

Modeling pattern formation on zebrafish

by

Alexandria Volkening

B.S., University of Maryland Baltimore County, Baltimore, MD, 2011

M.Sc., Brown University, Providence, RI, 2012

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in the Division of Applied Mathematics at Brown University

PROVIDENCE, RHODE ISLAND

May 2017

© Copyright 2017 by Alexandria Volkening

Abstract of “ Modeling pattern formation on zebrafish ” by Alexandria Volkening, Ph.D., Brown University, May 2017

The zebrafish (*Danio rerio*), a widely-studied model organism, is a small fish with distinctive black and yellow stripes on its body and fins. Numerous experimental studies have shown that these stripes, which form as the fish develops from a larva to an adult, emerge due to the interactions of pigment cells. Notably, many zebrafish mutations that disrupt cell interactions display altered patterns, including spots, widened stripes, or labyrinth curves. In this thesis, we work closely with the biological data to develop agent-based models of pattern formation on zebrafish, with the goal of helping to identify wild-type cell interactions and link genes to their functions by suggesting cell behaviors that are impacted in mutations. We model pigment cells as individual agents that interact through migration (coupled differential equations) and experience changes in population size based on a series of short- and long-range interactions (stochastic rules) on growing domains. In a first model, we consider two types of cells, black melanophores and yellow xanthophores, interacting through cell birth, death, and movement. Motivated by new experimental results, our next model incorporates five types of cell agents, and represents a predictive, current system consistent with a range of zebrafish dynamics, including wild-type, early-stage mutations, ablation experiments, and quantitative measurements. It suggests a candidate network of interactions governing cell behavior and proposes, among other findings, what interactions are altered in the spotted leopard zebrafish. Finally, our third model reconciles differences in the number of cells involved in body and fin patterning by showing that two types of cells are sufficient to create stripes on the caudal fin under certain conditions on growth and bone rays.

This dissertation by Alexandria Volkening is accepted in its present form
by the Division of Applied Mathematics as satisfying the
dissertation requirement for the degree of Doctor of Philosophy.

Date_____

Björn Sandstede, Ph.D., Advisor

Recommended to the Graduate Council

Date_____

Andrew Bernoff, Ph.D., Reader

Date_____

Martin Maxey, Ph.D., Reader

Approved by the Graduate Council

Date_____

Andrew Campbell, Dean of the Graduate School

Vitae

Alexandria Volkening

b. January 8, 1989

Arlington, VA, USA

Education

- Brown University, Providence, RI, 2011-2017

Ph.D. in Applied Mathematics, expected May 2017

M.Sc. in Applied Mathematics, 2012

- University of Maryland Baltimore County, Baltimore, MD, 2007-2011

B.S. in Mathematics, Summa cum laude, Honors, Honors in mathematics

Honors and Awards

- 2015 Publication [2] selected as cover image for Interface, Volume 12 (113).
- 2015 Publication [2] featured in Brown News, Fusion News, Science Daily, and Futurity.
- 2015, 2016 Conference Travel Fund, Brown University.

- 2015 Research Travel Grant, Brown University.
- 2015 Graduate Council Travel Award, Brown University.
- 2014, 2015, 2016 International Travel Grant, Brown University.
- 2014, 2015 SIAM Student Travel Award.
- 2011 National Science Foundation Graduate Research Fellowship.
- 2011 Valedictorian, UMBC.
- 2011 Phi Beta Kappa Honors Society.
- 2011 Outstanding Senior in Mathematics, UMBC.
- 2010 Outstanding Teaching Assistant in Mathematics, UMBC.
- 2010 Undergraduate Research Assistantship, UMBC.
- 2007 Premier Scholarship, UMBC.

Teaching Experience

- Instructor & course developer, Multivariable calculus, Catalyst Summer Bridge Program, Brown University, Summer 2015
- Co-Instructor, Business math, RI Correctional Facility, Community College of RI Program, Spring 2014
- Co-Instructor, Basic college math, RI Correctional Facility, Community College of RI Program, Fall 2013
- Teaching Assistant, Methods of applied mathematics I, Brown University, Spring 2013, Fall 2013

- Grader, Real analysis II, UMBC, Spring 2011
- Teaching Assistant, Honors calculus I, UMBC, Fall 2009, Fall 2010

Service and Professional Activities

- Secretary, SIAM Student Chapter, Brown University, 2016–Current.
- Panelist, AWM Panel on REUs, Brown University, 2016.
- Research Group Leader, Math Biology, APMA Graduate Student Retreat, Brown University, 2016.
- Panelist, AWM & Rose Whelan Graduate School Panel, Brown University, 2016.
- Participant, Opening workshop: Issac Newton Institute programme on stochastic dynamical systems in biology, Cambridge, UK. January 18-22, 2016.
- Judge, Brown Mathematical Contest for Modeling, Brown University, 2015, 2016.
- Organizer of minisymposium “Stripe formation on zebrafish: a collection of biological and mathematical perspectives”, SIAM Conference on the Life Sciences, Boston, MA, July 2016.
- Participant, Young Scholars Conference, Brown University. May 22-24, 2016.
- President & Lead Founder, SIAM Student Chapter, Brown University, 2015–2016.
- Judge, AWM National Essay Contest: Biographies of Contemporary Women in Math, 2016, 2017.

- Co-Organizer of special session “Diff. Equations, Probability, & Sea Ice”, Joint Math Meetings, Seattle, WA, January 2016.
- Co-Organizer, Alumni Panel: Jobs in Academia & Industry, Brown University, 2015.
- Member, Honorary Degree Committee, Brown University, 2015–Current.
- Member, Department of Public Safety Oversight Committee, Brown University, 2015–Current.
- Member, Graduate Student Council Finance Board, Brown University, 2015–2016.
- Participant, AMS Math Research Community “Differential equations, probability, and sea ice”, Snowbird, UT. June 21-27, 2015.
- Co-organizer of minisymposium “The behavior of autonomous agents in diverse applications”, SIAM Conference on Applications of Dynamical Systems, Snowbird, UT, May 2015.
- Representative for Applied Mathematics, Graduate Student Council, Brown University, 2014–2016.
- Participant, Sheridan Center Certificate V: Academic Advising Track, Brown University, 2014–2015.
- Co-Organizer of LCDS Workshop on Agent-Based Modeling, Brown University, March 2015.
- Participant, BU/Keio Workshop on Dynamical Systems, Boston, MA. Sept. 15-19 2014.

- Participant, Centro di Ricerca Matematica Ennio De Giorgi workshop “Stability of solitary waves”, Pisa, Italy. May 26-30, 2014.
- Lead-Organizer, Bi-annual finals prep sessions for applied math courses, Brown University, 2013–Current.
- Participant, IMA special workshop: “WhAM! A research collaboration for women in applied math and dynamical systems”, Minneapolis, MN. September 9-13, 2013.
- Secretary, AWM Chapter, Brown University, 2013–2014.
- Experienced Resource Person, NSF Graduate Research Fellowship Program, 2011–Current.
- Vice President, Math Club and Pi Mu Epsilon, UMBC, 2010–2011.
- Undergraduate Student Representative, Promotion and Tenure Committee, UMBC, 2008, 2010.

Publications

- J Abrams, A Schwartz, V Ciocanel, A Volkening, B Sandstede. *Influenza spread on context-specific social networks*. In revision (December 2016).
- A Volkening, B Sandstede. *Modelling stripe formation in zebrafish: an agent-based approach*. Journal of the Royal Society Interface, 12 (2015): 20150812.
- JL Gevertz, Z Aminzare, KA Norton, J Pérez-Velázquez, A Volkening, KA Rejniak. *Emergence of anti-cancer drug resistance: exploring the importance of the microenvironmental niche via a spatial model*, in “Applications of Dynamical Systems in Biology and Medicine”, Springer, 2015: 1–34.

Presentations

- “Agent-based models of pattern formation on zebrafish.” Seminar talk. Numerical Methods for PDEs Seminar, MIT, Cambridge, MA. March 15, 2017.
- “How the zebrafish got its stripes.” Contributed talk. WINRS New England Meeting, Brown University, Providence, RI. March 4, 2017.
- “Agent-based models of pattern formation on zebrafish.” Seminar talk. Widely Applied Math Colloquium, SEAS, Harvard University, Cambridge, MA. January 26, 2017.
- “Agent-based models of pattern formation on zebrafish.” Ignite talk. Dynamics Days, Silver Spring, MD. January 4-6, 2017.
- “Agent-based models of zebrafish stripes.” Poster presentation. Dynamics Days, Silver Spring, MD. January 4-6, 2017.
- “Agent-based models of pattern formation on zebrafish.” Seminar talk. Theoretical Biology Seminar, Penn State, State College, PA. November 8, 2016.
- “Modeling stripe formation on zebrafish.” Minisymposium talk. SIAM Conference on Nonlinear Waves and Coherent Structures, Philadelphia, PA. August 8-11, 2016.
- “Modeling stripe formation in zebrafish: an agent-based approach.” SIAM Annual Meeting, Boston, MA. July 11-15, 2016.
- “Agent-based models of stripe formation on zebrafish.” SIAM Conference on the Life Sciences, Boston, MA. July 11-14, 2016.
- “Agent-based models of zebrafish stripes.” Poster presentation. Conference on Analysis of PDEs using Dynamical Systems Techniques, Boston University,

Boston, MA. June 1-3, 2016.

- “Agent-based and continuum models of stripe formation on zebrafish.” Seminar talk. Brown/BU Dynamics and PDE Seminar, Brown University, Providence, RI. April 25, 2016.
- “From A to Z: Arctic sea ice and zebrafish stripes.” Seminar talk. Graduate Student Seminar, Brown University, Providence, RI. April 11, 2016.
- “Agent-based models of stripe formation on zebrafish.” Contributed talk. Applied Math Days, RPI, Troy, NY. April 1-2, 2016.
- “Agent-based models of zebrafish stripes.” Poster presentation. Opening Workshop: Isaac Newton Institute Programme on Stochastic Dynamical Systems, Cambridge, UK. January 18-22, 2016.
- “Agent-based and continuum models of stripe formation on zebrafish.” Minisymposium talk. SIAM Conference on Analysis of Partial Differential Equations, Scottsdale, AZ. December 7-10, 2015.
- “Modeling the formation of stripes in zebrafish.” Contributed talk. Workshop on Pattern Formation in Differential Equations, Dalhousie University, Halifax, Canada. July 18-22, 2015.
- “Modeling stripe formation in zebrafish.” Minisymposium talk. SIAM Conference on Applications of Dynamical Systems, Snowbird, UT. May 17-21, 2015.
- “Modeling the formation of stripes in zebrafish.” Seminar talk. Max Planck Institute for Developmental Biology, Tübingen, Germany. March 30-April 21, 2015.
- “Modeling the formation of stripes in zebrafish.” Math slam talk. Workshop on Agent-Based Modeling, Brown University, Providence, RI. March 21-22, 2015.

- “Modeling stripe formation in zebrafish: agent-based and continuum approaches.” Seminar talk. Graduate Student Seminar, Brown University, Providence, RI. February 24, 2015.
- “An agent-based model for the formation of stripes in zebrafish due to the reaction and diffusion of pigment cells.” Stability of Solitary Waves, Centro di Ricerca Matematica Ennio De Giorgi, Pisa, Italy. May 26-30, 2014.
- “Agent-based and continuum models of stripe formation on zebrafish.” Seminar talk. Brown/BU PDE Seminar, Brown University, Providence, RI. September 24, 2014.

Professional Associations

- American Mathematical Society (AMS)
- Association for Women in Mathematics (AWM)
- Society for Industrial and Applied Mathematics (SIAM)
- Society for Mathematical Biology (SMB)

Dedication

For Mom, Dad, Faith, and Clair.

Acknowledgements

This thesis is written with gratitude always to my advisor Björn. I am fortunate to be part of a wonderful community of mentors and friends in applied math, and Björn has been the center and, in many ways, the catalyst of that community for me. He has opened up opportunities, been a constant source of support and advice, served as a team member and advocate, and guided my growth as a researcher. It has been a joy and an honor to work with you the past several years, Björn, and the fundamental highlight of my graduate experience.

I am grateful to Andrew Bernoff and Martin Maxey for taking the time to be part of my thesis defense and for their mentorship. A special thank you to the group members of the Nüsslein-Volhard lab, especially April Dinwiddie, Prateek Mahalwar, Ajeet Pratap Singh, and Christiane Nüsslein-Volhard, for the invaluable opportunity to visit the Max Planck Institute and for helpful suggestions from an experimental perspective, and to undergraduate students Madeline Abbott, Emily Briggs, Dorothy Catey, Neil Chandra, Bethany Dubois, and Francesca Lim, who contributed to the work in Chapter 5.

More broadly, there have been many people in the Division of Applied Math, at UMBC, and in the wider applied math community who have supported me. To name a few, I am grateful to Jonathan Bell, Matthew Harrison, Kavita Ramanan, and Walter Strauss, and to collaborators, particularly Yuxin Chen, John Gemmer,

and Mary Silber. I have been very fortunate to be part of Björn's research group, a community of mentors and friends that grows and remains even after graduation.

Few people have had better officemates than me; I have been surrounded by good friends every day, and I am grateful for the encouragement and experiences shared with friends here in Providence, especially Amanda, Chao, Christian, Eirini, Ivana, Paul, Veronica, and Winnie, and afar, especially Andrea, Chelsea, and Nadejda.

Lastly, I am especially thankful to my sisters, Faith and Clair, and my parents, my first teachers and mentors, for their constant support and examples of strength, perseverance, and service - you taught me anything is possible and have been there every step of the way.

Contents

Vitae	iv
Dedication	xii
Acknowledgments	xiii
1 Introduction	1
1.1 Pattern formation and self-organization	2
1.2 Zebrafish patterns	6
1.2.1 Modeling approaches and previous work	9
1.3 Overview and motivation	14
1.3.1 Outline and main results	15
2 Preliminary biological background	19
2.1 Introduction to zebrafish	20
2.1.1 Anatomy	20
2.1.2 Staging and measurements	21
2.2 Pigment cells	23
2.2.1 Interactions	25
2.2.2 Precursors	25
2.3 Overview of pattern dynamics	26
2.4 A note on quantitative measurements	28
2.5 Summary of terminology	28
3 A two-cell model of pattern formation on zebrafish	30
3.1 Introduction	31
3.2 Summary of results	33
3.3 Overview of model	34
3.3.1 Cell migration	36
3.3.2 Cell differentiation and death	38
3.4 Results	40

3.4.1	Cellular interactions	40
3.4.2	Development	43
3.4.3	Ablation	53
3.4.4	Effects of domain growth	55
3.4.5	Mutations	59
3.4.6	Roles of birth, death, and migration	63
3.5	Detailed model description	65
3.5.1	Parameter fitting and biological data	66
3.5.2	Domain growth	67
3.5.3	Length scales	68
3.5.4	Cell migration	69
3.5.5	Cell differentiation	72
3.5.6	Cell death	74
3.6	Continuum limit	76
3.7	Discussion	78
4	A five-cell model of pattern formation on zebrafish	81
4.1	Introduction	82
4.2	Summary of results	85
4.3	Overview of model	87
4.3.1	Cell migration	90
4.3.2	Cell birth and death	92
4.3.3	Cell form changes	95
4.4	Results: part 1	97
4.4.1	Approach	98
4.4.2	Wild-type development	102
4.4.3	Early-stage mutations	107
4.4.4	Quantitative measures	116
4.4.5	Choker mutation	124
4.4.6	Puma mutation	127
4.4.7	Regeneration experiments	129
4.4.8	Temperature-sensitive <i>fms</i> / <i>pfeffer</i>	134
4.5	Results: part 2	136
4.5.1	Predicting the rules governing iridophore form	137
4.5.2	Predicting the rules governing xanthophore form	151
4.5.3	Predicting melanophore behavior	155
4.5.4	Late-stage mutations	158
4.6	Detailed model description	164
4.6.1	Length scales	165
4.6.2	Domain growth	167
4.6.3	Cell migration	168
4.6.4	Cell birth	173
4.6.5	Melanophore death	177
4.6.6	Xanthophore and iridophore form transitions	180
4.7	Discussion	182

5	Extensions to the caudal fin	185
5.1	Introduction	186
5.1.1	Differences between the body and fins	189
5.2	Summary of results	193
5.3	Brief model description	194
5.3.1	Cell migration	196
5.3.2	Cell differentiation and death	197
5.4	Results	198
5.4.1	Pattern formation on a static fin domain	201
5.4.2	Wild-type development on a growing fin	201
5.5	Detailed model description	216
5.5.1	Growth and bone rays	217
5.5.2	Cell migration	220
5.5.3	Cell differentiation	223
5.5.4	Cell death	227
5.6	Discussion	229
6	Conclusion	232
	Bibliography	236

List of Figures

1.1	Zebrafish (<i>Danio rerio</i>)	2
1.2	Examples of pattern formation in nature	4
1.3	Examples of self-organization	5
1.4	Horizontal myoseptum on zebrafish and wild-type development	6
1.5	Motivation for studying zebrafish patterns: regeneration	7
1.6	Motivation for studying zebrafish patterns: diverse mutations	9
1.7	Previous modeling work on zebrafish patterns	10
1.8	Modeling approaches	11
2.1	Zebrafish anatomy	21
2.2	Stripe arrangement and pigment cell ordering on the body of zebrafish	24
2.3	Cell pseudopodia extensions (long-range)	26
3.1	Summary of model length scales	36
3.2	Evaluation of different migration interactions	42
3.3	Wild-type development	43
3.4	Simulated wild-type pattern formation	47
3.5	Cell-to-cell distances and cell speeds in wild-type simulations	50
3.6	Stripe width in wild-type simulations	51
3.7	Melanophore coefficient of variation	52
3.8	Simulated ablation compared with ablation <i>in vivo</i>	54
3.9	Simulated ablation on a static domain	55
3.10	Impact of domain growth on patterning	56
3.11	Simulated choker mutation compared with choker zebrafish	60
3.12	Impact of migration forces on stripe width	61
3.13	Pattern formation without cell migration or without cell birth/death	64
3.14	Experiments used for model fitting and comparison with simulated data	66
4.1	Schematic of wild-type patterning with iridophores	83
4.2	Example range of simulated patterns	86
4.3	5-cell model schematic	88
4.4	Summary of model length scales	89
4.5	Schematic of model cell birth and death	94
4.6	Flowchart for modeling approach	100
4.7	Simulation conditions for wild-type, early stage mutations, choker, puma, and experiments	102
4.8	Initial condition in comparison with empirical images	104

4.9	Wild-type development, example 1	106
4.10	Wild-type development, example 2	107
4.11	Additional examples of simulated wild-type stripes at stage J+	108
4.12	Pfeffer development	110
4.13	Pfeffer with iridophores at stage CR	111
4.14	Nacre development	112
4.15	Shady development	114
4.16	Additional examples of simulated nacre, pfeffer, and shady	115
4.17	Cross mutations between nacre, pfeffer, and shady	117
4.18	Alternative shady and nacre; shady without dense xanthophores	118
4.19	Statistics on cell-to-cell distances	119
4.20	Distribution of melanophore-xanthophore distances; stripe widths	120
4.21	Statistics on melanophore migration	121
4.22	Statistics on melanophore coefficient of variation	122
4.23	Average cell density in wild-type, pfeffer, nacre, and shady	123
4.24	Maximum melanophore density	124
4.25	Choker mutation development	126
4.26	Puma mutation and effect of growth speed	128
4.27	Ablation Experiment 1	130
4.28	Ablation Experiment 2	132
4.29	Ablation at a later stage	135
4.30	Temperature-sensitive fins: pattern degeneration	136
4.31	Building blocks of candidate mechanisms governing iridophore form	141
4.32	Candidate mechanisms governing iridophore form	142
4.33	Results of candidate mechanisms for iridophore loose-to-dense form changes	145
4.34	Results of candidate mechanisms for iridophore dense-to-loose form changes	146
4.35	Csfl-overexpressing zebrafish and <i>Danio albolineatus</i>	148
4.36	Predicted dynamics governing iridophore form transitions	150
4.37	Predicted dynamics governing xanthophore form changes	152
4.38	Testing an alternative mechanism for xanthophore form changes	154
4.39	Pfeffer without local support of melanophores by loose iridophores	156
4.40	Wild-type stripes without short-range inhibition of melanophores by xanthophores	156
4.41	Candidate leopard mutation	159
4.42	Candidate schachbrett mutation	161
4.43	Candidate idex mutation	162
4.44	Crosses between late-stage mutations	163
4.45	Migration forces	170
4.46	Final schematic of proposed cell interactions	184
5.1	Motivation: mutations with altered body stripes but normal fin patterns	186
5.2	Timeline of patterning on the caudal fin	187
5.3	Caudal fin initial condition in comparison with empirical images	200
5.4	Pattern formation on a static caudal fin domain	202
5.5	Fin patterning under distal epithelium growth	204
5.6	Schematic showing similarities between fin patterning and ablation	205
5.7	Fin patterning under uniform epithelium growth	206
5.8	Fin patterning with melanophore migration along bone rays	209
5.9	Fin patterning with melanophore differentiation along bone rays	210
5.10	Fin patterning with density-dependent effects of bone rays	212

5.11	Fin patterning with intermingled cells at the lobes	213
5.12	Fin patterning under frequent cell birth and migration	215
5.13	Methods: caudal fin domain and bone ray specification	218
5.14	Methods: cell position stretching along bones under uniform growth .	220

CHAPTER ONE

Introduction



Figure 1.1: The zebrafish (*Danio rerio*) is characterized by black and yellow stripes across its body and fins. Photo by Azul reproduced under a CC-0 public domain license (<https://creativecommons.org/licenses/publicdomain/>).

This thesis is a study of wild-type and mutated pattern formation on zebrafish using an agent-based modeling approach.

The zebrafish is a small black and yellow striped fish that is widely studied in many different applications [57], including developmental biology [9], cancer [8], and genetic disease [28] (Figure 1.1). With a fully sequenced genome and a nearly transparent embryo, the zebrafish lends itself to the study of vertebrate development [9, 46], and it is particularly noteworthy that over 70% of human genes have an orthologue in the zebrafish genome [28]. Numerous experimental studies (for example, [43, 54, 89]) have shown that the characteristic stripes emerge due to the interactions of pigment cells on the skin, making patterning on zebrafish a self-organization problem. This, combined with the extensive biological and evolutionary applications of zebrafish studies, broadly motivate and frame our work.

1.1 Pattern formation and self-organization

Patterns are ubiquitous in biological and ecological settings, occurring at a range of different time and length scales. As shown in Figure 1.2, vegetation can form patterns

[37, 72], sea ice on the surface of the Arctic ocean may break into spots and labyrinth curves, and shells display both structural patterns in their ridges and decorative patterns on their surface [48]. Patterns on animal and insect coats are particularly widespread (examples include zebras, cheetahs, and butterflies; see Figure 1.2), and here the striking stripes, spots, labyrinth curves, and other skin patterns may fulfill mating and survival functions [68]. Mathematical studies of how these patterns initially form and are maintained in nature can contribute to very real applications. In the ecological setting, differences in the patterns displayed in vegetation in arid ecosystems, for example, can alert researchers to impending desertification [37, 72]. A knowledge of what drives patterns to form on animal skins, in turn, contributes to a better understanding of the mechanisms underlying development, particularly those related to the establishment of direction (e.g. a dorsal-ventral axis) in embryos. Additionally, variations in patterns between species can help explain how organisms evolve in response to their environment [84].

Of particular interest to this work are patterns that emerge due to the self-organization of independent agents. Collective motion and self-organization are visible in many settings: at the level of organisms, swarming locusts, migrating birds, schooling fish, and drivers in traffic all display macroscale behaviors that emerge from microscale interactions (see Figure 1.3). Studying the dynamics that emerge from these agents has applications to control and prevention: for example, a better understanding of why locusts swarm can help scientists combat the negative impact these insects have on crops [77]. Similarly, exploring how drivers behave under different road conditions can suggest methods for countering the formation of traffic jams. Self-organization is not limited to the organism level, however: collective motion of cells in biological systems plays a critical role in wound healing, cancer metastasis, and many other processes [1]. As shown in Figure 1.3c, for example, endothelial cells

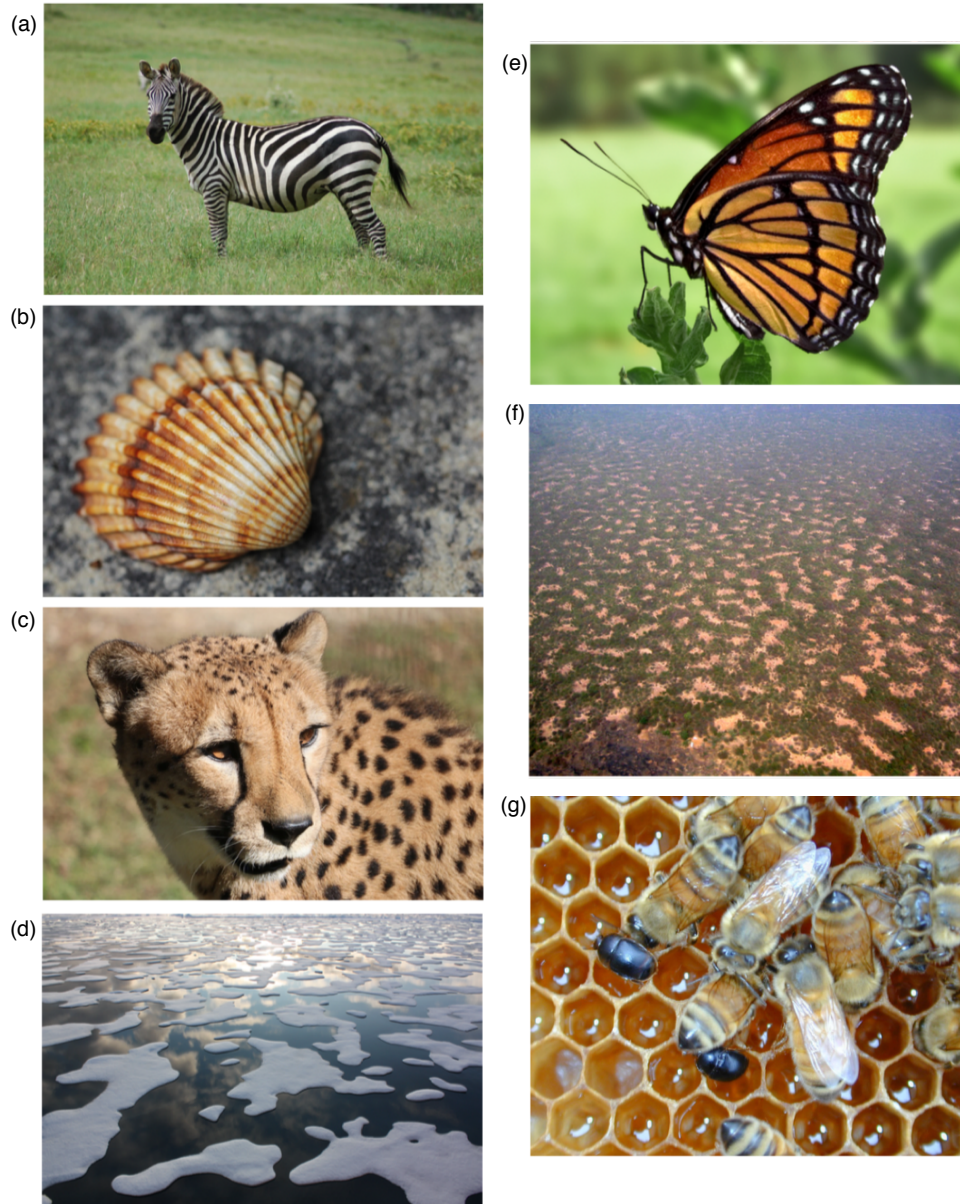


Figure 1.2: Examples of patterns in nature. (a) Zebra stripes; image by Joachim Huber (DSC_1426) reproduced under CC BY-SA 2.0 (<http://creativecommons.org/licenses/by-sa/2.0/>), via Wikimedia Commons. (b) Shell patterns; image reproduced from Max Pixel under CC0 1.0 (<https://creativecommons.org/publicdomain/zero/1.0/deed.en>). (c) Cheetah spots; image reproduced from Pixabay under CC0 1.0. (d) Sea ice patches; image reproduced from Nasa Goddard Space Flight Center's Flickr photo stream, under CC BY 2.0 (<https://creativecommons.org/licenses/by/2.0/>). (e) Butterfly wing pattern; image by PiccoloNamek (English wikipedia) reproduced under GFDL (<http://www.gnu.org/copyleft/fdl.html>) or CC BY-SA 3.0 (<http://creativecommons.org/licenses/by-sa/3.0/>), via Wikimedia Commons. (f) Vegetation pattern; image by Nicolas Barbier reproduced under CC BY-SA 3.0, via Wikimedia Commons. (g) Honey comb pattern; image from CSIRO reproduced under CC BY 3.0 (<https://creativecommons.org/licenses/by/3.0/>).

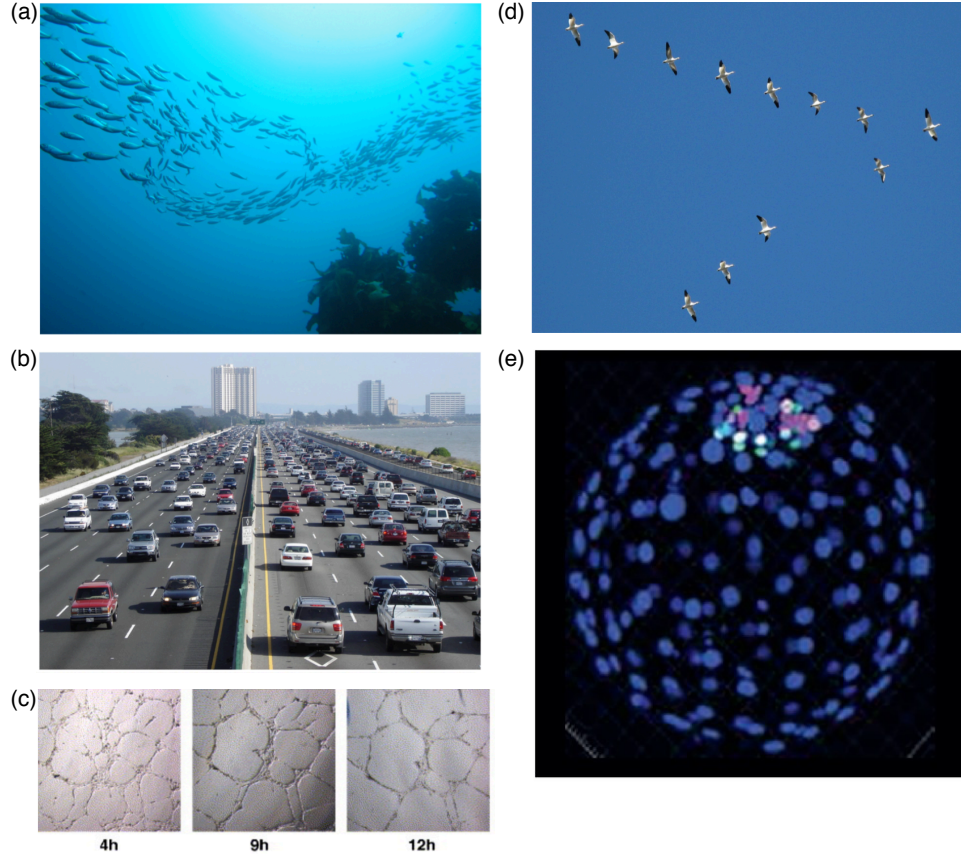


Figure 1.3: Examples of macroscopic dynamics that emerge from the self-organization of independent agents. (a) Schooling fish; image by Joanna Penn reproduced under CC BY 2.0 (<https://creativecommons.org/licenses/by/2.0/>). (b) Traffic flow; image by Minesweeper reproduced under CC BY-SA 3.0 (<http://creativecommons.org/licenses/by-sa/3.0/>). (c) *In vitro* vasculogenesis reprinted from Merks *et al.* [51], with permission from Elsevier. (d) Flocking geese; image by Gilles Gonthier from Canada (Volée d’oies – Flight of geese; uploaded by Amqui) reproduced under CC BY 2.0, via Wikimedia Commons. (e) Self-organization of cells in a developing embryo; image reproduced from Deglincerti *et al.* [10], with permission from Elsevier.

self-organize during vasculogenesis to produce new blood vessels [51]. Most related to this work, self-organization also takes place during the early development of organisms (Figure 1.3e). The characteristic stripes that emerge on zebrafish, in particular, are an example of directional information laid down during early development that depends on self-organization. The reliable appearance of directional information in embryos, including the establishment of dorsal-ventral (top-bottom) axes, is critical for normal development and survival.

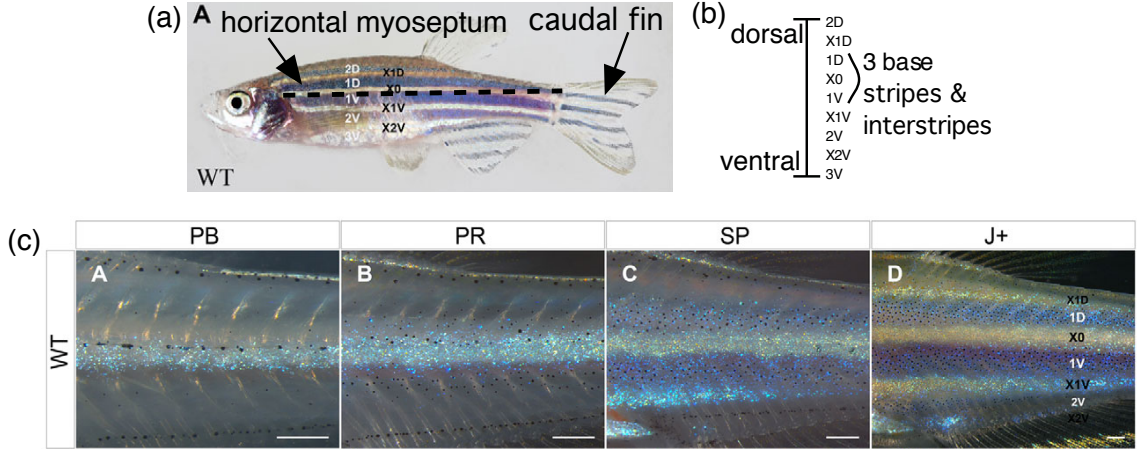


Figure 1.4: (a) Wild-type adult zebrafish. (b) Yellow stripes (hereto referred to as *interstripes*) are labelled X0, X1D, X1V, X2V from the center outward; black stripes (hereto referred to as *stripes*) are labelled 1V, 1D, 2V, 2D. (c) Images of wild-type development. Stripes and interstripes are added sequentially outward from the horizontal myoseptum [17, 75]. PB, PR, SP, and J+ refer to developmental stages; for details see §2.1.2. Scale bar is 250 μm . Images (a) and (c) are adapted and reproduced, respectively, from Frohnhöfer et al. [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd.

1.2 Zebrafish patterns

The zebrafish (*Danio rerio*) features striking black stripes and yellow interstripes on its body and caudal and anal fins, as shown in Figure 1.1. These namesake stripes form on the skin as zebrafish develop from larvae to adults, following the timeline in Figure 1.4 [58]. Briefly, adult stripe formation begins with the appearance of a strip of pigment cells at the center of the fish, and then over time, as the organism grows, the pattern extends outward from the center, with stripes and interstripes added sequentially to the dorsal and ventral edges of the existing pattern [75]. After the initial appearance of the central strip of pigment cells at the horizontal myoseptum (Figure 1.4) when the fish is roughly 3 weeks old, the remainder of the patterning process seems to continue autonomously, orchestrated by the self-organizing interactions of pigment cells [89].

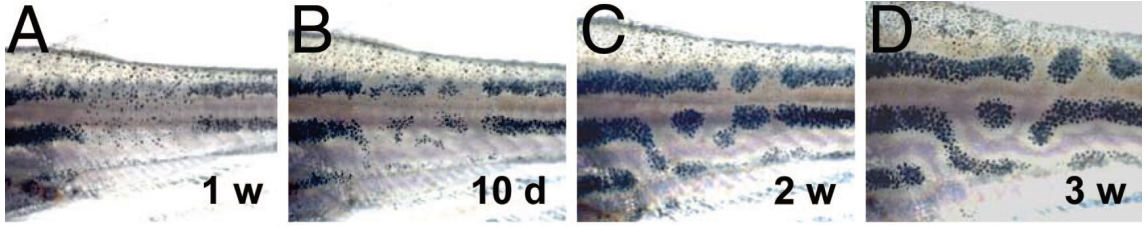


Figure 1.5: Zebrafish patterns regenerate (with lost directionality but maintained width) after artificial disturbance through laser ablation, demonstrating the autonomous nature of stripe development [89]; images are reproduced from Yamaguchi *et al.* [89] with permission; Copyright (2007) National Academy of Sciences, U.S.A.

Zebrafish stripes are formed by three main types of cells: black melanophores, yellow/orange xanthophores, and silver/blue iridophores [17, 25, 41, 54, 74]. While the black cells appear only in dark stripes, xanthophores and iridophores are spread all over the fish, though in different colors and forms in the light and dark regions [45, 75]. These cells appear in multiple layers, forming a mosaic that represents the wild-type pattern [26, 27], with stripes and interstripes about 7-12 cells wide [54]. The cells can be individually discernible on the fish skin, making zebrafish an excellent place to investigate how their self-organization leads to the establishment of directional information in developing organisms.

In addition to wild-type stripes, the rich dynamics that emerge from pigment cells on zebrafish include pattern regeneration [89] and a host of mutations [17, 33, 43, 84] that arise due to altered cell behavior. For example, Yamaguchi *et al.* [89] used a laser to remove a large region of cells on a young zebrafish and observed that the pattern regenerated, albeit with lost directionality, in response to this externally-applied perturbation (see Figure 1.5). This shows that the pigment cells maintain the ability to self-organize into distinct light and dark regions, and the study [89] provided early evidence that zebrafish patterning is caused not by the filling in of some existing pre-pattern but rather by the dynamic and autonomous interactions of cells.

While it was originally thought that only melanophores and xanthophores in interstripes were responsible for pattern formation, new work with mutations in which one pigment cell fails to develop has started to uncover the importance of interactions between all three types of cells on the body of zebrafish [17, 33, 41, 45, 64, 65, 74, 75]. A surge of experimental work (for example, [17, 33, 45, 65, 75]) from 2013 on has shown melanophores, xanthophores, and iridophores work together through migration, birth, death, and changes in form to pattern the body. In contrast to the body, melanophores and xanthophores are still expected to be the key players in pattern formation on the fish fins [86]. Notably, these interactions take place at both short and long range: neighboring cells seem to communicate through direct contact (e.g. gap junctions [33] and short dendrite extensions [29]), but long-range interactions spanning up to half a stripe width have also been identified [12, 25]. The cellular behaviors that drive pattern formation are elucidated using a variety of experimental and modeling techniques, but much remains unknown about cell interactions, especially regarding iridophores. Experimental techniques range from perturbing the skin patterns by removing pigment cells *in vivo* (see, for example, [25, 54, 76, 89]; see Figure 1.5) to observing pairs of cells removed interacting *in vitro* (see, for example, [29, 90]) and studying time-lapse images of wild-type and mutated development (see, for example, [17, 29, 30, 33, 35, 41, 43, 45, 59, 61, 62, 64, 75, 76]).

The wealth of altered phenotypes present in mutations supports the versatility of zebrafish as a model organism for the study of pattern formation and early development. As shown in Figure 1.6, mutated phenotypes include spots, widened stripes, and labyrinth curves, with some fish displaying disrupted patterns on the body but normal patterns on the fins. While the genes associated with these mutations are known, it is unclear how changes in these genes lead to functional differences in how the pigment cells behave. Thus, zebrafish offer a range of dynamics to explore as well

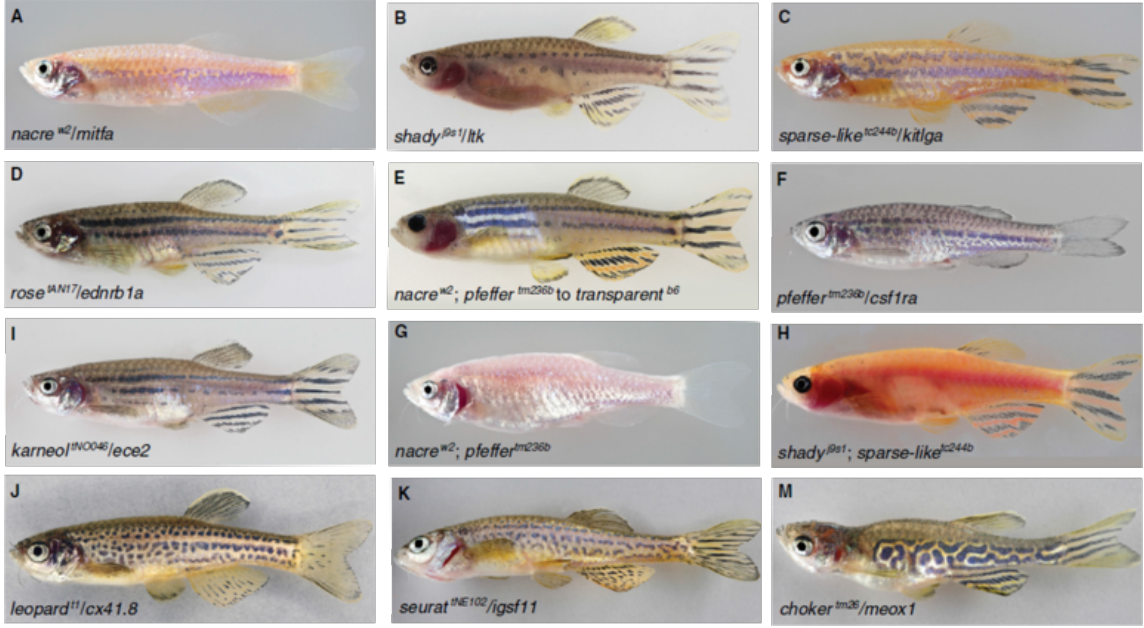


Figure 1.6: A sample of the diverse mutations arising in zebrafish; images reproduced from Singh and Nüsslein-Volhard [74] with permission from Elsevier.

as an opportunity to contribute to a better understanding of a widely studied model organism [57] with biological and medical applications. Modeling the development and regeneration of zebrafish skin patterns, both wild-type and mutations, is the focus of this thesis.

1.2.1 Modeling approaches and previous work

The patterns that appear on zebrafish have been studied in the past using both microscopic and macroscopic approaches. Because up until recently it was thought that only black melanophores and yellow xanthophores were involved in patterning, these previous studies have focused on two-cell models on a rectangular domain (see Figure 1.7). They have included different subsets of the known types of pigment cell interactions at the time of publication (in particular, no models have directly included changes in form or type for iridophores and xanthophores, as this is a

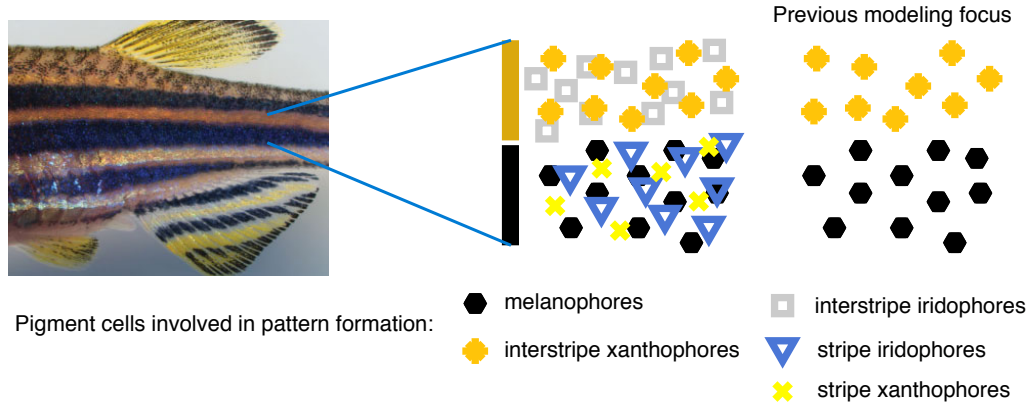


Figure 1.7: Based on the prevailing biology at the time, previous work has focused on two types of pigment cells: melanophores and xanthophores in interstripes. A two-cell model is also how we first approached zebrafish patterns (see Chapter 3), as the importance of iridophores arose during the time of this thesis work [75]. Our later work in Chapter 4 includes iridophores. Image of a wild-type zebrafish is reproduced with adaption from Parichy *et al.* [58], with permission from John Wiley & Sons, Inc.

recent experimental finding [45, 75]). The PDE models of [18, 54, 89] include cell migration through diffusion or convolution terms, in addition to fluctuations in cell densities (birth and death) through reaction terms. The studies of Bloomfield *et al.* [3], Caicedo-Carvajal and Shinbrot [7], Woolley *et al.* [88], Painter *et al.* [56], and Bullara and De Decker [5] involve reduced models in which cells interact only by differentiation/death or only by migration.

As illustrated in Figure 1.8, microscopic models are cell-based and come in two broad varieties: we differentiate between cellular automaton models, in which space is discrete, and agent-based approaches that allow cells to move continuously in the domain. In a cellular automaton framework, cells occupy spaces on a lattice, and discrete-time, stochastic rules control how the dynamics on the lattice evolve over time. Agent-based models, in turn, are built on a series of ordinary or stochastic differential equations that govern agent movement (each agent has its own differential equation). Lastly, macroscopic models offer a zoomed-out view of patterning, with individual agents replaced by continuous densities; the resulting models generally take the form of integro-differential equations or partial differential equations for the

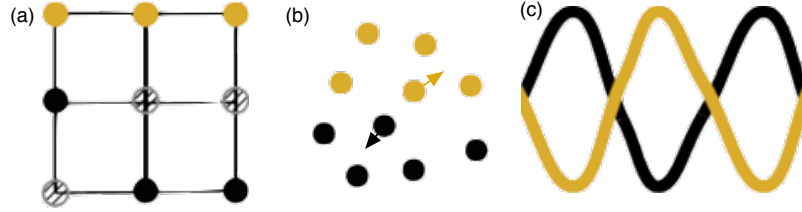


Figure 1.8: The modeling approaches applied to zebrafish patterns include (a) cellular automaton (discrete space); (b) agent-based (continuous space); and (c) continuum (density-based).

evolution of cell densities.

Microscopic and macroscopic approaches each contribute differently to our understanding of pattern formation on zebrafish. Because discrete (cellular automaton or agent-based) models work on the level of individual cells, they are often closer to the underlying application and allow for more detailed descriptions of and predictions about cell behavior. Nevertheless, the proximity to the application comes at the cost of additional parameters, making these simulation-type models time-consuming to fit (and researchers must be especially cognizant of not over-fitting). Furthermore, when these models involve stochastic rules, they are very challenging to analyze. Macroscopic systems, in turn, lend themselves to analysis and offer a broader view of the overarching mechanisms governing patterning (for example, short-range activation and long-range inhibition [19, 49, 80]). These high level approaches suffer in their abstract nature, however, and it is less clear how their results are concretely related to real dynamics on the fish skin at the level of cells.

To our knowledge there are two previous cellular automaton studies of zebrafish patterns: Moreira and Deutsch [53] in 2005 and, more recently, Bullara and De Decker [5] in 2015. In [53], Moreira and Deutsch modeled two types of cells, black melanophores and yellow xanthophores, interacting through birth, death, and movement on growing domains. They were interested in understanding wild-type development and the leopard (spots) and obelix (wide stripes) mutations [43], with a

particular focus on chromatophore precursors. While long-range cell interactions are now known to play an important role in zebrafish patterning, this was not the case in 2005, and, based on the prevailing biology at the time, Moreira and Deutsch did not include long-range communication. More recently, Bullara and De Decker [5] described the birth and death of melanophores and xanthophores on a static domain, and with the benefit of the new experimental data (for example, [54]), included both short- and long-range effects in their stochastic rules. This work [5] took a minimal approach, and was largely interested in understanding the role of the Turing mechanism [80] without diffusion or cell movement. Considering a continuous approximation, they proved that a Turing bifurcation does occur in their model, yet it arises not because of different diffusion coefficients but through nonlocal effects in cell dynamics [5].

We are aware of two agent-based models of patterning on zebrafish. The first [7], by Caicedo-Carvajal and Shinbrot in 2008, modeled the movement of black melanophores and yellow xanthophores through symmetric spring-like forces between cells (birth and death were not included). More recently, Yamanaka and Kondo [90] found melanohores and xanthophores extracted from fins do not exhibit symmetric interactions *in vitro*: instead, they observed chase-run movement, with melanophores moving away from xanthophores, which slowly follow in pursuit [90]. Motivated by this new finding [90], Woolley, Maini, and Gaffney [88] put forward a brief mathematical response in 2014, showing through an agent-based model for the movement of black and yellow cells that chase-run migration is not sufficient to explain patterning.

Continuous-density studies, in turn, have spanned systems of ordinary differential equations (ODEs)[3], integro-differential equations [3, 56], and traditional reaction-diffusion models [18, 54, 89]. In many cases, these works are concerned with patterning in general, using zebrafish as a test case, and the models considered are not

zebrafish-specific, in contrast to the microscopic approaches [5, 7, 53, 88]. The work [3] of Bloomfield, Painter and Sherratt in 2011 involves integro-differential equations for the evolution of black and yellow cell densities due to cell birth and death in 1D (in contrast to experimental data [54], cell death is not regulated by competition between unlike cells in this work); cell migration is not included, and their focus is instead on exploring different types of differentiation. They achieve their best results when cell birth depends on a community effect, with precursors differentiating into black or yellow cells depending on the cells in their neighborhood. More recently, Painter, Bloomfield, Sherratt, and Gerisch considered a general PDE model with integral terms in [56]; when applied to the zebrafish problem, a linear stability analysis showed that chase-run migration [90] on its own does not create stripes, in agreement with the agent-based model [88].

Kondo and his collaborators [54, 89] have both conducted and simulated ablation experiments, in addition to modeling stripe formation from a larval initial condition using reaction-diffusion equations. Special attention has been paid to role of the Turing mechanism in zebrafish patterning, where here the diffusing morphogens proposed by Alan Turing in [80] in 1952 are hypothesized to be replaced by pigment cells. In particular, in the striking work [89] published in 2007, Yamaguchi, Yoshimoto, and Kondo simulated two ablation experiments (see Figure 1.5) using a generic Gierer-Meinhardt reaction-diffusion equation [19, 49], supporting the idea that short-range activation and long-range inhibition are active on the skin of zebrafish. In 2009, Nakamasu, Takahashi, and Kondo followed this work with a 3-factor reaction-diffusion model (the three factors represent the densities of melanophores, xanthophores, and a long-range factor expressed by melanophores). They were able to simulate wild-type development, as well as leopard (spots) and obelix (wider stripes) mutations [43] and the regeneration experiment [89] in Figure 1.5. Lastly,

in 2015 Gaffney and Seirin Lee [18] evaluated how several reaction diffusion models behave with the addition of delays, showing that self-organization dynamics are vulnerable to delay and raising questions about how to combine the Turing mechanism with delays that arise naturally due to cell interactions.

1.3 Overview and motivation

Zebrafish patterning is an active field in the biological community now, and empirical views of cell dynamics on the fish skin have rapidly evolved and changed significantly during this thesis. In 2012, when we started this work, the focus of experimental research was still on melanophores and xanthophores in interstripes (this changed in 2014 with [45, 75], leading to debate in the biological literature [73, 85]). Even before iridophores entered the picture, however, a large amount of new data had emerged since the publication of previous mathematical studies, and some of it contradicted past modeling assumptions. For example, experiments [90] indicated that interactions between melanophores and xanthophores are not symmetric, in contrast to what was assumed in [7, 53], and, furthermore, that long-range communication occurs between cells in adjacent stripes and interstripes [25, 54].

Informed by these advances, we began this project with the goal of building an agent-based model for the interactions of melanophores and xanthophores based as closely as possible to the new biological literature available at the time; this is the subject of Chapter 3. We completed this model around the time the importance of iridophores on the fish skin came to light, and our new goal became producing a predictive model that could address questions surrounding iridophores, mutations, and cell form-changes concurrently with experimental approaches; we stress that this

work, presented in Chapter 4, proceeded alongside the biological literature. Lastly, in Chapter 5, motivated by the differences in growth, development, and the role of iridophores on the body and fins of zebrafish, we are interested in understanding if and how pattern formation is influenced by different growth mechanisms in these two regions. Contributing to a better understanding of patterning on fins can help reconcile lingering questions about the role of iridophores on the body, as mutational analysis seems to show these cells are not needed to produce striped fins.

1.3.1 Outline and main results

We begin in §2 by establishing some preliminary biological background and notation, particularly on zebrafish staging (because growth rates differ significantly between labs, pattern development is best tracked in stages rather than in time [58]). Here we also overview the kinds of experiments and data available, and briefly describe pigment cell interactions. (Most of the biological background we discuss is provided in each chapter alongside our model results, however).

In Sections 3, 4 and 5, we present our agent-based models of zebrafish pattern formation. While our three models differ, they share the same general form: we describe pigment cells as independent agents, modeled as point masses, interacting on growing 2D domains. These models couple cell migration by ordinary differential equations (ODEs) with noisy, discrete-time rules that account for changes in cell population size. Whenever possible, model rules and parameters are specified by biological data.

Two-cell body model. In Chapter 3, we present our initial agent-based model of pattern formation on the zebrafish body. This model includes the birth, death,

and migration of melanophores and xanthophores on growing rectangular domains (some features of dense iridophores are accounted for indirectly). We simulate the development of wild-type patterns, reproduce a regeneration experiment [89], and explore mutations that impact the width of zebrafish stripes. Our main results include:

- repulsion between melanophores and xanthophores maintains stripe separation and boundaries;
- sequential spreading and gathering of iridophores may promote directionality of additional stripes and interstripes; and
- growth supports shorter long-range interactions and widen stripes.

To explore the stability of zebrafish patterns and help limit the parameter space in our two-cell agent-based models, we also formally derive a continuum limit. Our analysis shows that some combinations of cell repulsion and attraction will not lead to Turing instabilities.

The results of Chapter 3 appear as joint work with Björn Sandstede in publication [81]. We are grateful to experimentalists Christiane Nüsslein-Volhard and David Parichy for providing feedback on an earlier form of this work.

Five-cell body model. Motivated by our initial model in Chapter 3 and new experimental data [75] suggesting iridophores play a crucial role in zebrafish pattern formation, we develop an agent-based model incorporating iridophores in Chapter 4. In addition to including cell birth, death, and migration, this model considers xanthophores of two forms (stellate/neutral or compact) and iridophores of two forms (loose or dense). The main contribution of this chapter is a predictive model that is consistent with the known biology and addressing the same questions as the experi-

mentalists. We present a set of rules to govern the transitions in form of xanthophores and iridophores that can account for the development of wild-type patterns, early-stage mutations, and a range of experiments and quantitative measurements. In preliminary work, we also adjust model rules to simulate late-stage mutations, with the goal of identifying candidate cell interactions that may be altered in these mutated phenotypes. In this way, we can help link genetic mutations to their functional difference in how cells behave.

The results of Chapter 4 are in preparation for submission as joint work with Björn Sandstede. We are very grateful to experimentalists April Dinwiddie, Anastasia Eskova, Andrey Fadeev, Hans Georg Frohnhofer, Uwe Irion, Prateek Mahalwar, Ajeet Pratap Singh, and Christiane Nüsslein-Volhard for providing feedback on this model at an earlier stage.

Extensions of two-cell model: fins. In Chapter 5, we adjust our two-cell model of pattern formation on the zebrafish body to explore how wild-type stripes form on the caudal (back) fin. While the horizontal myoseptum seems to initially align stripes horizontally on the body [17], with further directions given by spreading iridophores [75], these cues do not seem to be active on the fin. Instead, our results point to alternative mechanisms for specifying stripe alignment in this region, namely:

- epithelium growth at the distal end of the caudal fin does not account for stripe directionality unless cell birth keeps pace with fin growth;
- uniform epithelium growth can account for stripe directionality on the caudal fin; and
- birth and movement of melanophores along the fin bone rays can align stripes and support wild-type patterning.

At the present time, the results of Chapter 5 appear only in this thesis. This is joint work with Björn Sandstede and undergraduate students Madeline Abbott, Dorothy Catey, Neil Chandra, Bethany Dubois, and Francesca Lim. We are particularly grateful to April Dinwiddie for providing feedback and suggestions from a biological perspective.

CHAPTER TWO

Preliminary biological background

2.1 Introduction to zebrafish

In Sections 2.1.1 and 2.1.2, we provide some preliminary background on zebrafish that will be useful in the remaining chapters of our work. We note that most of this background, and our main focus, is on patterning on the zebrafish body; thus, we will explicitly mention when we are discussing fin stripes and will otherwise have zebrafish body patterns and cells in mind. Furthermore, it should be noted that the citations given represent examples drawn from the biological literature and not comprehensive lists of all experimental work on the topic.

2.1.1 Anatomy

As described in Figure 1.1 and shown in Figure 2.1, wild-type zebrafish display striped patterns on their body and two of their fins (anal and caudal). Stripe formation on the body is aided by the horizontal myoseptum [17, 75], which seems to serve as the only external source of direction specifying that stripes form horizontally. The horizontal myoseptum, shown in Figure 2.1, separates the fish body into dorsal (top) and ventral (bottom) regions. Stripes and interstripes form sequentially outward from this central line, and are often labelled in relationship to it: following the example in [17], we refer to the central (and first to form) yellow interstripe as X0, its two neighboring black stripes as 1V and 1D, the next interstripes moving outward as X1V and X1D, and so on (see Figure 2.1).

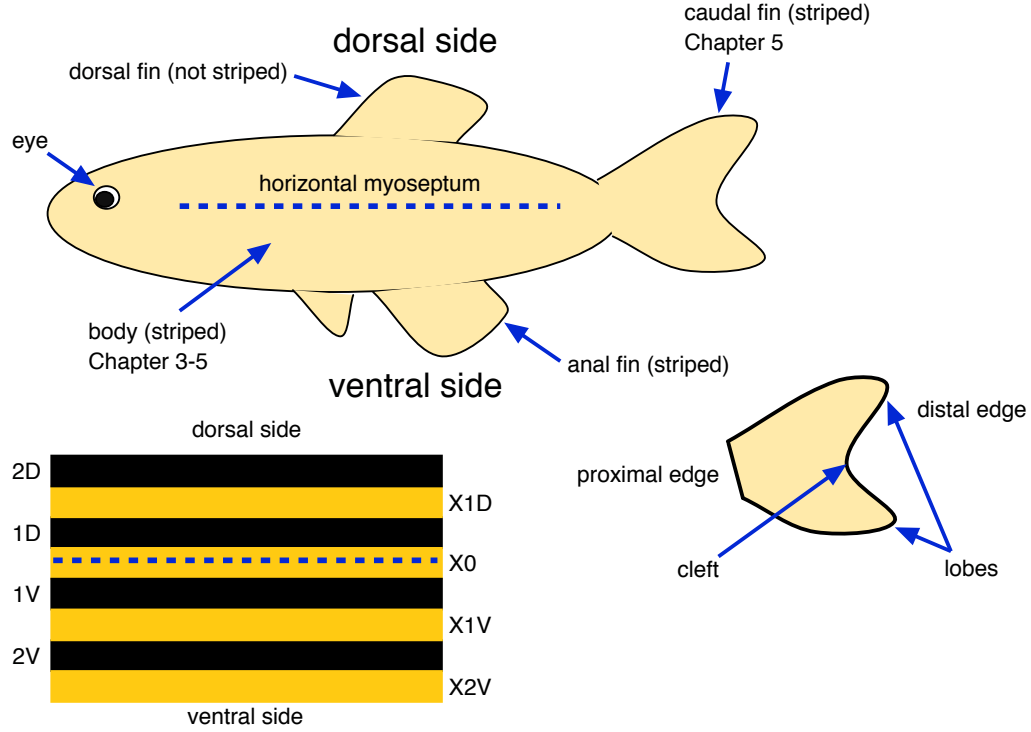


Figure 2.1: Summary of zebrafish anatomy related to this work. Wild-type zebrafish feature horizontal stripes on their body, caudal fin, and anal fin. On the body, the horizontal myoseptum at the center of the fish helps align the pattern horizontally [17]. Black stripes and yellow interstripes on the body are labelled outward from the horizontal myoseptum toward the dorsal and ventral edges of the fish, as X0, 1D and 1V, X1V and X1D, 2D and 2V, and so on [17]. The caudal fin, the subject of Chapter 5, features two lobes and a central cleft.

2.1.2 Staging and measurements

As the pattern forms on the surface of zebrafish, the fish develops from a larva to an adult, and experimentalists classify this development according to different stages [58]. We summarize the stages and corresponding pattern milestones relevant to our work in Table 3.2.

During the timeframe of patterning we are interested in, zebrafish grow in size from roughly 7 mm to 16 mm in length (excluding the caudal fin) [58], and their width, measured roughly at the beginning of the anal fin, varies from about 1 mm to 3 mm (based on our approximations of data in [58]). Outlined in the work [58]

Stage	Acronym	SSL	Pattern milestone
Pelvic fin bud	PB	7.2	Faint stripe of iridophores appears; some larval melanophores dispersed
Pelvic fin ray	PR	8.6	1D and 1V are discernible
Squamation onset posterior	SP	9.6	1D and 1V become more distinct
Squamation through posterior	SA	10.4	
Juvenile	J	11.0	Base stripes are nearly complete
Juvenile+	J+	13.0	2V forms
Juvenile++	J++	16.0	2D develops

Table 2.1: Stages of development related to pattern formation on the body of zebrafish. Standard length (SL) is a measurement of the fish from its snout to the beginning of its caudal fin; standardized standard length (SSL) is an approximation of SL based on representative zebrafish [58].

by Parichy *et al.*, experimentalists rely on several carefully defined measurements of zebrafish size, notably standard length (SL), a measurement from the tip of the snout to the proximal edge of the caudal fin, and height at the anterior edge of the anal fin (HAA), a measurement of body width.

While the most natural means of tracking zebrafish growth and the formation of stripes is time (measured in days post fertilization, or dpf), age turns out to be a poor measurement [46]. This is because growth rates differ significantly depending on lab conditions, as well as on stochastic and genetic effects [46, 58]; for example, Tübingen fish grow particularly fast [46], and McMenamin *et al.* point out that 20 dpf fish can measure anywhere from 5.1 to 9.8 mm SL, and have reached different developmental stages. (We note that this presented a challenge in our modeling work, as comparing results to experiments done by different labs is never straightforward in terms of marking time.) The recommended way of tracking development is using a combination of named stages and a measurement called standardized standard

length (SSL) [46].

Standardized standard length (SSL) is a measurement motivated by standard length, but based on the development of reference zebrafish [46, 58]. Instead of quibbling over whether a fish is 7.2 or 7.3 mm SL, for example, it is simply compared to a population of reference zebrafish and, depending on its development state, given an SSL equal to the archetypical SL for that stage in development [46]. To highlight that SL and SSL are not always equal and SSL is not directly a measurement of the size of the fish in question, SSL is reported without a unit of measurement (so a fish could be 7.2 SSL, but 7.3 mm SL) [46]. In our modeling work, we track a combination of time in days, developmental stages, and SSL, and it was often a challenge identifying length scales and growth rates from the experimental data (the growth rates used for each of our three models are different, and are discussed in each model chapter). The caudal fin domain came with further challenges because the major measurements collected on zebrafish do not include caudal fin measurements.

