryonic locations. These ventrally migrating TNCCs give rise to multiple derivatives including peripheral sensory and glial lineages, neurons associated with sympathetic autonomic circuits, and endocrine cells within the adrenal gland. The disruption of TNCC migration or differentiation can lead to congenital disorders affecting pigmentation and peripheral nervous system development. One such condition is Waardenburg syndrome, which is characterised by hypopigmentation and sensorineural deafness resulting from the absence of neural crest-derived melanocytes in the inner ear (Read and Newton, 1997).

1.2.1.4 Sacral neural crest

Sacral neural crest cells (SNCCs) emerge from the caudal-most region of the neural tube and contribute to the innervation of the distal hindgut and pelvic organs in the vertebrate embryo (Wang et al., 2011). SNCCs migrate later than their vagal counterparts and enter the hindgut from the caudal end of the embryo (Nagy and Goldstein, 2017). Within the distal intestine, SNCCs contribute enteric neurons and glia, supplementing the populations established earlier by VNCCs (Burns and Le Douarin, 1998; Burns et al., 2000). In addition to their role in forming the enteric nervous system, SNCCs give rise to the pelvic parasympathetic ganglia that innervate the urogenital system and distal colon (Wang et al., 2011).

1.2.2 Mechanisms of neural crest migration

A broad range of biological mechanisms are implicated in the collective migration of neural crest cells. Before providing a detailed review of the mechanisms underpinning CNCC migration in Chapter 2, here, we briefly review the conserved mechanisms of migration among the axial subpopulations of the neural crest.

1.2.2.1 Chemotaxis

Neural crest cells migrate directedly in response to chemical cues within the tissue through which migration occurs (Shellard and Mayor, 2016). In chick, CNCCs have been shown to migrate up gradients in vascular endothelial growth factor (VEGF), which is also associated with phenotypic transitions in CNCCs (McLennan et al., 2010, 2015a). In *Xenopus* and zebrafish, CNCCs migrate in response to stromal cell-derived factor 1 (Sdf1) signalling via expression of the receptor CXC chemokine receptor 4 (CXCR4) (Olesnick Killian et al., 2009; Theveneau et al., 2010). In *Xenopus*, this interaction gives rise to a *chase-and-run* mechanism, wherein CNCCs migrate up gradients of placode-derived Sdf1 and, upon contact with placodal cells,

undergo heterotypic CIL (Section 1.2.2.2), which causes placodal cells to retreat and thereby sustains the Sdf1 gradient that drives persistent directional migration (Theveneau et al., 2013). In chick, there is also evidence for chemotactic guidance in TNCC populations. Chick TNCCs migrate directedly in response to Neuregulin 1 (NRG1) gradients via ErbB receptors (De Bellard et al., 2018) and a subset of ventrally migrating TNCCs expressing CXCR4 are attracted towards sources of Sdf1, which biases their migration towards sympathetic ganglion targets (Kasemeier-Kulesa et al., 2010).

1.2.2.2 Contact inhibition of locomotion (CIL)

Contact inhibition of locomotion (CIL) is the repolarisation of cells away from sites of cell–cell contact (Stramer and Mayor, 2017). In neural crest cell populations, homotypic CIL is a well-established behaviour both *in vitro* and *in vivo*, where interactions between neighbouring neural crest cells lead to a collapse of protrusions extended at their membrane and a subsequent change in their direction of movement (Carmona-Fontaine et al., 2008). Homotypic CIL contributes to collective polarity and directional migration by reinforcing front-rear polarity following cell–cell collisions (Stramer and Mayor, 2017). Additional studies have shown that CIL is regulated by changes in cell–cell adhesion and polarity, as platelet-derived growth factor (PDGF) signalling controls N-cadherin expression to enable effective CIL in *Xenopus* neural crest cells (Bahm et al., 2017), and the polarity protein Par3 is required for repolarisation and directional migration following cell–cell collisions (Moore et al., 2013). In addition to homotypic interactions, heterotypic CIL has been observed in specific developmental contexts, most notably during CNCC–placodal cell interactions, where it contributes to the *chase-and-run* mechanism discussed in Section 1.2.2.1 (Theveneau et al., 2013).

1.2.2.3 Extracellular matrix guidance

The ECM provides both permissive and restrictive cues that guide neural crest cell migration (Perris and Perissinotto, 2000; Tsang et al., 2010). Neural crest cells preferentially migrate on ECM components such as fibronectin and laminin (Bilozur and Hay, 1988; Perris et al., 1989), which promote cell–substrate adhesion and motility. Other matrix components including Versican act as non-permissive guidance cues that restrict invasion and help pattern migratory streams (Landolt et al., 1995; Perris and Johansson, 1990). TNCCs migrate in spatially separated streams through the anterior sclerotome, which is a pathway enriched by permissive ECM. The posterior sclerotome remains inhibitory, contributing to a stereotyped segmental pattern of migration (Kubota et al., 1999; Loring and Erickson, 1987). In CNCCs, fibronectin-rich substrates support migration through integrin-mediated adhesion. Disruption of ECM-integrin interactions impairs directed movement (Alfandari et al., 2003). Neural crest cells actively remodel their surroundings. For example, matrix metalloproteinase activity has been shown to facilitate neural crest migration by locally modifying ECM composition (Monsonigo-Ornan et al., 2012).

1.2.2.4 Co-attraction (CoA)

In addition to CIL-mediated repulsion, neural crest cells also exhibit CoA that helps to maintain group cohesion during migration (Scarpa and Mayor, 2016). In *Xenopus*, neural crest cells secrete the chemoattractant complement fragment C3a and express its receptor C3aR, which generates short-range cues that attract neighbouring cells and stabilise migrating collectives (Carmona-Fontaine et al., 2011). CoA thus partially offsets the dispersive effects of CIL by driving cells that transiently move away from the group to repolarise back towards it through C3aR-dependent Rac1 activation, which promotes renewed protrusive activity (Carmona-Fontaine et al., 2011). C3a/C3aR signalling is also required for an effective collective response to external chemoattractants such as Sdf1, such that CoA also supports robust directional

chemotaxis at the group level (Carmona-Fontaine et al., 2011). Beyond the cranial neural crest, C3a/C3aR-dependent co-attraction has been implicated in maintaining the cohesion of migrating VNCCs in the embryonic gut (Broders-Bondon et al., 2016).

1.3 Mathematical modelling in the study of neural crest cell migration

Mathematical modelling is an important tool in the study of neural crest migration, enabling both the evaluation of hypotheses derived from experimental observations and the formulation of novel, experimentally testable hypotheses. In this section, we review the existing mathematical modelling literature pertaining to neural crest migration, with a focus on studies in which mathematical modelling and experimentation have been used in tandem to generate novel insights and biological hypotheses. For a more comprehensive overview, we refer the reader to Giniūnaitė et al. (2020).

1.3.1 Agent-based models

ABMs of neural crest cell migration represent cells as discrete, interacting agents. By resolving neural crest collectives at the single-cell level, they are able to capture cell–cell interactions and phenotypic plasticity, enabling the investigation of the emergent behaviours that underpin collective migration.

ABMs encompass a range of modelling frameworks, including Cellular Potts models, where cells occupy multiple lattice sites, on-lattice models, in which cells occupy a single lattice site, and off-lattice models, where cell positions vary continuously within a bounded domain. Each of these approaches offers distinct advantages and limitations. On-lattice models are typically computationally efficient, facilitating the simulation of large cell populations (this is of particular utility in simulating tumour progression, where cell populations are typically of order 10^8 – 10^9

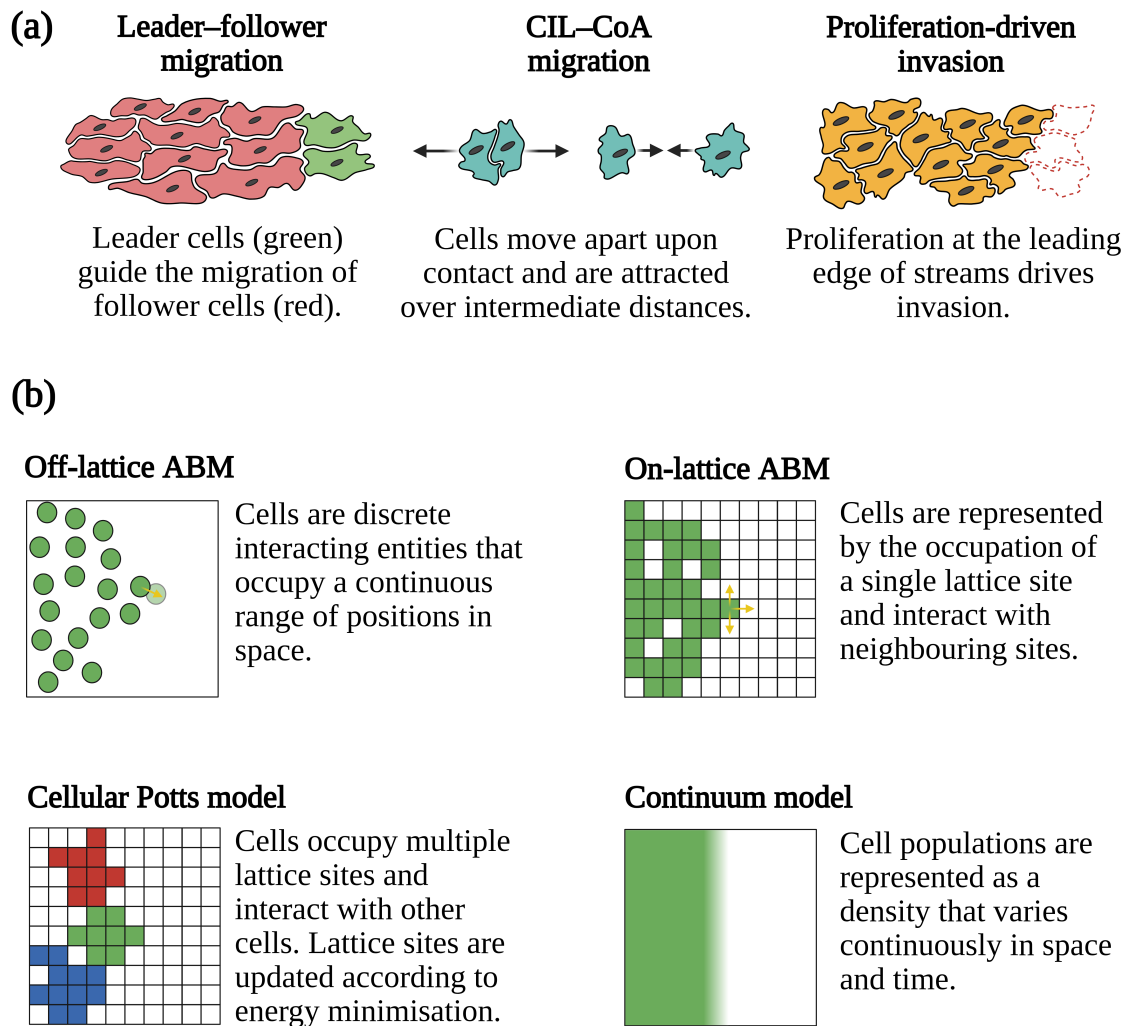


Figure 1.2: **(a)** Schematics of leader–follower, CIL–CoA, and proliferation-driven modes of collective neural crest cell migration. **(b)** Schematics of on-lattice ABMs, off-lattice ABMs, Cellular Potts models, and continuum models of neural crest migration. Created in BioRender by Samuel Johnson: <https://BioRender.com/1jtsruz>.

(Del Monte, 2009; Metzcar et al., 2019)). Conversely, off-lattice models are generally more computationally demanding but allow for more detailed representations of cell–cell interactions and provide a more natural framework for incorporating cell morphology and membrane interactions. Cellular Potts models offer a compromise between accuracy and complexity as a lattice-based framework that can explicitly represent cell shape and motility at the cost of increased computational complexity relative to traditional on-lattice models. In the study of neural crest migration, the number of cells within collectives is much lower than the number of cells in tumours (Morrison et al., 2017; Szabó et al., 2016). This affords a more detailed treatment of cell position without the constraints of computational complexity. As such, it is often the case that mathematical models of neural crest cell migration use an off-lattice framework, allowing cell-specific properties and cell–cell interactions to be studied in depth. A visual summary of each model framework is provided in Figure 1.2(b).

On-lattice ABMs have provided valuable mechanistic insights into neural crest cell migration by enabling the systematic testing of hypotheses that are difficult to disentangle experimentally. An on-lattice model has been used to show that persistent neural crest chains cannot be explained by movement along pre-existing ECM tracks alone, but instead require strong directional bias and coordinated cell–cell contacts to maintain cohesion and persistence (Wynn et al., 2012). Additionally, a more recent hybrid on-lattice biased random walk model incorporating intracellular Rac1 dynamics has demonstrated that collective migration can emerge from the interplay between cell–cell interactions, chemotactic cues, and spatial confinement (Freingruber et al., 2024).

Off-lattice ABMs of neural crest migration have highlighted CIL and CoA as sufficient mechanisms for directed, collective migration in neural crest cell populations. Based on experiments conducted in *Xenopus* and zebrafish that showed that CIL and CoA contribute to directional neural crest migration (Carmona-Fontaine et al.,

2008, 2011), an ABM was used to show that a balance between CIL-driven dispersal and CoA-driven cell–cell attraction leads to the self-organisation of neural crest streams and subsequent collective migration (Carmona-Fontaine et al., 2011) (Figure 1.2(a)). These predictions were supported and extended by a discrete element model parametrised with experimental cell data, which confirmed that both CIL and CoA are required for directional migration and further predicted that co-attraction contributes to stream guidance by preventing cells from invading restrictive regions (Woods et al., 2014). A later Cellular Potts model for neural crest cell migration, based on the CIL–CoA model paradigm, was also used to show that spatial confinement by Versican-rich boundaries *in vivo* enhances the persistence and efficiency of collective migration (Szabó et al., 2016). In addition, a recent off-lattice ABM of neural crest cell migration has predicted that CIL in isolation is a sufficient mechanism for directional collective migration when there is a sufficiently large influx of cells into a channel-like domain. In this context, population pressure paired with CIL-induced dispersal produces persistent, directed collective migration that propels cells along the domain (Khataee et al., 2021).

An off-lattice agent-based chemotactic model of TNCC migration demonstrated that a single long-range chemoattractant is insufficient to reproduce experimentally observed TNCC segregation into sympathetic and dorsal root ganglia (Section 1.2.1.3) (Dyson et al., 2018). In model simulations, robust patterning occurred only when cells responded to multiple, spatio-temporally distinct chemotactic cues along a shared migratory corridor. These results demonstrated that TNCC patterning can arise from heterogeneous chemotactic responses without invoking fixed ECM tracks or leader–follower hierarchies.

A recent two-dimensional off-lattice vertex model, casting neural crest ingression within a non-equilibrium free-energy framework, proposes that ingression can arise spontaneously, driven by myosin-dependent cell shape fluctuations, favourable adhesion energies, and entropic cell packing that together overcome an energy bar-

rier imposed by intercellular adhesion and the basement membrane, with directional cues primarily enhancing robustness rather than initiating ingression (Pasilio et al., 2025).

A force-based off-lattice ABM of neural crest migration has been used to hypothesise that leader neural crest cells (those at the leading edge of migrating streams) may interact with, and remodel, the ECM to prevent jamming during collective migration (Martinson et al., 2023). By modelling neural crest cell migration through a domain in which fibronectin puncta are initially uniformly distributed and isotropically oriented in the ECM, computational model simulations showed that contact guidance and fibronectin remodelling are important mechanisms in sustaining collective migration over biologically relevant distances.

A suite of off-lattice ABMs have also been used extensively in the study of CNCC migration (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2012, 2015a,b, 2017, 2020, 2023). Motivated by transcriptional analysis in the chick embryo (McLennan et al., 2012), these models postulate the existence of a binary leader–follower phenotypic division within streams, wherein leader CNCCs move according to self-induced gradients in VEGF and follower cells move according to cell–cell guidance cues (Figure 1.2(a)). These models have been used to generate insights into many of the mechanisms underlying CNCC migration, including the role of VEGF in inducing phenotypic transitions (McLennan et al., 2015a) and the role of factors expressed in the cranial microenvironment in maintaining separation between adjacent CNCC streams (Johnson et al., 2026b; McLennan et al., 2017, 2023). In Chapter 2, we discuss this class of models in greater depth, as the leader–follower model paradigm of CNCC migration forms the foundations for much of the work in this thesis.

1.3.2 Continuum models

Continuum models of cell migration represent collectives as continuously varying fields, such that the evolution of cell density, rather than individual cell positions, is modelled in time. As a result, these models are generally more mathematically tractable than ABMs, but sacrifice the ability to explicitly resolve individual cell–cell interactions and the dynamics of cell-specific properties within collectives.

A continuum model developed to study collective migration in the vagal neural crest has yielded substantial insights into the mechanisms underpinning the formation of the enteric nervous system in the vertebrate gut (Simpson et al., 2006, 2007b). In this work, a continuum partial differential equation (PDE) model describing the spatio-temporal evolution of cell density, with terms representing diffusion and proliferation, predicted that proliferation at the leading edge of vagal neural crest streams is a key driver of invasion of the gut (Figure 1.2(a)). This prediction was then validated experimentally using chick–quail tissue grafts, which demonstrated that cell proliferation at the leading edge of migrating streams is essential for successful colonisation of the gut (Simpson et al., 2007b). The same modelling framework was later used to generate insights into pathologies such as Hirschsprung’s disease and was also analysed using a related cellular automaton model of cell migration, though not explicitly in the context of the neural crest (Landman et al., 2007; Simpson et al., 2007a).

An additional non-local PDE framework has been employed to investigate the role of non-local repulsive cell–cell interactions, including CIL, in neural crest migration (Painter et al., 2015). In this approach, cell–cell repulsion is represented through a non-local interaction kernel that represents contact-mediated signalling over finite spatial ranges. Numerical simulations of the resulting model, which includes both non-local repulsion and proliferation, demonstrated that repulsive interactions significantly accelerate invasion relative to purely diffusive dynamics. These observations thus provided a mechanistic link between microscale cell–cell interactions and

emergent macroscale migratory behaviours.

Here, we note that all of these continuum representations of neural crest cell migration are predicated on the assumption that cell proliferation plays a role in driving invasion. In contrast, Chapter 6 of this thesis introduces a novel continuum framework for collective migration in the cranial neural crest, wherein robust stream-like invasion arises purely from short-range, directionally unbiased cell-cell interactions, without any reliance on proliferative growth. In doing so, this establishes a distinct class of continuum models for neural crest migration, demonstrating that coordinated large-scale invasion may emerge from interaction-driven dynamics alone.

Chapter 2

Collective cell migration in the cranial neural crest

Having reviewed the neural crest as a whole, we now focus on CNCCs that delaminate from the embryonic hindbrain during embryogenesis. We begin by reviewing experimental studies of collective CNCC migration in *Xenopus* and chick, before focusing on the leader–follower hypothesis derived from transcriptional analyses in chick. Following this, we then review mathematical models of neural crest cell migration and describe, in detail, a canonical ABM of leader–follower migration in chick, from which much of the work in this thesis is derived.

A large body of work studying collective CNCC migration has focused predominantly on *Xenopus* and chick, owing to the relative ease with which these systems may be experimentally perturbed and studied (Kulesa et al., 2004). In both systems, embryos can be cultured externally and are sufficiently large to permit direct experimental manipulation, targeted perturbation of gene function, and high-resolution live imaging of CNCC migration (de Bellard and Bronner-Fraser, 2005; DeSimone et al., 2005). These properties enable the precise spatio-temporal control of experimental interventions, including tissue grafting and explant culture. CNCC populations in both organisms also migrate along well-characterised pathways (Kulesa et al., 2010; Smith et al., 1997), which facilitates reproducible analyses of cell–cell interactions and responses to guidance cues at both the single-cell and collective scales. In addition, the conservation of molecular signalling pathways governing CNCC specification and migration ensures that insights gained from these models are broadly applicable to other vertebrates in which experimental perturbations are less feasible.

CNCCs delaminate from r1–7 of the vertebrate hindbrain following an epithelial-

to-mesenchymal transition (EMT), during which they acquire motility and subsequently migrate collectively towards the branchial arches, ba1–4 (Kulesa et al., 2010). Although numerous mechanisms are implicated in regulating CNCC migration (Section 1.2.2), experimental evidence indicates that their relative contributions vary between model organisms (Sections 2.1 and 2.2).

An important hallmark of collective CNCC migration is that cells are, to a large extent, mitotically quiescent prior to invasion of the branchial arches (Ridenour et al., 2014). Using *in vivo* cell cycle mapping, it has been shown that proliferative activity within the cranial neural crest is low during early migratory phases and increases only after CNCCs colonise the branchial arches (Ridenour et al., 2014). This temporal decoupling of migration and proliferation lies in contrast to that of other neural crest subpopulations in which proliferation plays a central role in driving tissue colonisation and an apparent directional advance. For instance, during enteric nervous system development, proliferation at the migratory wavefront underpins sustained invasion of the gut mesenchyme (Simpson et al., 2007b). The cranial neural crest, therefore, provides a uniquely advantageous system in which collective motility can largely be examined independently of population growth. As such, CNCC migration has emerged as a powerful model paradigm for analysing how coordinated, long-range collective movement can arise primarily from cell–cell and cell–environment interactions (Schumacher et al., 2016; Szabó and Mayor, 2018).

2.1 Collective cranial neural crest cell migration in *Xenopus*

Studies of CNCC migration in *Xenopus* have provided key insights into the cell–cell interactions that underpin collective migration. The prevailing model of collective CNCC migration in *Xenopus* is the contact inhibition of locomotion–co-attraction (CIL–CoA) paradigm (Section 1.2.2.4), in which short-range homotypic CoA pro-

motes cohesion within CNCC collectives, while homotypic CIL induces repolarisation upon collision, thus preventing excessive clustering. However, as CIL and CoA are intrinsically local and isotropic interactions, they are unlikely, in many contexts, to fully account for the robust long-range guidance of CNCC streams to distal targets *in vivo*. In *Xenopus*, additional directional guidance is provided by interactions with neighbouring tissues. In particular, CNCCs migrate chemotactically towards placode-derived Sdf1, and reciprocal CNCC–placode contact triggers heterotypic CIL that displaces placodal cells, yielding a chase-and-run mechanism (Section 1.2.2.1). In this setting, CIL and CoA govern intra-collective cohesion and rearrangements, while chase-and-run provides an external steering cue through CNCC–placode interactions. A parsimonious interpretation is, therefore, that directed cranial neural crest migration in *Xenopus* emerges from the interplay between these processes; placodal Sdf1 biases the net direction of advance, while homotypic CIL and CoA maintain a coherent, motile collective capable of sustained stream-like migration (Carmona-Fontaine et al., 2008, 2011; Olesnick Killian et al., 2009; Scarpa and Mayor, 2016; Theveneau et al., 2013).

2.2 The leader–follower hypothesis

In chick, experimental evidence suggests a different model of collective CNCC migration to that proposed for *Xenopus*. Previous experimental studies have shown that the transcriptome of chick CNCCs varies within streams in a manner correlated with their relative stream positions (McLennan et al., 2012; Morrison et al., 2017) (Figure 2.1(a)). The first of these studies divided streams migrating from the fourth rhombomere (r4) towards the second branchial arch (ba2) into leading and trailing subpopulations according to their distance from r4 (McLennan et al., 2012). Using both laser-capture microdissection (LCM) and fluorescence-activated cell sorting (FACS), 84 genes implicated in CNCC migration were screened across

the two subpopulations. Nineteen of these genes were found to be differentially expressed between leading and trailing CNCC subpopulations. In these experiments, CNCCs in leading positions were found to express genes associated with chemotaxis, ECM remodelling, and ECM attachment at levels higher than cells in trailing populations. Conversely, trailing cells were found to express cadherins distinct from those expressed by leading subpopulations (McLennan et al., 2012) (Figure 2.1).

These observations have been used to infer that collective CNCC migration in chick is facilitated by heterogeneous CNCC behaviours, wherein cells at the leading edge of streams move directedly in response to gradients in VEGF formed in the overlying ectoderm and follower cells use cadherin-mediated cell–cell adhesion to form loosely connected groups that are guided towards the branchial arches by leader cells at the invasive front. This type of leader–follower migration has been well-documented in many other biological systems, including epithelial cell migration (Vishwakarma et al., 2018) and angiogenesis (Chen et al., 2019).

2.3 Agent-based models of leader–follower cranial neural crest cell migration

Mathematical modelling has been widely used to complement experimental investigations of leader–follower CNCC migration in chick, providing a framework in which the proposed mechanisms of migration can be tested for sufficiency and robustness (Giniūnaitė et al., 2020). The majority of existing mathematical models of chick CNCC migration are derived from an ABM originally formulated in tandem with the leader–follower transcriptional analysis of r4–ba2 streams in chick (McLennan et al., 2012). In this modelling framework, CNCCs are assigned either leader or follower phenotypes with distinct behavioural rules (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2015a, 2017, 2020, 2023). Collectively, these ABMs have, in tandem with corresponding experiments, yielded many insights into

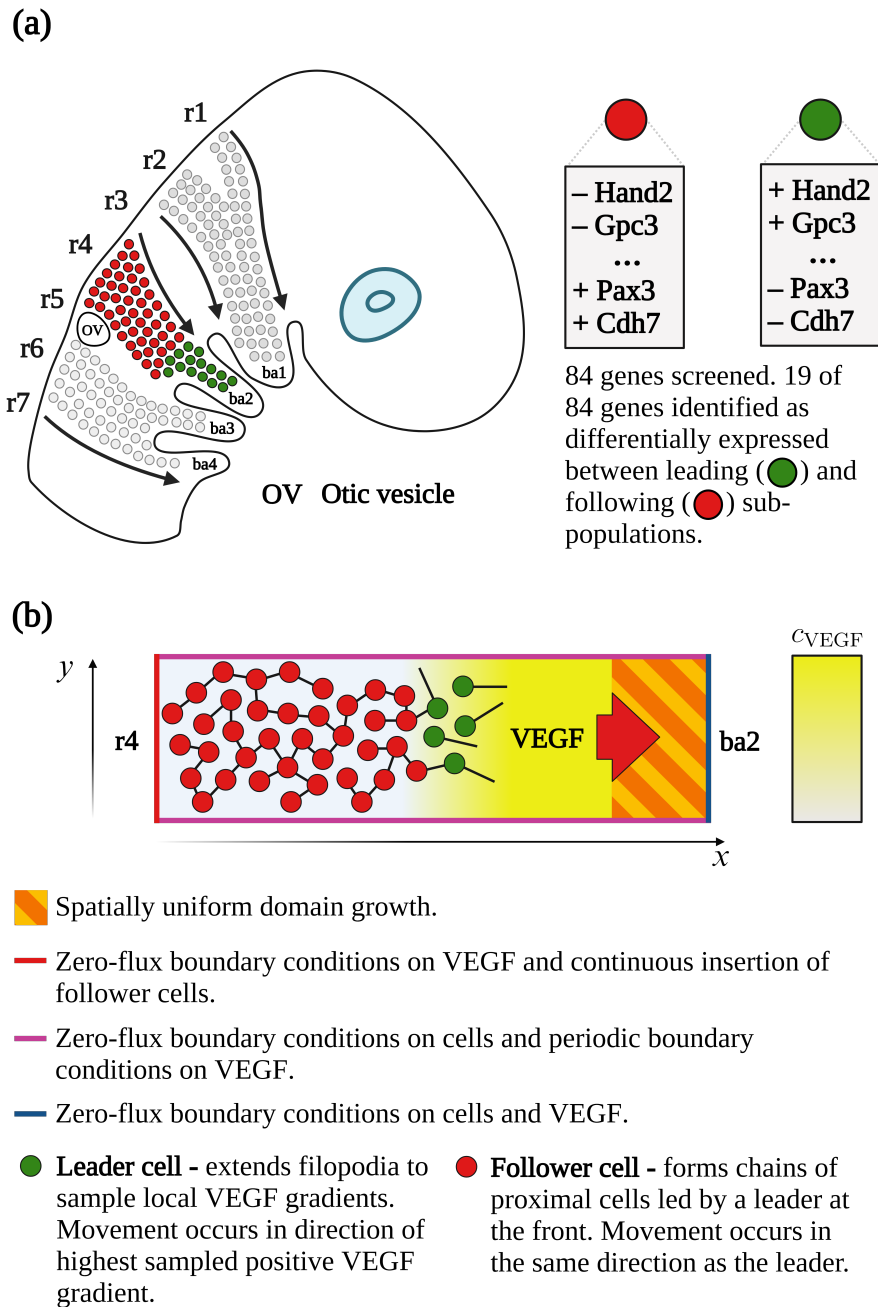


Figure 2.1: (a) Schematic of the leader–follower transcriptional analysis conducted in chick (McLennan et al., 2012). Using both LCM and FACS, CNCCs migrating towards ba2 were found to express genes correlated with their positions in streams. Leading cells (green) were found to express genes associated with VEGF guidance and cell–ECM interactions, whereas trailing cells were found to express genes associated with cell–cell adhesion at higher levels than leading cells. (b) Schematic of the leader–follower ABM of CNCC migration (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2012, 2015a, 2017, 2020, 2023). Leader cells migrate up gradients in VEGF detected by filopodia, while follower cells form cell–cell attachments, producing chains of CNCCs led by a leader at the front of the stream. Created in BioRender by Samuel Johnson: <https://BioRender.com/004nf6n>.

the cell-intrinsic and environmental factors that influence the collective migration of CNCCs. In what follows, we describe one implementation of this model, which forms the basis of subsequent chapters.

2.3.1 Model overview

The ABM for CNCC migration focuses on the migration of CNCCs through a region adjacent to r4, the r4–ba2 pathway (Figure 2.1(b)). The model is a two-dimensional representation of *in vivo* migration through the three-dimensional mesenchyme of the chick embryo, and as such, may be taken to represent the migration of a single layer of CNCCs within a stream. This two-dimensional representation should be interpreted as a dorsal-view projection, or equivalently as a representative planar layer, of a three-dimensional migratory process. Cells are represented as discrete two-dimensional non-overlapping discs whose migration depends on the concentration of VEGF in their neighbourhood.

In vivo, CNCCs migrate along curved trajectories (McKinney et al., 2020). Though the effect of domain curvature on migration is unknown for this ABM, it has recently been shown that for a purely deterministic continuum model, curvature does not significantly impact cell migration (Pollacco et al., 2025). As such, the r4–ba2 pathway is approximated as a two-dimensional rectangle in which VEGF is present everywhere. The r4–ba2 pathway is assumed to grow in length in a spatially uniform manner from approximately $300\ \mu\text{m}$ to $1100\ \mu\text{m}$ over a period of 24 h and its width is a constant value of $120\ \mu\text{m}$ throughout migration, corresponding to the anterior-to-posterior length of r4 in the chick (Section 2.3.2). The assumption of spatially uniform domain growth is made for computational simplicity, though it has been observed that the mesoderm grows non-uniformly in both space and time during chick head morphogenesis (McKinney et al., 2020). Relaxing this model assumption is a subject of future work.

Cells adopt one of two phenotypes, leader or follower. In leader–follower models

of CNCC migration, cells at the leading edge of collectives move up self-induced VEGF gradients sensed by filopodial protrusions. Cells behind the leading edge, where VEGF gradients are not detectable due to the degradation and dilution of VEGF by CNCCs and tissue growth, respectively, instead form chains of cells close to each other, in which all follower cells receive the same directional cues from a leader cell at the front of a chain. In the ABM, a follower cell joins a chain upon the detection of another cell currently in a chain. Once in a chain, follower cells move in the same direction as the leader cell at the front of their respective chain, to create a stream of cells that migrate as a collective. This mechanism is a coarse-grained representation of guidance propagation through a connected group of cells and is an abstraction motivated by *in vivo* evidence that chick CNCCs maintain frequent contacts with other CNCCs during migration. In particular, three-dimensional confocal imaging has shown both local contacts, mediated by lamellipodia or short filopodia over distances below approximately $20\text{ }\mu\text{m}$, and longer-range filopodial contacts of up to approximately $100\text{ }\mu\text{m}$, which can tether non-neighbouring cells and bias cell trajectories (Teddy and Kulesa, 2004). Thus, the model represents long-range stream coordination as the propagation of directional information through local and non-local cell–cell contacts.

In chick, VEGF signalling induces the expression of genes associated with a leader cell phenotype (McLennan et al., 2015a). To reflect this in the ABM, we implement a mechanism of phenotype switching wherein a follower cell that overtakes a leader cell adopts a leader phenotype and the leader cell that is overtaken adopts a follower phenotype, thus ensuring that leader cells remain at the leading edge of collectives where VEGF concentration is highest, on average. This mechanism is a computationally simple abstraction of VEGF-induced phenotype switching that has been used in a prior model of CNCC migration (McLennan et al., 2023) and in many models of CNCC migration produces qualitatively similar migratory profiles to those in which phenotype switching depends on time-averaged VEGF exposure

(McLennan et al., 2015a). This mechanism also fixes the number of leader cells in a simulation, which is another common simplification in models of CNCC migration. In Section 5.2, we discuss phenotypic plasticity in ABMs of CNCC migration in greater depth.

2.3.2 Domain growth

The domain through which CNCCs migrate is modelled as a two-dimensional rectangle of a fixed width ($L_y = 120 \mu\text{m}$) and a length that increases in time. We model the growth of the domain through which CNCCs migrate to be spatially uniform and logistic in time. Our implementation of domain growth is based on prior measurements in the chick cranial neural crest (McLennan et al., 2012). The length of the domain at time t during migration is given by

$$L_x(t) = \frac{L_0}{1 + e^{-k(t-t_0)}} + b, \quad (2.1)$$

where $L_0 = 855.8 \mu\text{m}$, $k = 0.294 \text{ h}^{-1}$, $t_0 = 14.98 \text{ h}$, and $b = 294.3 \mu\text{m}$. Parameter values were obtained by least squares fitting experimental data from the chick (McLennan et al., 2012) to Equation (2.1).

2.3.3 VEGF reaction–diffusion dynamics

The evolution of VEGF concentration is modelled with a reaction–diffusion PDE comprising terms for cell-induced degradation, production within the domain, dilution during domain growth, and diffusion. The concentration of VEGF, $c(x, y, t)$, where $(x, y) \in (0, L_x(t)) \times (0, L_y)$, is initially uniform throughout the domain ($c(x, y, 0) =$

c_0) and evolves according to

$$\underbrace{\frac{\partial c}{\partial t} + \frac{\partial(ac)}{\partial x}}_{\text{(I)}} = \underbrace{D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2}\right)}_{\text{(II)}} - \underbrace{c \sum_{i=1}^{N(t)} \frac{\lambda}{2\pi R^2} \exp\left[-\frac{(x-x_i)^2 + (y-y_i)^2}{2R^2}\right]}_{\text{(III)}} + \underbrace{\kappa c(1-c)}_{\text{(IV)}}, \quad (2.2)$$

where term (I) corresponds to advection and dilution due to growth of the domain, with $a = x\dot{L}_x(t)/L_x(t)$ the velocity field embedding the assumption of spatially uniform growth in the x -direction. Term (II) represents diffusion, (III) is an internalisation term representing the local degradation of chemicals by cells (indexed by i with a total population size $N(t)$), and term (IV) represents the production of the chemical within the domain. In solving Equation (2.2), we re-scale to a domain of unit length $((x, y) \in (0, 1) \times (0, L_y))$ for numerical convenience. The re-scaled equation is given by

$$\underbrace{\frac{\partial c}{\partial t} = D\left(\frac{1}{L_x(t)^2} \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2}\right)}_{\text{(Ia)}} - \underbrace{c \sum_{i=1}^{N(t)} \frac{\lambda}{2\pi R^2} \exp\left[-\frac{L_x(t)^2(x-x_i)^2 + (y-y_i)^2}{2R^2}\right]}_{\text{(IIa)}} + \underbrace{\kappa c(1-c)}_{\text{(IIIa)}} - \underbrace{\frac{c\dot{L}_x(t)}{L_x(t)}}_{\text{(IVa)}}, \quad (2.3)$$

where term (Ia) represents diffusion, term (IIa) represents internalisation by cells, term (IIIa) represents production, and term (IVa) represents dilution due to domain growth; a dot denotes differentiation with respect to time. In simulations, we solve Equation (2.3) using a custom forward-Euler solver implemented in `Python 3`. To approximate the rectangular domain as a representative section of a larger surrounding tissue, in this thesis, we impose periodic boundary conditions on VEGF in the y -direction. At the proximal and distal ends of the r4-ba2 pathway, we impose homogeneous Neumann boundary conditions, so that there is no diffusive flux

of VEGF through the x -boundaries. VEGF is initially of a uniform concentration, $c(x, y, 0) = c_0 = 1$, along the r4–ba2 pathway. Parameter names and values are given in Table 2.1.

2.3.4 VEGF sensing accuracy

Our implementation of the sensing accuracy of VEGF gradients by cells is based on prior work by Berg and Purcell (Berg and Purcell, 1977) and is as described in prior models of chick CNCC migration (McLennan et al., 2017). In the ABM, leader cells extend a fixed number of filopodia at uniformly and independently distributed orientations every timestep. A filopodial protrusion senses a VEGF gradient when

$$\frac{c_f(x, y, t) - c(x, y, t)}{c(x, y, t)} \geq \xi \sqrt{\frac{c_0}{c(x, y, t)}}, \quad (2.4)$$

where $c_f(x, y, t)$ is the average chemical concentration along the filopodium, $c(x, y, t)$ is the chemical concentration at the position of a cell’s current centre, c_0 is the initial uniform concentration of the chemical within the domain, and $\xi > 0$ is a dimensionless parameter that determines the sensitivity of chemotactic gradient detection by cells. If a gradient in VEGF is detected by multiple filopodia, then movement occurs in the direction of the highest detected gradient. Parameter names and values are given in Table 2.1.

2.3.5 Phenotype switching

The implementation of phenotype switching considered here is similar to that of a prior model for CNCC migration (McLennan et al., 2023). *In vivo*, VEGF signalling has been found to induce the expression of genes associated with leader cell behaviour in the chick cranial neural crest (McLennan et al., 2015a). To reflect these observations in the ABM, we approximate this mechanism by fixing the number of

leader cells in each simulation, and updating CNCC phenotypes after movement according to their position in the domain. If, after movement, a follower cell lies one cell length ahead of a leader cell in terms of their distance to r4, their phenotypes are switched, such that the follower cell adopts a leader phenotype and vice versa. This mechanism ensures that CNCCs with a leader phenotype are those which lie in the regions of highest VEGF concentration along the r4–ba2 pathway, on average.

2.3.6 Cell insertion and initial conditions

In chick, CNCCs do not begin migrating immediately upon specification (Theveneau and Mayor, 2012b). Following prior ABMs of chick CNCC migration (Johnson et al., 2026b; McLennan et al., 2017; Schumacher, 2019b), the domain is initially devoid of cells, with no migration occurring for $0 \text{ h} \leq t < 6 \text{ h}$. At $t = 6 \text{ h}$, a small number of leader cells ($n_{\text{lead}} = 5$) is introduced at the left boundary of the domain ($x = 0$), with transverse positions evenly distributed across $y \in [0, L_y]$. Thereafter, follower cells are proposed for insertion at the same boundary at fixed intervals of $1/60 \text{ h}$. Each proposed cell is assigned a transverse position drawn uniformly at random, and insertion is permitted only if there is no overlap with any other cell in the domain.

2.3.7 Leader cell movement

Leader cells migrate chemotactically by sensing local VEGF concentration gradients using filopodial protrusions. At each timestep, leader cells each extend n_{filo} filopodia, each of length $27.5 \mu\text{m}$, at uniformly and independently distributed orientations, before computing the average VEGF concentration along each protrusion. For each filopodium, this average concentration is compared with the VEGF concentration at the corresponding leader cell’s current position. If the relative increase along at least one filopodium exceeds the Berg–Purcell sensing threshold (Equation (2.4)), then the leader cell attempts to move in the direction of the highest detected VEGF gradient. If no filopodium satisfies this criterion, then the leader cell instead un-

dergoes an unbiased random displacement. All proposed movements are subject to volume exclusion. Any displacement that would result in overlap with another cell or movement beyond the domain boundaries is rejected.