2.2 Pigment cells

Body patterns: There are three main types of pigment cells present in adult zebrafish stripes: black melanophores (containing melanin), silver/blue iridophores (marked by reflecting platelets that make them iridescent), and yellow/orange xanthophores (containing carotenoid and pteridine pigment) [26, 36]. Each of the 4-5 black stripes on the body of the adult fish consists of melanophores spread over L-iridophores and blanketed by scattered xanthophores and a thin net of blue S-iridophores; the yellow interstripes, in turn, contain densely packed xanthophores over a compact layer of dense S-iridophores (from now on, we will refer to S-

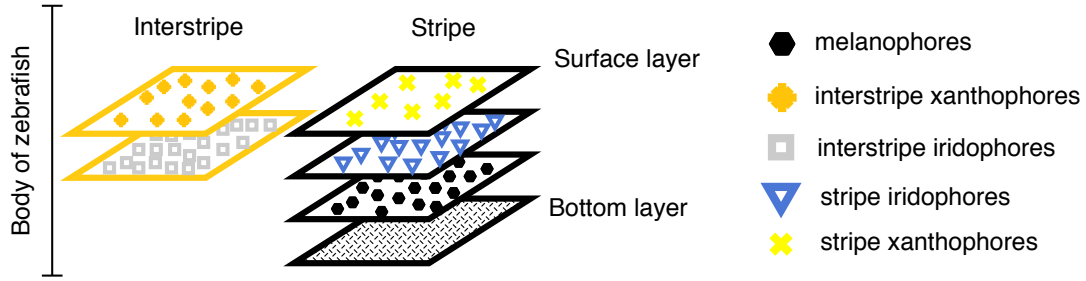


Figure 2.2: The zebrafish body features four layers of pigments cells in black stripes (only three seem to be involved in pattern formation, although L-iridophores in the bottom layer may play a role in stripe maintenance [17]). In dark stripes, stellate xanthophores are spread atop blue loose iridophores, which appear above black melanophores; interstripes feature compact orange xanthophores above silver dense iridophores [17, 27, 45, 75].

iridophores as iridophores) [17, 26, 27, 45, 47, 61, 74, 75]. These cell layers appear in multiple one-cell thick sheets, with the vertical ordering shown in Figure 2.2 [27]. For the remainder of this work, we will distinguish between compact xanthophores and dense iridophores in light interstripes, and stellate xanthophores and loose iridophores in dark stripes (in our two-cell models in Chapters 3 and 5, we refer to compact xanthophores simply as xanthophores). Because they appear on the skin only after the basic patterns have formed, L-iridophores do not seem to be involved in the stripe formation process, though they may be involved in pattern maintenance [17].

Fin patterns: On the striped fins of zebrafish, the arrangement of pigment cells is different: black stripes consist of a layer of xanthophores sandwiched between two sheets of melanophores, and light interstripes contain a single layer of xanthophores [27]. Whereas cells on the body and striped fins typically appear in gapless blankets, iridophores in the fins appear along the bone rays and do not form this structure [27]. We note that there has recently been a surge of experimental work (for example, [17, 44, 45]) detailing the various forms xanthophores and iridophores take on the zebrafish body. We are aware of less recent work on the fins of zebrafish, however, and suspect that the xanthophores there may also appear in different forms in the

light and dark stripe regions, though this seems to remain to be empirically shown.

2.2.1 Interactions

The importance of interactions between cells on the skin of zebrafish is firmly based in the experimental literature, yet the range of types of interactions and cells involved in patterning has evolved and expanded during this thesis work. Up until a few years ago, the focus of the experimental community was on melanophores and compact xanthophores, with iridophores generally considered to not be active in the patterning process. (We highlight the early work of Goodrich and Nichols [22] in 1931 on melanophores and xanthophores on the fins; early, but more modern, work focused on melanophores and xanthophores includes [35, 43]; examples of more recent findings in the last three years include [12, 44, 65, 74, 82]). Cell interactions involve both short- and long-range interactions [54], possibly mediated by direct cell contact, short dendrites, airnemes, filopodia, or long pseudopodia extensions [12, 25, 29, 39] (see Figure 2.3). These interactions will be described in detail during model development in Chapters 3, 4, and 5. Here we simply mention that they include cell birth by differentiation from precursors or division, competition/cell death [54], and cell migration [30, 76, 90]. More recently, it has also been shown that iridophores and xanthophores exhibit form changes between the light and dark regions on the fish skin [45, 75].

2.2.2 Precursors

Adult iridophores, melanophores, and xanthophores on the zebrafish body develop from different sources. Iridophores, arising from stem cells [4, 11, 74] first appear on

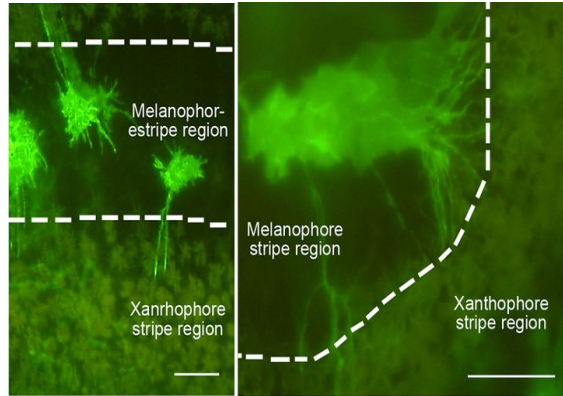


Figure 2.3: Pseudopodia extensions from melanophores extend into yellow interstripes and can reach up to half an adult stripe width in length [25]; these cell extensions do not seem to show any native dorsal/ventral directionality, as melanophores reach pseudopodia horizontally when there are breaks in black stripes. Images reproduced from Hamada *et al.* [25] and licensed under CC-BY 3.0; published by The Company of Biologists Ltd.

the skin at the horizontal myoseptum and then spread outward through considerable cell division and movement [74, 82]. Melanoblasts (unpigmented melanophore precursors [34]), in contrast, enter the skin along neurons and, for the most part, appear in place in the locations of future dark stripes, where they differentiate to form adult melanophores (note that melanophores themselves expand in size during patterning) [4, 11, 74, 82]. Lastly, xanthophores display an entirely different behavior: the adult form of these cells is largely a product of larval xanthophores already spread across the fish at the beginning of adult pattern formation (see §2.3) [45, 47]. These larval xanthophores divide and change their form depending on their environment: the resulting xanthophores become compact in light interstripes and stellate in dark stripes [44, 45].

2.3 Overview of pattern dynamics

Wild-type zebrafish display two separate patterns depending on their stage in development [65]: the first is an early larval pattern consisting of embryonic/early larval

melanophores and iridophores in thin stripes along the dorsal and ventral edges of the fish, as well as in a yolk sac stripe. Larval xanthophores are dispersed across the body of the fish, and early larval melanophores also mark the horizontal myoseptum at the center of the fish [41, 58, 65]. This is the early larval pattern, composed of larval cells. The process leading to adult patterns on zebrafish occurs during metamorphosis, beginning at roughly 21 dpf [82]. The initial adult interstripe begins forming with the appearance of dense iridophores at the horizontal myoseptum when the fish is about three weeks old [17, 75]. Remaining larval melanophores within the interstripe region either die off or migrate out of the X0 region [58]. In a recent paper [45] that led to an active debate (see [73, 85]) in the biological community, Mahalwar *et al.* demonstrated that larval xanthophores leftover from the larval pattern do not disappear but instead remain into metamorphosis, differentiating to cover the fish skin. This thesis is interested in understanding the formation of the adult pattern on zebrafish; thus, we focus on the dynamics immediately after the appearance of iridophores at the horizontal myoseptum, and discuss the adult patterning timeline in detail as we present our results in Chapters 3 and 4.

In addition to wild-type adult stripe development, there are two other broad types of dynamics we are interested in understanding from a modeling perspective: mutations and *in vivo* experiments. Mutations are separated into two classes: those in which certain types of pigment cells fail to develop (early-stage mutations), and those in which, despite normal early-stage development of cells, the fish still displays altered patterns (late-stage mutations) [35]. These mutations include spots, stripes of different widths, labyrinth curves, undulating stripes, and checkerboard patterns, and the wealth of patterns is increased by cross-mutations that combine phenotypes (see, for example, [14, 16, 17, 33, 35, 43, 84]). *In vivo* experiments, in turn, include ablation (for example, [25, 54, 65, 82, 89]) and temperature-sensitive mutation experiments

[61].

2.4 A note on quantitative measurements

Zebrafish patterning is studied experimentally both *in vivo* (see, for example, [17, 54, 76]) and *in vitro* (see, for example, [29, 30]). Much of the data generated by these studies is qualitative; here we briefly highlight a portion of the types of quantitative measurements collected (we will discuss measurements in detail as they relate to our model results in Chapters 3 and 4). Quantitative measurements available include cell speeds [76, 90], cell densities [4, 35, 45, 52, 63], and distances between neighboring cells [44, 76]. Additionally, measurements of xanthophore airneme [12], xanthophore filopodia [45], and melanophore pseudopodia [25] extensions have been collected, as well as measurements of cell size [44, 74] and stripe width [15, 25]. Pattern quality can be measured using the melanophore coefficient of variation (CV), a local measurement that quantifies how variable melanophore spacing is, with more uniform spacing associated with better formed patterns [61, 62]

2.5 Summary of terminology

For future reference, we summarize some terminology and conventions we will employ in the remainder of this work here:

- compact xanthophores := orange xanthophores in light regions on the fish skin (interstripes in wild-type)
- $\Delta_{mm} = 50 \mu\text{m}$ (the average distance between neighboring melanophores mea-

sured in [76])

- $\Delta_{xm} = 82 \mu\text{m}$ (the average distance between stripes and interstripes from [76])
- dense iridophores := silver iridophores in light regions
- dpf := days post fertilization, a measurement of time in zebrafish development
- interstripes := yellow stripes
- loose iridophores := blue iridophores in dark regions
- SL := standard length, a measure of fish length [58]
- SSL := standardized standard length, a measure of fish length [58]
- stellate/neutral (or simply stellate) xanthophores := yellow xanthophores in dark regions on the fish skin (stripes in wild-type)
- stripes := black stripes

CHAPTER THREE

A two-cell model of pattern formation on zebrafish

3.1 Introduction

Our original motivation for modeling pattern formation on zebrafish came largely from the work of Kondo and his collaborators: in [54, 89], they show that the stripes on zebrafish dynamically regenerate in response to perturbation, and they present a series of short-range activation and long-range inhibition interactions on the fish skin. Their experimental results are compared to simulations of a generic Gierer-Meinhardt reaction-diffusion equation [19], which shows the same kind of regenerative behavior. As discussed in Chapter 1, reaction-diffusion equations are a traditional and powerful tool for studying pattern formation, and, zebrafish stripes have been explored from this perspective by several studies [18, 54, 89]. The benefit of these models is that they can capture a wide range of different patterns and are analytically tractable; furthermore, the ablation experiments [89] by Kondo and his collaborators suggest a Turing instability [80] may be at play.

The agreement between the PDE system and the fish dynamics is compelling, and certainly captures something inherent in the biology. Nevertheless, these macroscopic approaches suffer in their ability to comment on the level of individual cell interactions, and the relationship between the few parameters in a reaction-diffusion equation, which necessarily represent multiple cell interactions, and the real dynamics is unclear. In contrast, discrete models, whether agent-based or cellular automaton, allow modelers to describe cells in more detail, and there is a clearer relationship between the parameters in these models and cell behaviors (though the sheer number of parameters in discrete models is also one of their biggest drawbacks). In order to combine the benefits of both approaches and account for the wealth of new experimental data available since previous discrete models [7, 53] were published, the original motivation for this chapter was to put forward a biologically-current model

of zebrafish patterning starting from a cell-based perspective:

Goal: To better understand wild-type and mutated cell interactions, develop a discrete model of zebrafish stripes and then find a continuum limit.

In contrast to a top-down PDE approach, we are interested in understanding what kind of models naturally come out of the experimental data if we let the biology lead. Because patterning on zebrafish depends on the interactions of discrete pigment cell agents, we chose to use an agent-based approach, as it seems the most natural biologically. This choice allows us to work on the same time and length scales as the experimental data and directly compare our results with the biological literature. While iridophores are now known to play a critical role in patterning [65, 75], the focus of the experimental community was still on melanophores and compact xanthophores when we began our work in 2012. Thus, based on the known biology at the time, this model considers the migration, differentiation, and death interactions of black melanophores and yellow xanthophores, and takes some properties of iridophores into account indirectly (additional cells are incorporated directly into our model in Chapter 4). Motivated by the experiments [54], we specify rules for cell birth and death that take the form of short-range activation and long-range inhibition. Cell migration, in turn, is described using a kinematic model with Morse potentials, motivated by the mathematical literature on collective motion and swarming [2, 77]. To study the timeline of pattern development, we incorporated growth into our model, stretching the domain and cell positions at each time step.

Around the same time as we were completing this first model, an active debate began in the biological community about the role of Turing mechanisms on the fish skin [45, 73, 85]. This was motivated in part by the new finding that iridophores have a crucial role in wild-type stripe formation [17, 65, 75]. Additionally, it was

found that there is no diffusion on the fish skin [39], and, instead, the pigment cells communicate directly through long cell extensions [25]. Our model accounts for these extensions through long-range communication rules and supports the finding that iridophores are needed for wild-type development.

Outline: We summarize the results of this chapter in the next section and give a brief overview of the general form of the model in §3.3. For details on parameter specification, see §3.5. Model results are discussed in §3.4, with key findings in *italics* described in detail and biological data incorporated simultaneously. Section 3.7 provides a brief discussion of results, but focused mainly on explaining where our model can be improved and suggesting some future directions.

3.2 Summary of results

In this chapter, we present a model of pattern formation on the zebrafish body that incorporates two cell types: black melanophores and yellow (compact) xanthophores. At the time of model development, the biological community in general thought that these were the main cells involved in patterning. We use our model to study cellular interactions (§3.4.1), wild-type development (§3.4.2), ablation experiments (§3.4.3), fish growth (§3.4.4), and mutations (§3.4.5). Our main findings indicate that:

- fish growth widens stripes and interstripes (§3.4.4);
- repulsion between melanophores and xanthophores maintains stripe/interstripe separation and boundaries (§3.4.1);
- the horizontal myoseptum and spreading/aggregation of iridophores (tested indirectly in our model by the sequential appearance of associated interstripe

xanthophores) guide pattern directionality (§3.4.2).

In addition, in §3.4.3, we reproduce an ablation experiment [54]. We also include results on zebrafish mutations (§3.4.5) and investigate the importance of different aspects of pattern formation by removing cell migration or cell differentiation and death from the model (§3.4.6).

3.3 Overview of model

We begin by broadly overviewing the model and refer to §3.5 for a detailed description and a discussion of the data used for parameter fitting. We reduce the complicated dynamics on the fish skin to the interplay of two types of pigment cells, which we model as point masses (so we do not take into account cell size directly). We will refer to these two cell groups as melanophores and xanthophores, but it is important to note that the xanthophores, in particular, represent idealized particles that indirectly take into account some dense iridophore behavior. This modeling simplification is based on experimental observations (see, for example, [26, 65]) that xanthophores are closely associated with dense iridophores in interstripes, as well as the known biology at the time of model development. We do not take into account L-iridophores, and the remaining cell types (blue S-iridophores and scattered xanthophores in black stripes), which appear at much lower density on the zebrafish skin, are not discussed in this chapter, but are included in our five-cell model in Chapter 4 (note that since we do not include two types of xanthophores here, it is not necessary to distinguish between compact xanthophores in interstripes and stellate xanthophores in dark stripes as in Chapter 4, so we do not use the added adjective: the xanthophores described in this model are compact xanthophores in light interstripes).

We consider two set-ups: developing fish (growing rectangular domain) and adult fish (static rectangular domain). When included, growth takes the form of stretching the domain uniformly with rates inferred from [17, 53, 58] (for details, see §3.5.2). For simulations of stripe/interstripe development, we follow the experimental convention of indirectly tracking time using stages associated with developmental milestones rather than days, as growth/development rates are highly variable and do not lend themselves to direct comparison with empirical data [58, 65] (see Sections 2.1.2 and 3.4.2). Without growth (or in the case of ablation experiments, for which the data itself is given in days), time is measured in days from the initialization of the simulation at $t = 0$. Unless otherwise noted, boundary conditions are roughly Neumann on growing domains and periodic on static domains (see §3.5.2 for details).

Because stripe/interstripe width seems to vary with fish age (an observation we made from images found in [58]), we base our length scales on measurements of average distances between cell centroids, namely $\Delta_{mm} = 50 \mu\text{m}$ (the average distance between neighboring melanophores), $\Delta_{xx} = 36 \mu\text{m}$ (the average distance between neighboring xanthophores), and $\Delta_{xm} = 82 \mu\text{m}$ (the average distance between melanophores and xanthophores at stripe/interstripe boundaries) [76], as well as on the observation [54] that stripes/interstripes are about 7-12 cells wide. Throughout this chapter, we refer to interactions on three length scales that are measured between cell centroids: contact, short-range, and long-range. We call contact interactions those that occur on a scale less than Δ_{xx} ; short-range interactions, in turn, involve cell communication on the order of Δ_{xm} ; these interactions include the contact range but also extend past it (Figure 3.1). Contact and short-range communication may be mediated by direct contact or by short dendrites [29, 39, 43, 90]. Lastly, long-range refers to distances on the order of $\frac{9}{2}\Delta_{mm} + \Delta_{xm}$ (approximately half a stripe width, assuming stripes of width 10 cells). These interactions may be

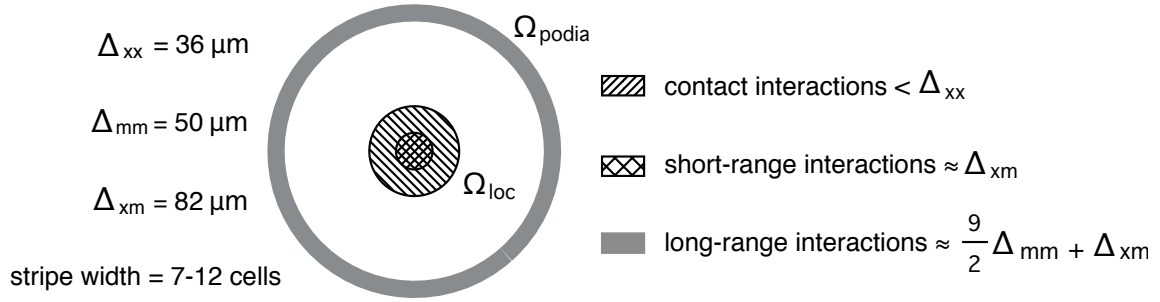


Figure 3.1: Summary of length scales. Δ_{xx} , Δ_{mm} , and Δ_{xm} refer to the average distances between cell centroids for two neighboring xanthophores, two neighboring melanophores, and melanophores and xanthophores at stripe/interstripe boundaries, respectively, as observed experimentally in [76]. Long-range interactions may represent communication by pseudopodia [25, 39]. Short-range and contact interactions may involve shorter dendrites or direct contact [29, 39, 43, 90].

governed by pseudopodia, which can reach a maximum of half an adult stripe width [25, 39].

3.3.1 Cell migration

The movement of each cell is governed by an ordinary differential equation for its position that specifies its interactions with the other cells in the underlying two-dimensional domain. The equations are

$$\frac{dM_i}{dt} = - \sum_{j=1, j \neq i}^{N_m} \nabla Q^{mm}(M_j - M_i) - \sum_{j=1}^{N_x} \nabla Q^{xm}(X_j - M_i), \quad (3.1)$$

$$\frac{dX_i}{dt} = - \sum_{j=1, j \neq i}^{N_x} \nabla Q^{xx}(X_j - X_i) - \sum_{j=1}^{N_m} \nabla Q^{mx}(M_j - X_i), \quad (3.2)$$

where M_i is the position of the i th melanophore; X_j is position of the j th xanthophore; N_m is the number of melanophores; N_x is the number of xanthophores; $\nabla = (\frac{\partial}{\partial x}, \frac{\partial}{\partial y})$

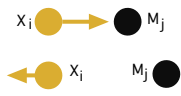
Action	Potential	Velocity of i th cell due to j th
M-M short-range repulsion	Q^{mm}	
X-X short-range repulsion	Q^{xx}	
X-M short-range repulsion	Q^{xm}	
M-X contact attraction & short-range repulsion	Q^{mx}	

Table 3.1: Summary of cell migration and notation.

is the gradient in two dimensions; and $Q^{k\ell}$ are Morse potentials of the form

$$Q^{k\ell}((x, y)) = R^{k\ell} e^{-\sqrt{x^2+y^2}/r^{k\ell}} - A^{k\ell} e^{-\sqrt{x^2+y^2}/a^{k\ell}}, \quad (3.3)$$

where $R^{k\ell}$, $r^{k\ell}$, $A^{k\ell}$, and $a^{k\ell}$ differ between the four types of pairwise cellular interactions and correspond to the strength and length scales of repulsion and attraction, respectively. Informed by [29, 39, 76, 90], we model the effects of melanophores on melanophores (Q^{mm}), xanthophores on xanthophores (Q^{xx}), and xanthophores on melanophores (Q^{xm}) as repulsive (see Table 3.1). The forces Q^{mx} on xanthophores due to melanophores are attractive at contact and repulsive at short range. This choice was based on *in vitro* experiments [90] that uncovered a chase-run mechanism: it was observed that melanophores extracted from the fins run away from short dendrites extended toward them by xanthophores, which slowly follow in pursuit; see 3.4.1 for details. We note that the chase-run mechanism observed in [90] has an anticlockwise bias; as a simplifying assumption, we chose not to include this bias in our model (for a simulation of spiraling chase-run movement, see [88]).

3.3.2 Cell differentiation and death

Our model is informed by a set of ablation experiments [54] performed by Nakamasu *et al.* to deduce when cells differentiate and die (see Figure 3.14). Their results suggest that xanthophores in adjacent interstripes promote both the differentiation and survival of melanophores over at least half a stripe width in distance. According to [25, 54], these long-range effects may be paired with short-range competition of xanthophores and melanophores (while we model short-range competition through cell death rules, it is also possible that this effect could arise due to cell movement). It should be noted here that significantly less data was recorded on xanthophores than on melanophores in [54], so we introduce the modeling assumption that xanthophores differentiate under analogous (opposite) conditions as do melanophores (we remove this assumption in our five-cell model; see Chapter 4). The resulting rules, outlined below, take the form of short-range activation and long-range inhibition.

We update populations for differentiation and death once per day based on the proportion of melanophores and xanthophores in two neighborhoods. A narrow annulus Ω_{podia} models long-range communication, possibly regulated by pseudopodia [25, 74], and a small disk Ω_{loc} represents short-range interactions (Figure 3.1). At the end of each day, every pigment cell is evaluated for possible death, with rules summarized below:

- Xanthophores die when the proportion of melanophores to xanthophores in their immediate vicinity (Ω_{loc}) is too high. Similarly, melanophores die when the proportion of xanthophores to melanophores in Ω_{loc} is too high.
- Melanophores may also die (with small probability [54]) if the proportion of melanophores to xanthophores in Ω_{podia} is too low. This reflects the experimental evidence [54] that xanthophores promote melanophore survival in adjacent

stripes.

It has been shown that melanophores and iridophores arise from a particular set of stem cells [4, 11, 74], and that xanthophores are derived from larval xanthophores widely scattered across the larval fish skin [45, 47, 60]. Based on these findings and on experimental images [89], we assume new cells appear in the fish skin domain by differentiation from precursor or stem cells, which we do not otherwise take into account. Differentiation is implemented each day by selecting a set number of random locations for possible melanophore differentiation and another equal number of random locations for possible xanthophore differentiation. At each of the selected positions, differentiation occurs according to the rules below:

- A new xanthophore appears in the selected location if it is locally surrounded by xanthophores in Ω_{loc} and the proportion of melanophores to xanthophores in Ω_{podia} is high enough.
- A melanophore differentiates under the opposite conditions: high melanophore to xanthophore ratio in Ω_{loc} and high xanthophore to melanophore ratio in Ω_{podia} .
- A small amount of random differentiation is also included.

With the exception of the small amount of random birth in our model, these conditions require that new cells differentiate only in locations near populated areas, as there must be nonzero numbers of melanophores and xanthophores in Ω_{loc} and Ω_{podia} for the rules to apply. Thus, though we randomly select locations for cell birth from the entire domain, we limit differentiation to areas where cells already exist, in effect reducing the probability for differentiation away from sources spreading outward from the center of the fish, where the larval pattern is initialized (see §3.4.2). This choice allows us to preserve the sequential nature of stripe development described in

[74, 75].

3.4 Results

We now present a study of pattern formation on zebrafish that includes development (§3.4.2), ablation (§3.4.3), and mutations (§3.4.5). We also include results on cellular interactions (§3.4.1) and a discussion of reduced forms of our model (§3.4.6) and the effects of domain growth (§3.4.4) on patterning.

3.4.1 Cellular interactions

Short-range repulsion preserves stripe/interstripe boundaries and separation

The experimental studies [54, 76] informed the repulsive form of the homogeneous interactions in our model. The asymmetric interactions between melanophores and xanthophores extracted from fins were studied *in vitro* by Yamanaka and Kondo [90] and Inoue et al. [30]. The results [90] suggest that heterogeneous interactions take the form of chase-run movement regulated by dendrites: melanophores move away from dendrites extended toward them by xanthophores, and xanthophores slowly follow in pursuit (note that this *in vitro* behavior have not yet been observed *in vivo*). In contrast, other studies [17, 74] indicate mutual repulsion between melanophores and xanthophores on the zebrafish body. To study these differing reports, which may represent the differences between body and fin cells, we model xanthophores as having a repulsive effect on melanophores and identify several possible mechanisms for the opposite interaction. The mechanisms of melanophore-on-xanthophore

interactions we tested are:

- A.** Attraction of xanthophores by melanophores at short range;
- B.** Contact repulsion and short-range attraction of xanthophores by melanophores;
- C.** Contact attraction and short-range repulsion of xanthophores by melanophores;
- D.** Repulsion of xanthophores by melanophores at short range.

Our results show that pure attraction, the simplest choice compatible with [90], leads to patterns with reduced separation between stripes and interstripes, as indicated in Figure 3.2a. Woolley et al. [88] also evaluated this mechanism in a minimal agent-based model for cell movement and found that chase-run behavior did not produce patterns. This result [88] was further supported in [56] by a stability analysis of a general nonlocal partial differential equation model: Painter et al. [56] found that chase-run behavior combined with repulsive homotypic interactions could not produce unstable wavelengths. We conducted a linear stability analysis of the continuum model that we obtained as the limit of our agent-based model (see §3.6) and, in agreement with [56], concluded that Mechanism A, unlike Mechanisms B-D, could not produce patterns from a uniform state.

We tested two ways of including attraction and repulsion in the forces that melanophores exert on xanthophores. Mechanism B, contact repulsion and short-range attraction, results in migration of xanthophores from interstripes into stripes (Figure 3.2b). Our simulations show that Mechanisms C and D, on the other hand, produce the correct cell-to-cell distances and support stripe/interstripe boundary separation and integrity, as shown in Figure 3.2a. While Mechanisms A and B do not seem to describe wild-type patterns, these interactions may be associated with mutations (see §3.4.5).

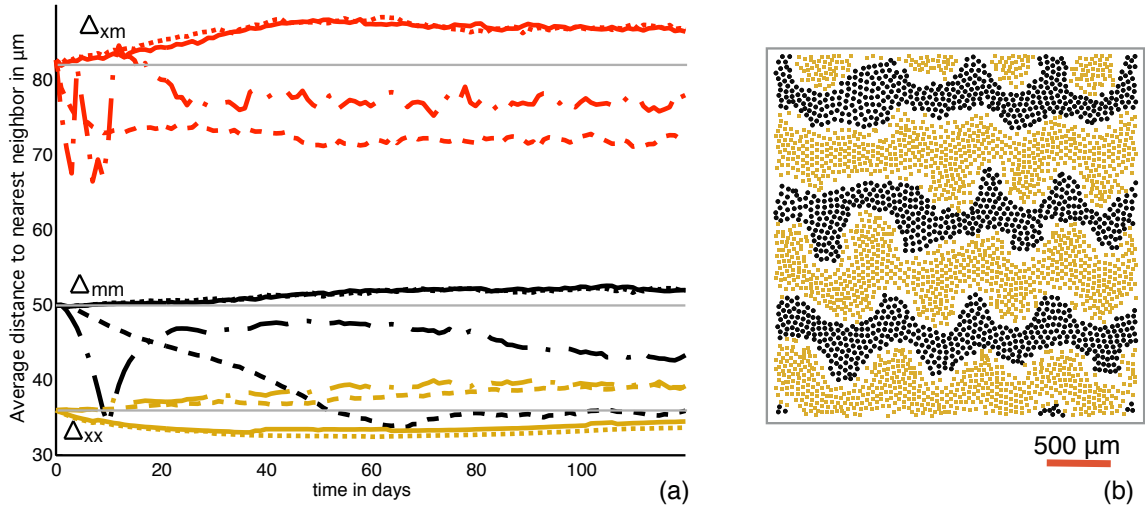


Figure 3.2: (a) Evolution of average cell-to-cell distances (measured between cell centroids) for Mechanisms A (dashed), B (dot-dashed), C (solid), and D (dotted) for representative simulations. Empirical measurements from [76] are represented as straight grey lines for comparison. (b) Pattern generated 100 days after simulation began under Mechanism B; xanthophores migrate toward stripes, disrupting alignment. The initial condition used for both images is a set of adult stripes and interstripes with cells at the experimentally-measured distances [76] apart (see Figure 3.12a). Note that the discrete cells in (b) and in the remaining images in this work may appear as a continuous density in some regions due to the scale of the figures, but this artifact disappears upon greater magnification.

In summary, our results indicate that net repulsion between xanthophores and melanophores maintains stripe/interstripe boundaries. Because more data is available on chase-run movement (in particular, *in vitro* measurements of cell speeds), we chose to use Mechanism C, rather than D, in our simulations. As mentioned above, chase-run behavior was linked in [39, 90] to dendrite communication. Frohnhöfer et al. [17] have suggested that attractive interactions between xanthophores and the dense S-iridophores underneath them may be responsible for confining interstripes. Furthermore, Patterson et al. [64] demonstrated that overexpression of xanthophores and reduction in iridophores on the fish skin is linked to intermingling of cells at stripe/interstripe boundaries. Thus, it appears that interactions between all three types of cells may be involved in preserving the sharp separation between melanophores and xanthophores (for proposed networks of such interactions, see [17, 65]). The short-range repulsion in our model could be an indirect way of ac-

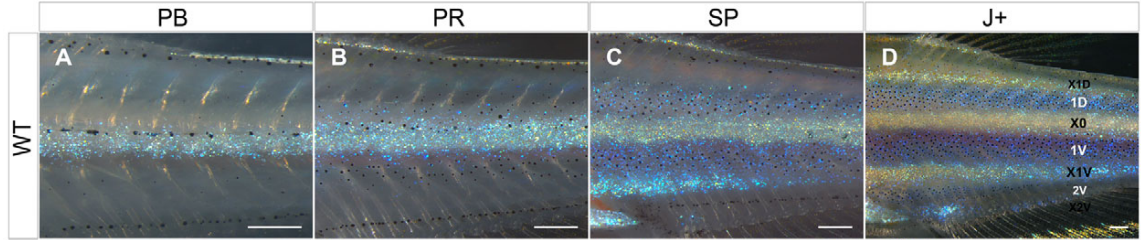


Figure 3.3: Images of wild-type development. Reproduced with permission from Frohnhöfer et al. [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd. PB, PR, SP, and J+ refer to developmental stages shown in Table 3.2. Scale bar is 250 μm .

counting for iridophores, which were not included in the *in vitro* experiments [90], or could support suggestions by Nüsslein-Volhard and her collaborators [17, 33, 74] that cellular interactions in the fins and body are intrinsically different.

3.4.2 Development

Adult pigmentation evolves from a larval pattern to a set of 4 interstripes and 4-5 stripes in the metamorphic period lasting from 20 to 45 days post fertilization (dpf), as reported in [45, 75] (it should be noted, however, that rates of development can vary significantly between labs [58]). As described in §2.3, the larval pattern consists of melanophores and iridophores in narrow stripes along the dorsal and ventral sides of the fish, as well as in a yolk-sac stripe (see, for example, [41]). Dense iridophores appear along the horizontal myoseptum and seem to orientate the first interstripe and first two stripes (see, for example, [17, 45]). Larval melanophores are also visible in a narrow strip along the horizontal myoseptum, but they withdraw from the prospective interstripe region by death or migration to make room for iridophores, which promote xanthophores (see, for example, [17, 58, 75]). This mechanism, shown in Figure 3.3, leads to the first interstripe X0 and adjacent stripes 1D and 1V.

Nüsslein-Volhard and her collaborators [33, 41, 45, 74, 75] have suggested that de-

layered iridophore localization (beyond the initial appearance at the horizontal myoseptum) may guide the formation of additional stripes and interstripes. By conducting iridophore ablation experiments, Parichy and his lab (see, for example, [64, 65]) have also shown that iridophores play an active organizational role in specifying stripe/interstripe position and stripe width, and, in particular, that iridophores promote and instruct xanthophore placement. The results [17, 33, 74, 75] indicate that iridophores spread dorsally and ventrally from the horizontal myoseptum and gather at the future locations of the next two interstripes, X1V and X1D. In this way, a repeated pattern of iridophore localization, attraction of xanthophores, and adjacent gathering of melanophores could account for the full development of the adult pattern. Our goal is to study this mechanism (newly proposed at the time of the work presented in this chapter), and determine how the timing of iridophore localization influences stripe/interstripe development (in Chapter 4, in contrast, we focus on helping identify what mechanisms drive iridophore localization).

As mentioned in §3.3 and described in §2.1.2, experimentalists often classify zebrafish development (Figure 3.3) into stages using measurements of length rather than days, as size varies significantly between fish [58]. The relevant stages for our study are reproduced from §2.1.2 in Table 3.2: standard length (SL) is a measurement from the snout of the fish to the point where its caudal fin begins [58]. Standardized standard length (SSL), in turn, denotes the standard length of a reference zebrafish and is used to approximate the length associated with developmental milestones despite individual variations [17, 58]. We follow the convention of tracking SL measurements and label images according to the developmental stages in Table 3.2; §3.5.2 contains a discussion of how our growth rates relate to these stages. Since zebrafish measure approximately 1 mm in height at 3 weeks when the metamorphic period begins, we begin with a 1 mm high by 2 mm long domain [45, 53, 75]. This

Stage	Acronym	SSL from [58]	Pattern milestone [58]
Pelvic fin bud	PB	7.2 mm	Faint stripe of iridophores
Pelvic fin ray	PR	8.6 mm	1D and 1V are discernible
Squamation onset poste- rior	SP	9.6 mm	Base stripes become more distinct
Juvenile	J	11.0 mm	Base stripes are nearly complete
Juvenile+	J+	13.0 mm	2V forms
Juvenile++	J++	16.0 mm	2D develops

Table 3.2: Stages of development. Standard length (SL) is a measurement of the fish from its snout to the beginning of its caudal fin; standardized standard length (SSL) is an approximation of SL based on a representative zebrafish [58].

field size enables us to track the full height of the zebrafish as it grows.

Sequential spreading and gathering of iridophores may promote directionality of additional stripes and interstripes

We initialize the fish skin domain with two narrow (1 cell wide) strips of melanophores along its dorsal and ventral edges and two strips of melanophores surrounding a strip of xanthophores along the center of the fish, as in Figure 3.4a. Thus, we model an initial pattern at a point between the PB and PR stages; we further assume any larval melanophores left in the prospective yellow interstripe have died or migrated out and that adult melanophores are faintly visible in two stripes. We incorporate iridophores indirectly by initializing a strip of xanthophores as part of the initial condition; this narrow strip of xanthophores takes on the role of iridophores by attracting new xanthophores and promoting their differentiation. (Note that more cell types, specifically larval pigment cells and iridophores, are needed to represent the larval pattern, and, as a two-population model, our approach is somewhat limited in initial pattern choice. Our initial pattern should be thought of as capturing features of the early zebrafish that are important for instructing later stripe/interstripe de-

velopment, rather than replicating the larval pattern or the PB and PR development stages. For an improved larval initial condition, see the work in Chapter 4.)

Our results show that, while our initial pattern (Figure 3.4a) aligns the three base stripes and interstripes with wild-type width, it rarely leads to completion of the full pattern. Instead, later stripes/interstripes form in part and with disrupted alignment, as in Figures 3.4b-3.4f. In about a third of our simulations, a narrow bridge of xanthophores extends out of the central interstripe (Figure 3.4l). Interestingly, Patterson and Parichy [65] observed that melanophores localize near iridophores and may even wrap around residual iridophores on fish skin subject to ablation of these cells; similarly, we find that melanophores wrap around partially formed yellow regions in our model as well (see Figure 3.4f). Furthermore, in the zebrafish mutations *rose*, which feature fewer iridophores, the stripes break up into spots, with only 1D and 1V visible (see, for example, [17, 41, 74, 75]). Thus, our simulations without the sequential spreading/aggregation of iridophores are qualitatively comparable to mutations and ablation experiments involving a reduction of iridophores.

While we can use our two-population model to test how the absence of iridophore spreading and aggregation negatively influences the pattern, it cannot be directly used to evaluate if delayed localization of iridophores positively impacts directionality. Instead, we take an indirect route based on empirical observations (see, for example, [64, 65]) that iridophores promote and instruct the placement of xanthophores. In particular, we model this effect of delayed iridophore localization by introducing two narrow (1 cell wide) strips of xanthophores at 20% and 80% of the current fish domain height at a delayed time. These xanthophores represent the result of iridophores spreading, accumulating there, and then attracting xanthophores. Though this modeling simplification leaves out a potential cause of xanthophores appearing, namely aggregation of iridophores, it captures one possible result, and allows us to

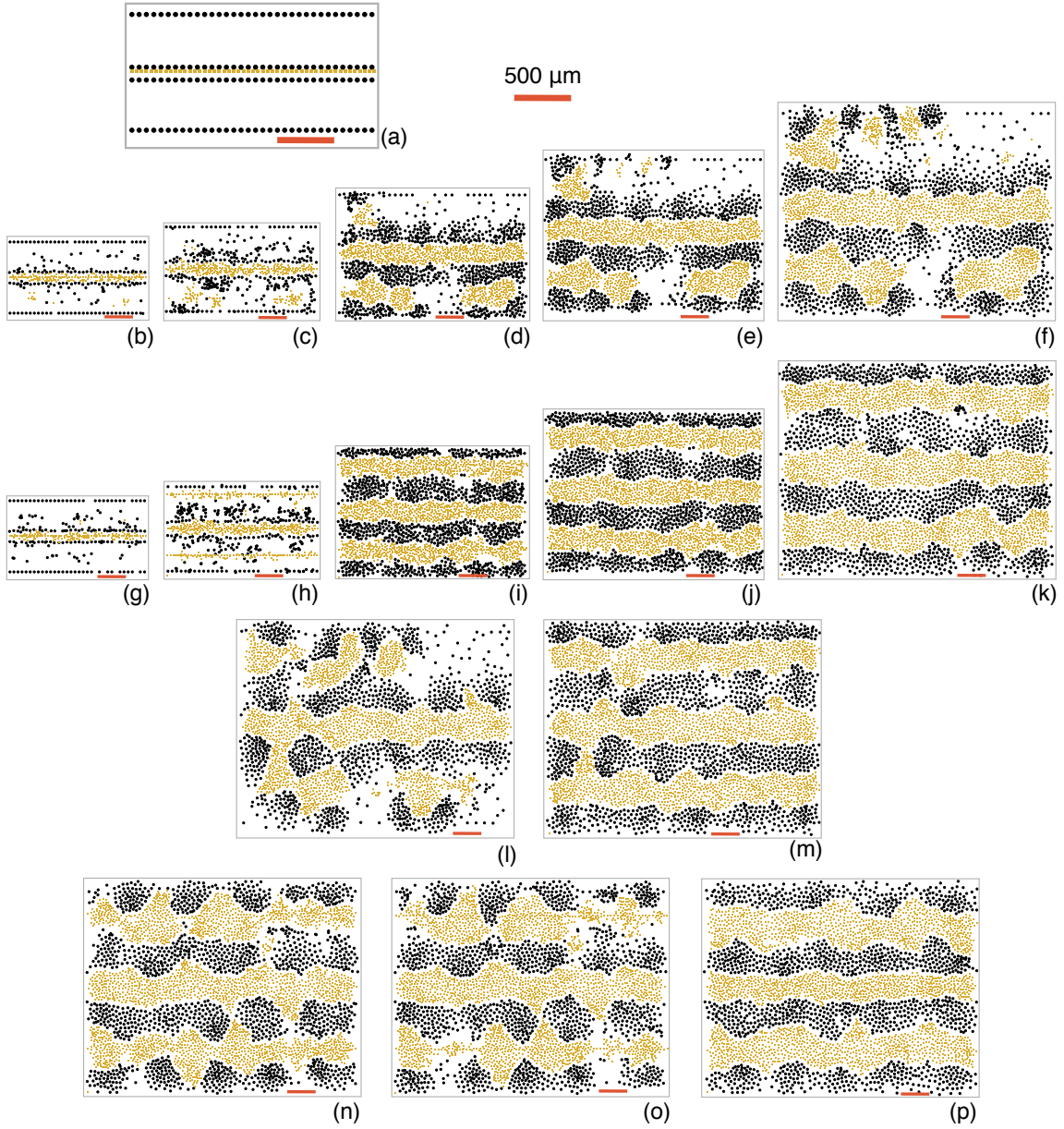


Figure 3.4: (a) Our initial pattern (initial condition) models features of the zebrafish pattern at a point between the PB and PR stages. (b-k) Simulated stripe pattern development from our larval pattern (Figure 3.4a) with (b-f) no additional iridophore localization and (g-k) iridophore localization at stage SP: shown at the (b, g) PR, (c, h) SP, (d, i) J, (e, j) J+, and (f, k) J++ stages, which correspond to $t = 2, 4, 9, 13$, and 20 days after initialization in our simulation. (l-m) Bridges between stripes or interstripes occasionally emerge: pattern shown at stage J++ for (l) no additional iridophore localization and (m) iridophore localization at stage SP. (n-p) Images at stage J++: (n) additional iridophore information introduced at stage J, (o) at stage J+, and (p) as part of the initial condition (between the PB and PR stages). PR, SP, J, J+, and J++ refer to developmental stages [17, 58] shown in Table 3.2.

study the impact iridophores have on pattern formation without adding complexity to our model. For the remainder of this section, we will talk about the localization

of iridophores (or appearance of iridophore alignment information) in interstripes X1V and X1D at different times: it is important to note, however, that though this symbolizes the dispersal and aggregation of iridophores behind the scenes, the only visible result in our model is the appearance of two narrow strips of xanthophores (for a direct study of iridophores without the need for the additional inclusion of 1 cell wide strips of xanthophores to model their effect, see Chapter 4).

We consider three delayed timings for the localization of iridophores in interstripes X1V and X1D (visible in our model by the appearance of two strips of xanthophores): 4, 9, and 14 days after we begin our simulation. According to our growth rates, these time points correspond to standard lengths associated with the SP, J, and J+ developmental stages, respectively. We find that alignment improves with earlier localization of iridophores at X1V and X1D. A delay to the SP stage typically produces stripes of wild-type width and correct alignment, as in Figures 3.4g-3.4k, though 30% of patterns (out of 30 simulations) display bridges (Figure 3.4m). At the J stage, quality decreases in every simulation and 40% of the resulting patterns contain bridges (Figure 3.4n). For iridophore localization at the J+ stage, early undirected differentiation away from our initial pattern leads to patches of cells in the prospective locations of interstripes X2D, X2V and stripes 2D, 2V. The introduction of alignment information at the J+ stage is not enough to smooth out these patches or reliably produce fully formed stripes of wild-type width (Figure 3.4o).

We further tested how the impact of iridophores depends on timing by initializing our model with two additional strips of xanthophores as part of the initial condition rather than at a delayed time. Our results show that this disrupts the natural sequential formation of stripes and interstripes. Instead, the full set of stripes/interstripes develops at the same time and at an accelerated rate. While zebrafish stripes/interstripes tend to narrow as they spread outward from the center

of the fish, the interstripes that develop in our simulations under no iridophore delay are equal in width if not wider away from the horizontal myoseptum, as shown in Figure 3.4p.

To evaluate the behavior of our model, we calculated the average distance between neighboring melanophores (Δ_{mm}), between neighboring xanthophores (Δ_{xx}), and between stripes and interstripes (Δ_{xm}). As shown in Figure 3.5a, regardless of the timing of iridophore localization, the model outputs measurements of the average distance between neighboring cells that match empirical results [76]. Furthermore, as indicated in Figures 3.5b and 3.5c, we find that melanophores move $\approx 10\text{-}15\ \mu\text{m}$ per day on average before joining complete stripes, in good agreement with the speed of $80\text{-}100\ \mu\text{m}$ per week estimated by Takahashi et al. [76]. (For both the empirical estimates [76] and our measurements of melanophore migration, movement due to growth has been accounted for separately, and the reported numbers represent change in position of melanophores due only to migration.)

While the average distance between xanthophores and melanophores at the boundaries between stripes and interstripes serves as a measure of the separation between stripes and interstripes, distributions of shortest xanthophore-to-melanophore distances across all xanthophores on the fish domain are related to interstripe width. As described in the caption of Figure 3.6, these distributions are obtained by calculating the distance from each xanthophore to its nearest melanophore. For xanthophores at the boundary of the interstripe, the nearest melanophore will simply be a distance $\approx \Delta_{xm}$ away; for xanthophores in the middle of an interstripe, however, the nearest melanophore will be half an interstripe width away. On average, for well-formed stripe patterns, cells are located not on the stripe boundary or in the middle of the stripe, but one quarter of a stripe width away from the boundary. Thus, the average shortest distance from each xanthophore to a melanophore serves as a rough esti-

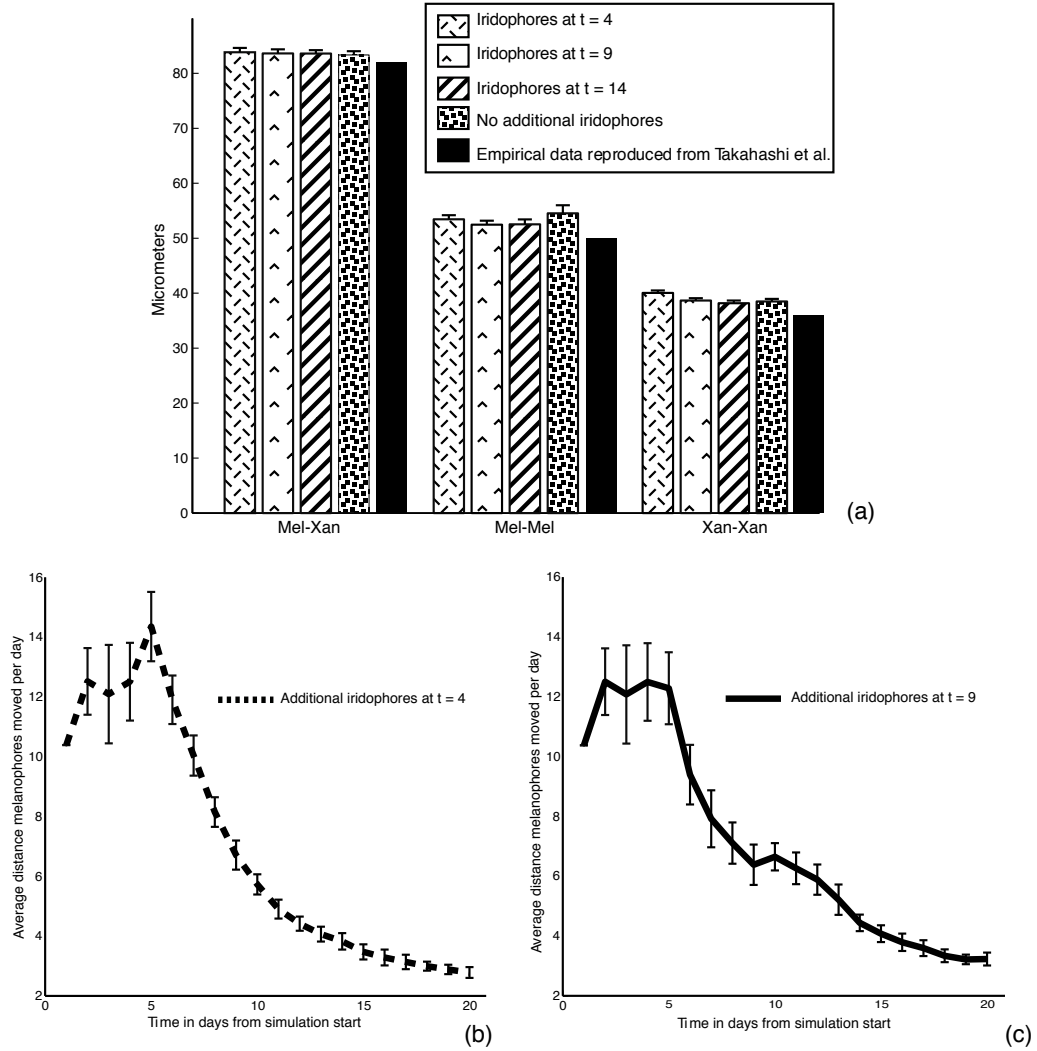


Figure 3.5: (a) Measured shortest cell-to-cell distances between neighboring melanophores and xanthophores (at stripe/interstripe boundaries), neighboring melanophores, and neighboring xanthophores at the J++ stage agree with empirical measurements by Takahashi et al. [76]. Bars represent mean cell-to-cell distances between neighboring cells averaged across 30 simulations; standard deviation shows how average shortest cell-to-cell distances between neighboring cells vary between simulations. (b-c) Takahashi et al. [76] report that, when active, melanophores move due to migration (growth is accounted for separately) roughly 80-100 μm per week *in vivo* (corresponding to $\approx 11\text{-}14 \mu\text{m}$ per day), and rarely move when densely packed in adult stripes. As shown in (b-c), melanophores in our model move $\approx 10\text{-}15 \mu\text{m}$ per day before slowing down and settling into complete stripes. Curves represent mean melanophore movement per day averaged across 30 simulations; standard deviation shows how mean melanophore movement varies between simulations. Note that melanophore movement is calculated before positions are updated for growth, so changes in position are due to migration alone.

mate of one quarter of an interstripe width. Using this method to approximate the average width of interstripes across 30 simulations, we find that interstripes develop to be roughly 392 μm wide when iridophores are introduced at the SP stage (for

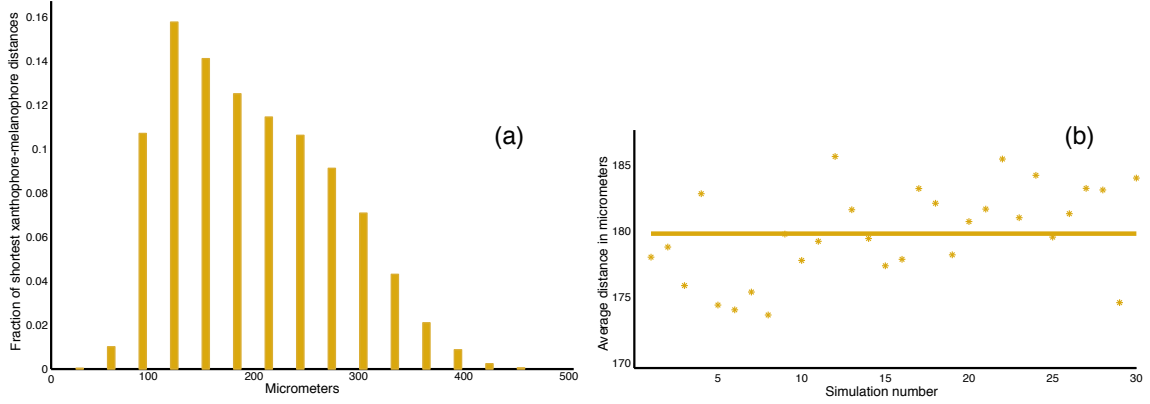


Figure 3.6: (a) Final distribution (stage J++) of xanthophore-to-melanophore distances, averaged across 30 simulations for iridophore localization at stage SP. The xanthophore-to-melanophore distribution is related to interstripe width. To assemble it, we calculated the shortest distance from each xanthophore to any melanophore on the fish skin domain. We then binned these distances in $30\ \mu\text{m}$ wide boxes for each simulation, divided by the number of xanthophores for each simulation, and averaged the results over 30 simulations. Bars are placed along the horizontal axis at $30\ \mu\text{m}$, $60\ \mu\text{m}$, $90\ \mu\text{m}$, and so on in $30\ \mu\text{m}$ increments; the height of each bar denotes, on average, what fraction of xanthophores are a distance of $0\text{--}30\ \mu\text{m}$, $30\text{--}60\ \mu\text{m}$, $60\text{--}90\ \mu\text{m}$, etc. away from any melanophore. As an illustrative example of this data, a bar of height 0.16 at $150\ \mu\text{m}$ would represent that, for an average of 16% of xanthophores on the fish skin, the nearest melanophore is between 120 and $150\ \mu\text{m}$ away. Note that the lower limit of the distribution support (roughly $60\ \mu\text{m}$ here) bounds the distance Δ_{xm} between stripes and interstripes from below. In addition, because xanthophores are furthest away from black cells when they are located in the middle of an interstripe (or at the edge of the domain), the upper limit of the distribution support ($\approx 450\ \mu\text{m}$ here) serves as an upper bound of half the width of interstripes. (b) For well-formed stripe patterns, calculating the average shortest xanthophore-to-melanophore distance for a single simulation and subtracting Δ_{xm} serves as a rough estimate of one quarter of an interstripe width. Data points show these averages for 30 different simulations at stage J++, and the solid line denotes the mean across 30 simulations. In particular, each data point is the result of one simulation and is related to the interstripe width generated by that simulation, while the solid line shows the average across 30 simulations. As shown, on average across the 30 simulations, the final width of interstripes is very roughly $(180\ \mu\text{m} - \Delta_{xm}) \times 4 = 392\ \mu\text{m}$, where $\Delta_{xm} = 82\ \mu\text{m}$ is taken from [76]. (Note that these estimates will in general be lower estimates of interstripe width, but serve as a means of comparing simulations and extracting a measurement of pattern width.)

details, see Figure 3.6).

An alternative quantitative means for evaluating pattern quality is to track the coefficient of variation (CV) for melanophore nearest-neighbor distances [61, 62]. The coefficient of variation, given by $\text{CV} = 100 \times \text{standard deviation}/\text{mean}$, is calculated by determining the average distance from each melanophore to its nearest-neighbor melanophore, and is a perceptive measure of pattern regularity [62]. Low CV values denote well-formed pattern elements (stripes or spots), while high

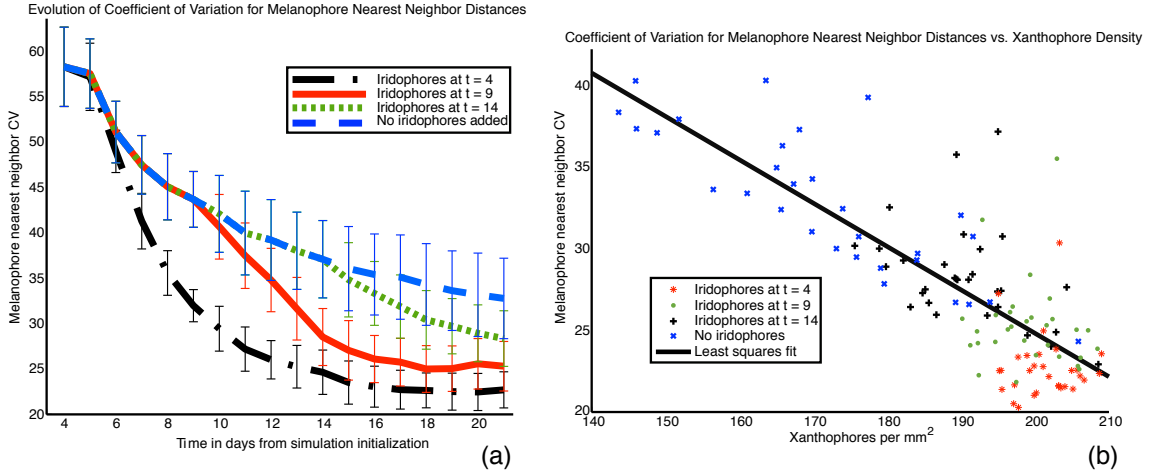


Figure 3.7: (a) Evolution of average coefficient of variation (CV) for melanophore nearest-neighbor distances; average is calculated across 30 simulations, and standard deviation is shown. CV decays in time, representing the development of better formed patterns, comparable with Parichy and Turner [62] (empirical measurements of CV take on values roughly between 100 and 30, by our approximation of the reported data [62]). (b) Scatter plot shows melanophore nearest-neighbor CV at stage J++ (for 30 simulations) as related to xanthophore density; in agreement with [61], we find that lower CV is associated with higher xanthophore density.

CV values are related to irregular patterns. Parichy and Turner [62] found that the CV for wild-type zebrafish decays during development and takes on values roughly between 100 and 30 (by our approximation of the reported data [62]). It has also been shown [61] that CV is correlated with xanthophore density, with high CV related to decreased xanthophore numbers. As shown in Figure 3.7a, we find that the coefficient of variation in our model (averaged across 30 simulations) is on the same order as empirical measurements [61, 62] and shows a similar decay over time. Furthermore, the CV reaches progressively lower values as iridophores are introduced nearer to the SP stage, providing quantitative support for the qualitative results observed above. In Figure 3.7b, we show a scatter plot of the CV relative to the xanthophore density for 30 trials; in good agreement with [61], we also observe a clear relationship between pattern regularity and xanthophore density.

In conclusion, our results support the model [45, 74, 75] that spreading and localization of iridophores helps guide differentiation [64, 65] and align stripes/interstripes

sequentially, and agrees with empirical observations [64, 65] that changing the timing of cell differentiation with respect to fish growth alters the resulting pattern. It has also been observed [64] that iridophores may have a role in bounding stripe width, and, in support of this empirical observation [64] we find that melanophores are more widely dispersed across the fish skin when additional iridophores are not introduced. Furthermore, our results suggest that some delay in the appearance of additional alignment information is important to preserve the sequential nature of stripe pattern formation. Nevertheless, too long of a delay leads to patchy stripes with disrupted alignment. Frohnhofer et al. [17] observed that iridophores accumulate in the future location of yellow interstripe X1V during the SP stage, as shown in Figure 3.3. In good agreement with [17], our results indicate that introducing additional alignment information during the SP stage is significantly more successful than longer delays to the J or J+ stages.

3.4.3 Ablation

Yamaguchi et al. [89] used laser irradiation to ablate a large central region of pigment cells on young (≈ 3 weeks) zebrafish and then observed the regeneration process. Their experiment led to stripes/interstripes with arbitrary direction but fairly normal width, as shown in Figure 3.8a. They further studied this phenomenon by simulating a reaction-diffusion model [89] on a static domain and obtained similar results. We simulated ablation [89] under two conditions: developing fish (growing domain) and adult fish (no growth). This choice enables us to compare results with both the *in vivo* work of Yamaguchi et al. [89] and their corresponding reaction-diffusion simulations on a static domain. Our results have interesting repercussions for the effects of growth on stripe/interstripe width and on the necessary scale for long-range

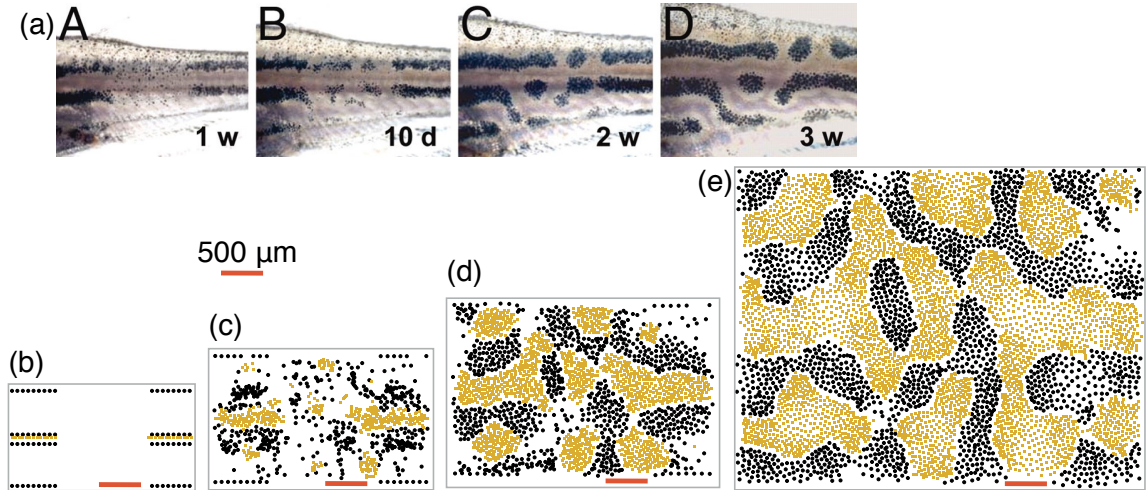


Figure 3.8: (a) Ablation on developing fish: images are reproduced from Yamaguchi et al. [89] with permission; Copyright (2007) National Academy of Sciences, U.S.A. (b-e) Corresponding simulated results: (b) initial ablation and stripe pattern development (c) 3, (d) 7, and (e) 20 days later. The regenerated pattern loses directionality.

interactions, as discussed in Section 3.4.4.

Ablation destroys directionality on developing fish

We model ablation [89] under the condition of growth by initializing the domain at roughly 20 dpf (between PB and PR developmental stages) with our standard initial pattern with one difference: a large central 1 mm^2 square region is removed, as indicated in Figure 3.8b. In good agreement with [89], we find that artificially disturbing the initial pattern results in regenerated stripes/interstripes with lost directionality but similar width (Figure 3.8).

Ablation on adult fish selects narrower stripes and interstripes

We also studied ablation on adult fish skin domains under the setting of no growth. The initial condition we used is a set of 6 perfectly formed 10-cell-wide stripes and interstripes with a large central region removed, as in Figure 3.9a. Our simulations show random differentiation followed by segregation of cells into stripes and inter-

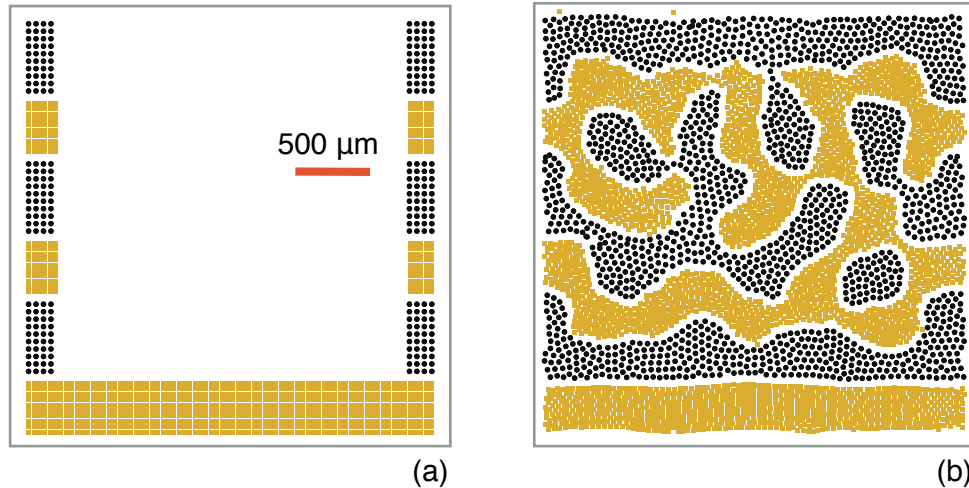


Figure 3.9: Simulated ablation on adult fish with no growth. (a) Initial condition: square region of stripes and interstripes is ablated. (b) Resulting stripes and interstripes (70 days later) are narrower than those initialized. (Note that groups of xanthophores in the initial condition may appear together as large squares: this is due to their initial ordered arrangement and is an artifact of the image size that disappears upon further magnification.)

stripes. This trajectory is similar to those observed in our simulations with fish growth and in the *in vivo* experiments [89]. The most interesting area of comparison is stripe/interstripe width: as shown in Figure 3.9b, 4 interstripes appear in the domain in place of the 3 interstripes initialized in Figure 3.9a. (To see this, it may be helpful to imagine drawing a line vertically across the domain in Figure 3.9b: in the process, the line will intersect 4 yellow regions, though the simulation began with 3 interstripes.) Thus, the width of the regenerated stripes/interstripes is roughly 75% the width of the initial stripes/interstripes. This result was unexpected, and it suggests that the relationship between fish growth and stripe/interstripe width should be further explored. We turn to this exploration in §3.4.4.