2.3.8 Follower cell movement

Follower cells do not sense VEGF gradients directly and instead move by forming transient chains led by a leader cell. At each timestep, free follower cells extend filopodia in n_{filo} random directions. If a filopodium contacts a cell that is already part of a chain, then the follower attaches to that chain and attempts movement in the direction of the cell it is attached to. Once attached, followers move in the same direction as the leader cell at the front, thereby forming elongated chains that propagate directional information through the collective. If a follower moves beyond a maximum interaction distance ($l_{\text{filo}}^{\text{max}}$) from the cell it is attached to, the chain is broken and all downstream followers detach. As in the case of leader cells, follower movement is constrained by volume exclusion and zero-flux boundary conditions at the borders of the domain, with any proposed displacement that would generate cell overlap or movement out of the domain being rejected.

2.4 Model pseudocode

Main ABM loop

```

1   initialise parameters
2   initialise leaders
3   for t from 6 h to 24 h:
4       insert_follower()
5       solve_VEGF()
6       grow_domain()
7       move_cells()

```

Main loop functions

```
1 FUNCTION insert_follower():
2   if t == insertion time and space available:
3     insert follower at LHS of domain (random y coordinate)
4
5 FUNCTION solve_VEGF():
6   solve Eq (2.3) for VEGF profile
7
8 FUNCTION grow_domain():
9   increase domain length, advect cells
10
11 FUNCTION move_cells():
12   for each cell in random order:
13     generate new filopodia directions
14     if cell is a leader:
15       move_leader(cell)
16     elif cell is a follower:
17       move_follower(cell)
18     check_phenotype(cell)
```

Helper functions

```
1 FUNCTION move_leader(cell):
2   if VEGF gradient detected by at least one filopodium:
3     bestVEGF ← direction of largest detected VEGF gradient
4   else:
5     bestVEGF ← random direction
6   move in direction of bestVEGF
7
8 FUNCTION move_follower(cell):
9   if cell is chained:
10    move_chained_follower(cell)
11  else:
12    move_free_follower(cell)
13
14 FUNCTION move_chained_follower(cell):
15   if cell within range of cell it is attached to in chain:
16     move in same direction as leader at front of chain
17  else:
18    detach cell and downstream followers from chain
```

```

19
20 FUNCTION move_free_follower(cell):
21     if within detection distance of chained cell:
22         join chain and move towards cell ahead in the chain
23     else:
24         move in random direction
25
26 FUNCTION check_phenotype(cell):
27     if cell is a follower and further from r4 than any leader by one cell length:
28         switch leader and follower phenotypes

```

Notes on model implementation and simulation logic: In each cell-movement step, proposed displacements have a fixed magnitude of $v_{\text{lead}}\Delta t$ for leader cells and $v_{\text{follow}}\Delta t$ for follower cells, with $\Delta t = 1$ min. This corresponds to approximately $1.04\ \mu\text{m}$ per successful leader cell movement and $1.38\ \mu\text{m}$ per successful follower cell movement. Cells are represented as hard discs of radius $R = 7.5\ \mu\text{m}$, corresponding to a diameter of $15\ \mu\text{m}$. Filopodia sample the environment over a distance $l_{\text{filo}} = 27.5\ \mu\text{m}$, and a follower loses contact with the cell ahead of it when their separation exceeds $l_{\text{filo}}^{\text{max}} = 45\ \mu\text{m}$.

Cell positions are updated sequentially in a random order without replacement at each timestep. This avoids artefacts that may arise from updating cells in a fixed order and provides a simple asynchronous approximation to cell decisions that are not synchronised *in vivo*. Proposed movements are evaluated one cell at a time using the current cell configuration. If a proposed movement would cause overlap with another cell or move the cell outside of the domain, then movement is aborted and the cell remains at its previous position.

The behavioural rules above define one implementation of the baseline leader–follower ABM that underlies much of the work in this thesis. In later chapters, several of these assumptions are relaxed or varied. Chapter 4 modifies cell speed, protrusive behaviour, chemotactic responses, and cell–cell interactions in response to microenvironmental factors, while Chapter 5 replaces the imposed leader–follower

chain-following rule with a spring–polarisation framework in which leader–follower organisation emerges from pairwise interactions and polarity dynamics.

2.5 Model parametrisation

We detail the parameters used in ABM simulations (Table 2.1). All parameter values are based on prior literature values.

	Description	Value	Reference
n_{filo}	directions sampled in one sampling interval	3	(McLennan et al., 2020)
n_{lead}	(fixed) number of leader cells	5	(McLennan et al., 2020)
Δt	simulation time-step	1 min	(McLennan et al., 2017)
R	cell radius (baseline)	$7.5 \mu\text{m}$	(McLennan et al., 2010)
v_{lead}	cell speed (leader cells)	$62.1 \mu\text{m h}^{-1}$	(McLennan et al., 2020)
v_{follow}	cell speed (follower cells)	$82.6 \mu\text{m h}^{-1}$	(McLennan et al., 2020)
L_y	height of domain	$120 \mu\text{m}$	(McLennan et al., 2017)
L_x	length of domain (Eq. (2.1))	$305 \mu\text{m}$ – $1094 \mu\text{m}$	(McLennan et al., 2012)
l_{filo}	cell sensing radius	$27.5 \mu\text{m}$	(McLennan et al., 2017)
$l_{\text{filo}}^{\text{max}}$	maximum separation of cells in contact	$45 \mu\text{m}$	(McLennan et al., 2017)
ξ	chemical sensing accuracy	0.1	(McLennan et al., 2017)
λ	VEGF internalisation rate	$2.75 \times 10^2 \mu\text{m}^2 \text{h}^{-1}$	(McLennan et al., 2017)
κ	VEGF production rate	10^{-4}h^{-1}	(McLennan et al., 2017)
D	VEGF diffusivity	$0.1 \mu\text{m}^2 \text{h}^{-1}$	(McLennan et al., 2017)

Table 2.1: Default parameter values are given. VEGF concentration is given in relative units, such that $c_0 = 1$.

Chapter 3

Cranial neural crest cell morphology

In this chapter, we develop a mathematical model to investigate the morphological variation of CNCCs within streams migrating from r4 to ba2 in chick (Teddy and Kulesa, 2004). *In vivo*, CNCCs at the leading edge of streams extend numerous short actin-based protrusions, including filopodia, in multiple directions along their membrane, whereas CNCCs in trailing positions are more elongated and extend fewer, longer protrusions that are aligned with their direction of migration. Hitherto, these observations have rarely been included in mathematical models of CNCC migration, and the mechanistic basis for this spatial variation in CNCC morphology within streams remains unclear.

To address this question, we develop a single-cell mathematical model that couples the protrusive dynamics of CNCCs to their ability to sense and migrate up gradients of VEGF within the overlying ectoderm of the cranial microenvironment. The model is built on the principle of energetic optimisation in chemotaxis, assuming that CNCCs extend actin-based protrusions in a manner that maximises their expected displacement along VEGF gradients, while accounting for the energetic costs associated with protrusion assembly and subsequent movement through the ECM. Within this framework, we then predict how the optimal number and length of protrusions to extend depend on the local steepness of microenvironmental VEGF gradients, which vary across streams of CNCCs *in vivo* (McLennan et al., 2010, 2015a) and may thus drive the emergence of distinct CNCC morphologies during migration.

In what follows, we begin by reviewing the biology of actin-based protrusions in eukaryotic cells and summarising previous mathematical frameworks used to model

their role in cell motility. We next discuss actin-based protrusions in the migration of CNCCs specifically, before deriving a novel mathematical model that describes their extension during CNCC chemotaxis. This model is then used to predict how a variation in the magnitude of local VEGF gradients may influence the optimal protrusive morphology of CNCCs for efficient migration. Using the ABM introduced in Chapter 2, we also test whether these single-cell predictions scale to the collective migration of CNCC streams *in vivo*. Finally, we discuss the biological implications of our results and propose future experiments to test the validity of the hypotheses generated here.

Comment on originality: Work in this chapter is based on the manuscript:

Johnson, S.W.S., Parsons, M., Baker, R.E. and Maini, P.K., 2025. An energy-based mathematical model of actin-driven protrusions in eukaryotic chemotaxis. *arXiv preprint arXiv:2509.20303*,

which is currently under peer review in *Biophysical Journal*.

Author contribution: The author of this thesis conceived the project and conducted the model formulation and analysis. The manuscript was written by the author of this thesis and revised by Philip Maini and Ruth Baker. Maddy Parsons provided support and guidance on the representation of biological processes and mechanisms within the model.

3.1 The biology of eukaryotic actin-driven protrusions

Eukaryotic cells employ a variety of actin-based membrane protrusions to navigate their surroundings, each distinguished by their morphology and underlying actin architecture (Mitchison and Cramer, 1996). The classification of these actin-based protrusions varies significantly across the literature and is, in general, poorly defined; the term *pseudopod* is often used as an umbrella term for various actin-based protrusions. Of the various types of pseudopod characterised in the literature, *lamellipodia* are perhaps the most clearly defined: broad, sheet-like protrusions composed of densely interwoven actin filaments (Small and Rottner, 2010). Lamellipodia are typically formed at the leading edge of cells and are most easily observed in two-dimensional migration assays in which cell spreading is more pronounced. The primary function of lamellipodia is to form adhesions to the surrounding ECM and to facilitate the movement of cells in the direction of microenvironmental (often chemical) gradients. In contrast, *filopodia* are slender protrusions that act primarily as sensory and exploratory structures for cells, allowing for the detection of external signals (Wood and Martin, 2002). These structures can also be used to form focal adhesions with the ECM that act as anchors to facilitate cell movement. For generality and simplicity, we abstract the various categories of pseudopodia referenced in the literature to consider a general class of protrusions extended at the membrane of cells that are used for both chemical gradient detection and the formation of focal adhesions with the ECM that facilitate movement.

The formation of many cellular protrusions is driven by actin polymerisation (Svitkina, 2018). However, the role of exogenous signals in their formation is a point of controversy in the experimental literature (Insall, 2010). In traditional *chemotactic compass* models, ligand binding at cell surface receptors induces the formation of protrusions by activating intracellular signalling pathways (Van Haastert and De-

vreotes, 2004). Signalling cascades stimulate the polymerisation of actin monomers into filaments that are bound by fascin (Jansen et al., 2011). As these actin filaments work to deform the inner cell membrane, an outward force is generated that leads to the emergence of a protrusion (Le Clainche and Carlier, 2008). In this regime, cells are polarised in the direction of a chemical gradient, as the formation of protrusions is favoured in regions of high chemical concentration where ligand-receptor binding occurs at a higher rate. Conversely, in *pseudopod-centred* models of chemical-induced motility, the initiation of protrusions is independent of external signalling (Insall, 2010). Instead, *de novo* protrusions are formed at random positions along the cell membrane. Once formed, exogenous signalling stabilises, grows, and divides pseudopodia preferentially in regions of high chemical concentration to polarise a cell in the direction of a chemical gradient, as in traditional compass-based models. Experimental evidence exists to support both of these conflicting frameworks. For a review of studies regarding compass- and pseudopod-centred models, we refer the reader to Insall (2010).

Once stabilised, cellular protrusions form focal adhesions to the ECM, anchoring them in place (Stephens et al., 2008). Following this, actomyosin contractions in the cell generate forces that propel the cell body in the direction of pseudopod-ECM focal adhesion sites (Ridley et al., 2003). Concurrently, the disassembly of rear focal adhesions facilitates the directed migration of the cell. Directionality and polarity in the cell are maintained through a combination of signalling pathways and cytoplasmic flow, resulting in a cycle of extension, adhesion, and retraction that facilitates biased motility in the direction of chemical gradients (Swaney et al., 2010).

3.2 Mathematical models of eukaryotic actin-driven protrusions

Extensive experimental work has revealed the complexity of the interplay between the dynamics of actin-based protrusions and environmental parameters. Cells modulate their protrusive activity based on multiple factors, including the mechanical properties of the substrate (Discher et al., 2005; Stroka and Aranda-Espinoza, 2009; Wang et al., 2020; Yeung et al., 2005) and the steepness of the chemical gradient along which they migrate (Andrew and Insall, 2007; Tweedy et al., 2013). However, while studies of this kind have helped to elucidate the environmental factors that influence the protrusive activity of cells, it is not clear mechanistically how these factors drive such morphological changes.

To move beyond associative relationships and probe the mechanistic basis of these phenomena, mathematical modelling offers a valuable complement to experimental methods. Mathematical models of cellular protrusions and motility span scales from single-filament dynamics to whole-cell behaviour (see Carlsson and Sept (2008), Holmes and Edelstein-Keshet (2012), and Danuser et al. (2013)). Foundational studies established the Brownian ratchet framework to explain how actin polymerisation generates protrusive forces at the cell membrane (Mogilner and Oster, 1996; Peskin et al., 1993). More recent three-dimensional and multiscale approaches have extended these foundations. For example, *in silico* models of mesenchymal cell chemotaxis couple cell–matrix interactions and chemosensing to study migration in three-dimensional environments (Ribeiro et al., 2017) and a Cellular Potts model integrates molecular-level actin assembly with cell mechanics to reproduce observed cell morphologies and migratory persistence (Marée et al., 2006). In recent studies, mathematical models of chemotaxis have been used to predict pseudopod splitting strategies in shallow gradients and optimal morphologies for chemotactic gradient detection (Alonso et al., 2025; Mou and Cao, 2025). However, no general mathemat-

ical framework has yet been constructed to study the energy-information trade-off in actin-based protrusions across a range of biological contexts and systems.

3.3 Cranial neural crest cell morphology in chick

In vivo, CNCCs generate actin-driven protrusions such as filopodia during migration (Genuth et al., 2018; Teddy and Kulesa, 2004). These protrusions are believed to facilitate the sensing of microenvironmental cues, including VEGF gradients that direct CNCC movement towards *ba2* (McLennan et al., 2015a). Within migrating CNCC streams, the spatial distribution and organisation of these protrusions vary markedly. Cells at the leading edge of streams typically extend numerous short protrusions oriented in multiple directions, whereas trailing cells extend fewer, longer protrusions that are typically aligned with their axis of migration (Teddy and Kulesa, 2004) (Figure 3.1). The mechanisms underlying this morphological heterogeneity remain unclear; however, one possibility is that the spatial heterogeneity of VEGF concentration across CNCC streams drives a differential response in actin polymerisation between CNCCs exposed to large VEGF gradients, where chemotaxis contributes substantially to migration, and in shallow gradients, where other mechanisms of movement likely play a larger role in migration.

To investigate this observed morphological heterogeneity, we develop a mathematical framework that quantifies chemotactic efficiency as a function of cell morphology, defined by the number, length, and orientation of actin-based protrusions extended by CNCCs. By analysing how the energetically optimal protrusive phenotype varies with the local VEGF landscape, this framework provides a means to assess whether energetic efficiency during migration can account for the morphological patterning exhibited by CNCC collectives in chick.

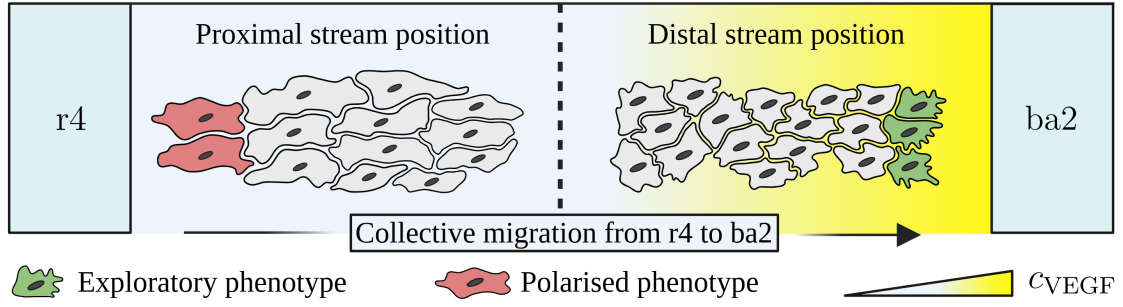


Figure 3.1: Schematic of the morphological heterogeneity observed within streams of CNCCs migrating from r4 to ba2 in chick. At the leading edge of streams, where gradients in VEGF are typically large, CNCCs extend many short protrusions around their membrane. In trailing stream positions, where gradients in VEGF are much shallower, CNCCs elongate in their direction of migration (Teddy and Kulesa, 2004). Created in BioRender by Samuel Johnson: <https://BioRender.com/lelu10g>.

3.4 Mathematical model formulation

In this section, we formulate a mathematical model for the optimal cell morphology in chemotactic movement. The model is agnostic to the type of eukaryotic cell considered, but in later sections, we will analyse this model in the context of CNCC movement to make cranial neural crest-specific inferences regarding the relationship between local VEGF landscapes and CNCC morphology during their invasion of ba2.

We consider a cell of radius l_0 in the two-dimensional plane and represent its membrane as a closed one-dimensional elastic loop (Hochmuth, 2000; Pontes et al., 2013). We wish to study the energy-motility trade-off in extending actin-rich protrusions at the cell membrane during chemotactic movement. In doing so, we begin by using linear elasticity theory to derive expressions for the energetic costs of cell movement and protrusion formation, before constructing a simple two-dimensional model for chemotaxis, in which we relate the number and length of protrusions extended to the probability of chemical gradient detection and subsequent movement (Figure 3.2). In the following derivation, we assume that cell movement occurs up concentration gradients, though the model is trivially adapted to consider

the converse.

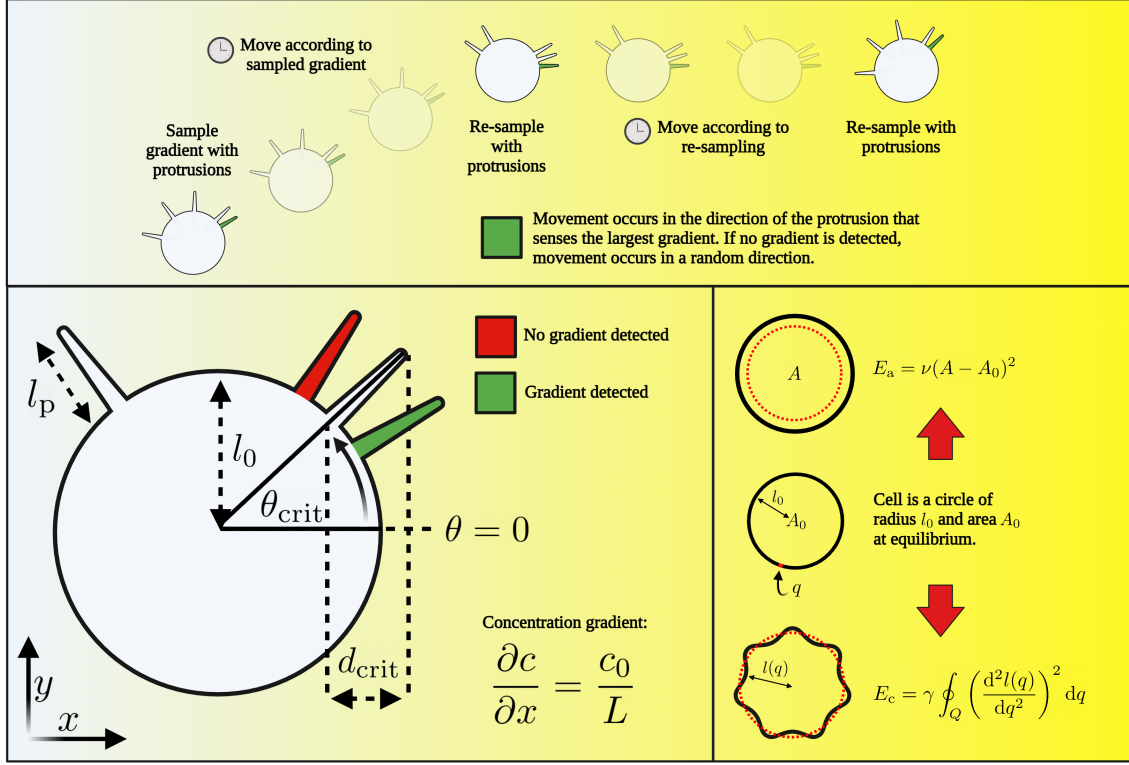


Figure 3.2: Schematic of the two-dimensional chemotaxis model and the energetic costs of protrusion extension. In the two-dimensional model of chemotaxis, a cell, initially of radius l_0 , samples its environment with n_p protrusions at a discrete number of time points (upper panel). Each protrusion is of length l_p , and protrusions are oriented at angles $\{\theta_i\}$, sampled independently from an underlying distribution, $f(\theta)$, for $\theta \in [0, \pi]$ (neglecting the lower half plane due to symmetry). If the smallest of the angles sampled, $\theta_{\min}$, satisfies $\theta_{\min} \leq \theta_{\text{crit}}(l_p)$, then movement occurs in the direction of the protrusion extended at $\theta_{\min}$. If this condition is not met, then the cell moves in a random direction, such that its expected distance moved along the chemical gradient is zero. The energetic costs of membrane deformation are partitioned into an energy cost due to area change in the cell and an energy cost due to increased membrane curvature, such that all possible perturbations on the circle within the two-dimensional plane are penalised. Created in BioRender by Samuel Johnson: <https://BioRender.com/8n29zcm>.

3.4.1 Energetic cost of cell movement

We begin by calculating the energetic cost of movement for a cell. For simplicity, we assume that the actin-based protrusions extended by a cell are all of equal length (l_p , say) and that they are extended instantaneously, without incurring an increased

energetic cost of movement (and hence, that the area of protrusions is small relative to the equilibrium area of the cell). We also assume a microenvironment of constant resistance (for a given value of substrate stiffness, β), such that the energy expenditure per unit distance moved is constant within the domain. In this context, energy expenditure due to cell movement upon the extension of protrusions may be quantified as

$$E_m = \alpha_m \beta s, \quad (3.1)$$

where s denotes the total distance migrated between protrusion extension and retraction, and α_m is the energetic cost of moving a unit length in a reference domain with $\beta = 1$. This formulation provides a coarse-grained representation of the mechanical work expended during cell motility; actin-based protrusions are extended and form focal adhesions with the ECM. Subsequently, actomyosin contractility generates traction forces that pull the cell body forward, while rear adhesions disengage which results in a net displacement, s . Equation (3.1) represents the corresponding energetic cost as the work performed against an environmental resistance, β , which aggregates adhesive dissipation and steric hindrance. This yields an energy expenditure that scales linearly with the distance travelled.

Here, we emphasise that the model derived in this section is a two-dimensional representation of three-dimensional migration through a microenvironment of constant resistance. By contrast, for chemotaxis *on* a substrate, where the substrate offers negligible resistance to motion, the energetic cost in Equation (3.1) would be effectively zero.

3.4.2 Energetic cost of membrane deformation

In calculating the energetic cost of protrusion extension, we adopt a framework similar to that of Ryan et al. (2017) and assume that the energetic cost of extending

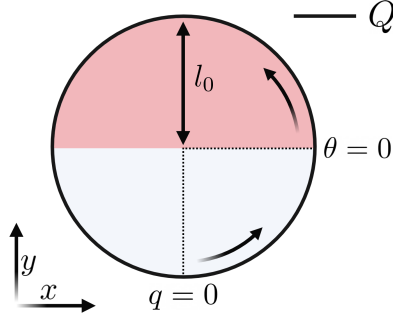


Figure 3.3: Schematic of the cell membrane coordinate system. $\theta = 0$ denotes the positive x -direction and corresponds to the point $q = \pi l_0/2$ on the membrane, Q . Protrusions are formed in the upper half-plane (shaded red region) to prevent discontinuities at the endpoints of the membrane, $q = 0, \mathcal{P}$. Created in BioRender by Samuel Johnson: <https://BioRender.com/u0dpscy>.

actin-based protrusions can be decomposed into a cost due to the area change of a cell and a cost due to bending of the cell membrane. At equilibrium, we assume that the outward force from actin polymerisation inside the cell is balanced by a tensile force from the membrane. From force balance, we then calculate the energy expended in actin polymerisation as the energy to deform the cell membrane.

The cell membrane, initially assumed to be circular with an equilibrium radius l_0 , is parametrised by a coordinate $q \in [0, \mathcal{P}]$, where $\mathcal{P} = 2\pi l_0$ is the initial circumference of the membrane. The coordinate q relates directly to the angular orientation θ around the cell, and is measured anticlockwise from a reference direction (chosen as $\theta = 3\pi/2$ at the bottom of the cell). Thus, we have

$$\theta = \left(\frac{q}{l_0} + \frac{3\pi}{2} \right) \bmod 2\pi. \quad (3.2)$$

To prevent discontinuities at the domain boundaries ($q = 0$ and $q = \mathcal{P}$), we select this convention so that protrusions are formed away from $q = 0$ and $q = \mathcal{P}$ (Figure 3.3).

When the cell membrane is deformed by extending a protrusion, its local radial curvature deviation from a circular reference shape, C , at a point q_0 , is given by

$$C(q_0) = \left. \frac{d^2 l(q)}{dq^2} \right|_{q=q_0}, \quad (3.3)$$

where $l(q) = l_0 + l_p(q)$ is the radial distance from the cell centre to point q on the membrane. We assume that protrusions are sufficiently small, allowing local segments of the curved membrane to be approximated as linear. This simplification justifies taking derivatives directly with respect to the membrane coordinate, q , without explicitly including higher-order curvature corrections.

Under these assumptions, the potential energy associated with bending the membrane is defined as

$$E_c = \gamma \oint_Q \left(\frac{d^2 l(q)}{dq^2} \right)^2 dq, \quad (3.4)$$

where Q is the cell membrane and γ is an elastic constant corresponding to the membrane bending modulus (Miura, 2020).

For analytical simplicity, we model protrusions as Gaussian in shape, so that

$$l(q) = l_0 + l_p \exp \left(- \left(\frac{q - q_c}{w_p} \right)^2 \right), \quad (3.5)$$

where l_p and w_p represent the characteristic protrusion length and width, respectively, and q_c denotes the central location of the protrusion along the membrane.

Calculating the second derivative of $l(q)$ with respect to q indicates that the curvature induced by a protrusion scales linearly with the protrusion length l_p . Consequently, the bending energy per protrusion scales as $E_c \sim l_p^2$. Furthermore, for well-spaced protrusions of equal length and width, the total bending energy increases linearly with the number of protrusions. Combining these scaling relations yields the total energetic cost associated with membrane bending due to n_p Gaussian protrusions, each of length l_p , as

$$E_c = \alpha_c n_p l_p^2, \quad (3.6)$$

where α_c represents the energy required to extend a single protrusion of unit length.

3.4.3 Energetic cost of membrane expansion

In calculating the energetic cost due to the change in area of a cell upon the extension of actin-based protrusions, we consider an expression that penalises both an increase and decrease in cell area relative to its equilibrium value

$$E_a = \nu(A - A_0)^2, \quad (3.7)$$

where A is the area of a cell after the extension of protrusions, A_0 is the baseline area of the cell (its area when no protrusions are extended), and ν is an elastic constant of expansion and contraction associated with membrane tension. Here, we have assumed the energetic cost to scale quadratically with an increase or decrease in cell area (and hence, cell strain to be sufficiently small that the assumption of linear elasticity holds (Hochmuth, 2000; Pontes et al., 2013)).

When a cell extends a protrusion of a fixed length and width, a constant value is added to the area of the cell (A_ϵ , say). We assume that when n_p protrusions of equal size are extended, the corresponding increase in area is given by $n_p A_\epsilon$. The energetic cost resulting from the area change in a cell after the extension of n_p protrusions of equal size, therefore, scales as $E_a \sim n_p^2$. The area of a Gaussian protrusion scales linearly with its length (for a fixed characteristic width). Consequently, the energetic cost of the area change in a cell also scales quadratically with the length of the protrusions; $E_a \sim l_p^2$.

Combining the scaling relations for n_p and l_p , the total energetic cost of extending n_p protrusions, each of length l_p , is

$$E_a = \alpha_a n_p^2 l_p^2, \quad (3.8)$$

where α_a is the energetic cost due to cell expansion upon extending a single protru-

sion of unit length.

3.4.4 Total energetic cost of chemical-induced cell motility

Combining Equations (3.1), (3.6), and (3.8), we obtain an expression for the total energy required for a cell to extend n_p protrusions, each of length l_p , and move a distance s in an ECM of stiffness β as

$$E = E_c + E_a + E_m = \alpha_c n_p l_p^2 + \alpha_a n_p^2 l_p^2 + \alpha_m \beta s. \quad (3.9)$$

3.4.5 Expected distance moved in chemical-induced cell motility

We now proceed to formulate a simple two-dimensional representation of eukaryotic chemotaxis to calculate the expected distance moved via chemotaxis as a function of n_p and l_p (Figure 3.2). In defining a chemoattractant landscape, we initially assume that, within the two-dimensional plane, the concentration of the chemical driving movement increases linearly with respect to x and with no dependency on y , such that $c(x, y) = c_0 x/L$. Limits on the accuracy with which cells can detect environmental gradients are well-studied (Berg and Purcell, 1977). As such, we also assume the existence of a threshold distance in the positive x -direction, d_{crit} , which is the minimum distance between the base and the tip of a protrusion that must be spanned in the positive x -direction for a chemical gradient to be detected (in Figure 3.2, the green protrusion meets this condition, whereas the red protrusion does not). From the model geometry, d_{crit} , therefore, imposes a minimum concentration difference of $d_{\text{crit}} c_0/L$ between the base and the tip of a protrusion, for a gradient to be detectable. The notion of a critical distance, d_{crit} , also affords the definition of a corresponding angle, $\theta_{\text{crit}} = \cos^{-1}(d_{\text{crit}}/l_p) \in [0, \pi/2]$, which depends on the length of a protrusion, l_p , and determines the maximum angle at which

a protrusion can be extended such that a gradient is detected (note here that if $l_p < d_{\text{crit}}$, then no such angle can be defined, meaning that a gradient is never detectable). We also assume that protrusions are extended perpendicular to the cell membrane and that the angles at which protrusions are extended are sampled independently from an underlying distribution, $f(\theta)$, for $\theta \in [0, \pi]$, where the lower half plane $\theta \in (\pi, 2\pi)$ is neglected due to up-down symmetry. Here, an angle of $\theta = 0$ corresponds to the extension of a protrusion in the positive x -direction, while an angle of $\theta = \pi/2$ corresponds to the extension of a protrusion in the positive y direction. When a cell extends n_p protrusions, we assume that all protrusions are the same length and are instantaneously extended in unison (the relaxation of the assumption of equal length across protrusions is the subject of future work within the same framework). Furthermore, we assume that a cell moves in the direction of the protrusion that senses the highest environmental concentration gradient (from the geometry of the system, this corresponds to the protrusion extended at the angle closest to $\theta = 0$). If a gradient is not detected due to no protrusion spanning a sufficient distance in the positive x -direction, then we assume that the cell moves in a random direction. We now calculate the expected distance moved by a cell upon the extension of n_p protrusions of length l_p .

To calculate the probability of gradient detection in cell movement (the probability that at least one protrusion is extended at an angle lower than θ_{crit} in Figure 3.2), we derive the probability density function of the minimum angle of n_p samples from the distribution $f(\theta)$. For n_p independent samples from this distribution, the probability that all angles are greater than a value θ is given by

$$\left(1 - \int_0^\theta f(\theta') d\theta'\right)^{n_p}. \quad (3.10)$$

From this, the cumulative distribution function of the minimum of these n_p samples is given by

$$P(\theta) = 1 - \left(1 - \int_0^\theta f(\theta') d\theta'\right)^{n_p}. \quad (3.11)$$

Differentiating Equation (3.11) with respect to θ , the probability density function of the minimum angle sampled is

$$p(\theta) = n_p f(\theta) \left(1 - \int_0^\theta f(\theta') d\theta'\right)^{n_p-1}. \quad (3.12)$$

From this expression, we obtain the probability of gradient detection upon the extension of n_p protrusions of length l_p as

$$\int_0^{\theta_{\text{crit}}} p(\theta'') d\theta'' = n_p \int_0^{\theta_{\text{crit}}} f(\theta'') \left(1 - \int_0^{\theta''} f(\theta') d\theta'\right)^{n_p-1} d\theta'', \quad (3.13)$$

where θ_{crit} is the maximum angle for which a protrusion of length l_p can be extended such that a gradient is detected:

$$\theta_{\text{crit}} = \cos^{-1} \left(\frac{d_{\text{crit}}}{l_p} \right). \quad (3.14)$$

We now calculate the expected distance travelled by a cell upon the extension of n_p protrusions of length l_p . If the smallest of the n_p angles sampled, θ_{min} , satisfies $\theta_{\text{min}} \leq \theta_{\text{crit}}$, then the distance moved by a cell in the positive x -direction (the direction of the environmental gradient) is given by

$$s \cos(\theta_{\text{min}}), \quad (3.15)$$

where s is the distance moved by the cell upon the extension of protrusions. If the minimum orientation of n_p protrusions does not meet the threshold for gradient detection ($\theta_{\text{min}} > \theta_{\text{crit}}$), then movement is unbiased, and hence, vanishes under expectation. Consequently, the expected distance travelled by a cell in the direction of an environmental gradient upon extending n_p protrusions, each of length l_p , is given by

$$sn_p \int_0^{\theta_{\text{crit}}} f(\theta'') \cos(\theta'') \left(1 - \int_0^{\theta''} f(\theta') d\theta' \right)^{n_p-1} d\theta'', \quad (3.16)$$

where we have restricted the integral to gradient-induced movement by integrating only from 0 to θ_{crit} .

A final consideration in the expression for distance moved is the effect of substrate stiffness and organisation on the speed at which cells migrate, and hence on the distance traversed between protrusion extension and retraction. In the model, we characterise the ECM by its stiffness, β , and by a parameter we refer to as the *topographical permissibility*, ϕ . For a given value of β , ϕ quantifies how permissive the ECM is to cell migration by capturing geometric and topological features of the matrix that are not encoded in stiffness alone, such as pore size and matrix connectivity. Additionally, ϕ could represent the degree to which fibres in the ECM are aligned. However, we do not consider this possibility here, as this would introduce a coupling between the direction of cell movement and the distance travelled per unit time. As such, we assume that the ECM is isotropic and focus specifically on how a variation in the parameter d_{crit} influences the optimal values of l_p and n_p during migration.

We now define a function, $g(\beta, \phi)$, that modulates the distance moved by a cell between protrusion extension and retraction, and which depends on both the stiffness and topographical permissibility of the surrounding ECM. Given the function $g(\beta, \phi)$, Equation (3.16) can be modified to account for substrate stiffness and permissibility as

$$g(\beta, \phi) sn_p \int_0^{\theta_{\text{crit}}} f(\theta'') \cos(\theta'') \left(1 - \int_0^{\theta''} f(\theta') d\theta' \right)^{n_p-1} d\theta''. \quad (3.17)$$

At this point, we do not prescribe a functional form for $g(\beta, \phi)$, though in subsequent sections, we make a phenomenological selection for $g(\beta, \phi)$ to study how substrate stiffness and topography affect protrusive activity in a range of biological

scenarios.

3.4.6 Final expression for the expected distance travelled per unit energy expended in environmental sensing and cell movement

Combining Equations (3.9) and (3.17), we arrive at an expression for the expected distance, d_x , travelled up a fixed chemical gradient in chemotaxis per unit energy (E) expended by a cell upon the extension of n_p protrusions, each of length l_p , as

$$\frac{\mathbb{E}(d_x)}{E} = \frac{g(\beta, \phi) s n_p \int_0^{\theta_{\text{crit}}} f(\theta'') \cos(\theta'') \left(1 - \int_0^{\theta''} f(\theta') d\theta' \right)^{n_p-1} d\theta''}{\alpha_c n_p l_p^2 + \alpha_a n_p^2 l_p^2 + \alpha_m \beta g(\beta, \phi) s}. \quad (3.18)$$

Without loss of generality, Equation (3.18) may be simplified by fixing $\alpha_c = 1$ and rescaling all other terms, such that

$$\frac{\mathbb{E}(d_x)}{E} = \frac{g(\beta, \phi) s n_p \int_0^{\theta_{\text{crit}}} f(\theta'') \cos(\theta'') \left(1 - \int_0^{\theta''} f(\theta') d\theta' \right)^{n_p-1} d\theta''}{n_p l_p^2 + \alpha_a n_p^2 l_p^2 + \alpha_m \beta g(\beta, \phi) s}. \quad (3.19)$$

We henceforth refer to Equation (3.19) as the distance-to-energy ratio (DTER) of cell movement.

3.5 Shallow VEGF gradients drive a transition from bet-hedging to speculative phenotypes

In this section, we study the effect of varying d_{crit} in Equation (3.19) on the optimal values of n_p and l_p during chemotactic movement. The parameter d_{crit} is inversely

related to the steepness of the chemical gradient in the domain, with higher threshold distances representing a shallower chemical gradient as protrusions must span a larger distance for a gradient to be detected.

In analysing the effect of d_{crit} on the optimal protrusive phenotype, we assume that $f(\theta)$, the probability distribution that governs the orientation of the protrusions extended at the cell membrane, is the uniform distribution $U[0, \pi]$, such that

$$f(\theta) = \frac{1}{\pi}. \quad (3.20)$$

This form of $f(\theta)$ represents a pseudopod-centred view of chemotaxis, where the generation of actin-driven protrusions is independent of the environmental chemical landscape within which a cell lies.

In selecting $g(\beta, \phi)$, we choose a phenomenological expression that penalises movement in substrates of extreme stiffness, and for which the topographical permissibility, ϕ , linearly increases the distance moved by a cell per unit of time. In very soft substrates, cells cannot generate sufficient traction forces for movement (Zaman et al., 2006). In contrast, in substrates of very high stiffness, cell motility is impeded by the physical barrier of the dense substrate (Wolf et al., 2013). A functional form of $g(\beta, \phi)$ that satisfies these constraints is

$$g(\beta, \phi) = \frac{\phi\beta}{k} \exp\left(-\left(\frac{\beta}{k}\right)^2\right), \quad (3.21)$$

where k is a constant that determines the peak of the function $g(\beta, \phi)$ at an intermediate substrate stiffness of $\beta = k/\sqrt{2}$. With these two assumptions, Equation (3.19) becomes

$$\frac{\mathbb{E}(d_x)}{E} = \frac{\phi\beta s n_p}{k\pi} \frac{\exp\left(-\left(\frac{\beta}{k}\right)^2\right) \int_0^{\theta_{\text{crit}}} \cos(\theta'') \left(1 - \frac{\theta''}{\pi}\right)^{n_p-1} d\theta''}{n_p l_p^2 + \alpha_a n_p^2 l_p^2 + \alpha_m \beta \left(\frac{\phi\beta}{k} \exp\left(-\left(\frac{\beta}{k}\right)^2\right)\right) s}. \quad (3.22)$$

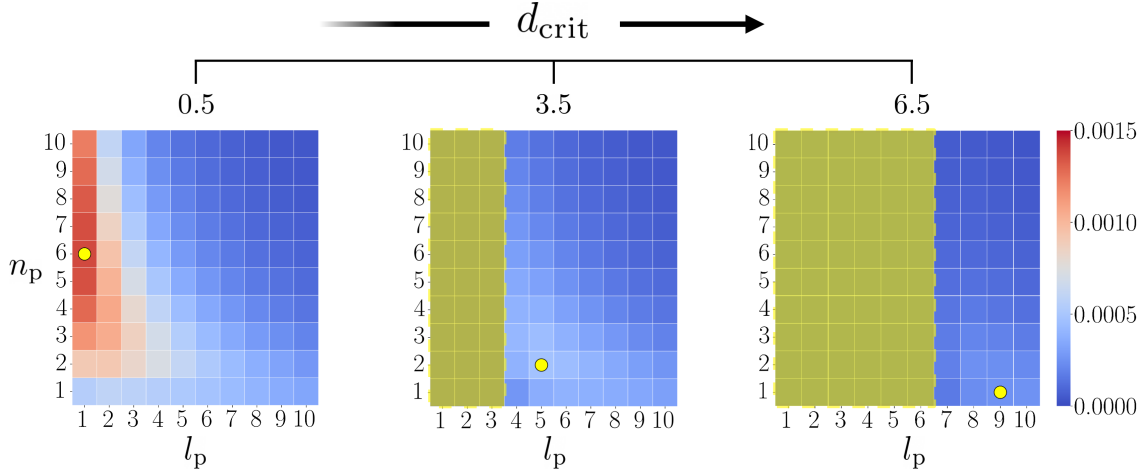


Figure 3.4: Heatmaps showing the evolution of the DTER (Equation (3.22)) with respect to d_{crit} (a proxy for the steepness of the chemical gradient within the domain). When $d_{\text{crit}} = 0.5$, the chemical gradient within the domain is of a large magnitude, such that the optimal strategy (yellow dot) is to sample multiple directions with short protrusions. Shaded green regions show parameter values in which gradient detection is not possible ($d_{\text{crit}} > l_p$). As d_{crit} is increased towards $d_{\text{crit}} = 6.5$, representing increasingly shallower chemical gradients, the optimal strategy shifts to the extension of a lower number of protrusions, each of a longer length. All figures show numerical solutions of Equation (3.22) with $\alpha_m = \alpha_a = \phi = s = 1$, $\beta = 500$, and $k = 500\sqrt{2}$.