3.4.4 Effects of domain growth

Fish growth plays a role in selecting stripe/interstripe width

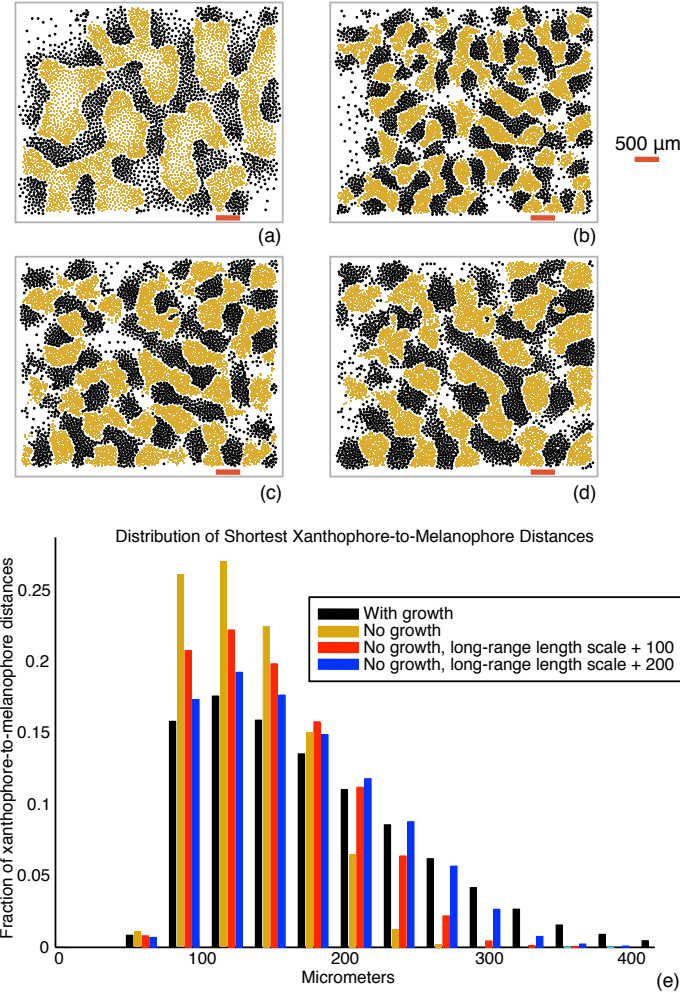


Figure 3.10: Pattern formation from an empty initial condition on (a) a growing domain; (b) a static domain; (c-d) a static domain with long-range interactions increased by 100 μm or 200 μm , respectively. Growing domains produce wider stripes and interstripes. Images are from 25 days into simulation; approximately Neumann boundary conditions are used (see §3.5.2 for details). (e) Bar graph shows the average distribution of shortest xanthophore-to-melanophore distances across 30 simulations; to assemble these distributions, we calculated the distance from each xanthophore to its closest neighboring melanophore, binned these distances in 30 μm wide boxes, and divided by the number of xanthophores for each simulation (at 35 days); we then averaged the results over 30 simulations. (Note that for simulations involving domain growth, growth was stopped at 25 days and distributions were calculated at 35 days, allowing 10 days for stripes/interstripes to relax and removing any artifact due to stretching of the cell positions.) For further details on this method of evaluating interstripe width, see Figure 3.6. As shown, bars drop off sharply without growth, but increasing long-range interactions by 200 μm on static domains widens the support of the distribution, bringing it closer to the results obtained on growing domains.

As discussed in §3.4.3, while stripes and interstripes of natural width form when growth is included, the stripes/interstripes that form on static domains after ablation are approximately 25% narrower. To test the idea that growth increases

stripe/interstripe width, we simulated stripe pattern formation from an empty initial condition (no cells, no initial pattern) on both static and growing domains. As shown in Figures 3.10a and 3.10b, the stripes and interstripes selected under conditions of growth are significantly wider. From a biological perspective, this suggests that fish growth is an integral part of the pattern formation dynamic: in particular, if a zebrafish were to grow to adult size and only then develop stripes and interstripes, our model indicates that these stripes/interstripes would be narrower than those that appear on fish for which pattern formation and growth occur concurrently. While our model shows that growth widens stripes and interstripes, it does not explain how growth does so. Interestingly, Parichy et al. [58] found that slowing body growth (and altering temperature) in wild-type fish led to a decoupling of melanophore pattern state from developmental stage. Taken together, these results suggest that the interplay of growth and pattern development should be further explored to elucidate how growth impacts patterns.

Growing domains support shorter long-range interactions

Because long-range interactions seem to be largely responsible for selecting and maintaining the width of stripes and interstripes (see, for example, [25, 74]), we hypothesized that simulations on a static domain with longer-range interactions could lead to the same widths as simulations on a growing domain with the original length scales. As expected, we find that the stripe and interstripe widths selected with no growth increase as the scale of long-range interactions increases. Lengthening these interactions by 100 μm (an increase of roughly 30%) widened stripes and interstripes, though not enough to match the widths found on growing domains (Figure 3.10c). An increase of 200 μm , however, generated interstripes on static domains of similar width to those that form with growth (Figure 3.10d). As an alternative means of presenting these results, we chart average distributions of shortest xanthophore-

melanophore distances in Figure 3.10e (as discussed in the caption of Figure 3.6, average distributions are related to interstripe width); the distribution obtained on a static domain with long-range length scales increased by $200\text{ }\mu\text{m}$ is close to that obtained with growth, while the distributions from static domains with shorter long-range length scales drop off more quickly, signifying narrower interstripes.

Intrigued by what this relationship between growth and stripe/interstripe width might mean for our long-range interaction scale, which was set using simulations on a static domain, we returned to studying development from our initial pattern, now with a reduced length scale. We find that decreasing the scale for long-range interactions by $50\text{ }\mu\text{m}$ (roughly 15%) produces slightly narrower stripes. In contrast, a decrease of $100\text{ }\mu\text{m}$ disrupts the pattern and enables long-range death signals to break up stripes. These long-range death signals, which may be regulated by pseudopodia [25], strongly control stripe width and enforce an interaction scale long enough for mid-stripe melanophores to be able to communicate with xanthophores.

From a mathematical perspective, we conclude that domain growth supports wider stripes/interstripes and, hence, shorter long-range interactions. Because our results indicate that a much larger interaction scale is needed on a static domain to obtain patterns similar to those on a growing domain, studying pattern formation on constant grids for problems involving growing organisms may not lead to accurate approximations of interaction distances, especially for problems that do not involve long-range death signals. Accounting for growth may bring the length scales that arise from mathematical studies closer to their biological counterparts.

3.4.5 Mutations

Zebrafish mutations are classified into two types: those that affect the pattern at the early development stage and those that lead to fish with disrupted adult stripes/interstripes despite initially normal pigment cell development [35]. We discuss both of these broad categories of zebrafish mutations, which we will refer to as early stage and late stage mutations, below.

Early stage mutations: directionality is lost without a horizontal myoseptum

The choker mutation lacks a horizontal myoseptum, resulting in adult stripe patterns with normal width but arbitrary direction, so that locally parallel stripes and interstripes curve and branch off on the fish skin [17]. To model choker development, we omit the initial pattern along the horizontal myoseptum and instead initialize the fish skin domain with only two narrow (1 cell wide) strips of melanophores at its dorsal and ventral edges. The resulting simulations agree with the patterns observed on choker fish, as shown in Figure 3.11. We conclude that horizontal stripes and interstripes do not form in the absence of a horizontal myoseptum. This finding is in agreement with experimental observations [17] suggesting that the timely presence of iridophores along the horizontal myoseptum may orientate stripes and interstripes.

Late stage mutations: disrupting heterotypic interactions alters stripe/interstripe width

Experimentalists have shown that many late stage mutations affect the interactions between melanophores and xanthophores [29, 90]. In the jaguar/obelix zebrafish [43], a mutation characterized by wider stripe patterns with ambiguous boundaries, fin melanophores seem to move around, instead of away from, fin xanthophores; fin

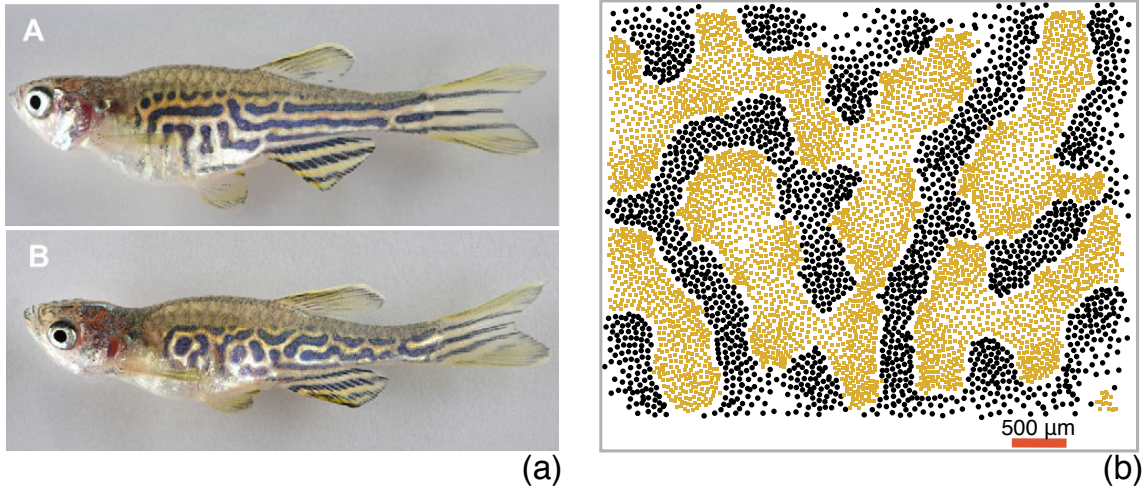


Figure 3.11: Choker zebrafish lack a horizontal myoseptum and feature locally parallel stripes and interstripes of arbitrary direction. (a) Images of two choker fish; reproduced with permission from Frohnhöfer et al. [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd. (b) Simulated results at stage J++.

xanthophores, in turn, exhibit reduced attraction toward fin melanophores *in vitro* [90]. As reported in [35], homozygous obelix zebrafish feature broad interstripes, and, as noted in [43], the heterozygous variation of this mutation displays fewer and wider stripes that lack strict separation from interstripe regions. The dali phenotype, which is similar in appearance to jaguar/obelix, is another example of a mutation displaying altered cell migration [30].

To better understand how changes in heterogeneous cell interactions are related to mutations, we simulated the evolution of fully formed adult stripes/interstripes initialized with natural width and correct cell-to-cell distances without growth; we then altered different aspects of migration and compared the results with simulations done using the original wild-type parameters (Figure 3.12b). Setting $R^{xm} = 0$ (see Equation 3.3) corresponds to removing the run part of chase-run movement; this alteration led to patterns featuring slightly widened stripes with less regular boundaries, as in Figure 3.12c. Disrupting the full chase-run movement by setting $A^{mx} = R^{xm} = 0$ gave similar results (Figure 3.12d). In contrast, removing all heterogeneous migration

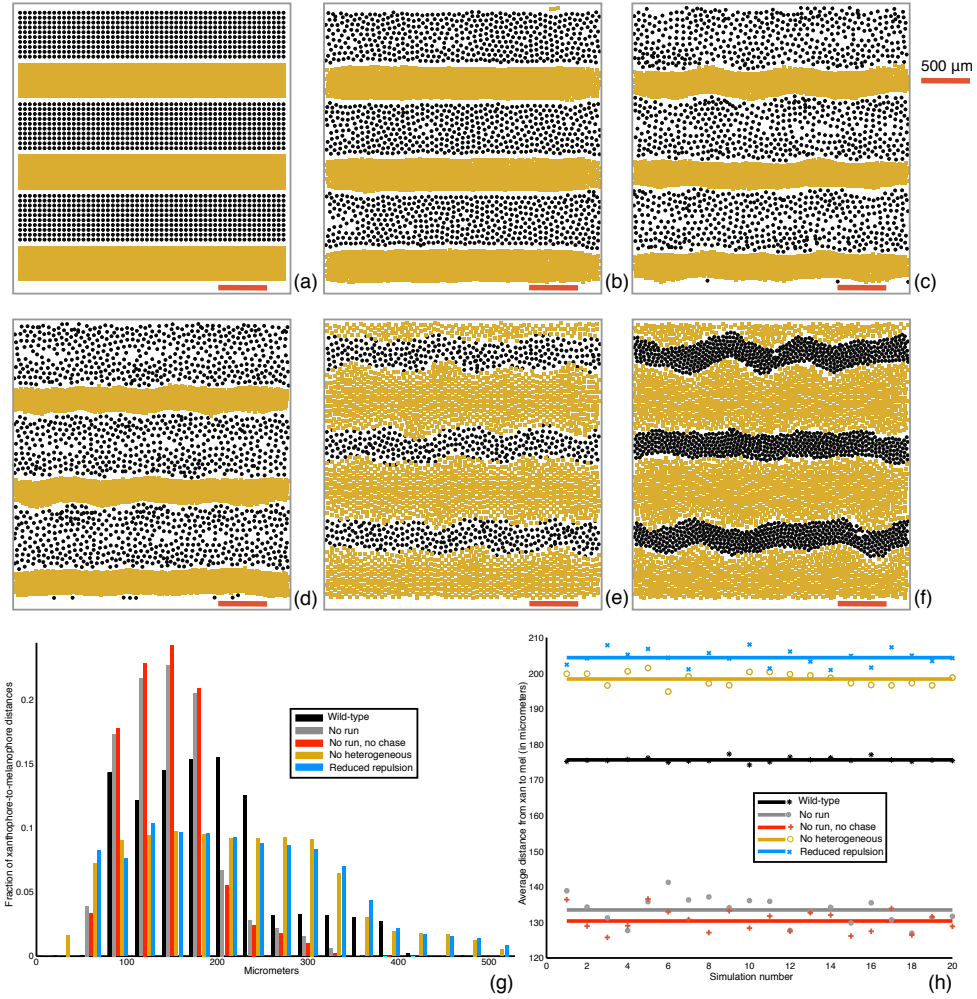


Figure 3.12: (a) Initial condition used for (b-f) is a set of fully formed adult stripes and interstripes of width 10 cells with the experimentally observed cell-to-cell distances [76]. Images are shown at 100 days after start of simulation. (b) Wild-type. (c) Run component of chase-run movement removed: $R^{xm} = 0$. (d) Full chase-run movement removed: $R^{xm} = A^{mx} = 0$. (e) All heterogeneous migration interactions disrupted: $R^{xm} = A^{mx} = R^{mx} = 0$. (f) Only repulsion of xanthophores from melanophores disrupted: $R^{mx} = 0$. (g) Average distribution of shortest xanthophore-to-melanophore distances across 20 simulations; as shown, altering heterogeneous migration interactions impacts both interstripe width and stripe/interstripe separation. Distributions were assembled by calculating the distance from each xanthophore to its closest melanophore (at 100 days), binning these distance in 30 μm wide boxes, and dividing by the number of xanthophores for each simulation; we then averaged these results over 20 simulations. (h) Scatter plot shows the average xanthophore-to-melanophore distances for each of 20 different simulations and solid line charts the mean across all simulations. For well-formed stripe patterns, calculating the average xanthophore-to-melanophore distance and subtracting the distance between stripes and interstripes serves as a rough estimate of one quarter of an interstripe width. For further details on this method of evaluating interstripe width, see Figure 3.6.

forces resulted in wide interstripes with reduced xanthophore-melanophore distances like those in homozygous obelix zebrafish (Figure 3.12e). Setting $R^{mx} = 0$ alone was

also enough to generate wider interstripes, shown in Figure 3.12f. As discussed in the caption of Figure 3.6, distributions of shortest xanthophore-melanophore distances are a quantitative means of describing interstripe width; as an alternative measure of how altered migration parameters impact stripe/interstripe width, we chart these distributions and their averages in Figure 3.12g and 3.12h.

Through transplantation experiments, Maderspacher and Nüsslein-Volhard [43] found that wild-type melanophores can form wild-type stripe patterns with obelix xanthophores, but obelix melanophores and wild-type xanthophores interact to form obelix-like patterns. This data [43] indicates that it is the genotype of melanophores that drives the obelix pattern. Our model generates wide interstripes under two different parameter tests and wide stripes (associated with narrower interstripes) under two parameter alterations as well. In particular, setting $R^{mx} = 0$ could be thought of as a weaker mutation than removing all heterogeneous interactions, though both parameter changes reduced repulsion between melanophores and xanthophores and widened interstripes. Likewise, disrupting only the run movement of melanophores had a similar impact on stripe/interstripe width as did the stronger alteration that removed the full melanophore-run and xanthophore-chase movement, lending support to the empirical observation in [43] that a mutation in melanophores alone is enough to generate an obelix-like pattern.

In conclusion, our results support experimental observations that changes in stripe pattern width are directly related to changes in heterogeneous migration interactions. We propose that the widening of interstripes and narrowing of stripes in the homozygous obelix mutation may be a product of the reduction in distance between melanophores and xanthophores: when boundaries are ambiguous and cells no longer repel (or repel less strongly in the case when R^{mx} alone is zero), short-range competition takes over, leading to the death of melanophores due to invading

xanthophores.

3.4.6 Roles of birth, death, and migration

Different roles: cell differentiation selects pattern width and migration maintains stripe/interstripe boundaries

One advantage of modeling is that we can systematically test which mechanisms of cell interaction are most important at different times during stripe pattern development. It has been suggested by Mahalwar et al. [45] that differentiation, rather than migration, drives stripe pattern formation. According to this viewpoint, cells appear in the appropriate locations and reorganize only locally to smooth out stripe/interstripe boundaries. In addition, Singh et al. [75] put forth that net repulsion of melanophores and xanthophores may maintain stripe/interstripe boundaries. We test these hypotheses by simulating our model under conditions of no migration or migration alone.

We first study how stripe pattern formation is affected when cell migration is removed. For simulations of our reduced model with no movement, we consider the evolution of an initially empty domain with fish growth. Because no starting pattern is initialized, the resulting stripes and interstripes form with arbitrary directionality. Interestingly, we find that similar stripe pattern widths are selected under the reduced and full model, as indicated in Figure 3.13a, but cell-to-cell distances vary dramatically. This result is in agreement with the minimal model [5]: Bullara and De Decker found that birth and death rules leading to an overall short-range activation and long-range inhibition dynamic could produce stripes and interstripes of wild-type width, a mechanism they call *differential growth* [5]. We further investigate

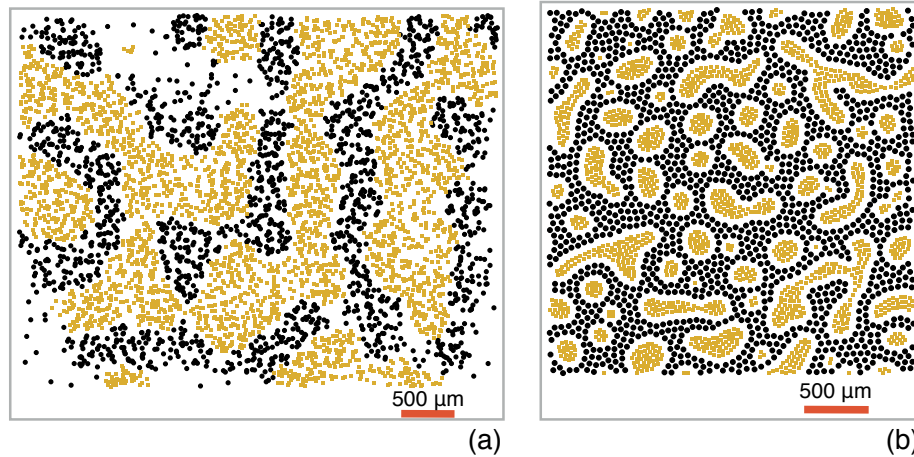


Figure 3.13: (a) Pattern formation from an initially empty growing domain without migration; image shown is 20 days from initialization. (b) Pattern formation on a fixed domain from a random distribution of pigment cells interacting by migration alone (no differentiation or death); image from 150 days after start of simulation.

the reduced model by simulating a random initial distribution of cells interacting on a static domain through movement alone. Shown in Figure 3.13b, the resulting patterns consist of small aggregates of xanthophores and melanophores. The pattern width selected is narrow, as the cells only migrate enough to gather into homogeneous conglomerates, but not far enough to reach natural stripe and interstripe widths. The average distances between pigment cells, however, are upheld.

We conclude, in agreement with Bullara and De Decker [5], that differentiation and death may be responsible for selecting stripe pattern width; in addition, our results indicate that migration supports cell-to-cell distances and ensures that short-range competition of melanophores and xanthophores does not degrade stripe/interstripe boundaries. As discussed in §3.4.5, we found that altering the net repulsion between melanophores and xanthophores disrupts stripe pattern width. Thus, while differentiation specifies stripe pattern width, migration may maintain it. These observations are instructive when compared to the results of reaction-diffusion models. As mentioned by Mahalwar et al. [45], and shown for an ablation simulation by Yamaguchi et al. [89], Turing-type models can achieve stripe pattern

formation from a random initial distribution of melanophores and xanthophores that separate into stripes and interstripes by lengthy movements. In contrast, simulations (Figure 3.13b) of our model indicate that movement alone cannot transform a random initial condition into stripes and interstripes of natural width, at least not on a biological time scale.

3.5 Detailed model description

In our model, black melanophores and yellow xanthophores interact in two dimensions on growing fish skin domains. These cells, which we model as independent agents, are represented by point masses marking cell centers and can interact through migration, differentiation, and death. Let

$M_i(t)$ = position of i th melanophore at time t ;

$X_j(t)$ = position of j th xanthophore at time t ;

$N_m(t)$ = number of melanophores at time t ;

$N_x(t)$ = number of xanthophores at time t .

Before describing in detail how we account for cell migration, differentiation, and death, we first discuss parameter fitting in §3.5.1 and make some notes about domain growth and boundary conditions in §3.5.2. We also highlight the length scales in our model (§3.5.3).

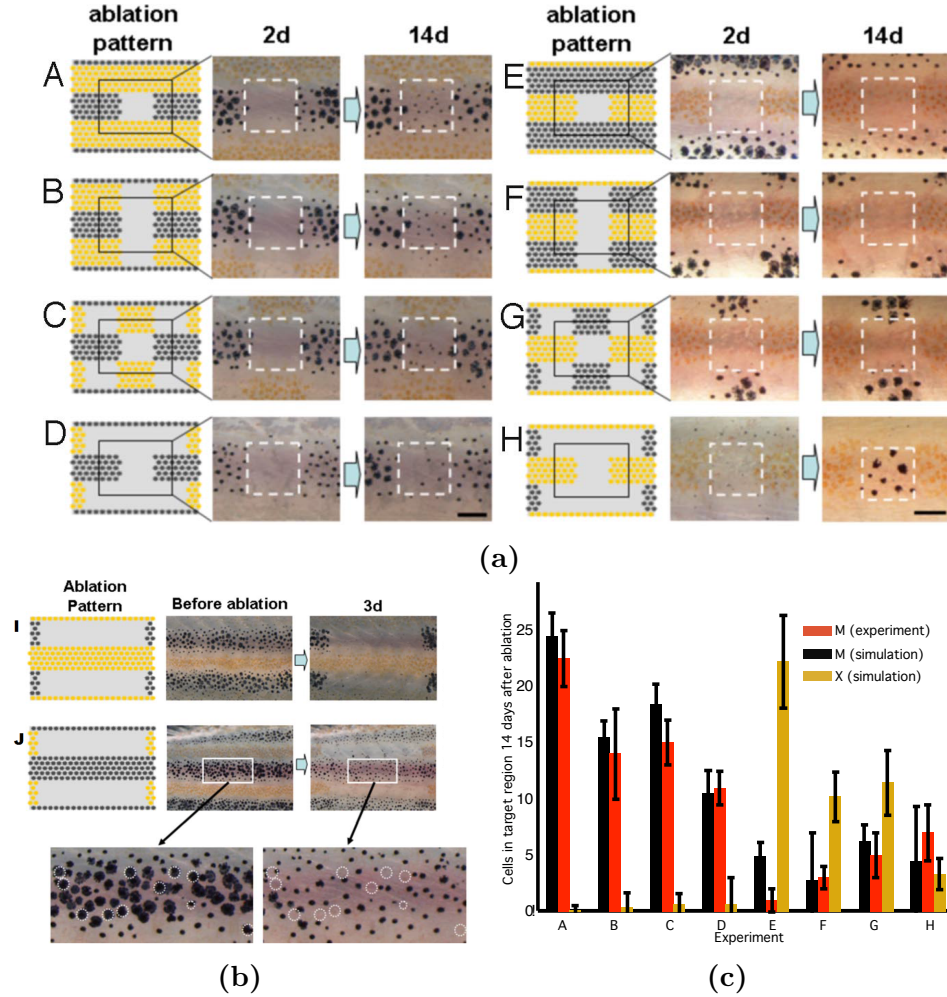


Figure 3.14: Figures (a-b) and red bars in (c) reproduced from Nakamasu, Takahashi, Kanbe, and Kondo [54]. (a) Exp. A-H by Nakamasu *et al.* [54] track the number of melanophores that appear in the target region (white dashed box) in order to deduce long-range mechanisms of differentiation. (b) Exp. I and J [54] focus on cell death in the central interstripe or stripe respectively; results for Exp. H [54] show that melanophore density decreases by $\approx 20\%$ in 6 days. (c) Our parameters were chosen to best fit the experimentally measured number of melanophores [54] in the target region 14 days after ablation for Exp. A-H. Bar graph shows the melanophore numbers reproduced from [54] and our model output for melanophores and xanthophores. The experimental averages from [54] are based on 5 tests for Exp. A-D and 8 for Exp. E-H; our results are averaged over 100 simulations.

3.5.1 Parameter fitting and biological data

We set aside three main sources [54, 76, 90] for parameter fitting and left the remaining experimental data for model testing. In particular, measurements of average

cell-to-cell distances from [76] and cell speeds from [76, 90] informed our choice of migration parameters. Our rules for differentiation and death (and the parameters involved), in turn, were guided by ablation experiments from [54]. Ten of these experiments, reproduced in Figure 3.14 from [54], demonstrate how long-range interactions influence cell differentiation and survival. Nakamasu *et al.* [54] tracked how many melanophores appeared in the white dashed boxes of Figure 3.14d 14 days after ablation; a comparison of their results with those given by our model is shown in Figure 3.14f. Other results from [54], not shown, indicate that melanophores and xanthophores compete at short range.

3.5.2 Domain growth

Domain growth is modeled as uniform stretching in both directions; each day we add $130\text{ }\mu\text{m}$ to the horizontal and vertical boundaries of the fish and then reposition each cell by multiplication with a growth factor. This can be visualized by imagining the cells sitting on a rubber sheet: when the domain grows we simply stretch the rubber sheet to a new size and the cell-to-cell distances stretch along with it, leading to new more spread out cell positions.

Our growth rates are approximations inferred from measurements of standardized standard length (SSL) [58], though size varies significantly between fish. Because our initial pattern is based on a point between the PB and PR stages of development, we assume the standard length (SL) of our model zebrafish is 7.9 mm, the average of the standardized standard lengths at these two stages. The fish height associated with this SL is roughly 1 mm [58]. Our simulations end at what we consider the J++ stage at 16 mm SL; at this point, the fish is ≈ 3 mm high [58]. Parichy *et al.* [58] observed that eye diameter varies from 1 to 2 mm between the PB and J++

stages, so we remove 1.5 mm to account for the eye region, leaving an initial length of 6.4 mm and a final length of 14.5 mm. The initial condition for our domain is 2 mm long by 1 mm high, so we evolve about a third of the fish length and its full height.

According to [45], 4 to 5 black stripes (now referred to simply as *stripes*) and 4 yellow interstripes (now referred to simply as *interstripes*) emerge on zebrafish over a period of 25 days. Since our initial condition is at a slightly delayed stage in development, we assume a simulation time of 20 days, leading to a horizontal growth rate of $\approx 130 \mu\text{m}$ for our domain size. Following the model of Moreira and Deutsch [53], we use a vertical growth rate of $130 \mu\text{m}$ as well. (Note that zebrafish of the Tübingen variety grow particularly fast [46]; our five-cell model in Chapter 4 uses slower growth rates).

3.5.3 Length scales

As described in §3.3.2, we specify discrete-time rules for cell death and differentiation based on the cell proportions in two regions, an annulus that accounts for long-range communication, potentially regulated by pseudopodia [25], and a short-range disk that models interactions between neighboring cells:

$\Omega_{\text{podia}}^{\bar{x}}$ = annulus of inner radius $L = 318 \mu\text{m}$ & width $w = 25 \mu\text{m}$ centered at $\bar{x}$;

$\Omega_{\text{loc}}^{\bar{x}}$ = short-range disk of radius $\ell = 75 \mu\text{m}$ centered at position $\bar{x}$.

The radius of the short-range disk is set to be slightly less than $\Delta_{xm} = 82 \mu\text{m}$. This value was chosen to concentrate short-range interactions either within stripes or within interstripes (unless stripe/interstripe boundaries have been degraded).

From Exp. J [54], we know long-range interactions must span at least half a stripe width. Actual measurements of stripe/interstripe width vary depending on fish age (an observation we made from images found in [58]); for example, by our estimate, the stripes/interstripes in Figure 4 (in the main manuscript) [17] at stage J+ are $\approx 340 \mu\text{m}$ wide and adult stripes can be 1 mm wide [53]. Thus, we base our approximation of stripe width off of cell-to-cell distances [76] and the observation [54] that stripes/interstripes are about 7-12 cell wide. Assuming 10-cell-wide stripes, we have:

$$\text{half the width of a stripe} \approx (9 \times \Delta_{mm})/2 + \Delta_{xm} = 307 \mu\text{m}.$$

Rather than set $L = 307 \mu\text{m}$, we chose $L = 318 \mu\text{m}$ to be half the diagonal of the square with width equal to 10 melanophores. This choice ensures that the proportion of melanophores to xanthophores in Ω_{podia} are different enough between stripes and interstripes to specify differentiation appropriately. The width of the annulus was again set based on fully formed stripes of width 10 cells: if the distance from the center of a stripe to the nearest xanthophore is $307 \mu\text{m}$ and xanthophores are roughly $36 \mu\text{m}$ apart, the distance to the next xanthophore in line is $343 \mu\text{m}$. Thus, we chose $w = 343 - 318 \mu\text{m} = 25 \mu\text{m}$ to give long-range interactions more flexibility.

3.5.4 Cell migration

As described in §3.3.1, the movement of each cell in our model is governed by an ordinary differential equation (ODE) that prescribes its interactions with all the other cells on the domain (the time step used to simulate our ODEs is 1 day with a forward Euler scheme; we tested smaller time steps post modeling and they do not strongly impact our results; in Chapter 4, in comparison, the time step is 0.1 day).

We assume inertial forces are negligible, so that velocity is proportional to force, and consider movement by Morse potential (see Equations 3.1, 3.2, and 3.3). While this choice of potential is not compactly supported, it decays exponentially and is smooth, making it easy to work with numerically. To ensure that this simplification did not influence our results, we also tested our model under Morse potentials with sharp, more biological cut-offs and found no difference in the simulations. We now describe the forces between cells in turn.

Melanophore-on-melanophore: Melanophores disperse when they have space to move [76], so we model the interactions between melanophores with a repulsive potential. In particular, Takahashi et al. [76] ablated a disk of radius $\approx 3\Delta_{mm}$ around an isolated melanophore in a stripe, thus creating a situation in which a single melanophore was separated from a cluster of melanophores by $3\Delta_{mm}$ ($\Delta_{mm} = 50 \mu\text{m}$ is the average distance between neighboring melanophores [76]). They found that, while the remaining densely packed melanophores spread outward, the isolated melanophore did not move [76]. This suggests that the length scale of repulsion should be less than $3\Delta_{mm}$ to account for the isolated melanophore not migrating away from the cluster.

Xanthophore-on-xanthophore: According to [90], xanthophores move significantly slower than melanophores, though a precise measurement of xanthophore speed *in vivo* is not available; in addition, Δ_{xx} , the average distance between neighboring xanthophores (measured as $36 \mu\text{m}$ by [76]), is less than Δ_{mm} [76]. Thus, we model xanthophore interactions as purely repulsive and choose the length scale and strength of repulsion to be shorter and weaker than the melanophore counterparts.

Xanthophore-on-melanophore: Melanophores move away from dendrites extended toward them by xanthophores [29, 76, 90]. To account for the large distance

between stripes and interstripes (the average distance between melanophores and xanthophores at the stripe/interstripe boundary, Δ_{xm} , is $82 \mu\text{m}$ [76]), while also recognizing that interactions between these cells seem to be generated at short range by dendrites, we chose a purely repulsive potential with the same length scale r^{xm} as for homogenous melanophore interactions but with increased strength R^{xm} .

Melanophore-on-xanthophore: Yamanaka and Kondo [90] found *in vitro* that melanophores and xanthophores harvested from fins exhibit chase-run movement: melanophores extracted from fins run away from short dendrites extended toward them by xanthophores, which slowly follow in pursuit (note that this *in vitro* behavior has yet to be observed *in vivo*). It has also been reported [17, 74] that melanophores and xanthophores from the zebrafish body repel each other. As discussed in §3.4.1, the potentials that best fit the experimental data are either purely repulsive or combine short-range attraction with mid-range repulsion. Because *in vitro* measurements of cell speeds [90] are available for chase-run movement, we chose this mechanism and set R^{mx} , A^{mx} , r^{mx} , and a^{mx} using average cell-to-cell distances [76]. We note that the chase-run mechanism observed in [90] has an anticlockwise bias; as a simplifying assumption, we chose not to include this bias in our model (for a simulation of spiraling chase-run movement, see [88]).

Boundary conditions are handled by discretizing the boundary of the domain into points and specifying that these points exert repulsive forces on all the cells in the domain (in the same way as the cells experience forces from the other cells). These forces are modeled by a repulsive Morse potential (Equation 3.3) and are simply added to the righthand side of Equations 3.1 and 3.2 for melanophore and xanthophore velocities. A summary of parameters involved in cell migration is shown in Table 3.3.

Name	Value	Description
R^{mm}	62 $\mu\text{m}/\text{day}$	Melanophore-on-melanophore repulsion
R^{xx}	50 $\mu\text{m}/\text{day}$	Xanthophore-on-xanthophore repulsion
R^{xm}	137 $\mu\text{m}/\text{day}$	Xanthophore-on-melanophore repulsion
R^{mx}	113 $\mu\text{m}/\text{day}$	Melanophore-on-xanthophore repulsion
A^{mx}	163 $\mu\text{m}/\text{day}$	No attraction between cells
r_{mm}	20 μm	Appears in Q^{mm}
r_{xx}	11 μm	Appears in Q^{xx}
r_{xm}	20 μm	Appears in Q^{xm}
r_{mx}	20 μm	Appears in Q^{mx}
a_{mx}	12 μm	Appears in Q^{mx}
R^b	137 μm	Boundary forces
r_b	20 μm	Appears in boundary force potential

Table 3.3: Summary of cell migration parameters.

3.5.5 Cell differentiation

We model differentiation from precursor or stem cells that we assume are uniformly distributed over the fish skin domain, so that new cells can appear anywhere with equal probability. At the end of each day, we select a set number of locations (N_{diff}) for possible melanophore differentiation and another equal number of locations for possible xanthophore differentiation. In contrast to how we model death, which is due to either short-range or long-range mechanisms, we specify differentiation only when conditions on both of these length scales are satisfied at the same time (in addition to an overcrowding condition). A small amount of random differentiation is also included.

Melanophore differentiation: Nakamasu *et al.* [54] suggested that xanthophores in adjacent interstripes may promote melanophore differentiation. We couple

this long-range mechanism with short-range activation of melanophores by like cells. This short-range activation, which serves to reinforce successful stripe pattern formation, is how we account for the differences in Exp. A and E or in Exp. C and G [54]. In particular, a melanophore appears at the randomly selected position $\bar{x}$ according to the rules below:

$$\begin{aligned} \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} M_i > \alpha \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} X_i, \quad \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} X_i > \beta \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} M_i, \quad \text{and} \\ \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i < \eta \quad \implies \quad \text{melanophore differentiation at } \bar{x}, \end{aligned}$$

where $\alpha = 1$ and $\beta = 3.5$ were fit to Exp. A-H and $\eta = 6$ was chosen based on experimentally measured melanophore densities [76]. The radius d_{crowd} of $\Omega_{\text{crowd}}^{\bar{x}}$ was set to Δ_{xm} .

Xanthophore differentiation: Because less data is available for xanthophore differentiation (notably, Exp. A-H and the associated bar graphs [54] only track the number of melanophores that appear in the ablated region), we model it as similar to melanophore differentiation. The rules for xanthophore differentiation at $\bar{x}$ are:

$$\begin{aligned} \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} X_i > \varphi \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} M_i, \quad \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} M_i > \psi \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} X_i, \quad \text{and} \\ \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i < \kappa \quad \implies \quad \text{xanthophore differentiation at } \bar{x}, \end{aligned}$$

where $\varphi = 1.3$ and $\psi = 1.2$ were fit to Exp. A-H and $\kappa = 10$ was chosen to match xanthophore densities [76].

Random differentiation: A small amount of random differentiation is also included. We enforce that random differentiation can only occur at a selected point if there are no cells in a disk of radius $d_{\text{rand}} = 100 \mu\text{m}$ around it; thus, cells do not

differentiate randomly in dense regions. The radius d_{rand} was chosen on the order of Δ_{xm} . Of the N_{diff} possible locations for melanophore differentiation selected every day, each position has probability $p_m = 0.03$ of being a site for random differentiation, assuming it is not densely populated already. Similarly, random xanthophore differentiation takes place at a selected location with probability $p_x = 0.005$. The value for p_m was fit to Exp. A-H [54], and the value of p_x was chosen smaller to match the observation [54] that xanthophores do not seem to develop in ablated stripe regions, suggesting that xanthophore differentiation is less random.

A summary of parameters involved in cell differentiation is shown in Table 3.4.

3.5.6 Cell death

Cell death can occur in our model due to two mechanisms: short-range competition or long-range survival signals, as we describe next.

Short-range competition: The experiments [54] suggest that melanophores and xanthophores compete at short range. We model this as

$$\begin{aligned} \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{M_j}} X_i &> \mu \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{M_j}} M_i \implies \text{death of } M_j, \\ \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{X_j}} M_i &> \nu \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{X_j}} X_i \implies \text{death of } X_j, \end{aligned}$$

where $\mu = \nu = 1$.

Long-range survival: Nakamasu *et al.* [54] demonstrated that ablating xanthophores in the two interstripes adjacent to a stripe results in melanophore death. The corresponding experiment (ablating stripes surrounding an interstripe), how-

Name	Value	Description	Source
N_{diff}	Varies	Number of locations selected each day for possible differentiation; depends on initial condition	Chosen to fill domain by appropriate time
α	1	Lower bound on $\frac{\sum_{i=1}^{N_m} M_i}{\sum_{i=1}^{N_x} X_i} _{\Omega_{\text{loc}}}$ for M differentiation	Fit to Exp. A-H [1]
β	3.5	Lower bound on $\frac{\sum_{i=1}^{N_x} X_i}{\sum_{i=1}^{N_m} M_i} _{\Omega_{\text{podia}}}$ for M differentiation	Fit to Exp. A-H [1]
η	6	Upper bound on $(\sum_{i=1}^{N_m} M_i + \sum_{i=1}^{N_x} X_i) _{\Omega_{\text{crowd}}}$ for M differentiation	Fit to Exp. A-H [1] & cell densities [2]
d_{crowd}	$82 \mu m$	Radius of overcrowding disk	Set to Δ_{xm} [2]
φ	1.3	Lower bound on $\frac{\sum_{i=1}^{N_x} X_i}{\sum_{i=1}^{N_m} M_i} _{\Omega_{\text{loc}}}$ for X differentiation	Fit to Exp. A-H [1]
ψ	1.2	Lower bound on $\frac{\sum_{i=1}^{N_m} M_i}{\sum_{i=1}^{N_x} X_i} _{\Omega_{\text{podia}}}$ for X differentiation	Fit to Exp. A-H [1]
κ	10	Upper bound on $(\sum_{i=1}^{N_m} M_i + \sum_{i=1}^{N_x} X_i) _{\Omega_{\text{crowd}}}$ for X differentiation	Fit to Exp. A-H [1] & cell densities [2]
d_{rand}	$100 \mu m$	Radius of ball for random differentiation	Chosen to be $\approx \Delta_{xm}$ [2]
p_m	0.03	Probability of random M birth	Fit to Exp. A-H [1]
p_x	0.005	Probability of random X birth	Fit to Exp. A-H [1]

Table 3.4: Cell differentiation parameters.

ever, has no negative effect. We include these observations by way of a stochastic long-range death signal for melanophores:

$$\sum_{i=1}^{N_m} \mathbf{1}_{\Omega_{\text{podia}}^{M_j}} M_i > \xi \sum_{i=1}^{N_x} \mathbf{1}_{\Omega_{\text{podia}}^{M_j}} X_i \implies \text{death of } M_j \text{ with probability } p_{\text{death}} \text{ per day,}$$

where $\xi = 1.2$ was fit to Exp. A-J. Because Nakamasu *et al.* [54] found that 6 days after ablation melanophore density had decreased by about 20% in comparison to

Name	Value	Description	Source
μ	1	Lower bound on $\frac{\sum_{i=1}^{N_x} X_i}{\sum_{i=1}^{N_m} M_i} _{\Omega_{\text{loc}}}$ for M death	Fit to Exp. A-H [1]
ν	1	Lower bound on $\frac{\sum_{i=1}^{N_m} M_i}{\sum_{i=1}^{N_x} X_i} _{\Omega_{\text{loc}}}$ for X death	Fit to Exp. A-H [1]
ξ	1.2	Lower bound on $\frac{\sum_{i=1}^{N_m} M_i}{\sum_{i=1}^{N_x} X_i} _{\Omega_{\text{podia}}}$ for M death	Fit to Exp. A-H [1]
p_{death}	0.0333	Probability of M death due to long-range effects	Given by [1]

Table 3.5: Cell death parameters.

control fish, we set $p_{\text{death}} = 0.2/6 = 0.0333$.

A summary of parameters involved in cell death is shown in Table 3.5.

3.6 Continuum limit

To derive a continuum limit, we follow the strategy outlined in [2] for a locust model.

Define the density functions for melanophores and xanthophores, respectively, as

$$m(x, t) = \sum_{j=1}^{N_m} P_m \delta(x - M_j); \quad \gamma(x, t) = \sum_{j=1}^{N_\gamma} P_x \delta(x - X_j),$$

where $P_m = O(\frac{1}{N_m})$ and $P_x = O(\frac{1}{N_x})$ are scaling factors. Taking the formal limit of our agent-based model leads to the left-hand side of the equations

$$m_t - \nabla \cdot [m(\nabla Q^{mm} * m + \nabla Q^{\gamma m} * \gamma)] = f(m, \gamma) \quad (6.1)$$

$$\gamma_t - \nabla \cdot [\gamma(\nabla Q^{\gamma\gamma} * \gamma + \nabla Q^{m\gamma} * m)] = g(m, \gamma), \quad (6.2)$$

where the potentials are the same as those in the agent-based model with γ replaced with x . Here $*$ denotes convolution, namely $f * g(x) = \int f(x - y)g(y)dy$. On the right-hand side Equations 6.1 and 6.2, the source terms f and g , obtained using a modeling argument, are continuous analogues of the differentiation and death rules in our discrete model. In particular,

$$\begin{aligned}
 f(m, \gamma) = & \underbrace{H\left(A * \left(\gamma - \tilde{\beta}m\right)\right)}_{\text{nonlocal differentiation}} \cdot \underbrace{H(m - \tilde{\alpha}\gamma)}_{\text{local differentiation}} \cdot \underbrace{(\tilde{\eta} - m - \gamma)}_{\text{overcrowding}} - \underbrace{C_1 m H(\gamma - \tilde{\mu}m)}_{\text{local death}} \\
 & - \underbrace{C_2 m H(A * (m - \tilde{\xi}\gamma))}_{\text{nonlocal death}}; \\
 g(m, \gamma) = & \underbrace{C_3 H\left(A * \left(m - \tilde{\psi}\gamma\right)\right)}_{\text{nonlocal differentiation}} \cdot \underbrace{H(\gamma - \tilde{\varphi}m)}_{\text{local differentiation}} \cdot \underbrace{(\tilde{\kappa} - m - \gamma)}_{\text{overcrowding}} - \underbrace{C_4 \gamma H(m - \tilde{\nu}\gamma)}_{\text{local death}},
 \end{aligned}$$

where $H(\cdot)$ is a smooth Heaviside function and $A(\cdot)$ selects an annulus neighborhood for long-range interactions. The constants C_1, C_2, C_3 , and C_4 are positive and specify growth and death rates; the parameters $(\tilde{\beta}, \tilde{\alpha}, \tilde{\eta}, \tilde{\mu}, \tilde{\xi}, \tilde{\psi}, \tilde{\varphi}, \tilde{\kappa}, \tilde{\nu})$ correspond to $(\beta, \alpha, \eta, \mu, \xi, \psi, \varphi, \kappa, \nu)$ in our agent-based model.

Following the work in [77], we performed a linear stability analysis of uniform density (time- and space-constant) equilibria (m_0, γ_0) for the case when $f(m, \gamma) = g(m, \gamma) = 0$ and, in agreement with the analysis in [56] for a more general nonlocal PDE model, found that unstable wave numbers cannot be achieved under Mechanism A (see §3.4.1). In contrast, chase-run movement or pure repulsion can lead to unstable wave numbers. We set $f(m, \gamma) = g(m, \gamma) = 0$ here because this analysis evaluates the stability of uniform steady states composed of a mixed population of melanophores and xanthophores, a condition that would not naturally arise in our discrete model under the influence of wild-type differentiation and death parameters. Thus, the focus of our stability analysis is on how perturbations to a uniform density effect pattern formation under the action of cell migration alone.

3.7 Discussion

We have presented a two-population model for the development of stripe patterns in zebrafish. Melanophores and xanthophores are modeled as independent agents interacting according to differential equations for migration and stochastic rules for differentiation and death. We account for both short-range cell communication, potentially regulated by dendrites, and long-range interactions, which may be governed by pseudopodia extensions [25].

We argue that fish growth and long-range interactions help select the width of zebrafish stripes and interstripes. The horizontal myoseptum and delayed localization of iridophores promotes stripe pattern alignment. At the same time, growth and development create a set-up in which stripes and interstripes can gradually appear, directed and informed by the existing pattern. Long-range interactions, the larval pattern, and iridophores work together to guide cell differentiation. Growth widens stripes/interstripes and thus supports a shorter, more biological scale for long-range interactions. Lastly, net repulsion of melanophores and xanthophores ensures that clear boundaries are maintained between stripes and interstripes. These clear boundaries prevent short-range competition from upsetting stripe pattern width.

Regarding the current Turing debate, it has been observed that the rules for differentiation and death suggested by Nakamasu *et al.* [54] take the form of short-range activation and long-range inhibition, a patterning mechanism first proposed by Gierer and Meinhardt [19]. In [25, 39], it was suggested that interactions by pseudopodia combined with repulsion of melanophores from xanthophores by dendrites could be considered a Turing-type mechanism [80] without diffusion. It is worthwhile to note that, in reaction-diffusion models, diffusion is responsible for dispersing in-

formation long range, whereas in our model it is the kinetics of differentiation and death that determine long-range interactions. Thus, though the abstract framework of two biological quantities interacting through short-range activation and long-range inhibition is present in both modeling approaches, the mechanisms at work are different.

As more biological results emerge about zebrafish, our approach toward xanthophores is one of the main places our model could be improved. Because less was known about these cells, which are hard to observe on zebrafish skin [58], at the time of this chapter's work, we modeled their differentiation as similar to melanophore differentiation. The ablation experiments of Nakamasu *et al.* [54] have demonstrated, however, that melanophores and xanthophores react very differently to long-range signals, both for differentiation and for survival. In addition, our initial pattern could be altered to better match the biology. We address both of these observations in our next model for pattern formation on the body of zebrafish, described in Chapter 4.

Regarding future work, one avenue a modeling perspective naturally lends itself to studying is the impact of domain growth on pattern formation, and many interesting questions can be raised in this area. In particular, Parichy and Turner [62] have shown that slowing body growth in the puma mutation can enable fish to recover wild-type stripes and interstripes (see §4.4.6). Our model of pattern formation on the caudal fin (Chapter 5) also invokes a discussion of the impact of growth on stripe formation. Interestingly, it is known that zebrafish stripes/interstripes do not form perfectly parallel to the horizontal myoseptum, but are actually tilted a few degrees from center [58], though the reason remains hidden. In the future, we plan to explore the interplay of growth and pattern development on the zebrafish skin.

In this study, we focused on late-stage mutations like jaguar/obelix and dali that

alter stripe pattern width, and we did not discuss the spotted leopard phenotype. Promisingly for future work, however, we found that one set of altered migration parameters (see §3.4.1) disrupted the stability of wild-type stripes and interstripes in such a way as to lead to an undulating stripe pattern with clear boundaries. This pattern, shown in Figure 3.2b, is akin to the undulating stripes/interstripes found in weak forms of the leopard mutation (see, for example, [43]). Newer experimental work [14, 33] has suggested that some spotted phenotypes may impact how iridophores change form, and we include results about the leopard mutation in the next chapter.

With only two cell populations and no direct precursor cells, our model successfully reproduces a wide range of experimental data. Nevertheless, many other types of pigment cells appear on zebrafish skin: sparse xanthophores in stripes, L-iridophores, blue S-iridophores, and dense S-iridophores [17, 33, 45, 74]. Because these cells are segregated into either stripes or interstripes, we were able to combine their known effects into two populations with good results. In the next chapter, we introduce iridophores into our model directly and investigate how their effectively directional diffusion influences stripe pattern alignment. By including melanophores, xanthophores, and iridophores there we are also able to more closely investigate early-stage mutations [17, 33, 74] in which one or two types of pigment cells fail to be present on the fish skin.

CHAPTER FOUR

A five-cell model of pattern formation on zebrafish

4.1 Introduction

Up until a few years ago, the experimental community focused largely on the role of melanophores and (compact) xanthophores in zebrafish patterns (see, for example, [35, 54, 89]) and it was thought that iridophores were not actively involved [40, 43]. By the time our first model [81] and the work of Bullara and De Decker [5] came out, though, it was becoming more clear that all three cell types have an important place in wild-type stripes [17, 65, 75]. This is supported strikingly by early-stage mutations in which one type of cell fails to develop: *pfeffer* (no xanthophores), *nacre* (no melanophores), and *shady* (no iridophores) all feature patterns that are very different than wild-type stripes, but experimental work has suggested these mutations act cell-autonomously to the missing cell types [17, 43]. This means, for example, that *shady* fish display black spots not because some cell interaction is altered, but simply because iridophores are not present on the fish skin. (In contrast, *shady* features normal stripes on the caudal fin, suggesting that iridophores are not required for fin patterning [33]; for a study of the tailfin, see Chapter 5).

While it has been known for a while that xanthophores and iridophores appear in both light and dark regions on the fish skin [27], the fundamentally different forms the cells take on in these two regions was only recently described: iridophores are loose in black stripes and dense in yellow; xanthophores, in turn, are stellate/neutral in dark regions and compact in light [17, 44, 45]. Most notably, by changing in form between dense and loose, iridophores mark black stripes and initialize new interstripes [75], directing other cells on the fish skin (Figure 4.1). Thus, as the importance of iridophores came to light, additional cell interactions also surfaced: it was shown that transitions between cell forms play a crucial role in patterning [45, 75]. New research [14, 33] is even suggesting that some mutations producing

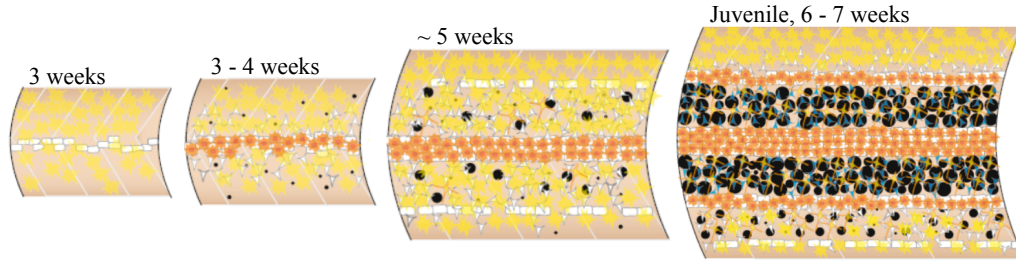


Figure 4.1: Schematic of wild-type patterning shows the importance and leading role of iridophores: these cells spread across the fish skin from the horizontal myoseptum, transitioning into stellate form (blue and white triangles) to mark dark stripes and aggregating in dense form (white squares) to produce new interstripes [74, 75]. These interactions seem to guide melanophores (black circles) and xanthophores (orange or yellow); xanthophores become compact (orange) after iridophores aggregate in dense form [45], but little is known about what controls iridophore behavior. This chapter focuses on contributing to this question from a mathematical modeling perspective. Images are reproduced from Singh and Nüsslein-Volhard [74] with permission from Elsevier with adaption.

spotted phenotypes may impact how iridophores changes form, further highlighting the importance of this new mechanism.

Because the importance of iridophores and transitions in cell form are very recent findings biologically, there is still little known about them experimentally [40]. Furthermore, while melanophores and compact xanthophores have been studied from a modeling framework in the past, we are aware of no models for zebrafish patterning incorporating iridophores and transitions in cell form. In particular, as discussed in §3.4.2, delayed iridophore localization in dense form is necessary for the sequential formation of new interstripes (for details see Figure 4.1). While the *if* and *if not* of normal iridophore dynamics are thus clear [75], the *why* remains largely unknown - correct iridophore behavior is crucial, but what instructs iridophores? This is a place where mathematical approaches can work alongside experimental studies to help elucidate cell interactions, and our overarching motivation for this chapter is to produce a predictive model that is current with the new experimental data and raising or addressing the same questions as the biologists.

Goals: As described earlier, some experimental data exists on what causes pig-

ment cells to differentiate, die off, or migrate; furthermore, new work [44, 45] is beginning to suggest interactions that drive xanthophores to change form between stellate and compact. Nevertheless, while new experimental hypotheses exist, very little is known about what causes iridophores to change form. Thus, the goal of our work is to use a mathematical modeling approach to identify a candidate set of minimal rules to govern iridophore and xanthophore form changes that is consistent with a breadth of dynamics on zebrafish skin. In this way, we can help predict what drives cells to change form and contribute to a better understanding of wild-type interactions. Once we have validated a candidate model for wild-type cell interactions, we can use it to explore late-stage mutations. In particular, experimentalists have uncovered a wide range of zebrafish mutations (for example, see [14, 33, 43]) in which all three types of pigment cell are present on the fish skin, but some cell interaction(s) are altered, leading to altered phenotypes (we refer to these mutations as late-stage mutations). While the genes associated with many mutations have been identified, their function in cell interactions remains largely unknown. By suggesting how a change in gene translates into a functional difference in how the pigment cells behave, we can help tie genes to their functions.

Outline: We summarize the results of this chapter in the next section and give a brief overview of the general form of the model in §4.3 (for details about model set-up, cell interactions, and domain growth, see §4.6). Our approach to fitting and testing the model is described in §4.4.1. Model findings are presented and discussed in relationship to the experimental literature, some of which was published during or after model development, in two parts: in §4.4, we present the dynamics our proposed model can account for without any changes to cell interactions (wild-type in §4.4.2; early-stage mutations in §4.4.3; choker and puma mutations in Sections 4.4.5 and 4.26; regeneration and degeneration experiments in Sections 4.4.7 and 4.4.8; and

quantitative measurements in §4.4.4). In part 2 of our results, §4.5, we motivate and derive the proposed model; this section results in predictions on wild-type dynamics governing iridophores (§4.5.1), xanthophores (§4.5.2), and melanophores (§4.5.3). In §4.5.4, we present preliminary work altering model interactions to help identify what cell interactions are affected in late-stage mutations. Finally, in §4.7, we present the full candidate network of interactions including iridophores predicted by the model, discuss places for improvement, and highlight some directions for future work.

4.2 Summary of results

Much experimental work has been done to uncover wild-type interactions, especially for black and yellow cells, but little is known about iridophores [14, 34, 40, 86]. New results [65, 75] suggest iridophores drive patterning: by changing form between dense and loose, they mark the appearance of new stripes and interstripes and guide melanophores and xanthophores. What drives iridophores, however, remains largely unknown and speculative. This is a place where mathematical methods can contribute to current biological unknowns, and this chapter is a first modeling step toward a better understanding of the interactions that govern iridophores and dictate cell form changes.

Using an agent-based approach that accounts for all three cell types, we identify interactions that instruct cells to form stripes and mutated patterns. While our results in §3.4.2 support work [65, 74, 75] suggesting iridophores direct stripes formation, we now suggest what drives iridophores. This model evolved alongside current biology, raising the same questions and hitting up against the same unknowns as the experimentalists. The crux of our work was developing an approach to balance the

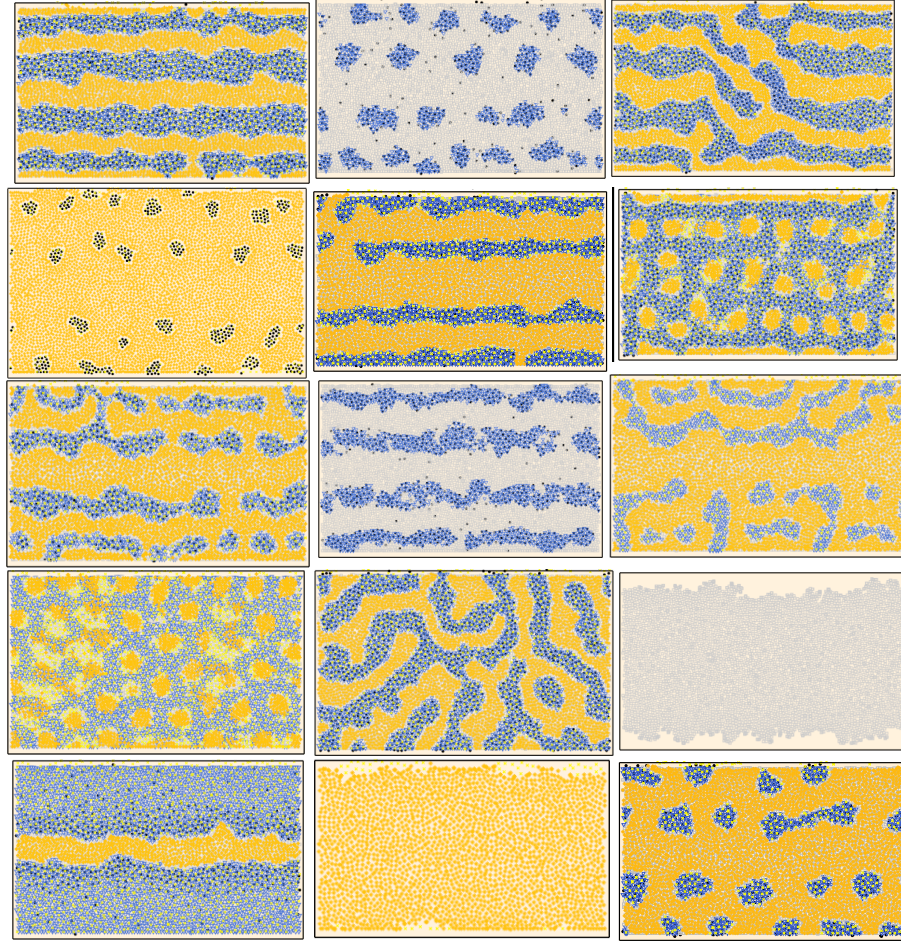


Figure 4.2: Overview of sample patterns, including wild-type, mutations, and experiments, developed from a larval initial condition by the model in this chapter. From left-to-right by row, we explore wild-type stripes (§4.4.2), pfeffer (§4.4.3), ablation experiments (§4.4.7), shady (§4.4.3), idfix (§4.5.4), light spots in a dark background possibly related to *Danio margaritatus* (§4.5.1), puma (§4.4.6), degeneration experiments (§4.4.8), nacre (§4.4.3), light spots in a blue background (§4.5.1), choker (§4.4.5), nacre;pfeffer (§4.4.3), altered conditions possibly related to *Danio albolineatus* (§4.5.1), nacre;shady, and leopard;schachbrett (§4.5.4).

open nature of current experimental questions on iridophores with sensitivity to not overfitting model rules (see §4.4.1). Our main contribution is a predictive and current model that has been validated on a broad range of experimental observations and can be used to predict communication pathways that may be impacted in late-stage mutations (§4.5.4). Our proposed network of cell interactions includes iridophores and mechanisms that instruct cells to change form (see Figure 4.46 in §4.7). Other results and predictions that came out of this work include questions about the pos-

sibility of yellow-spotted mutations and the relationships between zebrafish (*Danio rerio*) and other *Danio* fish (particularly *Danio albolineatus* and *Danio margaritatus*), suggestions for why zebrafish stripes are reliable and robust to stochasticity in nature, and support for recent October 2016 work [44] about presence of intermediate xanthophore forms. Figure 4.2 shows a sample of some of the patterns produced by this model.

4.3 Overview of model

We begin by giving an overview of the model and refer to §4.6 for a detailed description (cell form changes are discussed in §4.5). A summary of model interactions in network form is also provided in Figure 4.46 in §4.7. The model tracks cells of five types: melanophores, two forms of xanthophores, and two forms of iridophores. It should be noted that there are more cells that are part of zebrafish patterns: in particular, we knowingly exclude L-iridophores from our model, as these cells appear on the skin only after the patterns have formed. Thus, while it remains to be seen if L-iridophores are or are not involved in pattern maintenance [17], they are not active or present during the pattern formation timeframe we focus on. Additionally, the current experimental data offers an unclear perspective on larval cells: we initially put forward a model with six cell types (the additional cell type being larval xanthophores), but the feedback we received from the Nüsslein-Volhard group (personal communication) suggested we combine larval xanthophores and stellate xanthophores into one class of xanthophores (stellate or stellate/neutral). Furthermore, some experimental studies mention larval melanophores, which we do not directly include in our work, and [69] goes so far as to suggest cells may express a continuum of features as they differentiate from precursors over time, thus opening the possibility

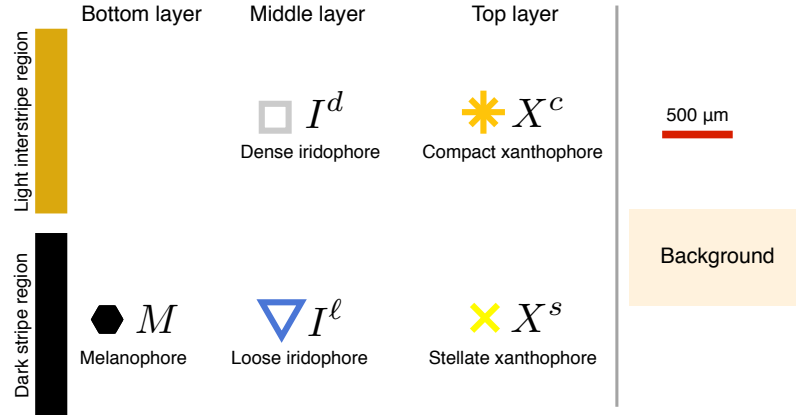


Figure 4.3: Schematic showing our simulation set-up. Dark stripes are represented by melanophores (black hexagons) under loose iridophores (blue triangles) and a final top layer of stellate/neutral xanthophores (yellow x's). Light interstripes consist of dense iridophores (silver squares) under compact xanthophores (orange asterisks). A tan background color is used for all of our simulations, and the scale bar in all images is 500 μm .

of many more cell types. Because we are aware of no previous models that include more than two types of cells, we believe that by including five cell types our model balances capturing new features of the experimental data with accounting for the right level of detail for the current knowns and unknowns in zebrafish patterning without overfitting or over-hypothesizing.