In the absence of experimental parameterisation, we set the parameters α_a , α_m , and ϕ to unity and select values of $\beta = 500$ and $k = 500\sqrt{2}$ that constrain the optimum values of n_p and l_p to lie between 1 and 10 for the parameter ranges considered. However, in Appendix A, we discuss the results of a general model parameter sweep to study the effect of each parameter choice on the optimal values of n_p and l_p and the robustness of subsequent findings.

Varying the threshold distance protrusions must span in the direction of the environmental gradient reveals a transition between phenotypes as chemical gradients become increasingly shallow (Figure 3.4). For low values of d_{crit} (i.e. steep chemoattractant gradients), the optimal protrusive phenotype is one in which cells extend multiple short protrusions. Only short protrusions are required due to the presence of easily detectable chemical gradients, and multiple protrusions are extended to ensure that the direction of movement is optimised with respect to chemical gradi-

ents. In contrast, in shallow gradients, where long protrusions must be extended in order to detect a chemical gradient, the optimum number of protrusions to extend is $n_p = 1$, due to the energetic costs of extending increasingly longer protrusions. This result highlights two distinct strategies for gradient detection: *bet-hedging* phenotypes, wherein risk is spread across multiple directions in steep chemical gradients, and *speculation* in shallow gradients, wherein all risk is placed in sampling a single direction.

In the cranial neural crest, there is strong experimental evidence for a transition between speculative and bet-hedging migratory strategies (Teddy and Kulesa, 2004). At the leading edge of migratory streams, where VEGF gradients are steep, CNCCs adopt so-called ‘hairy’ morphologies, characterised by the extension of many short protrusions in a wide range of directions. In contrast, at the rear of streams, where VEGF gradients are extremely shallow, CNCCs adopt ‘bipolar’ morphologies, becoming highly polarised and extending a smaller number of longer protrusions (Figure 3.1). The analysis presented here suggests that energetic efficiency may underlie this observed morphological variation. At the leading edge of streams, CNCCs actively invade regions of high VEGF concentration and there is strong evidence that they consume VEGF during migration (McLennan et al., 2010, 2015a), resulting in steep spatial gradients that drive migration towards *ba2*. In these stream positions, VEGF gradients are readily detectable, and a bet-hedging phenotype, characterised by the extension of multiple protrusions, would allow cells to sample their environment in many directions, thereby optimising their direction of movement. Conversely, trailing cell populations migrate into regions where VEGF has largely been depleted by leading cells, resulting in locally shallow VEGF gradients. Under these conditions, a speculative sampling strategy may be advantageous, whereby cells extend a small number of long, energetically costly protrusions to probe their environment more effectively and hence, to detect measurable gradients of VEGF in a limited number of directions.

In other contexts, however, the converse is true and cells in steep chemical gradients develop a single dominant leading edge and robust polarisation, while shallow gradients yield more exploratory, branched pseudopodial activity (e.g. in endothelial cells exposed to gradients in VEGF (Shamloo et al., 2008)). We propose that this discrepancy can be reconciled by considering the distance travelled by neural crest cells relative to other migratory cell populations. *In vivo*, CNCCs must travel distances upwards of $1000\ \mu\text{m}$ to colonise the branchial arches and facilitate proper facial patterning (McLennan et al., 2010). Neural crest cells are among the most extensively migrating cell populations in the vertebrate embryo (Bronner and Le Douarin, 2012), and as such, energetic constraints in gradient detection may be more of a consideration for neural crest cells than the other cell types studied in this context, for which the rapid detection of gradients and subsequent migration may be prioritised (e.g. in wound healing).

3.6 Assessment of model predictions in an agent-based model of cranial neural crest cell migration

In the study of CNCC migration, a pertinent question is whether predictions regarding CNCC morphology made at the single-cell level translate to the collective. To address this question, we now embed the DTER framework from Section 3.4 into the ABM of CNCC migration introduced in Chapter 2, to study how combinations of the leader cell filopodial number, n_p , and filopodial length, l_p , influence collective energetic efficiency during migration.

In the ABM of CNCC migration in chick (Chapter 2), the VEGF field is initially homogeneous along the r4–ba2 pathway, with gradients emerging dynamically through cell-induced VEGF degradation. Consequently, there is no direct analogue

of the threshold distance for VEGF gradient detection, d_{crit} , introduced in the single-cell model (Section 3.4). Instead, the internalisation rate, λ , governs the steepness of emergent VEGF gradients during migration; larger values of λ generate steeper localised gradients in VEGF at the leading edge of streams, while smaller values of λ lead to shallower gradients distributed over longer length scales. We now, therefore, execute simulations of the ABM of CNCC migration across multiple values of λ , varying n_p between 2 and 10 and l_p between $2\text{ }\mu\text{m}$ and $10\text{ }\mu\text{m}$ for leader cells to study the efficiency of various leader cell protrusive morphologies during collective migration. As follower cells do not actively sense VEGF gradients in this model, we fix n_p and l_p at their default values of 3 and $27.5\text{ }\mu\text{m}$, respectively, as described in Chapter 2.

To decouple active migration due to chemotaxis from passive migration due to domain growth, all simulations in this section are performed on a fixed domain of width $120\text{ }\mu\text{m}$ and length $1094\text{ }\mu\text{m}$, corresponding to the domain length at $t = 24\text{ h}$ in the growth model described in Chapter 2. In the original ABM, domain growth is assumed, without experimental support, to be spatially uniform, which leads to the passive advection of cells at a rate proportional to their x -coordinate at the time of domain growth. Alternative growth profiles would generate different advective relationships between cell position and advection, and, therefore, significantly impact the maximum distance of collective invasion over 24 h. To avoid confounding the analysis conducted here with our assumptions regarding the spatial nature of domain growth, we suppress domain growth and thus, restrict our attention to active collective migration on a stationary domain.

As simulations now comprise multiple cells, the definition of the DTER must also be adapted to a collective setting. For the distance metric (i.e. the analogue of $\mathbb{E}(d_x)$ in Equation (3.19)), we use the maximum x -coordinate attained by any cell when migration ends at $t = 24\text{ h}$ (d_x^{max}). For the energetic cost of collective migration, we consider only the energy expended by leader cells during environmental sampling

and movement, as leaders are the only cells in the model that actively probe VEGF gradients using filopodia. In the ABM, each leader cell samples the average VEGF concentration along each of its filopodia at every timestep. The total energetic cost of VEGF sampling is, therefore, taken to be proportional to the total number of leader cell sampling events aggregated over the simulation, weighted by the morphological costs associated with filopodial number and length.

Accordingly, the DTER for a single simulation is defined as

$$\text{DTER} \equiv \frac{d_x^{\max}}{E} = \frac{\max_{i \in \mathcal{C}} x_i(t = 24 \text{ h})}{n_p l_p^2 S_L + \alpha_a n_p^2 l_p^2 S_L + \alpha_m M_L}, \quad (3.23)$$

where $\mathcal{C}$ denotes the set of all cells in the simulation and $x_i(t = 24 \text{ h})$ is the x -coordinate of cell i when migration ends. Here, S_L denotes the total number of times that leader cells sample their environment with protrusions over the course of the simulation and M_L is the total number of times that leader cells move over the course of the simulation. As in the analytic model of Section 3.4, we once again rescale the energetic parameters of the model, such that $\alpha_c = 1$, and α_a and α_m represent the energetic costs of membrane area change and cell movement through the domain, respectively, relative to the energetic cost of increased membrane curvature. For each parameter combination $(n_p, l_p, \lambda, \alpha_m)$, we conduct 500 numerical model simulations and average the results to obtain a mean measure of efficiency during collective migration.

Numerical simulations of the ABM recover the same relationship between the optimal protrusive phenotype and the steepness of VEGF gradients as predicted by the single-cell analysis in Section 3.5 (Figure 3.4) and the relationship between protrusive activity and the energetic cost of movement discussed in Appendix A. We find a strong dependency between the cost of movement, α_m , and the extent to which the optimal phenotype samples the local microenvironment. For low values of α_m , the DTER is maximised at the lower boundaries of parameter space ($n_p = 2$, $l_p = 2 \mu\text{m}$) (Figure 3.5). In this regime, the energetic cost of movement is small

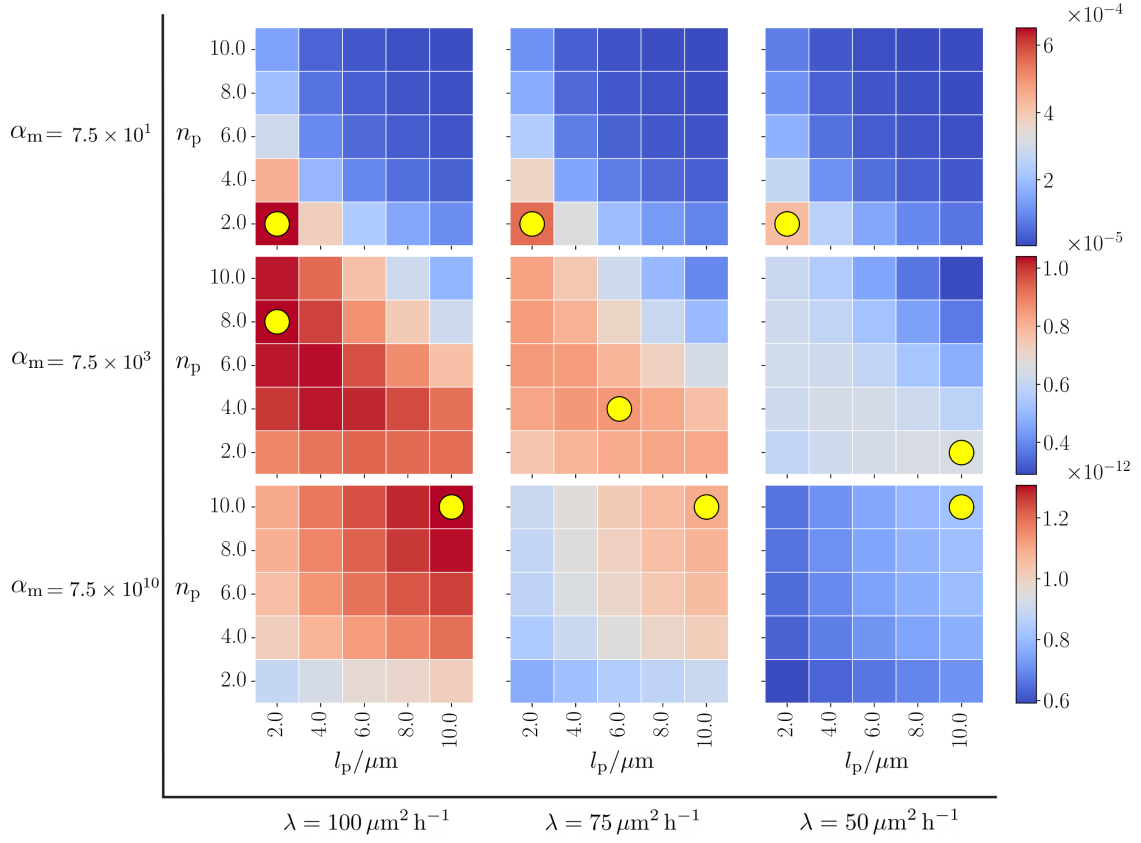


Figure 3.5: Heatmaps of the DTER in numerical model simulations of the ABM outlined in Chapter 2. Each heatmap shows the mean DTER (Equation (3.23)) as a function of leader cell filopodial length, l_p , and filopodial number, n_p , for a fixed combination of the VEGF internalisation rate, λ , and the movement cost, α_m . Yellow circles indicate the filopodial morphology that maximises the DTER for each parameter combination. Results are averaged over 500 simulation repeats for each $(n_p, l_p, \lambda, \alpha_m)$ quadruplet.

relative to the costs of cell membrane deformation. As such, energetic efficiency is linked primarily to a minimisation of protrusive activity, but not necessarily an optimised direction of movement up gradients in VEGF.

As α_m increases, the optimal leader cell protrusive phenotype becomes strongly dependent on λ , reflective of the single-cell analysis in Section 3.5. A relatively low value of $\lambda = 50 \mu\text{m}^2 \text{h}^{-1}$ means that the VEGF gradients generated at the leading edge of collectives are shallow. As such, leader cells must extend longer filopodia to detect gradients in VEGF and propel collectives along the r4–ba2 pathway. However, as the cost of extending each filopodium is high relative to the cost of movement, few of these are extended in the regime of optimal energetic efficiency. As λ is increased to $75 \mu\text{m}^2 \text{h}^{-1}$, gradients in VEGF at the leading edge of collectives grow steeper, meaning shorter protrusions are required to detect gradients in VEGF at the leading edge of streams and more energy can be spent optimising the direction of movement via more extensive environmental sampling. As λ is further increased to a value of $100 \mu\text{m}^2 \text{h}^{-1}$, this trend continues, until a higher number of very short filopodia optimise the DTER. This directly recapitulates the transition between bet-hedging and speculation observed previously in the analytic DTER model (Section 3.5), suggesting that this morphological transition is conserved when considering collective, rather than single-cell, CNCC migration.

For sufficiently large values of α_m , the DTER is maximised at the upper boundaries of the explored parameter space ($n_p = 10$ and $l_p = 10 \mu\text{m}$). In this high movement cost regime, energy expenditure occurs primarily due to movement, which means that energetic efficiency is maximised by protrusive strategies that most strongly optimise the direction of movement along the r4–ba2 pathway. Consequently, leader cells maximising energetic efficiency adopt morphologies that best sample their surrounding environment to reduce the frequency of energetically expensive movement that does not optimise movement towards ba2.

Taken together, these results demonstrate that the bet-hedging to speculation

transition predicted by the single-cell DTER model (Section 3.5) is preserved at the collective scale. Within the DTER framework, bet-hedging and speculative morphologies arise as distinct energy-efficient responses to different local VEGF landscapes, providing a biologically interpretable mechanism for the emergence of protrusive heterogeneity observed *in vivo* (Teddy and Kulesa, 2004). Moreover, the model predicts that increases in the energetic cost of movement can promote greater investment in protrusive sampling, as cells trade higher sensing expenditure for improved directional accuracy and reduced expenditure on poorly directed motion relative to the emergent cell-induced VEGF gradients in the cranial microenvironment.

3.7 Discussion

In this work, we developed a novel theoretical framework that casts the dynamics of actin-driven protrusions in eukaryotic chemotaxis as an energetic optimisation problem, wherein cells balance the benefits of chemical gradient detection against the energetic costs of protrusion extension and subsequent movement.

In the context of CNCC migration, the analytical single-cell model offers an explanation of the observed morphological heterogeneity within CNCC collectives, suggesting that energetic efficiency in chemotactic migration may underpin the transition between bet-hedging and speculative phenotypes observed in chick (Teddy and Kulesa, 2004). By embedding this model in the ABM for CNCC migration described in Chapter 2, we also found that this transition is conserved when considering the collective migration of streams.

This work could be extended by embedding additional biological realism into the model, such as dynamic protrusion formation and retraction, the consideration of unequal protrusion lengths, and biochemical feedback loops linking local VEGF concentration to membrane-localised VEGF receptor availability (McLennan et al.,

2015a). These enhancements would allow for the exploration of transient dynamics in CNCC chemotactic sensing and provide insight into how feedback mechanisms interact with energetic constraints to shape protrusive behaviour.

In conclusion, our work in this chapter provides a novel theoretical lens through which to interpret and predict eukaryotic cell behaviours in chemotaxis. By elucidating how cells balance energetic constraints with information acquisition in complex environments, we have contributed to a deeper understanding of cell migration, and in particular, an understanding of the distinct morphologies observed within streams of CNCCs in chick.

Understanding how CNCC morphologies emerge from energetic and environmental constraints may aid in identifying morphological signatures of aberrant migration. This, in turn, may provide a mechanistic link between altered cell shape and downstream developmental pathologies.

Appendix

A DTER model parameter sweep

Variation of the parameters of Equation (3.19) for $f(\theta) = 1/\pi$ with

$$g(\beta, \phi) = \frac{\phi\beta}{k} \exp\left(-\left(\frac{\beta}{k}\right)^2\right) \quad (3.24)$$

demonstrates that the optimal protrusive phenotype depends quantitatively on the energetic balance between environmental sampling and cell movement (Figures 3.6–3.8). For a fixed threshold distance, d_{crit} , sweeping the energetic parameters of the DTER model reveals a systematic variation in the optimal number of protrusions, $n_{\text{p}}^{\text{opt}}$. In particular, $n_{\text{p}}^{\text{opt}}$ increases when cells become more motile (larger ϕ) or when the energetic cost of movement is increased, reflecting a greater importance of accurately determining the direction of migration under conditions where cell displacement is energetically expensive. Conversely, increasing the energetic cost associated with membrane deformation, α_{a} , leads to a reduction in $n_{\text{p}}^{\text{opt}}$ as the energetic penalty for extending multiple protrusions outweighs the benefit of improved directional sensing.

Additionally, varying the threshold distance for gradient detection reveals a clear and robust trend. As d_{crit} is increased from 0.5 to 3.5 and 6.5 (Figures 3.6, 3.7, and 3.8, respectively), the optimal number of protrusions, $n_{\text{p}}^{\text{opt}}$, decreases monotonically across the parameter space as each protrusion must be longer to sample measurable gradients, and, therefore, is of a substantial energetic cost. Across all parameter regimes explored, we further find that the optimal protrusion length, $l_{\text{p}}^{\text{opt}}$, varies significantly less than $n_{\text{p}}^{\text{opt}}$ and is determined primarily by the threshold condition for reliable gradient detection, with the optimal protrusion number encoding the energetic strategy adopted by the cell.

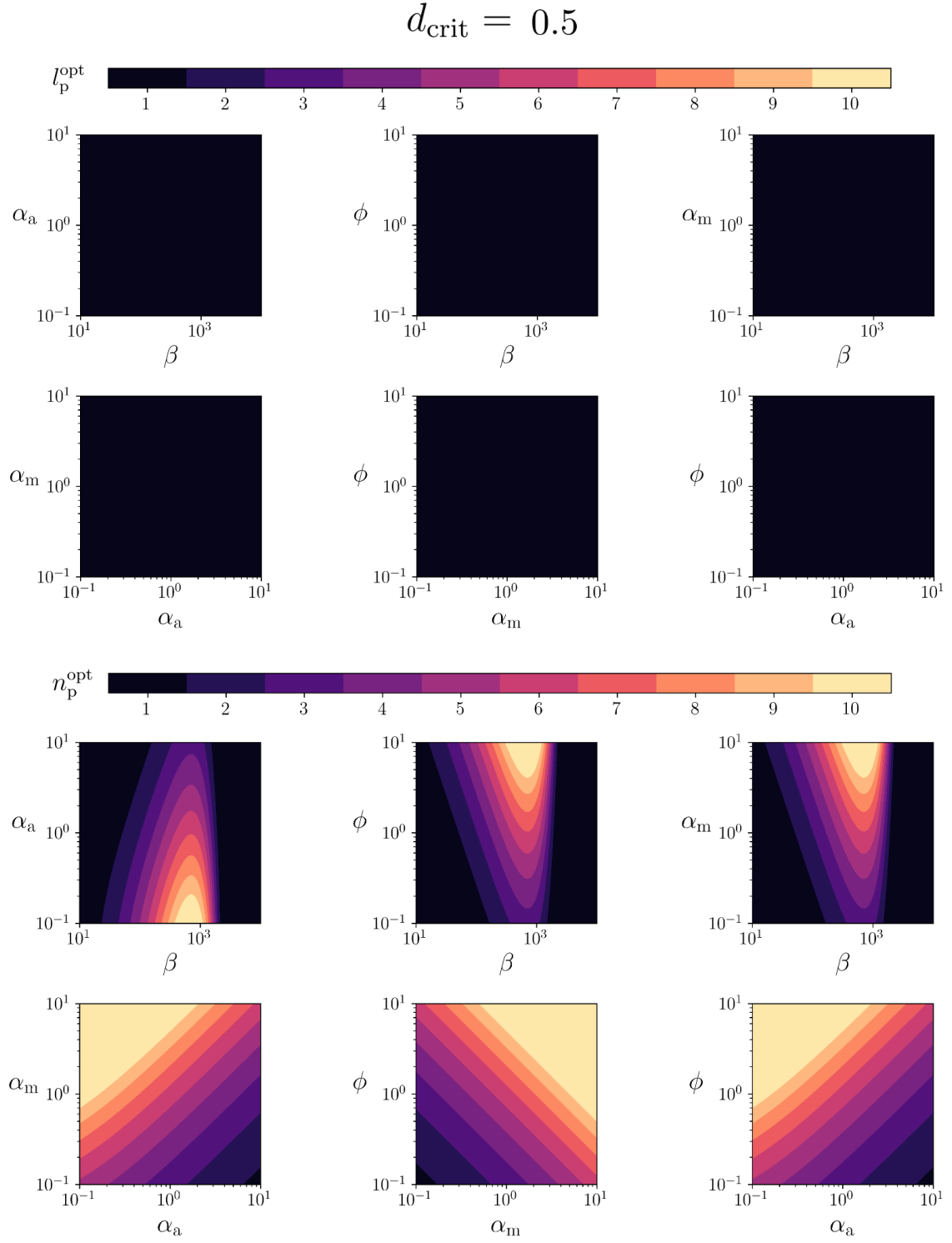


Figure 3.6: Parameter sweep of the DTER model for a steep chemical gradient, $d_{\text{crit}} = 0.5$. Heatmaps show the optimal number of protrusions, n_p^{opt} , and the optimal protrusion length, l_p^{opt} , as functions of pairs of energetic parameters. For each parameter pair, the DTER is maximised over $n_p, l_p \in \{1, \dots, 10\}$. In all heatmaps, we choose $\alpha_m = \alpha_a = \phi = s = 1$, $\beta = 500$, and $k = 500\sqrt{2}$ as baseline values, which are perturbed in parameter sweeps.

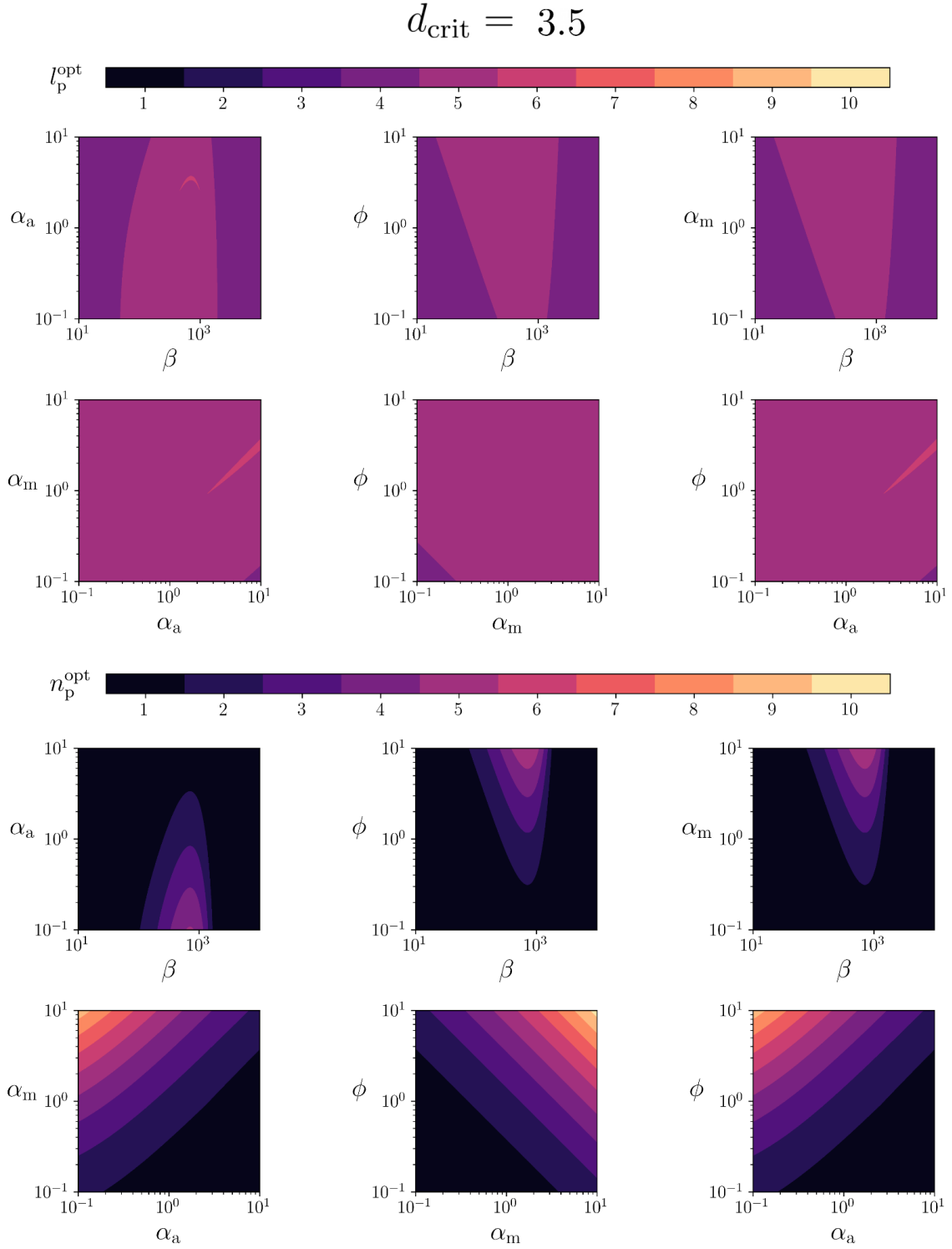


Figure 3.7: Parameter sweep of the DTER model for an intermediate chemical gradient, $d_{\text{crit}} = 3.5$. Heatmaps show the optimal number of protrusions, n_p^{opt} , and the optimal protrusion length, l_p^{opt} , as functions of pairs of energetic parameters. For each parameter pair, the DTER is maximised over $n_p, l_p \in \{1, \dots, 10\}$. In all heatmaps, we choose $\alpha_m = \alpha_a = \phi = s = 1$, $\beta = 500$, and $k = 500\sqrt{2}$ as baseline values, which are perturbed in parameter sweeps.

$$d_{\text{crit}} = 6.5$$

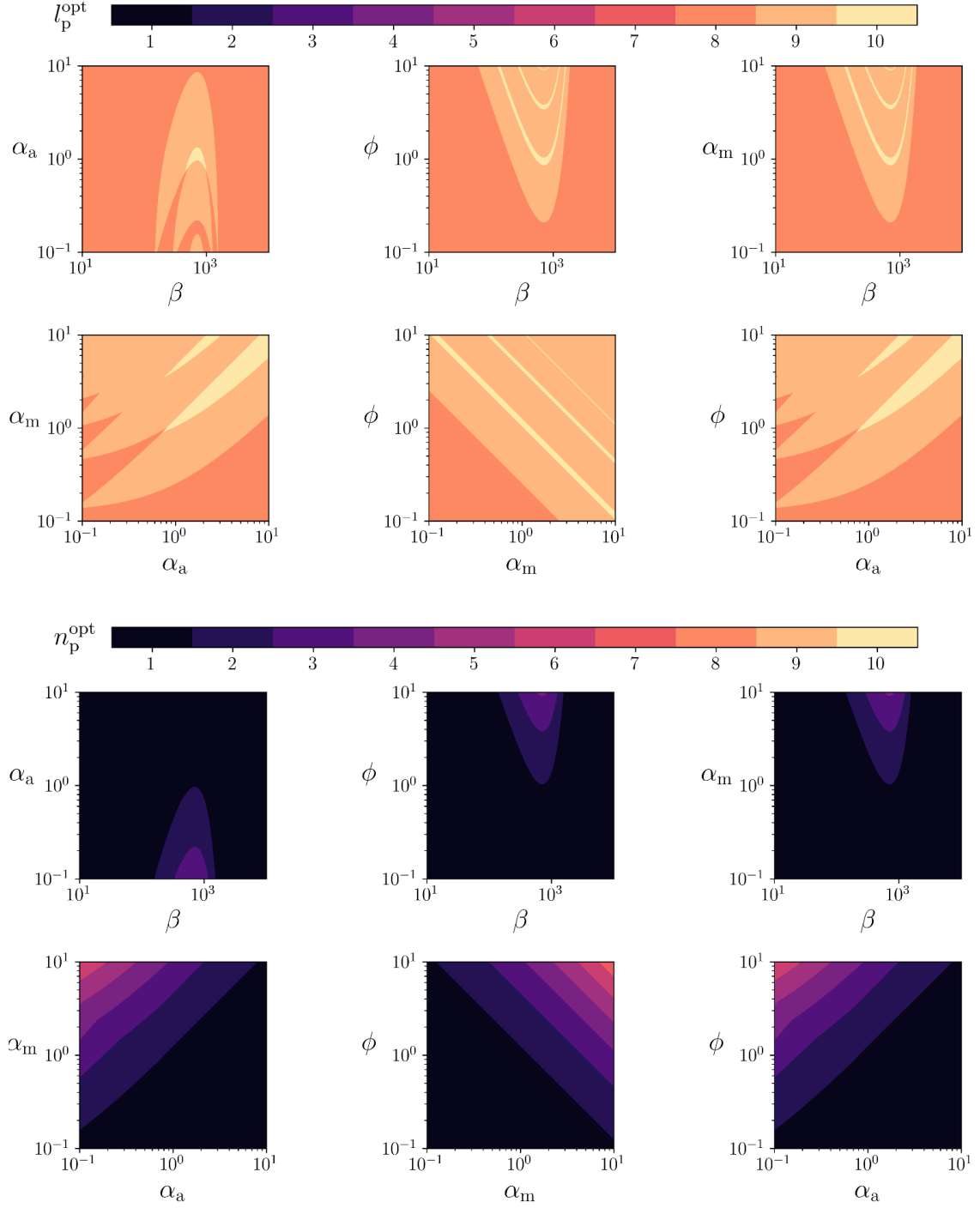


Figure 3.8: Parameter sweep of the DTER model for a shallow chemical gradient, $d_{\text{crit}} = 6.5$. Heatmaps show the optimal number of protrusions, n_p^{opt} , and the optimal protrusion length, l_p^{opt} , as functions of pairs of energetic parameters. For each parameter pair, the DTER is maximised over $n_p, l_p \in \{1, \dots, 10\}$. In all heatmaps, we choose $\alpha_m = \alpha_a = \phi = s = 1$, $\beta = 500$, and $k = 500\sqrt{2}$ as baseline values, which are perturbed in parameter sweeps.

Chapter 4

Cranial neural crest cell confinement during collective migration

In this chapter, we use mathematical modelling to investigate the biological mechanisms by which streams of CNCCs remain spatially discretised during migration, which is crucial in ensuring proper craniofacial patterning during development (McLennan et al., 2023). If mixing between adjacent streams occurs, a range of birth defects, termed *neurocristopathies*, may result (Sato et al., 2019; Vega-Lopez et al., 2018). As such, a better understanding of the mechanisms governing the confinement of CNCC trajectories to distinct stereotypical migratory pathways could provide insights into craniofacial anomalies and identify therapeutic opportunities to minimise costly repair surgeries (Pilon, 2021).

Analyses in chick have identified key molecular candidates for the confinement of CNCC migration to stereotypical pathways as *Colec12*, *Trail*, and *Dan* (McLennan et al., 2017, 2023). Although the effects of these factors on CNCC behaviour *in vitro* have been characterised, their roles in maintaining stream separation *in vivo* remain poorly understood. In what follows, we develop and analyse a set of hypothetical mechanisms through which these factors may regulate stream confinement and sustain coherent migration, building on the ABM introduced in Chapter 2 by explicitly integrating the effects of chemical signalling on cell speed, direction, and cell–cell interactions.

We begin by summarising existing experimental evidence concerning the molecular regulators implicated in CNCC stream confinement. Then, drawing on *in vitro* data (McLennan et al., 2017, 2023), we formulate mechanistic hypotheses regarding how *Colec12*, *Trail*, and *Dan* may influence CNCC movement during migration, before testing these proposed mechanisms in an expanded ABM for CNCC migration.

Finally, based on the results of numerical simulations of the model, we outline future experiments that may be used to test our proposed mechanisms of confinement to strengthen the validity of our conclusions.

Comment on originality: Work in this chapter is based on the manuscript:

Johnson, S.W.S., Kulesa, P.M., Baker, R.E. and Maini, P.K., 2026. Mathematical modeling predicts novel mechanisms of stream confinement from Trail/Colec12/Dan in the collective migration of cranial neural crest cells. *Developmental Dynamics*.

Author contribution: The author of this thesis conceived this project alongside Paul Kulesa, Ruth Baker, and Philip Maini, all of whom also contributed to the development of the model. The author of this thesis conducted the model analysis and wrote the manuscript, which was revised by Paul Kulesa, Ruth Baker, and Philip Maini.

Code availability: Code used for the generation of the results in this chapter can be found at <https://github.com/SWSJChCh/leaderFollower>.

4.1 The biology of cranial neural crest cell confinement

During migration, CNCCs form streams adjacent to rhombomeres r1/2/4/6 that remain separated by regions void of CNCCs adjacent to rhombomeres r3/5 (Graham et al., 1993) (Figure 4.1). The spatial separation of adjacent CNCC streams is a crucial aspect of migration; there are many birth defects associated with the mixing of CNCC streams and a subsequent breakdown in migration (Sato et al., 2019; Vega-Lopez et al., 2018). The experimental literature highlights many poten-

tial mechanisms for the confinement of CNCC streams to discrete pathways *in vivo*, though this process is still incompletely understood. Here, we review previous experimental investigations of the factors confining CNCC migration to stereotypical pathways in chick and other model systems.

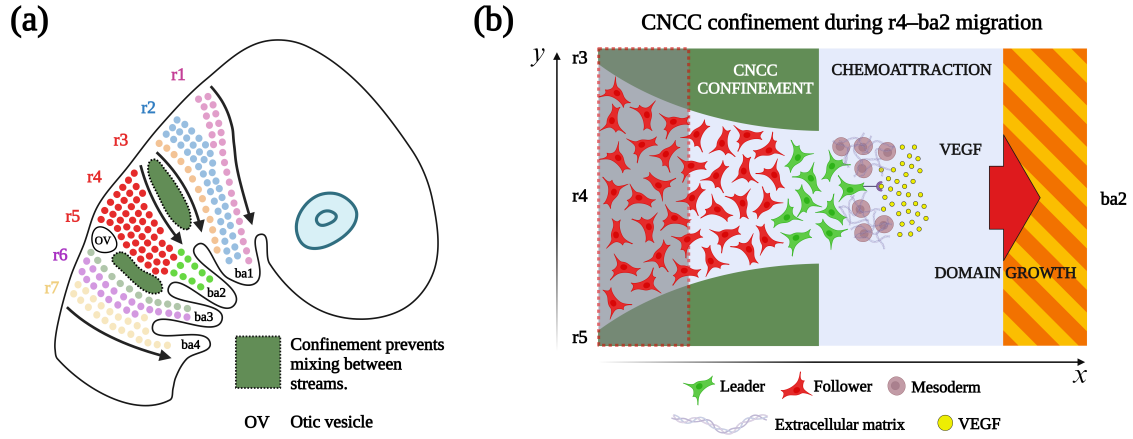


Figure 4.1: **(a)** Schematic of CNCC migration in chick (based on prior works (Carstens, 2023; Johnson et al., 2026b; Kuo and Erickson, 2010)). CNCCs emerge from r1–7 and are sculpted into discrete streams that remain spatially separated during invasion of ba1–4. The majority of CNCCs originate from r2/4/6 and populate ba1–3, respectively. CNCCs emerging from r3/5 are diverted into the regions adjacent to r2/4/6 due to the presence of inhibitory signals in the paraxial mesoderm adjacent to r3/5 (dark green). **(b)** Schematic of CNCC r4–ba2 migration *in vivo*. CNCCs emerge from r3–r5 and are sculpted into a single stream adjacent to r4 that populates ba2. VEGF guides migration towards ba2, whilst inhibitory signalling in the CNCC-free zones adjacent to r3/5 (dark green) prevents migration into the regions adjacent to r3/5. Additional inhibitory signalling in the regions proximal to the hindbrain adjacent to r3–5 (translucent grey) modulates CNCC speed after delamination from the rhombomeric hindbrain. Created in BioRender by Samuel Johnson: <https://BioRender.com/a3ivhog>.

The factors influencing the exit locations of newly-delaminated CNCCs onto stereotypical migratory pathways in the paraxial mesoderm have been well-studied. Apoptosis is thought to play a role in regulating the number of CNCCs exiting from the odd-numbered rhombomeres, r3 and r5 (Ellies et al., 2002; Smith and Graham, 2001). However, it is unlikely that apoptosis alone is responsible for maintaining spatial separation between adjacent streams for the duration of migration towards the branchial arches, which suggests the existence of other biological mechanisms

of CNCC confinement during migration. After emergence from the hindbrain in the regions adjacent to r2–5, the over-expression of soluble Neuropilin-1/2 Fc, a Neuropilin-1/2 decoy receptor that binds class-3 semaphorins (primarily Sema3A and Sema3F), in the chick cranial microenvironment has been observed to result in the movement of CNCCs into the CNCC-free zone adjacent to r3 (Osborne et al., 2005). This observation is suggestive of a role for Neuropilin-1/2 and their class-3 semaphorin ligands in the early confinement of CNCCs in regions adjacent to the hindbrain ($\approx 0\text{--}200\ \mu\text{m}$ from the dorsal mid-line of the neural tube). However, it is also likely that other factors beyond regions of semaphorins adjacent to the hindbrain are responsible for confinement in the subsequent stages of migration where CNCCs collectively migrate long distances through the mesoderm between the dorsal neural tube and the branchial arches.

In *Xenopus*, Versican has been shown to confine CNCC collectives by forming inhibitory boundaries in placodal tissues near the branchial arches that prevent the mixing of adjacent streams in late-stage migration (Szabó et al., 2016). However, Versican loss-of-function experiments show that reduced Versican expression does not result in extensive mixing between adjacent streams *in vivo*, meaning that other factors must also be responsible for the confinement of CNCCs to distinct stereotypical migratory pathways prior to invasion of the branchial arches. Similarly, CNCCs have also been found to avoid regions of EphB2 and Ephrin-B1 expression in the chick cranial microenvironment (Küry et al., 2000). However, the expression of EphB2 and Ephrin-B1 lateral to the otic vesicle and ba2 means that these factors are also unlikely to be responsible for the confinement of CNCCs to discrete streams as they migrate away from the hindbrain towards targets in the branchial arches. These observations have led to more recent investigations of the factors expressed in the mesoderm that confine CNCCs to discrete streams for the duration of migration. Expression analyses conducted in the chick cranial microenvironment have highlighted Dan (NBL1, a Dan family BMP antagonist), Trail (tumour necro-

sis factor-related apoptosis-inducing ligand, CD253), and Colec12 (Collectin-12, a transmembrane scavenger receptor C-type lectin) as factors expressed along, and adjacent to, stereotypical migratory pathways that confine CNCCs to discrete, spatially separated streams during migration (McLennan et al., 2017, 2023). However, the mechanisms through which these factors confine CNCC movement to stereotypical migratory pathways in the cranial neural crest remain unclear.

Dan has been found to restrict CNCC movement in stripe assays, and average cell speed and displacement are reduced *in vitro* when Dan protein is added to the culture media (McLennan et al., 2017). Furthermore, Dan is expressed in the paraxial mesoderm adjacent to the hindbrain and its concentration is reduced in regions through which CNCCs have previously migrated. These observations are suggestive of a mechanism where migrating CNCCs degrade Dan in chick, though this hypothesis has not yet been validated experimentally. The addition of Dan into a computational model of CNCC migration showed that cell speed modulation in the presence of Dan is a potential mechanism by which the frequent break-up of collectives observed in prior models can be prevented (McLennan et al., 2017). However, this model neglected the spatial variations in Dan concentration along the r4-ba2 pathway that are observed *in vivo*, instead assuming that CNCC speed was reduced uniformly across streams exposed to Dan. In reality, it is likely that the reduced concentration of Dan in regions through which CNCCs have previously migrated results in a spatially varying effect on CNCCs, with a stronger influence on cells at the leading edge where Dan concentration is, on average, higher.

Subsequent analyses in the chick cranial microenvironment have also revealed high expression of Trail and Colec12 in the CNCC-free zones adjacent to CNCC streams. *In vitro* assays have highlighted the effects of Trail and Colec12 on chick CNCC morphology, migratory dynamics, and cell-cell interactions (McLennan et al., 2023). Trail has been found to enhance cell-cell adhesion in chick CNCCs, resulting in clustered amoeboid-like motion rather than movement as individuals, as observed

in control media. Moreover, a significant reduction in both the average speed and displacement of CNCCs has been observed in cells cultured in the presence of Trail. Conversely, equivalent assays have highlighted a change in chick CNCC morphology in the presence of Colec12 through the formation of longer, non-retracting filopodial protrusions with increased branching relative to assays in control media. *In vitro*, chick CNCCs in regions of Colec12 expression have also been observed to reverse direction and migrate back towards the neural tube explant. The mechanism driving this movement back towards the neural tube remains unclear, though it is possible that CNCCs migrate down gradients of Colec12 expression via chemotaxis, which is a well-documented mechanism of migration in CNCC populations (Shellard and Mayor, 2016). Experiments in chick show that loss-of-function of either Trail or Colec12 results in the increased deviation of migrating CNCCs away from stereotypical migratory pathways and into neighbouring CNCC-free zones. However, as in the case of Dan, the biological mechanisms through which Trail and Colec12 restrict CNCC movement to stereotypical migratory pathways in collective invasion of the branchial arches remain unclear. An ABM for chick CNCC migration has been used to predict that the confinement of CNCCs to the r4–ba2 pathway by factors expressed adjacent to r3/5 is only necessary for the first third of the migratory domain ($\approx 400\mu\text{m}$) to prevent a breakdown in the collective invasion of ba2 (McLennan et al., 2023). This prediction guided subsequent experiments, which found the enhanced expression of both Trail and Colec12 for approximately the first $400\mu\text{m}$ of typical CNCC-free zones adjacent to r3/5. However, this model and other previous models of CNCC migration (McKinney et al., 2020; McLennan et al., 2012, 2015a,b, 2017, 2020) considered a simple representation of inhibitory signalling via the imposition of zero-flux boundary conditions on CNCCs at the axial borders of the regions adjacent to r3/5 and, as such, did not permit an exploration of the mechanisms through which Trail and Colec12 confine CNCC migration to the r4–ba2 pathway *in vivo*.

In this chapter, we use mathematical modelling to study the extent to which the effects of Colec12, Trail, and Dan on CNCCs observed *in vitro* can constrain the collective migration of CNCCs *in vivo*. By expanding the ABM described in Chapter 2, we explicitly represent the effects of these factors on the movement and behaviour of CNCCs and, using numerical simulations of this revised model, quantify the extent to which these mechanisms are sufficient to confine CNCC streams *in vivo*. In doing so, we generate a range of testable biological hypotheses regarding the roles of these factors during collective CNCC migration which may be used to validate the predictions made by the model.

4.2 Incorporating Colec12, Trail, and Dan into an agent-based model of cranial neural crest cell migration

The expanded ABM for CNCC migration again focuses on the migration of CNCCs through the r4–ba2 pathway that is enclosed by CNCC-free zones adjacent to r3 and r5. Cells are represented as discrete two-dimensional non-overlapping discs whose migration depends on the concentration of VEGF, Dan, Trail, and Colec12 in their neighbourhood. The r4–ba2 pathway is approximated as a two-dimensional rectangle in which VEGF is expressed everywhere and Dan is expressed for a certain proportion of the pathway in the x -direction (Figure 4.2). As in the model described in Chapter 2, the r4–ba2 pathway is assumed to grow in length in a spatially uniform manner from approximately $300\text{ }\mu\text{m}$ to $1100\text{ }\mu\text{m}$ over a period of 24 h and its width is a constant value of $120\text{ }\mu\text{m}$ throughout migration, corresponding to the anterior-to-posterior length of r4 in chick. The r4–ba2 pathway is also enclosed by two CNCC-free zones adjacent to r3 and r5, each of width $120\text{ }\mu\text{m}$, in which VEGF is expressed everywhere and Trail, Colec12, and Dan are expressed for a certain proportion of the

domain in the x -direction, and that grow in length at the same rate as the r4–ba2 pathway. For the chick embryo, there is currently no explicit discussion of VEGF expression adjacent to r3/5 in the experimental literature. Here, we assume that VEGF is expressed in these regions as this is likely to drive the invasion of leader cells into CNCC-free zones and hence presents the best test of robustness for our proposed mechanisms of CNCC confinement. The expanded model geometry considered here is outlined in Figure 4.2 and the model parametrisation is as described in Table 4.1, with notes on parametrisation given in Section 4.2.5. Model pseudocode is provided in Appendix A.

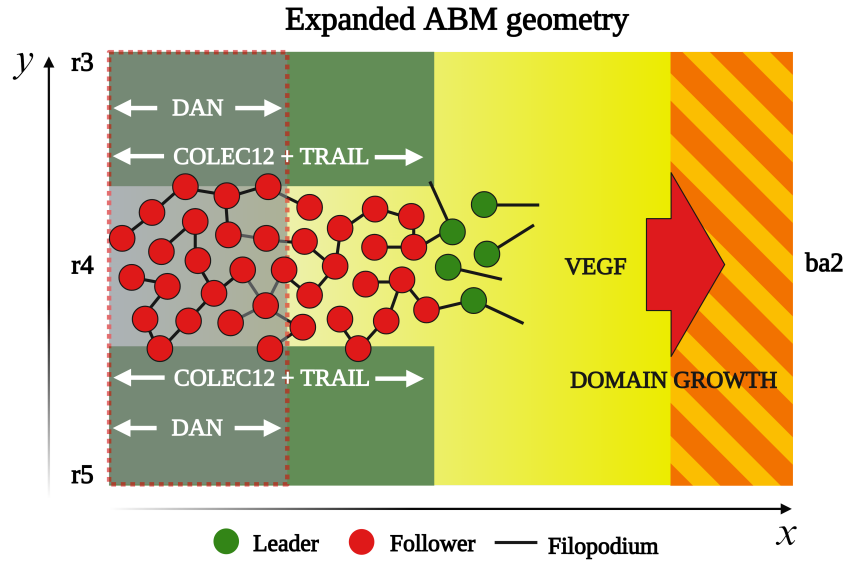


Figure 4.2: Schematic of CNCC migration in the expanded model. CNCCs migrate along the r4–ba2 pathway for a period of 24 h. Leader cells (green) migrate up self-induced VEGF gradients towards ba2, while follower cells (red) move according to directional cues from cell–cell contacts, facilitating migration as a collective. Trail and Colec12 (dark green) are expressed for a portion of the regions adjacent to r3 and r5 in the x -direction, as observed in chick (McLennan et al., 2017, 2023). Dan (translucent grey) is also expressed for a portion of the regions adjacent to r3–5 and modulates the speed of CNCCs during migration. The domain through which CNCCs migrate grows in the x -direction, but remains at a constant width in the y -direction. Created in BioRender by Samuel Johnson: <https://BioRender.com/x8o6m85>.

4.2.1 Chemical reaction–diffusion dynamics

We model the evolution of VEGF, Dan, Trail, and Colec12 concentrations with four reaction–diffusion PDEs, comprising terms for cell-induced degradation, production within the domain, dilution during domain growth, and diffusion. The concentration of chemical j ($j \in \{\text{VEGF}, \text{Dan}, \text{Trail}, \text{Colec12}\}$), $c_j(x, y, t)$, where $(x, y) \in (0, 1) \times (0, L_y)$, evolves according to the same re-scaled reaction-diffusion equation for VEGF outlined in Chapter 2:

$$\begin{aligned} \frac{\partial c_j}{\partial t} = & D_j \left(\frac{1}{L_x(t)^2} \frac{\partial^2 c_j}{\partial x^2} + \frac{\partial^2 c_j}{\partial y^2} \right) - c_j \sum_{i=1}^{N(t)} \frac{\lambda_j}{2\pi R^2} \exp \left[-\frac{L_x(t)^2 (x - x_i)^2 + (y - y_i)^2}{2R^2} \right] \\ & + \kappa_j(x, y) c_j (1 - c_j) - \frac{c_j \dot{L}_x(t)}{L_x(t)}, \end{aligned} \quad (4.1)$$

where $\kappa_j(x, y)$ is a spatially non-uniform production rate that is constant within the regions where chemical j is produced and zero elsewhere. In solving Equation (4.1), we impose zero-flux boundary conditions for all chemicals on the re-scaled domain boundaries at $x = 0$ and $x = 1$ and periodic boundary conditions for all chemicals at $y = 0$ and $y = L_y$, to represent the presence of adjacent migratory pathways in the axial direction. The initial conditions are uniform concentrations of $c_j(x, y, 0) = c_0 = 1$ ($j \in \{\text{VEGF}, \text{Dan}, \text{Trail}, \text{Colec12}\}$) in the regions where each chemical is expressed. Parameter names and values are given in Table 4.1, with some parameters set to zero to omit certain terms in Equation (4.1) for certain chemicals.

4.2.2 Cell movement in the presence of Dan

We assume that the speed of migrating CNCCs is modulated in the presence of Dan protein according to

$$v_i(t) = v_0 \frac{c_{\text{Dan}}^0 - c_{\text{Dan}}(x_i, y_i, t)}{c_{\text{Dan}}^0}, \quad (4.2)$$

where $v_i(t)$ is the speed of CNCC i at time t ($i \in \{1, \dots, N(t)\}$), where $N(t)$ is the

number of CNCCs in the domain at time t). In Equation (4.2), c_{Dan}^0 is the initial concentration of Dan in the domain and $c_{\text{Dan}}(x, y, t)$ is the concentration of Dan at a position (x, y) in the domain at time t . This representation of speed modulation by Dan means that CNCC speed decreases linearly with the local concentration of Dan, and that movement ceases completely in regions where Dan is at the initial maximum concentration of c_{Dan}^0 . A similar representation of CNCC speed modulation in regions of Dan expression was considered in a prior model of CNCC migration (McLennan et al., 2017), though in that model, Dan was not degraded by CNCCs, and hence was of a spatially uniform, time-varying concentration in a sub-region of the domain adjacent to r4.

4.2.3 Cell movement upon the detection of Trail

We represent the effects of Trail protein on CNCCs *in vitro* through multiple modifications to the behaviour of CNCCs. When a CNCC detects Trail (Section 4.2.5), its speed is reduced by 25%, in line with *in vitro* observations (McLennan et al., 2023). To represent increased cell–cell adhesion in the presence of Trail, we modify the primary mechanism of movement for CNCCs in chains. When Trail is not detected, follower cells in the model move by forming chains of cells with a leader at the front (as described in Chapter 2). All cells in a given chain move in the same direction as the leader cell at the front of the chain. In CNCCs that detect Trail, we modify this mechanism of movement, such that follower cells instead move towards the cell directly in front of them in their respective chain, meaning that movement in detectable concentrations of Trail is directed towards areas of higher cell density, on average.

4.2.4 Cell movement upon the detection of Colec12

We represent the effects of Colec12 protein on CNCCs *in vitro* through multiple modifications to the behaviour of CNCCs. When a CNCC detects Colec12 (Sec-

tion 4.2.5), the length of the filopodial protrusions it extends is increased by 50%, in line with *in vitro* observations (McLennan et al., 2023). Furthermore, we represent the increased branching of filopodia by the extension of a higher number of filopodia in a given time interval. To represent the slower rate of filopodia retraction in CNCCs exposed to Colec12 protein, we also increase the time interval between the extension and retraction of new filopodia for CNCCs, such that a given filopodium remains extended for a longer time interval. In simulations where the effects of Colec12 protein on CNCCs were limited to the morphological changes observed *in vitro*, the expression of Colec12 in CNCC-free zones adjacent to r3/5 was insufficient to confine CNCCs to the r4–ba2 pathway (Section 4.3). As such, we also added a mechanism of chemotaxis, in which CNCCs migrate down gradients in Colec12 protein and back towards the r4–ba2 pathway where its concentration is lower. The detection of gradients in Colec12 by CNCCs is a modification of the Berg-Purcell detection limit described in Chapter 2, such that a gradient in Colec12 is detected if

$$\frac{c_f^{C12}(x, y, t) - c^{C12}(x, y, t)}{c^{C12}(x, y, t)} \leq -\xi \sqrt{\frac{c_0^{C12}}{c^{C12}(x, y, t)}}, \quad (4.3)$$

where $c_f^{C12}(x, y, t)$ is the average concentration of Colec12 integrated along the filopodium, $c^{C12}(x, y, t)$ is the concentration of Colec12 at the current position of the cell centre, c_0^{C12} is the initial uniform concentration of Colec12 within the domain, and ξ is a dimensionless parameter that determines the accuracy of Colec12 gradient detection by cells.

4.2.5 Model parametrisation

We detail the parameters used in simulations of the ABM (Table 4.1), and briefly discuss the values of new parameters in the expanded ABM.

Table 4.1: Default parameter values are given. When a range is given, model outputs are robust to a variation of the parameter in the stated range, and bracketed values were used as default. Chemical concentrations are given in relative units, such that for Dan, Colec12, and Trail, $c_0 = 1$.

	Description	Value
λ_{DAN}	Dan internalisation rate	$2.75 \times 10^2 \mu\text{m}^2 \text{h}^{-1}$
κ_{DAN}	Dan production rate	$0\text{--}1 \text{h}^{-1}$ (0)
D_{DAN}	Dan diffusivity	$0\text{--}10 \mu\text{m}^2 \text{h}^{-1}$ (0.1)
λ_{C12}	Colec12 internalisation rate	$0 \mu\text{m}^2 \text{h}^{-1}$
κ_{C12}	Colec12 production rate	$0\text{--}1 \text{h}^{-1}$ (0)
D_{C12}	Colec12 diffusivity	$0\text{--}10 \mu\text{m}^2 \text{h}^{-1}$ (0.1)
λ_{TRAIL}	Trail internalisation rate	$0 \mu\text{m}^2 \text{h}^{-1}$
κ_{TRAIL}	Trail production rate	$0\text{--}1 \text{h}^{-1}$ (0)
D_{TRAIL}	Trail diffusivity	$0\text{--}10 \mu\text{m}^2 \text{h}^{-1}$ (0.1)
β_{C12}	fold increase in filopodia extended per unit time in regions of Colec12 expression	$2\text{--}10$ (5)
α_{C12}	fold decrease in rate of filopodia retraction in regions of Colec12 expression	$2\text{--}20$ (10)
ϕ	threshold relative concentration for Colec12/Trail detection	$0.05\text{--}0.2$ (0.1)

Diffusivity of Trail, Colec12, and Dan

The diffusivity of Trail, Colec12, and Dan has not been quantified experimentally. In the absence of experimental parametrisation, we assume that the diffusivities of Colec12, Trail, and Dan are the same as that of VEGF. In general, we find that VEGF, Trail, Colec12, and Dan must all be of a diffusivity $D \lesssim 10 \mu\text{m}^2 \text{h}^{-1}$ for typical stream patterning to occur.

Internalisation rate of Trail, Colec12, and Dan

The degradation of Trail and Colec12 by CNCCs has not been observed *in vivo*. As such, CNCCs in the model do not degrade Trail or Colec12 as they migrate. However, in chick, Dan has been observed to exhibit a dynamic expression profile during CNCC migration, with Dan concentration lower in regions through which CNCCs have previously migrated, compared with regions through which migration has not occurred (McLennan et al., 2017). As such, we hypothesise that CNCCs degrade Dan during migration. In the absence of experimental studies that allow us to parameterise this rate of degradation, we assume Dan to be degraded at the same rate as VEGF ($2.75 \times 10^2 \mu\text{m}^2 \text{h}^{-1}$).

Production rate of Trail, Colec12, and Dan

There is no *in vivo* evidence to suggest that Dan, Trail, and Colec12 are produced in the paraxial mesoderm during CNCC migration. As such, our default production rates of Dan, Trail, and Colec12 are $\kappa_{\text{DAN}} = \kappa_{\text{TRAIL}} = \kappa_{\text{C12}} = 0 \text{h}^{-1}$. However, our findings are robust to variations in these parameters provided that $\kappa_{\text{TRAIL}} \lesssim 1 \text{h}^{-1}$, $\kappa_{\text{C12}} \lesssim 1 \text{h}^{-1}$, and $\kappa_{\text{DAN}} \lesssim 1 \text{h}^{-1}$.

Fold increase in the number of filopodia extended when CNCCs detect Colec12

In vitro, CNCCs that detect Colec12 exhibit increased branching in the filopodia they extend when compared with control media (McLennan et al., 2023). We represent increased branching in filopodia by the extension of a higher number of filopodia in regions of enhanced Colec12 expression. There is no way to directly link the increased branching observed *in vitro* to the fold increase in filopodia extended in our model. As a default value, the fold increase in filopodia extended by CNCCs that detect Colec12 protein expression is $\beta_{C12} = 5$, but our findings are robust to large variations in this parameter ($\beta_{C12} = 2$ to 10).

Fold decrease in the rate of filopodia retraction when CNCCs detect Colec12

In vitro, CNCCs that detect Colec12 retract filopodia at a slower rate than in control media (McLennan et al., 2023). However, the fold decrease in the rate at which filopodia are retracted in regions of Colec12 expression has not been quantified experimentally. We chose a default value of $\alpha_{C12} = 10$, but our findings are robust to large variations in this parameter ($\alpha_{C12} = 2$ to 20).

Threshold relative concentration for the detection of Trail or Colec12

There is no direct way to parametrise the relative concentration threshold at which CNCCs detect Trail or Colec12. In simulations, we therefore prescribe an arbitrary threshold concentration of $\phi = 0.1$ for the detection of Trail or Colec12. This value ensures that, throughout migration, CNCCs consistently detect Trail and Colec12 within their typical regions of expression, while remaining insensitive to the minute concentrations that diffuse into the r4-ba2 pathway. As all chemical concentrations are diluted by the growth of the migratory domain, our detection threshold must be chosen below the maximum attainable relative concentration of Trail and Colec12

when migration ends, to ensure that detection is possible throughout migration. In our simulations, this maximum is approximately $c^{\max}(t = 24 \text{ h}) = 0.25$.

4.3 Trail and Colec12 confine cranial neural crest cells to the r4–ba2 pathway during collective migration

Expression profile analyses of the paraxial mesoderm through which CNCCs migrate have revealed enhanced levels of Trail and Colec12 expression in typically CNCC-free zones adjacent to r3 and r5. Furthermore, *in vitro*, CNCCs have been found to avoid regions of either Trail or Colec12 expression (McLennan et al., 2023). These findings suggest that both Trail and Colec12 play a role in the confinement of CNCCs to stereotypical migratory pathways during migration.

In vitro experiments have shown that CNCCs exposed to Colec12 protein extend longer, more branched protrusions that retract at a slower rate than in control media (McLennan et al., 2023). To investigate the effects of Colec12 on the spatial confinement of CNCCs, we used the ABM to study CNCC migration along the r4–ba2 pathway with Colec12 expressed in the CNCC-free zones adjacent to r3 and r5. In the ABM, Colec12 is expressed for a certain proportion of the CNCC-free zones adjacent to r3 and r5 (Figure 4.3(a)). The proportion of CNCC-free zones for which Colec12 is expressed remains constant during domain growth, such that the distance over which Colec12 is expressed in CNCC-free zones increases as the domain is extended. CNCCs that detect Colec12 extend filopodial protrusions that are 1.5 times longer than at baseline. We choose to represent the increased branching of filopodia in CNCCs exposed to Colec12 by the extension of five times the number of filopodia in a given time interval. Furthermore, we represent the slower retraction of filopodia by CNCCs by increasing the time interval over which cells extend new filopodia by a factor of 10 (such that a given filopodium remains extended for a longer period of time). A visual summary of the effects of Colec12 protein on CNCCs in the ABM can be seen in Figure 4.3(c).

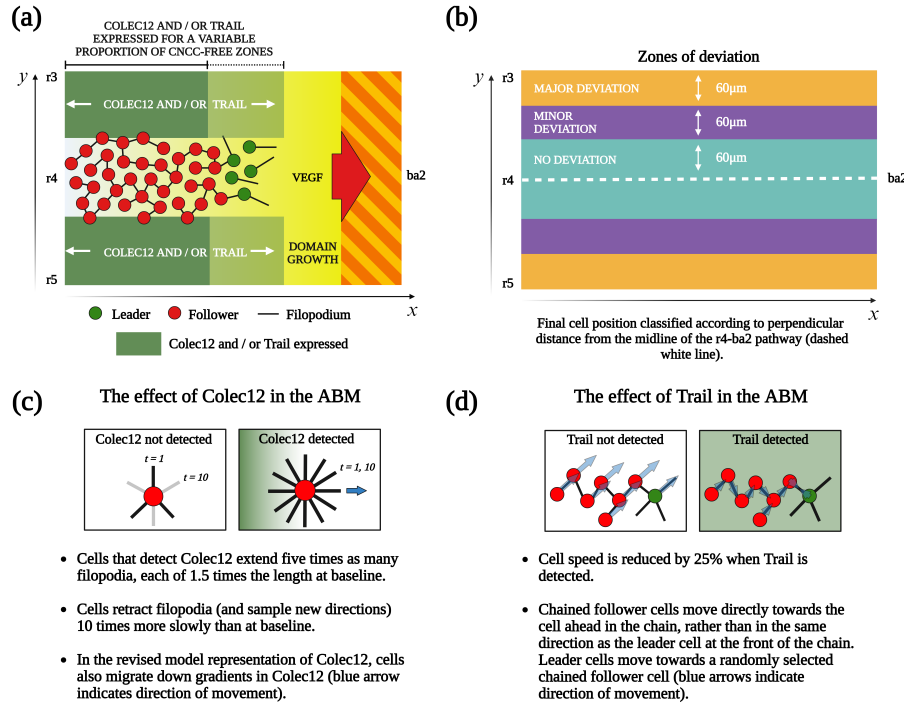


Figure 4.3: **(a)** Schematic of the expression patterns of Trail and Colec12 in the expanded ABM. Trail and/or Colec12 are expressed for a certain proportion of the CNCC-free zones adjacent to r3/5 in the x -direction, which is varied to study the effects of Trail and Colec12 expression on CNCC confinement. In the initial computational experiments where Trail and Colec12 are expressed for variable proportions of CNCC-free zones, Dan is not expressed anywhere in the domain, and VEGF is expressed everywhere in the domain. **(b)** Schematic showing zones of deviation in the ABM. The final position of CNCCs in the domain is classified by their perpendicular distance from the mid-line of the r4-ba2 pathway at $t = 24$ h. If a cell remains in the r4-ba2 pathway after 24 h, it lies in a zone of no deviation. Cells in CNCC-free regions within 60–120 μ m of the mid-line of the r4-ba2 pathway after migration are classified as having exhibited a minor deviation. All other cells are classified as having exhibited a major deviation in migration. **(c)** Schematic for the effects of Colec12 in the ABM. CNCCs that detect Colec12 extend five times as many filopodial protrusions that are each 1.5 times longer than at baseline. Filopodia are also retracted 10 times more slowly than at baseline. In the revised model representation of the interaction between Colec12 protein and CNCCs, CNCCs also undergo chemotaxis down concentration gradients in Colec12, driving movement away from regions of Colec12 expression back towards the r4-ba2 pathway. **(d)** Schematic for the effects of Trail in the ABM. Movement speed is reduced by 25% when cells detect Trail. Follower CNCCs that detect Trail also move directly towards the cell in front of them in a chain to represent an increase in cell-cell adhesion. Created in BioRender by Samuel Johnson: <https://BioRender.com/iggd3d9>.

In order to quantify the extent to which Colec12 confines CNCCs to the r4–ba2 pathway in model simulations, we define three regions of the r4–ba2 pathway and adjacent CNCC-free zones according to their distance from the central mid-line of the r4–ba2 pathway (Figure 4.3(b)). At $t = 24$ h, we classify CNCC positions in zones of no, minor, or major deviation according to the end point of their trajectory in the domain.

Simulations of the ABM show that CNCC interactions with Colec12 protein have a minimal effect on the overall confinement of migrating CNCCs to the r4–ba2 pathway. By varying the proportion of CNCC-free zones for which Colec12 is expressed (Figure 4.3(a)), we found no correlation between the proportion of CNCC-free zones for which Colec12 is expressed and the extent to which CNCCs remain confined to the r4–ba2 pathway for the 24 h period of migration (Figure 4.4). For all distances of Colec12 expression in CNCC-free zones, we found that approximately 35% of CNCCs lay in regions of minor or major deviation after migration ended. Furthermore, we found that this lack of confinement to the r4–ba2 pathway led to the frequent break-up of collectives and a subsequent breakdown in collective migration. This finding is suggestive of an additional mechanism of interaction between Colec12 and CNCCs that is responsible for their confinement during migration.

Motivated by the observation that CNCCs exposed to Colec12 *in vitro* frequently change direction and migrate back towards the neural tube explant (McLennan et al., 2023), we added a mechanism of chemotaxis in CNCCs that detect Colec12 protein, such that they migrate down concentration gradients of Colec12 in the model (Figure 4.3(c)). When this mechanism of interaction was added to the model representation of Colec12, a strong dependence between the extent of CNCC confinement to the r4–ba2 pathway and the proportion of CNCC-free zones for which Colec12 is expressed in CNCC-free regions emerged. We found that as the proportion of CNCC-free zones for which Colec12 is expressed increases, the overall confinement of CNCCs to the r4–ba2 pathway after the 24 h period of migration

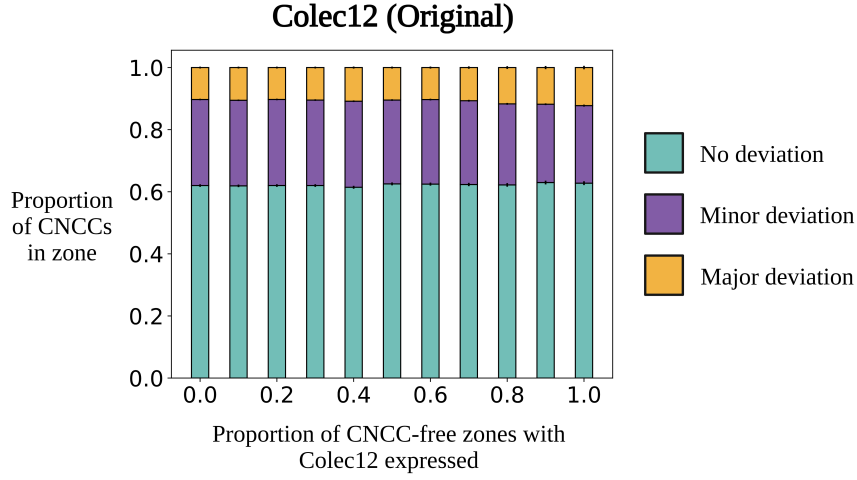


Figure 4.4: The confinement of migrating CNCCs to the r4–ba2 pathway as a function of the fraction of CNCC-free zones for which Colec12 is present in the original model representation of Colec12. Results are averaged over 500 simulations.

increases from approximately 65% confinement for no Colec12 expression in CNCC-free zones to approximately 95% confinement when Colec12 is expressed for the full length of CNCC-free zones (Figure 4.5(a)). Based on these observations, we hypothesise that chemotaxis down gradients in Colec12 protein is the primary mechanism through which Colec12 confines CNCCs to the r4–ba2 pathway during migration. The morphological effects of Colec12 on CNCCs observed *in vitro* may, therefore, be interpreted as mechanisms that increase the likelihood of gradient detection through the extension of longer, more branched filopodia.

To study the effect of Trail expression in CNCC-free zones adjacent to r3/5, we conducted a similar investigation by varying the fraction of CNCC-free zones for which Trail is expressed. *In vitro*, Trail reduces the speed at which CNCCs move and increases adhesion between CNCCs, resulting in an amoeboid-like movement (McLennan et al., 2023). To represent the effects of Trail on CNCCs in the ABM, we reduce cell speed upon the detection of Trail protein by 25% and alter the mechanism through which follower CNCCs move when they detect Trail. In the absence of Trail, follower cells in the ABM move by forming chains of cells with a leader at the front. If a follower cell lies in a chain at the time of movement, it moves in the same

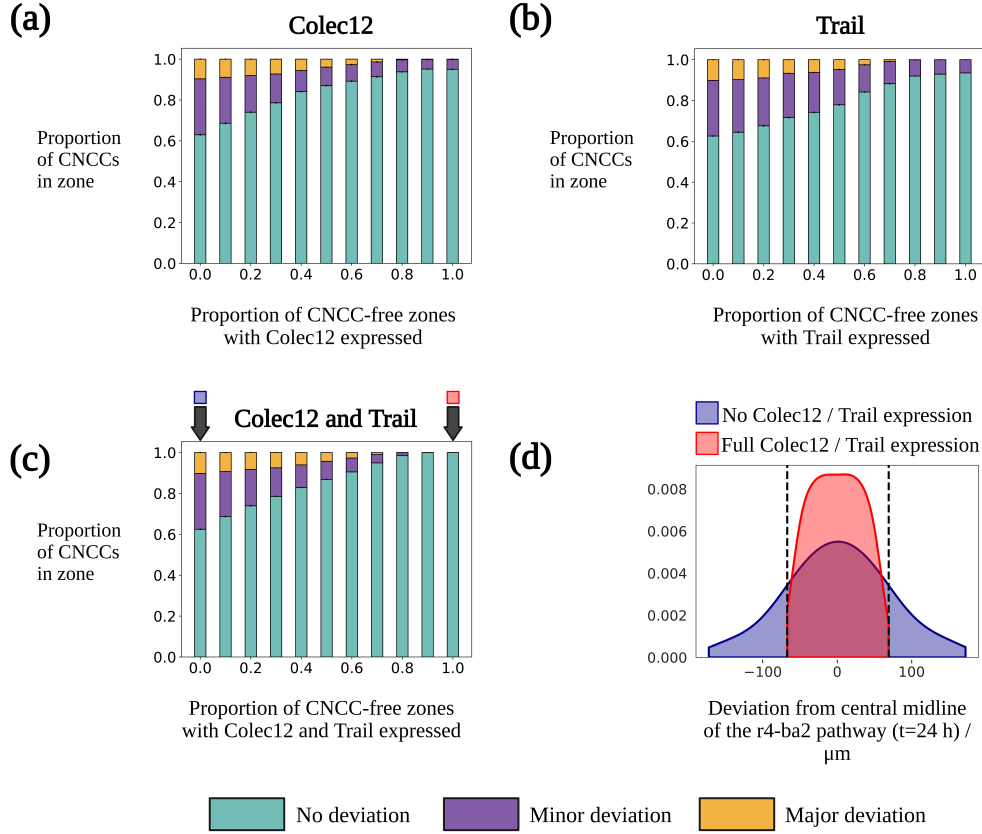


Figure 4.5: (a)-(c) The confinement of migrating CNCCs to the r4–ba2 pathway as a function of the fraction of CNCC-free zones for which Colec12, Trail, or Colec12/Trail are expressed. In all simulations, Dan is not present anywhere in the domain and VEGF is present everywhere in the domain. (d) Truncated kernel density estimates for the distribution of final CNCC y coordinates relative to the mid-line of the r4–ba2 pathway when Trail and Colec12 are not expressed and when they are expressed for the full length of CNCC-free zones. Dashed lines indicate the maximum and minimum deviations when Trail and Colec12 are expressed for the full length of CNCC-free zones. Both kernel density estimates are displayed only between the maximum and minimum values in each respective data set to avoid displaying non-zero densities for distances beyond extremal data points. The results for each parameter value in each plot are averaged over 500 simulations.

direction as the leader cell at the front of its respective chain. In regions where Trail is detectable, we represent an increase in cell–cell adhesion by altering the mechanism of follower cell movement, such that follower cells instead move directly towards the cell in front of them in their respective chain, directing CNCC movement towards areas of higher cell density, on average. A visual summary of the effects of Trail expression on CNCCs in the ABM can be seen in Figure 4.3(d).

As in the case of our modified model for *Colec12* expression in CNCC-free zones, we find a strong dependence between the proportion of CNCCs confined to the r4–ba2 pathway at 24 h and the fraction of CNCC-free zones adjacent to r3 and r5 for which Trail is expressed. Model simulations suggest that increasing the length over which Trail is expressed in CNCC-free zones increases the overall confinement of CNCCs to the r4–ba2 pathway from approximately 65% confinement for no Trail expression to approximately 90% confinement for expression along the entire length of CNCC-free zones (Figure 4.5(b)). These observations suggest that the effects of Trail on CNCCs noted *in vitro* are mechanisms through which Trail may confine CNCCs to the r4–ba2 pathway during migration. By increasing cell–cell adhesion, Trail may drive CNCC movement into regions of high cell density, and hence, towards the stream along the r4–ba2 pathway.

We concluded our investigation of the effects of Trail and *Colec12* expression in CNCC-free zones by investigating the combined effects of both Trail and *Colec12* expression in CNCC-free zones adjacent to r3 and r5 on the confinement of CNCCs to the r4–ba2 pathway. As in our prior investigations of the effects of either Trail or *Colec12* expression in CNCC-free zones, we conducted simulations in which both Trail and *Colec12* are expressed for a fraction of the CNCC-free zones adjacent to r3/5. Simulations of this model reveal that the proportion of CNCCs confined to the r4–ba2 pathway with both Trail and *Colec12* expressed is higher than in the cases of either Trail or *Colec12* expression in CNCC-free zones (Figure 4.5(c)). We find that the confinement of CNCCs to the r4–ba2 pathway increases from 65%

for no Trail or Colec12 expression in CNCC-free zones adjacent to r3 and r5 to almost 100% confinement for the expression of both Trail and Colec12 along the full length of CNCC-free zones. The expression of both Trail and Colec12 in the CNCC-free zones adjacent to the r4–ba2 pathway is, therefore, a mechanism that mitigates against large migratory defects by preventing the invasion of CNCC-free zones (Figure 4.5(d)).

To summarise, model simulations of Trail and/or Colec12 expression in CNCC-free zones adjacent to stereotypical migratory pathways suggest that Trail and Colec12 are complementary factors for the confinement of CNCCs to stereotypical migratory pathways during migration. In simulations, the confinement of CNCCs to stereotypical migratory pathways increases with the fraction of CNCC-free zones for which either factor is expressed. Furthermore, the confinement of CNCCs to stereotypical migratory pathways in model simulations is maximised when both Trail and Colec12 are expressed in CNCC-free zones.

4.4 Cranial neural crest cell-induced degradation of Dan increases stream coherence through differential speed modulation

Dan is a bone morphogenetic protein antagonist that has been found to exhibit a dynamic expression profile along stereotypical migratory pathways in chick (McLennan et al., 2017). Prior to migration, Dan is present throughout the regions parallel to the body axis adjacent to r1–7. However, after CNCCs populate the branchial arches, Dan concentration is lower in regions through which CNCCs have previously migrated. This observation suggests that Dan may be degraded by CNCCs during their migration, though this has not yet been verified experimentally. *In vitro*, CNCCs avoid regions of Dan expression and move more slowly when they detect Dan protein, suggesting that Dan may restrict the movement of CNCCs during mi-

gration by lowering their average speed and displacement. Simulations of a previous computational model support this hypothesis by suggesting that Dan prevents the escape of cells at the leading edge of CNCC collectives by modulating their speed (McLennan et al., 2017), which is necessary to ensure proper development in chick (McLennan et al., 2012). However, this computational model did not fully account for the dynamic concentration profile of Dan, in that the potential degradation of Dan by CNCCs was not accounted for and its concentration was assumed to be spatially uniform in a subregion of the domain through which migration occurs. This, in turn, did not permit a study of the effects of preferential, rather than uniform, speed modulation in cells at the leading edge of collectives which may arise due to interactions between Dan and CNCCs.

In order to investigate the mechanisms through which Dan promotes collective migration in the cranial neural crest, we represent Dan as a degradable chemical that is initially present for approximately the first one-third of the regions adjacent to r3–5 (Figure 4.2). As CNCCs move through the domain, they locally degrade Dan to produce a dynamic concentration profile in which the concentration of Dan is lower in regions through which CNCCs have previously migrated (Figure 4.6(a)). The speed of CNCCs is lowered in the presence of Dan according to Equation (4.2). We then repeated previous model simulations in which Trail and Colec12 are expressed for a certain fraction of CNCC-free zones adjacent to the r4–ba2 pathway (Figure 4.2) to study the effect of Dan on the frequency of leader escape during collective migration.

Model simulations show that the presence of Dan in the regions through which CNCCs migrate is essential for collective migration to occur by preventing the break-up of collectives. Furthermore, we find that the degradation of Dan is a mechanism that confers robustness on migration, in that dynamic Dan concentration profiles increase the range of CNCC-induced VEGF degradation rates for which collective migration is preserved (Figure 4.6(c)). When Dan is degraded by CNCCs, we find that it functions as a preferential modulator of speeds at the leading edge of CNCC

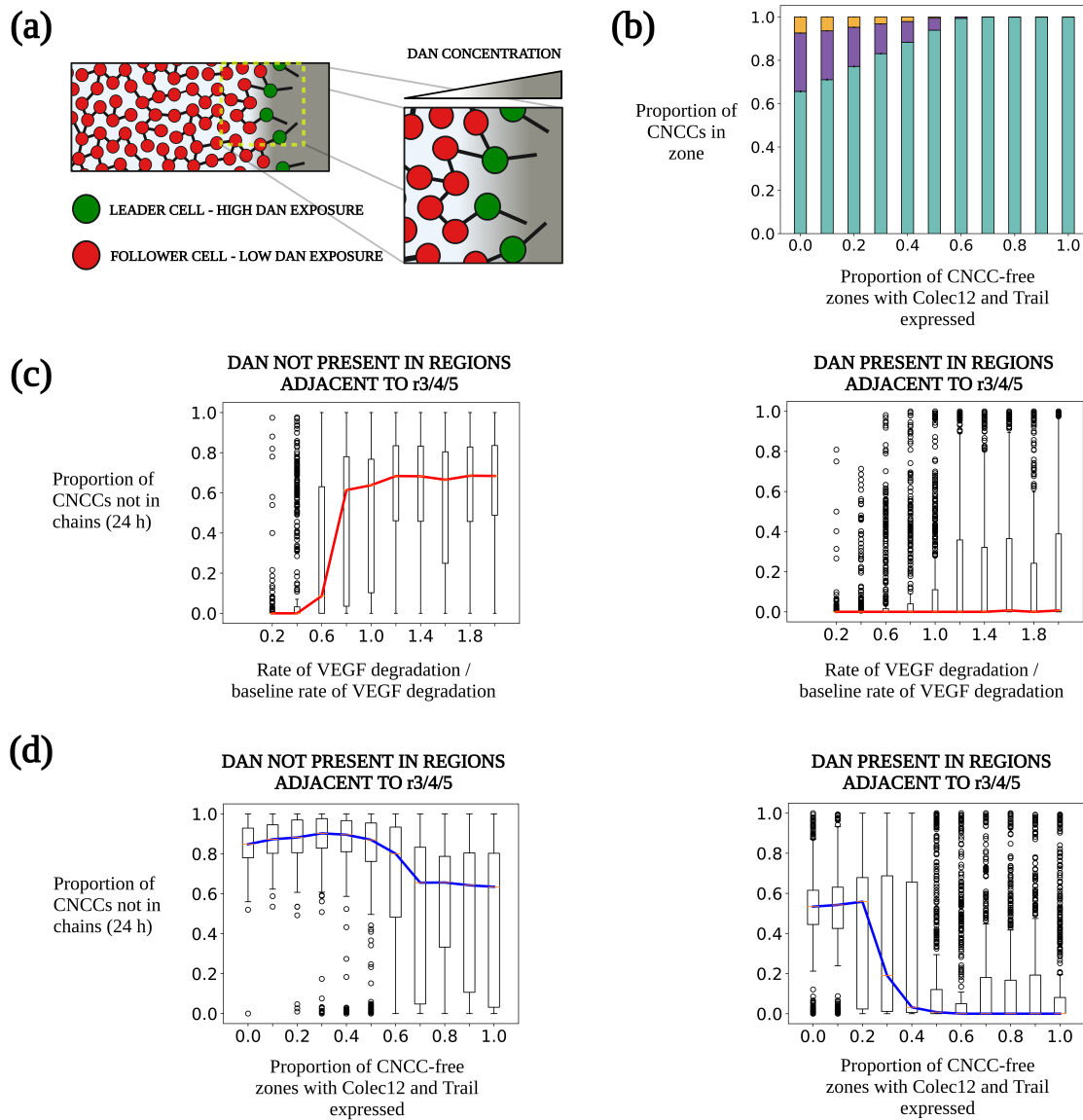


Figure 4.6: Caption overleaf.