As with our previously described agent-based models, cells are modeled as point masses, so we do not directly take into account cell size. It should be noted that the cells we consider are separated into 3 distinct layers on the fish skin [26, 27]. Rather than consider a 3D domain to directly account for these layers, we use a 2D domain where cells may sit on top of each other. Thus, this model produces pictures in which black stripes are a layered mosaic of stellate yellow xanthophores on top, blue loose iridophores, and finally black melanophores; yellow regions, in turn, are represented by orange compact xanthophores spread atop silver dense iridophores (Figure 4.3).

We are interested in understanding the timeline of pattern formation from when the fish is roughly 3 weeks old, the point at which dense iridophores appear along the

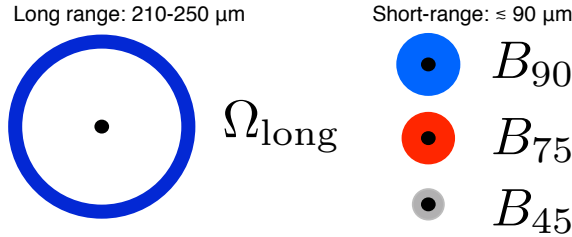


Figure 4.4: Summary of length scales used in our five-cell model. Long-range interactions are modeled by an annulus Ω_{long} with an inner radius of $210 \mu\text{m}$ and an outer radius of $250 \mu\text{m}$; we think of interactions at this scale as involving cell communication via pseudopodia (measured to be up to half an adult stripe width or $\approx 300 \mu\text{m}$ in length [25]) or airnemes (measured to be up to $\approx 250 \mu\text{m}$ in length [12]). While we differentiated between cell interactions at short-range (possibly regulated by dendrites [29]) and contact-range (possibly regulated by direct cell-cell contact through gap junctions [44], for example) in our two-cell model (compare to Figure 3.1), here we do not make this distinction. Instead, we will refer to short-range or local dynamics as interactions occurring on length scales between roughly 30 and $90 \mu\text{m}$. We think of these interactions as governed by gap junctions or potassium channels (direct contact), short dendrites, or short airnemes [12, 29, 33].

horizontal myoseptum, until juvenile stages, when the fish is 2-3 months old. Thus, all simulations are done on growing domains (this is in contrast to our two-cell model in Chapter 3 and represents an improvement to our work on mutations there, as we now describe the timeline of their development). We simulate the full width of the zebrafish and roughly a third of its length (excluding a region around the eye), with boundary conditions that are periodic in x (in a small neighborhood of the top and bottom of the domain, we remove the component of cell velocity perpendicular to these boundaries).

Our cells, separated into five distinct classes, exhibit short and long-range interactions: short-range refers to length scales between roughly 30 and $90 \mu\text{m}$ in our model, and we think of these dynamics as governed by direct cell contact or short dendrite extensions [29, 44]. Our long-range interactions, possibly regulated by pseudopodia [25] or airneme extensions [12], occur on a shorter scale here than in our two-cell model, with length scales reaching up to $250 \mu\text{m}$ (in comparison, adult stripes have been measured to be roughly $600 \mu\text{m}$ wide [25]). Our length scales are summarized in Figure 4.4.

We include all four known types of cell behavior: migration, birth, death, and changes in form. While our previous models included cell death for both black and yellow cells, this model allows for melanophore death only. Instead, xanthophores and iridophores undergo form changes between stellate and compact or loose and dense, respectively. As much as possible, we make use of the known experimental data to specify cell interactions, but the role of iridophores and the mechanisms that determine form changes are not well understood, so this is a place where the model has an opportunity to be predictive. We overview how we model cell interactions next (for a detailed description, see §4.6, and for a discussion of form change rules in particular, see §4.5).

4.3.1 Cell migration

Analogous to Chapter 3, cell migration takes the form of coupled ODEs. All cells experience migration, but our forces on stellate xanthophores and iridophores are primarily for spreading these cells out to maintain appropriate densities. We model stellate xanthophores as interacting through migration only with other xanthophores (that is, cells in the same layer on the fish skin), and iridophores experience forces only from other iridophores. We highlight the differential equations used for melanophore and compact xanthophore migration here, as they include the most interactions with other cells, and refer to §4.6 for a full description.

The movement of each melanophore is given by:

$$\begin{aligned} \frac{dM_i}{dt} = & \underbrace{\sum_{j=1, j \neq i}^{N_M} F^{MM}(\|M_j - M_i\|) \frac{M_j - M_i}{\|M_j - M_i\|}}_{\text{repulsion from melanophores}} + \underbrace{\sum_{j=1}^{N_X^c} F^{X^c M}(\|X_j^c - M_i\|) \frac{X_j^c - M_i}{\|X_j^c - M_i\|}}_{\text{repulsion from compact xanthophores}} \\ & + \underbrace{\sum_{j=1}^{N_I^d} F^{I^d M}(\|I_j^d - M_i\|)}_{\text{weak repulsion from dense iridophores}} ; \end{aligned}$$

and the ODE for the movement of each compact xanthophore is:

$$\begin{aligned} \frac{dX_i^c}{dt} = & \underbrace{\sum_{j=1, j \neq i}^{N_X^c} F^{X^c X^c}(\|X_j^c - X_i^c\|) \frac{X_j^c - X_i^c}{\|X_j^c - X_i^c\|}}_{\text{repulsion from compact xanthophores}} + \underbrace{\sum_{j=1}^{N_X^{sn}} F^{X^s X^c}(\|X_j^s - X_i^c\|) \frac{X_j^s - X_i^c}{\|X_j^s - X_i^c\|}}_{\text{repulsion from stellate xanthophores}} \\ & + \underbrace{\sum_{j=1}^{N_M} F^{MX^c}(\|M_j - X_i^c\|) \frac{M_j - X_i^c}{\|M_j - X_i^c\|}}_{\text{repulsion from melanophores}} + \underbrace{\sum_{j=1}^{N_I^d} F^{I^d X^c}(\|I_j^d - X_i^c\|) \frac{I_j^d - X_i^c}{\|I_j^d - X_i^c\|}}_{\text{attraction to dense iridophores}}, \end{aligned}$$

where M_i , X_j^c , X_k^s , and I_m^d are the positions of the i th, j th, k th, and m th melanophore, compact xanthophore, stellate xanthophore, and dense iridophore respectively; N_M , N_X^c , N_X^s , and N_I^d are the corresponding numbers of these cells; and the force F^{**} has the general form

$$F^{**}(d) = -R^{**} \left(\frac{1}{2} + \frac{1}{2} \tanh\left(\frac{r_{**} - d}{\delta}\right) \right),$$

where $\delta = 5$ serves to smooth out the hyperbolic tangent and $**$ denotes that the parameters used depend on the migration interaction. These forces are plotted in Figure 4.45.

The repulsive and attractive interactions we specify between cells are summarized in Table 4.1. These forces are motivated by experimental studies [17, 29, 30, 59, 76,

82, 90], and we consider them well-based in the biology. Note that the experimental data is often challenging to reconcile, however, and it is not always clear to us what results are hypotheses and which interactions are empirically proven; the biological literature is also rapidly advancing and changing. Additionally, *in vivo* results on cell spreading could be explained in different ways: cells could spread into vacant space through movement, differentiation, or a combination of both. *In vitro* studies of pairs of isolated cells are thus tempting to base our work on, yet these studies come with their own challenges. For example, the chase-run movement between melanophores and xanthophores [30, 90] (discussed in §3.4.1) raises questions about the form the xanthophores were in (compact or stellate) and whether fin and body cells behave the same.

4.3.2 Cell birth and death

As discussed in §3.7, the approach we used to model xanthophore birth in our two-cell model was one of the main places our work could be improved. In particular, there we described compact xanthophores as differentiating due to short-range activation and long-range inhibition, similar to melanophore birth, yet this assumption was based on ablation experiments [54] only done for black cells. Here we include a more biologically faithful description of xanthophore differentiation: based on [45, 47, 82], we model xanthophore birth by differentiation from larval xanthophores (stellate xanthophores in our model) and cell division of existing cells.

Singh *et al.* showed that initially iridophores differentiate from stem cells and appear at horizontal myoseptum, but from there they seem to actively divide [47, 75, 82]. Thus, we model iridophore birth as occurring via cell division of existing iridophores. From a modeling framework, this means xanthophores and iridophores

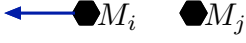
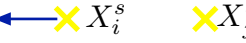
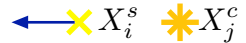
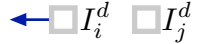
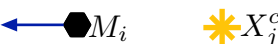
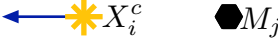
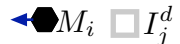
Action	Force	Impact of jth cell on ith	Source*
MM short-range repulsion	F^{MM}		☒
$X^c X^c$ short-range repulsion	$F^{X^c X^c}$		☒
$X^s X^s$ short-range repulsion	$F^{X^s X^s}$		☒
$X^s X^c$ short-range repulsion	$F^{X^s X^c}$		☒
$X^c X^s$ short-range repulsion	$F^{X^c X^s}$		☒
$I^d I^d$ weak short-range repulsion	$F^{I^d I^d}$		☒
$I^\ell I^\ell$ short-range repulsion	$F^{I^\ell I^\ell}$		☒
$I^\ell I^d$ short-range repulsion	$F^{I^\ell I^d}$		☒
$I^d I^\ell$ short-range repulsion	$F^{I^d I^\ell}$		☒
$X^c M$ short-range repulsion	$F^{X^c M}$		☒
$M X^c$ short-range repulsion	$F^{M X^c}$		☐
$I^d M$ weak short-range repulsion	$F^{I^d M}$		☐
$I^d X^c$ short-range attraction	$F^{I^d X^c}$		☐

Table 4.1: Summary of cell migration interactions in our model. We note that the number of parameters involved is fewer than this table indicates: in particular, $F^{X^c X^c} = F^{X^c X^s} = F^{X^s X^c}$ and $F^{I^\ell I^d} = F^{I^d I^\ell}$. We check the source box to signify that the corresponding behavior has roughly been observed *in vivo* or *in vitro* [29, 30, 76, 82, 90]. It is important to note that this distinction is not sharp, as described in the text; for example, [17, 75] have suggested that xanthophore-iridophore attraction, repulsion between melanophores and dense iridophores, and repulsion between compact xanthophores and melanophores are present on the fish skin. See §4.6 for details.

promote their own birth at short-range, and each day we evaluate every such cell for possible division:

- if an xanthophore is not overcrowded by other xanthophores, a new xan-

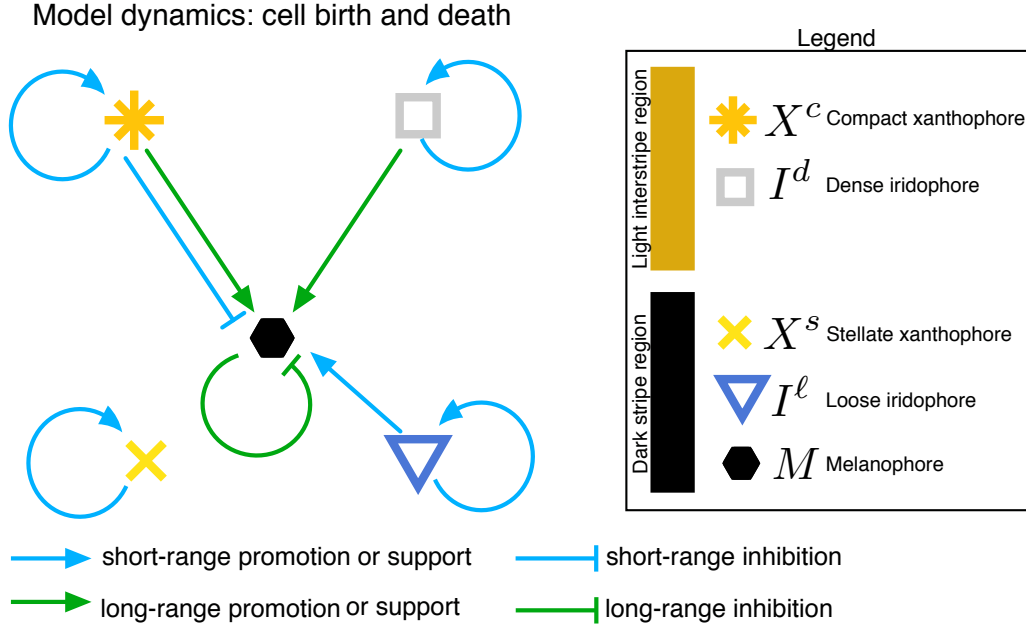


Figure 4.5: Summary of pigment cell birth and death dynamics in our model.

thophore is added to the domain next to its parent (stellate or dense form is determined by the parent cell); and

- if an iridophore is not overcrowded by other iridophores, a new iridophore is added to the domain next to its parent (loose or dense form is determined by the parent cell).

A small amount of random differentiation of stellate xanthophores is also included.

Melanophore differentiation, in contrast to xanthophore birth, maintains some similarities with the two-cell model in Chapter 3. Recent work has shown that melanophores differentiate from precursors or stem cells, often first appearing in the locations of future black stripes and exhibiting little movement from there [4, 11, 45, 75, 82]. Based on the ablation experiments [54] that demonstrate melanophores inhibit melanophore birth at long-range and xanthophores in neighboring interstripes promote melanophore survival, we model melanophore birth as due to long-range signals:

- new melanophores appear at randomly selected locations when there are more dense iridophores and compact xanthophores than melanophores in Ω_{long} (a small amount of random birth is also included).

We do not include short-range activation because melanophores exhibit much less cell proliferation than iridophores and xanthophores [82].

We model melanophore death in much the same way as in our two-cell model (Chapter 3), with one change (*italicized below*) to account for the pfeffer mutation. Every day each black cell is evaluated for possible death according to the rules:

- short-range competition with xanthophores: melanophores die when there are too many compact xanthophores locally;
- long-range inhibition: *when there are not enough loose iridophores nearby*, melanophores die with small probability informed by [54] if there are more melanophores than xanthophores in Ω_{long} .

The dynamics governing cell birth and death are summarized in Figure 4.5. Our xanthophore and iridophore birth rules are largely dictated by the experimental data [45, 47, 75, 82], so we do not view them as modeling assumptions. Our rules for melanophore death, survival, and birth involve more modeling choices but are strongly motivated by [17, 25, 45, 54, 59, 65].

4.3.3 Cell form changes

A role for iridophores and cell form changes in patterning has come to light in the last few years, so comparatively less is understood about these dynamics. The exper-

imental papers [17, 45, 65, 75] in 2013 and 2014 first drove our model development; the biological literature has rapidly increased since then, and we will discuss results on this topic from as recent as October 2016 [44] in Sections 4.4 and 4.6. Thus, the model rules that govern iridophore (and, to a lesser extent, xanthophore) form are the crux of our work and the mechanism that required the most modeling. This is a place where our mathematical study was progressing alongside the experimental work. Broadly, we model cell form changes according to rules that depend on the number of cells in short-range and long-range regions. We summarize these mechanisms here, but refer to Sections 4.5.1 and 4.5.2 for a motivation of our modeling choices and a discussion of alternatives.

Hypotheses for the interactions that govern iridophore form have been put forward by the experimental community, but much remains unknown and speculative. For example, Irion *et al.* [33] suggest communication channels on the surface of xanthophores and melanophores could play a role in directing iridophores. We model iridophore form changes by evaluating each iridophore on the fish skin domain for possible transitioning according to the following mechanisms:

- dense iridophores become loose when melanophores are nearby;
- if they not locally surrounded by compact xanthophores, dense iridophores can also become loose when the number of compact xanthophores at a distance becomes too high;
- loose iridophores transition to dense if there are compact xanthophores nearby but few melanophores; and
- loose iridophores are also instructed to change to dense form when there are too few compact xanthophores at a distance and melanophores nearby;

While iridophores respond to both long- and short-range signals in our model, xan-

thophores are instructed entirely by local communication. Each day we evaluate every xanthophore on the domain for form changing:

- compact xanthophores transition to stellate when there are more loose than dense iridophores nearby; and
- stellate xanthophores become compact when they are locally surrounded by more dense iridophores and compact xanthophores than loose iridophores and melanophores.

These rules were motivated by work [45] suggesting compact xanthophores strongly associate with iridophores in the dense form. More recently, [44] has shown melanophores influence xanthophore form locally.

4.4 Results: part 1

In this section and §4.5, we present the results (predictions and validations) generated from our agent-based model of zebrafish patterning. As discussed in §4.3, the model includes five types of cells and accounts for four types of interaction: migration, birth, death, and loose-dense or stellate-compact changes in form. The majority of our work has focused on identifying candidate rules for cell form changes, as this is the place where experimental data is most scarce and mathematical modeling has the opportunity to make a timely contribution to biology. Our approach to predicting these cell interactions (separation of data for fitting and testing) is detailed in the next section §4.4.1. Rather than presenting the many different mechanisms considered for these cell interactions next, we instead focus first on the results of the final model identified. This serves to introduce the reader to the dynamics that

we are looking for in the different simulation set-ups, so that when we compare our identified model to other candidate mechanisms it is clearer where these alternative choices fail to replicate the observed dynamics.

Thus, we first provide a series of validations of the model interactions we identified: wild-type development is discussed in §4.4.2, early-stage mutations in §4.4.3, choker in §4.4.5, puma in §4.4.6, and regeneration and degeneration experiments in Sections 4.4.7 and 4.4.8. Quantitative measurements are shown in relationship to empirical data in §4.4.4. We stress that all cell interactions are the same in every simulation presented in these sections. Once we have validated the identified model, we turn to a detailed discussion of the various mechanisms we considered to govern form changes: this motivates the model we chose and culminates in our predictions for the rules that govern iridophores (§4.5.1), xanthophores (§4.5.2), and melanophores (§4.5.3). Finally, in §4.5.4, we present some preliminary work on late-stage mutations: here we alter cell interactions to suggest possible functional effects of genetic mutations leopard, luchs, and idefix.

Note: regardless of whether a timeline of simulated pattern development or only the final image is shown for each example discussed in this chapter, all figures were produced by simulating the timeline of development from stage PB, roughly 21 dpf (with one exception simulated from an earlier stage at ≈ 14 dpf).

4.4.1 Approach

To accomplish our goals described in §4.1, we split the zebrafish biology into two groups and differentiate between two classes of mutations: set-ups in which cell interactions are wild-type (wild-type condition) and cases where one or more cell

behaviors are altered (altered condition). Similarly, we distinguish between early-stage mutations (namely *nacre*, *pfeffer*, *shady*, and crosses between these mutations) in which one or more types of cell fails to develop, and late-stage mutations (including *leopard*, *schachbrett*, *luchs*, and *obelix* [14, 33, 43]) in which all the pigment cells are present. For the former, based on the experimental data, we assume no cell interactions are altered and the mutated phenotypes arise solely because cells are missing on the skin; in other words, we assume early-stage mutations should be modeled using wild-type cell interactions. Additionally, because the *choker* mutation involves an altered initial condition (no horizontal myoseptum) and the *puma* mutation reduces melanophore birth [63], but neither seems to impact cell behavior otherwise, we consider these *middle-stage* mutations of sorts: we assume *choker* and *puma* should be accounted for with wild-type cell interactions, but with an altered initial condition and altered melanophore birth rate, respectively (in contrast to our work in Chapter 3, the same birth rate is used across all of our simulations except *puma*). In contrast, our work on late-stage mutations focuses on identifying altered interactions responsible for altered phenotypes.

Because more is known about cell differentiation, death, and migration, the main challenge in modeling was identifying a set of rules for cell form changes, particularly for iridophores. Very little is known about what causes iridophores to change form, so this is a place where our model was hitting against the same questions and walls as the experimentalists. To address the challenge that iridophore form changes is a wide open question, while being sensitive to not overfitting our model, we determined cell interaction rules based on wild-type, *pfeffer*, *nacre*, *shady*, *pfeffer/nacre*, *shady/nacre*, and *nacre/pfeffer*. Our approach was to put forward one set of wild-type cell interactions that could cohesively reproduce wild-type development, measurements of melanophore speed and cell-cell distances, and these early-stage

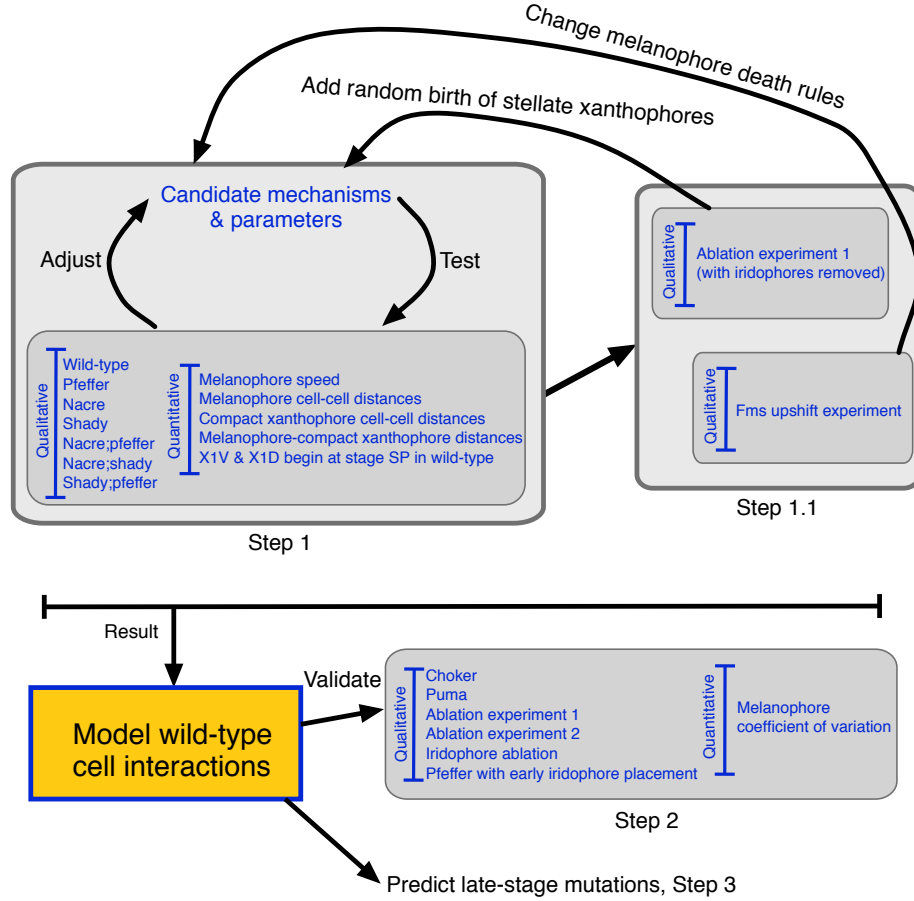


Figure 4.6: Flowchart showing modeling approach and output. The majority of work went into Step 1, which was repeated many times to account for the particularly open nature of the problem under consideration. This is illustrated best in comparison with our two-cell model in Chapter 3: there we specified rules of short-range activation and long-range inhibition, motivated by [54], between melanophores and xanthophores, and the modeling challenges for specifying cell birth and death were limited to determining the length scales and parameters (proportionality constants) to use in these rules. In contrast, when iridophores and additional cell forms are introduced, many of the rules themselves are unknown. Thus, we had to identify both mechanisms to govern cell form changes and the parameters within these rules; because of this, we focused largely on obtaining qualitative fits. At one point (Step 1.1) in Step 1, we tested the model on an approximation of an ablation experiment in [89], and this motivated the inclusion of a small amount of random birth of stellate xanthophores in our model. Additionally, simulating an *fms* temperature-upshift experiment from [61] led us to reframe our rules for melanophore death. After this side-step to Step 1.1, we continued to fine-tune in Step 1. The result is a model of wild-type cell interactions that is consistent with the experimental set-ups and quantitative measurements in the flowchart.

mutations without any changes or adjustments to parameters.

While many combinations of parameters and cell interactions are able to reproduce wild-type development or any single early-stage mutation, capturing these

dynamics with a single set of rules was challenging. Once a rough candidate set of interactions was identified, an ablation experiment [89] and a temperature-adjustment [61] experiment were used to test the model and motivated two minor changes (in particular, the ablation experiment led us to include some random stellate/neutral xanthophore differentiation, and experiments with a temperature-sensitive *fms*/*pfeffer* mutation informed our melanophore survival rules). After this initial test, our focus returned to wild-type and early-stage mutations, now with the goal of accounting for the finer details of these patterns and quantitative measurements. Many rounds of modeling were needed to produce wild-type and early-stage mutations reliably; once a reasonable model was produced, we then worked backward, removing assumptions to minimize the model; this resulted in further rounds of modeling.

The result is our final model of cell interactions (see Figure 4.46), which we then test on new cases, including *choker*, *puma*, *pfeffer* with earlier iridophore appearance, additional ablation experiments, and the quantitative measurement melanophore CV. We stress that for wild-type, early-stage mutations, middle-stage mutations (with the exception described above), and ablation/temperature experiments, all results shown are simulated using the same set of cell interactions and model parameters (see Figure 4.7). The only change made to simulate *pfeffer*, for example, is to turn off xanthophore birth. Thus, our approach to the scarcity of data on iridophores without overfitting was to put forward one set of model rules that is consistent with many different dynamics without any changes. While our model is not a proof and certainly is not unique, by accounting for a wide range of dynamics on the fish skin without alterations to cell interactions, we are confident it captures some biological truth and can be used to make predictions (§4.5.4).

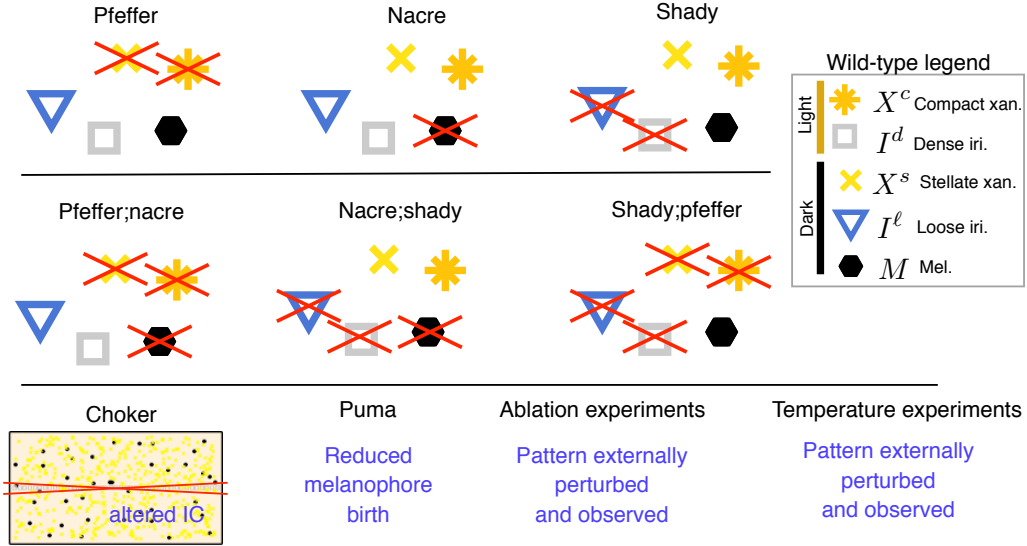


Figure 4.7: Summary of simulation conditions for early-stage mutations, middle-stage mutations (choker and puma), and experiments. These dynamics are accounted for using the same set of cell interactions, and the only changes are described pictorially: early-stage mutations lack pigment cells and are thus simulated by turning off birth of the cells impacted; the choker mutation arises due to an altered initial condition; the puma mutation reduces melanophore birth; and ablation and temperature experiments are simulated by externally perturbing the pattern or altering growth rates.

4.4.2 Wild-type development

At ≈ 20 dpf, adult patterning begins with the appearance of a light strip of dense iridophores at the center of the fish, along the horizontal myoseptum [34, 75]. Stellate/neutral (or larval) xanthophores are already spread across the body at this point [45]. Thus, for wild-type and mutations, we simulate development from stage PB (≈ 21 dpf), a natural starting point marking when adult pattern formation begins on zebrafish. Our initial condition approximates this set-up: we specify a uniform random distribution of melanophores and stellate/neutral xanthophores across the domain and a single stripe of iridophores (one cell wide, cells $100 \mu\text{m}$ apart) at the center of the fish skin domain (each initial condition is random; one example set-up is compared with experimental images and schematics in Figure 4.8).

We quantify qualitative success in our wild-type simulations according to two

measures. First, we consider a candidate model successful if it produces timelines of development that match up with several key times in zebrafish patterning. In particular, we aim to produce simulations in which interstripes X1V and X1D begin to form at stage SP (≈ 39 -44 dpf in our model), with dense iridophores first emerging from black stripes and stellate xanthophores becoming compact in response; stripes 2V and 2D, in turn, should appear faint at stage J (beginning at ≈ 51 dpf in our model) and be fully formed by J+ (beginning at ≈ 70 dpf; these points are chosen based on our approximations from [17, 58, 74], noting that, as discussed in Chapter 2, matching days and stages is not an exact science. Our second measurement of success is concerned with bulk simulations: we want a model that produces a low number of simulation runs leading to breaks/interruptions of yellow interstripes. Notably, wild-type patterning occasionally produces breaks or interruptions in dark stripes, particularly later dark stripes, so we do not consider infrequent breaks in black regions a model failure. Light interstripes, in contrast, seem to be largely uninterrupted, so we focus on obtaining a low percentage of breaks in yellow regions. Additionally, we collect several quantitative measures, namely distances between cells, cell velocity, stripe width, and melanophore coefficient of variation (see §4.4.4 for a quantitative evaluation of wild-type development).

Two representative simulations of wild-type development are shown in Figures 4.9 and 4.10; additionally, we provide the first 21 random simulations in Figure 4.11 to give a more complete view of the variance in our wild-type results. Based on 100 simulations, we find that 11% feature small breaks in yellow interstripes (e.g. a bridge connecting to black stripes): in 4% this break occurs in X1V, in 6% it is present in X1D, and in one simulation we find a break in X0. We consider this an 89% success rate in wild-type stripe development according to our second measurement of success (quantitative measurements are discussed in §4.4.4).

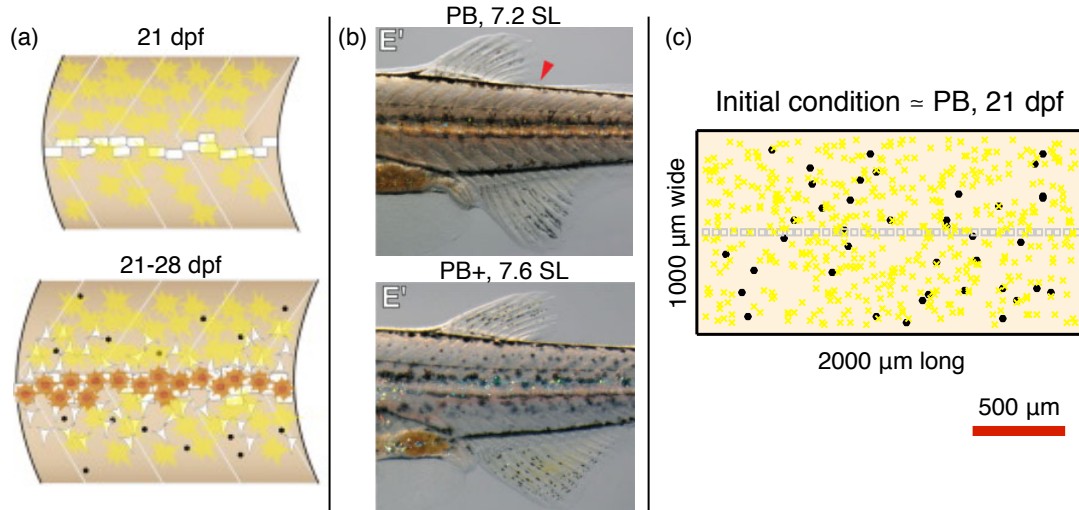


Figure 4.8: Adult skin patterning on zebrafish begins with the appearance of dense iridophores along the horizontal myoseptum at roughly 3 weeks post fertilization [74, 75]. Stellate/neutral (or larval) xanthophores already fill the body trunk [45]. (a) Schematic reproduced from Singh and Nüsslein-Volhard [74] and (b) images of wild-type development [58] motivate the (c) initial condition we use for development simulations. Images in (a) are reproduced from Singh and Nüsslein-Volhard [74] with permission from Elsevier. Images in (b) are reproduced from Parichy *et al.* [58] with permission from John Wiley & Sons, Inc.

Spreading iridophores align stripes horizontally

As shown in Figures 4.9 and 4.10, patterns form by the sequential appearance of stripes and interstripes, with dense iridophores initializing each new interstripe and stellate xanthophores becoming compact in response. In contrast to our two-cell model in Chapter 3, no additional interstripes need to be introduced into the domain indirectly: instead, the dynamics are entirely cell-autonomous. The outward spreading of iridophores through division and movement and associated form changes between dense and loose directs further stripe formation. These results are interesting when viewed in relationship to our two-cell model simulations of wild-type development (see Figure 5.8); there we observed that early random birth of pigment cells away from the developing pattern could hijack stripe formation. In contrast, here the spreading iridophores control the timeline of wild-type development: xanthophores respond locally to the spreading iridophores to form interstripes and melanophores react at long-range, tightly controlling cell differentiation and en-

Stage	SSL [58]	Start day (dpf)	Domain length (μm)	Domain width (μm)	Target milestone (during stage)
PB	7.5	21	2000	1000	Faint strip of iridophores at horizontal myoseptum [58]
PR	8.5-8.7	30-32	2333-2400	1243-1297	Iridophores spread outward from X0 in loose form [17, 75]; 1D and 1V are discernible
SP	9.5-9.6	39-40	2667-2700	1486-1513	Dense iridophores in X1V and X1D [17]
SA	10.1-10.4	44-47	2867-2967	1621-1702	X1V and X1D forming
J	10.9-11.7	51-58	3133-3400	1810-1999	2V and 2D faint
J+	13.0	70	3833	2323	2V and 2D forming
J++	16.0	96	4833	3025	(Only simulated to this point in shady mutation)

Table 4.2: Stages of development associated to the timeline of our simulations and target pattern milestones we identify and strive to meet (approximations based largely on images in [17, 58]). Standard length (SL) is a measurement of the fish from its snout to the beginning of its caudal fin; standardized standard length (SSL) is a version of SL based on representative zebrafish [58]. Note that we consider the full width of the zebrafish and roughly a third of its length (excluding a region around the eye)

sureing that the bulk of cell birth occurs sequentially across the domain. This allows the existing horizontal pattern to guide the alignment of each new stripe or inter-stripe.

We note that our model produces a large amount of melanophore birth in the positions of prospective stripes 1V and 1D before loose iridophores have spread across these regions, suggesting melanophore birth does not depend on loose iridophores. Our view of the experimental literature [74, 75] is that it is currently unclear what comes first (and possibly drives the other), either the appearance of

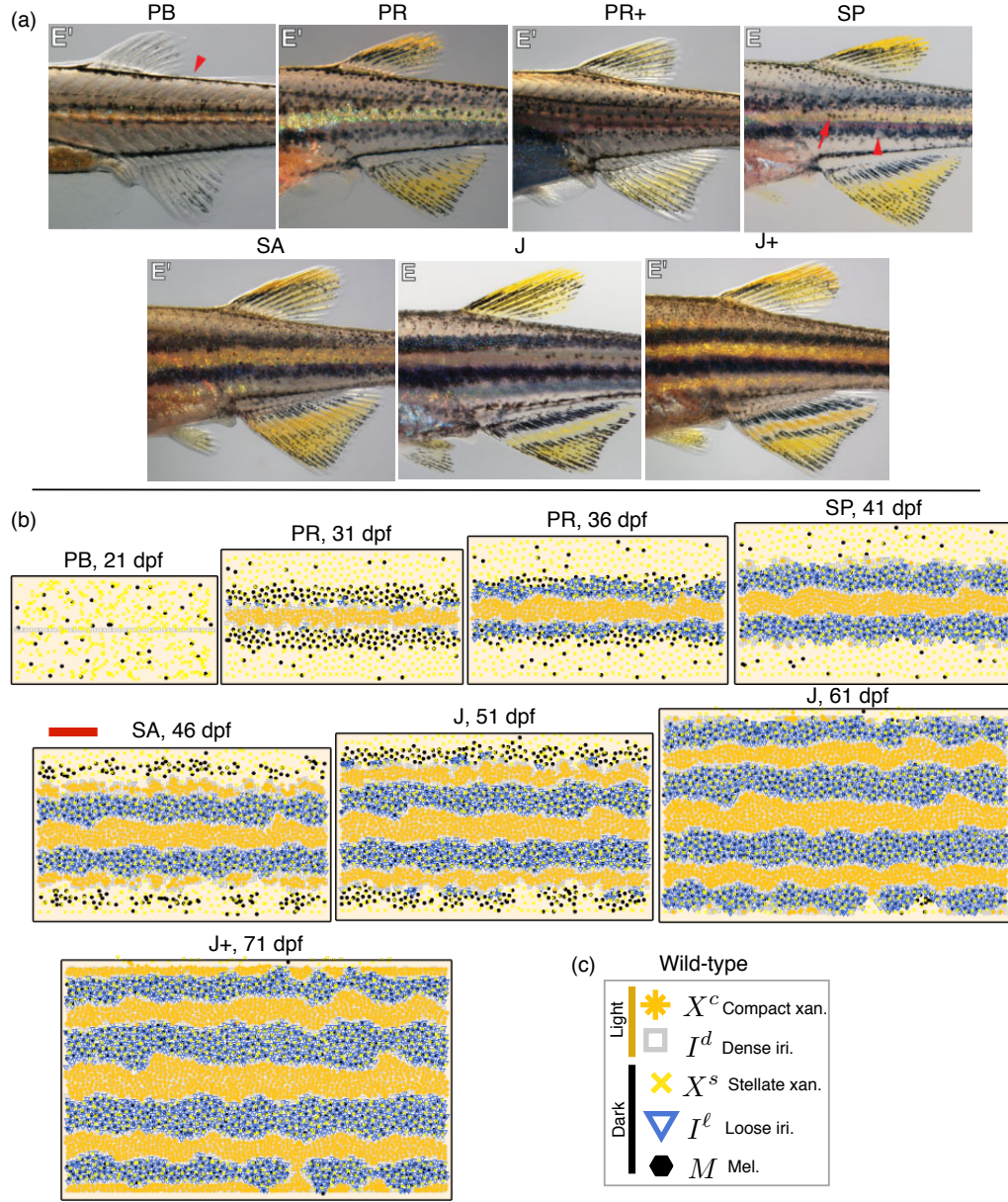


Figure 4.9: Wild-type development. It is useful to compare these images with the schematic in Figure 4.1: as described there, wild-type patterning is driven by iridophores that spread across the fish skin from the horizontal myoseptum (center line) [75]. These cells, initially dense, change their form to loose (blue) to mark black stripes and transition back to dense (silver) to begin new interstripes [74, 75]. (a) Images of zebrafish at different stages reproduced from Parichy *et al.* (b) Sample simulated wild-type development (scale bar is 500 μm). Stripes and interstripes are added sequentially outward from the horizontal myoseptum [17, 75]. PB, PR, SP, and J+ refer to developmental stages described in §2.1.2. (c) Guide to cell organization in light and dark regions. Images in (a) are reproduced from Parichy *et al.* [58] with permission from John Wiley & Sons, Inc.

adult melanophores in 1V and 1D or the spreading of loose iridophores across the future locations of these stripes. To test the alternative, we are currently working

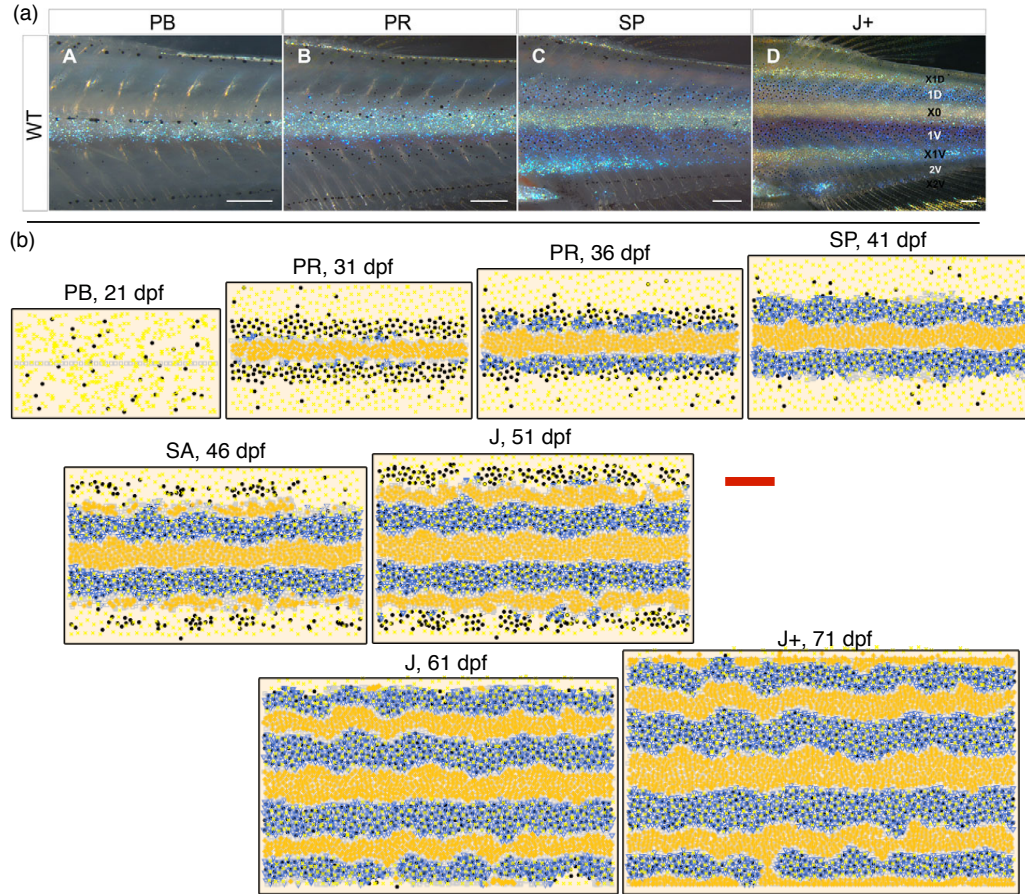


Figure 4.10: As a comparison, we include a second timeline of wild-type development, as one of the challenges throughout this work was the relatively small number of images and sparse sampling of time-points available for zebrafish development. (a) Timeline of patterning on a single fish (scale bar is 250 μm) is reproduced from Frohnhofer et al. [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd. (b) Simulated wild-type patterning (scale bar is 500 μm).

on adjusting our model to slow melanophore birth, but at this time our results with these adjustments (not shown) do not reliably account for wild-type development and early-stage mutations.

4.4.3 Early-stage mutations

As discussed in §4.4.1, we refer to early-stage mutations as those in which one or more type(s) of adult pigment cells fail to develop [35]. These phenotypes are nacre

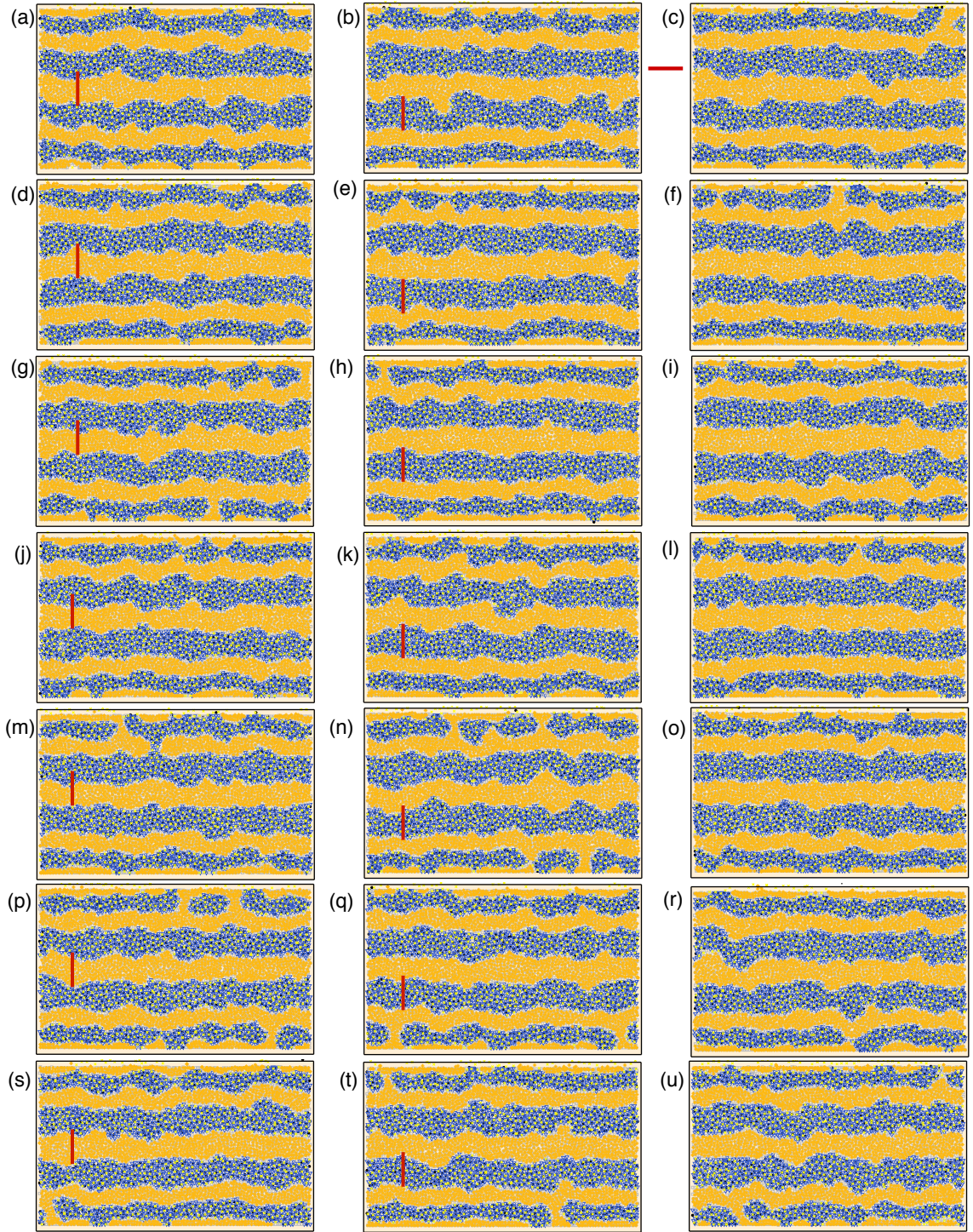


Figure 4.11: To give a sense of the variability in our wild-type simulations, we include example wild-type patterns at stage J+ (roughly 71 dpf). Vertical scale bars ($500 \mu\text{m}$) are provided to visualize pattern width.

(no melanophores), pfeffer (no xanthophores), shady (no iridophores), and cross mutations (pfeffer;nacre, nacre;shady, and pfeffer;shady) resulting in fish with only

one type of pigment cell on their body [17]. Experiments with chimeric zebrafish combining mutated and wild-type cells together indicate that *nacre*, *pfeffer*, and *shady* act autonomously to melanophores, xanthophores, and iridophores, respectively [17, 43, 61]. For example, Maderspacher and Nüsslein-Volhard found that introducing wild-type melanophores into *nacre* is enough to generate stripes, suggesting that it is simply the lack of melanophores that causes this phenotype, not inherent differences in how xanthophores behave [43]. Thus, we assume these mutated phenotypes result entirely because cells are missing; in particular, all the mutations discussed in this section are done without changing any model parameters or cell interactions with one necessary exception (that we turn off birth of the cell(s) missing from each mutation). This means that to simulate *pfeffer*, for example, we simply turn off xanthophore birth in our model (we note that the *shady* mutation involves an additional change, which we discuss when we present our results on *shady*). When viewing the next results, it is important to keep in mind that early-stage mutations are significantly more variable [17] than the dependable stripes that form on wild-type zebrafish, and the experimental images reproduced for comparison are examples of what these mutations can look like. Thus, our goal is to capture broad qualitative features of these mutations (we include a discussion of quantitative measurements in §4.4.4).

Pfeffer: As shown in Figure 4.12a, *pfeffer* (also referred to as *fms* and *pfe* [17, 43, 61]), a mutation lacking xanthophores, features messy black spots (roughly aligned horizontally) and peppered melanophores spread across a sea of compact iridophores [17]. When a high enough density of melanophores is present locally, dense iridophores become loose and blue above the black cells, but single melanophores are also found in the interstripe regions [17]. Frohnhöfer *et al.* [17] report that the stripes are roughly normal width, and interstripe X0 expands into the locations where 1V

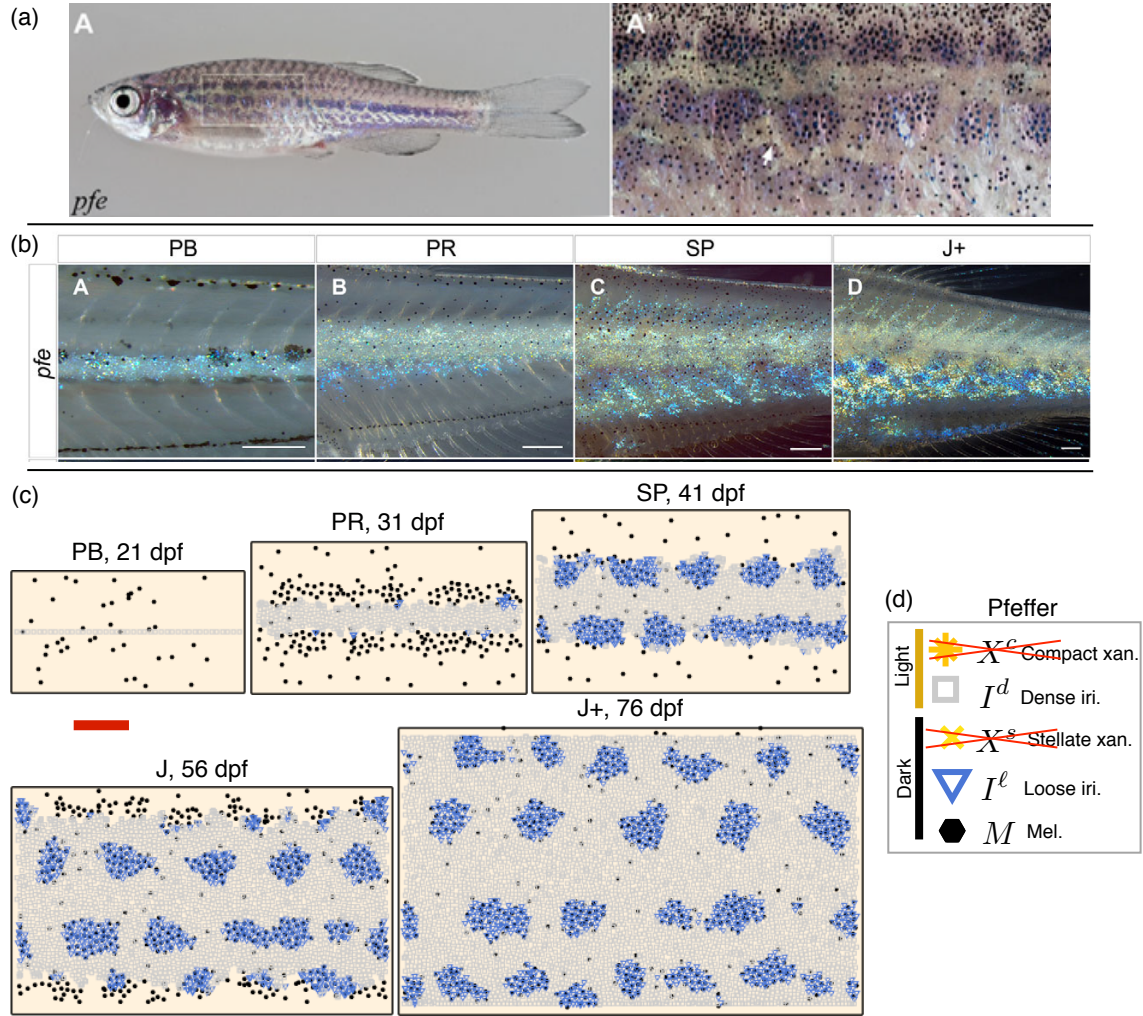


Figure 4.12: Pfeffer (pepper in German) is an early-stage mutation characterized by the absence of xanthophores [17]. The result is a messy spotted phenotype with black/blue spots roughly aligned in stripes. (a) Pfeffer zebrafish from [17]. (b) Timeline of pfeffer development. (c) Simulated pfeffer development. (d) Guide to simulation set-up. Empirical images are reproduced from Frohnhöfer *et al.* [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd.

and 1D are in wild-type. An example simulated pfeffer zebrafish is shown in Figure 4.12, and seven additional images are provided in Figure 4.16 to give a sense of the variance present in our simulations. (Note that the initial condition used for pfeffer simulations lacks stellate xanthophores, as these cells are missing in pfeffer).

According to [17], the appearance of dense iridophores along the horizontal myoseptum is earlier in some pfeffer zebrafish (occurring at stage CR, which is at

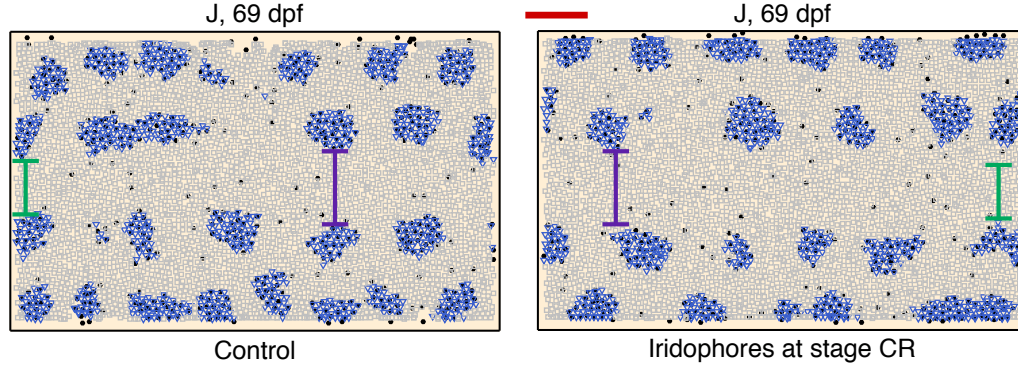


Figure 4.13: Left: control pfeffer with iridophores present in our initial condition at stage PB. Right: pfeffer with dense iridophore placement 7 days earlier at stage CR. Frohnhofer *et al.* [17] report that pfeffer has a widened X0 region when iridophores appear earlier; this may be reproduced by our simulations, but additional statistics should be computed to verify this. Green bars are the same length in both figures; longer purple bars are also the same length in both figures.

roughly 14 dpf by our approximation of the data in [58]). When this occurs, the central X0 area occupied by dense iridophores on the fish skin is larger [17]. We test this by simulating pattern formation from stage CR (in this case, informed by [58], our initial domain is $811 \mu\text{m}$ high and $1734 \mu\text{m}$ wide). Here we specify the appearance of dense iridophores at the horizontal myoseptum as our initial condition and hold melanophore differentiation off until 7 days later at 21 dpf, the normal starting point of simulations. An example result, in comparison to a control pfeffer simulation, is shown in Figure 4.13 (preliminary work: statistics need to be computed to determine if we obtain wider stripes in general).

Nacre: Next we turn to simulating nacre (nac), a phenotype lacking melanophores [17]. This zebrafish features an expanded X0 interstripe with rough borders, and compact xanthophores, associated with dense iridophores, are separated from regions of loose blue iridophores and stellate xanthophores [17, 44]. In the example image in Figure 4.14a, the dorsal side (top half) of the fish seems to be covered in compact xanthophores above dense iridophores, while the ventral side contains messy, elongated spots of orange against the surrounding blue iridophore background. These orange patches appear in this loose iridophore background at stage J [17]. An

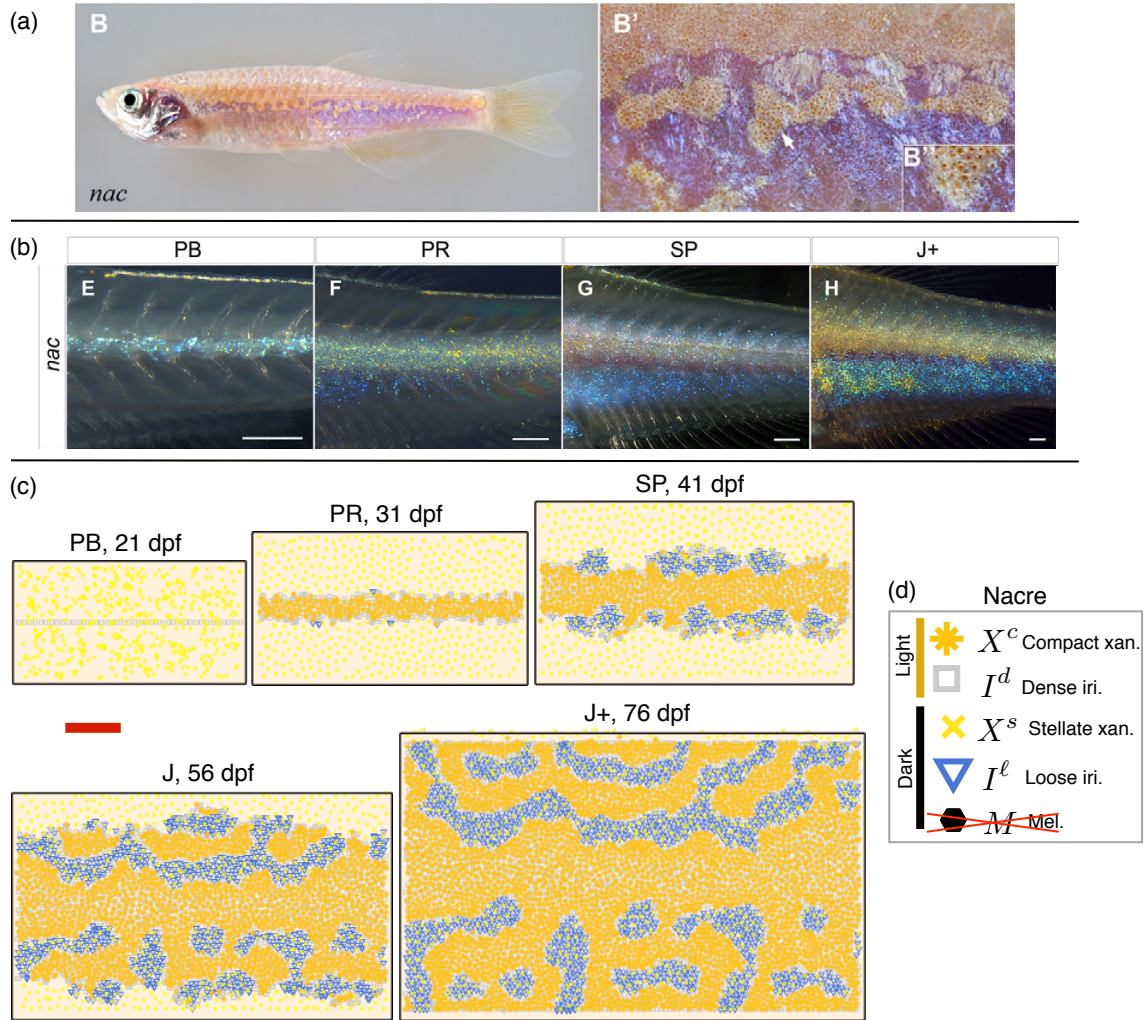


Figure 4.14: Nacre is an early-stage mutation featuring no melanophores [17]. (a) Nacre fish. (b) Timeline of nacre development. (c) Simulated nacre development. (d) Guide to simulation set-up. Empirical images are reproduced from Frohnhöfer *et al.* [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd.

example simulated nacre mutation is shown in Figure 4.14c, in comparison to the timeline of nacre development reproduced from [17] in Figure 4.14b. To give a sense of the variability of patterns produced by our model, we also provide 7 randomly selected example images in Figure 4.16.

Our nacre represents a compromise between the different patterns on the dorsal and ventral sides of the nacre fish in Figure 4.14a. We see an expanded X^0 region with messy borders, with a combination of orange and blue spots away from the

horizontal myoseptum. Note that, because we do not make any distinction between ventral and dorsal dynamics in terms of domain growth or cell interactions, we cannot account for differences in these two regions. This is a simplifying assumption, and our simulations expectedly produce the same kind of patterns in the top and bottom portions of the domain (this is also visible in our wild-type patterns: while 2V and 2D appear at slightly different times [58] in wild-type, we make no effort to reproduce this difference in timing between dorsal and ventral patterning).

Shady: The shady mutation is extremely variable and depends on the strength of the mutation. Notably, while shady is characterized by clean black spots of melanophores across an expanded xanthophore background, 20% of shady fish have a random distribution of melanophores instead of spots ventrally [17]. The black spots often seem to be arranged in strips dorsal and ventral to the X0 region, and, compared to pfeffer, there are even fewer melanophores present on the skin (20%-30% of the number on pfeffer) [17]. Because shady lacks iridophores, we simulate this mutation without the strip of dense iridophores in our initial condition. Without these dense iridophores, there is no reason for xanthophores to become compact in X0; nevertheless, Frohnhofer *et al.* describe xanthophores appearing in X0 at a delayed time. Thus, we introduce one additional change (mentioned earlier) to our shady fish: at 22 dpf, we place a strip of compact xanthophores in the center of the domain (note: we could do the same across all our simulations and it would not impact the results, so the same model can reproduce the dynamics discussed in this chapter without changes). A representative timeline of simulated shady development is shown in Figure 4.15, and additional examples are shown in Figure 4.16.