Figure 4.6: **(a)** Schematic of leader cell speed modulation by Dan in CNCC migration. CNCCs at the leading edge of collectives invade regions in which Dan protein concentration is high, leading to a reduction in their average speed and displacement. Trailing cells in collectives migrate through regions of lower Dan concentration and hence move with a higher average speed, thus preventing a loss of contact with cells closer to the leading edge of streams. **(b)** The confinement of migrating CNCCs to the r4–ba2 pathway as a function of the fraction of CNCC-free zones for which Trail and Colec12 are expressed with Dan expressed for approximately the first one-third of the domain in the proximal-to-distal direction and VEGF expressed everywhere in the domain. **(c)** Boxplots of the proportion of CNCCs not in chains at $t = 24$ h as a function of VEGF degradation rate. When Dan is not present, high rates of VEGF degradation (and hence, the formation of VEGF gradients along which leader cells migrate) lead to a break-up of streams due to leader escape. When Dan is present in the first one-third of the domain, migration is robust to rapidly induced VEGF gradients. Here, Trail and Colec12 were expressed for the full length of the CNCC-free zones adjacent to r3/5. Red lines indicate the median proportion of CNCCs not in chains after 24 h, boxes indicate the upper and lower quartile values, and circles indicate outliers. The baseline rate of VEGF degradation in simulations is $2.75 \times 10^2 \mu\text{m}^2 \text{h}^{-1}$. **(d)** Boxplots of the proportion of CNCCs not in chains at $t = 24$ h as a function of the proportion of CNCC-free zones for which Trail and Colec12 are expressed. When Dan is not present in the domain, the median fraction of CNCCs not in chains after migration ends at $t = 24$ h remains above a value of 0.6 for all Trail/Colec12 expression lengths along CNCC-free zones adjacent to the r4–ba2 pathway. For Dan expression along the first one-third of the domain, the proportion of CNCCs not in chains after migration ends at $t = 24$ h exhibits phase transition-like behaviour as the length of CNCC-free zones for which Trail/Colec12 are expressed increases. When Trail/Colec12 are expressed for at least half of CNCC-free zones in the proximal-to-distal direction, the median proportion of CNCCs in chains when migration ends is 1. Blue lines indicate the median proportion of CNCCs not in chains after 24 h, boxes indicate the upper and lower quartile values, and circles indicate outliers. The results in figures **(b)**, **(c)**, and **(d)** are each aggregated over 500 simulations. Created in BioRender by Samuel Johnson: <https://BioRender.com/5v2aard>.

collectives (Figure 4.6(a)), such that it prevents the break-up of collectives when leader cell movement along VEGF gradients occurs too rapidly for coherent stream migration to occur in follower populations. In simulations where Dan is not present in the regions adjacent to r3/4/5, on average, at least 60% of CNCCs are not in chains when migration ends after a period of 24 h (Figure 4.6(d)). However, when Dan is present for the first one-third of the domain in the proximal-to-distal direction, we find that for Trail/Colec12 expression distances of greater than approximately one-half of the CNCC-free zones adjacent to r3/5, the median proportion of CNCCs in chains when migration ends is 1, in line with observations from a previous computational model that collective migration towards the branchial arches can occur for only partial expression of Trail and Colec12 in the regions adjacent to r3/5 (McLennan et al., 2023) (Figure 4.6(d)).

In summary, model simulations suggest that Dan expression along the r4–ba2 pathway is necessary to prevent the break-up of CNCC streams during migration due to the escape of cells at the leading edge of collectives. As CNCC collectives move along stereotypical migratory pathways, cells at the leading edge of collectives invade regions of high Dan expression, and, as a result, their speed is significantly reduced. Due to the degradation of Dan by CNCCs in the ABM, trailing cells migrate into regions of lower Dan concentration, such that their speed of movement is higher than that of leading cells, on average, thus preventing the escape of leader cells that results in a breakdown of collective migration. When Dan, Trail, and Colec12 are expressed in their typical patterns (Figure 4.1(b)), CNCC migration in the model is both highly confined to the r4–ba2 pathway due to the effects of Colec12 and Trail, and coherent in the sense that all cells, on average, remain in chains throughout migration towards ba2 due to speed modulation from Dan.

4.5 Trail and Colec12 expression in CNCC-free zones facilitates the exchange of cranial neural crest cells between adjacent collectives

Time-lapse imaging of chick cranial neural crest migration has revealed the exchange of a small number of CNCCs between streams emerging from regions adjacent to r4 and r6 (Kulesa and Fraser, 2000). Whilst streams adjacent to r4 and r6 remain largely separated during invasion of ba2 and ba3 *in vivo*, a small number of CNCCs within these streams invade the region lateral to the otic vesicle (OV) lying between the typical migratory pathways from r4 and r6 to ba2 and ba3, respectively. This results in the exchange of a small number of CNCCs between the streams along the r4–ba2 and r6–ba3 pathways over distances of 50–100 μm , without the breakdown of collective migration. Furthermore, Neuropilin 2 or Semaphorin 3F loss-of-function assays have demonstrated the formation of single-cell-wide bridges between streams invading ba1 and ba2, without large-scale disruption to migration (Gammill et al., 2007). Hitherto, the mechanisms underpinning these phenomena have not been explained through experiments or computational modelling.

Using the ABM for CNCC migration, we investigated the effects of Trail and Colec12 expression in the CNCC-free zone adjacent to r3 on the exchange of cells between streams invading ba1 and ba2. In order to study the exchange of CNCCs between streams, we modified the model geometry to include two streams adjacent to r2 and r4 that are separated by a CNCC-free zone adjacent to r3 in which Trail, Colec12, and Dan are expressed (Figure 4.7(a)). As before, VEGF is present everywhere in the domain (McLennan et al., 2010) and Dan is expressed for approximately the first one-third of the regions adjacent to all rhombomeres. In this revised model geometry, Trail and Colec12 are also expressed for the full length of the regions adjacent to r1/5 along the migratory pathway. The expression of Trail and Colec12 in the regions adjacent to r1/5 has not been confirmed experimentally,

but is included to keep streams confined to the regions adjacent to r2/4 during migration. However, the mechanistic inferences made in this section depend only on the expression of Trail/Colec12 in the region adjacent to r3, which has been observed *in vivo* (McLennan et al., 2023).

As before, migration occurs over a period of 24 h, and the rules governing cell movement in the ABM are unchanged. In simulations, we characterise the exchange of a cell between streams as having occurred when a cell migrates from its original stream, crosses the mid-line of the CNCC-free zone adjacent to r3, and joins the neighbouring stream. For exchange to have occurred, this cell must then remain in this new stream until migration ends after 24 h. We adopt this definition of cell exchange to prevent over-counting cell exchange when a CNCC fluctuates about the mid-line of the CNCC-free zone adjacent to r3.

Model simulations indicate that the probability of CNCC exchange between adjacent collectives depends primarily on the distance of separation between adjacent collectives (Figure 4.7(c)). In simulations, cells are exchanged between the streams adjacent to r2 and r4 when either Trail, Colec12, or Trail and Colec12 are present in the CNCC-free zone adjacent to r3. When only Colec12 is present, exchange occurs due to single-cell chemotaxis across the region adjacent to r3, when cells move into regions of Colec12 expression and extend filopodia into the adjacent stream where Colec12 is not expressed. However, in this context, we also find that both streams often develop diffuse edges which occasionally merge with one another, disrupting migration. Conversely, when Trail is present in the CNCC-free zone (by itself or in addition to Colec12), the mechanism of CNCC exchange is different and leads to the formation of CNCC bridges between the two streams without a large-scale disruption to migration. In this instance, the exchange of cells between the r4–ba2 and r2–ba1 pathways is observed when a cell loses directional information in its current stream due to a loss of cell–cell contact during migration (Figure 4.7(b)(i)). When this occurs, it is possible that filopodial protrusions extended by a cell proximal

to the CNCC-free zone adjacent to r3 detect a cell in the adjacent stream (Figure 4.7(b)(ii)). If a cell in an adjacent stream is detected, then movement towards this cell is induced by the increase in cell–cell adhesion in regions of enhanced Trail expression within the CNCC-free zone adjacent to r3 (Figure 4.7(b)(iii)). Any other cells in this chain that also lie in the CNCC-free zone move towards the cell they are connected to, forming a chain that migrates across the CNCC-free zone.

If only Trail is expressed in the CNCC-free zone adjacent to r3, we find that CNCC bridges form consistently for stream separation distances of approximately $24\text{ }\mu\text{m}$ or less (Figure 4.7(c)), which corresponds to the approximate maximum radius of communication for a cell in the model (the maximum length of a filopodial protrusion). If both Trail and Colec12 are expressed in the CNCC-free zone adjacent to r3, the frequency of exchange is slightly lower than in the case of only Trail, as a smaller number of cells initially invade the region adjacent to r3. However, exchange occurs over a larger range of separation distances (approximately $36\text{ }\mu\text{m}$), which corresponds to the increase in the length of filopodia extended by CNCCs exposed to Colec12 (Figure 4.7(c)). If only Colec12 is expressed in the CNCC-free zone adjacent to r3, exchange between streams occurs for a similar range of separations, but only due to single-cell migration down gradients in Colec12, and can lead to a disruption in migration when the diffuse edges of the two adjacent streams make contact.

Overall, simulations in this revised geometry suggest that CNCC exchange between adjacent streams can occur due to the hypothetical Trail and Colec12 mechanisms of action considered here. An increase in cell–cell adhesion upon the detection of Trail may cause the movement of cell chains across CNCC-free zones due to a loss of contact guidance in a cell’s current stream together with connections with the adjacent stream. The morphological effects of Colec12 noted *in vitro* may increase the likelihood of contact with adjacent streams due to the formation of longer filopodial protrusions that span the region separating adjacent streams. Cells can

also be exchanged between streams due to the effects of Colec12, though these exchanges represent single-cell migration across the CNCC-free zone, rather than the collective migration of chains observed *in vivo*. Here, we also note that Trail and Colec12 protein stripe assays analysed in prior studies of CNCC migration show cells stretching to lengths well beyond the maximum value prescribed in the ABM considered here (McLennan et al., 2023). However, CNCC lengths in these stripe assays were not precisely measured or quantitatively analysed, such that their values remain undetermined. If we were to reflect these observations in the ABM and increase the maximum radius of cell communication to larger values, then the width of the CNCC-free zone adjacent to r3 for which swapping occurs would likely increase to values comparable to the width of the CNCC-free zone adjacent to r3 *in vivo*. We highlight further study of the sizes of CNCCs exposed to Trail and Colec12 as a key experimental means of validating our proposed mechanism of CNCC exchange between adjacent streams.

4.6 Discussion

In this chapter, we used an ABM for CNCC migration to generate and test mechanisms for maintaining the coherence and spatial confinement of migrating CNCC streams. Our main objective was to study the effects of Trail, Colec12, and Dan on the confinement of CNCC streams to the stereotypical r4–ba2 pathway during migration. Prior *in vitro* studies have highlighted a change in the migratory dynamics and morphology of CNCCs cultured in the presence of Trail and Colec12 (McLennan et al., 2023). Furthermore, *in vivo* experiments have shown that loss-of-function of either Trail or Colec12 results in the increased diversion of chick CNCCs away from the stereotypical r4–ba2 migratory pathway into CNCC-free zones adjacent to r3 and r5. However, prior work in this context had not yet elucidated the mechanisms through which Trail and Colec12 confine CNCC streams to stereotypical migratory

pathways *in vivo*. To this end, we conducted model simulations of CNCC responses to Trail and Colec12 mechanisms of action on CNCC behaviours derived from *in vitro* observations.

Model simulations predict that the expression of Trail in CNCC-free zones confines CNCC streams to stereotypical migratory pathways by enhancing cell–cell adhesion, resulting in the migration of CNCCs that detect Trail back towards areas of higher cell density in the r4–ba2 migratory pathway. Conversely, equivalent simulations of the expression of Colec12 in CNCC-free zones adjacent to the r4–ba2 pathway predict that the effects of Colec12 on the protrusive dynamics of CNCCs observed *in vitro* (McLennan et al., 2023) have a negligible effect on the confinement of CNCCs to stereotypical migratory pathways. We then hypothesised a mechanism of chemotaxis in CNCCs, motivated by observations that CNCCs exposed to Colec12 migrate back towards the neural tube in chick, and found that this revised model increased the proportion of CNCCs confined to the r4–ba2 pathway throughout migration. As such, we predict that chemotaxis down gradients in Colec12 is the primary mechanism through which Colec12 restricts CNCC trajectories to stereotypical migratory pathways *in vivo* and that the morphological effects of Colec12 on CNCCs that have been observed *in vitro* are mechanisms that increase the likelihood of gradient detection in chemotaxis.

Model simulations also predict a synergy between Trail and Colec12, in that the confinement of CNCCs to stereotypical migratory pathways is maximised when both Trail and Colec12 are expressed in CNCC-free zones adjacent to the r4–ba2 pathway. In good agreement with prior studies (McLennan et al., 2023), we also find that high levels of spatial confinement of CNCCs to the r4–ba2 pathway are achieved by expressing Trail and Colec12 in approximately the first third ($\approx 400\ \mu\text{m}$) of the CNCC-free zones adjacent to r3 and r5. Collectively, model simulations suggest that both Trail and Colec12 are each factors that reduce the invasion of CNCCs into typically CNCC-free zones adjacent to r3 and r5. The Colec12 mechanism of action

hypothesised here acts on individual cells to drive movement along external chemical gradients and re-connections with streams along the r4–ba2 pathway. Conversely, the Trail mechanism of action proposed here alters cell–cell interactions within collectives, and hence primarily acts to keep CNCC streams as a whole within the region adjacent to r4. These two distinct mechanisms, therefore, maximise CNCC confinement in a complementary manner by driving both individual and collective CNCC migration towards the r4–ba2 pathway.

Previous experiments have highlighted the importance of Dan in collective chick CNCC migration (McLennan et al., 2017). *In vitro*, Dan has been found to modulate the speed at which CNCCs move. Moreover, *in vivo* experiments in the chick cranial neural crest have shown that Dan loss-of-function results in increased speed and directionality in CNCCs. Unlike Trail and Colec12, the expression profile of Dan extends parallel to the body axis alongside r1–7 and, intriguingly, the concentration of Dan is reduced in regions through which leader CNCCs have previously migrated. In the ABM, these observations were incorporated by modelling Dan as a degradable factor expressed adjacent to the neural tube, both along the stereotypical r4–ba2 migratory pathway and in the CNCC-free regions adjacent to r3 and r5. Simulations predict that while Trail and Colec12 prevent invasion into CNCC-free zones, they cannot prevent the frequent escape of cells at the leading edge of streams, resulting in the breakdown of directional guidance cues in streams and a subsequent separation of the stream. However, simulations in which Trail, Colec12, Dan, and VEGF are expressed in CNCC-free zones, and VEGF and Dan are expressed in the regions adjacent to r3–5, suggest that Dan decreases the frequency of break-up of CNCC collectives by preferentially reducing the speed of cells at the leading edge of collectives to prevent their escape. In the model, Dan differentially modulates CNCC speeds as a result of its degradation by CNCCs, in that cells at the leading edge of collectives are exposed to higher levels of Dan expression, and hence, have their speeds reduced by a greater amount than trailing cells that migrate through regions

in which Dan has previously been degraded. Whilst previous models of CNCC migration have reproduced collective migration without considering the effects of Dan (McLennan et al., 2012, 2015a, 2023), successful migration in these models required VEGF degradation by CNCCs (and hence, the formation of VEGF gradients) to occur at sufficiently slow rates to prevent leader escape. The addition of Dan into the ABM considered here increased the range of feasible VEGF degradation rates for collective migration to occur. As such, we predict that both the expression of Dan along stereotypical migratory pathways and the degradation of Dan by CNCCs are mechanisms that confer robustness in collective invasion of the branchial arches. If Dan is expressed along stereotypical migratory pathways, and Trail/Colec12/Dan are expressed in adjacent CNCC-free zones, we find that CNCCs remain in streams confined to stereotypical migratory pathways that collectively invade the branchial arches for a wide range of VEGF degradation rates (and hence, maximum speed of movement of CNCCs at the leading edge).

A final prediction of the ABM is that Trail and Colec12 can drive a small number of cells to divert into CNCC-free zones and connect neighbouring streams through single-cell-wide strands without disrupting the overall migratory pattern. The exchange of single cells between streams has previously been observed in chick, where contact between CNCCs in adjacent streams is found to occur infrequently but consistently throughout migration (Kulesa and Fraser, 2000). Furthermore, in mice carrying null mutations of either *Npn2* or *Sema3F*, single-cell-wide bridges have been observed to form between streams invading *ba1* and *ba2* (Gammill et al., 2007). Hitherto, this phenomenon has not been explained either with experiments or computational models. We predict that for adjacent collectives within approximately four CNCC radii ($30\ \mu\text{m}$) of one another (the approximate range of cell-cell interactions in regions of Colec12 expression), the effects of Trail and Colec12 on CNCC migratory behaviour facilitate contact between cells in adjacent streams. When a cell loses directional information in its current stream, it is possible that a cell in an

adjacent stream is detected through the extension of filopodial protrusions. If this occurs, then cells that detect Trail begin to move towards the cell detected in an adjacent stream, leading to movement across a CNCC-free zone and into a neighbouring collective. We find that the exchange of CNCCs between adjacent streams increases as the distance between collectives is reduced, as contact between CNCCs in adjacent collectives occurs at a higher frequency. This effect is also observed when only Trail is expressed in CNCC-free zones, though adjacent streams must be closer to one another for this to occur at the same frequency as when Colec12 is also expressed (due to the reduced range of interactions in CNCCs). We believe that this mechanism presents a possible explanation for the formation of CNCC bridges between adjacent collectives observed *in vivo* that is, hitherto, without a mechanistic explanation.

Each of the biological hypotheses generated here merits further experiments to validate our predictions. Our hypothesis that Trail confines CNCC movement to stereotypical migratory pathways may be tested experimentally through studying the secretome of CNCCs cultured in the presence of Trail. If an upregulation of chemokines associated with cell clustering is observed in the secretome of CNCCs exposed to Trail, then it is likely that enhanced cell clustering is a primary mechanism through which Trail confines CNCCs to stereotypical migratory pathways *in vivo*. Furthermore, the exposure of CNCCs to Colec12 gradients in culture would provide a simple means of validating our hypothesis of CNCC chemotaxis down Colec12 gradients. Additionally, Trail and Colec12 stripe assays in which the width of Colec12/Trail-positive stripes separating adjacent CNCC streams is varied would allow for the validation of the prediction that the distance at which adjacent CNCC streams are separated by regions of enhanced Trail and Colec12 expression is the major determinant in the formation of single-cell-wide bridges between adjacent streams. It would also be desirable to measure the maximum cell length in these stripe assays, to determine if the putative mechanisms of CNCC exchange in the

ABM are sufficient for the exchange observed over longer distances *in vivo*. A final testable hypothesis generated by this model is that the degradation of Dan by CNCCs would result in a higher number of CNCC exchanges between adjacent streams in regions of the domain where exchanges have previously occurred, due to a localised loss of speed modulation from Dan. This hypothesis could be easily tested in modified Trail and Colec12 protein stripe assays where Dan protein is also present.

Appendix

A Expanded model pseudocode

Below is revised model pseudocode for the ABM including explicit representation of Colec12, Trail, and Dan expression in CNCC migration.

A.1 Main ABM loop

```
1  initialise parameters
2  initialise leaders
3  for t from 6 h to 24 h:
4      insert_follower()
5      solve_chemicals()
6      grow_domain()
7      move_cells()
```

A.2 Main loop functions

```
1  FUNCTION insert_follower():
2      if t == insertion time and space available:
3          insert follower at LHS of domain (random y coordinate)
4
5  FUNCTION solve_chemicals():
6      solve Eq (4.1) for Colec12, Trail, Dan, VEGF
7
8  FUNCTION grow_domain():
9      increase domain length, advect cells
10
11 FUNCTION move_cells():
12     for each cell in random order:
13         generate new filopodia directions if required
14         if cell is a leader:
15             move_leader(cell)
16         elif cell is a follower:
17             move_follower(cell)
18             check_phenotype(cell)
```

A.3 Helper functions

```
1 FUNCTION is_detected(chemical):
2   if chemical concentration at cell centre  $\geq$  threshold:
3     return True
4   else:
5     return False
6
7 FUNCTION move_leader(cell):
8   hasC  $\leftarrow$  is_detected(Colec12)
9   hasT  $\leftarrow$  is_detected(Trail)
10  if VEGF gradient detected by at least one filopodium:
11    bestVEGF  $\leftarrow$  direction of largest detected VEGF gradient
12  else:
13    bestVEGF  $\leftarrow$  random direction
14  if negative Colec12 gradient detected by at least one filopodium:
15    bestColec12  $\leftarrow$  direction of largest detected negative Colec12 gradient
16  else:
17    bestColec12  $\leftarrow$  random direction
18  if hasC and hasT:
19    move in average direction of bestColec12 and direction of a random attached follower
20  elif hasC and not hasT:
21    move in direction of bestColec12
22  elif not hasC and hasT:
23    move in direction of a random attached follower cell
24  else:
25    move in direction of bestVEGF
26
27 FUNCTION move_follower(cell):
28   hasC  $\leftarrow$  is_detected(Colec12)
29   hasT  $\leftarrow$  is_detected(Trail)
30   if cell is chained:
31     move_chained_follower(cell, hasC, hasT)
32   else:
33     move_free_follower(cell, hasC, hasT)
34
35 FUNCTION move_chained_follower(cell, hasC, hasT):
36   if negative Colec12 gradient detected by at least one filopodium:
37     bestColec12  $\leftarrow$  direction of largest detected negative Colec12 gradient
38   else:
39     bestColec12  $\leftarrow$  random direction
40   if cell too far from cell it is attached to in chain:
41     detach cell from chain
```

```

42     elif hasC and hasT:
43         move in average direction of bestColec12 and direction of cell ahead in chain
44     elif hasC and not hasT:
45         move in direction of bestColec12
46     elif hasT and not hasC:
47         move towards cell ahead in chain
48     else:
49         move in same direction as leader at front of chain
50
51 FUNCTION move_free_follower(cell, hasC, hasT):
52     if negative Colec12 gradient detected by at least one filopodium:
53         bestColec12 ← direction of largest detected negative Colec12 gradient
54     else:
55         bestColec12 ← random direction
56     if hasC:
57         move in direction of bestColec12
58     elif within detection distance of chained cell:
59         join chain and move towards cell ahead in the chain
60     else:
61         move in random direction
62
63 FUNCTION check_phenotype(cell):
64     if cell is a follower and further from r4 than any leader by one cell length:
65         switch leader and follower phenotypes

```

Chapter 5

The spontaneous emergence of leaders and followers during cranial neural crest cell migration

In this chapter, we formulate a novel ABM of CNCC migration to investigate the spontaneous emergence of phenotypic heterogeneity in CNCC streams during migration. Transcriptional analyses of genes essential for CNCC migration in chick have revealed distinct gene expression profiles between cells in leading and trailing stream positions (McLennan et al., 2012) (Section 2.2). These findings have been used to motivate many leader–follower ABMs of CNCC migration, in which the behaviour of cells is determined by a binary phenotypic classification.

However, a later whole-transcriptome analysis in chick demonstrated that CNCC gene expression varies in a continuous, rather than binary, fashion within streams invading ba2 (Morrison et al., 2017; Schumacher, 2019b). Furthermore, an ABM based on these findings was used to demonstrate that a continuous phenotype ABM of CNCC migration can reproduce streams consistent with those observed *in vivo*, under certain conditions (Schumacher, 2019b). These findings challenge the assumption of the leader–follower phenotypic division in many mathematical models of CNCC migration and suggest that models accounting for continuous phenotypic variation may more accurately capture the spatial and behavioural heterogeneity between CNCCs observed *in vivo*.

In what follows, we begin by discussing the experimental and mathematical evidence for continuous CNCC phenotypic variation within streams invading ba2. Then, to study the emergence of distinct phenotypes during migration, we formulate a *spring–polarisation* ABM of CNCC movement, in which cell phenotype is contin-

uous and determined by time-averaged VEGF exposure. Subsequently, we conduct numerical model simulations to study phenotypic variation within CNCC streams as a function of various environmental and cell-intrinsic parameters. After analysing the model and demonstrating the robustness of an approximate leader–follower phenotypic division within streams, we also demonstrate the consistency of this model with previous CNCC ablation and transplantation experiments (McLennan et al., 2012; Richardson et al., 2016) and propose additional biological mechanisms that could be incorporated into the model.

Comment on originality: Work in this chapter is based on the manuscript

Johnson, S.W.S., Baker, R.E. and Maini, P.K., 2026. The spontaneous emergence of leaders and followers in a mathematical model of cranial neural crest cell migration. *arXiv preprint arXiv:2601.00374*,

which is currently under peer review in *Journal of Theoretical Biology*.

Author contribution: The author of this thesis conceived this project, carried out the model formulation and analysis, and wrote the associated manuscript, which was revised with Philip Maini and Ruth Baker.

Code availability: Code used for the generation of the results in this chapter can be found at <https://github.com/SWSJChCh/cranialEmergence>.

5.1 Cranial neural crest cell transcriptomic heterogeneity and plasticity *in vivo*

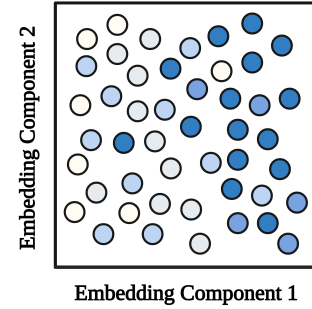
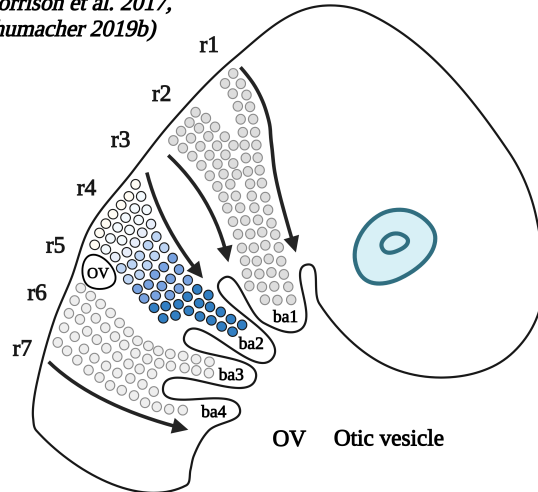
We begin by reviewing the existing experimental evidence regarding transcriptomic and phenotypic heterogeneity and plasticity during CNCC migration *in vivo*.

5.1.1 Cranial neural crest cell transcriptomic heterogeneity

In chick, previous experimental studies have shown that the transcriptome of CNCCs varies within streams in a manner correlated with their relative stream positions (McLennan et al., 2012; Morrison et al., 2017) (Section 2.2). These experiments were originally used to argue in favour of discrete classifications of CNCCs into leaders and followers (McLennan et al., 2012) and later, leaders, followers and trail-blazers (Morrison et al., 2017). However, a subsequent mathematical modelling study re-examining the same transcriptomic data demonstrated that the underlying transcriptomic variation within CNCC streams is continuous rather than discrete (Schumacher, 2019b) (Figure 5.1). In this study, principal component analysis (PCA) was used to project whole-transcriptome data into a high-dimensional feature space, which was then embedded into two dimensions using a force-directed layout of a k -nearest-neighbour graph (Weinreb et al., 2018). This embedding revealed a continuum of transcriptional states that were correlated with stream position *in vivo*. These observations were, in turn, used to motivate a continuous phenotype ABM of CNCC migration, which reproduces typical CNCC stream migration under specific parameter regimes (Schumacher, 2019b) (Section 5.2).

These experimental findings raise questions regarding the validity of the leader–follower structure of many previous ABMs of CNCC migration; if transcriptomic heterogeneity is continuous within streams, then it remains unclear as to whether collective behaviour *in vivo* can be appropriately captured by mathematical models that project phenotypic heterogeneity onto a binary leader–follower division.

(Morrison et al. 2017,
Schumacher 2019b)



12,909 genes screened. 59-component principal component analysis (PCA). PCA embedded in two dimensions. Cell position in embedding space varies continuously.

Figure 5.1: Schematic of the full-transcriptome (14,299 gene) single-cell RNA sequencing analysis (Morrison et al., 2017) and subsequent continuous transcriptomic analysis in chick (Schumacher, 2019b). In this study, CNCCs were isolated using FACS and analysed transcriptomically. Subsets of genes were found to be correlated with relative stream position; a novel transcriptional signature of ~900 genes was detected for trailblazer cells at the leading edge of streams. Cells at the trailing edge expressed fewer differentially expressed genes overall, while those in frontal regions just behind the leading edge exhibited transcriptomic profiles that were intermediate between trailblazers and trailing cells. In a later reanalysis of the same dataset (Schumacher, 2019b), principal component analysis (PCA) of the 14,299 measured genes (12,909 after preprocessing) revealed that transcriptional variation among cells was best captured by 59 principal components. When projected into a two-dimensional embedding, cells from trailing, leading, and invasive-front stream positions occupied a continuous manifold in gene expression space, in contrast to the discrete classifications in earlier studies. This continuum recapitulated their approximate spatial order within the migratory stream, thereby supporting the interpretation that CNCC molecular identities vary gradually in space rather than as discrete positionally-correlated states. Created in BioRender by Samuel Johnson: <https://BioRender.com/o93f7u5>.

5.1.2 Cranial neural crest cell transcriptomic plasticity

There is substantial experimental evidence that the transcriptomic variation observed within CNCC streams is not pre-determined or fixed, but instead arises due to differences in the local microenvironment of CNCCs during migration. For example, CNCCs cultured *in vitro* do not exhibit leader-like or follower-like transcriptional profiles when deprived of the cues they normally encounter *in vivo* (McLennan et al., 2015a). However, exposure to VEGF in culture induces the up-regulation of genes characteristic of leading CNCCs *in vivo*, suggesting that VEGF promotes the acquisition of leader-like and trailblazer phenotypes observed in the transcriptional analyses.

Furthermore, transplantation experiments conducted in chick (McLennan et al., 2012) in which a small number of follower-like cells were transplanted to the leading edge of streams showed that transplantation into VEGF-rich environments induces a transcriptomic transition in follower-like cells to leader-like transcriptomes, wherein genes associated with VEGF signalling, ECM attachment, and ECM degradation are upregulated. Additionally, experiments in which cells at the leading edge of CNCC streams were ablated during migration demonstrated that collective migration can persist upon the removal of cells in leading positions (Richardson et al., 2016), suggesting that all CNCCs within streams share an equal capacity for leader-like behaviour that guides streams towards the branchial arches.

Given these observations, it remains an open question as to whether phenotypic heterogeneity arises spontaneously during collective CNCC migration, whether this variation remains broadly stable over the period of migration, and whether it can be reasonably approximated by a leader–follower phenotypic division.

5.2 Cranial neural crest cell phenotypic plasticity in mathematical models

Many ABMs of collective CNCC migration incorporate mechanisms of phenotypic plasticity. In several formulations of the ABM outlined in Chapter 2 (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2020, 2023), a fixed number of cells are selected as leaders prior to migration (n_1 , say). As cells migrate, their phenotypes are updated according to their relative position in streams; the n_1 cells with the largest x -coordinate (or a similar statistic indicative of relative stream position) are selected as leaders, and all others as followers. This positional rule constrains leader cell behaviours to regions of maximal VEGF concentration, on average, and thereby approximates the follower-to-leader transition observed in CNCCs exposed to VEGF *in vitro* (McLennan et al., 2015a).

An alternative implementation of phenotypic plasticity replaces positional switching rules with an *integrate-and-switch* mechanism (McLennan et al., 2015a, 2017). In this formulation, the size of the leader cell subpopulation is not predetermined. Instead, each cell maintains an internal variable that increases when sensed VEGF gradients exceed a detection threshold and decays when they do not. The rate of increase and decay of this cell-specific variable is governed by two independently specified timescales corresponding to follower-to-leader and leader-to-follower transitions. As the threshold values for leader-to-follower and follower-to-leader transitions differ, this model formulation introduces hysteresis into phenotypic transitions; cells switch phenotype only when sustained exposure (or lack thereof) to a detectable VEGF gradient drives the internal state variable across the appropriate boundary. This memory-based rule yields variable, rather than preassigned, numbers of leader cells during migration, more accurately capturing VEGF-dependent phenotype switching during migration. However, this formulation, and previous positional switching formulations, divide CNCC streams into strict leader and follower

subsets and hence, do not represent the intermediate phenotypes observed in prior transcriptomic analyses (Schumacher, 2019b).

An alternative ABM of CNCC migration relaxes the assumption of discrete leader and follower cell states entirely (Schumacher, 2019b). In this continuous-phenotype framework, all CNCCs are governed by a single set of movement rules based on local cell–cell guidance cues and instantaneous exposure to VEGF gradients. The relative contribution of these signals is determined directly by the magnitude of the VEGF gradient sensed by each cell. This is implemented either by allowing cells to stochastically select between chemotactic and cell–cell guidance cues at the point of movement or by combining the two through a gradient-dependent weighting. Under certain conditions, this model reproduces the behaviour of prior leader–follower ABMs of CNCC migration, with invasion occurring over biologically relevant distances in a 24 h period. However, as cell movement occurs due to instantaneous cues, this model loses the notion of stable subpopulations during migration, such that it is not possible to identify cells as leader-like or follower-like over the course of migration.

Here, we build on the principles underlying both continuous-state and leader–follower model formulations to develop a unified *spring–polarisation* framework in which all cells obey an identical set of movement rules but exhibit emergent behavioural heterogeneity through a cell-specific internal vector that evolves according to their time-averaged exposure to VEGF gradients. The magnitude of this vector modulates the relative influence of chemotactic and cell–cell guidance cues in the movement of each cell and thereby generates a continuum of behaviours within an otherwise homogeneous rule set. As such, the model provides a means of quantitatively characterising phenotypic variation across CNCC streams and enables a systematic investigation of how phenotypic heterogeneity arises and propagates during collective migration.

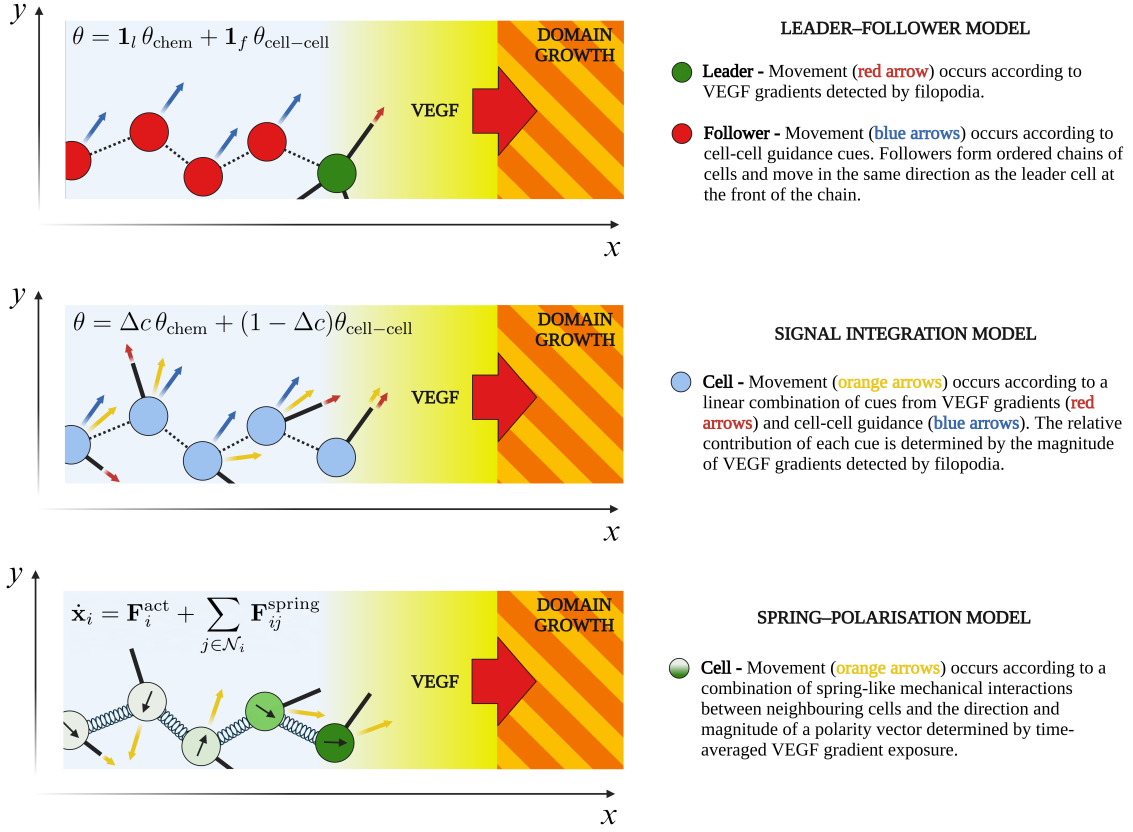


Figure 5.2: Schematics of leader–follower (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2012, 2015a, 2017, 2020, 2023) and signal integration (Schumacher, 2019b) models, together with the spring–polarisation model of CNCC migration formulated here. In leader–follower models, a fixed subset of leader cells at the front of streams move according to VEGF gradients detected in the cranial microenvironment. Follower cells, in weaker gradients of VEGF, move according to cell–cell guidance cues, forming connected chains of follower cells led by a leader cell at the front. In the signal integration model, all cells migrate according to a combination of VEGF and cell–cell guidance cues. The movement of each cell is determined by a linear combination of these two mechanisms of movement, with the relative contribution of each mechanism governed by the magnitude of the VEGF gradient detected at the time of movement. In the spring–polarisation model, all cells interact with their neighbours via spring-like mechanical interactions and carry a cell-specific polarity vector that evolves in time according to VEGF gradient sensing. Movement occurs according to a combination of forces from spring-like interactions and a force determined by the orientation and magnitude of the polarity vector. Created in BioRender by Samuel Johnson: <https://BioRender.com/qmkcncz>.

5.3 A spring–polarisation agent-based model of cranial neural crest cell migration

We formulate a stochastic ABM representing the migration of chick CNCCs over a

period of 24 h. In the model, CNCCs are represented as point particles whose movement is governed by spring-like pairwise mechanical interactions with their neighbours and the magnitude and orientation of a cell-specific polarity vector that evolves according to time-averaged exposure to VEGF gradients. Here, the cell-specific polarity vector represents the response of CNCCs to timed exposure to VEGF, which has been shown to induce rapid phenotypic transitions *in vitro* (McLennan et al., 2015a). In this model formulation, we avoid the *a priori* prescription of cell phenotypes used in prior ABMs of CNCC migration (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2012, 2015a, 2017, 2020, 2023), in that all cells are governed by the same set of movement rules. However, we allow for the emergence of distinct phenotypes through differences in the time evolution of VEGF-induced polarity in each cell, which determines the relative contributions of chemotaxis and cell-cell interactions towards movement. As in the prior signal integration ABM of CNCC migration (Schumacher, 2019b), cells move according to a combination of cues from cell-cell interactions and VEGF gradients, though in the model considered here, cell movement depends on the results of environmental sampling over a finite time interval, rather than on instantaneous cues detected at the time of movement. In doing so, we introduce a notion of memory into CNCC movement, which, in turn, allows individual CNCCs to integrate fluctuating local cues into migratory behaviours that persist over time. This memory-dependent movement mechanism enables transient heterogeneities in VEGF exposure or cell density to generate sustained collective migration patterns without the explicit assignment of leader or follower phenotypes. Consequently, the model demonstrates how the emergence of coordinated CNCC migration and phenotypic variation can be viewed as emergent properties of the underlying VEGF and mechanical movement mechanisms. A visual summary of previous leader-follower and signal integration models, alongside the spring-polarisation model formulated here, is shown in Figure 5.2.

5.3.1 Migratory domain and VEGF dynamics

The domain through which CNCC migration occurs and the dynamics of VEGF within the domain are primarily as described in the leader–follower ABM of CNCC migration outlined in Section 2.3. However, to avoid artificial VEGF-induced polarisation of CNCCs at the left-hand boundary of the migratory domain, we impose zero-Dirichlet, rather than zero-flux, boundary conditions on VEGF at $x = 0$ and $x = L_x(t)$.

5.3.2 Cell sensing, polarity, and heading dynamics

Each cell, indexed by i , is represented as an autonomous agent that samples its local environment through a fixed number of filopodia, which are used to infer the direction of the steepest local positive VEGF gradient.

As in the ABM described in Chapter 2, no cells are present in the domain between $0 \text{ h} \leq t < 6 \text{ h}$. At $t = 6 \text{ h}$, a small cohort of cells is initialised along the domain boundary at $x = 0$, with evenly spaced y -coordinates spanning $y \in [0, L_y]$. Thereafter, new cells are proposed for insertion at $x = 0$ in intervals of $1/60 \text{ h}$, each with a randomly assigned y -coordinate. A proposed insertion is accepted only if the inserted cell lies at least two cell radii ($15 \mu\text{m}$) from all other cells.

At each time step, every cell samples $n_f = 5$ orientations, $\{\phi_k\}_{k=1}^{n_f}$, within a circular region of a radius equal to the filopodial length, $\ell_f = 27.5 \mu\text{m}$. These values of n_f and ℓ_f are comparable with previous models of CNCC migration (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2012, 2015a, 2017, 2020, 2023; Schumacher, 2019b).

For each orientation ϕ_k at each time step, each cell computes the mean VEGF concentration along the corresponding filopodium

$$c_f(\phi_k) = \frac{1}{\ell_f} \int_0^{\ell_f} c(\mathbf{x}_i + r(\cos \phi_k, \sin \phi_k)) \, dr, \quad (5.1)$$

and compares this to the concentration of VEGF at its current position, $c(\mathbf{x}_i)$. The direction that maximises $c_f(\phi_k)$, $\phi_{\max} = \arg \max_{\phi_k \in \{\phi_1, \dots, \phi_{n_f}\}} c_f(\phi_k)$, defines an instantaneous chemotactic direction $\hat{\mathbf{u}}_{\text{chemo}} = (\cos \phi_{\max}, \sin \phi_{\max})$.

A relative cue, σ , and detection threshold, v , based on prior ABMs (Section 2.3.4), are then computed as

$$\sigma = \frac{c_f(\phi_{\max}) - c(\mathbf{x}_i)}{c(\mathbf{x}_i)}, \quad v = \xi \sqrt{\frac{c_0}{c(\mathbf{x}_i)}}, \quad (5.2)$$

where c_0 is the initial concentration of VEGF in the domain, and $\xi > 0$ is a dimensionless parameter that controls the sensitivity threshold for gradient detection. The chemotactic activation variable, $\chi \in [0, 1]$, quantifies the strength of a cell's response to detected VEGF cues. It is computed from the relative concentration signal, σ , and the sensitivity threshold, v , such that chemotactic responses are inactive when the chemotactic signal is below a threshold value and increase linearly when the signal is above it, before saturating at unity

$$\chi = \begin{cases} 0, & \sigma \leq v, \\ \min\left(1, \frac{\sigma - v}{\gamma_{\text{sat}} v}\right), & \sigma > v. \end{cases} \quad (5.3)$$

The parameter γ_{sat} , therefore, determines how rapidly chemotactic responses saturate at a maximum once the threshold for gradient detection is exceeded. In simulations, we fix $\gamma_{\text{sat}} = 0.5$, meaning a maximum chemotactic response is achieved when detected gradients are at least 1.5 times the magnitude of the threshold for gradient detection. This is an arbitrary parameter choice used to reduce the dimensionality of the free model parameter space. However, all trends discussed in later sections are robust to variations in this parameter.

Each cell stores a two-dimensional polarity memory vector, $\mathbf{m}_i$, that evolves according to an ODE with a characteristic relaxation time, $\tau_{\text{sense}} > 0$,

$$\dot{\mathbf{m}}_i(t) = \frac{1}{\tau_{\text{sense}}} (\chi \hat{\mathbf{u}}_{\text{chemo}} - \mathbf{m}_i(t)), \quad (5.4)$$

where $\hat{\mathbf{u}}_{\text{chemo}}$ is the direction vector of the highest measured VEGF gradient. Similar representations of cell polarity dynamics have been implemented in other mathematical models of collective cell chemotaxis (e.g. Camley et al. (2016)). In simulations, we set $\tau_{\text{sense}} = 0.1 \text{ h}$, corresponding to a characteristic timescale of approximately six minutes for polarity reorientation. This parameter choice reflects the rapid VEGF-dependent transcriptional and behavioural responses observed *in vitro*, where gene expression changes were detected within less than ten minutes of VEGF withdrawal or re-exposure (McLennan et al., 2015a).

At each time step, the magnitude of the memory vector, $\|\mathbf{m}_i\|$, determines the magnitude of active forces generated by chemotactic gradients, while the memory angle, $\theta_{\text{mem}} = \arg(\mathbf{m}_i)$, sets the preferred heading for chemotactic movement when $\|\mathbf{m}_i\| > 0$. The instantaneous cell orientation, θ_i , relaxes towards θ_{mem} with an angular rate k_{chemo}

$$\dot{\theta}_i(t) = k_{\text{chemo}} \sin(\theta_{\text{mem}}(t) - \theta_i(t)), \quad \theta_i(t) \in \mathbb{R}/2\pi\mathbb{Z}, \quad (5.5)$$

which is an angular relaxation model that has previously been used in models of cell migration and polarity (Sliusarenko et al., 2007; Zhang et al., 2018). In simulations, we set $k_{\text{chemo}} = 10 \text{ h}^{-1}$, corresponding to a characteristic relaxation timescale of approximately six minutes. This value once again reflects the rapid transcriptional responses observed in CNCCs exposed to ectopic sources of VEGF *in vitro* (McLennan et al., 2015a) and is also consistent with ABM simulations that predict maximal migratory efficiency in collectives when phenotype switching occurs on the order of minutes (McLennan et al., 2015a). Further details on the implementation of VEGF polarisation in numerical model simulations are given in Appendix A.

5.3.3 Over-damped active mechanics

Cells are represented as point particles located at positions $\mathbf{x}_i = (x_i, y_i)$ within the two-dimensional migratory domain, $L_x(t) \times L_y$. Movement occurs in the over-damped limit, where inertial effects are negligible. The instantaneous velocity of each cell satisfies

$$\dot{\mathbf{x}}_i = \mathbf{F}_i^{\text{act}} + \sum_{\substack{j \in \mathcal{N}_i \\ j \neq i}} \left(\mathbf{F}_{ij}^{\text{spring}} + \mathbf{F}_{ij}^{\text{rep}} \right), \quad (5.6)$$

where $\mathbf{F}_i^{\text{act}}$ is the polarity-driven active force, $\mathcal{N}_i$ is the neighbourhood of cell i , $\mathbf{F}_{ij}^{\text{spring}}$ represents an effective adhesive interaction between neighbouring cells (for example, cadherin-mediated adhesion between CNCCs (McLennan et al., 2012; Morrison et al., 2017)), and $\mathbf{F}_{ij}^{\text{rep}}$ is a short-range interaction force that imposes an effective exclusion zone around cells (and thus represents the finite size of cells *in vivo*). In numerical simulations, stochasticity in movement is introduced through an additive positional perturbation applied at each discrete time step of the overdamped cell movement (Appendix B.1.4).

The active force on a cell due to chemotaxis is proportional to the magnitude of the cell-specific polarity vector, $||\mathbf{m}_i||$, and is directed along the current heading

$$\mathbf{F}_i^{\text{act}} = f_0 ||\mathbf{m}_i|| (\cos \theta_i, \sin \theta_i), \quad (5.7)$$

where f_0 is a parameter that defines the characteristic chemotactic force strength per cell.

Adhesion between neighbouring cells is modelled using dynamic Hookean springs of stiffness k_{spring} that represent transient adhesive bonds between cells. When the distance between the centres of two cells falls below a linking radius corresponding to the maximum distance of cell–cell interactions, ℓ_f^{max} , an adhesive bond is formed and a spring is introduced between the cells. The rest length of the spring, L_0 , is

set to the inter-cell distance at the time of bond formation.

If r_{ij} denotes the distance between cells i and j , and $\hat{\mathbf{n}}_{ij}$ is the unit vector from cell i to cell j , the adhesive force acting on cell i due to cell j is given by

$$\mathbf{F}_{ij}^{\text{spring}} = k_{\text{spring}} (r_{ij} - L_0) \hat{\mathbf{n}}_{ij}. \quad (5.8)$$

Bonds are broken when the inter-cell distance exceeds a prescribed breaking radius, taken to be slightly larger than the linking radius (Appendix B.1.5). This implementation provides a coarse-grained approximation of cell–cell adhesive interactions arising from effects such as cadherin-mediated contacts (Taneyhill, 2008) and cell–cell interface deformation (Maître and Heisenberg, 2011).

Volume exclusion is represented by a soft-core repulsive force when cells overlap

$$\mathbf{F}_{ij}^{\text{rep}} = \begin{cases} -k_{\text{rep}} (2R - r_{ij}) \hat{\mathbf{n}}_{ij}, & r_{ij} < 2R, \\ \mathbf{0}, & r_{ij} \geq 2R. \end{cases} \quad (5.9)$$

Zero-flux boundary conditions are imposed on cells, such that they remain within the domain throughout migration, thus representing in a simple way the presence of inhibitory signals adjacent to the r4–ba2 pathway *in vivo* (Johnson et al., 2026b; McLennan et al., 2017, 2023) (Chapter 4).

Here, it is important to highlight that all mechanisms of environmental and inter-cellular interactions in this model are isotropic, in that there is no prescribed spatial bias in cell sensing or movement. This represents a relaxation of assumptions made in previous ABMs of CNCC migration (Section 2.3) where movement cues must be taken from cells “ahead” in follower cell chains (i.e. those closer to leader cells in terms of chain indexing), but not from those “behind” (those further away from leader cells in terms of chain indexing), in order for directional collective migration to occur. With the model formulated here, we show in later sections that the biased migration of CNCC collectives is a phenomenon that can occur solely due to an

emergent asymmetry in VEGF profiles, with no prescribed orientational asymmetry in cell-cell interactions. In the model, asymmetric cell-induced VEGF gradients induce a non-zero polarity in the positive x -direction for cells at the leading edge of streams, which, through spring-like mechanical couplings, induces a subsequent directional force on trailing cells. This effect results in migration as a collective without an in-built spatial bias in cellular responses to environmental cues. This, in turn, suggests that polarisation and directional movement are qualities that can propagate along streams of CNCCs from cells at the leading edge of collectives through local intercellular interactions that transmit alignment cues during migration.

5.4 Collective cranial neural crest cell migration requires balanced intercellular adhesion, active polarity-driven forces, and cell-induced VEGF degradation

In model simulations, the three parameters that most strongly influence collective CNCC migration are f_0 , which scales the magnitude of active forces generated by VEGF-induced polarisation, k_{spring} , the Hookean spring constant governing intercellular adhesion, and λ , the rate at which VEGF is degraded by cells.

Numerical simulations of the spring-polarisation model demonstrate that moderate values of λ are required for robust sustained collective CNCC migration over biologically relevant distances (Figures 5.3(b), (c)). If λ is too small, then cells at the leading edge of streams are exposed only to shallow gradients in VEGF, and thus, remain weakly-polarised throughout migration, generating active forces that are insufficient to drive the migration of collectives over biologically relevant distances. Conversely, if λ is too large, then rapid VEGF degradation frequently erodes the local chemotactic gradients that are required to maintain non-zero polarity at the

leading edge of streams, resulting in a transient period of high polarity before a loss of polarisation at the leading edge and a subsequent cessation of active motility. Only when λ takes intermediate values are stable VEGF gradients consistently preserved that sustain sufficient polarity in cells at the leading edge of streams, and thus, continued active migration towards ba2.

For intermediate values of λ that facilitate persistent polarisation at the leading edge of streams, the model exhibits three distinct migratory behaviours determined by the interplay between the magnitude of intercellular adhesion, k_{spring} , and the active chemotactic force scale, f_0 . Too small a value of f_0 means that invasion is inhibited irrespective of the value of k_{spring} as there is an insufficient chemotactic drive at the leading edge of streams to propel collectives forward (Figure 5.4(a)). As f_0 is increased, two distinct phases of migration emerge. Too small a value of k_{spring} relative to f_0 leads to the escape of a small subset of cells at the leading edge, when strong chemotactic polarisation induces a high active force away from the weakly-polarised and loosely-connected bulk (Figure 5.4(c)). However, for sufficiently high f_0 and k_{spring} , the active force generated by highly polarised cells is sufficient to propel collectives along the domain without compromising intercellular adhesions that are essential to maintain a cohesive stream (Figure 5.4(b)).

Thus, model simulations indicate that balanced levels of active chemotactic force generation and intercellular adhesion are required for robust, sustained, coherent migration as a collective. Similar trends have been observed both experimentally in chick and in a corresponding ABM of CNCC migration (McLennan et al., 2020). *In vivo*, gain-of-function of Aquaporin-1, a gene linked to filopodial stabilisation and persistent migration, increases the distance of CNCC invasion, while a loss-of-function results in failed invasion of ba2. In the aforementioned ABM, these perturbations manifest as leader cell escape under gain-of-function and failed invasion under loss-of-function, respectively.

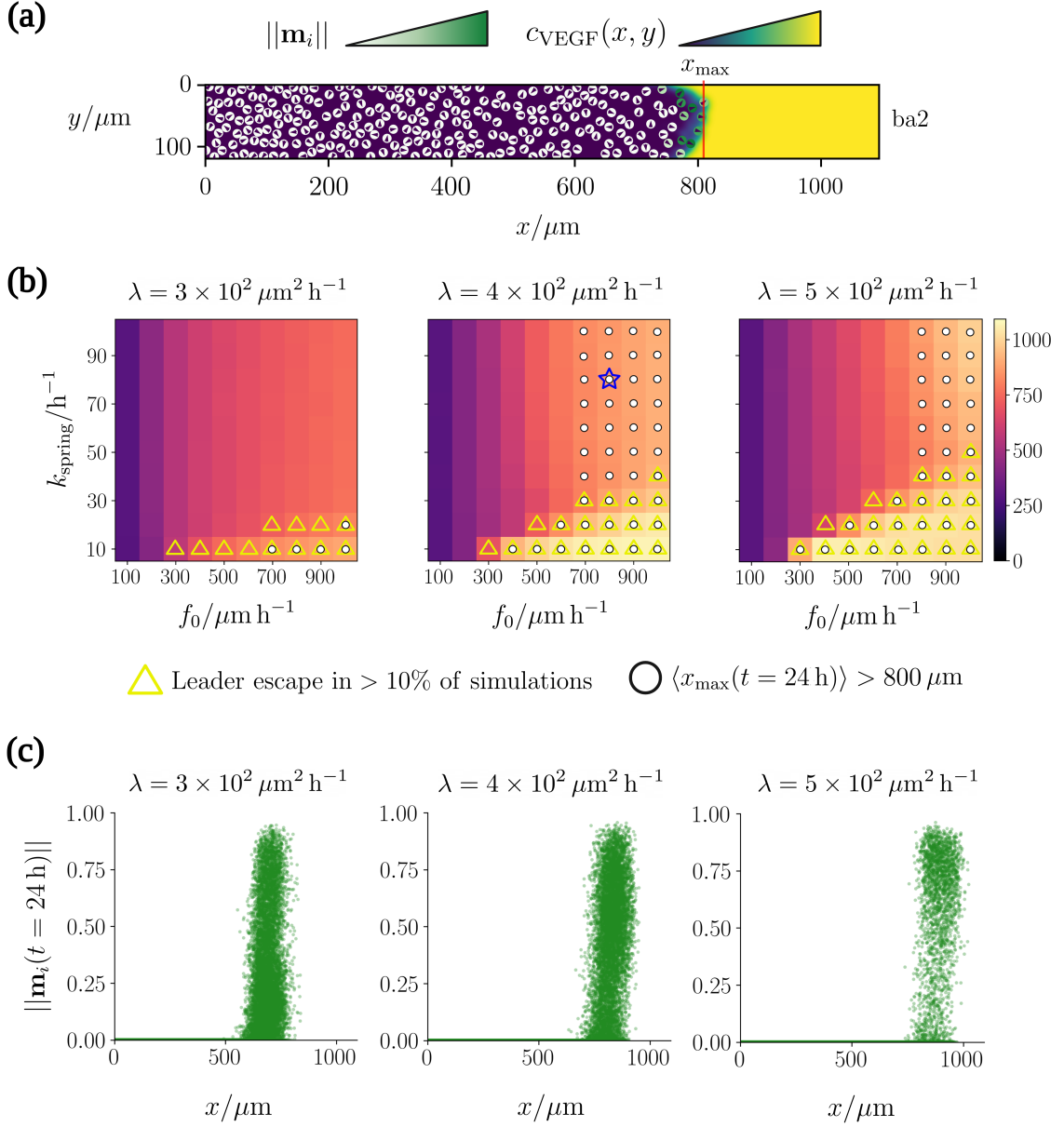


Figure 5.3: **(a)** Example model simulation ($t = 24$ h) for the parameter set $\{f_0 = 800 \mu\text{m h}^{-1}, k_{\text{spring}} = 80 \text{ h}^{-1}, \lambda = 400 \mu\text{m}^2 \text{ h}^{-1}\}$. Non-zero polarisation ($\|\mathbf{m}_i\| > 0$) at the leading edge of the stream is maintained due to strong local VEGF gradients, driving collective migration towards ba2 over a biologically relevant distance. The statistic $x_{\max}$ denotes the maximum x -coordinate across all cells in the stream when migration ends. **(b)** $\langle x_{\max} \rangle$ as a function of λ , f_0 , and k_{spring} . Yellow triangles denote regions of parameter space in which $> 10\%$ of model simulations result in leader escape, where at least one cell lies more than three cell radii ahead of the bulk when migration ends. White circles denote regions of parameter space in which $\langle x_{\max} \rangle$ exceeds $800 \mu\text{m}$. The blue star denotes the representative parameter set that is used to analyse model behaviour in Sections 5.5 and 5.6. **(c)** Scatter plots of $\|\mathbf{m}_i\|$ as a function of x at $t = 24$ h for small, intermediate, and large values of λ for the representative parameter set $\{f_0 = 800 \mu\text{m h}^{-1}, k_{\text{spring}} = 80 \text{ h}^{-1}\}$. Results for each parameter combination in **(b)** are averaged over 500 simulations and the data in **(c)** are aggregated over 500 simulations.

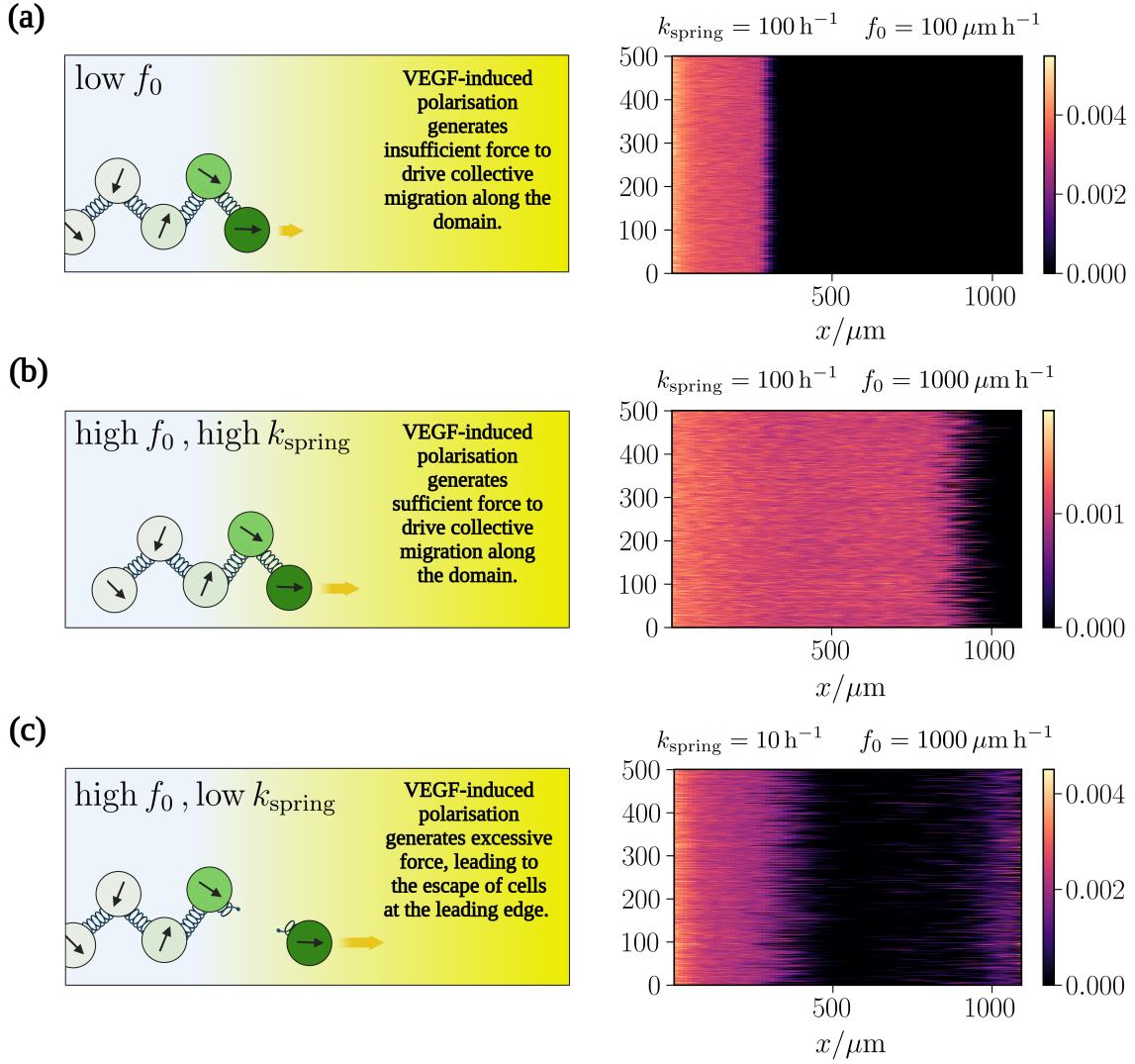


Figure 5.4: Schematics of the relationship between f_0 and k_{spring} and their effect on CNCC migration in the model. **(a)** When f_0 is low, the active force induced by VEGF gradients is insufficient to drive migration along the domain. **(b)** When both f_0 and k_{spring} are sufficiently large, the force induced by VEGF gradients is sufficient to drive migration along the domain, leading to typical CNCC migratory formations. **(c)** If f_0 is much larger than k_{spring} , the active force induced by VEGF gradients leads to the escape of highly polarised cells at the leading edge of collectives, resulting in aberrant migration. Adjacent to each schematic are heatmaps displaying the density of cells as a function of x in 500 numerical model simulations where $\lambda = 400 \mu\text{m}^2 \text{h}^{-1}$ when migration ends at $t = 24 \text{ h}$. Created in BioRender by Samuel Johnson: <https://BioRender.com/by4a4j>.

5.5 Leader and follower phenotypes emerge spontaneously in a spring–polarisation agent-based model of cranial neural crest cell migration

In further investigating the behaviour of the model formulated here, we select a combination of $\{f_0, k_{\text{spring}}, \lambda\}$ that results in invasion over biologically relevant distances and that produces coherent CNCC streams which remain connected throughout migration. A model parameter sweep (Figure 5.3(b)) reveals that a range of $\{f_0, k_{\text{spring}}, \lambda\}$ triplets yield the desired outcome, in which collective migration is sustained over a period of 24 h, and the maximum invasion distance of the stream is comparable to observations *in vivo* ($x_{\text{max}} > 800 \mu\text{m}$). As such, we select the parameter values $\{f_0 = 800 \mu\text{m h}^{-1}, k_{\text{spring}} = 80 \text{ h}^{-1}, \lambda = 400 \mu\text{m}^2 \text{ h}^{-1}\}$, which lie well within these intervals, to serve as a representative regime for further analysis. This regime supports sustained, cohesive migration over biologically relevant timescales and distances, enabling further investigation of the model and its emergent behaviour.

Studying the magnitude of the cell-specific polarity vector, $\|\mathbf{m}_i\|$, as a function of the distance migrated away from the embryonic hindbrain at $x = 0$ reveals the emergence of a phenotypic division between cells at the front of streams and trailing cells behind. As migrating CNCCs locally degrade VEGF, cells at the leading edge consistently invade regions where VEGF gradients are large (Figure 5.5(a)). These gradients drive stronger chemotactic activation in cells at the leading edge, resulting in higher VEGF-induced polarity and larger active forces that propel the movement of collectives. In contrast, trailing cells migrate through regions of depleted VEGF, and therefore, remain weakly-polarised, moving primarily through mechanical interactions with their neighbours. This effect results in a subset of cells at the leading edge of streams becoming polarised upon entry into the domain and remaining polarised ($\|\mathbf{m}_i\| > 0$) thereafter, while all other cells remain largely unpolarised ($\|\mathbf{m}_i\| \approx 0$) (Figure 5.5(b)).

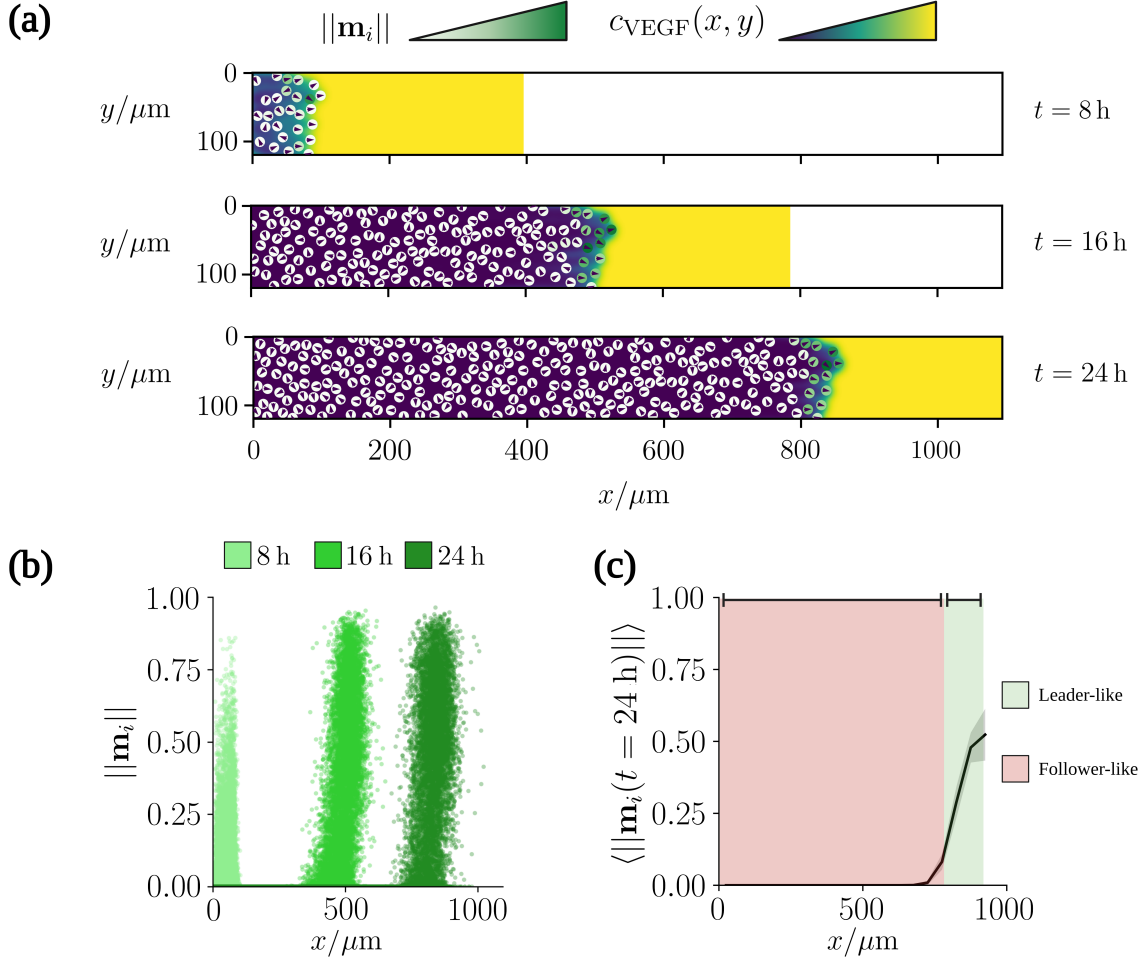


Figure 5.5: **(a)** Typical model simulation for the representative parameter combination $\{f_0 = 800 \mu\text{m h}^{-1}, k_{\text{spring}} = 80 \text{ h}^{-1}, \lambda = 400 \mu\text{m}^2 \text{ h}^{-1}\}$. Initially, CNCCs emerge into regions of high VEGF concentration, and thus, begin to generate large VEGF gradients as VEGF is degraded. The generation of large VEGF gradients induces a strong VEGF polarisation in CNCCs, which results in their movement along the domain. Cells emerging at later times emerge into regions of depleted VEGF, and therefore, remain largely unpolarised. As such, their movement is primarily governed by intercellular attraction–repulsion mechanisms. Throughout migration, CNCCs at the front of the collective remain highly polarised, thus creating a persistent subset of cells for which chemotaxis is the primary determinant of directional movement. **(b)** Scatter plot of $\|\mathbf{m}_i\|$ for $\{f_0 = 800 \mu\text{m h}^{-1}, k_{\text{spring}} = 80 \text{ h}^{-1}, \lambda = 400 \mu\text{m}^2 \text{ h}^{-1}\}$ at $t = 8$ h, 16 h, and 24 h. Cells at the leading edge of streams are highly polarised due to consistent exposure to large VEGF gradients. Cells behind the leading edge remain unpolarised. **(c)** $\langle \|\mathbf{m}_i(t = 24 \text{ h}) \rangle$ as a function of x for the same data set as **(b)**. Non-zero mean polarisations are restricted to the green region at the front of the stream. All other cells in the pink region are largely unpolarised when migration ends. The data in **(b)** and **(c)** are aggregated over 500 model simulations.

Interpreting these results biologically suggests that leader-like and follower-like phenotypes emerge spontaneously during the collective migration of CNCCs. Cells that remain strongly polarised during migration move primarily according to VEGF-induced motility, mirroring leader-like cells in chick for which transcriptomic analysis suggests an increased tendency to move according to gradients in VEGF (McLennan et al., 2012). Cells that remain largely unpolarised behind the leading edge of streams receive movement cues primarily from neighbouring cells, reflective of follower-like cells in chick that up-regulate genes associated with cell–cell adhesion (e.g. cadherins (McLennan et al., 2012)). Defining cells with $||\mathbf{m}_i|| > 10^{-1}$ as leader-like cells, and all other cells as follower-like (Figure 5.5(c)), model simulations demonstrate the emergence of strong spatial correlations in phenotypes, which reflects previous observations in chick. These findings lend credibility to existing models of leader–follower migration, but demonstrate the existence of a narrow spatial region of continuous, rather than binary, phenotypic variation at the leading edge of streams, and that these phenotypic differences may emerge spontaneously from both environmental and intercellular guidance cues that are detected throughout migration.

5.6 A spring–polarisation agent-based model of cranial neural crest cell migration produces migratory behaviour consistent with experimental perturbations in chick and zebrafish

Ablation and transplantation experiments conducted in chick and zebrafish (McLennan et al., 2012; Richardson et al., 2016) have yielded a range of testable predictions regarding the behaviour of CNCC streams in response to environmental and cellular perturbations. To test the validity of the model formulated here, we

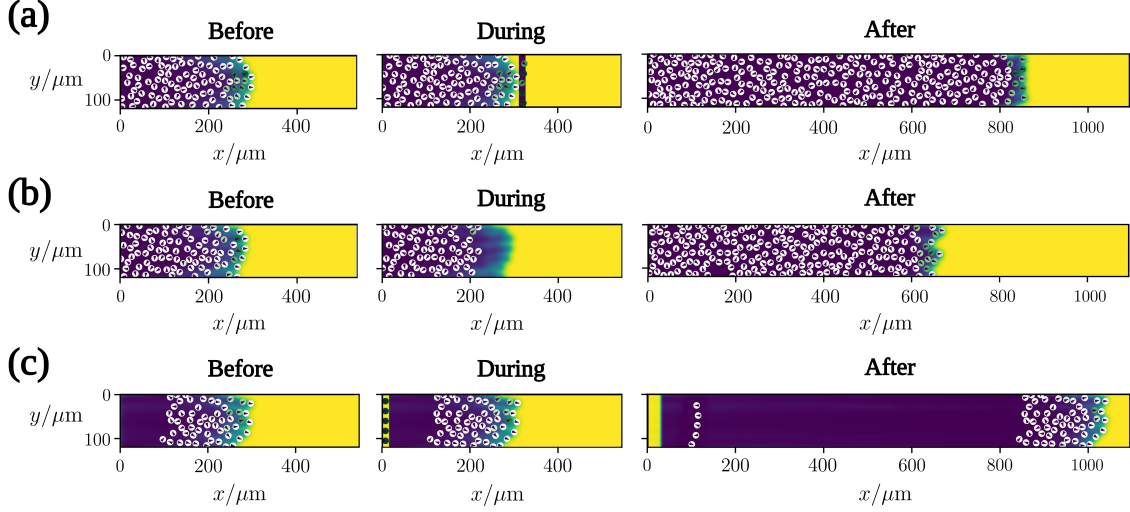


Figure 5.6: The results of perturbations to numerical model simulations based on prior experiments conducted in chick and zebrafish (McLennan et al., 2012; Richardson et al., 2016). **(a)** Upon the transplantation of tissue containing $n = 5$ unpolarised cells ahead of the leading edge of CNCC streams at $t = 12$ h, migration transiently ceases, as there are no longer polarised cells at the leading edge to guide migration. After a short transient period, cell-induced VEGF gradients form in the region of transplantation. Subsequently, transplanted cells become polarised, such that migration resumes in a typical stream formation. **(b)** Upon the ablation of $n = 20$ cells at the leading edge of CNCC streams at $t = 12$ h, the remaining cells at the leading edge of the post-ablation stream are exposed to local gradients in VEGF, which drives their polarisation and a subsequent resumption in active migration. **(c)** When polarised cells are transplanted into the region of the r4–ba2 pathway adjacent to the embryonic hindbrain at $t = 12$ h, they migrate into a region of degraded VEGF and, therefore, cannot sustain the polarisation required to invade ba2.

now compare numerical model simulations with the outcomes of these experiments.

The results of each experiment are visualised in Figure 5.6. As before, we take representative values of the parameters governing cell–cell and chemotactic forces $\{f_0 = 800 \mu\text{m h}^{-1}, k_{\text{spring}} = 80 \text{ h}^{-1}, \lambda = 400 \mu\text{m}^2 \text{ h}^{-1}\}$ with other parameter values as described in Appendix B.

5.6.1 Collective cranial neural crest cell migration persists upon the transplantation of trailing cells to the leading edge of streams

In experiments where unpolarised follower-like cells are transplanted at the leading edge of streams, it has been observed that collective migration stalls for a short transient period, before further migration towards ba2 (McLennan et al., 2012).

In model simulations where $n = 5$ unpolarised cells are transplanted ahead of the leading edge of CNCC streams at $t = 12$ h, a similar phenomenon is observed (Figure 5.6(a)). Initially, the unpolarised cells cannot generate sufficient active forces to propel the collective towards ba2. However, as VEGF is degraded in the region of transplantation, transplanted cells begin to gain polarisation, which induces migration towards ba2, as observed *in vivo*.

In a previous ABM of CNCC migration, this phenomenon could not be fully captured (McLennan et al., 2012), with further modifications to the model required to recapitulate the behaviour observed *in vivo*. In the spring-polarisation model studied here, this behaviour is robust to both the number of cells transplanted and the time of transplantation, provided that the leading edge of the stream after transplantation lies in regions where VEGF has not been degraded. Biologically, these results support a self-organised model of CNCC streams, in which directed collective movement arises from local, context-dependent interactions rather than from the fixed leader-follower hierarchy considered in prior ABMs of CNCC migration, as discussed in Section 2.3.

5.6.2 Collective cranial neural crest cell migration persists upon the ablation of cells at the leading edge of streams

In zebrafish, collective CNCC migration persists upon the ablation of CNCCs at the leading edge of streams (Richardson et al., 2016). This observation was used to argue

that leader-like CNCCs do not constitute a fixed subpopulation and that all CNCCs within a stream share an equivalent capacity to adopt leader-like behaviours. Here, we test this hypothesis by ablating $n = 20$ cells at the leading edge of the stream at $t = 12$ h.

Numerical simulations of this experiment align with observations in zebrafish (Figure 5.6(b)). When the twenty front-most cells in CNCC streams are ablated, the cells at the leading edge of the post-ablation stream lie in regions of detectable VEGF gradients. As such, after a transient period without active migration, these cells become polarised and once again propel the collective towards ba2.

However, varying the number of cells ablated at the leading edge reveals that this behaviour does not persist when larger numbers of cells are ablated. For migration to persist upon leading cell ablation, cells at the leading edge of the ablated stream must remain within regions where a detectable VEGF gradient is present, such that they can become polarised. This is true for cells immediately behind the original leading edge of the stream, but not in positions closer to the embryonic hindbrain, where VEGF has largely been degraded. Consequently, extensive frontal cell ablation shifts the leading edge of the stream into a region void of chemotactic cues, resulting in a persistently unpolarised bulk that cannot reinitiate movement. Biologically, these findings indicate that, although all CNCCs possess the capacity to acquire leader-like properties, this transition is not determined solely by intrinsic cellular properties, but rather by cell–environment interactions. The results of varying the number of leading cells ablated at $t = 12$ h are shown in Appendix C.

Here, we note that, although this experiment was performed in zebrafish rather than in chick (Richardson et al., 2016), the conclusion that leadership is not a fixed property is also supported by quantitative cell-tracking analysis of chick CNCCs in the same study, where cells exhibit similar migratory parameters irrespective of their position within the stream. As such, the model is compared against a phenomenon that is directly documented in chick, the system for which it is parameterised.

5.6.3 Cells at the leading edge of streams transplanted to the region adjacent to the embryonic hindbrain fail to invade ba2

In experiments, leader-like cells transplanted into the region adjacent to the embryonic hindbrain fail to invade ba2, and instead remain in place (McLennan et al., 2012). This phenomenon is also observed in numerical model simulations, provided that the transplanted cells lie outside of the range of cell–cell interactions of the stream (Figure 5.6(c)).

When highly polarised cells are transplanted behind the stream, they migrate into regions where VEGF has been eroded, and thus, begin to lose polarisation after a transient period of migration. As they are sufficiently far from the stream, they also cannot receive cell–cell guidance cues from cells within the main stream. As such, they remain in place, and are unpolarised when migration ends.