Crosses of early-stage mutations: Crosses of early-stage mutations result in fish that feature only one main type of cell: iridophores (nacre;pfeffer), melanophores (shady;pfeffer), or xanthophores (nacre;shady). Nacre;pfeffer features dense irido-

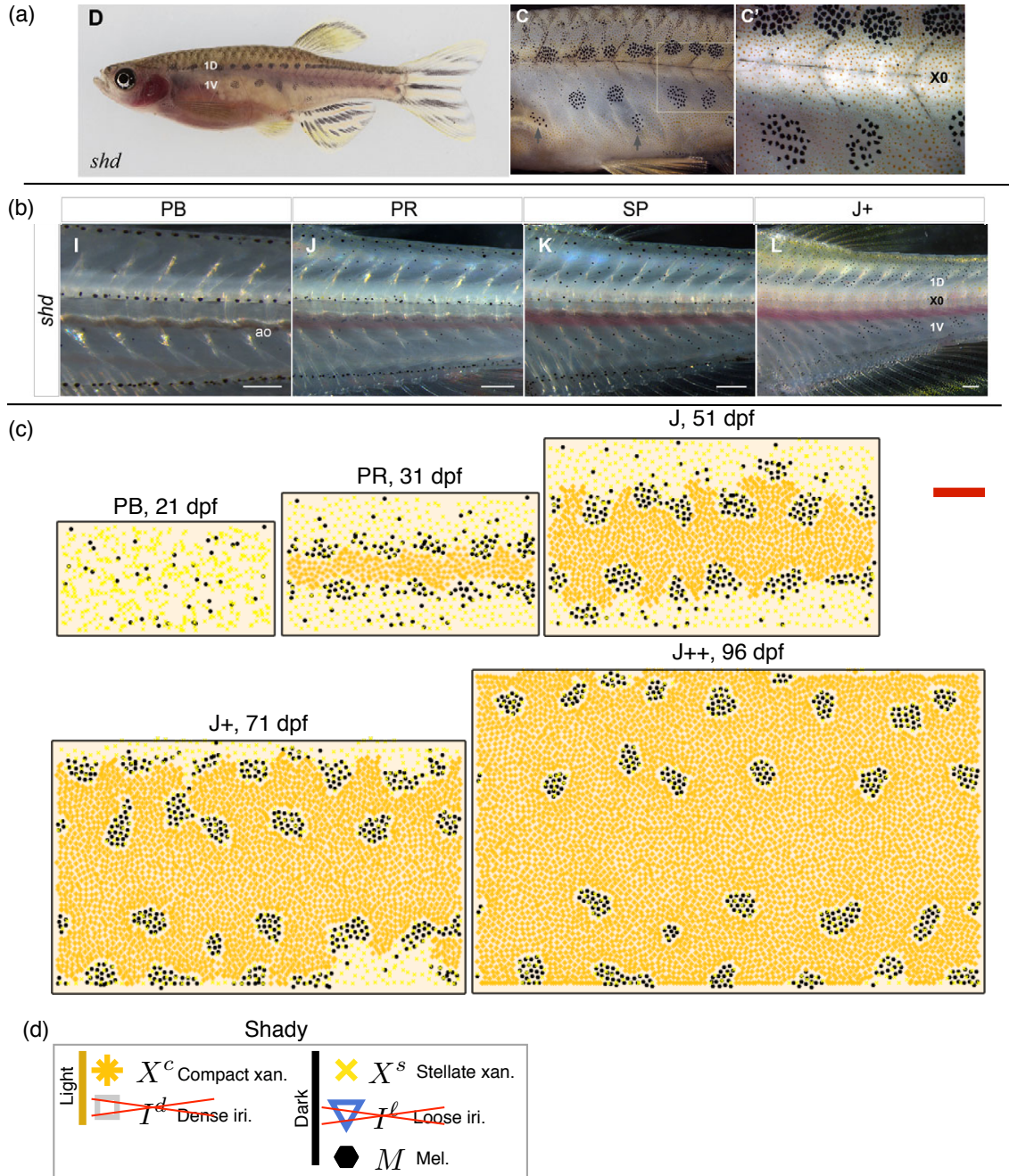


Figure 4.15: Shady is an early-stage mutation lacking iridophores, and its phenotype is particularly variable [17]. It provides strong evidence that iridophores are necessary for wild-type stripe formation on the body, as fish lacking these cells form spotted patterns [17]. (a) Shady fish. (b) Timeline of shady development. (c) Simulated shady development. (d) Guide to simulation setup. Empirical images are reproduced from Frohnhofer *et al.* [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd.

phores across the body, and *shady;pfeffer* is characterized by dispersed melanophores [17]. Example simulations of these crosses, in relationship to experimental images

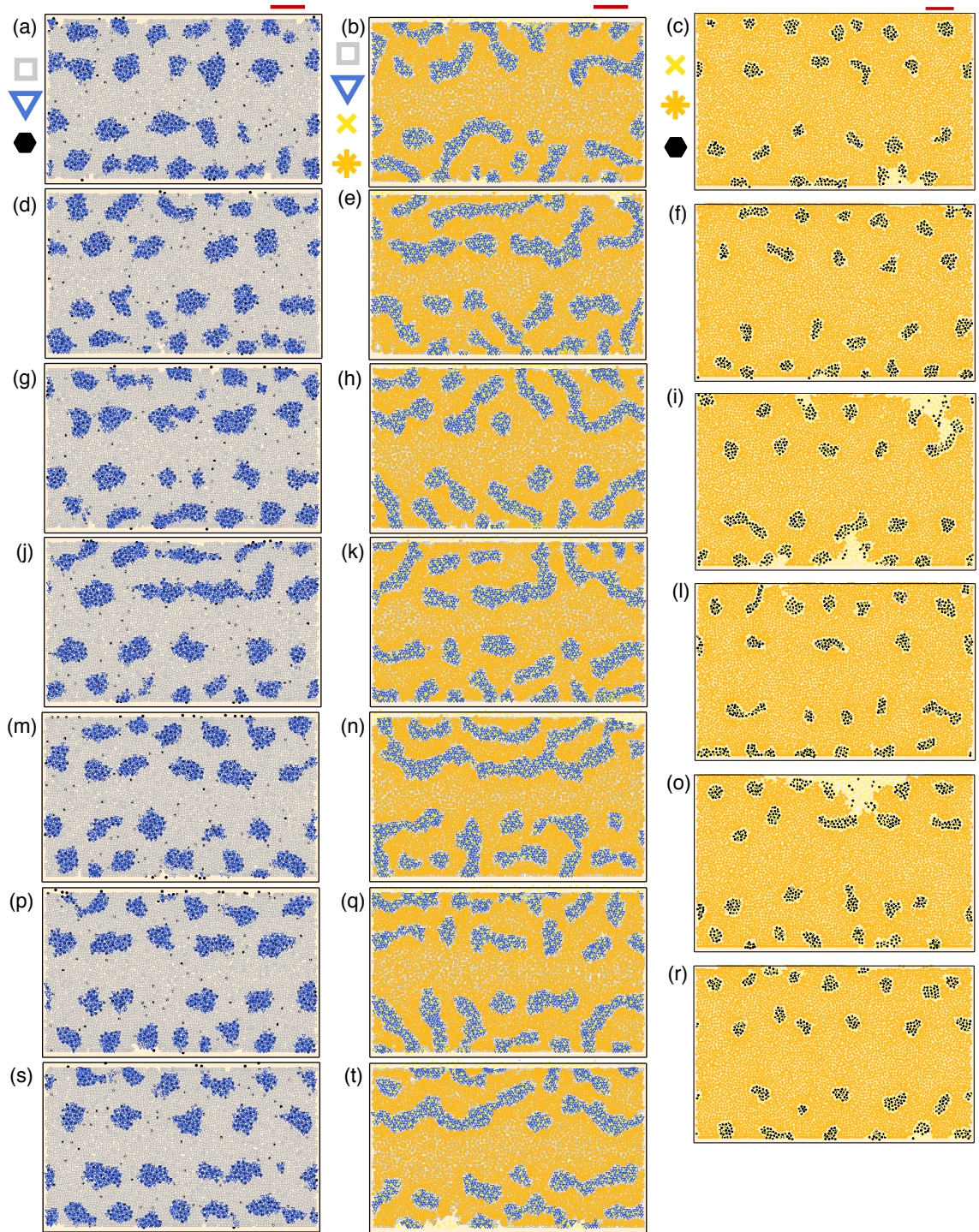


Figure 4.16: Additional sample simulations of pfeffer, nacre, and shady to show the variability produced by our model. Pfeffer and nacre are shown at J+ (roughly 71 dpf). Shady is shown at J++ (96 dpf).

reproduced from [17], are shown in Figure 4.17. Note that we simulate nacre;pfeffer by turning off melanophore and xanthophore death, but both forms of iridophores

are allowed, so the model self-selects iridophores in the dense form. The remaining cross mutation (nacre;shady) is described in [17] as featuring a uniform dense layer of xanthophores, but we are aware of no images of this fish in the biological literature (see §4.5.2 for further discussion). Thus, we model nacre;shady by turning off melanophore and iridophore birth, and, similar to shady, we introduce a strip of compact xanthophores in the center of the domain at 22 dpf (see Figure 4.17).

Note that, if we do not introduce a strip of compact xanthophores at the horizontal myoseptum in place of the missing dense iridophores in shady and shady;pfeffer, our simulations of these mutations lack compact xanthophores (see Figure 4.18). It is possible that the variance in shady mentioned in [17] may be related to whether or not compact xanthophores appear at the horizontal myoseptum.

4.4.4 Quantitative measures

Here we present quantitative measurements collected on wild-type and early-stage mutations (note that because iridophores are largely an open question biologically, the focus of our work was mainly qualitative). Measurements of cell-to-cell distances, where available, shaped our model, as did melanophore speeds and pattern widths. As described in Figure 4.6, our approach was to fit our model to wild-type, early-stage mutations, and these three quantitative measurements in wild-type. Melanophore CV, a local measurement of pattern quality [62] also used in Chapter 3, was reserved for final model validation. In all figures presented, wild-type statistics are based on 100 simulations, nacre and pfeffer statistics are based on 80 simulations, and shady statistics are computed using 50 simulations.

The cell-to-cell distances produced by our model, in comparison to empirical

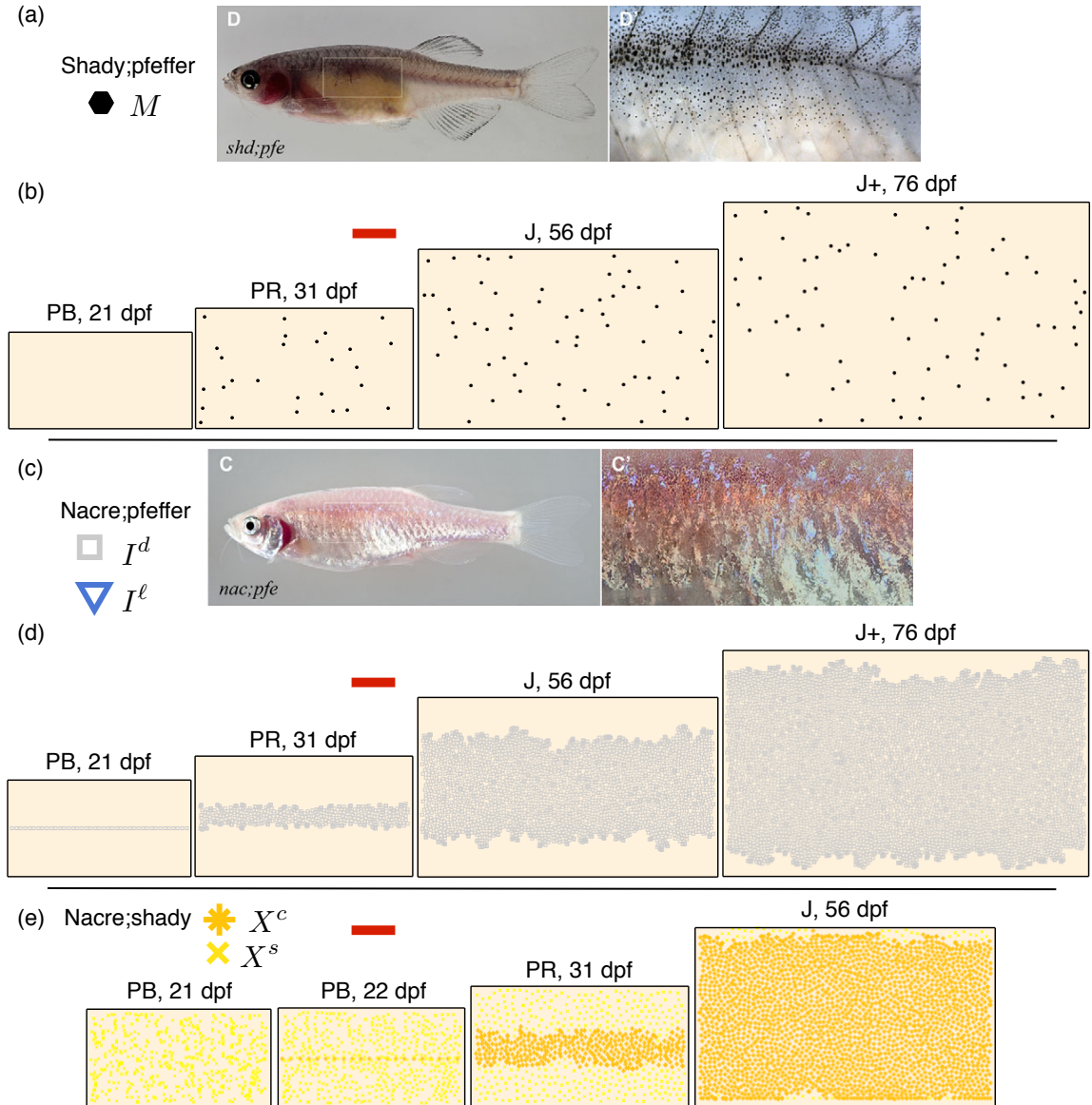


Figure 4.17: Cross mutations between nacre, pfeffer, and shady result in phenotypes featuring only one of the 3 main cell types: melanophores, xanthophores, and iridophores [17]. (a-b) Empirical image of *shady;pfeffer* (only melanophores) and simulated development. (c-d) Empirical image of *nacre;pfeffer* (only iridophores) and simulated development. (e) Simulated development of *nacre;shady* (only xanthophores; experimental image not available). Note that our *shady;pfeffer* features uniform melanophore distributions, instead of heavier density near the horizontal myoseptum, because we introduced no preference for location of melanophore precursors into our model. One way to account for the appearance of more melanophores near the horizontal myoseptum in *shady;pfeffer* would be to use a normal distribution (instead of a uniform distribution) for selecting random locations for black cell birth. Fish images (a) and (c) are reproduced from Frohnhöfer *et al.* [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd.

measurements [76] are shown in Figure 4.19 (not all cell-to-cell distances have been measured on the fish skin; we only include simulated measurements when there is

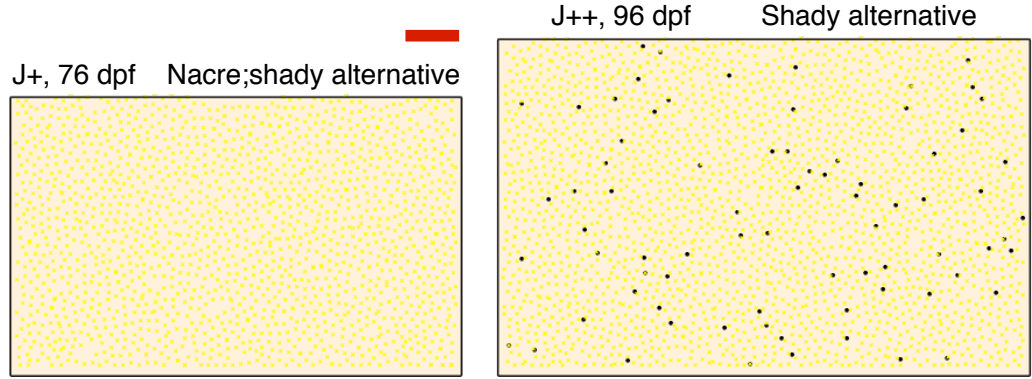


Figure 4.18: Frohnhofer *et al.* describe xanthophores as appearing at the horizontal myoseptum at a delayed time in the shady mutation [17]. As an alternative set-up, if we do not introduce a strip of compact xanthophores in place of dense iridophores in the center of the fish when iridophores are not present, nacre;shady is made up entirely of stellate xanthophores and shady fails to produce any black spots. Note that we could include the strip of compact xanthophores in all of our simulations (not just shady and nacre;shady), and it would not strongly impact our results.

experimental data available for comparison). Note that the distances reproduced from Mahalwar *et al.* [44] are not strictly for compact and stellate xanthophores, but rather for xanthophores associated with and without dense iridophores. The October 2016 work [44] showed that the xanthophores in shady are in an intermediate form, not quite compact or stellate (in contrast, nacre features compact xanthophores atop dense iridophores). In particular, shady xanthophores are more spread out than compact xanthophores in wild-type. As shown in Figure 4.19, our model produces xanthophores that are slightly more spread out in shady (grey bars) than in wild-type, capturing features of the new work [44].

Zebrafish stripes and interstripes grow with age (an observation we made from [58]); stripes on adult fish are roughly $600 \mu\text{m}$ wide [25], while adult interstripes are on the order of $400 \mu\text{m}$ wide [15] (adult is the stage after J++ [58]). As discussed in §3.4.2, we can roughly quantify stripe widths using the distribution of distances from each melanophore to its nearest xanthophore on the domain (here we focus on stripe widths at stage J+, 51 dpf). This is done for each simulation by finding the closest xanthophore to each melanophore on the domain, binning these distances in

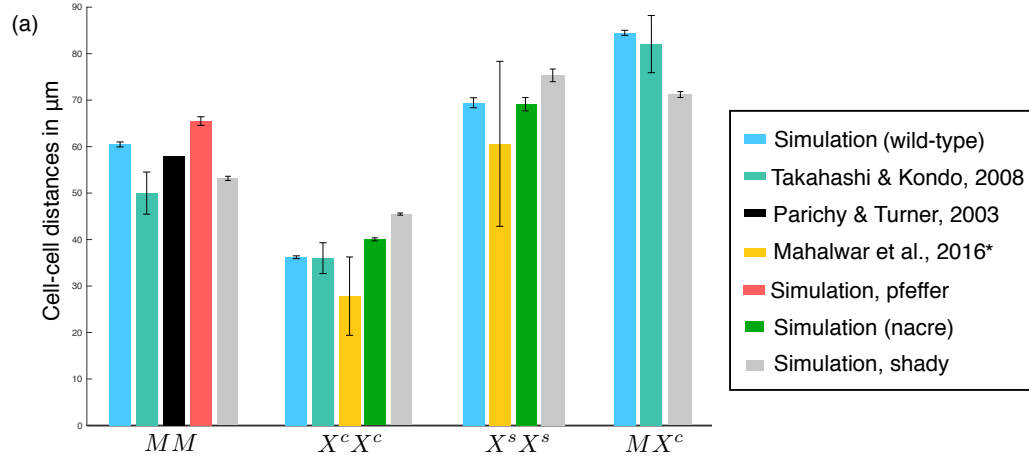


Figure 4.19: Simulated distances are reported at J+ (71 dpf) in wild-type, nacre, and pfeffer, and at J++ (96 dpf) in shady. Empirical measurements are from [44, 62, 76]. Note that the distances reproduced from Mahalwar *et al.* [44] are for xanthophores with and without iridophores (not strictly xanthophores in compact and stellate form, as presented here).

15 μm wide bins, and scaling by the number of melanophores present to produce a distribution. The average of these distributions across 100 simulations is shown in Figure 4.20a. If instead we calculate the distance from each xanthophore to its nearest melanophore, the result is related to interstripe width (Figure 4.20b). We caution that these distributions are not as reliable as those in §3.4.2, as the newer stripes and interstripes at the edges of our domain are much narrower than the older pattern at the center of the fish (vertical scale bars are provided in the wild-type simulations in Figure 4.11 as an alternative means of quickly gauging pattern width).

Data on melanophore speeds varies in the experimental literature. Takahashi *et al.* report melanophores moving 80-100 μm in one week during patterning *in vivo* [76]; Walderich *et al.* describe melanophores as typically differentiating from precursors near their final position and moving little. Our melanophore speeds are based on [76], and, while select black cells in our simulations do move on the order of 15 μm per day, this is not visible in bulk statistics, as shown in Figure 4.21. There we plot the average distance melanophores move per day, averaged across 100 simulations, at 5 different time points in development. Different bars distinguish between different

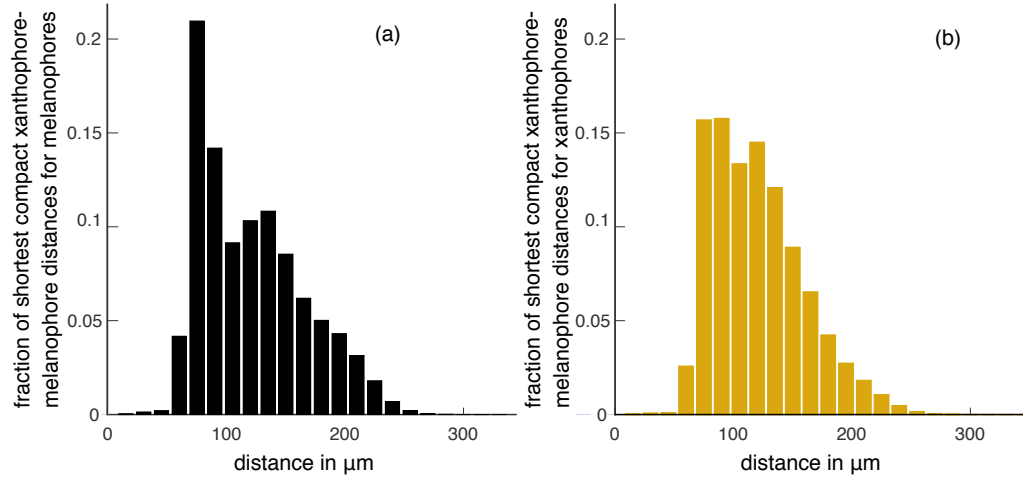


Figure 4.20: Average distributions of shortest melanophore-xanthophore distances provide information about pattern width. (a) Average distribution of shortest distance to compact xanthophores for each melanophore; (b) Average distribution of shortest distance to melanophores for each compact xanthophore. See the caption of Figure 5.10 for details of how these distributions should be interpreted in relationship to pattern width. Data is shown for wild-type patterns (100 simulations) at stage J+, 71 dpf.

local environments (the number of cells in a Δ_{xm} neighborhood surrounding the moving cell). Figure 4.21a shows how average melanophore movement during wild-type patterning varies depending on the number of melanophores nearby (for comparison, we show the corresponding measurements for pfeffer in Figure 4.21c); Figure 4.21b, in turn, shows this data depending on the number of compact xanthophores nearby.

The coefficient of variation, defined as $CV = 100 \times \text{standard deviation}/\text{mean}$, for the distance between neighboring melanophores serves as a local measurement of pattern quality [61, 62]. As discussed in §3.4.2, low CV values are associated with better formed patterns and decay during wild-type patterning from roughly 100 to 30 (based on our approximation of data in [62]). The average CV for 100 wild-type simulations is shown in Figure 4.22: our values range between roughly 85 to 10 (modeled results are more regular than biology). Parichy and Turner [61] found that CV increases as the number of xanthophores decreases; shown in Figure 4.22, our CV measurements for melanophores in pfeffer, where no xanthophores are present, capture this [61], as well as the messy nature of pfeffer described in

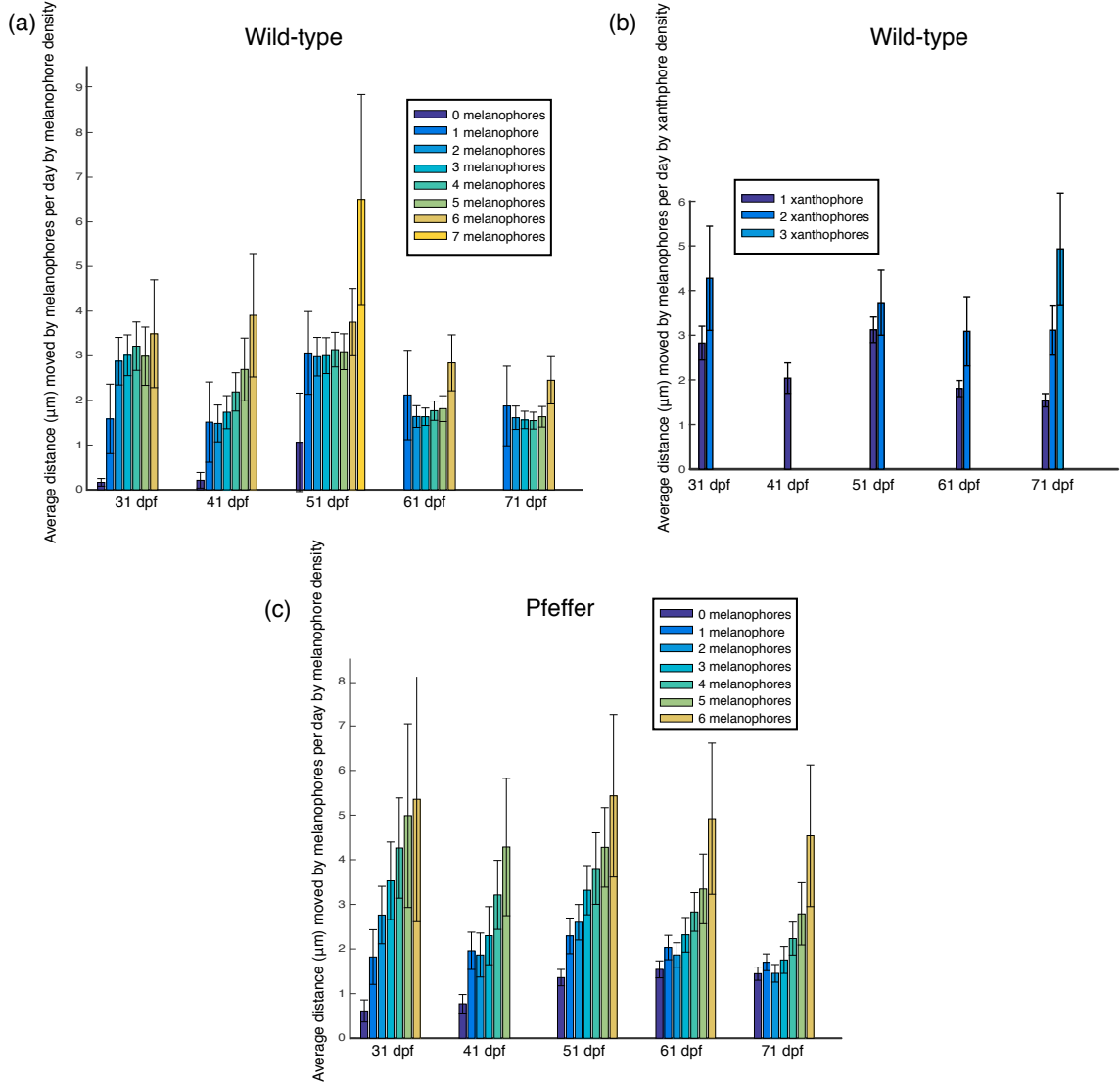


Figure 4.21: Statistics on melanophore migration (distance moved per day in μm) at 5 different sample times in development (given in dpf, days post fertilization; note our simulations begin at 21 dpf). Values are averaged for each simulation; bars provide means of these averages and standard deviation across 100 simulations for wild-type in (a-b) or 80 simulations for pfeffer in (c). Note that distance moved is due to migration alone (after the previous cell positions are stretched due to growth, we calculate the difference in position pre- and post- migration step). In comparison, [76] measured active melanophores moving roughly 80-100 μm in a week.

§4.4.3. In contrast, because shady contains xanthophores, we obtain low CV values for melanophores in shady (albeit with higher standard deviation, possibly reflecting the higher variability of shady phenotypes [17]).

Lastly, we provide averages of cell density for melanophores, compact xantho-

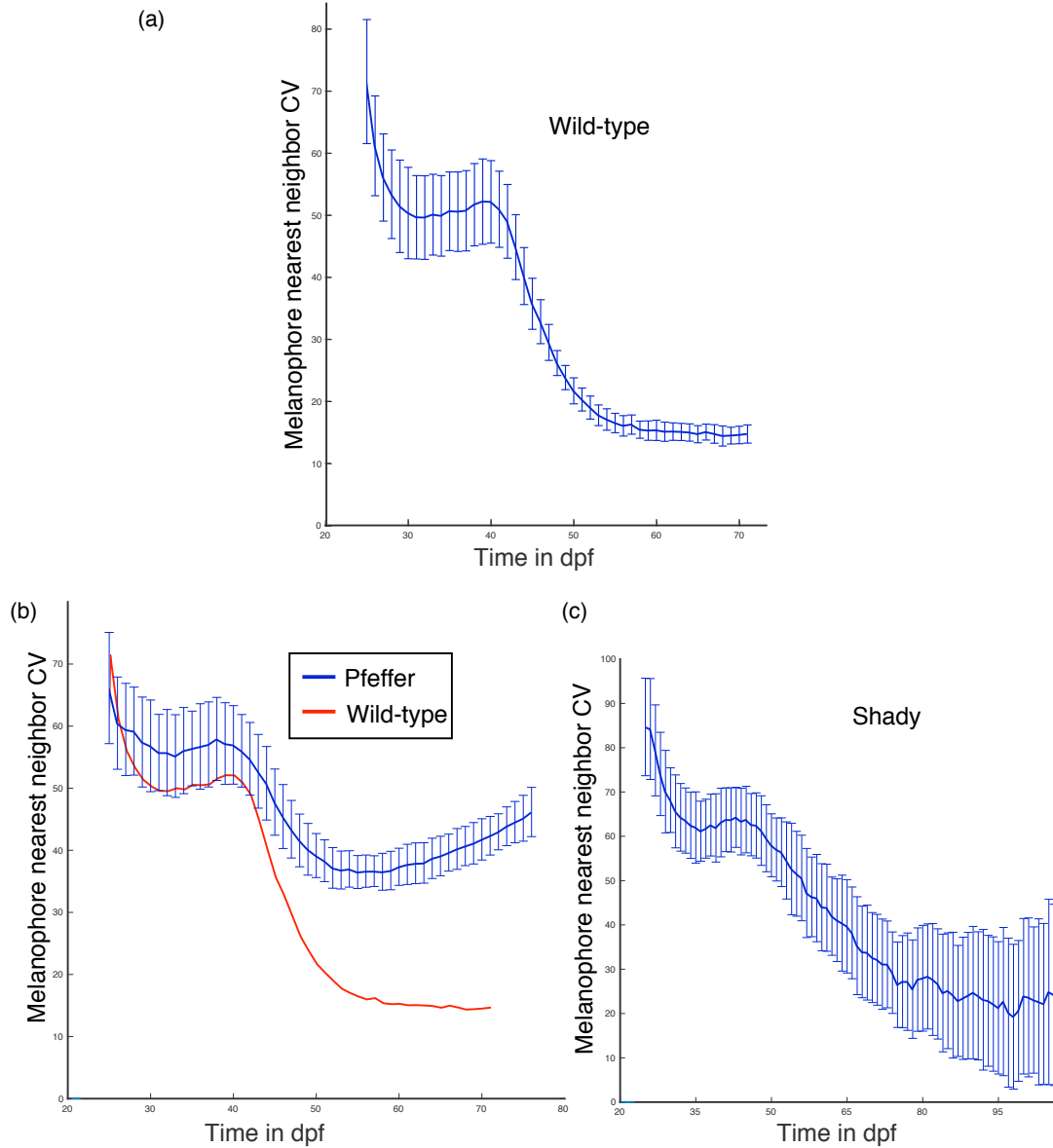


Figure 4.22: CV during (a) wild-type, (b) pfeffer, and (c) shady pattern development. The coefficient of variation (CV) for the distance between neighboring melanophores is a local measurement of pattern quality [61, 62]. It should decay in time, symbolizing better formed patterns, and ranges between roughly 100 and 30 during wild-type development (our approximation based on [62]). Shown are average values across simulations (100 for wild-type, 80 for pfeffer, and 50 for shady) with standard deviation. [61] showed CV increases with reduced xanthophores: in agreement with this, we find higher CV values for pfeffer compared to wild-type.

phores, and dense iridophores across our simulations in Figure 4.23. These heat maps are computed by calculating the local density of cells in $\Delta_{xm} = 82 \mu\text{m}$ -wide squares centered at grid points spaced $50 \mu\text{m}$ apart on our domain. Wild-type, nacre, and pfeffer densities are reported at stage J+ and shady is shown at J++.

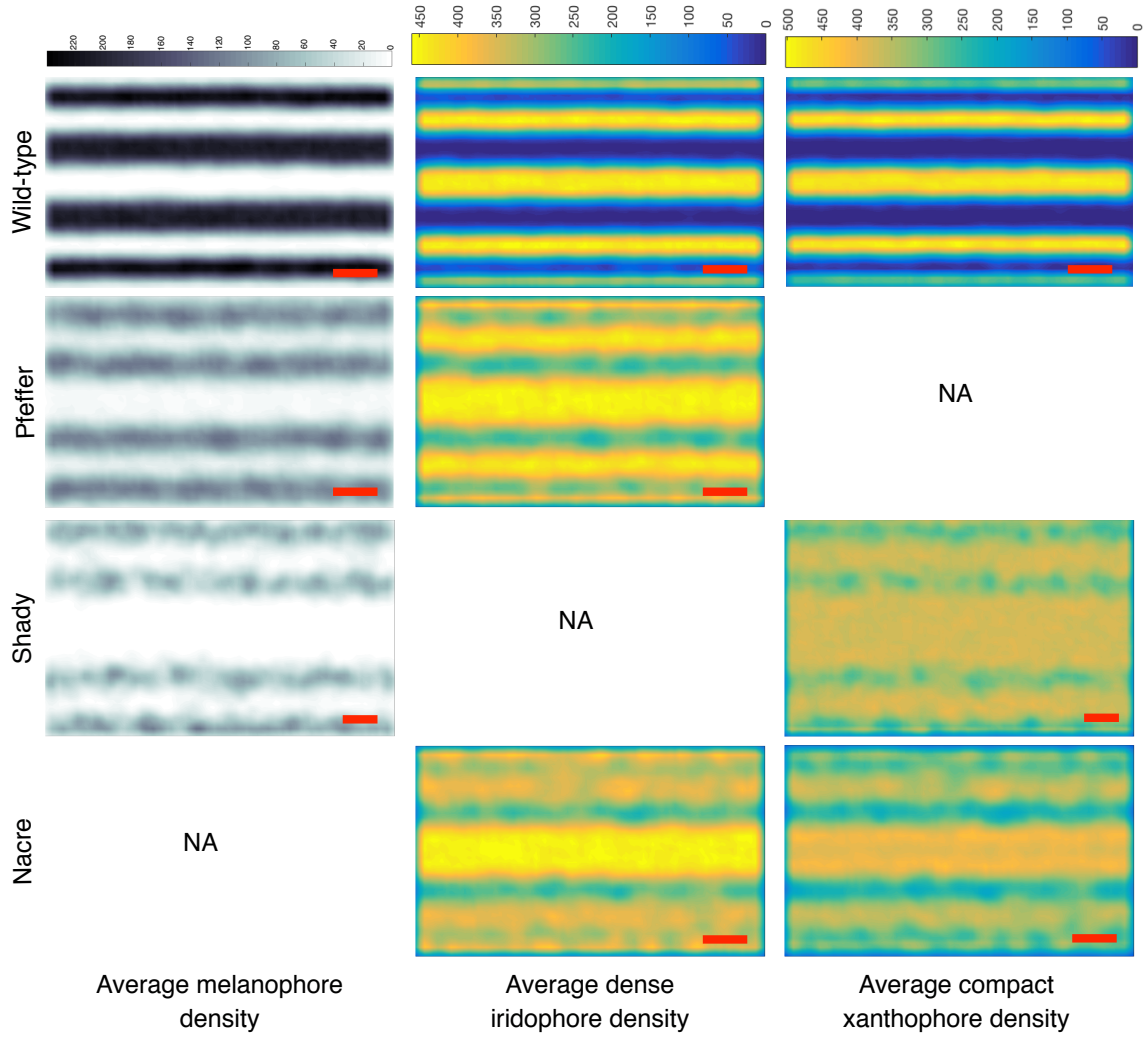


Figure 4.23: Heat maps showing average cell density for melanophores, compact xanthophores, and dense iridophores averaged across 100 simulations for wild-type, 80 for pfeffer and nacre, and 50 for shady.

As a measure of stripe quality that is as harsh as possible on patterning errors, we report the maximum melanophore densities obtained in wild-type, pfeffer, and shady in Figure 4.24: for example, we see a break in X0 there based on 1% of 100 simulations.

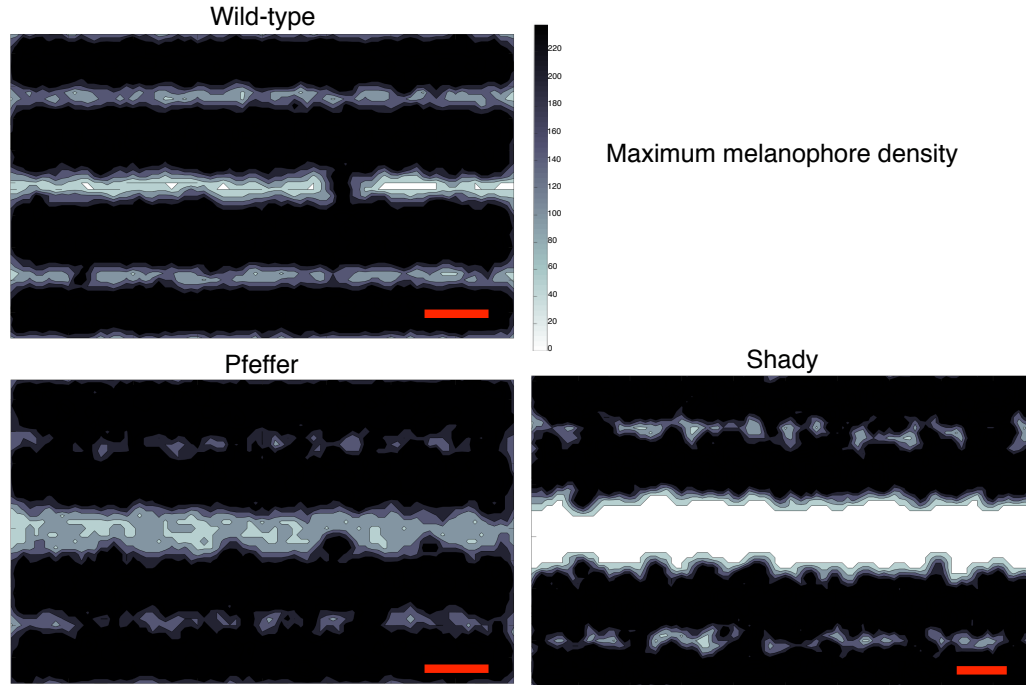


Figure 4.24: Heat map showing maximum density of melanophores across 100 simulations for wild-type, 80 for pfeffer, and 50 for shady. This is the harshest possible measure of pattern quality: in particular, the break in X0 in wild-type is the result of 1 out of 100 simulations.

4.4.5 Choker mutation

The choker zebrafish mutation features a labyrinth pattern of curves stripes with normal width, as shown in Figure 4.25 [17]. Notably, choker zebrafish lack a horizontal myoseptum and, thus, the strip of dense iridophores visible along this morphological marker at stage PB [58] is also missing [17]. The resulting altered pattern could conceivably involve altered cell interactions, but a minimal hypothesis is that it only impacts patterning indirectly by disrupting the initial condition and timing of cell differentiation. We assume the latter option here.

In detail, choker development disrupts the timing and placement of cell differentiation in two main ways: melanophores appear randomly across the zebrafish body at stage PB, but iridophores do not emerge until stage PR, and, when they do so, they arise in patches with arbitrary locations [17]. Frohnhofer *et al.* describe xanthophores

(we assume compact xanthophores) appearing within the new iridophore patches at stage PR, but they do not mention stellate/neutral xanthophores (most likely because the importance of larval xanthophores spread across the skin of zebrafish prior to patterning was a later experimental result from [45]). Thus, we simulate choker development by introducing a small uniform random distribution of dense iridophores (15 dense iridophores) to our domain at stage PR (31 dpf), and consider two possible initial conditions at stage PR (21 dpf): in the first, we assume stellate/neutral xanthophores are missing from the choker skin, so only random melanophores are present. In the second, we assume choker does not impact the initial distribution of xanthophores; in this case, our initial condition is the same as Figure 4.8 without the strip of dense iridophores. We stress that no changes are made to our wild-type parameters in any way: cell interaction rules, as well as the number of random locations evaluated for melanophore birth per day, are kept constant. The only change is the initial condition and the appearance of dense iridophores randomly at 31 dpf to simulate choker development.

Choker mutation does not impact cell interactions, only time and location of initial iridophore emergence

As shown in Figure 4.25, regardless of whether we assume choker alters the initial distribution of larval xanthophores, our model reproduces the timeline of choker development. We see compact xanthophores emerging within patches of dense iridophores by stage SP; similar to the iridophore ablation experiments in [65], melanophores appear next to and wrap around these interstripe patches. The simulated patterns at stage J+ feature distinct light and dark regions of roughly normal width but entirely lost directionality. Because these simulations were done without adjusting cell interactions, they suggest the effect of the choker mutation is limited to disrupting the initial appearance of iridophores in time and space.

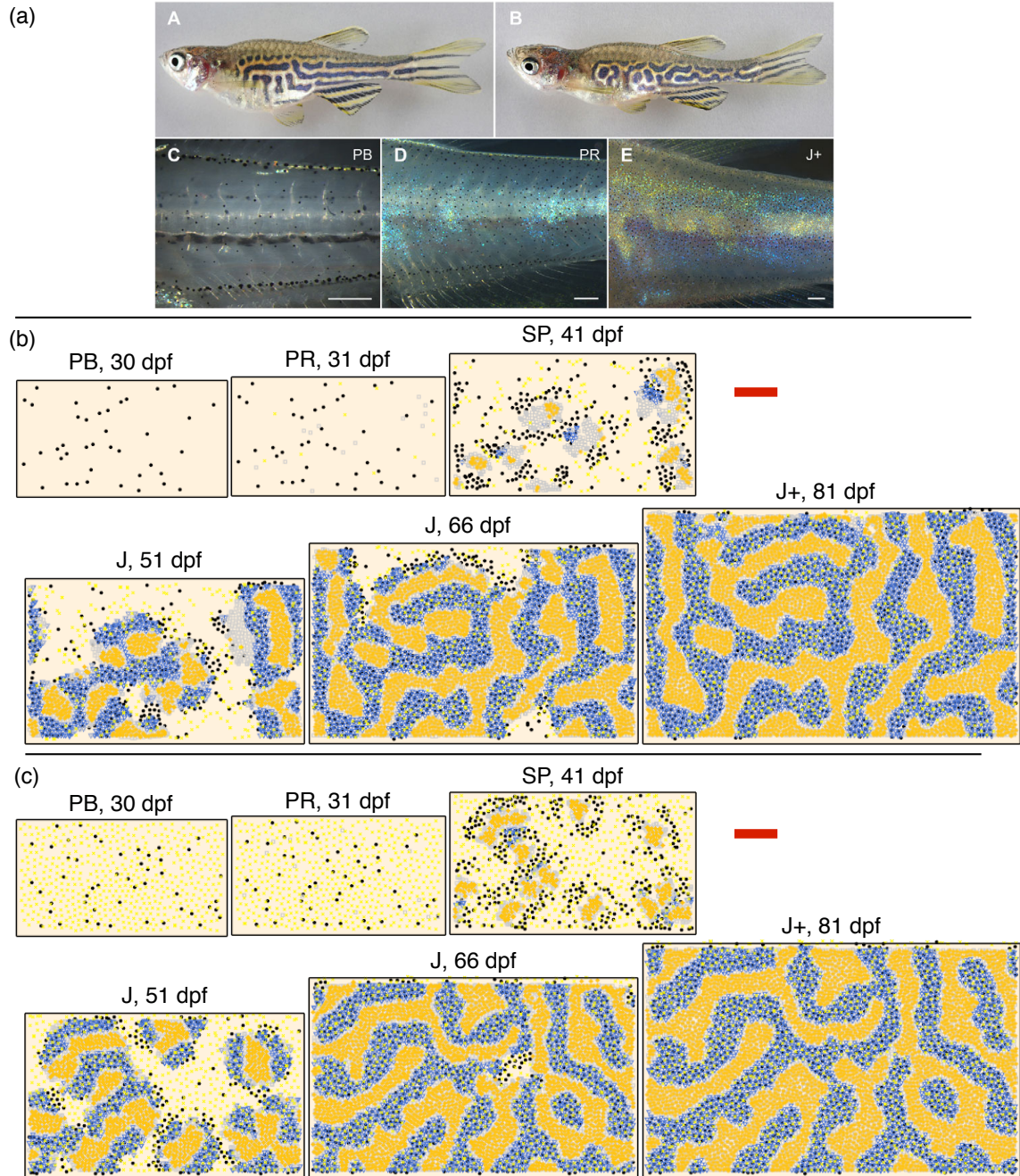


Figure 4.25: The choker zebrafish lacks a horizontal myoseptum (and the strip of dense iridophores associated with it), leading to labyrinth-style patterns [17]. (a) Example choker fish and phenotype development reproduced from Frohnhofer *et al.* [17] and licensed under CC-BY 3.0 (<http://creativecommons.org/licenses/by/3.0>); published by The Company of Biologists Ltd. (b) Simulated choker development assuming this mutation also impacts the time stellate xanthophore first appear. (c) Simulated choker development assuming no impact to stellate xanthophores. In both cases, motivated by [17], dense iridophores are not present in our larval initial condition at 21 dpf, but are instead introduced at random locations at stage PR. (Images can be compared to Figure 3.11).

Model supports the role of the horizontal myoseptum in specifying pattern directionality

By reproducing the timeline of choker development without altering cell interactions, our work provides additional evidence to [17] that the horizontal myoseptum (and associated dense iridophores) have an important role in specifying the horizontal nature of zebrafish patterns. Removing this morphological marker is sufficient to severely alter stripe alignment.

4.4.6 Puma mutation

Puma mutation does not impact cell interactions, only timing and amount of melanophore differentiation

The puma zebrafish mutation, reproduced from [63] in Figure 4.26, features messy stripes and dark patches. This phenotype involves a severe reduction in melanophore birth during patterning [62, 63]. Thus, like choker (§4.4.5), we assume the altered pattern does not involve altered cell interactions, so this mutation serves as an opportunity for model validation. As a rough approximation of the puma effects described in [62, 63], we simulate puma by simply reducing the number of random locations selected each day for possible melanophore birth (from 350, used throughout our other simulations, to 50). Biologically, this could correspond to reducing the number of melanophore precursors in the skin. An example simulated puma fish is compared with an empirical image from [63] in Figures 4.26a-b; as shown, the puma phenotype can be accounted for without changing cell interactions.

Careful interplay between cell birth and fish growth is necessary for wild-type

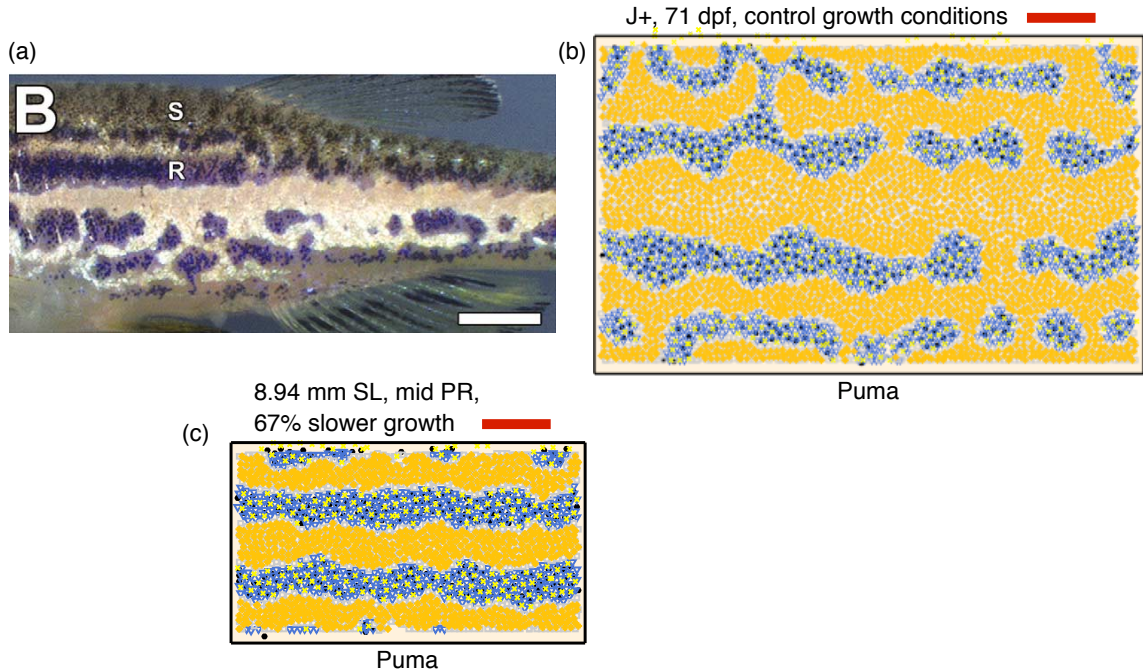


Figure 4.26: The puma mutation features heavily reduced melanophore birth [62, 63]. (a) Puma mutation; image reproduced from Parichy, Turner, and Parker [63] with permission from Elsevier. (b) Puma phenotype simulated by reducing the number of random locations selected for melanophore birth each day from 350 to 50. (c) Reducing the speed of domain growth reclaims wild-type stripes despite the same reduction in melanophore birth, in agreement with [63].

patterning

Parichy and Turner [62] showed that raising puma fish in conditions associated with reduced growth rates led to wild-type patterns. To simulate this condition, we reduce our growth rates by 67% (the developmental stages associated with the resulting domain sizes are then approximated using SSL from [58]). As shown in Figure 4.26c, slowing growth can reclaim wild-type stripes (though interstripes X1V and X1D form more quickly, relative to the domain size, in comparison to our wild-type simulations in §4.4.2). This highlights the careful interplay between cell birth and fish growth, and echoes empirical findings [64, 65], also discussed in §3.4.2, that altering the timing of differentiation relative to fish growth disrupts patterning.

4.4.7 Regeneration experiments

Ablation or regeneration experiments involve ablating a portion of the wild-type pattern on zebrafish and observing the dynamics that result from this perturbation *in vivo*. This can be done by treating larvae with a drug [82] or using a laser [54, 89]. To simulate ablation, we remove a portion of the cells in our domain to emulate the experimental set-ups in [89]. Note that we have not yet simulated the experiments of Nakamasu *et al.* [54] that our two-cell model in §3.4.3 is based on, and this would be an interesting test for future work.

Yamaguchi *et al.* [89] conducted two ablation experiments, the first of which we studied in §3.4.3, and here we simulate both. In their first set-up, which we will refer to as Experiment 1, they describe ablating *all of the melanophores and xanthophores* in a large region of the body, as shown in Figure 4.27a. As the focus on iridophores in the experimental community took off post the 2007 study [89], no mention of iridophores is made. With the benefit of newer data, a natural question now is how this externally applied perturbation impacts iridophores, as well as the skin of zebrafish. Thus, in a review article in 2015 [86], Watanabe and Kondo clarify that, while the type of laser ablation performed in the study by Nakamasu *et al.* is meant to kill cells with colored pigments, meaning iridophores are less impacted, roughly 20% of iridophores beneath xanthophores die. We expect that the kind of laser ablation the Kondo lab used in [54] and [89] is the same, but this point was not made directly, so we test several options for the impact of these experiments [89] on iridophores below.

While Yamaguchi *et al.* conducted Experiment 1 on a 20 dpf zebrafish, it took time for the debris resulting from laser ablation to clear [89]. Thus, we begin our simulated Experiment 1 by clearing the cells in a rectangular region 1000 μm long

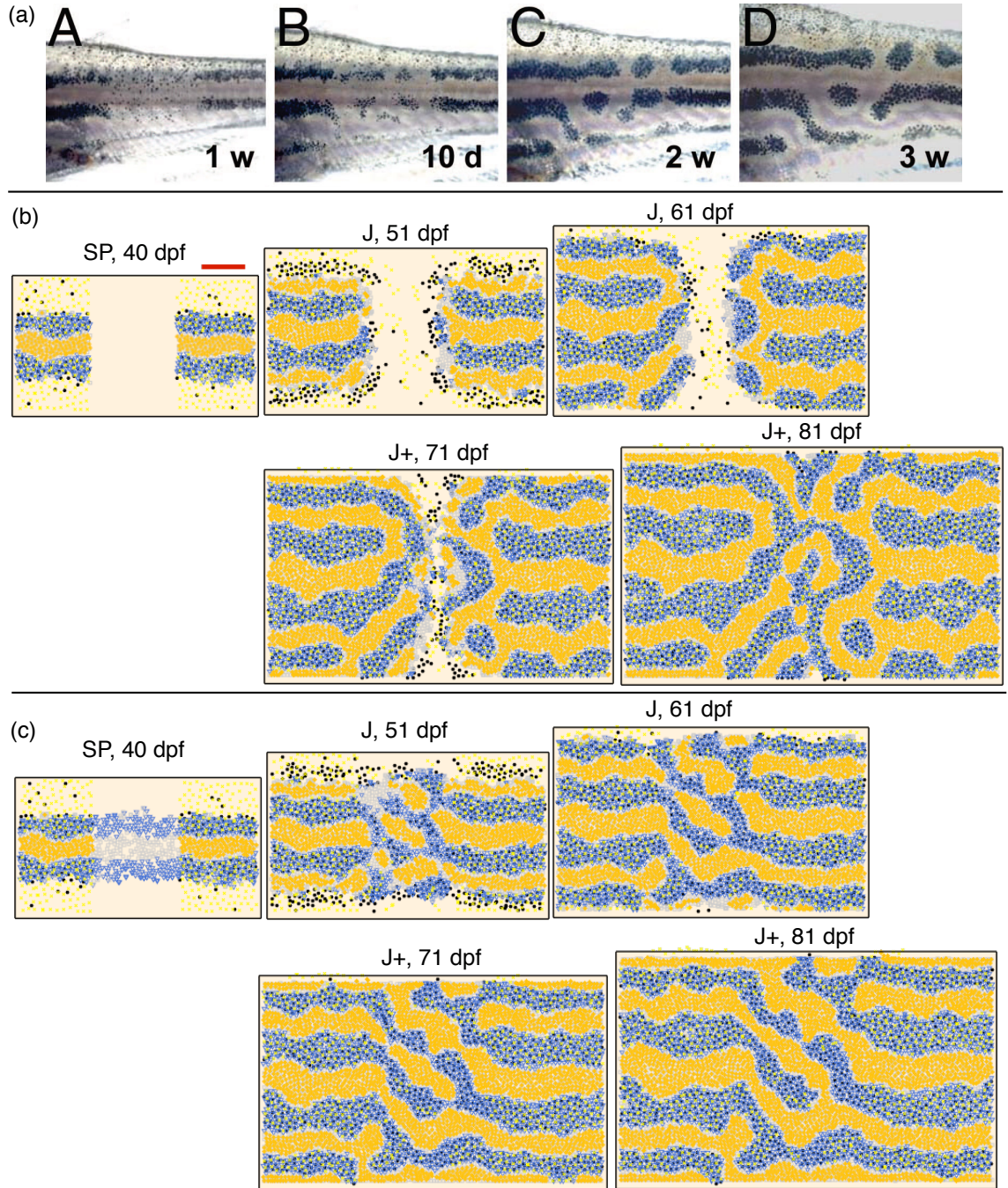


Figure 4.27: Experiment 1: ablation of all melanophores and xanthophores in a wide region. (a) Timeline of ablation reproduced from Yamaguchi *et al.* [89] with permission; Copyright (2007) National Academy of Sciences, U.S.A. Note that time points in the empirical images are given in weeks (w) post initial ablation. (b-c) Simulated ablation of a 1000 μm -wide region at 40 dpf: in (b) we remove all cells in this region; in (c) based on new clarification about the impact laser ablation has on iridophores in [86], we remove all melanophores, all xanthophores, and 20% of the iridophores.

(and the full height of the fish domain) at 40 dpf, when our pattern seems to most closely resemble the first image in the timeline of Figure 4.27a. We test two cases: in Figure 4.27b, we assume the laser destroys all cells in the $1000\text{ }\mu\text{m}$ long area, and, in Figure 4.27c, we specify that it kills all xanthophores and melanophores, as well as 20% (randomly selected) each of the iridophores in loose and dense form. As shown, regardless of the impact of laser ablation on iridophores, the result is patterns with lost directionality but maintained width, in agreement with [89]. We note that the second set-up, in which only 20% of iridophores die, seems to produce results that are closer to the experimental data [89]. Thus, our work with Experiment 1 supports the observation that laser ablation does not strongly harm iridophores.

Next we turn to Experiment 2, shown in Figure 4.28a, conducted by Yamaguchi *et al.* [89]. In this set-up, they consider a more complicated experiment: melanophores in stripes 1V and 1D were continually ablated for a week, while other pigment cells were not purposely harmed [89]. This experiment was done on three zebrafish, and the resulting dynamics led to stripe 2V moving upward to produce a downward parabola shape (one fish, Figure 4.28) or connecting with 1V to form a bridge across the X1V interstripe (two fish, results presented as SI animations in [89]). We note that scale bars, stages, and zebrafish ages are not present or reported for this experiment [89], making it more challenging to determine the conditions. Thus, based on the first image in the timeline of Experiment 2 (Figure 4.28), we begin removing melanophores in the area of stripes 1V and 1D (in a $1000\text{ }\mu\text{m}$ long region) at 41 dpf. (The vertical span of stripes 1V and 1D used for this ablation experiment is approximated based on our wild-type simulations). We continue removing black cells each day in these regions for 7 days; because laser ablation disrupts pigmented cells [86], we also remove all stellate xanthophores in these regions each day. As with Experiment 1, we test two options for the impact of the laser on iridophores,

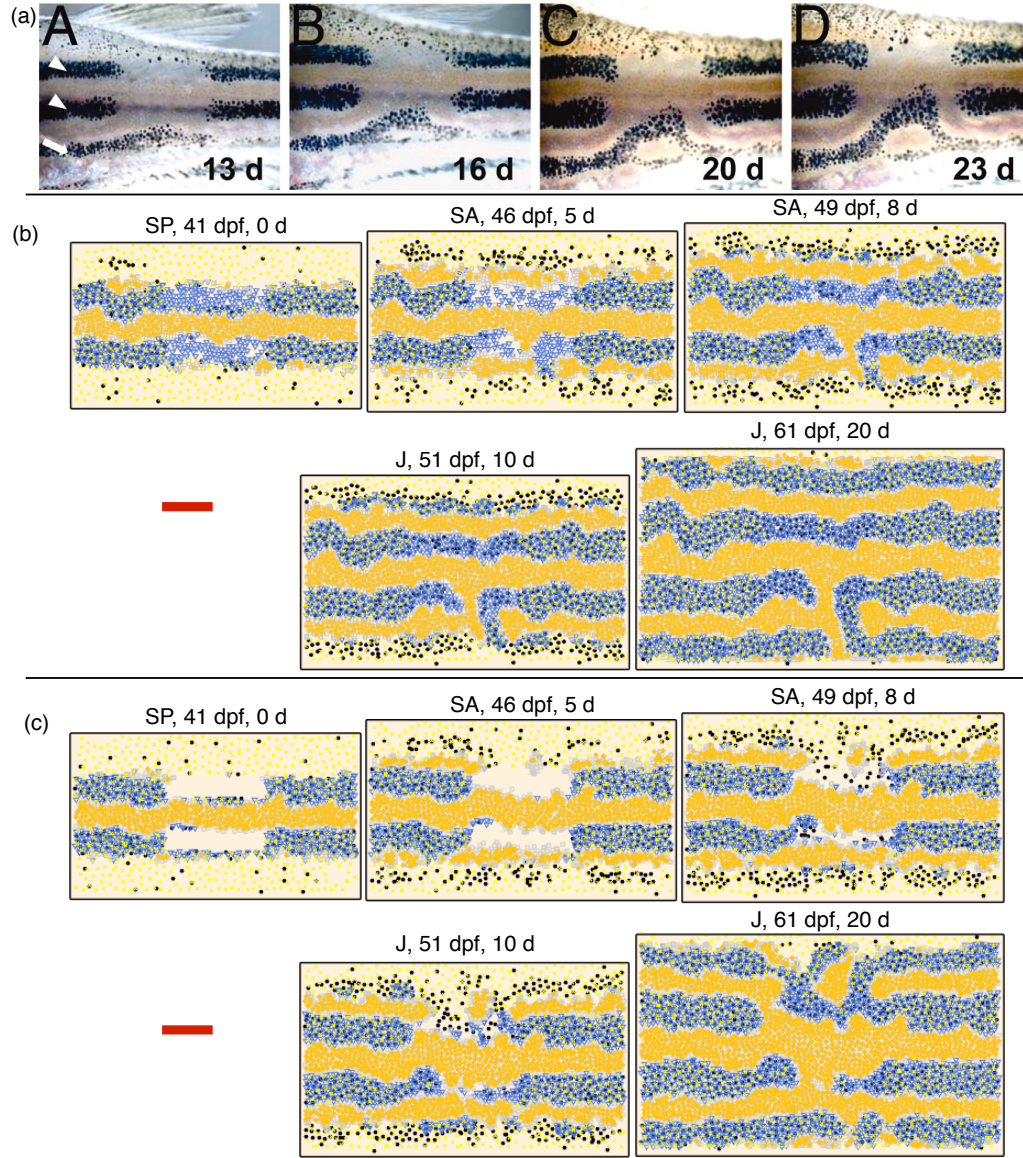


Figure 4.28: Experiment 2: continual ablation of melanophores in stripes 1V and 1D for one week. (a) Example timeline of ablation reproduced from Yamaguchi *et al.* [89]; Copyright (2007) National Academy of Sciences, U.S.A. Note that time in the empirical images is given in days (d) post initial ablation. Additional examples of Experiment 2 (as animations in SI of [89]) show 2V (bottom black stripe) moving upward and connecting with 1V, more similar to our simulated results in (b-c). We simulate this experiment by removing all melanophores and xanthophores in a 1000 μm -wide region in 1V and 1D; in (b), motivated by [86], we remove 20% of loose blue iridophores as well; in (c) we remove all loose iridophores in this region.

either removing all loose iridophores in the ablated region, or killing off a randomly selected 20% each day.

The results of our simulated version of Experiment 2 are in Figure 4.28b-c. As

shown, we remove melanophores, stellate xanthophores, and 20% or all of the loose iridophores (Figures 4.28c and 4.28b, respectively) in the regions of 1V and 1D until 47 dpf; at this point we allow cells to differentiate without external perturbation. In these example simulations, we observe stripe 2V connecting to 1V or stripe 2D bridging across X1V to connect with 1D. Though qualitatively similar to the corresponding dynamics on the fish skin [89] (more so to the animations in the SI of [89] than the figure provided), we consider these results not as clean as our work with Experiment 1. This could provide a place for model improvement or be a by-product of the complicated nature of this experiment; it is important to keep in mind, for example, that the experimental sample size (3 fish) used in [89] is small. Additionally, it would be useful to test different lengths for the ablation region used in our simulations, as the length of the region ablated was not reported in [89]. Furthermore, we expect that the continued laser ablation of pigment cells across 7 days has other impacts on the fish skin not accounted for.

As a last study of the regeneration experiments in [89], we test simulating Experiment 1 on an older fish, removing cells in a wide area at 50 dpf instead of 40 dpf. The main difference in these two time-points is that the initial development of interstripes X1V and X1D has already started at 50 dpf. As shown in Figure 4.29, the result of our simulated version of Experiment 1 is stripes with lost directionality but relatively normal width regardless of the time of initial ablation. It is interesting to note that black stripe 1V in the unperturbed region of the domain features a small orange spot in its center at stage J++ in Figure 4.29b (red arrow). This is most likely due to the width of the stripe at 86 dpf (longer than we usually simulate wild-type patterning). Our model considers long-range survival signals from compact xanthophores to melanophores as acting on a length scale of $250\text{ }\mu\text{m}$; as the domain continues growing and cell positions continue stretching across it, stripes

widen. Adult stripes measure roughly $600\text{ }\mu\text{m}$, and pseudopodia extensions, possibly involved in carrying survival signals from melanophores to xanthophores, have been measured to be up to half this width [25]. Thus, our long-range length scales may need to be longer to produce wild-type patterning at later stages in development; because individual melanophores grow in time, this could be modeled using a long-range length scale that grows with cell age (we tested this option, but chose to exclude it for simplicity and instead focus on patterning until stage J+; results not shown). Alternatively, the L-iridophores (not included in this model) that spread under melanophores after pattern formation could provide stripe maintenance and survival cues to black cells; this possibility has been suggested in [17].