Taken together, numerical simulations of experiments conducted in chick and zebrafish show concurrence with behaviour *in vivo*. As such, the model formulated here provides a unified mechanistic mathematical framework that is consistent with both experiments and unperturbed migration, suggesting that it is an appropriate model framework within which to further study collective CNCC migration.

5.7 Discussion

We have introduced a polarity-based ABM for CNCC migration that captures the spontaneous emergence of leader–follower behaviour without imposing phenotypic differences *a priori*. In this framework, all cells evolve according to identical rules, interact mechanically through spring-like couplings, and respond to time-averaged self-induced gradients in VEGF according to an ODE for chemical-induced polarisation. Numerical simulations of the model show that, given these mechanisms, distinct leader-like and follower-like behaviours emerge spontaneously from the collective dynamics of the system according to differences in chemical-induced polarity.

These findings demonstrate that persistent heterogeneity in migratory behaviour can arise solely from the interplay of chemotactic sensing, persistence in polarisation, and mechanical cell–cell couplings. As such, we predict that pre-determined cell identities are not required at the point of delamination from the embryonic hindbrain for successful migration to occur.

The model reproduces a range of experimental observations from *in vivo* and *in vitro* studies of CNCC migration. Leader-like cells at the front of streams align with local VEGF gradients and direct collective movement, while follower-like cells behind them maintain stream cohesion through mechanical coupling and contact guidance. Stream-like structures remain coherent over biologically relevant timescales and respond adaptively to heterogeneous chemical landscapes. As such, directed collective migration is achieved without imposing an intrinsic spatial bias or anisotropic motility parameters, which has been a necessary assumption in previous ABMs for them to reproduce the stream-like migratory patterns observed *in vivo*. This, in turn, suggests that emergent polarity dynamics and isotropic cell–cell interactions alone are sufficient to sustain coordinated migration in the cranial neural crest.

From a modelling perspective, this work extends previous ABMs of CNCC migration by relaxing the assumption of both a leader–follower phenotypic dichotomy and also of instantaneous responses to environmental perturbations made in a later signal integration ABM of CNCC migration (Schumacher, 2019b). Incorporating persistence in polarity provides a mechanistic link between finite-interval environmental sensing and cell migration, allowing the model to capture both transient environmental adaptation and long-term behavioural stability within a unified framework.

Future work could extend the present model in several directions. Coupling polarity dynamics to intracellular signalling networks may help connect molecular-scale regulation to emergent migratory behaviours (Welf and Haugh, 2011). Additionally, incorporating interactions with the ECM or heterogeneous mechanical environments could further improve biological realism, as cell–ECM interactions are

a well-documented mechanism of migration for CNCCs (Bilozur and Hay, 1988; Peris et al., 1989). Finally, the framework developed here could be generalised to study other instances of collective migration where leader–follower organisation emerges spontaneously, such as cancer metastasis (Deisboeck and Couzin, 2009) or wound healing (Li et al., 2013).

Appendix

A Model implementation

We outline implementation details of the spring–polarisation model, before listing the values of fixed model parameters in numerical simulations.

A.1 VEGF gradient sensing accuracy

Our implementation of VEGF gradient detection is based on work by Berg and Purcell (Berg and Purcell, 1977) and is as described in previous ABMs of CNCC migration (Chapter 2). In the model, a filopodial protrusion extended at an angle ϕ senses a VEGF gradient when

$$\frac{c_f(\phi) - c(\mathbf{x}_i)}{c(\mathbf{x}_i)} \geq \xi \sqrt{\frac{c_0}{c(\mathbf{x}_i)}}, \quad (5.10)$$

where $c_f(\phi)$ is the average VEGF concentration sensed by a filopodium extended at an angle ϕ , $c(\mathbf{x}_i)$ is the VEGF concentration at cell i 's current position, ξ is a dimensionless parameter governing gradient detection, and c_0 is the initial uniform VEGF concentration in non-dimensional units.

An issue with this implementation in numerical model simulations is that both sides of Equation (5.10) diverge as $c(\mathbf{x}_i) \rightarrow 0$, which leads to unrealistically high chemotactic sensitivity at very low VEGF concentrations. To prevent this phenomenon in simulations, we impose a lower bound on the effective concentration used in the detection criterion and modify the condition for gradient detection to

$$\frac{c_f(\phi) - c(\mathbf{x}_i)}{\max\{c(\mathbf{x}_i), \epsilon\}} \geq \xi \sqrt{\frac{c_0}{\max\{c(\mathbf{x}_i), \epsilon\}}}, \quad (5.11)$$

	Description	Value	Reference
R	cell radius (nuclear)	$7.5 \mu\text{m}$	(McLennan et al., 2010)
L_y	height of domain	$120 \mu\text{m}$	(McLennan et al., 2012)
L_x	length of domain	$305 \mu\text{m}$ – $1094 \mu\text{m}$	(McLennan et al., 2012)
ℓ_f	cell sensing radius	$27.5 \mu\text{m}$	(McLennan et al., 2017)
$\ell_f^{\max}$	maximum distance for intercellular bonding	$45 \mu\text{m}$	(McLennan et al., 2017)
D	VEGF diffusivity	$0.1 \mu\text{m}^2 \text{h}^{-1}$	(McLennan et al., 2017)
κ	VEGF production rate	10^{-4}h^{-1}	(McLennan et al., 2017)
ξ	VEGF sensing accuracy	0.25	(McLennan et al., 2017)
τ_{sense}	relaxation time for polarity vector	0.10 h	(McLennan et al., 2015a)
k_{chemo}	angular relaxation rate	10h^{-1}	(McLennan et al., 2015a)
n_f	number of directions sampled in one sampling interval	5	N/A – Appendix B
k_{rep}	strength of short-range repulsive interaction	10^3h^{-1}	N/A – Appendix B
γ_{sat}	chemotactic detection saturation parameter	0.5	N/A – Appendix B
η	stochastic positional noise amplitude	$0.1 \mu\text{m}$	N/A – Appendix B

Table 5.1: Fixed parameter values in the ABM, along with references, where appropriate.

where $\epsilon = 10^{-2}$ is an effective lower bound on the sensed concentration that prevents the detection of small gradients in low VEGF concentrations.

B Model parameterisation

Parameter values in the PDE governing VEGF dynamics and the properties of cells are primarily derived from prior ABMs for CNCC migration, as described in Chapter 2. Table 5.1 lists model parameters that are fixed in numerical model simulations, with Sections B.1.1–B.1.5 providing justification for parameter choices made in the absence of prior mathematical or biological parameterisation.

B.1 Notes on model parameterisation

We provide brief notes on fixed model parameters that cannot be derived from the existing biological and mathematical modelling literature.

B.1.1 Number of directions sampled in one sampling interval

In vivo, CNCCs project many filopodia along their membranes (Genuth et al., 2018). These filopodia typically persist for time intervals that are longer than the interval between successive environmental sampling events in the model. Accordingly, we set $n_f = 5$ in the model, which is selected to balance the smaller number of filopodia extended by CNCCs in the model against the higher rate of sampling compared with CNCCs *in vivo*. This parameter value is similar to, but slightly higher than, values from previous ABMs of CNCC migration (Johnson et al., 2026b; McLennan et al., 2012, 2015a, 2017, 2020, 2023; Schumacher, 2019b). Here, we select a slightly higher value of this parameter to drive the VEGF-induced polarisation of cells that highlights the emergence of distinct CNCC phenotypes during migration.

B.1.2 Strength of short-range repulsive interactions

There is no way to directly parameterise k_{rep} , the Hookean spring constant governing the force between overlapping cells. As such, we fix $k_{\text{rep}} = 10^3 \text{ h}^{-1}$ in model simulations, which is sufficiently large to prevent cells from overlapping during migration. However, all model predictions are robust to large variations in this parameter.

B.1.3 Chemotactic detection saturation parameter

The parameter γ_{sat} governs how rapidly the intracellular signals induced by VEGF gradients saturate at a maximum. There is no way to parameterise this biologically, but we find that the qualitative trends observed in numerical model simulations are robust to variations in this parameter ($\gamma_{\text{sat}} \in [0.25, 0.75]$).

B.1.4 Stochastic positional noise

The parameter η sets the amplitude of the stochastic positional perturbations applied during each numerical substep of the overdamped mass-spring update. In the implementation, a two-dimensional random vector with independently and uni-

formly sampled components in $[-1, 1]$ is generated at every time step. This vector is then multiplied by the scalar amplitude, η , before being added to the cell's current position. This stochastic contribution represents unresolved microscopic variability in the microenvironment through which migration occurs. In simulations of the model, we fix $\eta = 0.1 \mu\text{m}$. However, model predictions remain consistent provided the noise amplitude remains small compared to deterministic displacements arising from chemotactic polarisation and cell–cell mechanical interactions.

B.1.5 Adhesive bond formation and removal

Cell–cell adhesion is implemented via transient adhesive bonds that form and break dynamically during migration. When the distance between two cells falls below the interaction radius, ℓ_f^{max} , an adhesive bond is formed and represented as a Hookean spring with a rest length equal to the inter-cell separation at the time of bond formation. The choice of ℓ_f^{max} is informed by prior agent-based models of CNCC migration (McLennan et al., 2017).

Adhesive bonds are removed once the inter-cell distance exceeds a prescribed breaking radius, taken to be slightly larger than the interaction radius ($1.1 \ell_f^{\text{max}}$). Introducing a distinct breaking threshold reduces spurious bond turnover for cell pairs fluctuating near the maximum range of cell–cell interaction and ensures stable adhesive coupling over biologically relevant timescales.

C Varying the number of leading cells ablated

at $t = 12 \text{ h}$

Ablating cells at the leading edge of streams at $t = 12 \text{ h}$ reveals that the subsequent migratory behaviour of streams varies with the number of leading cells ablated. For small numbers of ablated cells, cells at the leading edge of the ablated stream lie in regions of large VEGF gradients, such that they subsequently become polarised and reinitiate migration (Figure 5.7). However, the ablation of a larger number of cells

pushes the leading edge into regions where VEGF has largely been eroded. As such, all cells remain unpolarised and invasion does not occur over biologically relevant distances.

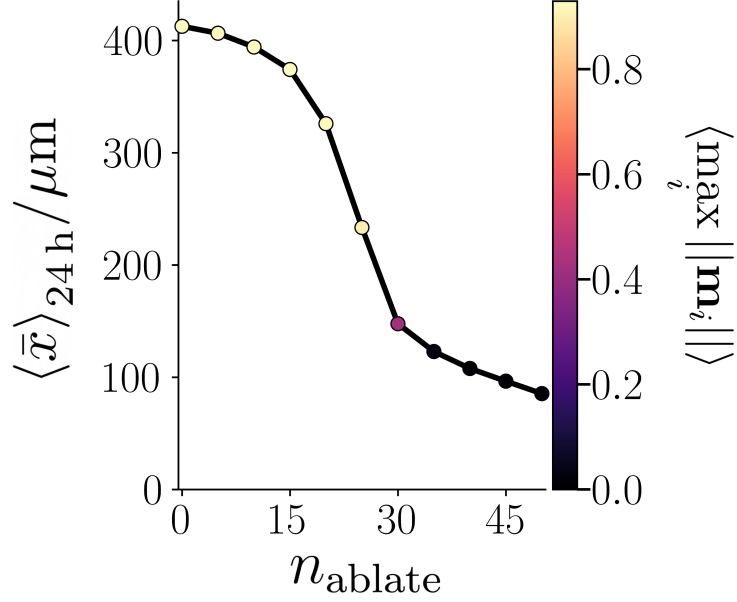


Figure 5.7: Mean stream invasion distance (average x -coordinate of stream centroids) and maximum VEGF-induced polarisation as a function of the number of leading cells ablated. Points show the mean over 500 simulations and point colour denotes the mean maximum polarisation magnitude within streams. When only a small number of cells are ablated, migration persists over biologically relevant distances, giving an x -centroid comparable to unperturbed migration. As the number of cells ablated increases, cells at the leading edge lie in regions of eroded VEGF, fail to polarise, and invasion is inhibited.

Chapter 6

Towards a continuum model of collective cranial neural crest cell migration

In Chapter 5, we demonstrated, with a novel ABM, that an approximate leader–follower phenotypic division can emerge spontaneously during collective CNCC migration. Owing to the pertinence of leader–follower dynamics in a vast array of other biological systems (Qin et al., 2021), we now aim to formulate a continuum representation of generic collective leader–follower migration due to the mathematical tractability of continuum models and the relative ease with which they may be analysed at the tissue scale.

Existing continuum models of neural crest cell migration have largely focused on enteric nervous system development, where proliferation plays a central role in driving population-level advance (Section 1.3.2). In contrast, CNCCs invading *ba2* are largely mitotically quiescent on the time scale over which migration occurs (Ridenour et al., 2014). This motivates the development of a continuum mathematical model in which chemotaxis-driven leader cell migration and cell–cell interaction-driven follower cell behaviour can give rise to sustained collective leader–follower migration. As continuum descriptions of chemotactic leader cell-like migration are well-established (Arumugam and Tyagi, 2021), here, we place our focus on modelling follower cell behaviour, and in particular on studying the role of cell–cell adhesion in maintaining long, cohesive streams that migrate behind a narrow leader subpopulation.

We begin by formulating a phenomenological continuum model in which follower cells respond to short-range attractive cues generated by both leaders and other

followers, providing a natural analogue of the adhesion-based interactions commonly used in ABMs of CNCC migration. However, numerical simulations of this canonical model of cell–cell adhesion demonstrate that, while this model can reproduce the migration of small clumps of follower cells, long, spatially persistent streams cannot be sustained.

Motivated by this model limitation, we then consider whether extended follower cell transport requires a mechanism by which directional information can propagate through collectives. In the adhesion-based model, directional cues from leader cells are transmitted through short-range taxis-like interactions, which constrains follower recruitment to a finite interaction length scale. We therefore introduce a second continuum model that augments the follower density with an internal polarity variable and incorporates non-local polarity alignment between neighbouring cells. This provides a phenomenological way to represent the propagation of directional signals through chains of follower cells. Numerical simulations of this model show that this extension can sustain long, coherent follower streams behind a small leader subpopulation, thereby overcoming a limitation of the adhesion-only continuum formulation.

Our results highlight a limitation of a canonical model of cell–cell adhesion and suggest that signal propagation and polarity-mediated alignment may underpin sustained collective migration in the cranial neural crest, where streams of cells extend well beyond the typical length scale of cell–cell interactions.

Comment on originality: Results in this chapter are synthesised from two related but distinct research projects. Sections 6.1 and 6.2 are adapted from the manuscript

Jewell[†], T.J., Johnson[†], S.W.S., Baker, R.E. and Maini, P.K., 2026. Cell-cell adhesion cannot sustain extended follower streams in a minimal non-local model of leader-follower migration. *arXiv preprint arXiv:2604.15283*,

which is currently under peer review in *Journal of Theoretical Biology*. The material in Section 6.3 is entirely original to this thesis and presents a new model and analysis developed by the author.

Author contributions: For the work presented in Sections 6.1 and 6.2, Thomas Jun Jewell and the author of this thesis jointly formulated the mathematical model, performed the analysis, and wrote the manuscript from which these sections are adapted. The manuscript was revised by Philip K. Maini and Ruth E. Baker. The author of this thesis independently formulated the model presented in Section 6.3 and carried out all associated analytical and numerical work.

Code availability: Code used for the generation of the results in this chapter can be found at <https://github.com/SWSJChCh/continuumLeaderFollower>.

6.1 Modelling background

A canonical continuum framework for representing cell–cell adhesion lies in non-local aggregation PDE models, wherein cells migrate in response to a finite-ranged, spatially averaged neighbour interaction term (often expressible as a convolution or interaction kernel). This produces a directed flux driven by non-local interactions. In biological applications, these models are routinely interpreted as coarse-grained descriptions of short-range adhesion and co-attraction mediated by membrane-scale contacts (Huang et al., 2016), actin-based protrusions (Teddy and Kulesa, 2004), or locally secreted cues (Carmona-Fontaine et al., 2011). This body of work is strongly influenced by a foundational non-local PDE model of cell–cell adhesion (Armstrong et al., 2006), alongside earlier related studies in which non-local adhesion kernels were introduced to model cell rearrangement and adhesion-driven pat-

turning (Sekimura et al., 1999). Subsequently, non-local aggregation models have been generalised and applied broadly across developmental and pathological settings, including differential adhesion-driven cell sorting and mixing, and a variety of clustering and invasion phenomena (Carrillo et al., 2019; Painter et al., 2024b). Moreover, recent work has elucidated mathematical connections between non-local adhesion models and their related local limits, further supporting their role as canonical continuum representations of short-range adhesive cell–cell interactions (Falcó et al., 2024).

In parallel, there is a growing body of continuum representations of leader–follower dynamics, particularly in the contexts of animal swarming and vascular cell sprouting. At the macroscopic level, leader–follower PDE frameworks have been developed to describe how a small informed subpopulation can bias the motion of a much larger uninformed population and steer collective behaviour at the group scale (Bernardi et al., 2021a,b). In angiogenesis, where migratory *tip* endothelial cells guide proliferative *stalk* cells, the coarse-graining of hybrid and lattice-based models, building on classical continuum *snail-trail* formulations, has produced analytically tractable PDE systems for the spatio-temporal evolution of tip and stalk cell densities (Martinson et al., 2021; Terragni et al., 2023).

However, despite the maturity of non-local continuum models for cell–cell adhesion and the parallel development of continuum representations of leader–follower dynamics, it remains unclear as to whether finite-ranged, isotropic non-local adhesion is sufficient to maintain and transport follower populations over arbitrarily long distances when modelled as a continuum. This issue is particularly relevant in the context of chick CNCC migration, where a small population of leader cells responds to micro-environmental guidance cues (such as gradients in VEGF), and a much larger population of followers migrates collectively behind them (Kulesa et al., 2010). Although cell–cell adhesion and co-attraction are frequently hypothesised to maintain stream cohesion in CNCC collectives, CNCC streams persist over

distances that far exceed the direct physical range of cell–cell interactions. It therefore remains an open question as to whether purely isotropic, short-range non-local adhesion mechanisms alone can generate and sustain long, coherently migrating streams of follower cells.

Non-local aggregation models are known to support a rich set of collective behaviours, including stationary clusters, travelling pulses, and other coherent structures under suitable interaction kernels and parameter regimes (Burger and Di Francesco, 2008; Jewell et al., 2023; Topaz et al., 2006). Nevertheless, long, spatially extended follower streams that migrate persistently behind leaders have not, to our knowledge, been reproduced within a non-local adhesion framework. As such, in what follows, we begin by formulating a minimal non-local model of cell–cell adhesion, and study whether short-range adhesive interactions are sufficient to maintain long, cohesive cohorts of follower cells during collective migration.

6.2 A minimal model of cranial neural crest cell migration based on cell–cell adhesion

We study a minimal continuum model of leader–follower migration on a fixed one-dimensional domain of $2000\ \mu\text{m}$ in length. The model comprises two cell populations, leaders and followers, each represented as continuously varying densities along the length of the domain. Leaders and followers each evolve in time according to a system of coupled non-local PDEs. The leader PDE prescribes movement in the positive x -direction at a fixed speed, v_0 , representing migration according to local micro-environmental cues during chemotaxis. Conversely, the follower PDE contains terms representing movement according to random diffusive motion, reflecting undirected motility with diffusivity, D_F , and short-range, non-local attraction to both leaders and other followers, representing the adhesive and co-attractive interactions commonly cited as key mechanisms underlying follower cell migration (Carmona-

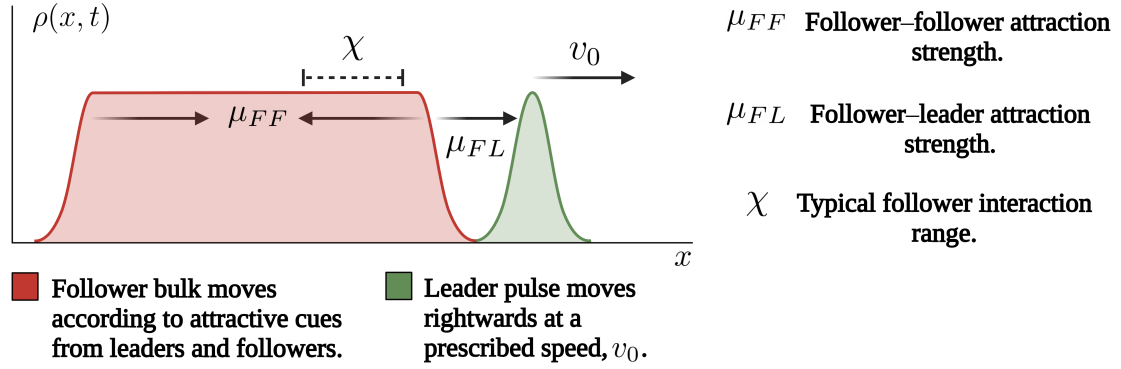


Figure 6.1: Schematic of the adhesion-based continuum model of CNCC migration. The model represents leader and follower CNCC populations as continuous densities on a one-dimensional domain of a fixed length, $L = 2000 \mu\text{m}$. Leader cells migrate in the positive x -direction with a constant speed, v_0 , representing migration according to gradients in external cues. Follower cells move according to a non-local PDE, with terms representing diffusion and attractive cues from leader cells and other follower cells. Follower–follower and follower–leader interaction strengths are modulated by the parameters μ_{FF} and μ_{FL} , respectively, whilst the typical length over which follower–follower and follower–leader interactions occur is represented by the parameter χ . Created in BioRender by Samuel Johnson: <https://BioRender.com/wq7kjiz>.

Fontaine et al., 2011; McLennan et al., 2012). Follower–follower and follower–leader interactions are parametrised by the non-negative interaction strength coefficients, μ_{FF} and μ_{FL} , respectively, and by an interaction length, χ , that determines the typical distance over which cells can sense and interact with one another. Here, we take χ to be invariant between follower–follower and follower–leader interactions, as such cell–cell interactions in the model are mediated by intrinsic properties of follower cells, such as the length of actin-based protrusions extended at their membrane or the density of ligand receptors along their membrane. This assumption has also been made in a previous model of non-local leader–follower interactions (Painter et al., 2024a). Follower motion is also subject to a volume-filling constraint, which prevents overcrowding when the combined non-dimensional leader–follower density approaches a maximum, $\kappa = 1$.

Under these assumptions, the evolution of the leader and follower cell densities,

$\rho_L(x, t)$ and $\rho_F(x, t)$, on the domain $x \in [0 \mu\text{m}, 2000 \mu\text{m}]$, is governed by the PDEs

$$\frac{\partial \rho_L}{\partial t} = -v_0 \frac{\partial \rho_L}{\partial x}, \quad (6.1)$$

$$\frac{\partial \rho_F}{\partial t} = D_F \frac{\partial^2 \rho_F}{\partial x^2} - \frac{\partial}{\partial x} \left(\rho_F (\kappa - \rho_L - \rho_F) \frac{\partial C}{\partial x} \right), \quad (6.2)$$

where C is the non-local attraction potential, incorporating follower–follower and leader–follower interactions,

$$C(x, t) = \mu_{FF} \int_0^L K_\chi(x - y) \rho_F(y, t) dy + \mu_{FL} \int_0^L K_\chi(x - y) \rho_L(y, t) dy, \quad (6.3)$$

and $L = 2000 \mu\text{m}$ is the length of the domain. The kernel, $K_\chi(x)$, specifies how the strength of co-attraction or adhesion changes with the distance between cells, and is taken as an even, non-negative Gaussian function such that

$$K_\chi(x) = \begin{cases} \alpha(\chi) \exp\left(-\frac{x^2}{2\chi^2}\right), & |x| \leq 3\chi, \\ 0, & |x| > 3\chi, \end{cases} \quad (6.4)$$

where $\alpha(\chi)$ is a factor ensuring that

$$\int_{\mathbb{R}} K_\chi(x) dx = \chi. \quad (6.5)$$

Unlike many continuum representations of non-local adhesion, we do not normalise the kernel $K_\chi(x)$ independently of χ . Rather, we allow the total mass of the kernel to scale in a manner proportional to χ , thus representing the modelling assumption that an increased range of cell–cell interactions does not dilute interactions over shorter length scales. Further details of the model and the numerical scheme with which these equations are solved can be found in Appendix A.

At the beginning of each simulation, leaders are initialised as a narrow cluster positioned closely ahead of a bulk of follower cells that extend over several hun-

dred micrometres (Figure 6.1). Leaders then advance with a constant velocity of $v_0 = 1 \mu\text{m min}^{-1}$ for a period of 12 h, while followers disperse, interact, and reorganise according to Equation (6.2). Parameter values of the model are chosen to represent typical cell dynamics *in vivo*. Follower diffusivity is fixed at $D_F = 10 \mu\text{m}^2 \text{min}^{-1}$, leader speed at $v_0 = 1 \mu\text{m min}^{-1}$, and the characteristic follower interaction length, χ , ranges from approximately one to ten cell lengths ($10 \mu\text{m}$ to $100 \mu\text{m}$). The interaction strengths, μ_{FF} and μ_{FL} , are then varied systematically to explore the extent to which follower–follower and follower–leader adhesion facilitate the formation of extended streams of follower cells migrating as a collective behind the small subpopulation of motile leader cells. To isolate advection arising from cell–cell interactions from advection driven by population pressure, we do not prescribe a positive follower cell flux at $x = 0$, despite the continuous delamination of CNCCs from r4 in chick (Theveneau and Mayor, 2012b). Consequently, all collective transport in the model emerges solely from follower–follower and follower–leader interactions.

To quantify the extent to which each parameter set, $(\chi, \mu_{FF}, \mu_{FL})$, facilitates the formation of follower streams, we compare the mass of the main contiguous region of follower density lying directly behind the leaders after migration ends (M_F) to the initial mass of followers in the domain (M_0) (Figure 6.2(a)). This ratio provides a measure of leader–follower migratory efficiency, quantifying the fraction of follower mass that is successfully transported across the tissue segment by leaders. Our analysis compares follower stream sizes across parameter sets and thus, investigates whether the model considered here is sufficient to reproduce the long streams of followers observed in chick (McKinney et al., 2020).

6.2.1 Model analysis

We systematically explore the parameter space defined by the interaction strengths (μ_{FF}, μ_{FL}) and interaction length, χ , quantifying migratory success by the ratio, M_F/M_0 , of follower mass transported along the domain with leaders, relative to the

total follower mass in the domain. The parameter sweep covers wide ranges of μ_{FF} and μ_{FL} for $\chi = 10 \mu\text{m}$, $50 \mu\text{m}$, and $100 \mu\text{m}$, representing typical interaction lengths of approximately one, five, and ten cell lengths, respectively.

For all values of χ considered, two distinct dynamical regimes consistently emerge (Figure 6.3). In the first regime, follower–follower attraction, μ_{FF} , is sufficiently strong, such that attraction from leader cells is unable to detach follower cells from the initial bulk, and thus, the entire follower mass remains in place. An example simulation in this regime is shown in the upper panels of Figure 6.2(a) and corresponds to the region denoted by $*$ in the binary heatmap shown in Figure 6.2(b). In the second regime, strong follower–leader attraction, μ_{FL} , enables followers initially positioned within range of the leaders to move towards them. However, because in this regime, follower–follower attraction is comparatively weak, these migrating cells are unable to attract additional followers from the middle and rear of the bulk, leaving the majority of the population behind. An example simulation in this regime is shown in the lower panels of Figure 6.2(a) and corresponds to the region denoted by $\dagger$ in the binary heatmap shown in Figure 6.2(b). Figure 6.2(b) shows a heatmap of M_F/M_0 for $\chi = 50 \mu\text{m}$ with regions in which $M_F/M_0 < 10^{-2}$ for large values of μ_{FF} relative to μ_{FL} and $M_F/M_0 > 10^{-2}$ for smaller values of μ_{FF} relative to μ_{FL} , corresponding to the two regimes described above, respectively. Adjacent to this heatmap is a binary heatmap indicating the demarcation of these two regimes.

As shown in Figure 6.3(b), the fraction of transported follower mass increases monotonically with follower–leader attraction strength, μ_{FL} . However, for larger values of this parameter, this increase plateaus, indicating a fundamental limit on the proportion of followers that can be transported along the domain with leaders. The precise saturation level depends on the typical follower interaction range, χ . For $\chi = 10 \mu\text{m}$, only a very small fraction of followers, below 0.05, migrate behind the leader subpopulation. For $\chi = 50 \mu\text{m}$, the transported fraction does not exceed 0.2. For $\chi = 100 \mu\text{m}$, the transported fraction reaches a maximum of approximately

0.35, and consistently produces the largest mass of follower cells transported for a given set of interaction strengths (μ_{FF}, μ_{FL}) (Figure 6.3). Thus, for all parameter ranges considered, the majority of follower cells consistently fail to migrate. Here, we note that further increasing χ may lead to the transport of a larger mass of follower cells. However, such lengths would correspond to cell–cell communication over lengths that are far larger than the typical range of short-range communication *in vivo* (Teddy and Kulesa, 2004).

Taken together, these results show that while this continuum formulation of short-range adhesion can generate small cohorts of followers trailing leaders, it cannot sustain extended streams typical of those observed in chick. Instead, follower transport is limited by the maximum length over which followers may interact with other cells.

6.2.2 Discussion

The minimal non-local continuum model analysed in this section indicates that, within the isotropic adhesion formulation considered here, long, migratory follower cell streams, such as those formed during CNCC migration, are not sustained by adhesive and co-attractive follower–follower and follower–leader interactions alone. Numerical simulations of two coupled one-dimensional non-local advection-diffusion PDEs show that follower transport remains limited by the length scale over which follower cells can interact. The underlying reason for this depends on the relative magnitudes of the attraction strength parameters, μ_{FF} and μ_{FL} . When μ_{FF} is sufficiently large, follower–follower adhesion generates a cohesive cluster that resists leader attraction and subsequent advection. In contrast, when μ_{FL} is large, followers in closest proximity to the leader pulse move rapidly towards it, but relatively weaker follower–follower attraction is insufficient to propagate advection to more distant followers, resulting in the detachment of a small cohort of follower cells that is advected with the leaders. We find that, across all parameter sets examined,

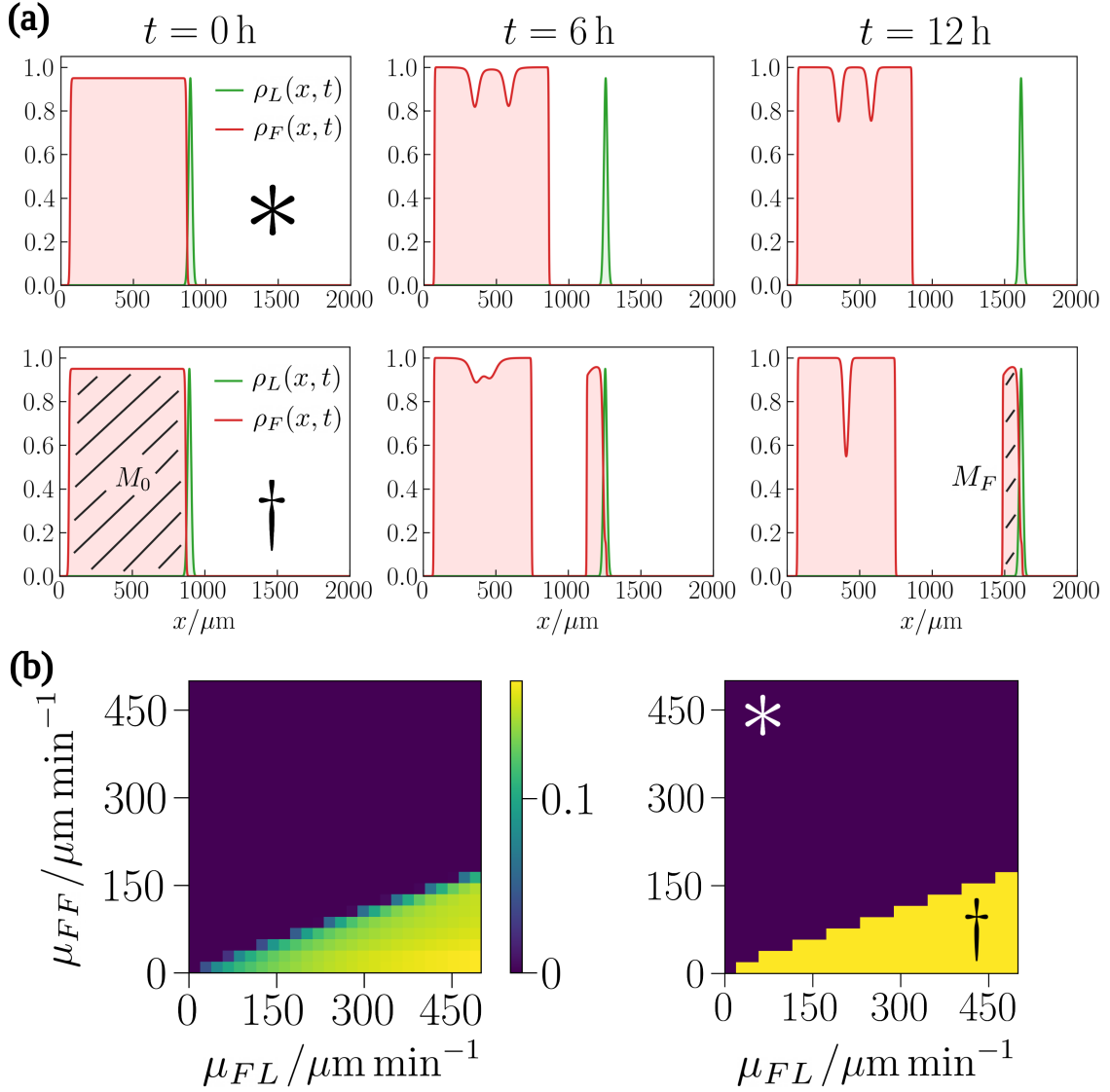


Figure 6.2: **(a)** Model simulations for $(\chi, \mu_{FF}, \mu_{FL}) = (50\text{ }\mu\text{m}, 20\text{ }\mu\text{m min}^{-1}, 20\text{ }\mu\text{m min}^{-1})$ (upper) and $(50\text{ }\mu\text{m}, 20\text{ }\mu\text{m min}^{-1}, 200\text{ }\mu\text{m min}^{-1})$ (lower), shown at 0 h, 6 h, and 12 h. In the upper panel, follower–follower and leader–follower attraction strengths are equal. Under these conditions, follower cells (red) remain tightly bound, and the attraction exerted by leader cells (green) is insufficient to detach followers from the initial cluster. In the lower panel, follower–leader attraction is increased to ten times the follower–follower attraction. As a result, followers located within the initial interaction range of leaders are rapidly advected towards leaders, while those outside of this range remain in the bulk due to low follower–follower attraction strength. In the lower panel, M_0 denotes the initial mass of the follower cluster and M_F , the final mass advected with the leaders. **(b)** M_F/M_0 for $\chi = 50\text{ }\mu\text{m}$ across a range of follower–follower and follower–leader interaction strengths. The left heatmap reveals two distinct regimes: (*) a regime in which follower–follower attraction is sufficiently strong to maintain cluster integrity throughout migration, and (†) a regime in which strong leader–follower attraction advects only those followers within the leaders’ initial interaction radius. The right heatmap provides a visualisation of this binary classification.

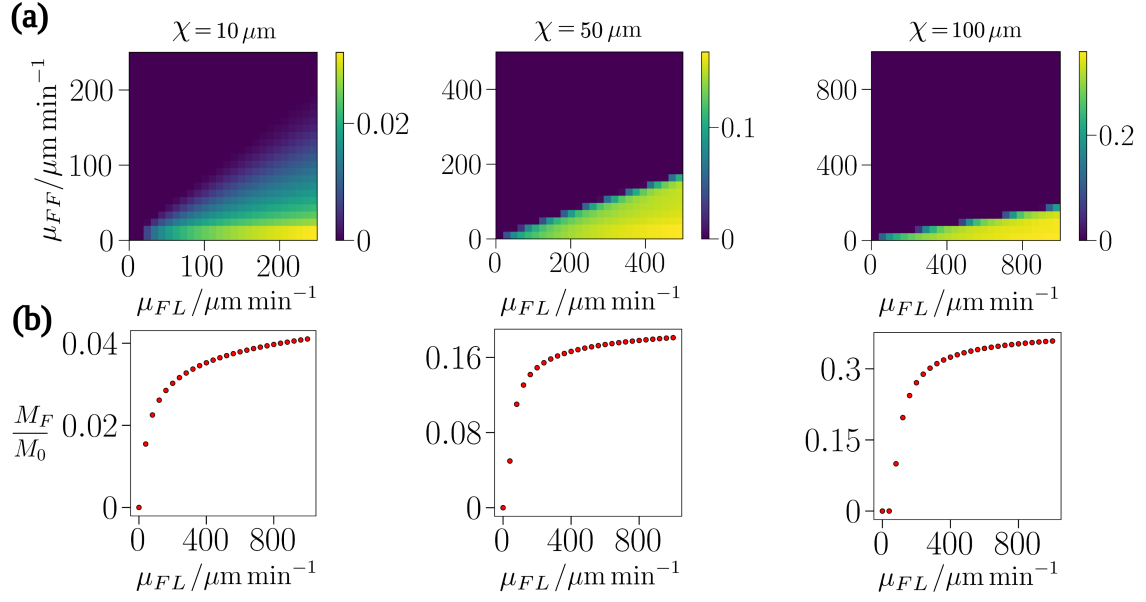


Figure 6.3: Heatmaps of M_F/M_0 for $\chi = 10 \mu\text{m}$, $50 \mu\text{m}$, and $100 \mu\text{m}$ for a range of interaction strengths and corresponding scatter plots of M_F/M_0 as a function of μ_{FL} for fixed $\mu_{FF} = 10 \mu\text{m min}^{-1}$. Heatmaps show the existence of two regimes in (μ_{FF}, μ_{FL}) space. The first is a regime in which follower-follower attraction is sufficiently strong to prevent follower advection with leaders and the second is a regime in which strong leader-follower attraction advects only those followers within the leaders' initial interaction radius. Scatter plots show a gradual plateau in M_F/M_0 as μ_{FL} is increased, indicating a fundamental limit on the degree to which long follower chains may be created.

simulations of the non-local PDE system consistently yield outcomes corresponding to one of the two regimes described above.

As these results are numerical and based on a finite sampling of parameter space, we cannot exclude the possibility of a narrow parameter range in which neither effect dominates and the formation of long follower streams becomes possible. However, even if such a range were to exist, the absence of a broad parameter regime supporting extended follower transport would suggest that this behaviour is not structurally robust within this model class. Such a finely balanced regime would, therefore, be unlikely to provide a satisfactory continuum-level explanation for the sustained formation of long follower streams observed across a diverse range of systems *in vivo*.

It is important to emphasise that these results should not be interpreted as ruling out adhesion or co-attraction as biologically important contributors to follower cell stream cohesion during collective migration. Rather, they indicate a limitation of the canonical isotropic non-local model of cell–cell adhesion considered here. In this model, directional cues from leader cells are transmitted to followers only through a density-dependent attractive potential with finite spatial support. Consequently, the model does not contain an explicit mechanism by which directional information can be stored by follower cells and propagated through the follower population. Followers outside the interaction neighbourhood of the leader pulse are, therefore, influenced only indirectly through their attraction to intermediate followers, and this indirect coupling is either sufficiently strong to maintain the follower bulk in place, or too weak to transmit advection through the population.

As such, these results identify the need to enrich this continuum description in order to recapitulate the desired mode of collective migration. Possible extensions include spatially anisotropic cell–cell interactions, longer-range signalling, feedback from follower cells to leader cells, or coupling to external fields or substrates. In the context of CNCC migration, an additional possibility is that a finite-ranged directional variable, such as actin-based polarity, is propagated along streams of

follower cells through local cell–cell interactions. As such, we now formulate an additional continuum model of leader–follower CNCC migration to investigate whether the explicit inclusion of follower cell polarity and non-local polarity alignment can overcome the transport limitation of the adhesion-based continuum formulation.