Note that, as described in §4.4.1, Experiment 1 with all iridophores removed was used during early model development and motivated the inclusion of some random stellate xanthophore birth in our model. Experiment 1 with only 20% of iridophores removed and Experiment 2, in contrast, was reserved for testing of the final model.

4.4.8 Temperature-sensitive *fms*/*pfeffer*

As discussed in §4.4.1, ablation Experiment 1 [89] from §4.4.7 was one of two set-ups that were used for initial tests and led to adjustments to our model. The second, described here, is an experiment [61] on a temperature-sensitive *fms* (e.g. *pfeffer*, no xanthophores) zebrafish. Parichy and Turner used this temperature-sensitive mutation to tune when xanthophores disappear: increasing the temperature essentially transforms the fish from wild-type to *pfeffer* conditions, destroying xanthophores after temperature upshift [61]. As shown in Figure 4.30a, reproduced from [61], the result is gradual destruction of wild-type stripes over time. We simulate this experiment by first developing wild-type stripes until 64 dpf; we then remove all

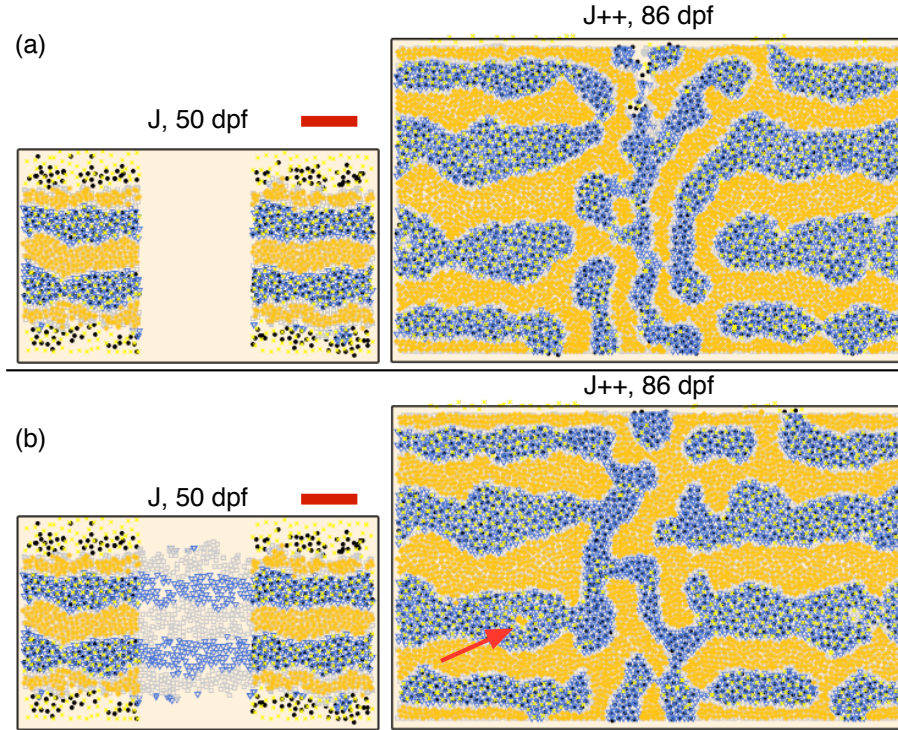


Figure 4.29: Ablation at 50 dpf. Simulations of Experiment 1 from [89] (a) assuming all iridophores are lost in the ablated region and (b) assuming only 20% of iridophores are lost in the ablated region. Images shown are at the time of ablation and the final simulation time. Note that in (b) we see a small orange spot (red arrow) in black stripe 1V. This may be because we have run the timeline of development longer than usual (to stage J++ instead of stage J+). As the domain grows, our cell positions are stretched and stripes widen (stripes also widen on the fish skin with age, based on our view of images in [58], for example). Because our long-range length scale is $250\ \mu\text{m}$, stripes stretched too wide may develop spots in the center. To simulate wild-type development to adult stages, we would need to increase this length scale (adult stripes measure roughly $600\ \mu\text{m}$ and long-range cell pseudopodia extensions have been measured to be up to half a stripe width in length [25]). Black melanophores grow in time [75], so it is conceivable that their long-range pseudopodia extensions grow as well.

xanthophores from the domain and halt their further birth (Figure 4.30b), reproducing the dynamics observed in [61]. For a discussion of the way this experiment shaped model development, see §4.5.3.

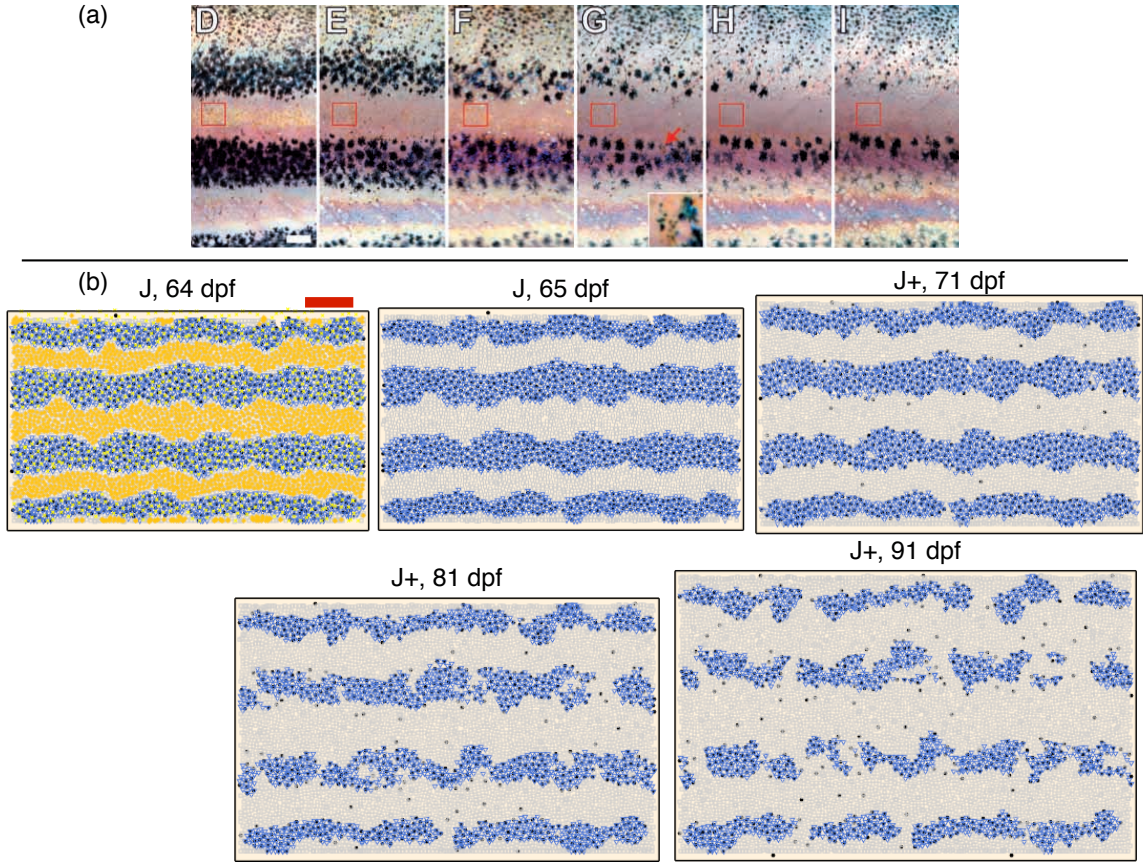


Figure 4.30: Increasing the temperature of the environment for the temperature-sensitive *fms* mutation essentially transforms the fish from wild-type to *pfeffer* (no xanthophore) conditions [61]. (a) Temperature upshift experiment on the fish skin, leading to stripe degeneration [61]. (b) Simulated upshift experiment (xanthophores are removed from the domain at 65 dpf and their further birth is halted). Images in (a) are reproduced from Parichy and Turner [61] with permission of The Company of Biologists, Ltd. via Copyright Clearance Center.

4.5 Results: part 2

Section 4.4 presented validations of (and some predictions generated by) our final model. By outlining some of the various dynamics on the fish skin our model can account for under wild-type cell interactions, it also served to introduce the reader to the kinds of patterns we want to produce. The results presented next are of a different nature than §4.4 and consist of two main classes: in the first class (Sections 4.5.1, 4.5.2, and 4.5.3), we take a step back and consider alternative modeling choices. In particular, our main work, presented in §4.5.1, is a detailed modeling derivation and

motivation of the interactions governing iridophores present in our final model (the model used in §4.4). Sections 4.5.2 and 4.5.3, in turn, present alternative modeling choices for xanthophores and melanophores, respectively. These sections serve to provide another source of validation of our model interactions by showing how some alternative mechanisms fail, and result in several predictions.

The second class of work presented in this section (in §4.5.4) is a preliminary study of late-stage mutations. After validating our model in §4.4 and explaining where the alternative models we considered failed in Sections 4.5.1, 4.5.2, and 4.5.3, we consider the model to have captured something inherent in the biology. While our model is most likely not unique, the range of dynamics it can reproduce suggests it can be used to contribute meaningfully to a better understanding of late-stage mutations. These are mutations in which all cells are present, but some interactions are altered, and represent an opportunity for mathematical modeling to help shed light on how changes in genes are related to functional differences in cell behavior.

4.5.1 Predicting the rules governing iridophore form

During the time of model development, a burst of biological literature commenting on iridophores [14, 44] emerged, and the papers [17, 64, 65] put forward the first proposed networks of cell interactions including three cell types. The work [44, 65, 75] has resulted in a better understanding of how iridophores impact melanophores and xanthophores, but the reciprocal cellular communication that directs iridophores remains largely unknown and hypothetical [14, 34, 40, 86]. Notably, Patterson *et al.* have suggested an abundance of xanthophores can repress the appearance of dense iridophores [64]; this is based on experiments showing fish that overexpress a factor called Csf1, leading to intermingled cells and numerous xanthophores, are associated

with a lack of dense iridophores localizing in additional interstripes past X0 (we discuss this more below).

While it is clear that transitions in iridophore form from loose to dense mark the beginning of each new interstripe [74, 75], what drives this important dynamic remains to be determined (see Figure 4.1). Interestingly, new research on late-stage mutations (leopard [83, 84], luchs [33], schachbrett [14]) featuring spots or interrupted stripes [14, 33] suggest these phenotypes arise because dense iridophores fail to change form to loose when they encounter black stripe regions, further supporting the importance of iridophore behavior. Because leopard and luchs are required in melanophores and xanthophores, but not iridophores, this suggests these spotted phenotypes arise because iridophores fail to receive wild-type signaling from melanophores and xanthophores [33]. The communication mechanisms at work between different cells to drive shape transitions are complex and possibly nonlinear [14, 34]. Studying the signals iridophores receive from other cells is thus particularly exciting because it has the potential to point to new connexins in iridophores [33, 44]. To contribute to a better understanding of iridophores from an iridophore perspective, here we test a range of possible mechanisms that could drive iridophores to transition in form between loose and dense, with the goal of helping shed light on the cells and length scales involved in these dynamics.

Based on wild-type and early-stage mutations *nacre*, *pfeffer*, and *nacre;pfeffer*, we can deduce some bounds on the possibilities for the rules governing iridophore form changes:

- Because the iridophores in *nacre;pfeffer* (a cross mutation with only iridophores) are in dense form [17], dense iridophores, first emerging from the horizontal myoseptum [75], should remain dense when melanophores and xanthophores

are not present;

- Because pfeffer features loose and dense iridophores, melanophores should be involved in iridophore form-change rules;
- Similarly, because nacre features loose and dense iridophores, xanthophores should have a place in iridophore form-change rules (a place independent of the role of melanophores).

The loose iridophores above black spots in pfeffer [17] support the first mechanism we propose: [A] Melanophores induce dense iridophores to become loose at short range. The simplest explanation for loose iridophores becoming dense once they pass across the black spots in pfeffer leads to a complementary mechanism: [$\tilde{A}$] Without melanophores present at short range (e.g. without communication with melanophores, or when they lose a signal from melanophores), loose iridophores become dense. Related to these mechanisms, Frohnhöfer *et al.* have suggested local repulsion between dense iridophores and melanophores [17].

Nacre is more complicated to explain than pfeffer. Because dense iridophores eventually transition to loose and halt the expanding X0 interstripe in nacre, we suggest iridophores respond to a long-range signal from the interstripe region. This signal could conceivably come from compact xanthophores or dense iridophores. Interestingly, density-dependent motion, first proposed by Cahn and Hilliard [6], can explain the self-organization of mussels into patterns [42]. Thus, it is conceivable that the different densities and speeds of iridophores in loose and dense forms could explain their separation on the fish skin. Nevertheless, the cross mutation pfeffer;nacre shows that iridophores alone cannot induce their own change to the loose form, so we conclude the long-range signal should come from compact xanthophores. Frohnhöfer *et al.* have also suggested that xanthophores are needed to halt expanding interstripes, creating an inhibitory effect on iridophores by xanthophores at long-range

[17]. As mentioned earlier, Patterson *et al.* have proposed that an overabundance of xanthophores can repress iridophores. Thus, the next mechanism is: [B] Once dense iridophores sense too many compact xanthophores at a distance, they change form to loose. The counter mechanism explains the appearance of later spots of dense iridophores in nacre: $[\tilde{B}]$ When loose iridophores sense too few compact xanthophores at a distance, they become dense. This also explains the appearance of new interstripes in wild-type.

Lastly, the close appearance of compact xanthophores in association with dense iridophores [17, 44, 45] motivates a local signal from compact xanthophores to promote dense iridophores: [C] Dense iridophores do not become loose atop compact xanthophores. Similarly, we propose: $[\tilde{C}]$ Compact xanthophores induce loose iridophores to become dense. Note that this mechanism cannot account for the appearance of new interstripes, as it is dense iridophores that first instruct xanthophores to become compact [45]. Frohnhöfer *et al.* have also suggested mutual attraction between interstripe xanthophores and iridophores [17].

A summary of the interactions we test for governing when iridophores become loose or dense is given in Figure 4.31. These mechanisms are building blocks: based on nacre and pfeffer, we know both xanthophores and melanophores are involved in determining when iridophores change form (sets of combinations of these mechanisms are presented in Figure 4.32). Thus, we considered different combinations of mechanisms $[A], [B], [C]$ and $[\tilde{A}], [\tilde{B}], [\tilde{C}]$, with the goal of capturing the qualitative features of wild-type, nacre, pfeffer, and pfeffer;nacre with the same set of unchanged rules (in the bigger picture, these rules also need to complement the rest of the interactions in our model, so that we can reproduce shady, shady;nacre, and shady;pfeffer, as well as quantitative measurements).

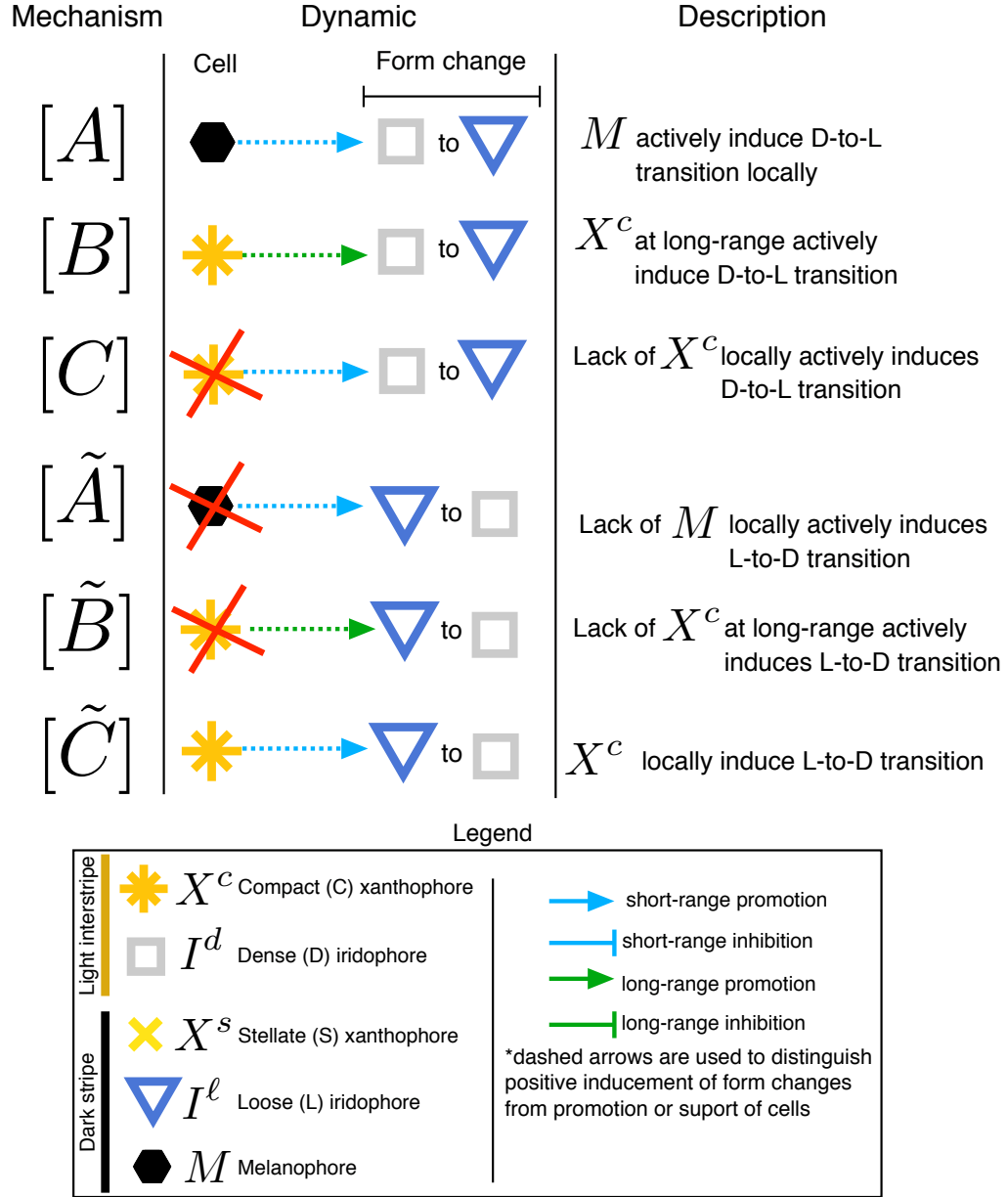


Figure 4.31: Summary of possible dynamics identified to account for iridophore form changes in wild-type, pfeffer, nacre, and pfeffer;nacre. These are building blocks that we will base our candidate cell interactions on. As an example, $[A]$ prescribes that iridophores change from dense to loose when melanophores are present nearby (e.g. local melanophores induce iridophore form change). Note that, for the mechanisms involving *lack of* cells (corresponding to the lack of a signal from these cells to iridophores), we do not strictly require complete lack of cells in our model rules: instead, we require *too few* cells (see §4.6). Biologically, *too few* cells could correspond to *lack of* cell communication due to stochastic effects or delays in interactions, for example.

First we note that $[A]$ and $[\tilde{A}]$ are necessary (among the mechanisms proposed) for pfeffer, as the other mechanisms, involving xanthophores, cannot account for this phenotype. Similarly, we suggest $[\tilde{C}]$ is necessary to account for the appearance of

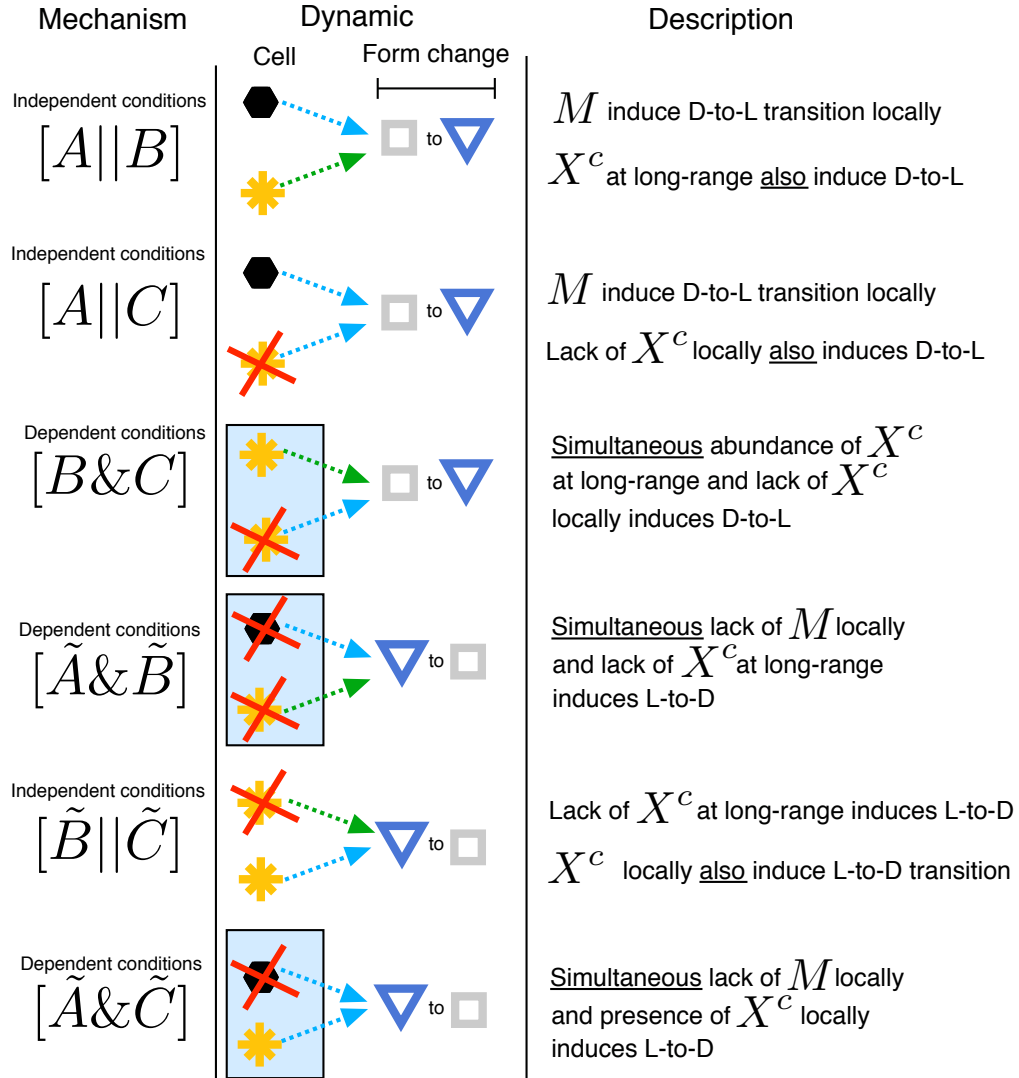


Figure 4.32: Summary of alternative mechanisms tested for governing iridophore form changes (results are shown in Figures 4.33 and 4.34). These two-part mechanisms are based on the building blocks in Figure 4.31. The minimal interactions we identified to account for patterning dynamics are, in turn, a combination of these two-part mechanisms. Note that we use $\&$ to mean both conditions must be met simultaneously; $||$ signifies independent interactions that can induce form change if one *or* the condition (or both) is met.

new interstripe patches at a distance from the expanded X0 region in nacre. Since compact xanthophores seem to follow dense iridophores, $[\tilde{B}]$ cannot explain these patches in nacre. Thus, we propose a minimal rule for governing loose-to-dense iridophore transitions: $[\tilde{A}\&\tilde{C}]$ iridophores become dense when the local melanophore density is low *and* there are too few compact xanthophores at long-range (e.g. they lose a signal from compact xanthophores). In contrast, using $[\tilde{A}||\tilde{C}]$ does not work

because iridophores in pfeffer, without xanthophores, would constantly be instructed to turn dense through $[\tilde{C}]$. Note that we use $\&$ to mean both conditions must be met simultaneously and $||$ to signify that iridophores will change form if one *or* the other condition (or both) are met.

While $[A]$ alone can explain wild-type patterning if dense iridophores become loose in the locations of 1D and 1V only after the appearance of melanophores (in fact, which dynamic comes first does not seem to have been experimentally elucidated yet), it cannot account for nacre, where no melanophores are present. We found the best results when we use $[A||(B\&C)]$. This means dense iridophores turn into the loose blue form found in dark stripes under one of two conditions: either they encounter melanophores nearby, locally inducing the change to loose; or they sense too many compact xanthophores at a distance and transition to loose, assuming they are not directly below compact xanthophores on the fish skin.

The mechanisms $[A||(B\&C)]$ and $[\tilde{A}\&\tilde{C}]$ represent the minimal rules we could identify to produce wild-type and early-stage mutations. In the end, we added one additional component, instead considering $[\tilde{A}\&(\tilde{B}||\tilde{C})]$. This last tweak makes our rules for loose-to-dense and dense-to-loose transitions logical complements. More importantly, while it does not impact high-level features of the patterns that form, it produces a small improvement in nacre, discussed below. Thus, our predicted wild-type (control) rules, used for the simulations in §4.4, are $[A||(B\&C)]$ and $[\tilde{A}\&(\tilde{B}||\tilde{C})]$. These rules are summarized pictorially in Figure 4.36. In Figures 4.33 and 4.34, we contrast the different wild-type, nacre, and pfeffer patterns that emerge under our selected mechanisms with simplified alternatives (we present the best simulations we obtained for these alternatives). We next describe the mechanisms tested that led to reasonable wild-type patterns; this is followed by a series of predictions and a discussion of the mechanisms that fail at wild-type stripes.

First, in Figure 4.33, we leave the control mechanism $[A|(B\&C)]$ for dense-to-loose transitions unchanged and explore alternative dynamics that could govern loose-to-dense changes. As shown, $[\tilde{A}]$, $[\tilde{B}]$, and $[\tilde{B}|\tilde{C}]$ produce reasonable wild-type patterns on first view, but fail on nacre or pfeffer. On closer inspection, it is worth noting that $[\tilde{B}]$ and $[\tilde{B}|\tilde{C}]$, which are both mechanisms that do not account for melanophore interactions with loose iridophores, lead to some silver dense iridophores coexisting with the blue cells in black stripes. $[\tilde{A}\&\tilde{C}]$ produces reasonable patterns across wild-type, pfeffer, and nacre, and does so fairly reliably. It differs from our control mechanism in the finer details: these simplified rules lead to occasional blue iridophores trapped within interstripe regions. Because they are surrounded at long-range by a high density of compact xanthophores and receive no local signal from the orange cells around them, these blue iridophores persist in interstripes.

In Figure 4.34, we maintain our control mechanism for loose-to-dense transitions but adjust the rules governing how iridophores change from dense to loose. We find that $[A]$, $[C]$, $[A|C]$, and $[B\&C]$ are capable of producing wild-type stripes, but, with the exception of $[A|C]$, these mechanisms fail at nacre or pfeffer. That $[A|C]$, a rule specifying iridophores transition to the loose form of dark stripes entirely based on local signals (when melanophores are present or compact xanthophores are not), can result in stripes was surprising and led to further study. It turns out that, while $[A|C]$ can produce stripes, it does so extremely unreliably, with 86% of 100 simulations featuring curvy patterns with breaks in interstripes. It is also useful to note that $[A|C]$ is not able to reproduce the dense iridophores in pfeffer;nacre, as it would instruct all iridophores to turn loose.

The timeline of wild-type development is iridophore-led

Simplifications $[\tilde{C}]$ and $[\tilde{A}\&\tilde{C}]$ in Figure 4.33 entirely disrupt wild-type, pfeffer,

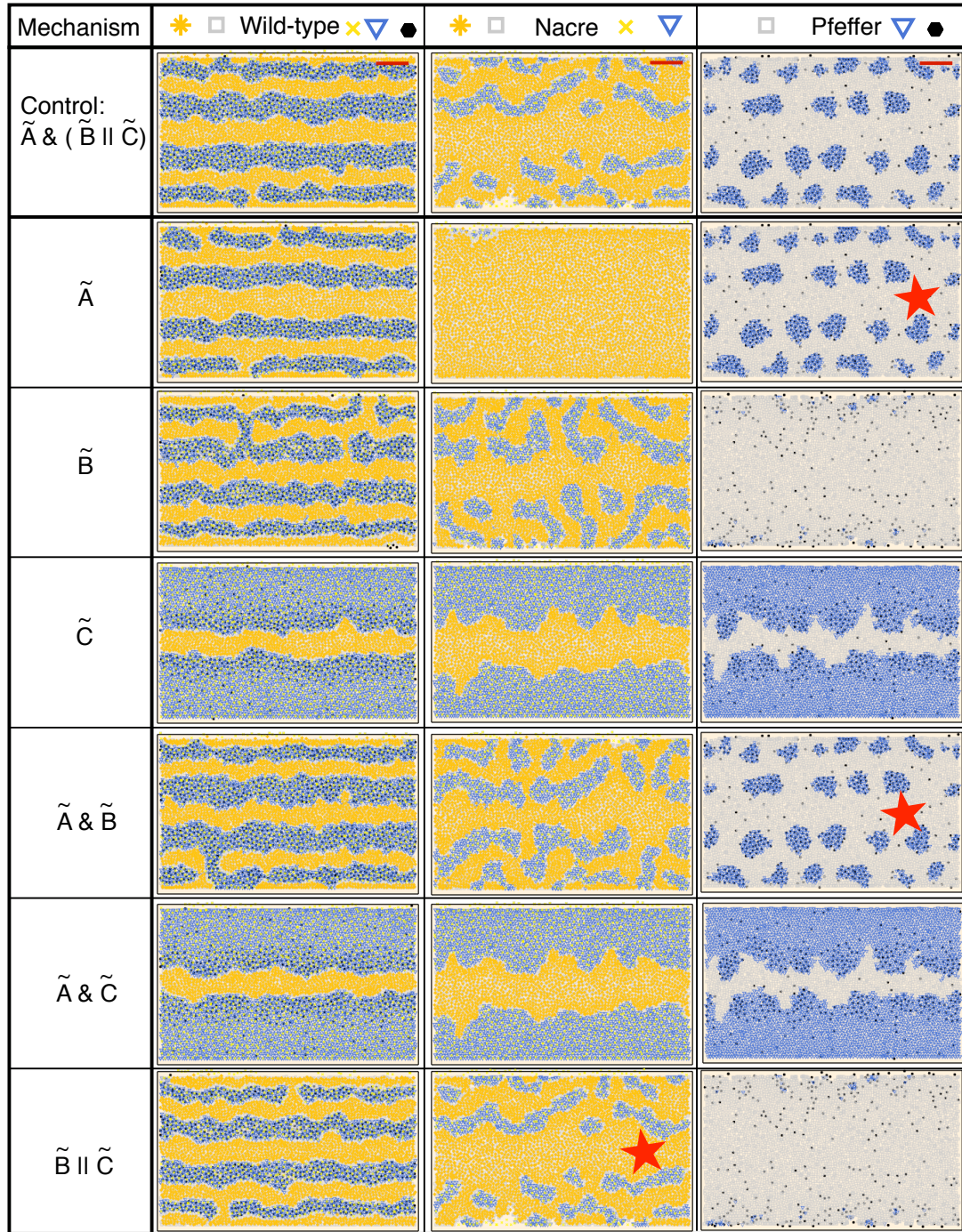


Figure 4.33: Comparison of some candidate mechanisms for specifying when iridophores change from loose to dense. Red stars denote cases where the mechanism tested will necessarily not change the indicated pattern (for example, removing xanthophore interactions $[\tilde{B} \parallel \tilde{C}]$ to obtain mechanism $[\tilde{A}]$ will not change pfeffer from our control case because pfeffer has no xanthophores). We do not show how these mechanisms behave on the cross mutation pfeffer;nacre because it can be more easily deduced how the rules will impact this phenotype with only iridophores.

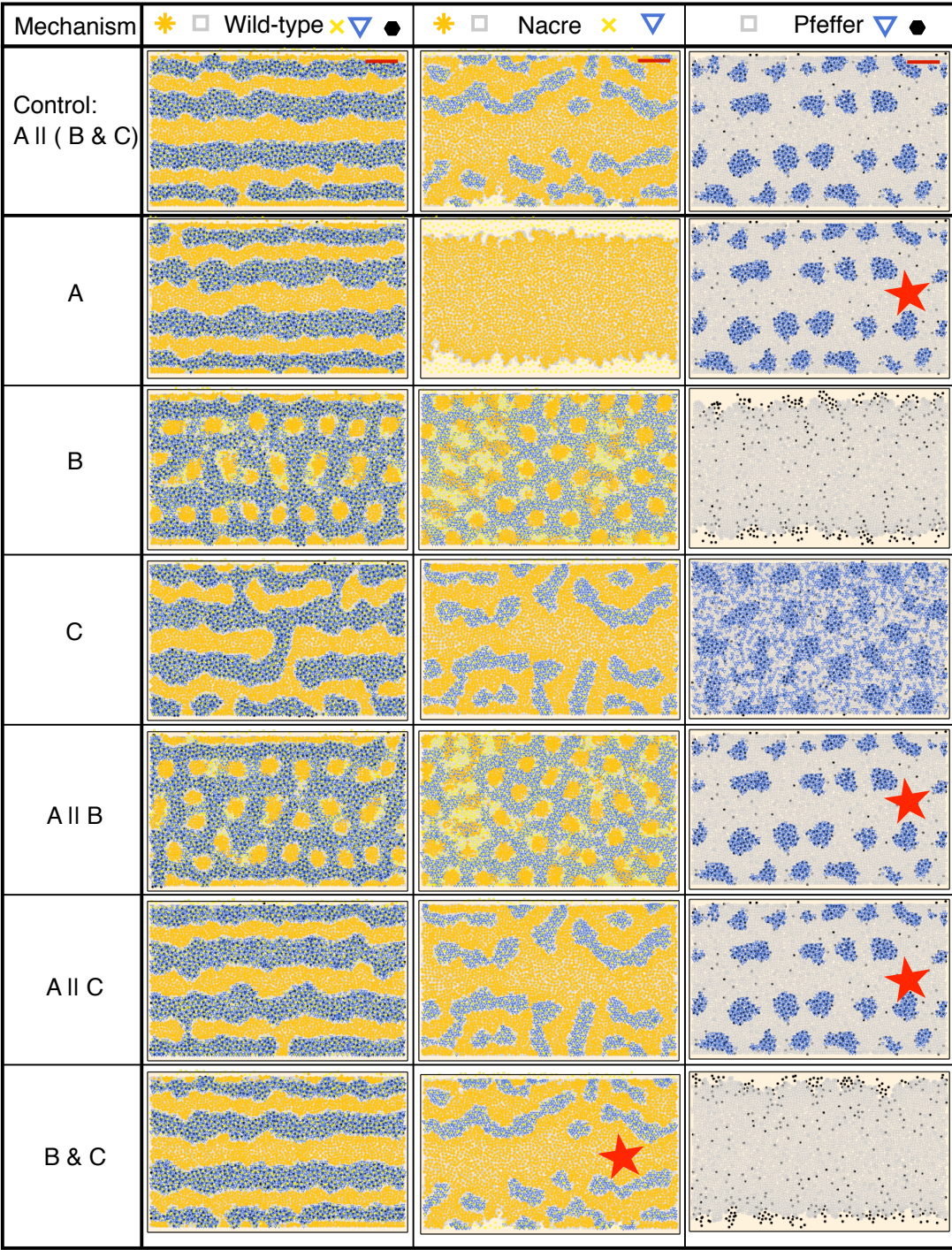


Figure 4.34: Comparison of some candidate mechanisms for governing iridophore form transitions from dense to loose. Red stars denote cases where the mechanism tested will necessarily not change the indicated pattern (for example, removing xanthophore interactions [B&C] to obtain mechanism [A] will not change pfeffer from our control case because pfeffer has no xanthophores).

and nacre, and point to the crucial ordering of events in time present in wild-type patterning. In both of these settings, we are assuming loose iridophores require

compact xanthophores locally to turn dense. However, experimental work [75] has shown that iridophores emerge from the black stripes, become dense, and only then do xanthophores become compact on top to produce light interstripes. Thus, that $[\tilde{C}]$ and $[\tilde{A}\&\tilde{C}]$ fail supports this sequential timeline as well as findings [45] that xanthophores adopt a compact shape in response to dense iridophores: wild-type stripe formation is iridophore-led. These mechanisms may also be related to work [64] on *Csfl*-overexpressing zebrafish and *Danio albolineatus*: as shown in Figure 4.35, these fish fail to produce additional interstripes beyond X0. Patterson *et al.* have suggested this is due to widespread differentiation of xanthophores leading to the inhibition of iridophores [64]. Here we show that when local interactions $[\tilde{C}]$ with compact xanthophores are kept intact, but long-range communication $[\tilde{B}]$ is removed, new interstripes do not form. This is a possible direction for future work: it would be interesting to specify widespread birth of xanthophores across the domain to relate our work more closely to the situation in [64] and *Danio albolineatus*.

Model raises questions about the possibility of yellow-spotted zebrafish and the relationship of zebrafish with other Danio fish

The orange spotted patterns produced under $[B]$ and $[A||B]$ are particularly interesting. While zebrafish display a wealth of spotted/broken stripe late-stage mutations (for example, leopard, luchs, schachbrett [14, 33, 43]), these phenotypes always feature black spots on light backgrounds, and the opposite has not been observed. However, a relative of zebrafish, namely *Danio margaritatus*, does feature light spots on a black background [55] (note: zebrafish is *Danio rerio*). This suggests a venue for future work, as we are not sure how to explain these light spot simulations and it would be interesting to explore a possible relationship to *Danio* evolution. The mechanisms $[B]$ and $[A||B]$ differ from our control interactions in that they remove a local signal between compact xanthophores and dense iridophores; in particular,

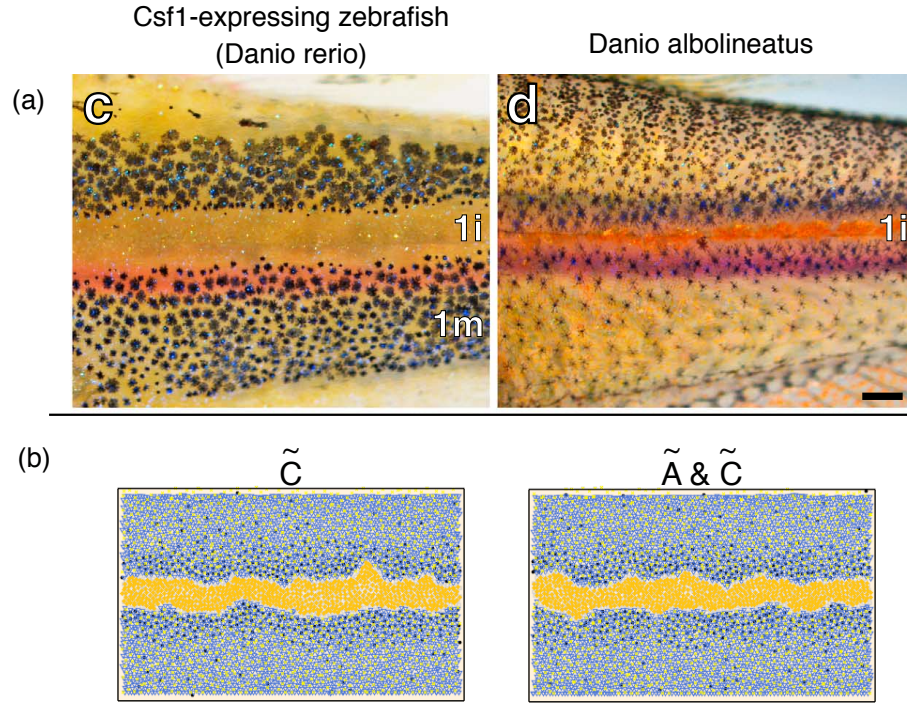


Figure 4.35: Fish images (a) reproduced from Patterson *et al.* [64] and reprinted by permission from Macmillan Publishers Ltd: Nature Communications [64], copyright Macmillan Publishers Ltd. (2014). Simulations (b) with reduced loose-to-dense form transition rules for iridophores may be related to Csf1-overexpressing zebrafish and *Danio albolineatus*.

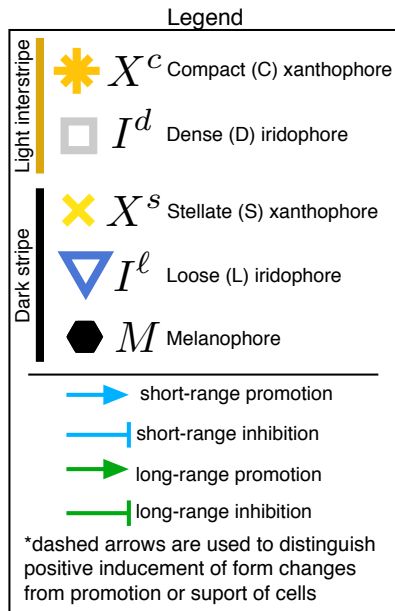
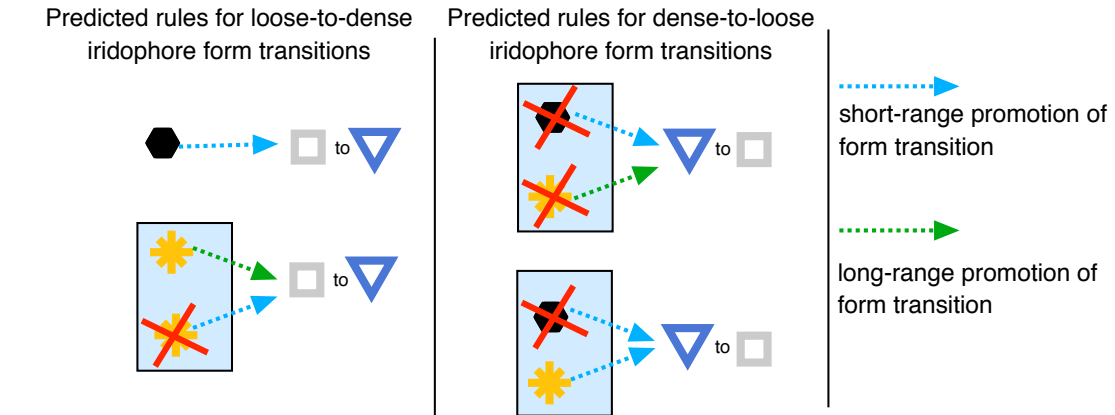
we see spots because dense iridophores, sensing compact xanthophores at a distance but not concerned with their local environment, break interstripes perpendicularly in the same way they normally halt the parallel advancement of yellow regions. Thus, this supports findings that dense iridophores and compact xanthophores are strongly associated locally [17]. Furthermore, it is possible that the orange spots produced are strong evidence against mechanisms $[B]$ and $[A||B]$, adding support to the control interactions we identified. Alternatively, these patterns may be a by-product of our model rules, which may capture dynamics in a different way than they appear on the zebrafish skin (e.g. interaction $[B]$ in our model could be indirectly accounting for a delay in communication between cells). Regardless, the orange spots present interesting questions for future work, and we see a few possible relationships to the biology, discussed next.

Because $[B]$ and $[A||B]$ are simplifications of our predicted mechanism, if our predictions are correct, we would expect a mutation would exist that breaks down cell interactions to these simplified cases. (The alternative is that nature is particularly robust to preventing that specific mutation). If this is the case, a light-spotted zebrafish remains to be discovered. Alternatively, the light spots could hint at how *Danio rerio* differs from *Danio margaritatus* and be related to *Danio* evolution. Another possibility is that the same gene encodes communication between dense iridophores and compact xanthophores locally and long-range; here the reason we do not see yellow-spotted zebrafish could be explained by local and long-range communication of dense iridophores with xanthophores always being knocked out concurrently. A final possibility is related to the work [64] on *Csfl*-overexpressing zebrafish (Figure 4.35): while these fish do not produce secondary interstripes, Patterson *et al.* found that tuning this mutation so that it only arises after X1V and X1D are formed does not destroy these interstripes. Thus, our work could support the idea of a ‘priority effect’ [64] in which developing interstripes win out, resisting long-range signals from compact xanthophores to break into spots.

Wild-type pattern formation on zebrafish may be robust to altered cell behavior because of back-up mechanisms

Lastly, the fact that 10 of the 14 mechanisms considered in total between Figures 4.33 and 4.34 can produce stripes is a testament to the resiliency of wild-type patterning, and could explain why, in contrast to early-stage mutations, there is so little variability in wild-type stripes [17]. In comparison, reasonable nacre and pfeffer patterns are produced in 8 and 7 of the 14 cases considered here. From a mathematical perspective, our intuition may be to search for one clear rule that governs patterning, but biology is after resilience to mutation. We suspect that wild-type pattern formation on zebrafish is robust and dependable because evolution has led

(a) Dynamic-based view of predicted iridophore form change rules



(b) Cell-based view of predicted iridophore form change dynamics

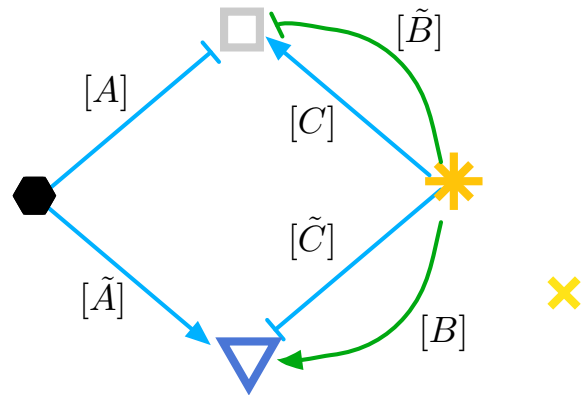


Figure 4.36: Final model identified for governing iridophore form changes. (a) Dynamic-based view of what induces transitions of iridophores between loose and dense. (b) Alternative view showing activation and inhibition effects of other cells on iridophores.

to back-up mechanisms at work on the fish skin: when one interaction fails, the remaining cellular dynamics can still support wild-type patterning.

4.5.2 Predicting the rules governing xanthophore form

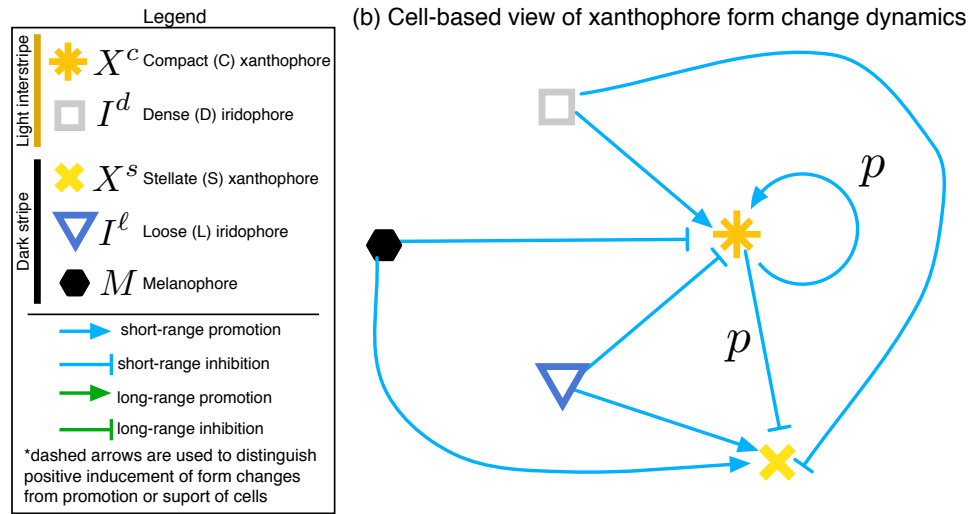
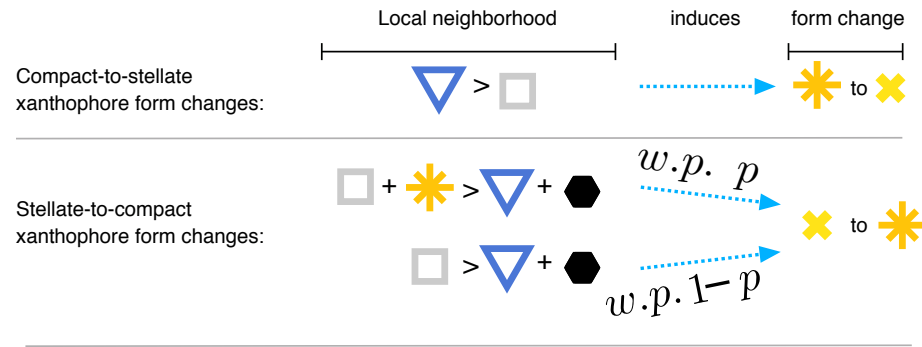
Compared to iridophores, the interactions governing xanthophore transitions between compact in light regions and stellate in dark are more understood. During wild-type pattern formation, larval xanthophores across the skin seem to respond to local cues, becoming compact in response to the appearance of dense iridophores and appearing stellate in black regions [44, 45] (note [44] was published in October 2016). This is at least in part because of local expression of *Csf1* by dense iridophores, a factor that promotes compact xanthophores [64, 65]. With one italicized addition (below), our model rules, summarized in Figure 4.37, support these findings:

[Compact-to-stellate] compact xanthophores become stellate when locally surrounded by more loose than dense iridophores;

[Stellate-to-compact] stellate xanthophores transition to compact when locally surrounded by more dense iridophores *and/or compact xanthophores, with 50% probability*, than loose iridophores and melanophores.

The second rule represents a linear relationship: in particular, stellate xanthophores become dense when the sum of interstripe cells nearby is greater than the corresponding sum of stripe cells (note that compact xanthophores are only factored into the interstripe sum 50% of the time; see §4.6). Furthermore, in contrast to our nonlinear iridophore dynamics, which seem to necessarily include long-range interactions to account for the presence of orange spots in nacre, here we predict xanthophore form is guided entirely by local communication. This supports experimental suggestions [44] that cell form involves gap junction-dependent interactions. (Note that we also tried including a local signal from melanophores for compact xanthophores to become stellate, but it was not needed; furthermore, we found fewer breaks in interstripes when xanthophores depended more strongly on iridophores than melanophores).

(a) Dynamic-based view of predicted mechanisms driving xanthophore form changes



p controls what form xanthophores are naturally in

Figure 4.37: Final model used to govern xanthophore form changes between stellate and compact. (a) Dynamic-based view of requirements on the local neighborhood to induce form changes (note we do not include parameters in this cartoon, but overarching effects; see §4.6 for details). Xanthophores become stellate when their local environment has more loose than dense iridophores; they become compact when their local neighborhood has more dense iridophores and compact xanthophores than loose iridophores and melanophores. p regulates how strongly xanthophores are impacted by other xanthophores; it controls what form they self-select to be in. This parameter can be adjusted once empirical images of nacre/shady are available.

Model development can account for altered xanthophore forms present in shady

An October 2016 paper by Mahalwar *et al.* [44] complicates the xanthophore picture: xanthophores cannot be sharply categorized into stellate (and/or larval) and compact; instead, intermediate forms are found in the shady and leopard mutations [44]. We expect this is related to the presence of the italicized addition in

our [Stellate-to-compact] rule. In particular, during model development, the shady mutation was the phenotype we struggled most to account for while simultaneously producing wild-type and the other early-stage phenotypes. The work [45, 64, 65, 75] had established that dense iridophores, when they reach prospective interstripe locations, induce stellate (or larval) xanthophores, spread across the skin, to become compact. Without directions from iridophores, it is unclear how to account for shady, and we were unsure what our target phenotype should be: should shady have stellate or compact xanthophores or some combination? This question is further complicated by the lack of experimental images of the cross mutation *nacre;shady* (only xanthophores); we know of no images of this mutation, suggesting it is particularly sensitive or not viable. Without images of *nacre;shady*, it is unclear what form xanthophores self-select to appear in (see Figure 4.37; changing the parameter p controls this in our simulations).

Based on the distinct black spots present in *shady* [17] (as well as a brief early 2013 statement, without data shown, that xanthophores appear in a dense sheet in *shady;nacre* [17]), we made the modeling choice to produce *shady* phenotypes with orange compact xanthophores. Without compact xanthophores, there is nothing present to gather melanophores into spots. The question then became how to signal stellate xanthophores, present across the skin, to become compact without the deduced signals from iridophores [45, 64, 65]. We address this by introducing the italicized addition in our [Stellate-to-compact] rule: each stellate xanthophore, with 50% probability each day, responds to local signals from its compact neighbors to become compact (note this is the only place in our form change rules where we have stochasticity). In effect, this means compact xanthophores are weakly preferred over the stellate form, and will win out on *shady;nacre*. Without this addition, we obtain the alternative images in Figure 4.38, which feature a mix of xanthophores

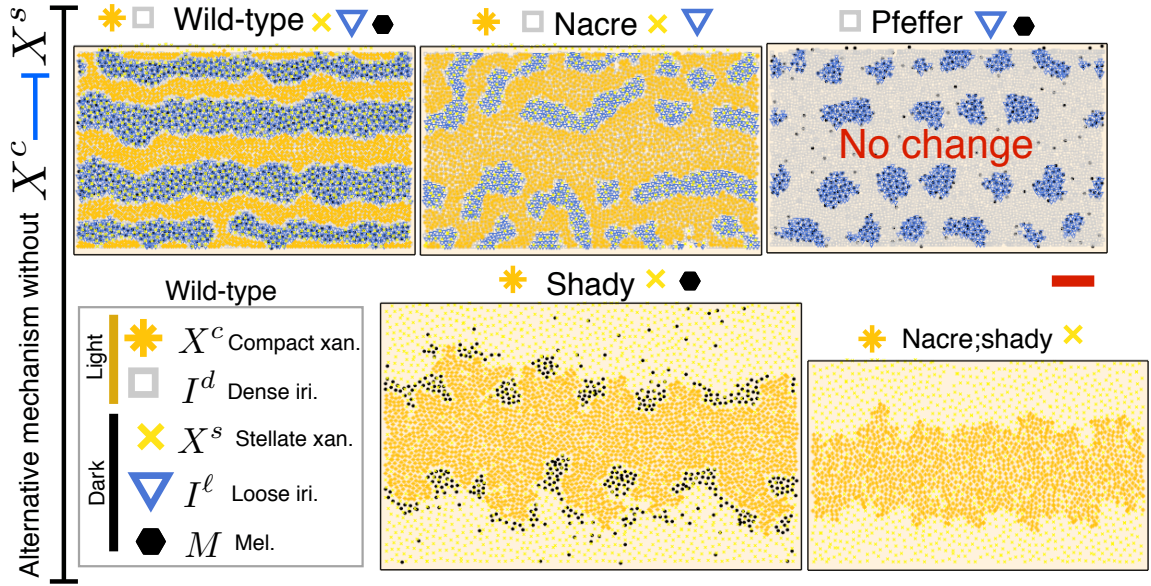


Figure 4.38: Alternative xanthophore form change mechanism. When we remove the local signal from compact xanthophores to stellate xanthophores to become compact (e.g. $p = 0$ in Figure 4.37), there is necessarily no difference in pfeffer, and nacre and wild-type are not negatively impacted. This change is visible only in shady and shady;nacre: without a signal from compact to stellate xanthophores, these mutations feature a mixture of xanthophores of both types, possibly related to the October 2016 work [44].

in both stellate and compact forms in shady and shady;nacre. We consider this mixture of xanthophore forms to be one way our model, with two distinct classes of xanthophores, could account for the intermediate xanthophores now known to be present in shady [44]. Because removing our signal between xanthophores does not impact wild-type, nacre, or pfeffer, we thus present it as an alternative candidate model.

Shady xanthophores, despite altered morphology, may retain compact cell function

Lastly, it is useful to note that without compact xanthophores present in shady in our model (see Figure 4.18), melanophores will have no reason to gather into spots. Thus, we predict that either melanophores have an intrinsic ability to adhere together, or at least some of the intermediate-shaped xanthophores present in shady

[44] maintain the function of compact xanthophores. As evidence against the former, we point to the pfeffer;shady cross mutation (Figure 4.17): without other cell types present, melanophores are randomly distributed.

4.5.3 Predicting melanophore behavior

While we have focused on suggesting what drives cell form changes and studying iridophores from an iridophore perspective, our model also produces predictions about wild-type effects of iridophores on melanophores. We describe two below.

Loose iridophores support melanophore survival

The ablation experiments [54] have shown that melanophores depend on signals from interstripe regions for survival: Nakamasu *et al.* removed compact xanthophores in interstripes surrounding a black stripe and observed that 20% of melanophores died in response. Newer work [86] has clarified that, although not targeted, roughly 20% of iridophores also die in these experiments. Thus, it is unclear if the deduced survival signals come from the xanthophores or the iridophores in yellow interstripes. At first, to account for the fact that the pfeffer mutation maintains melanophores despite lack of xanthophores, we assumed both cell types in interstripes provide long-range survival signals. When we tested our model on the temperature sensitive mutation experiments [61], however, we were not able to reproduce the results (instead of stripes deteriorating upon upshift as shown in §4.4.8, the long-range survival signals from iridophores maintained wild-type patterns without xanthophores in our simulations). This suggests that the long-range survival signals [54] are the result of xanthophore-melanophore interactions alone.

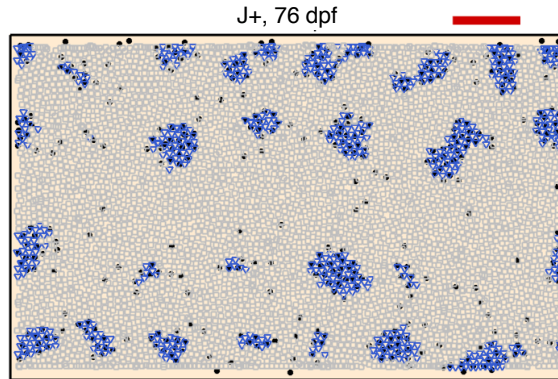


Figure 4.39: If we remove the proposed local survival signal from loose (blue) iridophores, melanophores continually die on the pfeffer fish skin (because there is nothing to protect them from the dynamic observed in [54]: xanthophores promote melanophore survival at long-range).

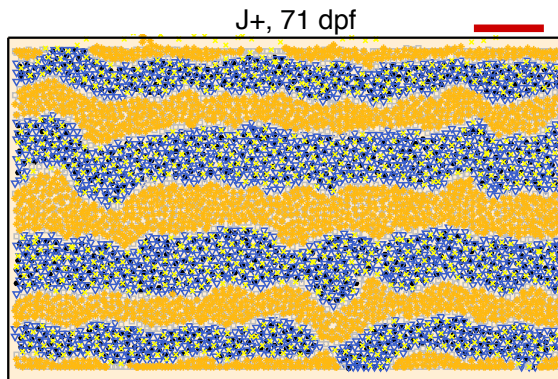


Figure 4.40: Removing melanophore cell death signals does not strongly impact wild-type patterning. This suggests the short-range inhibition of melanophores by xanthophores, observed by Nakamasu *et al.* [54], primarily affects early melanophores leftover from the larval pattern. Supporting this interpretation, the death signal in [54] was observed in young fish when melanophores were present in X0. In contrast to these melanophores in the X0 interstripe, new work [4, 11, 75, 82] suggests most melanophores enter the skin *in situ*. Our model does not account for larval melanophores currently (see §4.7 for future work).

Without long-range survival signals from iridophores, it was unclear how to account for pfeffer. As mentioned in §4.3, we thus introduced one additional component to our melanophore death rules that differs from our earlier work in Chapter 3. We predict that loose blue iridophores atop melanophores offer weak local support, partially maintaining melanophores even when compact xanthophores are not present at long-range (alternatively, L-iridophores, which appear below melanophores after patterning, could provide this signal). This supports suggestions by Frohnhöfer *et al.* [17].

Cell death mainly affects larval melanophores; model supports differentiation of adult melanophores in situ

Recent work has shown that melanophores tend to differentiate from precursors *in situ*, appearing in the prospective locations of future stripes and migrating little [82]. In contrast, much work [59] in the past has described early melanophores, leftover from the larval pattern and located near X0, disappearing from this region through death or outward migration as adult patterning begins. In its current form, our model does not take into account these larval melanophores (doing so is related to setting the parameter $\alpha = 0$, in effect removing an indirect delay between iridophore appearance and melanophore birth; see §4.6). When we do account for larval melanophores (results not shown), including repulsion from compact iridophores to melanophores becomes less crucial for preventing breaks in X0 (in contrast, removing this repulsion in our current model produces only 15% of 100 wild-type simulations with breaks in light interstripes; this is comparable to the 11% breaks we see with our control model, §4.4). This motivates a test of the impact of short-range competition on melanophore patterning in our model. As shown in Figure 4.40, we find that short-range competition between melanophores and compact xanthophores is not needed to account for wild-type stripes. At first surprising in relationship to the empirical evidence [54] supporting competition, this can be explained by the lack of larval melanophores in our simulations. Because adult melanophores typically appear *in situ* [4, 11, 75, 82], local competition is not heavily needed: instead, we suggest this signal is more active in clearing black cells from interstripe regions when they are present in the wrong location (e.g. larval melanophores in the X0 region).

4.5.4 Late-stage mutations

This chapter has focused on developing a predictive model that is keeping pace with the experimental work and prepared to raise and contribute meaningfully to the same questions the biological community is currently asking from a mathematical modeling perspective. While our model (particularly the parameters) is likely not unique, the range of zebrafish dynamics it is consistent with suggests the overarching network of interactions is biologically relevant. This means we can now use the model, defined as wild-type base-case, to explore late-stage mutations. These are mutations in which a genetic upset, leading to a functional difference in how cells behave, results in an altered phenotype (including wider stripes and a range of spots [16, 33, 43]). Mathematical models are particularly suited to shedding light on these altered interactions, and this is the goal of our future work. Here we present some preliminary results to overview our approach for commenting on late-stage mutations and give a sense of what our model can account for. We do not attempt to tie the altered interactions in the images below with the biological literature as closely as in the rest of this chapter, but leave more detailed discussions for future work.