6.3 A polarisation-based continuum model of cranial neural crest cell migration

The analysis of Section 6.2 demonstrates that while a continuum representation of isotropic, short-range cell–cell adhesion is sufficient to generate local cohesion amongst follower cells, it is fundamentally unable to sustain long, coherently migrating follower streams behind a narrow leader subpopulation. In such models, leader cell directionality is transmitted to followers only indirectly through a non-local, density-dependent, attractive potential acting over a fixed interaction radius. Consequently, directional information cannot propagate beyond this intrinsic length scale and follower recruitment remains spatially bounded, unless interaction lengths far exceeding biologically realistic scales are assumed.

These findings motivate the consideration of an alternative biological mechanism for extended stream formation *in vivo*, namely the local propagation of directional information through cell–cell contact-mediated alignment of polarity. During cranial neural crest migration, follower cells remain in close proximity and frequently maintain persistent cell–cell contacts within streams (Teddy and Kulesa, 2004). A growing body of experimental evidence suggests that such contacts can actively transmit mechanical forces and polarity cues between neighbouring cells. In particular, force transmission through cadherin-based junctions has been shown to reorient protrusive activity and align migratory orientations within collectives, enabling directional information sensed locally to propagate over distances far exceeding those of direct cell–cell contact (Boutillon et al., 2022; Cai et al., 2014; Theveneau and

Mayor, 2012a). These observations suggest that sustained follower streams may arise not from cell–cell adhesion alone, but from the local, contact-mediated alignment of cell polarity that enables directional information to propagate through collectives.

To investigate this hypothesis, we introduce a continuum model in which follower cell density is distributed in both physical space and also an internal polarity space. In explicitly modelling the evolution and transport of polarity within the follower population, we allow for the propagation of directional information through contact-mediated interactions without invoking unrealistically long-range forces or anisotropy in cell–cell interactions. In doing so, the model provides a minimal continuum representation of contact-mediated polarity alignment that may enable the formation of long, coherent CNCC streams *in vivo*.

6.3.1 Model formulation

We formulate a continuum model for CNCC migration in which follower cells transmit directional information through non-local, contact-mediated alignment in polarity. As in Section 6.2, the CNCC population is divided into leaders and followers. Both subpopulations are again represented by densities in physical space, but also now in polarity space.

Let $\rho_L(x, p, t)$ and $\rho_F(x, p, t)$ denote the densities of leader and follower cells, respectively, where $x \in [0 \mu\text{m}, 2000 \mu\text{m}]$ denotes position along a fixed one-dimensional tissue segment and $p \in [-1, 1]$ is a scalar polarity variable representing the actin-based polarity of cells (where $p = 1$ denotes fully rightwards polarised and $p = -1$, fully leftwards polarised). The total cell density at position x and time t is given by

$$\rho(x, t) = \int_{-1}^1 (\rho_F(x, p, t) + \rho_L(x, p, t)) \, dp. \quad (6.6)$$

Both leader and follower cells undergo polarity-dependent advection in physical space, with an instantaneous velocity $v_0 p$, where v_0 determines the speed at which

fully polarised cells migrate. Follower cells additionally undergo interaction-driven dynamics in polarity space.

To recover the leader advection described in Section 6.2, we assume that leader polarity is fixed at $p = 1$ throughout migration, such that leader cells are advected at a constant speed $v_0 = 1 \mu\text{m min}^{-1}$. This formulation means that the leader cell density, $\rho_L(x, p, t)$, satisfies

$$\partial_t \rho_L(x, p, t) + \partial_x (v_0 p \rho_L(x, p, t)) = 0, \quad (6.7)$$

where

$$\rho_L(x, p, t) = \rho_L(x, t) \delta(p - 1). \quad (6.8)$$

The evolution of the polarity-resolved follower density is governed by

$$\begin{aligned} \partial_t \rho_F(x, p, t) + \partial_x (v_0 p \rho_F(x, p, t)) + \partial_p (A_{p,F}(x, p, t) \rho_F(x, p, t)) \\ = \partial_x (D_F \partial_x \rho_F(x, p, t)) + D_p \partial_{pp} \rho_F(x, p, t), \end{aligned} \quad (6.9)$$

where $D_F > 0$ is the follower diffusion coefficient in physical space and $D_p > 0$ is the follower diffusion coefficient in polarity space. In this formulation, follower cells are advected at a speed proportional to their instantaneous polarity. Biologically, D_F represents stochastic unbiased motion within the follower cell population, while D_p represents small fluctuations in cell polarity due to spontaneous actin polymerisation and de-polymerisation. Unlike the adhesion-based model of Section 6.2, we omit an explicit carrying capacity in Equation (6.9), as we assume that polarity is a property of cells that fully characterises their motile capacity and propensity for directed motion.

Polarity alignment between cells is encoded through the drift term

$$A_{p,F}(x, p, t) = \lambda(1 - p^2)(\tilde{p}(x, t) - p), \quad (6.10)$$

where $\lambda > 0$ is the rate of non-local polarity alignment. The factor $(1 - p^2)$ suppresses alignment dynamics near maximal polarisation and ensures that polarity remains bounded within the interval $[-1, 1]$. The target polarity, $\tilde{p}(x, t)$, towards which the follower polarity distribution relaxes, is defined as a non-local, density-weighted average

$$\tilde{p}(x, t) = \frac{\int_0^L W_\chi(x - y) c(y, t) q(y, t) dy}{\int_0^L W_\chi(x - y) c(y, t) \rho(y, t) dy}, \quad (6.11)$$

with the convention that $\tilde{p}(x, t) = 0$ whenever the denominator of Equation (6.11) is zero. Here, $W_\chi(x)$ is an interaction kernel defining the spatial neighbourhood over which polarity information is aggregated,

$$W_\chi(x) = \begin{cases} \beta(\chi) \exp\left(-\frac{x^2}{2\chi^2}\right), & |x| \leq 3\chi, \\ 0, & |x| > 3\chi, \end{cases} \quad (6.12)$$

with $\beta(\chi)$ chosen such that

$$\int_{\mathbb{R}} W_\chi(x) dx = 1. \quad (6.13)$$

The function $q(x, t)$ denotes the first moment of the combined CNCC population polarity,

$$q(x, t) = \int_{-1}^1 p \rho_F(x, p, t) dp + \rho_L(x, t), \quad (6.14)$$

and $c(x, t)$ is a polarity-weighting factor defined as

$$c(x, t) = \frac{\int_{-1}^1 |p| \rho_F(x, p, t) dp + \rho_L(x, t)}{\rho(x, t)}, \quad (6.15)$$

with the convention that $c(x, t) = 0$ whenever $\rho(x, t) = 0$. This weighting reflects the fact that directional mechanical cues emerge only once cells develop a pronounced front–rear asymmetry. Weakly polarised cells (those with lower values of $|p|$) exert

largely isotropic forces and thus transmit little directional information across cell-cell contacts. Conversely, strongly polarised cells generate sustained anisotropic traction and cytoskeletal tension that biases the polarity of neighbouring cells.

Zero-flux boundary conditions are imposed in polarity space for the follower cell density, preventing unphysical polarity flux at $p = \pm 1$. In physical space, zero-flux boundary conditions are imposed on followers to ensure conservation of follower mass. Leaders are permitted to freely outflow at $x = L$, with no inflow at $x = 0$. Spatial initial conditions mirror those used in Section 6.2 and are as described in Appendix A.2. Leaders are initialised as a narrow spatial pulse with all mass concentrated at $p = 1$, while followers are initially distributed as a bulk trailing the leaders, with their polarity initialised as a narrow mean-zero Gaussian distribution. Further details regarding the numerical implementation of this model can be found in Appendix B.

6.3.2 Model analysis

We simulated the polarity-alignment model over a period of 12 h, varying the alignment rate, λ , and the characteristic interaction length, χ , to study their effects on the capacity of leaders to transport followers along the domain.

Simulations reveal that, in contrast to the adhesion-based model of Section 6.2, the polarity-integrated follower density consistently remains contiguous throughout migration for the parameter values we considered (Figure 6.4), in that the spatial follower cell density does not split into a population immediately behind the leaders and a population that remains in place (as in Figure 6.2). As such, the statistic M_F/M_0 used to analyse follower transport in Section 6.2 is not informative in this context, as it fails to distinguish between genuine advection behind leaders and the qualitatively distinct failure mode in which the spatial follower cell density at $t = 12$ h is diffuse and non-zero throughout the domain.

For the parameter ranges considered, leader-driven follower cell transport in-

creases with both the rate of polarity alignment, λ , and the interaction length, χ (Figure 6.4). For sufficiently large λ , polarity relaxes rapidly towards the neighbourhood-averaged polarity, $\tilde{p}$, yielding robust, coherent follower transport behind the leaders over the period of migration considered (Figure 6.5).

The interaction length χ modulates these behaviours in a manner that depends on the alignment rate, λ . For intermediate and large interaction lengths ($\chi = 50 \mu\text{m}, 100 \mu\text{m}$), robust transport of followers is observed across the range of λ values considered. By contrast, for short interaction lengths ($\chi = 10 \mu\text{m}$), follower transport exhibits a dependence on λ . For the lowest alignment rate considered ($\lambda = 2 \text{ min}^{-1}$), followers closest to the leaders polarise quickly, forming a long, continuous follower mass that initially lags behind as polarity propagates slowly rearward along the stream. As λ is increased to 6 min^{-1} and 10 min^{-1} , this transient lag is attenuated, resulting in polarity alignment that propagates rapidly and hence maintains coherent follower transport with the leader subpopulation.

These simulations demonstrate that polarity and contact-mediated alignment provide mechanisms for sustained follower transport that depends jointly on the rate and spatial extent of polarity transmission. This mechanism is absent from the isotropic short-range adhesion framework of Section 6.2, where directional information is conveyed only through a density-dependent attractive potential acting over a fixed finite length scale, which intrinsically limits follower recruitment. In the polarity-based model, directional information is stored in a dynamical internal variable and transmitted through local alignment which enables extended follower streams to migrate coherently behind a small leader subpopulation.

6.4 Discussion

In this chapter, we have analysed two minimal continuum models of collective CNCC migration, with the aim of assessing whether short-range, non-local interactions

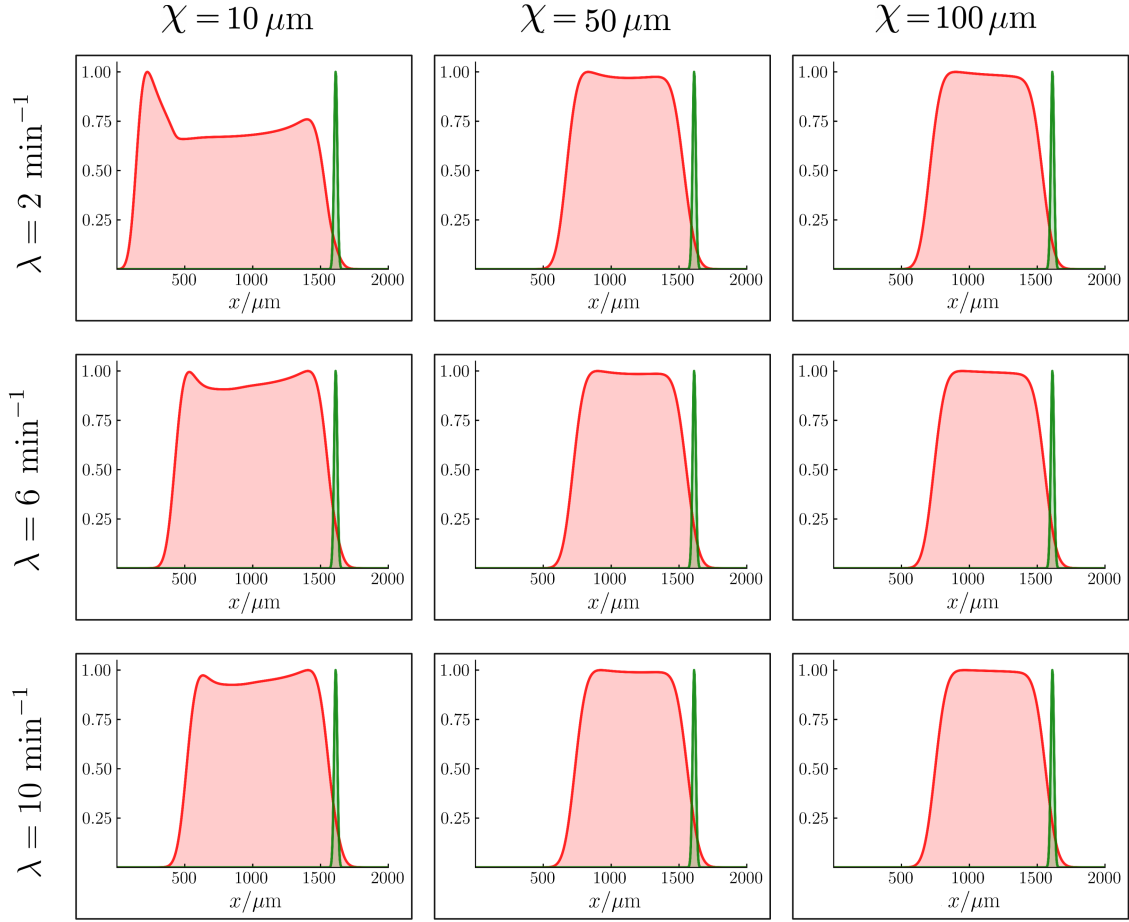


Figure 6.4: Simulations of the polarity alignment model for $\lambda \in \{2 \text{ min}^{-1}, 6 \text{ min}^{-1}, 10 \text{ min}^{-1}\}$ and $\chi \in \{10 \mu\text{m}, 50 \mu\text{m}, 100 \mu\text{m}\}$ at $t = 12 \text{ h}$. Polarity alignment means that for sufficiently high rates of alignment, follower cells (red) are advectively transported behind the continuously advected leader subpopulation. Spatial initial conditions for leader and follower densities are as described in Section 6.2 and visualised in Figure 6.2. Initial follower cell polarisation is distributed as a mean-zero Gaussian distribution with a standard deviation $\sigma = 0.05$.

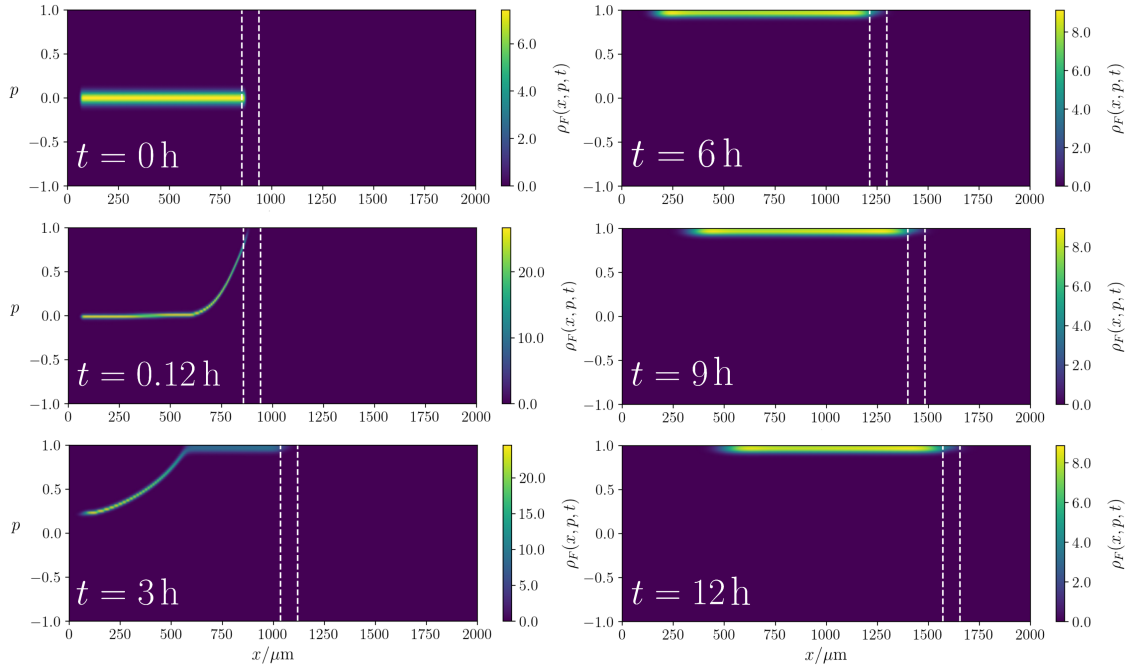


Figure 6.5: Example polarity alignment model simulation for $\chi = 10 \mu\text{m}$ and $\lambda = 10 \text{ min}^{-1}$. At $t = 0 \text{ h}$, follower cells are distributed along the x -axis with a mean-zero Gaussian polarity distribution. Shortly after migration begins ($t = 0.12 \text{ h}$), positive leader cell polarity propagates to follower cells within the interaction range of the leader subpopulation. As time progresses, alignment propagates through the follower subpopulation, until all follower cells are highly polarised. This results in follower cells being advected with a constant velocity along the domain. White dashes indicate the spatial extent of leader cell density as a function of time.

alone are sufficient to generate and sustain the long, coherent streams of follower cells observed *in vivo*. By systematically comparing an adhesion-based model with a polarity alignment-based model, we identified a fundamental limitation of a canonical model of isotropic non-local adhesion in its ability to sustain follower cell transport and demonstrated that contact-mediated propagation of polarity provides a viable alternative mechanism to recapitulate this behaviour at the tissue scale.

In Section 6.2, we examined a canonical continuum formulation of non-local cell-cell adhesion, motivated by its widespread use as a coarse-grained representation of short-range adhesive and co-attractive interactions in multicellular systems (Armstrong et al., 2006). While this model successfully captures local cohesion and the formation of small cohorts of followers migrating behind leaders, follower transport remains bounded by the intrinsic interaction length scale of cell-cell interactions. Across all parameter regimes explored, the follower population consistently separated into either a static cohesive bulk or a small cohort advected with leaders. No regime produced extended streams of followers comparable to those observed *in vivo*.

This suggests that directional information from leader cells cannot propagate efficiently through large follower populations in this isotropic non-local adhesion model as follower recruitment is inherently local and constrained by the finite spatial support of adhesive interactions. Although non-local aggregation models can admit travelling pulses and other coherent structures under suitable conditions (Carrillo et al., 2019; Painter et al., 2024b), our results indicate that such behaviour does not readily translate into long, coherent streams of followers without biologically implausible assumptions.

Motivated by this limitation, we introduced a second continuum model in which follower cells possess an internal polarity variable that governs their speed and direction of motion, and that evolves through non-local, contact-mediated alignment with neighbouring cells. In contrast to the adhesion-based model, this framework allows

directional information to propagate sequentially along chains of followers, rather than being transmitted solely through a density-dependent attractive potential. Follower recruitment is, therefore, no longer constrained by a fixed interaction radius in physical space. With sufficiently fast alignment dynamics, the model reproduces long streams of follower cells led by a highly polarised leader subpopulation.

These results distinguish between mechanisms that promote local cohesion and those required for long-range collective transport. While a model of isotropic adhesion is sufficient to maintain cluster integrity over short distances, the sustained migration of extended follower populations requires an additional mechanism capable of transmitting directional information through the collective. Polarity-mediated alignment provides a biologically plausible mechanism for the generation of this phenomenon.

More broadly, these results highlight the need to examine the implicit assumptions of continuum interaction models when extrapolating from local cell behaviour to tissue-scale dynamics. Mechanisms that are effective at generating local order do not necessarily support long-range coordination. The polarity-based framework introduced here offers a foundation for future modelling extensions incorporating additional biological features such as cell–ECM coupling and a continuous representation of cell phenotypes, as in the ABM in Chapter 5. Such extensions may further clarify how robust collective migration emerges in the cranial neural crest and in other leader–follower systems across development and disease.

Appendix

A Cell–cell adhesion model implementation

We detail the numerical implementation of the cell–cell adhesion model outlined in Section 6.2.

A.1 Numerical scheme

We discretise the domain into $N = 10^4$ mesh points, $\{x_i\}_{i=1}^N$ with uniform spacing $h = L/(N - 1)$. The system is integrated explicitly in time using a forward Euler scheme. The time step Δt is chosen conservatively to satisfy a diffusion Courant-Friedrichs-Lewy (CFL) constraint

$$\Delta t \leq \frac{h^2}{2D_F}, \quad (6.16)$$

with additional safety factor $\theta \in (0, 1)$ (here $\theta = 0.05$) to ensure accurate results, giving a final time step of

$$\Delta t = \theta \frac{h^2}{2D_F}. \quad (6.17)$$

A.1.1 Numerical leader update

Leaders are transported at a speed v_0 using a discrete shifting algorithm. The displacement increment per time step is

$$\delta s = \frac{v_0 \Delta t}{h}. \quad (6.18)$$

Let S denote the accumulated displacement in the x -direction. At each time step, S is increased by δs , and whenever $\lfloor S \rfloor$ increases by one, the leader density profile ρ_L is shifted by one mesh point in the positive x -direction.

A.1.2 Numerical follower update

Followers evolve via explicit forward Euler updates of the form

$$\rho_F^{n+1} = \rho_F^n + \Delta t \left(D_F \Delta_h \rho_F^n - \nabla_h \cdot J^n \right), \quad (6.19)$$

where superscripts denote time and Δ_h denotes a second-order finite-difference Laplacian with homogeneous Neumann boundary conditions.

The taxis flux is written in conservative form as

$$J = \rho_F P \nabla C, \quad P = \kappa - (\rho_L + \rho_F), \quad (6.20)$$

where C is the non-local attraction potential. Zero-flux boundary conditions are imposed on ρ_F at $x = 0 \mu\text{m}$ and $x = 2000 \mu\text{m}$, such that the integrated follower mass remains constant throughout migration.

A.1.3 Discretisation of the taxis flux

The divergence of the taxis flux is discretised using a finite-volume formulation. Gradients of the attraction potential, C , are evaluated at cell faces using centred differences,

$$(\partial_x C)_{i+\frac{1}{2}} = \frac{C_{i+1} - C_i}{h}, \quad (6.21)$$

and the corresponding face velocities are defined by

$$u_{i+\frac{1}{2}} = P_{i+\frac{1}{2}} (\partial_x C)_{i+\frac{1}{2}}, \quad (6.22)$$

where i indexes the centres of mesh points and $P_{i+\frac{1}{2}}$ is obtained by arithmetic averaging of P between adjacent mesh points.

The numerical flux through each cell face is computed using an upwind discretisation,

$$J_{i+\frac{1}{2}} = \begin{cases} \rho_{F,i} u_{i+\frac{1}{2}}, & u_{i+\frac{1}{2}} \geq 0, \\ \rho_{F,i+1} u_{i+\frac{1}{2}}, & u_{i+\frac{1}{2}} < 0, \end{cases} \quad (6.23)$$

which yields a conservative update and preserves non-negativity of the follower density.

The discrete divergence of the flux is then given by

$$(\nabla_h \cdot J)_i = \frac{J_{i+\frac{1}{2}} - J_{i-\frac{1}{2}}}{h}. \quad (6.24)$$

A.1.4 Non-local convolution

Given a kernel, K , and a discrete density, Z , the convolution is given by

$$(K * Z)(x_i) \approx h \sum_j K_\chi(x_i - x_j) Z(x_j), \quad (6.25)$$

and is computed using a fast Fourier convolution with zero-padding beyond the domain boundaries.

A.1.5 Follower cell diffusion constant

In simulations, we choose a moderate diffusion constant of $D_F = 10 \mu\text{m}^2 \text{min}^{-1}$ for follower cells. For one hour of migration, this results in a characteristic diffusive length-scale of

$$\ell(60 \text{ min}) = \sqrt{2D_F t} = \sqrt{2 \times 10 \times 60} \mu\text{m} = \sqrt{1200} \mu\text{m} \approx 35 \mu\text{m}. \quad (6.26)$$

Thus, with this choice of D_F , the expected spread of a cell over one hour is approximately 3.5 cell lengths ($\sim 35 \mu\text{m}$), though this diffusion is largely attenuated by the follower–follower attraction that is also present in the model.

A.2 Initial conditions

The initial condition consists of a follower bulk trailing a leader pulse

$$\rho_F(x, 0) = \frac{f_0}{2} \left(\tanh \frac{x-x_a^F}{\delta} - \tanh \frac{x-x_b^F}{\delta} \right), \quad (6.27)$$

$$\rho_L(x, 0) = l_0 \exp\left(-\frac{(x-x_0)^2}{2\sigma^2}\right), \quad (6.28)$$

with $0 < x_a^F < x_b^F < x_0 < L$, where $f_0 = l_0 \leq \kappa$. In simulations we take $[x_a^F, x_b^F] = [65 \mu\text{m}, 870 \mu\text{m}]$, $x_0 = 895 \mu\text{m}$, $\delta = 5 \mu\text{m}$, $\sigma = 12 \mu\text{m}$, and $f_0 = l_0 = 0.95$, yielding a narrow leader peak located just ahead of an extended follower train (Figure 6.2(a)).

B Polarisation alignment model implementation

We detail the numerical implementation of the polarisation alignment model outlined in Section 6.3.

B.1 Numerical scheme

We discretise the spatial domain into $N_x = 500$ mesh points, $\{x_i\}_{i=1}^{N_x}$, with uniform spacing $h = L/(N_x - 1)$, and the polarity domain $[-1, 1]$ into $N_p = 100$ mesh points, $\{p_j\}_{j=1}^{N_p}$, with uniform spacing $\Delta p = 2/(N_p - 1)$. The system is integrated explicitly in time using a forward Euler scheme.

The time step Δt is chosen conservatively to satisfy stability constraints arising from both advective and diffusive transport in physical and polarity space. Specifically, we require

$$\max\left(\frac{v_0 \Delta t}{h}, \frac{|A_{p,F}| \Delta t}{\Delta p}, \frac{2D_F \Delta t}{h^2}, \frac{2D_p \Delta t}{\Delta p^2}\right) \leq 0.1, \quad (6.29)$$

ensuring stability of the upwind advection and diffusion operators in both dimensions. In simulations we take a fixed time step $\Delta t = 10^{-4} \text{ min}$, which satisfies all four constraints for the parameter ranges considered.

B.1.1 Numerical leader update

The numerical scheme for leader cell advection is as described in Appendix A.1.1.

B.1.2 Numerical follower update

Follower cells evolve according to explicit forward Euler updates of the form

$$\rho_F^{n+1} = \rho_F^n + \Delta t \left(-\nabla_h \cdot J_x^n - \nabla_p \cdot J_p^n + D_F \Delta_h \rho_F^n + D_p \Delta_p \rho_F^n \right), \quad (6.30)$$

where superscripts denote time and Δ_h and Δ_p denote second-order finite-difference Laplacians in physical and polarity space, respectively.

The physical-space advective flux is given by

$$J_x = v_0 p \rho_F, \quad (6.31)$$

while the polarity-space flux is

$$J_p = A_{p,F} \rho_F, \quad (6.32)$$

with $A_{p,F}$ as defined in Section 6.3.1. Zero-flux boundary conditions are imposed at $x = 0 \mu\text{m}$, $x = 2000 \mu\text{m}$, and at $p = \pm 1$, ensuring conservation of follower mass during migration.

B.1.3 Discretisation of advective fluxes

Both physical and polarity advection terms are discretised using a finite-volume formulation with first-order upwind fluxes. For physical-space transport, velocities are evaluated at cell faces by arithmetic averaging, and the numerical flux through the face $x_{i+\frac{1}{2}}$ is given by

$$(J_x)_{i+\frac{1}{2},j} = \begin{cases} v_{i+\frac{1}{2},j} \rho_{F,i,j}, & v_{i+\frac{1}{2},j} \geq 0, \\ v_{i+\frac{1}{2},j} \rho_{F,i+1,j}, & v_{i+\frac{1}{2},j} < 0, \end{cases} \quad (6.33)$$

with an analogous construction used for polarity-space transport, where i indexes the centres of mesh points. This yields a conservative update and preserves non-negativity of the follower density.

B.1.4 Non-local convolution

Given a kernel, W_χ , and a discrete density, Z , the convolution is given by

$$(W_\chi * Z)(x_i) \approx h \sum_j W_\chi(x_i - x_j) Z(x_j), \quad (6.34)$$

with values outside the spatial domain treated as zero. Here, the compact support of W_χ is exploited and this zero-padded convolution is computed by direct finite-support summation over mesh points satisfying $|x_i - x_j| \leq 3\chi$, rather than by a fast Fourier transform. This is algebraically equivalent to the zero-padded discrete convolution described in Appendix A.1.4, but is implemented directly for compatibility with the Numba Python package.

B.1.5 Diffusion coefficients

In simulations we take a follower spatial diffusion coefficient of $D_F = 1 \mu\text{m}^2 \text{min}^{-1}$, which is one order of magnitude lower than that of Section 6.2, as there is no longer a mechanism of direct follower–follower adhesion to bind follower cohorts together. Polarity diffusion primarily regularises the polarity distribution, and its net effect is strongly attenuated by alignment. For diffusion alone in polarity space, the characteristic spread in p after time t is

$$\ell_p(t) = \sqrt{2D_p t}. \quad (6.35)$$

With $D_p = 10^{-3} \text{min}^{-1}$ and $t = 60 \text{min}$ this gives

$$\ell_p(60 \text{min}) = \sqrt{2 \times 10^{-3} \times 60} = \sqrt{0.12} \approx 0.35, \quad (6.36)$$

such that diffusion produces moderate smoothing across polarity space on a time scale of hours.

B.2 Initial conditions

The initial condition consists of a follower bulk trailing a leader pulse. Followers are initialised as a spatial slab,

$$\rho_F(x, p, 0) = f_X(x) g(p), \quad (6.37)$$

where

$$f_X(x) = \frac{f_0}{2} \left(\tanh \frac{x-x_a^F}{\delta} - \tanh \frac{x-x_b^F}{\delta} \right), \quad (6.38)$$

and $g(p)$ is a narrow mean-zero Gaussian distribution normalised on $[-1, 1]$. Leaders are initialised as a Gaussian pulse,

$$\rho_L(x, 0) = l_0 \exp\left(-\frac{(x-x_0)^2}{2\sigma^2}\right), \quad (6.39)$$

with $0 < x_a^F < x_b^F < x_0 < L$. In simulations we take $[x_a^F, x_b^F] = [65 \mu\text{m}, 870 \mu\text{m}]$, $x_0 = 895 \mu\text{m}$, $\delta = 5 \mu\text{m}$, $\sigma = 12 \mu\text{m}$, and $f_0 = l_0 = 0.95$, yielding a narrow leader peak positioned just ahead of an extended follower population.

Chapter 7

Conclusion

In this thesis, we have used mathematical modelling to gain insights into the biological mechanisms that guide CNCCs as they migrate collectively during chick embryogenesis. To this end, we developed a suite of mathematical models ranging from the single-cell to the continuum scale that allowed us to investigate the origins of morphological and phenotypic heterogeneity within CNCC streams and the factors that confine these streams during migration. Where possible, we also compared model predictions with experimental data from the chick embryo, enabling validation and iterative refinement of the models we developed. In what follows, we summarise the key findings of this thesis and identify directions for future work.

7.1 Summary

In Chapter 1, we introduced collective cell migration as a fundamental biological process and presented the neural crest as a powerful model system for studying its underlying mechanisms. We then reviewed the conserved mechanisms of collective neural crest cell migration, alongside the mathematical models that have been developed to describe these processes. Building on this foundation, Chapter 2 focussed specifically on the cranial neural crest, outlining the principal model organisms used to study CNCC migration and the prevailing models of migration associated with each. We highlighted the chick embryo as a powerful model system within which to study the collective migration of CNCCs, owing to the relative ease with which CNCC migration may be studied and experimentally perturbed in chick. We then reviewed existing mathematical models of CNCC migration and the biological insights they have generated, often in tandem with corresponding experiments. A mathematical model of particular importance was an ABM that, supported by

transcriptional analysis of CNCCs in chick, divides CNCC phenotypes into leaders and followers (McLennan et al., 2012). This model, and the broader leader–follower paradigm, formed the conceptual foundations for the work carried out in this thesis.

In Chapter 3, we developed a novel energy-based mathematical framework to investigate how microenvironmental VEGF gradients influence the morphology of CNCCs migrating from r4 to ba2 in chick (Johnson et al., 2025). By explicitly coupling the number, length, and orientation of actin-driven protrusions extended by CNCCs to both the probability of VEGF gradient detection and the energetic costs of cell membrane deformation and movement through the ECM, the model yielded an expression for the expected chemotactic displacement per unit energy expended by CNCCs, which we referred to as the distance-to-energy ratio (DTER). Within this framework, assuming that CNCCs will maximise the DTER during migration predicted a morphological transition from a *bet-hedging* phenotype in steep VEGF gradients, where cells extend many short protrusions in multiple directions, to a *speculative* phenotype in shallow gradients, where cells extend fewer, longer protrusions, directly recapitulating the leading versus trailing CNCC morphologies observed *in vivo* (Teddy and Kulesa, 2004). Embedding this model in the leader–follower ABM outlined in Chapter 2 showed that this prediction regarding single-cell morphology also translates to collective migration. As such, we hypothesised that CNCCs *in vivo* adopt morphologies that make them maximally efficient during collective migration.

Following this, in Chapter 4, we expanded the ABM in Chapter 2 to formulate mechanistic hypotheses for how three factors expressed in the cranial microenvironment, *Colec12*, *Trail*, and *Dan* (McLennan et al., 2017, 2023), regulate the confinement and coherence of CNCC streams during collective migration (Johnson et al., 2026b). By integrating experimentally-informed effects of these factors on cell speed, chemotactic responses, protrusive morphology, and cell–cell interactions into the ABM, we predicted a synergy between these factors in forming coherent

streams that remain separated by regions devoid of CNCCs during collective migration. Numerical simulations of the expanded ABM predicted that Trail enhances confinement by increasing cell–cell adhesion and drives CNCC movement towards high-density regions along the r4–ba2 pathway, while Colec12 primarily restricts the invasion of CNCC-free zones via chemotaxis down gradients in Colec12. Moreover, we predicted that Dan maintains the coherence of streams by differentially reducing leader-cell speed through its degradation by CNCCs. Model simulations also highlighted a plausible mechanism for the exchange of cells between adjacent streams via Trail- and Colec12-mediated bridging, which is observed *in vivo*, but is, hitherto, without a mechanistic explanation (Gammill et al., 2007). Our analysis in this context generated a series of experimentally testable predictions regarding how these factors jointly maintain typical CNCC migratory patterns that we hope will be the subject of future experimental work.

In Chapter 5, we introduced a *spring–polarisation* ABM framework to investigate the mechanisms by which phenotypic heterogeneity can emerge spontaneously during CNCC migration, and whether the emergent heterogeneity can be appropriately projected onto a leader–follower dichotomy (Johnson et al., 2026a). Building on experimental evidence that CNCC gene expression varies continuously within streams and adapts to local VEGF landscapes (Richardson et al., 2016; Schumacher, 2019b), the model coupled isotropic pairwise mechanical interactions, filopodial VEGF gradient sensing, and a time-averaged polarity variable that relaxes on experimentally motivated timescales. Numerical simulations of the model showed that sustained, coherent migration requires a balance between polarity-driven active forces, intercellular adhesion, and VEGF degradation, which jointly maintain persistent chemotactic cues at the invasive front. Under these conditions, a robust spatial gradient in cell-specific polarity arises; CNCCs at the leading edge of streams acquire and maintain strong VEGF-induced polarity throughout migration, whereas trailing cells remain weakly polarised and move primarily through pairwise mechanical

couplings, yielding an emergent leader–follower stream organisation consistent with prior ABMs (Johnson et al., 2026b; McKinney et al., 2020; McLennan et al., 2012, 2015a, 2017, 2020, 2023; Schumacher, 2019b). The model further reproduced key outcomes of *in vivo* transplantation and ablation experiments (McLennan et al., 2012; Richardson et al., 2016), including transient stalling and the re-establishment of migration when follower-like cells are transplanted to the front, the resumption of collective migration upon leader cell ablation, and the failure of isolated leader-like cells to invade ba2 upon transplantation to regions adjacent to the embryonic hindbrain. These findings demonstrated that CNCC leader–follower behaviour can emerge spontaneously from local environmental sensing and mechanical interactions alone without predetermined phenotypic identities. In doing so, they lend support to existing leader–follower ABMs of CNCC migration by showing that a leader–follower dichotomy can arise spontaneously from a population of cells that are initially phenotypically indistinguishable. In Chapter 6, we formulated a continuum leader–follower representation of CNCC migration in which cell densities evolve in both physical and polarity space. The inclusion of a polarity dimension was motivated by preliminary simulations of a canonical continuum cell–cell adhesion model (Armstrong et al., 2006), which failed to generate the long stream-like formations characteristic of CNCC migration *in vivo*. This limitation stemmed from the inability of a non-local cell–cell adhesion framework to capture the propagation of directional signals from CNCCs at the front of streams, where the local distribution of cell density is anisotropic, to cells at the rear, where the microenvironment of cells is locally symmetric. By embedding a mechanism for finite-range, non-local polarity alignment into the continuum framework, we reproduced chains of follower cells led by a small pulse of leader cells. This model provides a promising foundation for future theoretical work, enabling the incorporation of additional biological complexity to investigate the conditions under which robust travelling waves are biologically feasible in a continuum framework.

7.2 Open questions and future work

As with all mathematical representations of complex biological processes, there are many ways in which the work conducted here could be extended to gain further insight into the mechanisms underlying collective CNCC migration. In this section, we outline three directions that we consider particularly important for advancing our understanding of CNCC migration in chick using mathematical modelling.

7.2.1 Relax the assumption of equal filopodial lengths for the DTER model

In the DTER model derived in Chapter 3, we constrained the space of potential protrusive phenotypes by assuming that all filopodia extended by CNCCs are of equal length. This assumption allowed us to sweep over other parameters of the model, and predict the transition between bet-hedging and speculation that is consistent with observations in chick (Teddy and Kulesa, 2004). *In vivo*, it is not the case that all filopodia extended by CNCCs are equal in length; filopodia vary in their length, lifetime, orientation, and density along the cell membrane (Genuth et al., 2018; Teddy and Kulesa, 2004). Relaxing the assumption of equal filopodia lengths would allow us to explore a wider range of protrusive strategies, and may highlight non-trivial environmental sampling strategies in which longer filopodia probe more distant regions of the microenvironment while shorter filopodia sample the immediate surroundings, potentially modifying the balance between bet-hedging and speculation discussed in this thesis.

7.2.2 Include CNCC-induced mesoderm proliferation in an ABM of CNCC migration

Many of the ABMs of chick CNCC migration hitherto have modelled the growth of the domain through which CNCCs migrate as spatially uniform (Johnson et al.,

2026b; McLennan et al., 2012, 2015a, 2017, 2020, 2023; Schumacher, 2019b). No ABM of chick CNCC migration has yet explicitly modelled the observation that CNCCs may influence the proliferation of mesoderm through which migration occurs (McKinney et al., 2020). A previous ABM of CNCC migration showed that when different segments of the r4–ba2 pathway grow at different rates during migration, the distance over which invasion occurs is affected (McKinney et al., 2020). However, this model did not explicitly represent the hypothesised CNCC-induced mesoderm proliferation *in vivo*. This relationship could be incorporated into an ABM by allowing the local rate of domain growth to depend on CNCC density. This, in turn, would enable the model to capture potential feedback between CNCC advance and tissue expansion and, thus, to assess how this coupling influences migratory speed and stream morphology.

7.2.3 Derive a continuum limit of the *spring–polarisation* ABM and verify consistency with the polarity alignment PDE

The continuum representation of collective CNCC migration we formulated in Chapter 6 is phenomenological, and was formulated in a way that allows directional signals to propagate along streams of CNCCs through advection in polarity space. Although this model reproduces streams of CNCCs similar to those observed *in vivo*, it was not formally derived from a model taking into account biological mechanisms of CNCC–CNCC and CNCC–environment interactions at the single-cell level. In order to verify that our biological inferences made from the polarity alignment PDE are valid, it would be informative to take the continuum limit of the spring–polarisation ABM using well-established techniques for discrete mass–spring models (Murphy et al., 2019, 2020; Murray et al., 2009). Predictions of the resulting PDE could then be compared with those of the simpler polarity alignment model in order

to verify the validity of the conclusions we have drawn from its analysis.

7.3 Final remarks

We conclude by stating that this thesis has contributed to a broader understanding of collective cell migration in the cranial neural crest, and as such, has further highlighted the value of mathematical modelling in the field of developmental biology.

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