The Nüsslein-Volhard lab approaches the study of late-stage mutations using cross mutations, and we imitate their approach from a mathematical perspective. In particular, the papers [14, 16, 33, 43] involve careful crosses of late-stage mutations, first with early-stage mutations, and then with each other. By crossing late-stage mutations with early-stage mutations, which lack one or more cell types, the cells that do or do not play a role in the altered phenotype can be largely identified: for example, if late-stage mutation A is crossed with *pfeffer* (no xanthophores) and leads to a more severe phenotype, we know mutation A must impact either (or both) iridophores and melanophores (note this experiment would say nothing conclusive

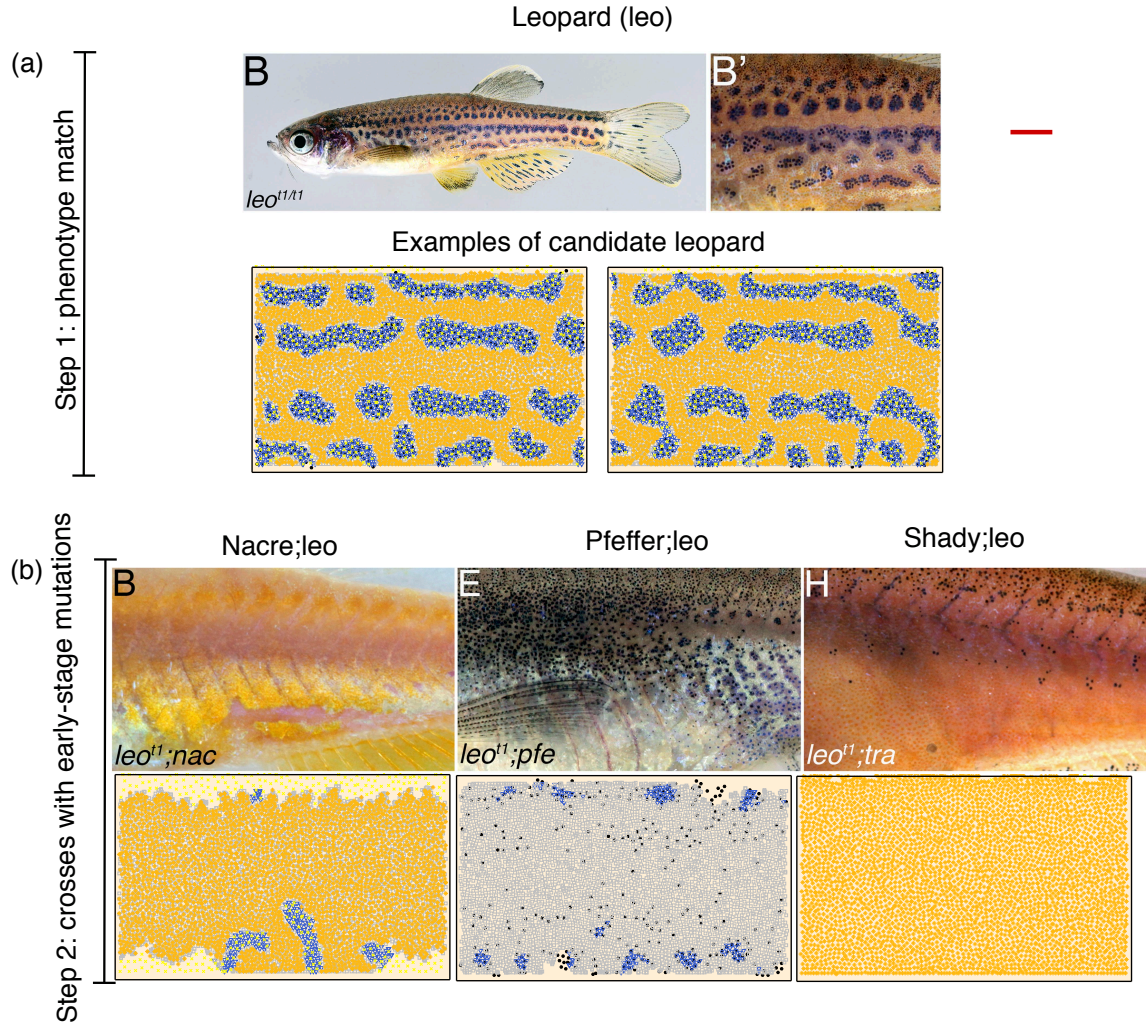


Figure 4.41: Leopard zebrafish [33, 36, 43, 83] feature striking spotted and curved stripe phenotypes that arise due to altered pigment cell interactions (late-stage mutation). Preliminary work: to help identify these altered interactions, (a) we first match the leopard phenotype; (b) crosses with early-stage mutations (simulated by using leopard parameters combined with removing the appropriate cells) provide a second step for validation. Empirical images reproduced from [33] are shown in comparison to simulated images. Final simulated images are shown at 71 dpf, but simulated from a larval initial condition. Images of leopard zebrafish in (a) and cross mutations in (b) are reproduced from Irion *et al.* [33] and licensed under CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>); copyright Irion *et al.* Note 500 μm scale bar applies only to simulated images.

about xanthophores). In contrast, if mutation A crossed with pfeffer does not alter the pfeffer phenotype, this suggests this late-stage mutation affects xanthophores. Crosses of late-stage mutations with each other provide additional cues about which cell communication pathways may be altered.

From a modeling perspective, this is a puzzle: we are faced with a multiplication table of different late-stage mutations, crossed with each other and with early-stage mutations. Our goal is to produce predictions for each phenotype that are consistent with our other predictions across the full table. We approach this by first matching a single phenotype (e.g. leopard in Figure 4.41a); next we test whether crosses of this candidate leopard with early-stage mutations produce results that match the empirical images (e.g. Figure 4.41b). These steps are repeated until we obtain consistency across the candidate leopard and crosses with early-stage mutations. Finally, we cross the candidate leopard with our other candidate late-stage mutations (Figure 4.44), and go back to the first step as necessary. Our preliminary work presents this process for leopard (Figure 4.41) and schachbrett (Figure 4.42). Additionally, we include the newly described mutation idfix [16] (April 2016), as an example of a mutation for which early-stage crosses and some late-stage crosses do not seem to be available yet. By crossing schachbrett and idfix, as in Figure 4.44, we produce results that can be empirically tested.

Note: we model crosses of late-stage and early-stage mutations by using the candidate altered late-stage parameters together with turning off cell birth for the appropriate cell types missing (see §4.4.3). Crosses of late-stage mutations A and B, in turn, are done by using the union of both sets of altered parameters (if the same parameter is altered in both cases, we take the more severe adjustment).

This is preliminary work and represents candidate predictions. As we account for more late-stage mutations and associated crosses (for example, obelix, which has wider stripes [43]), the predictions produced will evolve and become more strongly supported by zebrafish dynamics. An additional means of strengthening predictions is by exploring if slowly varying the same parameters can reproduce different strengths of the same mutation (for example, leopard can vary from curved stripes

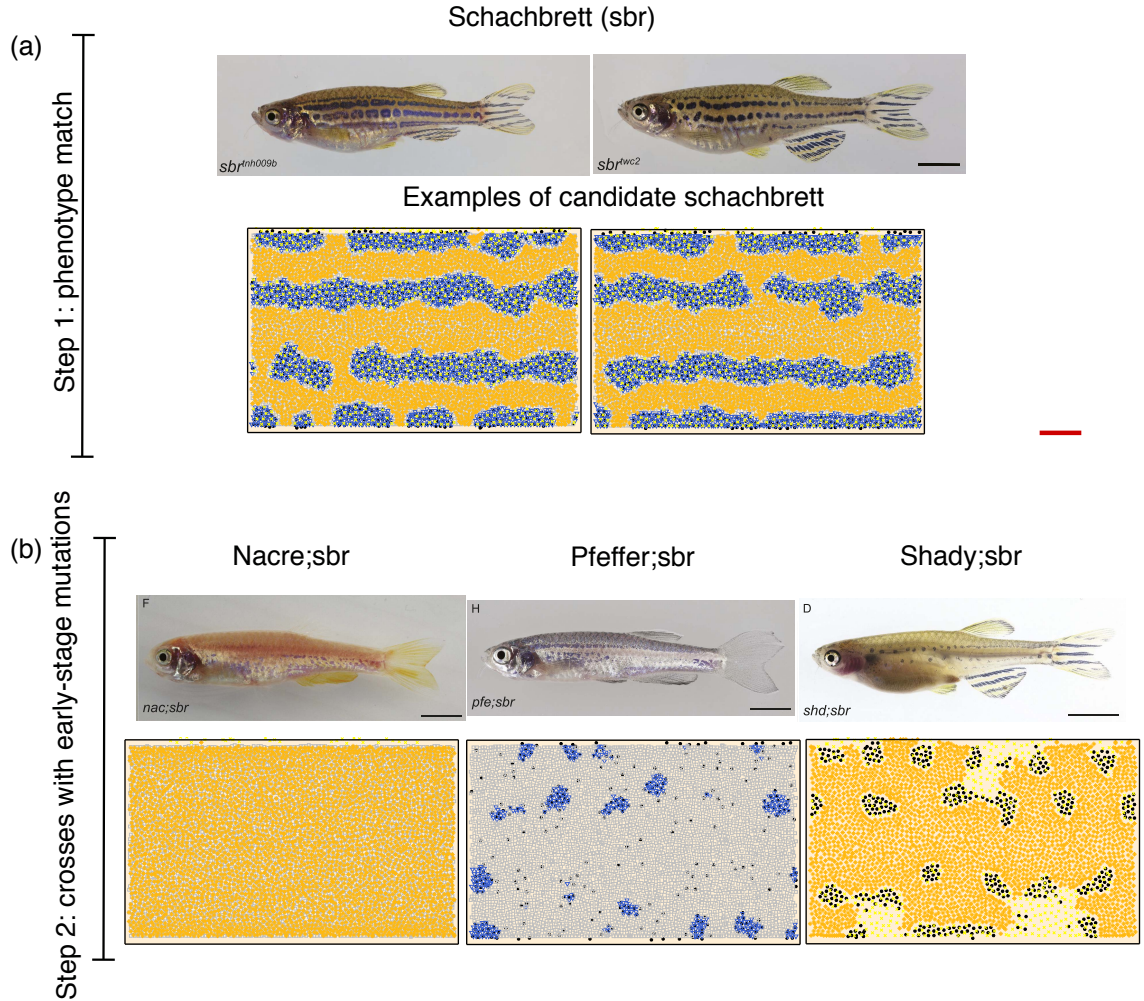


Figure 4.42: Schachbrett, a late-stage mutation described in 2015 in [14], features a checkerboard pattern. Preliminary work: to help identify altered interactions that lead to this pattern, (a) we first match the schachbrett phenotype; (b) crosses with early-stage mutations (simulated by using sbr parameters combined with removing the appropriate cells) provide a second step for validation. Empirical images from [14] are shown in comparison to simulated images. Final simulated images are shown at 71 dpf, but simulated from a larval initial condition. Images of schachbrett zebrafish in (a) and cross mutations in (b) are reproduced from Fadeev *et al.* [14] and licensed under CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>); copyright Fadeev *et al.*

to spots to a uniform pattern [33]).

In contrast to our intuition that would search for a single parameter that, when adjusted, could control the appearance of an altered phenotype, this does not seem to be the case biologically and was surprising at first. We briefly illustrate this here: one means of local communication between cells is through gap junctions, which are

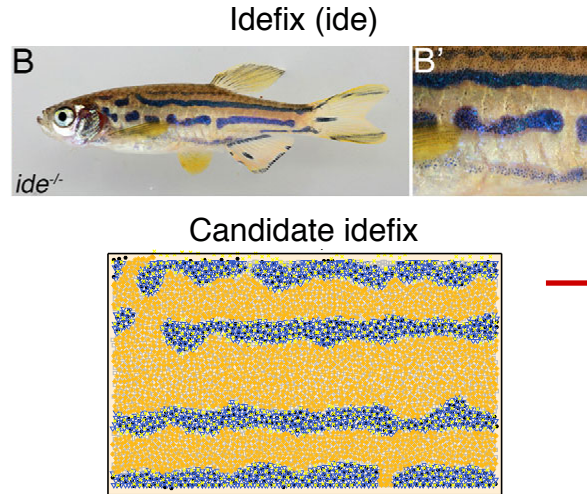


Figure 4.43: Idefix, a mutation described in April 2016 in [16], features thin, somewhat curved black stripes and wide yellow interstripes. To our knowledge, crosses of early-stage mutations with idfix are not experimentally available yet. Preliminary work: candidate idfix simulation at 71 dpf (simulated from a larval initial condition). Image of idfix zebrafish reproduced from Frohnhofer *et al.* [16] and licensed under CC BY 3.0 (<https://creativecommons.org/licenses/by/3.0/>); published by The Company of Biologists, Ltd.

made up of two connexons (connexons, in turn, are made up of 6 connexins) [87]. We think of gap junctions as the handshake between two cells, connexons as the hands, and connexins as the fingers. Notably, connexons can be made up of all the same connexins or more than one type, and the same connexin (finger) can play a role in many different gap junctions (handshakes) [87]. The leopard zebrafish gene, for example, encodes a connexin called Connexin41.8, which corresponds to a connexin in mammals [33, 87]: thus, this single genetic mutation could alter the function of multiple cell communication pathways and multiple parameters in our model (this may also open up the possibility for our model to help shed light more broadly on the structure of the pathways through which cells talk to each other in future work).

Briefly, leopard (Figure 4.41) is currently simulated by removing the signal from xanthophores to melanophores to differentiate (we suggest [36] as possible evidence for this choice, as well as the fact that leopard;shady phenotypes feature few or no black spots [33]); we also reduce how strongly melanophores inhibit dense iridophores

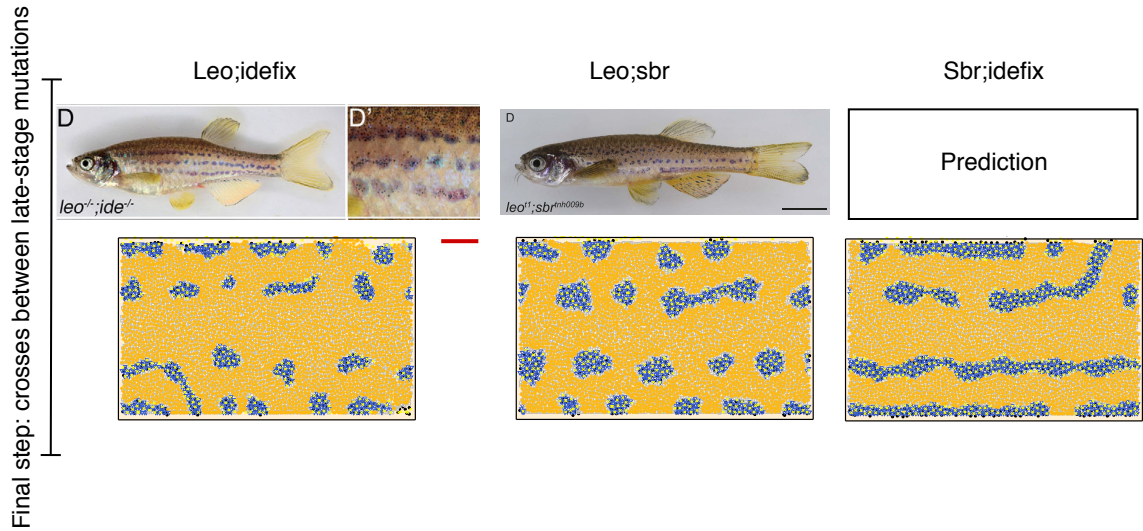


Figure 4.44: Empirical images from [14, 16] are shown in comparison to simulated results. Preliminary work: crossing our candidate late-stage mutations with each other provides the final test of whether our prediction is consistent with the dynamics arising from the altered genes. An additional test of predictions (not shown) is to vary mutation parameters slowly to reproduce varying strengths of the same genetic mutation. Leo;idefix zebrafish mutation is reproduced from Frohnhöfer *et al.* [16] and licensed under CC BY 3.0 (<https://creativecommons.org/licenses/by/3.0/>); published by The Company of Biologists, Ltd. Leo;sbr zebrafish mutation is reproduced from Fadeev *et al.* [14] and licensed under CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>); copyright Fadeev *et al.*

and compact xanthophores. Schachbrett (Figure 4.42), in turn, involves increasing dense iridophores speeds and making these cells less sensitive to cues to become loose. This is motivated, for example, by work showing iridophores spread across black stripe regions in dense form and become loose at a delayed time in schachbrett [33]. Lastly, idefix is simulated by removing the local attractive forces dense iridophores exert on compact xanthophores. We note this is preliminary work, and these candidate alterations may change as we account for more late-stage mutations and crosses.

4.6 Detailed model description

We consider independent cells of five types interacting on growing rectangular domains; while these pigment cells appear in three distinct 1-cell thick layers on the skin of zebrafish [27], we use a 2D domain as a modeling simplification and instead account for the additional dimension indirectly by allowing some cells to occupy the same point in space. Each cell position is thus given by its (x, y) coordinates on the domain. Our cells are separated into five distinct classes and modeled as point-masses, with the following notation:

$M_i(t)$ = position of i th melanophore at time t ;

$X_j^c(t)$ = position of j th compact xanthophore at time t ;

$X_i^s(t)$ = position of i th stellate/neutral xanthophore at time t ;

$I_j^d(t)$ = position of j th dense iridophore at time t ;

$I_i^\ell(t)$ = position of i th loose iridophore at time t ;

$N_M(t)$ = number of melanophores at time t ;

$N_X^c(t)$ = number of compact xanthophores at time t ;

$N_X^s(t)$ = number of stellate/neutral xanthophores at time t ;

$N_I^d(t)$ = number of dense iridophores at time t ;

$N_I^\ell(t)$ = number of loose iridophores at time t .

Because many experiments track pattern development in days, we measure time in days to quantify cell speeds and set our time step for cell differentiation, death and form changes. Space, in turn, is measured in micrometers (μm) or millimeters (mm).

Our cells interact by both local and long-range interactions, and we include four

types of cell behavior: migration, birth, death, and changes in form. Before describing the details of these interactions, we first overview the length scales present in our model and describe how our growth rates relate to zebrafish SSL and stages (see Chapter 2 for an introduction to zebrafish staging).

4.6.1 Length scales

Broadly, zebrafish patterning includes both short-range and long-range interactions. We think of short-range communication as occurring on the order of the distance between neighboring cells; these interactions may be regulated by short dendrite extensions or by direct contact (e.g. through gap junctions or potassium channels) [29, 33, 44]. Long-range interactions, in contrast, occur on the scale of multiple cell diameters, and may be regulated by pseudopodia or airneme extensions [12, 25]). Finally, it should be noted that the cells on the skin of zebrafish are arranged in three layers; this opens up the possibility for a third length scale to be present in the biology.

Throughout our models, we are sensitive to maintaining biologically reasonable long-range length scales; during early modeling, we found that longer length scales were easier to work with, so maintaining shorter long-range length scales introduces a challenge into our work. We model long-range interactions, possibly through pseudopodia or airneme extensions [12, 25], as occurring at length scales between 210 and 250 μm . As with our two-cell model in Chapter 3, long-range interactions involve an annulus, namely

$$\Omega_{\text{long}}^{\bar{x}} = \text{annulus centered at } \bar{x} \text{ of inner radius } 210 \mu\text{m} \text{ and outer radius } 250 \mu\text{m},$$

where $\bar{x}$ represents the location of the pigment cell or precursor reaching extensions outward toward its neighbors.

In comparison, Hamada *et al.* [25] report adult stripe width as roughly $600\ \mu\text{m}$, suggesting an upper bound of $\approx 300\ \mu\text{m}$ for our long-range length scales (airneme extensions have been reported to be up to $\approx 250\ \mu\text{m}$ in length [12]). We chose to use a length scale under $300\ \mu\text{m}$ in part because zebrafish stripes widen during growth (our observation from images in [58]); furthermore, melanophores expand over time [75], so we suspect their cell extensions also increase in time. Thus, because we focus on pattern formation until juvenile stages, we use a long-range length scale that is slightly narrower than half an adult stripe width (Adult is the stage after J++ [58]). By choosing an annulus to model long-range dynamics, we assume no directionality is inherent in cell communication; as an alternative, we could specify that dorsal cells extend dendrites ventrally, and ventral cells reach their extensions dorsally. Because an annulus domain includes cells in both stripes and interstripes, we expect it would be less challenging to fit model parameters to domains with inherent directionality.

What we consider short-range communication in our model involves length scales between about 30 and $90\ \mu\text{m}$. We think of these interactions as occurring by direct cell contact, possibly mediated by gap junctions or potassium channels, or through short dendrite or airneme extensions [12, 29, 33, 39, 43, 90]. According to Takahashi *et al.* [76], the average distance between neighboring melanophores is $\Delta_{mm} = 50\ \mu\text{m}$, between xanthophores is $\Delta_{xx} = 36\ \mu\text{m}$, and between stripes and interstripes is $\Delta_{xm} = 82\ \mu\text{m}$, so our short-range length scales are on the order of the distance between neighboring cells.

We recognize that it would be more elegant to include only one short-range length scale for our cell differentiation, death, and form change rules, more akin to the role

of Ω_{loc} in our two-cell models in Chapters 3 and 5, but we found our five-cell model required more length scales for these rules. Thus we introduce the notation below:

$$B_{45}^{\bar{x}} = \text{disc of radius } 45 \text{ } \mu\text{m centered at } \bar{x},$$

$$B_{75}^{\bar{x}} = \text{disc of radius } 75 \text{ } \mu\text{m centered at } \bar{x}, \text{ and}$$

$$B_{90}^{\bar{x}} = \text{disc of radius } 90 \text{ } \mu\text{m centered at } \bar{x},$$

where $\bar{x}$ is the location of the cell of interest. We expect that the need for these different length scales reflects two things: first, because some cells that occupy different layers on the fish skin can appear in the same location on our 2D domain, B_{45} could account for very local interactions between cell layers (the range of short-range length scales was particularly important in our cell form change rules). Second, while there are measurements of the average distance between two melanophores, between two xanthophores, and between melanophores and compact xanthophores available, we do not know of data on other cell-to-cell distances. We expect the short-range length scales used could be reduced by rescaling the model to account for new data on cell-to-cell distances.

The length scales present in our cell differentiation, death, and form change rules (as well as the notation used) are summarized in Table 4.3.

4.6.2 Domain growth

Analogous to our two-cell model in Chapter 3, each day cell positions are stretched across the domain in the vertical and horizontal directions in response to domain growth (zebrafish growth is uniform on the body, in contrast to the fins; see Chapter 5). Our simulations track patterning on the full width of the fish and roughly a

Name	Description	Source/Motivation
$\Omega_{\text{long}}^{\bar{x}}$	Annulus with outer radius $250 \mu\text{m}$ and inner radius $210 \mu\text{m}$ centered at $\bar{x}$	Chosen on the order of half a stripe width
$B_{90}^{\bar{x}}$	Disc of radius $90 \mu\text{m}$ centered at $\bar{x}$ accounts for short-range communication at light-dark boundaries	Chosen slightly larger than Δ_{xm}
$B_{75}^{\bar{x}}$	Disc of radius $75 \mu\text{m}$ centered at $\bar{x}$	Chosen slightly less than Δ_{xm}
$B_{45}^{\bar{x}}$	Disc of radius $45 \mu\text{m}$ centered at $\bar{x}$	Chosen to account for local communication, possibly between cells in different layers
$B_{\Delta_{xm}}^{\bar{x}}$	Disc of radius $\Delta_{xm} = 82 \mu\text{m}$ [76]	Appears only in cell birth over-crowding conditions

Table 4.3: Notation and values for length scales present in our cell differentiation, death, and form change rules.

third of its body length (after removing a $1500 \mu\text{m}$ region for the eye), beginning at stage PB (onset at 7.2-7.5 mm SSL) [58]. Thus, we assume a starting length of 7.5 mm SSL, corresponding to roughly 21 dpf (see Chapter 2 for a discussion of how caution should be used in associating measurements of time with stages and size). Our growth rates are slower than those used in §3 here: we specify that domains expand by $38 \mu\text{m}/\text{day}$ length-wise and $27 \mu\text{m}/\text{day}$ in height.

4.6.3 Cell migration

The movement of each cell is specified by an ODE that governs its interactions with other cells in the domain. Our ODEs are first order, so we consider a kinematic model in which velocity is directly proportional to the force between cells, a reasonable simplifying assumption since we are interested in the movement of small agents. As summarized in Table 4.1, all forces prescribed are purely repulsive, with the exception of an attractive force exerted by dense iridophores on compact xanthophores. We

first present the ODEs in our model and then give a detailed description of our motivation for each migration interaction included. In particular, the migration of melanophores is given by:

$$\begin{aligned} \frac{dM_i}{dt} = & \underbrace{\sum_{j=1, j \neq i}^{N_M} F^{MM}(\|M_j - M_i\|) \frac{M_j - M_i}{\|M_j - M_i\|}}_{\text{repulsion from melanophores}} + \underbrace{\sum_{j=1}^{N_X^c} F^{X^cM}(\|X_j^c - M_i\|) \frac{X_j^c - M_i}{\|X_j^c - M_i\|}}_{\text{repulsion from compact xanthophores}} \\ & + \underbrace{\sum_{j=1}^{N_I^d} F^{I^dM}(\|I_j^d - M_i\|)}_{\text{weak repulsion from dense iridophores}} ; \end{aligned}$$

Similarly, the ODEs for xanthophore migration, which depends on cell form, are:

$$\begin{aligned} \frac{dX_i^c}{dt} = & \underbrace{\sum_{j=1, j \neq i}^{N_X^c} F^{X^cX^c}(\|X_j^c - X_i^c\|) \frac{X_j^c - X_i^c}{\|X_j^c - X_i^c\|}}_{\text{repulsion from compact xanthophores}} + \underbrace{\sum_{j=1}^{N_X^{sn}} F^{X^sX^c}(\|X_j^s - X_i^c\|) \frac{X_j^s - X_i^c}{\|X_j^s - X_i^c\|}}_{\text{repulsion from stellate xanthophores}} \\ & + \underbrace{\sum_{j=1}^{N_M} F^{MX^c}(\|M_j - X_i^c\|) \frac{M_j - X_i^c}{\|M_j - X_i^c\|}}_{\text{repulsion from melanophores}} + \underbrace{\sum_{j=1}^{N_I^d} F^{I^dX^c}(\|I_j^d - X_i^c\|) \frac{I_j^d - X_i^c}{\|I_j^d - X_i^c\|}}_{\text{attraction to dense iridophores}} ; \\ \frac{dX_i^s}{dt} = & \underbrace{\sum_{j=1, j \neq i}^{N_X^s} F^{X^sX^s}(\|X_j^s - X_i^s\|) \frac{X_j^s - X_i^s}{\|X_j^s - X_i^s\|}}_{\text{repulsion from stellate xanthophores}} + \underbrace{\sum_{j=1}^{N_X^c} F^{X^cX^s}(\|X_j^c - X_i^s\|) \frac{X_j^c - X_i^s}{\|X_j^c - X_i^s\|}}_{\text{repulsion from compact xanthophores}} ; \end{aligned}$$

and the ODEs for iridophore movement are:

$$\begin{aligned} \frac{dI_i^d}{dt} = & \underbrace{\sum_{j=1, j \neq i}^{N_I^d} F^{I^dI^d}(\|I_j^d - I_i^d\|) \frac{I_j^d - I_i^d}{\|I_j^d - I_i^d\|}}_{\text{repulsion from dense iridophores}} + \underbrace{\sum_{j=1}^{N_I^\ell} F^{I^\ell I^d}(\|I_j^\ell - I_i^d\|) \frac{I_j^\ell - I_i^d}{\|I_j^\ell - I_i^d\|}}_{\text{repulsion from loose iridophores}} ; \\ \frac{dI_i^\ell}{dt} = & \underbrace{\sum_{j=1, j \neq i}^{N_I^\ell} F^{I^\ell I^\ell}(\|I_j^\ell - I_i^\ell\|) \frac{I_j^\ell - I_i^\ell}{\|I_j^\ell - I_i^\ell\|}}_{\text{repulsion from loose iridophores}} + \underbrace{\sum_{j=1}^{N_I^d} F^{I^d I^\ell}(\|I_j^d - I_i^\ell\|) \frac{I_j^d - I_i^\ell}{\|I_j^d - I_i^\ell\|}}_{\text{repulsion from dense iridophores}} . \end{aligned}$$

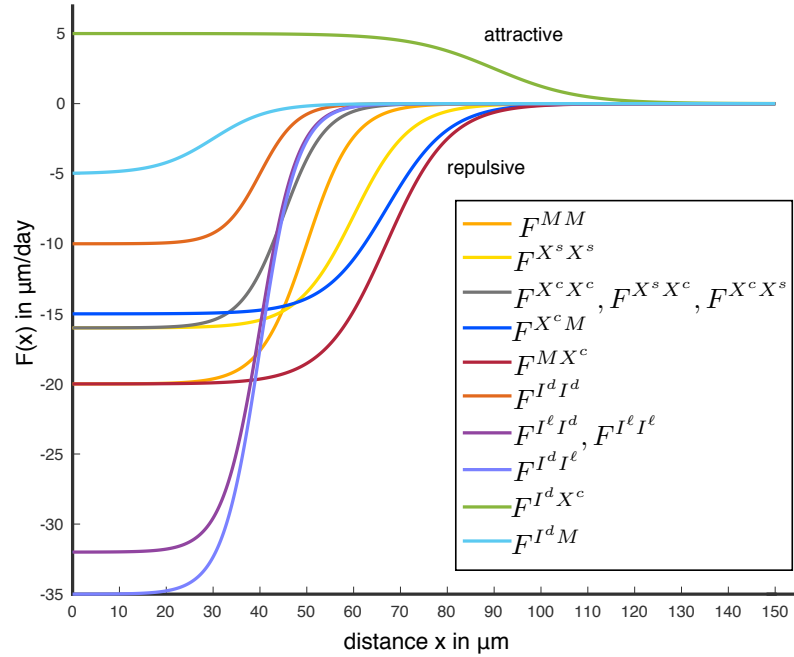


Figure 4.45: Forces used to model migration.

In all cases, the forces take the general form

$$F^{**}(d) = -R^{**} \left(\frac{1}{2} + \frac{1}{2} \tanh\left(\frac{r^{**} - d}{\delta}\right) \right),$$

where $\delta = 5$ serves to smooth out the hyperbolic tangent and $**$ denotes that the parameters used depend on the migration interaction. We chose to use hyperbolic tangents, rather than Morse potentials as in our two-cell models, because we expect a compactly supported force strength is more biologically faithful; these forces operate over a short scale, representing direct cell contact or dendrite extensions (see Figure 4.45). We use a forward Euler scheme with time step $\Delta t = 0.1$ days to simulate the ODEs.

Forces on melanophores: In our model, we assume melanophores exhibit two migratory interactions on the fish skin: they are repelled from other melanophores (to maintain appropriate cell densities) and from compact xanthophores. As discussed in §3.5.4, experiments [76] have shown that melanophores tend to spread out when they

have space to move; furthermore, because a single melanophore located $3\Delta_{mm}$ away from a group of other melanophores did not move away from the group in the experiments [76], we have an upper bound on the length scale over which repulsive forces act between melanophores. Nevertheless, the experimental data is complicated, and other studies have shown alternative perspectives on melanophore movement. In particular, Walderich *et al.* [82] transplanted wild-type melanophores into nacre and albino fish, and their data suggests melanophores tend to clump together to reach normal densities and cell-to-cell distances, even when there is open space available. Nüsslein-Volhard has also supported this perspective on melanophore-melanophore interactions; she suggested we model melanophore migration not through repulsive forces, but rather through a rule that requires that no two melanocytes occupy the same position (personal communication, 2015). (Note: the apparent disagreement between the findings on migration in [76] and [82] may result from the complexity of cell dynamics: many interactions are going on at the same time, and it is difficult to determine what underlying behaviors - for example cell competition or cell migration - are driving the observed macroscopic dynamics).

We account for these various perspectives on melanophore forces by specifying a short-range force that acts between neighboring cells (on the order of Δ_{mm}) apart. This force serves to separate the melanophores and ensure that normal black cell densities are achieved on the fish skin. In this way, we address both the work of Takahashi *et al.* [76] showing that melanophores repel each other and the studies of the Nüsslein-Volhard lab [82] that observe melanophores remaining in aggregated conditions. The average distance between two melanophores, Δ_{mm} determines the length scale of melanophore-melanophore forces in our model, and melanophore speeds, in turn, are based on experimental data from [76] suggesting melanophores migrate roughly 80-100 μm per week ($\approx 11\text{-}14 \mu\text{m}$ per day). Motivated by suggestions in

[17], we also specify weak repulsive forces on melanophores due to dense iridophores at short-range.

Forces on compact xanthophores: We model compact xanthophores as interacting through migration with xanthophores (both stellate and compact), dense iridophores, and melanophores. We assume compact xanthophores experience the same short-range repulsive force from neighboring xanthophores, regardless of cell form. This short-range force serves to spread the xanthophores out so that they evenly cover the fish skin domain while maintaining wild-type cell densities. It agrees with experiments [82] suggesting xanthophores tend to move into empty space as a cohesive net through differentiation and migration. It should be noted that Walderich *et al.* [82] describe this behavior as *attraction between xanthophores*, but we model this as a local repulsive force that accounts for experimentally measured cell-to-cell distances and then decays rapidly to zero to achieve correct cell-to-cell distances. Motivated by suggestions in [17], we also include repulsive forces from melanophores to compact xanthophores to account for the clear boundaries between stripe and interstripe regions, and attraction by dense iridophores. As described in §4.5.4, this attraction is crucial in our model, and removing it is sufficient to produce altered phenotypes resembling the *idfix* mutation [16].

Forces on stellate xanthophores: The forces on stellate xanthophores are simply to spread these cells out within the same layer at appropriate densities on the fish skin. Stellate xanthophores repel across a slightly longer length scale than compact xanthophores do to account for the lower density of these cells [44, 45].

Forces on dense and loose iridophores: We assume iridophores experience forces only from other iridophores. Walderich *et al.* [82] ablated iridophores in a region of X0 and found that iridophores filled these gaps by migrating horizontally

and differentiating. This shows that there is no dorsal-ventral directionality inherent in iridophore interactions; instead, it seems these cells spread outward from the horizontal myoseptum during development simply because this is where there is space for expansion [75, 82].

To keep our cells in the domain we use periodic boundary conditions in the x direction. At the top and bottom boundary of the fish skin domain, however, periodic boundary conditions did not seem the most natural. Instead, we implement an alternative rule: in a small $50\text{ }\mu\text{m}$ wide neighborhood of these boundaries we simply remove the component of a cell's velocity that is normal to the boundary. We doubt the choice of boundary conditions has a strong impact on model results, but it would be interesting to test this in future work.

4.6.4 Cell birth

Cell birth is implemented once per day at random locations based on deterministic rules (some random birth is also included).

Melanophores: We assume melanophores appear *in situ* on the fish skin through differentiation from precursor cells that we do not directly take into account (this is similar to our two-cell models). This choice is supported by experimental data showing melanophore progenitors arrive approximately in place in the locations of future dark stripes [4, 11, 75]. In particular, each day we randomly selected N_{diff} locations

on the fish skin domain and evaluate them for possible melanophore differentiation:

$$\underbrace{\sum_{i=1}^{N_X^c} \mathbb{1}_{\Omega_{\text{long}}^{\bar{x}}} X_i^c + \sum_{i=1}^{N_I^d} \mathbb{1}_{\Omega_{\text{long}}^{\bar{x}}} I_i^d}_{\text{long-range differentiation signal}} > \alpha + \beta \sum_{i=1}^{N_M} \mathbb{1}_{\Omega_{\text{long}}^{\bar{x}}} M_i \quad \text{and} \\
 \underbrace{\sum_{i=1}^{N_X^c} \mathbb{1}_{B_{\Delta_{xm}}^{\bar{x}}} X_i^c + \sum_{i=1}^{N_M} \mathbb{1}_{B_{\Delta_{xm}}^{\bar{x}}} M_i + \sum_{i=1}^{N_I^d} \mathbb{1}_{B_{\Delta_{xm}}^{\bar{x}}} I_i^d}_{\text{overcrowding condition}} \leq \eta \quad \implies \quad \text{melanophore birth at } \bar{x},$$

We considered several mechanisms for melanophore differentiation, setting $\alpha = 0$, $\beta = 0$, or leaving both $\alpha, \beta > 0$. Ultimately, we specified $\beta > 0$ based on the same ablation experiments [54] that informed our two-cell models. These ablation experiments [54] showed that xanthophores at a distance support melanophore birth *and* that melanophores at a distance inhibit melanophore birth. We chose $\beta = 3.5$, the same value used for our two-cell models. We think of $\alpha > 0$ as introducing a time delay between xanthophore and iridophore birth and melanophore precursor response; $\alpha = 3$ is used in our current model. Setting $\alpha = 0$ produces reasonable results (not shown), but leads to more frequent breaks in yellow stripes across many simulations. This is because melanophores immediately differentiate at X0 when $\alpha = 0$, in effect reproducing the presence of larval melanophores along the horizontal myoseptum; we are currently working on adjusting cell death and migration forces to remove α and account for larval melanophores.

Because melanophores appear within their own layer on the fish skin, but only in dark stripes, it is unclear how to account for melanophore overcrowding; thus, our overcrowding condition also introduces an assumption that melanophores are only overcrowded by dense iridophores and compact xanthophores (this may indirectly play the role of the local signal in our two-cell model that black cells prefer to be born near more black cells than yellow cells). Lastly, we place melanophores at 1%

of the randomly selected locations when the neighborhood is empty:

$$\sum_{i=1}^{N_M} \mathbb{1}_{B_{\Delta_{xm}}^{\bar{x}}} M_i + \sum_{i=1}^{N_X^c} \mathbb{1}_{B_{\Delta_{xm}}^{\bar{x}}} X_i^c = \gamma \quad \implies \quad \text{random melanophore birth at } \bar{x},$$

where here we assume melanophores are only overcrowded by compact xanthophores and other melanophores ($\gamma = 0$). This accounts for a small amount of noise (it would be interesting to test how much this value can be increased without destroying patterning; note that melanophores generally appear in place on the skin [82]; also see Figure 4.1).

Note: we explored a variety of alternative melanophore differentiation mechanisms, including making the number of random locations selected time-dependent (so that the number of precursors increases with the size of the domain); adjusting the conditions for overcrowding (so that melanophores are only overcrowded by other melanophores); introducing a short-range signal directly as in our two-cell models; specifying growing long-range length scales; choosing random locations based on a distribution with more weight at the center of the domain; and removing signals from iridophores or xanthophores, in turn.

Compact and stellate xanthophores: Our approach to xanthophore differentiation was one of the main places the cell interactions in our two-cell model could be improved, and we have accounted for this observation in our five-cell model. Instead of modeling xanthophore birth as occurring under similar (but opposite) conditions as melanophore birth, we now specify that xanthophores are born through cell differentiation from existing xanthophores on the fish skin. This choice is strongly supported by experimental data showing xanthophores differentiate from larval xanthophores (stellate xanthophores in our model), which are spread across the fish skin when pattern formation starts [45, 47]. Furthermore, [82] reports that xanthophores

divide at a rate of one cell division per 10 days. Thus, based on conversations with Prateek Mahalwar (personal communication, April 2015), we assume compact and stellate xanthophores differentiate by cell splitting from existing compact and stellate xanthophores, respectively, on the fish skin domain. In particular, each day we loop through every xanthophore present and determine if it is overcrowded; if it is not overcrowded, we place a new xanthophore of the same type randomly in a square of side length $10 \mu\text{m}$ centered at the current xanthophore:

$$\begin{aligned} \sum_{j=1}^{N_x^c} \mathbb{1}_{B_{\Delta_{xm}}^{X_i^c}} X_j^c + \sum_{j=1}^{N_x^s} \mathbb{1}_{B_{\Delta_{xm}}^{X_i^c}} X_j^s \leq \varphi &\implies \text{compact xanthophore appears near } X_i^c; \\ \sum_{j=1}^{N_x^c} \mathbb{1}_{B_{\Delta_{xm}}^{X_i^s}} X_j^c + \sum_{j=1}^{N_x^s} \mathbb{1}_{B_{\Delta_{xm}}^{X_i^s}} X_j^s \leq \psi &\implies \text{stellate xanthophore appears near } X_i^s, \end{aligned}$$

where $\varphi > \psi$ because xanthophores appear at higher densities in compact form [45]. These rules model differentiation of xanthophores by cell splitting from existing xanthophores when there is space (for example, [82] suggests increased distance between cells may trigger division). Note that we only consider the presence of other xanthophores when evaluating for overcrowding: this is because xanthophores occur in a separate layer than the other pigment cells, and, furthermore, it has been suggested that competition with other xanthophores regulates xanthophore number [26, 27, 82]. We also include a small amount (N_{rand}) of random differentiation of stellate xanthophores in empty locations:

$$\sum_{j=1}^{N_x^c} \mathbb{1}_{B_{\Delta_{xm}}^{\bar{x}}} X_j^c + \sum_{j=1}^{N_x^s} \mathbb{1}_{B_{\Delta_{xm}}^{\bar{x}}} X_j^s = \gamma \implies \text{stellate xanthophore born at } \bar{x},$$

where $\bar{x}$ is a location selected uniform randomly on the domain ($\gamma = 0$).

Dense and loose iridophores: Singh *et al.* [75] showed that iridophores differentiate from stem cells and enter the skin at the horizontal myoseptum; from there,

they spread across the fish as a sheet through birth and movement. Thus, similar to xanthophores, iridophores are born by differentiating from existing iridophores in our model (with the loose or dense form of the daughter cell determined by the parent cell). Analogous to our xanthophore differentiation rules, each day we loop through all the iridophores present on the fish skin domain and evaluate if they are overcrowded; if not overcrowded, a new cell of the same form is placed randomly in a square of side length $10 \mu\text{m}$ centered at the current iridophore:

$$\begin{aligned} \sum_{j=1}^{N_I^d} \mathbb{1}_{B_{\Delta_{xm}}^{I_i^d}} I_j^d + \sum_{j=1}^{N_I^d} \mathbb{1}_{B_{\Delta_{xm}}^{I_i^d}} I_j^\ell \leq \rho &\implies \text{dense iridophore appears near } I_i^d; \\ \sum_{j=1}^{N_I^d} \mathbb{1}_{B_{\Delta_{xm}}^{I_i^\ell}} I_j^d + \sum_{j=1}^{N_I^\ell} \mathbb{1}_{B_{\Delta_{xm}}^{I_i^\ell}} I_j^\ell \leq \rho &\implies \text{loose iridophore appears near } I_i^\ell. \end{aligned}$$

Based on the observation that iridophores divide more quickly than xanthophores [45], $\varphi, \psi < \rho$. Finally, unlike our rules for xanthophores, we do not include any random iridophore birth; this choice is based on experimental observations that iridophores spread outward from the horizontal myoseptum [75].

4.6.5 Melanophore death

Melanophores are the only cell type that experiences death through competition in our five-cell model (in place of cell death, we model xanthophores and iridophores as changing cell form). We account for black cell death here very similarly to how we modeled it in our two-cell body model; in particular, each day we evaluate each melanophore M_i on the fish skin domain for possible cell death through local com-

Name	Value	Description	Source/Motivation
N_{diff}	350	Number of random locations selected each day for possible melanophore birth according to deterministic rules	Reproduces dynamics
N_{init}^M	40	Number of melanophores on the domain in our initial condition	
N_{init}^X	500	Number of stellate xanthophores on the domain in our initial condition	Stellate xanthophores cover the fish skin [45]
N_{rand}	10	Number of random locations selected each day for possible random birth of stellate xanthophores	Motivated by testing ablation experiment 1 [89]
β	3.5	Lower bound on $\frac{\sum_{i=1}^{N_X^c} X_i^c + \sum_{i=1}^{N_I^d} I_i^d}{\sum_{i=1}^{N_M} M_i} _{\Omega_{\text{long}}}$ for M differentiation	Same value used in §3
η	4	Upper bound on $(\sum_{i=1}^{N_M} M_i + \sum_{i=1}^{N_X^c} X_i^c + \sum_{i=1}^{N_I^d} I_i^d) _{B_{\Delta_{xm}}}$ for M differentiation	Chosen based on cell-cell distance measurements [76]
φ	6	Upper bound on $(\sum_{i=1}^{N_X^c} X_i^c + \sum_{i=1}^{N_X^s} X_i^s) _{\Delta_{xm}}$ for X^c differentiation	Chosen based on cell-cell distance measurements [76]
ψ	1	Upper bound on $(\sum_{i=1}^{N_X^c} X_i^c + \sum_{i=1}^{N_X^s} X_i^s) _{B_{\Delta_{xm}}}$ for X^s differentiation	Chosen based on cell-cell distance measurements
ρ	7	Upper bound on $(\sum_{i=1}^{N_I^d} I_i^d + \sum_{i=1}^{N_I^\ell} I_i^\ell) _{\Delta_{xm}}$ for I^d or I^ℓ differentiation	Chosen because iridophores proliferate more and are smaller than other cells
γ	0	Appears in conditions for random melanophore and stellate xanthophore birth	Birth due to noise occurs only in empty regions

Table 4.4: Cell birth parameters.

petition with compact xanthophores by:

$$\sum_{j=1}^{N_X^c} \mathbb{1}_{B_{90}^{M_j}} X_j^c > \mu \sum_{j=1}^{N_M} \mathbb{1}_{B_{90}^{M_i}} M_i \implies \text{death of } M_i,$$

Name	Value	Description	Source/Motivation
μ	1.25	Lower bound on $\frac{\sum_{i=1}^{N_X^c} X_i^c}{\sum_{i=1}^{N_M} M_i} _{B_{75}}$ for M death	Short-range competition [54]
ξ	2	Lower bound on $\frac{\sum_{i=1}^{N_M} M_i}{\sum_{i=1}^{N_X^c} X_i^c} _{\Omega_{\text{podia}}}$ for M death	Long-range survival signals [54]
η	3	Upper bound on $\sum_{i=1}^{N_I^d} I_i^d _{B_{45}}$ for M death	Set to account for freezing experiments; reproduces dynamics
p_{death}	0.0333	Probability of random M death	Based on [54]

Table 4.5: Melanophore death parameters.

where $\mu = 1.25$. This rule accounts for the short-range competition described by Nakamasu *et al.* [54]. Additionally, we include the observation from [54] that xanthophores in interstripes provide a long-range survival signal to melanophores:

$$\sum_{j=1}^{N_M} \mathbb{1}_{\Omega_{\text{long}}^{M_i}} M_i \geq \xi \sum_{i=1}^{N_x^c} \mathbb{1}_{\Omega_{\text{long}}^{M_i}} X_j^c \quad \text{and}$$

$$\sum_{j=1}^{N_I^d} \mathbb{1}_{B_{45}^{M_i}} I_j^d < \nu \implies \text{death of } M_j \text{ with probability } p_{\text{death}} \text{ per day,}$$

where $p_{\text{death}} = 0.0333$ based on [54], $\xi = 2$, and $\nu = 3$. The inclusion of a local survival signal from dense iridophores to melanophores accounts for the pfeffer mutation: because there are no xanthophores present in pfeffer, yet melanophores survive, we needed to include some reason for them not to continually die. We initially modeled this as a long-range survival signal from dense iridophores, akin to the one from compact xanthophores, but this did not account for experiments in which xanthophores, but not dense iridophores, are removed from the fish skin and melanophore densities decrease strongly (see §4.5.3). Introducing a local survival signal from loose iridophores gave good agreement with these results (see §4.5.3 for details).

4.6.6 Xanthophore and iridophore form transitions

We model xanthophores changing form between compact and stellate and iridophores transitioning between dense and loose through rules that depend on the number of cells in the discs B_{45} , B_{75} , and B_{90} and the long-range annulus Ω_{long} . Each day each xanthophore and iridophore on the fish skin is simultaneously evaluated for form transitions according to the rules summarized below. Because our motivation for these rules is discussed in detail in §4.5, we do not address motivation in this section.

Parameters are summarized in Table 4.6; note that we expect that these parameters can be rescaled and reduced. Because of the open nature of questions surrounding iridophore interactions, it is the overarching structure of our cell form change rules, not the parameters in them, that we suggest have biological meaning. In future work, we would like to further explore the possibility of reducing the number of short-range length scales or parameters in our cell form rules.

Xanthophores: Compact xanthophore X_i^c becomes stellate when

$$\sum_{j=1}^{N_I^\ell} \mathbb{1}_{B_{75}^{X_i^c}} I_j^\ell > a + \sum_{j=1}^{N_I^d} \mathbb{1}_{B_{45}^{X_i^c}} I_j^d,$$

where $a = 2$. Note that this parameter is not meant to suggest that an xanthophore requires signals from 2 more loose iridophores than dense iridophores to change form; instead, we think of this as capturing stochasticity on the fish skin (e.g. cell dendrites reach outward and successfully find another cell). The same note applies to our other parameters as well.

Stellate xanthophore X_i^s becomes dense when

$$\sum_{j=1}^{N_I^d} \mathbb{1}_{B_{45}^{X_i^s}} I_j^d + P_i \sum_{j=1}^{N_X^c} \mathbb{1}_{B_{75}^{X_i^s}} X_j^c > b + \sum_{j=1}^{N_I^\ell} \mathbb{1}_{B_{45}^{X_i^s}} I_j^\ell + \sum_{j=1}^{N_M} \mathbb{1}_{B_{90}^{X_i^s}} M_j,$$

where P_i is a Bernoulli random variable with mean $p = 0.5$, and $b = 1$.

Iridophores: Loose iridophore I_i^ℓ becomes dense when one (or both) of two conditions is satisfied. The first condition is given by the rule:

$$\sum_{j=1}^{N_M} \mathbb{1}_{B_{90}^{I_i^\ell}} M_j < c \text{ and } \sum_{j=1}^{N_X^c} \mathbb{1}_{\Omega_{\text{long}}^{I_i^\ell}} X_j^c < d \implies I_i^\ell \text{ transforms to dense.}$$

Alternatively, I_i^ℓ becomes dense based on the rule:

$$\sum_{j=1}^{N_M} \mathbb{1}_{B_{90}^{I_i^\ell}} M_j < c \text{ and } \sum_{j=1}^{N_X^c} \mathbb{1}_{B_{75}^{I_i^\ell}} X_j^c > h \implies I_i^\ell \text{ transforms dense,}$$

where $c = 3$, $d = 9$, and $h = 3$.

Dense iridophore I_i^d becomes loose when one (or both) of two conditions is satisfied. First, we specify that:

$$\sum_{j=1}^{N_M} \mathbb{1}_{B_{90}^{I_i^d}} M_j > e \implies I_i^d \text{ transforms to loose,}$$

where $e = c = 3$. Alternatively, I_i^d can become loose according to:

$$\sum_{j=1}^{N_X^c} \mathbb{1}_{\Omega_{\text{long}}^{I_i^d}} X_j^c > f \text{ and } \sum_{j=1}^{N_X^c} \mathbb{1}_{B_{75}^{I_i^d}} X_j^c < g \implies I_i^d \text{ transforms to loose,}$$

where $f = 5$ and $g = 2$.

Name	Value	Description
a	2	Appears in xanthophore compact-to-stellate form transition rule
b	1	Appears in xanthophore stellate-to-compact form transition rule
c	3	Appears in iridophore loose-to-dense form transition rule
d	9	Appears in iridophore loose-to-dense form transition rule
e	3	Appears in iridophore dense-to-loose form transition rule
f	5	Appears in iridophore dense-to-loose form transition rule
g	2	Appears in iridophore dense-to-loose form transition rule
h	3	Appears in iridophore loose-to-dense form transition rule
p	0.5	Probability of compact xanthophores affecting stellate-to-compact transitions

Table 4.6: Parameters in cell form change rules. We expect these parameters could be rescaled and stress that it is the structure of our form change rules, not the parameters in them, that we suggest have biological value.

4.7 Discussion

In this chapter we have put forward an initial step toward a more comprehensive picture of the cell interactions that lead to wild-type and mutated patterns on the skin of zebrafish. Melanophores, xanthophores in two forms, and iridophores in two forms (five cell types in total) are modeled as independent agents that interact through differential equations for migration and discrete-time, stochastic rules for birth, death, and change in cell form. To account for the current unknowns about iridophores in the experimental literature, we separate zebrafish dynamics into sets for model development and testing. The final minimal network of cell interactions we identified is shown in Figure 4.46. This network indicates that:

- (1a) Loose iridophores support melanophore survival locally;
- (1b) Melanophores select iridophores in loose form over dense, inducing their

form changes locally;

- (2) Dense iridophores are born by cell division;
- (3) Compact xanthophores are born by cell division; they also weakly induce stellate xanthophores to become compact locally;
- (4) Melanophores inhibit their own birth and survival at long-range;
- (5a) Compact xanthophores compete with melanophores at short-range;
- (5b) Melanophores prevent stellate xanthophores from becoming compact;
- (6) Compact xanthophores support melanophore birth and survival at long-range;
- (7) Dense iridophores support melanophore birth at long-range;
- (8a) Dense iridophores promote compact over stellate xanthophores locally, inducing form changes (and loose iridophores have the opposite effect); dense iridophores also exert an attractive force on compact xanthophores;
- (8b) Compact xanthophores select iridophores in dense form over loose, inducing form changes locally;
- (9) Compact xanthophores select loose iridophores over dense iridophores at long range, inducing form changes at a distance.

These interactions are consistent with wild-type development as well as early-stage mutations (pfeffer, nacre, shady, nacre;pfeffer, shady;nacre, and pfeffer;shady), choker and puma mutations, and degeneration/regeneration experiments that artificially perturb stripes.

One of the main places our model could be improved is the inclusion of larval melanophores: melanophores, leftover from the larval pattern, are initially present in the center interstripe region but are cleared through death and movement [76]. We are currently working to include these cells, as well as to slow the speed of our melanophore birth, so that black cells more gradually fill in stripes. It would

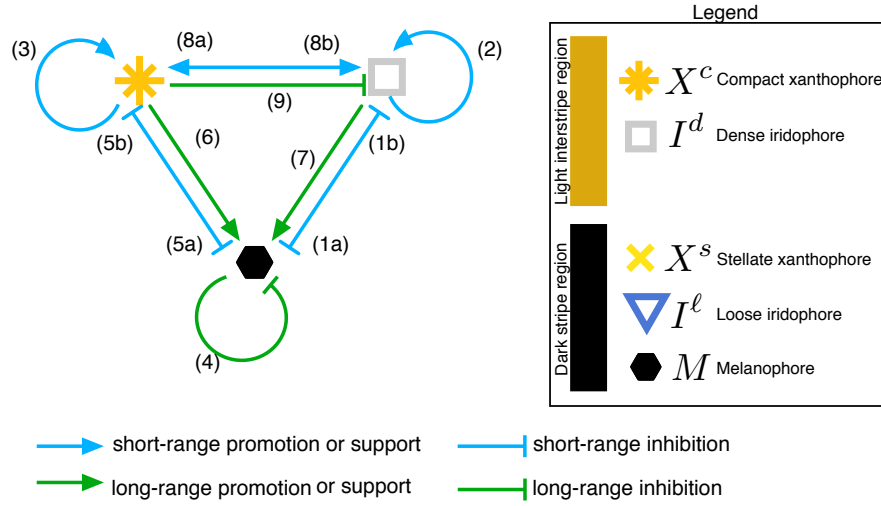


Figure 4.46: Final schematic showing cell interactions proposed in this chapter. To make our results more viewable, we do not include stellate xanthophores and loose iridophores in this network directly, but incorporate their effects in the promotion and inhibition influences described in the text. Note that two-sided edges denote mutual effects.

also be interesting to test how adding some noise to our parameter values impacts patterning: our initial work (not shown) shows that patterning is fairly robust to choosing long-range length scales from a distribution.

Regarding future work, there are two main directions: modeling and analysis. We are currently using the model developed to make predictions about late-stage mutations; as discussed in §4.5.4, our preliminary work suggests spotted phenotypes may involve alterations to the instructions melanophores and xanthophores give iridophores, in agreement with suggestions in [14, 33]. From an analytical perspective, it would be interesting to consider a PDE model of cell interactions based on the network in Figure 4.46. It is possible, for example, that the spreading of iridophores across the fish skin could be accounted for with density-dependent motion [6]. A stability analysis of the continuum limit, together with agent-based modeling, could be used to more comprehensively identify altered interactions in mutations.

CHAPTER FIVE

Extensions to the caudal fin

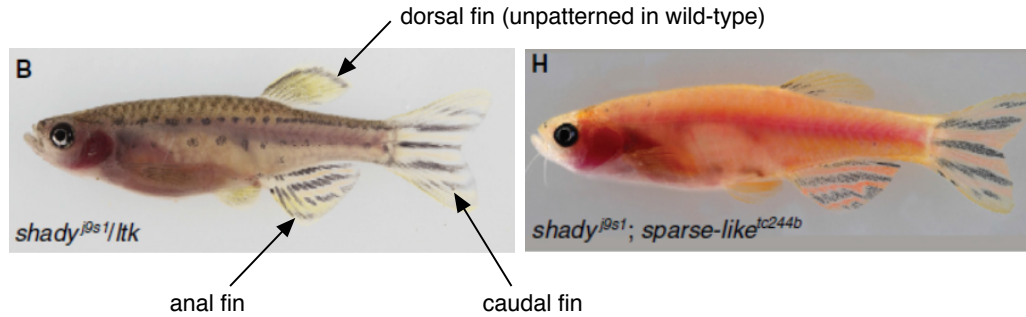


Figure 5.1: Left: the *shady* mutation lacks iridophores; it features disrupted body patterns, but the fins remain largely unchanged [17]. Right: the *shady*;*sparse-like* phenotype sharply highlights differences in the body and fin patterns. Images reproduced (with adaption: the addition of arrows and fin names) from Frohnhöfer et al. [17] and licensed under CC-BY 3.0 (<https://creativecommons.org/licenses/by/3.0/>); published by The Company of Biologists, Ltd.

5.1 Introduction

Striking black stripes and yellow interstripes appear not only across the body of the zebrafish but also on its anal (bottom) and caudal (back) fins, as shown in Figure 5.1. While it may seem natural for the same cell interactions to drive patterning in both regions, mutations [14] in which body patterns are altered but the fins remain unchanged complicate this picture. These phenotypes include both early- and late-stage mutations: most strikingly, *shady* mutations, which lack iridophores, feature spotted body patterns, but the fins maintain stripes [17]. This suggests iridophores are not actively involved in patterning on the fins [14, 17, 33], and that melanophores and xanthophores are enough to drive stripe formation there [86]. The timeline of pattern development on the body and fins is also very different, most notably with respect to how directionality is specified. While body stripes and interstripes are added sequentially to the edges of an existing horizontal marker at the horizontal myoseptum [17], caudal fin patterns seem to form outward as the fin grows, as shown in Figure 5.2.

While much work, spanning both biological and mathematical perspectives, has

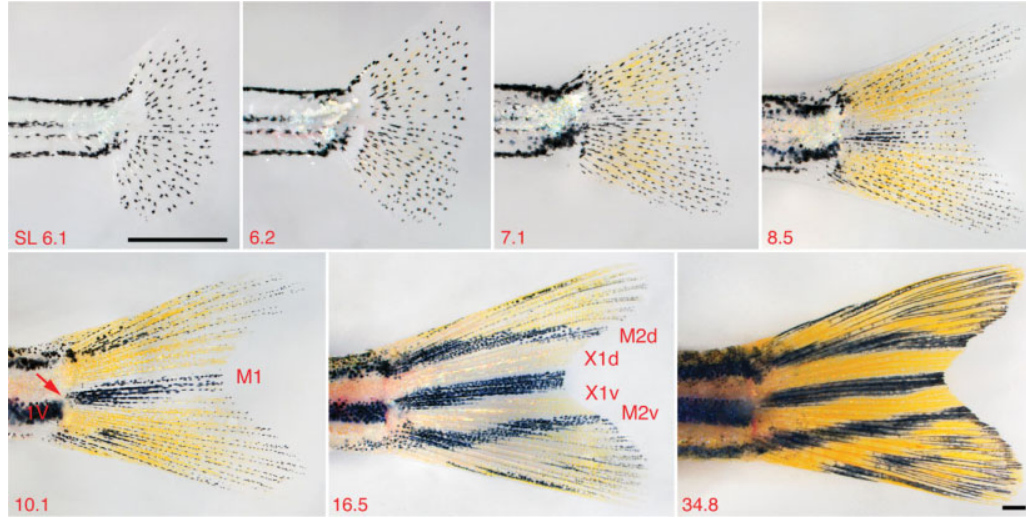


Figure 5.2: Timeline of wild-type pattern formation on the caudal fin of zebrafish shows outgrowth of stripes, in contrast to sequential addition of stripes at the edges of the existing pattern on the body [75]. Red arrow shows stripes becoming contiguous across the body and fin later in development [58]. Images are reproduced from Parichy *et al.* [58] with permission from John Wiley & Sons, Inc.

been done in the past to identify cells involved in zebrafish patterning and elucidate key interactions, we are aware of comparatively few results focused specifically on patterning on the fins. On the experimental side, melanophores and xanthophores extracted from fins are occasionally used in *in vitro* experiments [29, 30, 87, 89]. We highlight the work of Tu and Johnson [78] for its study of melanophore lineages, cell precursors, and the direction melanophores spread on the caudal fin; additionally, Parichy *et al.* [58] provide a wealth of images of stripe formation on zebrafish as a whole. The tailfin is particularly amenable to amputation experiments, and the studies [22, 23, 38, 67, 70, 79] all involve amputating the fin and observing regrowth. Furthermore, several studies (for example, [13, 35, 50, 52, 61, 68]) discuss the impact of mutations on the fins of zebrafish (*Danio rerio*) or other *Danio* fish. Much of the experimental work [20, 21, 24, 32, 66] is interested in understanding fins from the perspective of growth or skeleton development, however, not pigment patterning. From a mathematical perspective, in particular, Rolland-Lagan *et al.* [71] put forward a model of bone ray growth and joint patterning.

Opinions on the presence of differences in cell interactions between the body and fins diverge in the experimental community, with some researchers hypothesizing melanophores and xanthophores behave the same in both domains [85, 86]. Studying the tailfin is thus an important problem from the perspective of zebrafish patterning research because it allows for more broad comments on pigment cell interactions and, especially, on the role of iridophores, on the body and fins [40]. It gives a more comprehensive picture, as the fact that iridophores do not seem to be needed on the caudal fin is in some ways a sticking point that raises questions about their role on the body. A better understanding of fin patterns can also contribute to applications outside of zebrafish stripe research: most notably, the ability of the caudal fin (and associated pattern) to regenerate after amputation suggests possible applications to wound healing and tissue regeneration [60].

The previous mathematical studies [3, 5, 7, 18, 53, 54, 56, 88] of zebrafish patterning, overviewed in Chapter 1, have either focused on how stripes form on the body or have made no attempt at differentiating between patterning on the body and the fins. Nevertheless, the role of iridophores, the impact of genetic mutations, and the timeline of growth and development suggest key differences are present in these two regions. Thus, we turn to a short modeling study of the development of stripes on the caudal fin at the tail end of this thesis.

Based on the experimentally-accepted hypothesis that iridophores are not involved in stripe formation on the fins [86], we develop a two-population model of patterning on the caudal fin in this chapter. In this initial step toward a more comprehensive view of patterning on zebrafish from a mathematical perspective, we are interested in elucidating how horizontal stripe alignment is specified and identifying if there are necessary differences between the body and fin dynamics. Our fin model is based on the two-cell body model presented in Chapter 3, enabling us to directly

test if the same cell interactions that replicate pattern formation on the body can account for fin stripes.

Outline: In the next section, §5.1.1, we discuss differences between the zebrafish body and caudal fin, with the purpose of highlighting the unknowns and questions that drive our work. In §5.2, we summarize the results of this chapter, and, in §5.3, we present a brief overview of the different modeling mechanisms we explore. Our results on wild-type development are covered in §5.4. We summarize our short fin study and suggest some model improvements and directions for future work in §5.6. For more technical details on simulation set-up, model rules, and parameter values, see §5.5.

5.1.1 Differences between the body and fins

While the simplest explanation for patterning on the body and fins is that the same mechanisms are at work in both of these regions on zebrafish, we identify seven main differences that complicate the picture and motivate our study, as discussed below:

1. **Fewer cell types:** The observation that shady zebrafish, which lack iridophores, display spotted body patterns but unaltered fins suggests iridophores may not have an active role in caudal fin patterning [17]. Instead, present data suggests only melanophores and xanthophores participate in pattern formation on the caudal fin. Furthermore, the arrangement of cells on the body and tailfin is different [27] (see §2.2). Notably, iridophore placement on the fins is not correlated with stripe arrangement [86].

2. **Effect of late-stage mutations:** Differences between body and fin patterns

are not limited to the shady mutation. While some late-stage mutations (e.g. leopard and obelix [43]) lead to altered patterns on both the body and fins, others (e.g. luchs [33]) do not strongly impact fin patterning. This could suggest that pigment cells on the body and fins arise from different precursors [60], or could indicate differences in wild-type cell interactions in these two domains. If iridophores are indeed not active on the fin, one explanation could be that leopard and obelix only alter cell interactions involving iridophores. The specific interactions impacted in these mutations remain to be uncovered, however, so it cannot be ruled out that some late-stage mutations impact melanophores and xanthophores differently depending on their position on the fish.

3. Stripe directionality specification: It has been shown that the horizontal myoseptum [17] initially specifies stripes to form horizontally on the zebrafish body, and, as we discussed in Chapters 3 and 4, spreading iridophores play a crucial role in directing later interstripes to form [75] (see Figure 4.1). The horizontal myoseptum is not present on the caudal fin, nor do iridophores seem to be actively involved in fin patterning, making it unclear what directs stripes to form horizontally. Interestingly, experiments with a temperature sensitive mutation by Parichy and Turner [61] (see §4.4.8 for simulations of these experiments on the body) lead to vertical stripes on the caudal fin.

4. Timeline of development: On the zebrafish body, stripes are added sequentially to the edges of the existing pattern. As the boundary of free space is parallel to the developing stripes, each new stripe seems to emerge across the full length of the body at the same time. In contrast, on the caudal fin, images suggest stripes, which still seem to arise sequentially, grow outward from the proximal edge (where the fin connects to the body) toward the distal (posterior) end. In particular, Tu and Johnson [78] describe the melanocytes spreading from proximal to distal (we

suspect melanocytes and melanophores are interchangeable terms here). Thus, on the caudal fin the boundary of free space seems to be perpendicular to the developing stripes (see Figure 5.6).

5. Intermingling of melanophores and xanthophores: Notably, the distal end of the caudal fin features intermingled melanophores and xanthophores (see images in [58]), in contrast to what we would expect on the body, where short-range competition [54] discourages coexistence of these two cell types in the same region.

6. Growth: While the body grows uniformly, the caudal fin mainly grows by the addition of new segments to the distal ends of bone rays [20, 31, 32, 86]. Interestingly, the longfin zebrafish, a mutation featuring abnormally long fins [66], can display bifurcating stripes near the distal end of the fin (Nüsslein-Volhard, personal communication, January 2015). It is worthwhile to note that, together with the intermingling of black and yellow cells on the distal tip of the fin, this suggests that a more traditional Turing mechanism [80] could be present there.

7. Bone rays: Lastly, the fin features boney rays not present on the body. Iridophores appear along these rays, and, based on the images in [58], black melanophores seem to be associated with the bones. Notably, the early work of Goodrich and Nichols [22] mentions pigment cells lining up along the sides of these rays in the anal fin. Furthermore, Goodrich *et al.* [23] describe melanophores first invading light interstripes along fin rays (and only later appearing between fin rays) after freezing experiments that disrupt patterning on the caudal fin; their amputation experiments [23], in turn, led to an initial appearance of melanophores along and between bone rays.

These differences raise several questions:

- How is directionality specified on the caudal fin?
- Are different types of pigment cells involved? e.g. why do the cells intermingle at the distal end of the caudal fin?
- How do cells enter the caudal fin? Is there any dependency on or interaction with the body pattern?
- How does fin growth impact patterning? Do the fins grow only at the end or across the whole regime?
- What is the role of bone rays?

These questions serve as the driving motivation for our model. We focus specifically on the caudal fin, because there seems to be more experimental literature available on this region, and leave the anal fin for future studies. Our main goal is:

Goal: Help identify necessary differences and similarities between wild-type body and fin patterning and elucidate what mechanisms are capable of specifying horizontal stripe alignment on the caudal fin.

Because the shady mutation, which lacks iridophores, displays normal fin patterns [17], we approach this problem by considering a two-population model of black melanophores and yellow xanthophores interacting on growing fin domains. This model is based on our previous body model in Chapter 3: by testing an existing body model on a new domain, we can naturally comment on whether melanophore and xanthophore interactions present on the body are sufficient for explaining patterning on the fin.

After testing our body model on a caudal fin domain, we consider alternatives and slight adjustments to explore the role of growth and bone rays in fin patterning, as well as comment on the intermingling of melanophores and xanthophores at the

distal edge of the fin. In contrast to our studies of pattern formation on the body, where we are aware of more experimental data available, here we do not attempt to describe mutations and regeneration experiments; instead, our focus for this short initial study is entirely on wild-type patterning. This is because the questions we are asking about fins are at a more elementary level at this point, so our goal is to put an initial modeling frame of reference on caudal fin patterning, comparing dynamics on the fin to those on the body, suggesting some plausible mechanisms, and placing some delimiters around the realm of possibilities. We approach this problem by first putting forth several hypotheses for patterning mechanisms, and then comment on which are sufficient to reproduce the dynamics on the caudal fin.

5.2 Summary of results

Key differences exist in the timeline of development and the dynamics present on the zebrafish body and caudal fin, and these differences lead to several questions, described in §5.1.1, that motivate our work. We use our model to comment on these questions, relate our work to pattern formation on the zebrafish body, and raise questions for future research. Our main findings indicate that:

- caudal fin growth can align stripes if growth occurs uniformly, not limited to the distal edge;
- fin bone rays may be involved in directing stripes to form horizontally;
- different populations of xanthophores and melanophores may be needed to account for differences in patterning between the center and the distal edge of the caudal fin.

Furthermore, our model supports experimental evidence from mutational analysis that melanophores and xanthophores are sufficient for pattern formation on the caudal fin, and iridophores are unnecessary in this region. By beginning with a model of pattern formation on the zebrafish body and making only minor alterations, we also argue that the interactions between melanophores and xanthophores may be largely unchanged on the body and caudal fin.

5.3 Brief model description

An outgrowth of the two-cell model presented in Chapter 3, this model accounts for the interactions of melanophores and xanthophores on growing fin-shaped domains. As discussed in Chapter 2, Hirata *et al.* described how the organization of the zebrafish pigment pattern differs between the body and fins: on the fins, dark stripe regions are made up of a layer of xanthophores sandwiched between two sheets of black melanophores, and light interstripes contain a sheet of xanthophores [27]. While fins also contain iridophores, they do not match up with the stripes like on the body [86]. In this first model we do not consider multiple layers of melanophores and xanthophores in black stripes; instead, we describe a single layer of cells, with black melanophores in black stripes and yellow xanthophores in interstripes.

As with our previous work, we use an agent-based approach, modeling pigment cells as point masses that undergo deterministic migration and stochastic birth and death. The boundary conditions used for our simulations are roughly Neumann (see §5.5 for details), and we consider set-ups with and without domain growth, though most of our simulations are done on growing domains. When growth is included, we simulate development from when the fish is roughly ≈ 7.1 mm SL (18 dpf) to

when it reaches adult stages (150 dpf); on stationary domains, we use a caudal fin domain at adult stage. We track the full fin domain in our simulations, with the shape determined directly by tracing curves around images in [58] and interpolating between these curves.

In addition to domain shape, the main difference between the body model described in Chapter 3 and the current work is the presence of bone rays. Based on [71] and images in [58], we specify 18 rays, separated ≈ 3 degrees apart, on our domains (see §5.5 for details). As described in §5.4 (for implementation details, see §5.5), we consider different model set-ups in which cells may be affected by these rays. In a control set-up, we assume no interactions with the bones; we also test whether cell positions may stretch along the rays as the fish grows; finally, motivated by images in [58] that show melanophores in close association with the bones in some places on the caudal fin (see Figure 5.2) and personal communication with April Dinwiddie (March-April 2015), we test whether melanophore migration or differentiation may be influenced by the rays.

Biologically, zebrafish fins mainly grow at the distal end, with new bone segments added to the distal edge of each ray [20, 31, 32, 86]. As discussed in [20] and in §5.4.2, this could be accompanied by various different means of epithelium growth. Thus, we test two types: epithelial growth limited to the distal edge, which we think of as modeling skin growth by differentiation of cells at the distal edge (here we assume cell positions are not stretched or altered as the fin grows); and epithelial growth across the whole domain, with the skin stretching across the bones (here we assume cell positions are stretched with growth along the bones; for details see §5.5). As with our previous models, we track time using standardized standard length [58] when growth is included (though we also note time in days).

5.3.1 Cell migration

Cell migration is specified by Equations 3.1, 3.2, and 3.3, the same as for the two-cell body model in Chapter 3. As described in detail §5.5, the parameters used in these equations are different than their body equivalents in some of our simulations. More broadly, we continue to assume melanophores are repelled from xanthophores and other melanophores; xanthophores, in turn, are repelled from other xanthophores. This is motivated by *in vitro* experimental studies [29, 90] showing melanophores extracted from fins move away from dendrites extended toward them by xanthophores; while the *in vitro* study [90] did not find clear directional movement when pairs of melanophores or pairs of xanthophores were observed, we continue to include repulsive interactions between like cells to maintain cell-to-cell distances (for example, [76] indicates melanophores repel each other on the body). Xanthophores extracted from the fins seem to be attracted to fin melanophores *in vitro* [90], and, when included, this behavior is modeled the same way in our fin simulations and in our two-cell body model work: we specify that melanophores provide contact attractive forces and short-range repulsive forces on xanthophores (see §3.4.1 for a more detailed discussion of chase-run movement). As discussed in §5.5, however, we remove the attractive component of chase-run movement in some simulations and instead model xanthophores as simply repelled from melanophores. This choice was made to simplify parameter fitting in this first model, since it is less clear how changes made to the migration parameters will impact cell movement when interactions are both repulsive and attractive. These migration interactions are summarized in Table 5.1.

Action	Potential	Velocity of ith cell due to jth
M-M repulsion	Q^{mm}	
X-X repulsion	Q^{xx}	
X-M repulsion	Q^{xm}	
M-X attraction locally (when included) & repulsion	Q^{mx}	 

Table 5.1: Summary of cell migration and notation.

5.3.2 Cell differentiation and death

Our rules for cell birth and death take the same general form as in the two-cell body model in Chapter 3, and depend on the proportion of cells in a long-range annulus Ω_{podia} and a short-range disc Ω_{loc} . We update populations for differentiation once per time step (we consider different time step options here: as noted in each figure, some simulations use a time step of one day, others have a smaller time step); cell death, in turn, is implemented by evaluating every cell in the domain for possible death at the end of each day. In the different simulation set-ups discussed in §5.4, we consider a few adjustments to the baseline rules for cell birth and death used for the two-cell body model. Thus, rather than outline these adjustments here, we instead refer to §3.3.2 for a brief overview of the body model rules and note each alteration considered as it appears in §5.4. Details on adjustments to these rules and the parameters used for the fin domain can be found in §5.5.

While we actively made the choice to base our fin model rules on our two-cell body model to allow us to study whether or not the same cell interactions can account for pattern dynamics in both regions, it is worth noting where the rules used also have a basis in the biological data collected on fins. As discussed in §3.3.2, in our two-cell work we broadly model cell birth on the body as due to short-range

activation and long-range inhibition, with black cells differentiating in regions near other black cells and with yellow cells at a distance (opposite rules apply for yellow cells). This was based heavily on the ablation experiments [54] Nakamasu *et al.* performed on the zebrafish body. Unfortunately, ablation experiments severely damage the fins, so the same data is not available for the tailfin [86]. Instead, we remark that experiments with a temperature-sensitive mutation [61] indicate that xanthophores support melanophores at long-range on the fins [86] (see §4.4.8). Furthermore, Goodrich and Nichols [22] described melanophore death on the anal fin in an early study of fin regeneration and development, and [23] provides early evidence for competition between melanophores and xanthophores, melanophore migration, and a repulsive effect of xanthophores on melanophores.

5.4 Results

Zebrafish have three fins, two of which display striped patterns, and here we focus specifically on wild-type pattern formation on the caudal (back) fin. As discussed earlier, it is unclear what drives stripes to form horizontally on this fin and what, if any, differences exist between cell interactions in this region and on the fish body. In particular, it has been shown that the horizontal myoseptum [17] initially specifies stripes to form horizontally on the body, and our models in Chapters 3 and 4 agree with experimental studies [74, 75] suggesting iridophores play a crucial role in directing later interstripes to form. While body stripes and interstripes are added sequentially to the edges of an existing horizontal marker at the horizontal myoseptum [17], caudal fin patterns seem to extend outward as the fish grows (for example, see images in [58]; [78] also describes melanocyte patterning occurring from proximal to distal). This set-up is somewhat reminiscent of an ablation experiment (see Figures

1.5 and 5.6), and it is unclear what instructs the stripes to form horizontally on the caudal fin without a horizontal myoseptum or spreading iridophores [74, 75]. One could envision multiple mechanisms at work:

- A. Base case: melanophore and xanthophore interactions alone, or in addition to the initial condition, are enough to specify stripe directionality without growth (this is a short control case discussed in §5.4.1 on a static domain, and is less biologically relevant since the fin grows during pattern development);
- B. Distal growth: Fin epithelium growth occurs only at the distal edge, preferentially selecting horizontal stripe formation through the controlled appearance of open space for cells to proliferate in;
- C. Uniform growth: Fin epithelium growth occurs uniformly across the whole domain, stretching cell positions as the pattern forms; and
- D. Bone rays: Based on fin images [58], black pigment cells seem to appear associated with the bone rays [22], and this suggests that bones may impact cell movement or differentiation, particularly because the caudal fin is much thinner than the body (for connections between the bone rays and melanophore dynamics, we gratefully acknowledge conversations with experimentalist April Dinwiddie in March-April 2015 and September 2016).

It should be noted that Watanabe and Kondo have also hypothesized that fin growth through the addition of new segments at the distal tips of rays could be a source of directionality (on the body, in contrast, growth is uniform) [86]. Furthermore, the experiments [61] with a temperature-sensitive mutation led Parichy and Turner to suggest that melanophore and xanthophores interactions alone may not be enough to specify horizontality on the fins. Lastly, we highlight the work of Tu and Johnson [78], which we became aware of post model development: their work tracking melanocyte lineages in the caudal fin suggests most new melanocytes are added in the distal

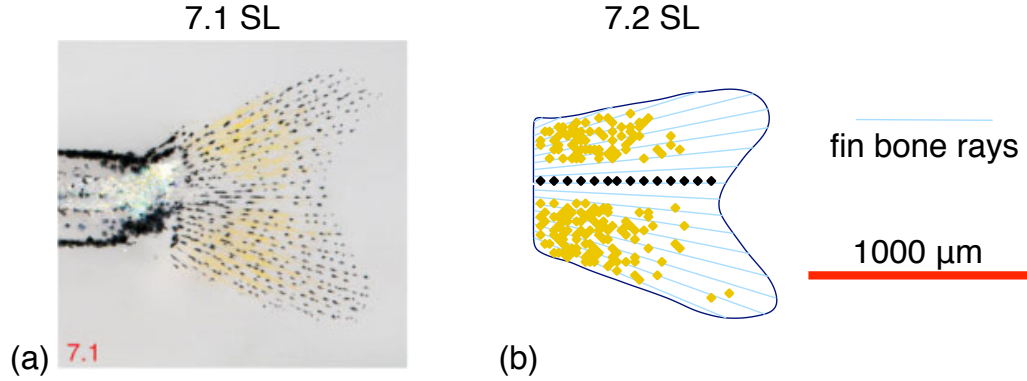


Figure 5.3: We simulate pattern formation from an initial condition approximating the caudal fin at 18 days post fertilization (dpf). (a) Caudal fin image reproduced from Parichy *et al.* [58] with permission from John Wiley & Sons, Inc. (b) Simulation initial condition features a strip of black melanophores at the center of the fin and a distribution of yellow xanthophores across the lobes.

third of the developing stripes, though they also observed some melanocyte birth across the entire pattern. Their findings could be accounted for by a combination of uniform and distal growth or by an interplay of cell repulsion and cell birth with growth, for example.

To comment on these different mechanisms, we now present a study of wild-type stripe formation on the caudal fin. With the exception of our evaluation of Mechanism A in §5.4.1, all simulations are meant to describe the timeline of wild-type stripe development from when the fish is ≈ 7.2 mm SL (18 dpf) to when it reaches adult stages (150 dpf). We start with an initial condition consisting of a central strip of melanophores along the center of the fin and a random distribution of xanthophores across the domain (see Figure 5.3 for a comparison with the pattern on the caudal fin at 7.1 mm SL). We think of this initial condition as representing the pattern at 18 dpf, but note that it would be useful to test other model approximations of the 18 dpf pattern in future work. In particular, we do not include a random distribution of melanophores across the full fin domain, though black cells can be seen randomly dispersed on the fin at 7.1 mm SL (see Figure 5.3a).

5.4.1 Pattern formation on a static fin domain

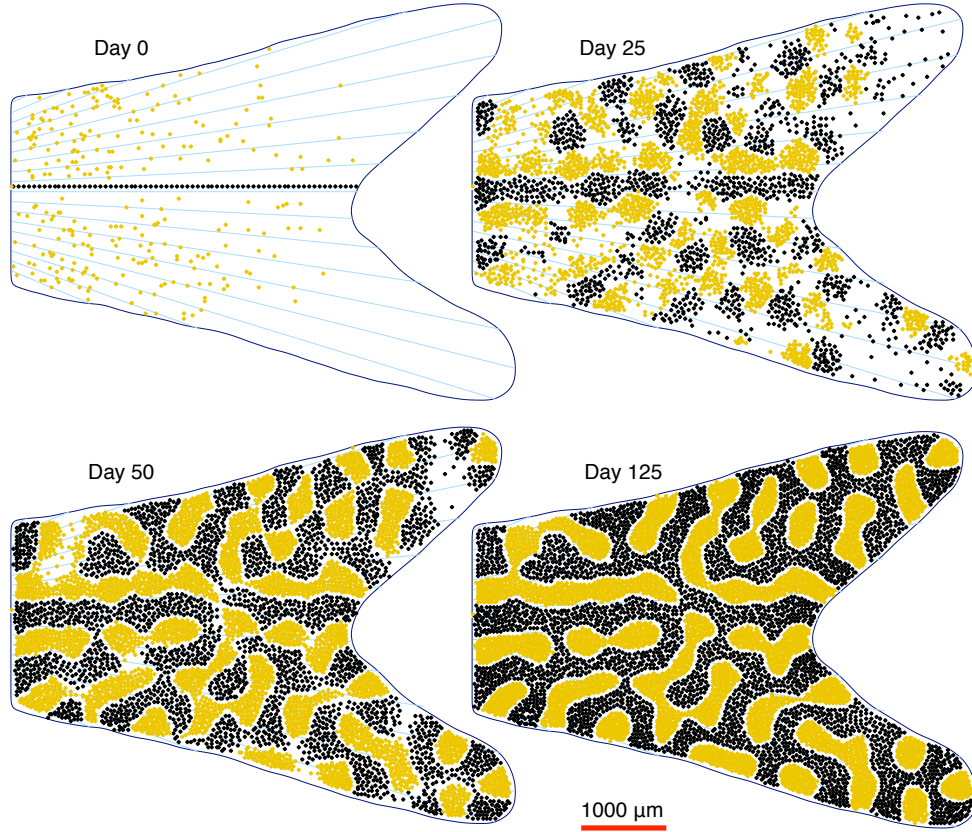
Without growth, the interactions of melanophores and xanthophores from the body cannot account for the timeline of horizontal fin patterning

We begin by simulating our original body model on a static fin domain as a first control experiment (Mechanism A). The caudal fin domain used is at J++ stage (roughly 100 dpf by our estimation of [58]), and to allow for more comparison with our results on wild-type stripe development on a growing tailfin, we start with an initial condition that represents our approximation of the fin pattern at 7.2 mm SL (≈ 18 dpf) scaled up to a larger fin size.

As shown in Figure 5.4, this naive approach leads to patterns with lost directionality away from the central stripe. In many ways this experimental set-up is similar to our simulations of wild-type development in §3.4.2 without additional iridophore localization: both settings provide alignment instructions at the center of the domain, but this is not enough direction to specify stripe horizontality away from this region. Undirected random differentiation away from the center disrupts the pattern, leading to the formation of spots in the upper and lower regions.

5.4.2 Wild-type development on a growing fin

Having completed a first control experiment on a static caudal fin domain, we now turn to a short study of wild-type stripe development on growing fins. Our focus is qualitative at this point, and we leave a closer quantitative study for future work. To evaluate Mechanisms B-D, we identify three broad measurements of success, based on our view of the timelines of wild-type development presented in [58]. In particular,



Simulation on a static non-growing domain (fin size corresponds to 100 dpf fish)

Figure 5.4: Hypothesis A: Without fin growth (static domain), the initial pattern is enough to specify directionality of the first black stripe and first two interstripes, but the rest of the pattern loses directionality. Parameter note: body parameters are used, the time step for simulations is 1 day, $D = 0 \mu\text{m}$, and $u = 1000$ cells (see §5.5).

we consider the model successful if (0) horizontal stripes develop; (1) stripes form by gradually extending from proximal to distal; and (2) three horizontal black stripes are fully formed (e.g. have roughly reached the distal edge of the domain) at stage J++ (≈ 100 dpf). While we learned of [78] post model development, success measurement (1) is also supported by this work, which shows that melanocyte growth occurs from proximal to distal. Furthermore, the early study of Goodrich *et al.* [23] describes xanthophore differentiation as occurring outward from the body.

As discussed in §5.3, the bone rays on the tailfin grow by adding new segments to the distal (back) ends of existing rays [20, 31, 32, 86]. One could think of two possible

ways this would impact the pigment cells on the skin: either the skin grows at the distal edge of the fin as well, so that cell positions are not altered with growth; or the skin accounts for the appearance of new bone segments by expanding everywhere, leading cell positions to be stretched with growth (this could be supported, for example, by the observation that skin is constantly forced to regenerate itself due to exposure). We begin here by testing the former hypothesis by simulating the body model on a growing fin domain without cell stretching: this is Mechanism B.

Epithelium growth at the distal end does not account for stripe directionality under body cell interactions

If we assume epithelium growth occurs only at the distal edge of the tailfin, we obtain the dynamics shown in Figure 5.5. The central strip of black melanophores at the PB stage is enough to specify horizontal alignment in the regions directly above and below it, but the pattern forming more dorsally entirely lacks horizontal directionality. In fact, most of our simulations show a preference for vertical patterns in this set-up. This loss of horizontal alignment is not unexpected when compared to the ablation experiments discussed in §3.4.3. In many ways, domain growth without stretching sets up similar conditions to an ablation experiment [89]: a region of empty space with a boundary perpendicular to the current stripes is present in both cases, and the result is lost directionality but maintained pattern width (see Figure 5.6).

The fact that the stripes continue to form horizontally near the proximal end of the domain is certainly motivated by the presence of the central black strip of cells in this region; nevertheless, this is not enough to fully explain the dynamics, as a central black strip across the whole domain was not enough to specify directionality in our simulations in §5.14. We expect the additional reason is related to the smaller

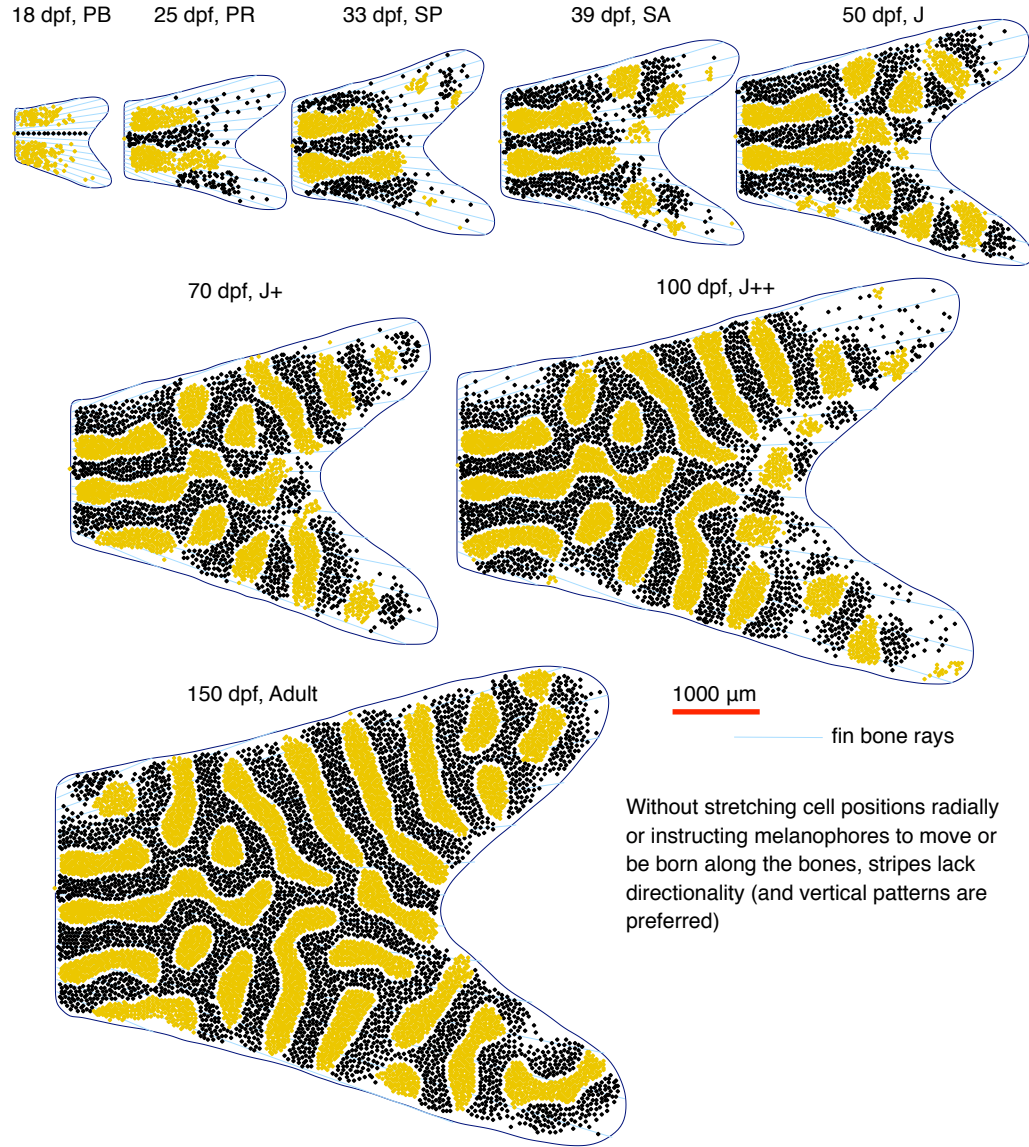


Figure 5.5: Mechanism B: Without any changes to the body model, stripes tend to form vertically. Parameter note: body parameters are used, the time step for simulations is 1 day, $D = 0 \mu\text{m}$ and $u = 1000$ cells (see §5.5).

width of the fin in this region: smaller domains reduce the space available for random differentiation to operate and take over the pattern. The preferential appearance of vertical stripes within the lobes is less clear and presents an avenue for future study.

Uniform epithelium growth can account for stripe directionality

Alternatively, the skin on the tailfin may stretch across the bone rays and grow

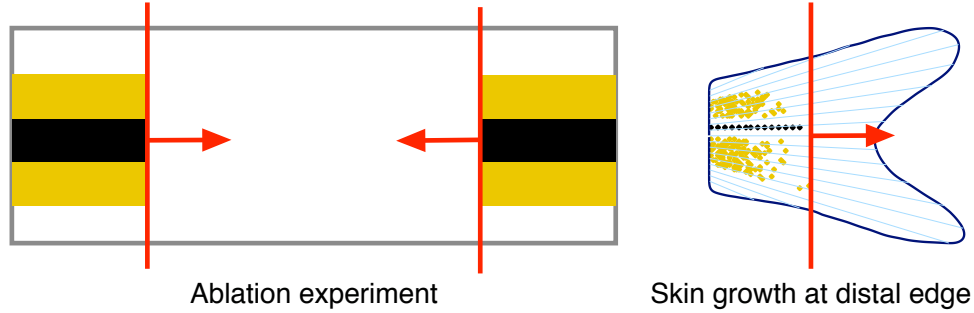


Figure 5.6: Cartoon image shows how skin growth at the distal edge of the tailfin sets up similar conditions as the initial ablation experiment in [89] (see §4.4.7): in both settings, open space appears in a region perpendicular to the boundary of current stripes and we see patterns forming with lost directionality but maintained width [89].

everywhere as additional segments are added to bone rays at their distal edges: this is Mechanism C. As shown in Figure 5.7, uniform epithelium growth can account for stripe directionality. Nevertheless, while this mechanism meets our success measure (0), the pattern forms too quickly and, in particular, does not grow outward from proximal to distal.

Note that in this setting we model growth as stretching cell positions radially as the fin grows outward (in particular, we stretch cell positions along the bone rays; see §5.5 for details). We further adjust the body model by introducing an additional mechanism for cell birth: when density is low, we allow black and yellow cells to differentiate in a small $150\text{ }\mu\text{m}$ wide region of the proximal boundary according to long-range interactions alone. This additional opportunity for cell differentiation is not necessary, and we obtain the same qualitative behavior without it (results not shown), but it models cells differentiating in a controlled way from precursors located at the base of the caudal fin.

Because random differentiation away from the developing pattern seems to disrupt stripe formation in our fin simulations and our body model in Chapter 3, we now step away from including this random birth (instead, we continue to include

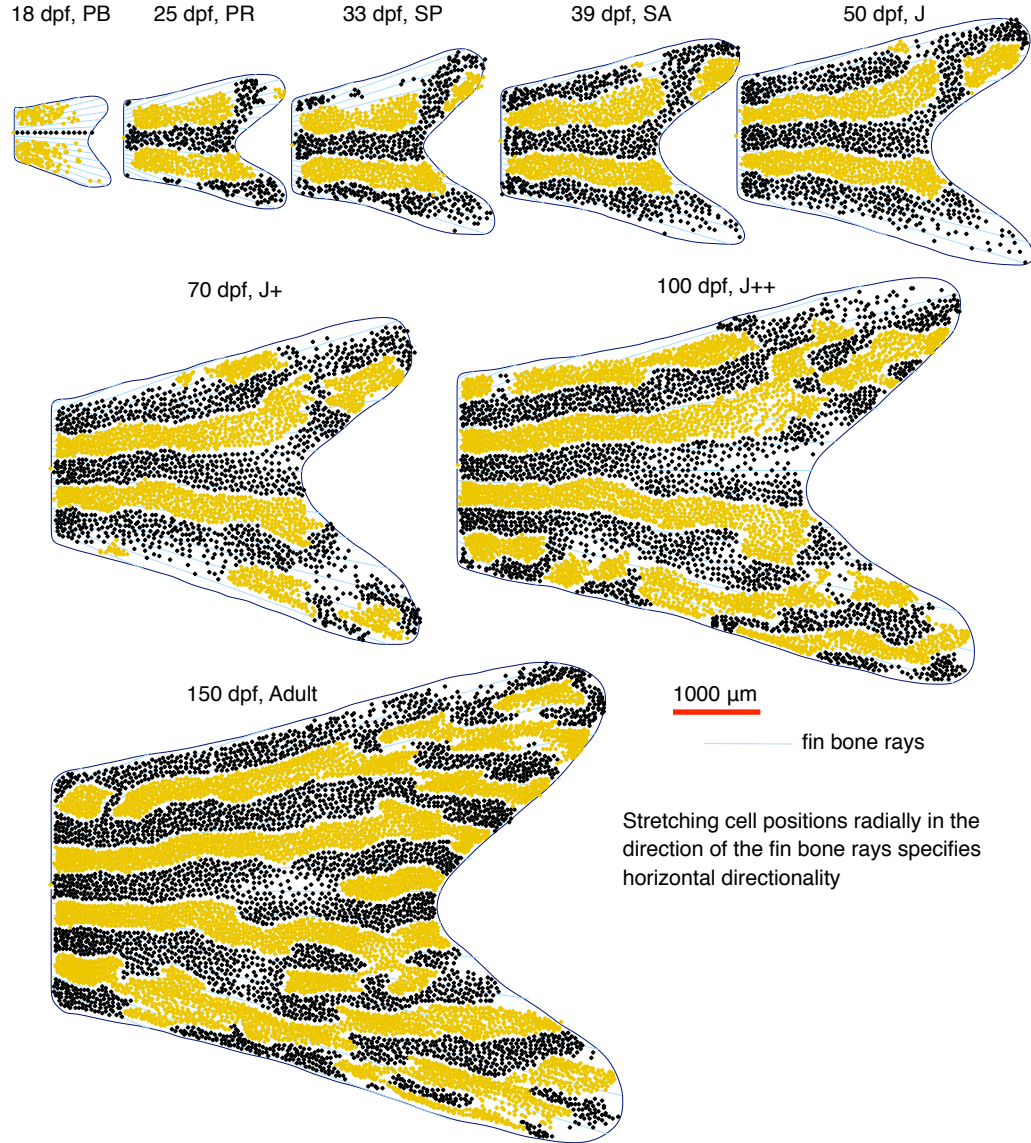


Figure 5.7: Hypothesis C: Stretching cell positions along the bones as the fin grows can specify directionality, but does not match the timeline of wild-type development. Parameter note: this simulation uses body parameters; in contrast to our body model, here we allow for cell birth at low densities near the proximal edge of the fin according to long-range interactions alone ($D = 150 \mu\text{m}$); $u = 1000$ cells, and the time step for simulations is 1 day (see §5.5).

the opportunity for cells to differentiate near the proximal edge of the domain due to long-range interactions alone; this allows for the appearance of new stripes when random birth is not included). By removing random birth, we will not be able to reproduce the random distribution of intermingled melanophores and xanthophores in the fin lobes (the short-range competition between black and yellow cells in our

two-cell body model does not support this intermingling anyway). Instead, our focus is on achieving horizontal stripes and success measurements (1) and (2), namely proximal to distal stripe development with 3 stripes reaching across the full domain at stage J++. We suspect that the intermingled cells present on the distal portion of the fin are of a different variety (perhaps larval) than those making up the completed stripes; the works [70, 78], for example, mention the presence of several types of black cells on the fin.

Additionally, while our previous models have always considered cell birth as occurring once per day, in the remaining simulations we test using a smaller time step for differentiation (evaluating our populations for cell birth between 2 and 5 times per day; the time step used is noted in the caption of each figure). In these settings, we use the same reduced time step for cell migration as well, but maintain our evaluation of cells for competitive death signals once per day. This choice allowed for more controlled cell birth and spreading across the fin domain in the right time frame: in particular, because the short-range component of our cell birth rules (see §5.5) requires that black cells are born in regions with strictly more black than yellow cells (opposite, respectively, for yellow cells), when random birth is not included this means that our simulated patterns extend due to cell birth very slowly, under Δ_{xm} per differentiation time step. Thus, for each of the model set-ups discussed next, we choose the time step that best generates three black stripes reaching the distal edge of the domain at roughly 100 dpf, stage J++. We now turn to an evaluation of Mechanism D.

Melanophore migration along the bone rays can preserve stripe alignment, but not dependably

Images from [58] show melanophores in close association with bone rays (see Fig-

ure 5.2). Because of the thinness of the caudal fin in comparison to the body of the fish, one possible means of accounting for this apparent association is that the bones of the caudal fin serve to corral pigment cells and hamper their dorsal-ventral movement across rays. As early as 1954, Goodrich *et al.* observed melanophores invading yellow regions *usually along fin rays*. This is supported by recent suggestions as well: melanophores, in particular, tend to move along the bones (personal communication, April Dinwiddie, March 2015). We implement this condition by projecting melanophore velocity on the nearest bone ray, and, as shown in Figure 5.8, the resulting dynamics are able to generate patterns that form horizontally and from the proximal to distal edge of the fin. Nevertheless, this mechanism is not reliable, as approximately 40% of our simulations do not produce horizontal patterns.

Melanophore birth on bone rays can preserve proximal to distal stripe formation

Alternatively, the appearance of melanophores in close association with the bone rays in Figure 5.2 could be explained by black cells arising from precursors or other sources along the bones (April Dinwiddie, personal communication, April 10, 2015). As shown in Figure 5.9, requiring melanophore birth along the bones does produce patterns that emerge from proximal to distal, and, in fact, does so dependably across many simulations. Nevertheless, the resulting stripes are strongly directed by the radial nature of the bones, resulting in stripes that diverge at the cleft. This is in contrast to the experimental work [78], which shows that, rather than exhibiting radial expansion, black cells spread from proximal to distal. Thus, requiring that melanophores always move along the rays too strongly imparts a radial nature to patterns.

One possible explanation is that the bone rays only interact with melanophores near the more distal regions of the developing stripes. This may be supported by the

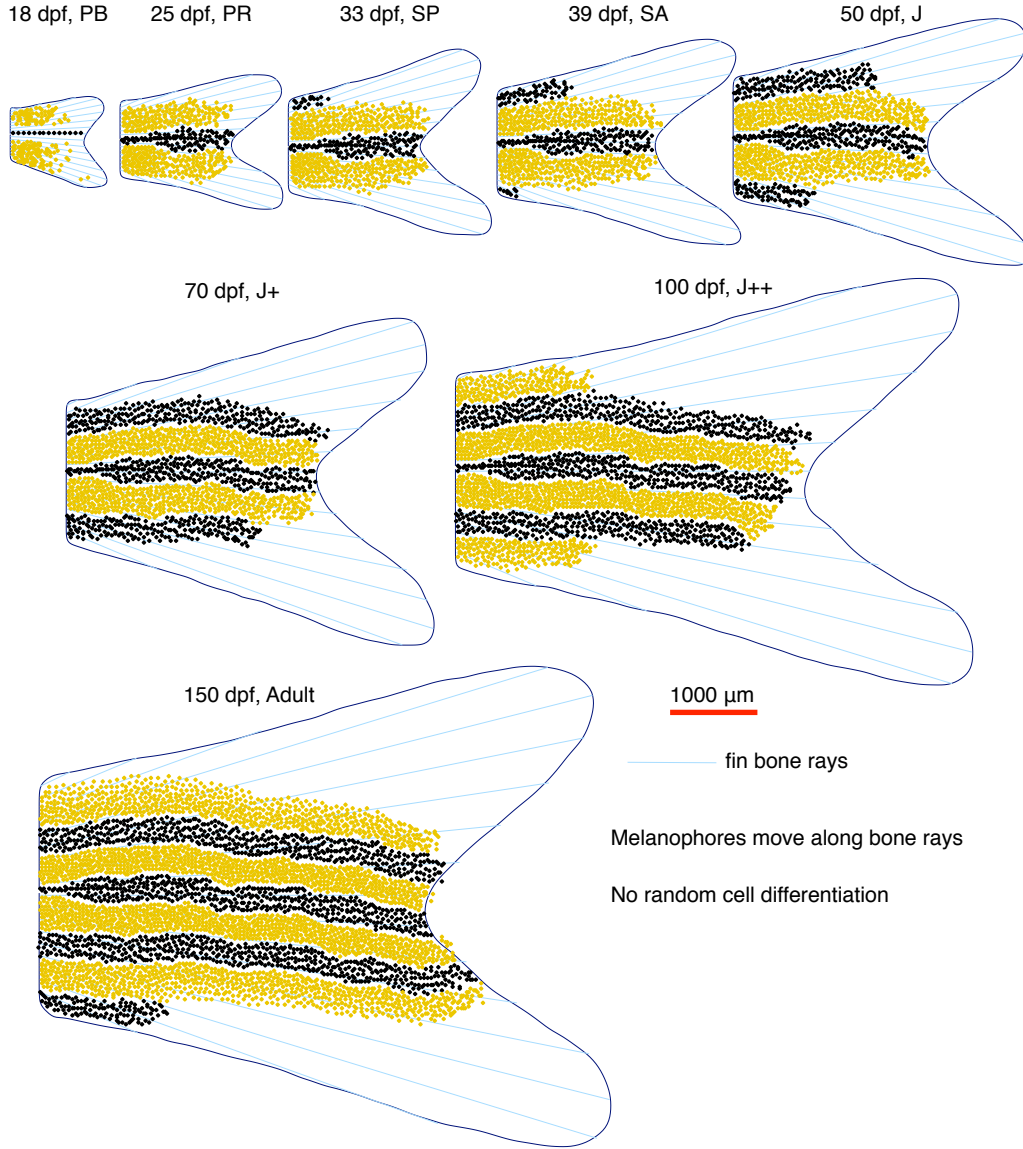


Figure 5.8: Hypothesis D: Restricting melanophore migration along the bones preserves directionality and the development timeline in $\approx 60\%$ of our simulations. (For now we focus on replicating the appearance of three black stripes and leave out random cell differentiation in the lobes). Parameter note: this simulation uses fin parameters; melanophore velocity is projected along the bones; no random cell birth is included, $D = 150 \mu\text{m}$, $u = 1000$ cells, and the time step for differentiation and migration is 0.5 days (see §5.5).

early work [23], showing melanophores initially present along rays and only later in areas between the fin bones during an experiment. Furthermore, comparing images in the timeline of wild-type development in Figure 5.2 seems to show melanophores initially appearing radially, but later retaining a horizontal arrangement. At the lobes of the caudal fin and the leading (distal) edges of the developing black stripes,

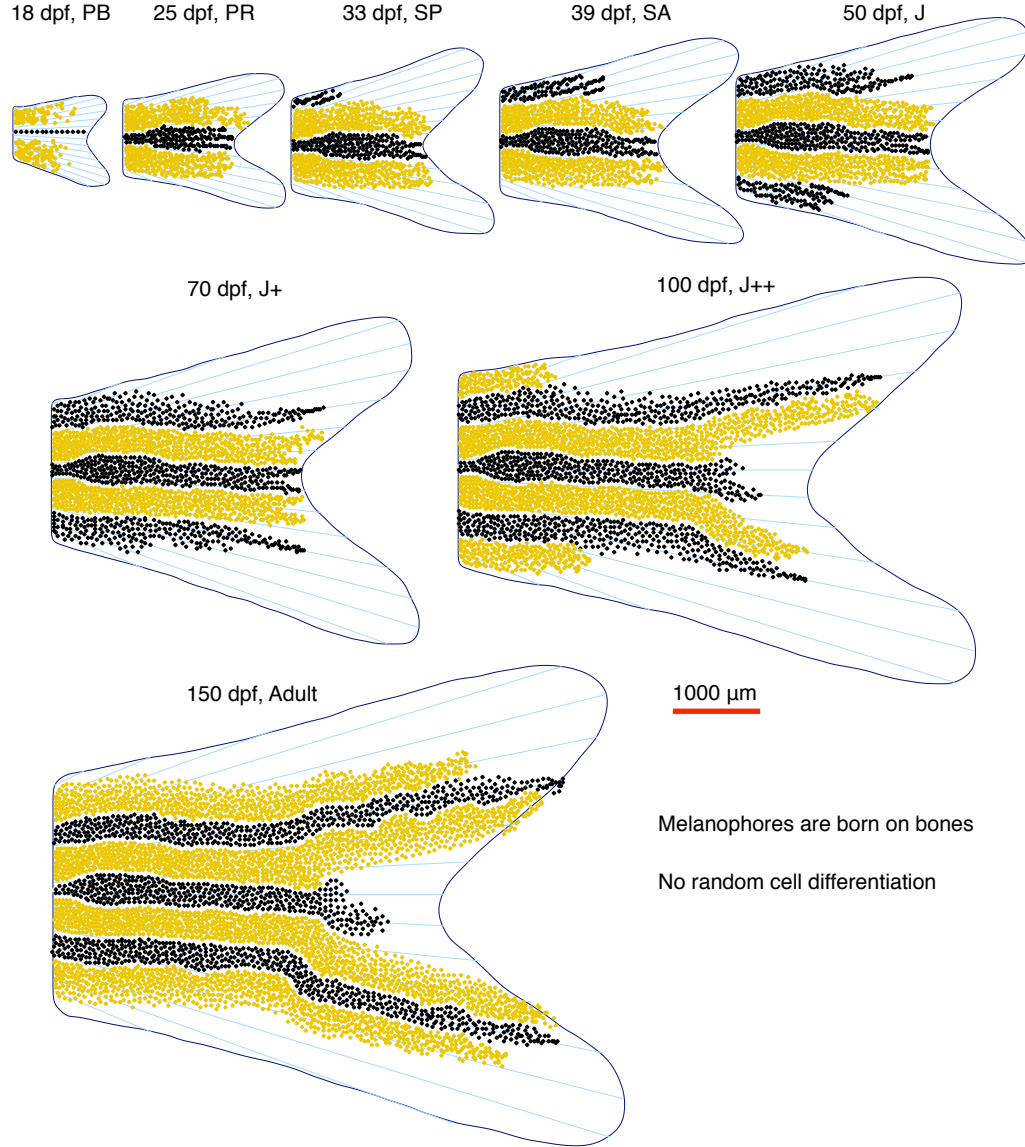


Figure 5.9: Hypothesis D: Requiring melanophores to emerge/differentiate on the bones ensures stripes never form vertically, but the black stripes too strongly follow the bone rays near the distal boundary of the fin. Parameter note: this simulation uses fin parameters; melanophore birth is along the bones; no random cell birth is included; $D = 150 \mu\text{m}$; $u = 1000$ cells; and the time step for differentiation and migration is 0.333 days (see §5.5).

in particular, melanophore arrangement appears radial, yet the three black stripes present at stage J++ are parallel. This difference could conceivably depend on the thickness of the fin, for example: when the fin is thin, cell dynamics could be tied to the bone rays; as the fin thickens, first at the proximal edge, cell interactions could become independent of the rays.

A density-dependent combination of melanophore birth and movement along the bones aligns stripes and supports proximal-to-distal pattern formation

To account for what appears to be an initial radial emergence of melanophores followed by horizontal alignment, we introduce a density-dependent rule: we assume melanophores are born on and move along the bones at low cell density. Once the density in their immediate neighborhood reaches a critical value, however, their behavior decouples from the rays and becomes autonomous. As shown in Figure 5.10, this produces our best results (and Monte Carlo simulations confirm this mechanism is dependable), with three horizontal black stripes, formed from proximal to distal, reaching the boundary of the caudal fin around stage J++. This density-dependent rule keeps black stripes from forming too radially, as they did when we required melanophore migration always occur along the bones; at the same time, it provides enough forcing to reliably produce wild-type stripes. This model also matches up with the finer details of wild-type patterning on the caudal fin visible in Figure 5.2: we see black stripes initially with a slight radial structure later dropping away from the bones and moving horizontally toward the cleft. The sequential addition of stripes, each from proximal to distal, is also visible.

Intermingled melanophores and xanthophores on the fin lobes may be in an earlier or different form than cells in stripes

Here we return to exploring the intermingling of melanophores and xanthophore on the lobes of the caudal fin (see Figure 5.2 for example). If we add a small amount of random birth back into the model just described (Figure 5.10) and introduce an additional assumption that competition is only active at high enough densities so that mutual competition between black and yellow cells does not destroy cells in the lobes, we obtain the results in Figure 5.11 (note the added assumption could

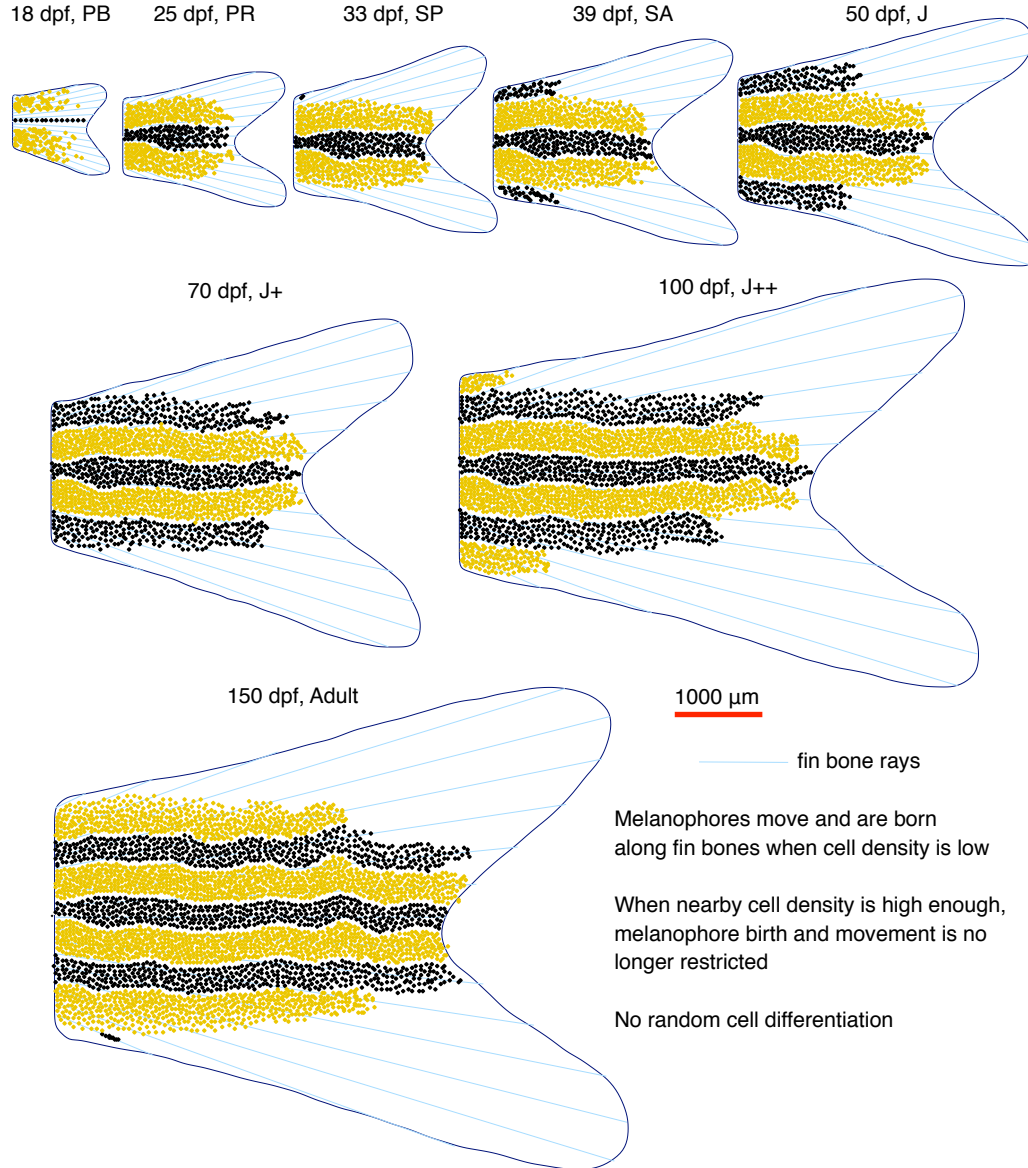


Figure 5.10: Hypothesis D: To keep black stripes from forming too radially, we incorporate a density dependent rule that restricts melanophore movement and birth along the fin rays only at low cell density. Parameter note: this simulation uses fin parameters; melanophore birth and movement is along the bones at low density; no random cell birth is included; $D = 150 \mu\text{m}$; $u = 1000$ cells; and the time step for cell differentiation and migration is 0.2 days (see §5.5).

account for the time it takes cells to fully differentiate and become competitive adult chromatophores, for example). As shown, even this small amount of random differentiation destroys the proximal to distal nature of patterning: this is particularly visible at stage SA in Figure 5.11, where we see a black stripe emerging from both the proximal and distal edges of the fin, later meeting in the middle.

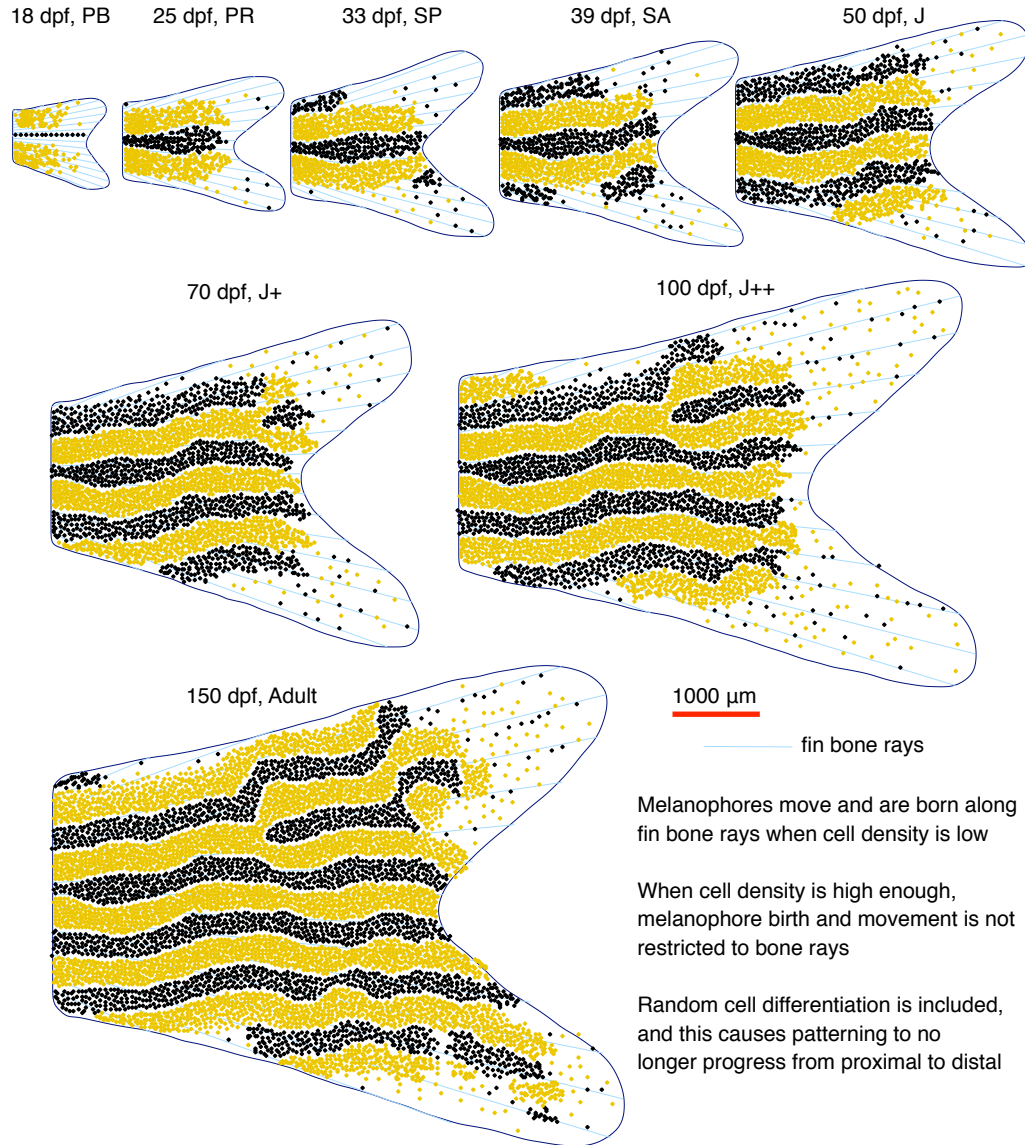


Figure 5.11: Adding random cell differentiation (to account for the mix of cells in the fin lobes) no longer preserves the distal formation of stripes, suggesting there may be different populations of cells in the fin. Parameter note: this simulation uses fin parameters; melanophore birth and movement is along the bones at low density; random cell birth is included; $D = 150 \mu\text{m}$; $u = 3$ cells; and the time step for differentiation and migration is 0.5 days (see §5.5).

Our inability to replicate the timeline of wild-type stripe formation in the main portion of the caudal fin and simultaneously reproduce intermingled black and yellow cells in the lobes suggests our model is missing something. From the beginning of our fin work, we suspected that we would not be able to reproduce clear stripes on the fin and intermingled cells in the lobes at the same time. The dynamics in

these two regions are extremely different, and, at the very least, the short-range competition present on the body of the fish [54] and on its caudal fin [23] must not be present to the same extent in the lobes. The intermingled cells in the lobes could be related to dynamics on the dorsal fin, which is not patterned and instead features intermingled cells [23]. This could be explained by different types of cells or by differences in the environment in these regions [23]. We suspect that the black and yellow cells on the lobes of the caudal fin are in an earlier or larval form, different from the adult pigment cells making up the developing and fully formed stripes.

Frequent cell migration and birth can align stripes under distal epithelial growth

Finally, because our use of a more frequent time step for cell birth is new to our larger work (in our body models in Chapters 3 and 4, we always used a time step of 1 day for birth and death), we turn to an evaluation of its impact on our simulations. In particular, here we return to the parameters used in our body model with two exceptions: we remove random birth and allow for new cells to appear in the proximal region of the fin domain due to long-range interactions alone (this is needed when random birth is not present to account for the initialization of new stripes). Additionally, we set a smaller time step for cell differentiation (and migration), evaluating the domain for possible cell birth 4 times per day. The result, shown in Figure 5.12, was unexpected: this strongly controlled cell birth and migration aligns stripes strikingly, without the need for cell position stretching or any interactions with the bone rays. Nevertheless, it struggles to replicate the more delicate nature of stripe formation in Figure 5.2: the yellow and black stripes form quickly across the domain, almost extending outward as a group. Increasing the time step of cell birth even slightly destroyed the horizontal alignment of the stripes, however. These findings may be a by-product of our model when it is too heavily coded. Alternatively, they point to the importance of cell interactions and suggest that

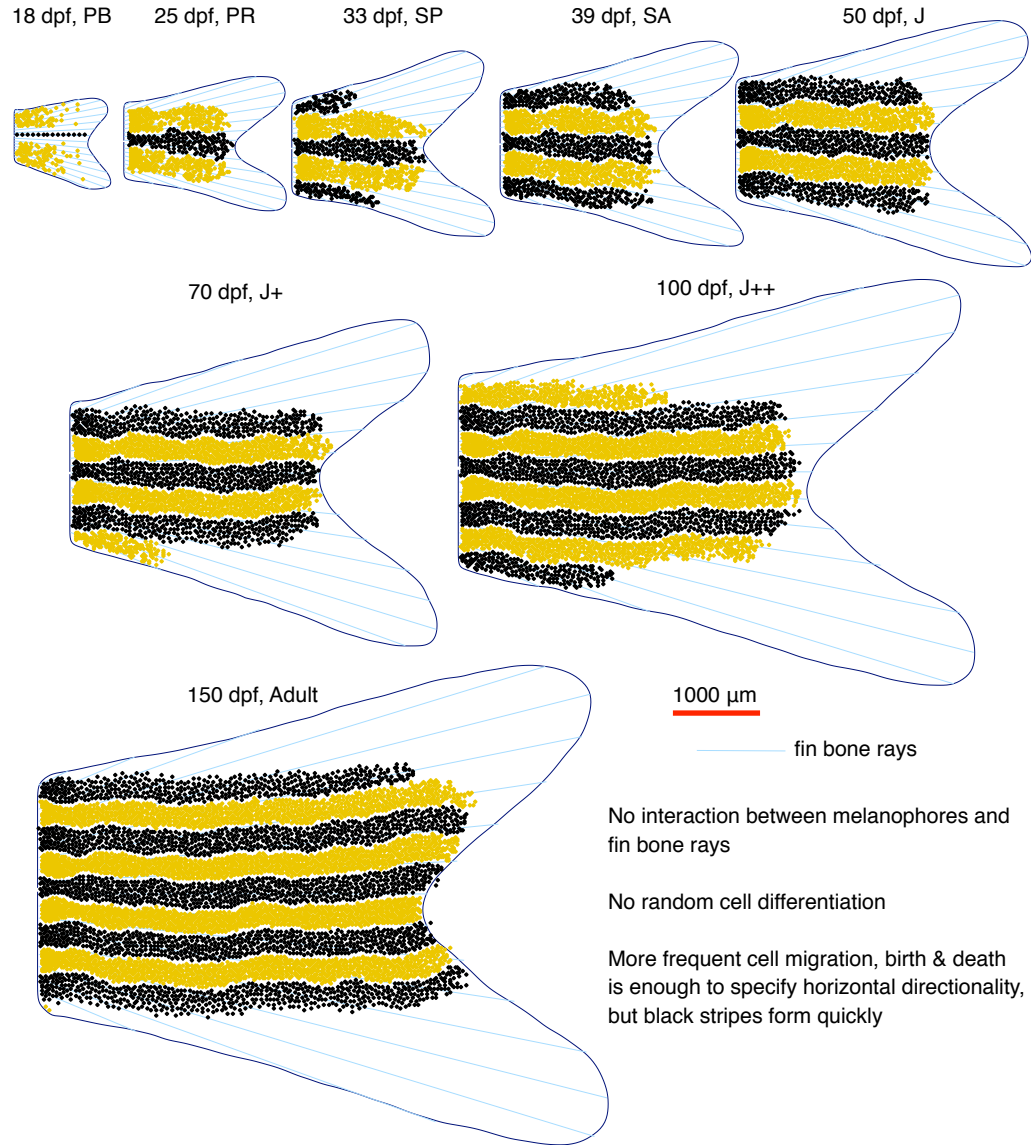


Figure 5.12: Hypothesis B: Our preliminary work shows that careful, frequent cell birth and migration can align stripes under distal epithelial growth (without cells stretching). Parameter note: this simulation uses body parameters; random cell birth is not included; $D = 150 \mu\text{m}$; $u = 1000$ cells; and the time step for differentiation and migration is 0.25 days (see §5.5).

careful timing of birth and movement are enough to align stripes without epithelial stretching on the fin. This could be better elucidated by more quantitative studies and presents directions for future work.

5.5 Detailed model description

Our fin model is an outgrowth of the two-cell body model presented in Chapter 3 and the majority of cell interactions remain the same in both set-ups. Notably, the ODEs have the same form as Equations 3.1 and 3.2 (except when we add an additional term to test whether melanophores experience forces from the rays). Similarly, cell differentiation rules are again broadly short-range activation and long-range inhibition, dependent on the proportion of cells in a local disc Ω_{loc} and a long-range annulus Ω_{podia} :

$\Omega_{\text{podia}}^{\bar{x}}$ = annulus of inner radius $L = 318 \mu\text{m}$ & width $w = 25 \mu\text{m}$ centered at $\bar{x}$;

$\Omega_{\text{loc}}^{\bar{x}}$ = short-range disk of radius $\ell = 75 \mu\text{m}$ (when body parameters are used)

and $\ell = 82 \mu\text{m}$ (when fin parameters are used) centered at position $\bar{x}$,

as well as a disc Ω_{crowd} with radius Δ_{xm} ($82 \mu\text{m}$) used to evaluate cell positions for crowding. We consider two general versions of the fin model: in the first version, all parameters used are the same as those specified for the body (we refer to this case as *using body parameters* in our figures); in the second version, which tests direct interactions between melanophores and the fin bones (through either migration or differentiation), we have adjusted the parameters slightly from their body equivalents, partly to help widen stripes when growth does not stretch cell positions (we distinguish this case in our figures as *using fin parameters*).

5.5.1 Growth and bone rays

The growing caudal fin domain and bone ray placement is the work of undergraduate students Madeline Abbott, Dorothy Catey, Neil Chandra, Bethany Dubois, and Francesca Lim, through a Summer@ICERM research program, and we are grateful for their involvement in this project. We overview their work specifying the caudal fin shape, domain growth, and bone ray positions below.

Because it contains a range of caudal fin images, we rely heavily on the work [58] of Parichy *et al.* to specify the fin domain shape and growth rates in our model. In particular, 10 caudal fin images spanning 6.2 mm SL to 34.8 mm SL (approximately 13 to 280 days post fertilization based on our measurements from [58]) were manually sharpened using Adobe Photoshop, the (x, y) coordinates of the fin boundaries in the cleaned images were identified using Matlab, and a smoothing algorithm was applied to remove rough edges in the resulting curves (see Figure 5.13a-b). We approximated the age of the zebrafish from measurements in [58] for each of the 10 fin images used. To account for the days in between each of these images, we calculated additional boundary curves for each day by assuming linear growth in between images and a continuous transformation to each new image.

The placement of bone rays in our fin domain is again based on the work of Parichy *et al.* [58]. Using a range of images from [58], we estimated the average angle between two consecutive rays to be between 2.5 and 4.5 degrees, with the average angle decreasing with zebrafish age. As a simplifying assumption, we specify bone rays as 2.833 degrees apart regardless of fish age, and, motivated by [31, 71], we consider 18 fin rays (see Figure 5.13c). This angle between bone rays is constant in time. In particular, each day we define the positions of the bones in five steps:

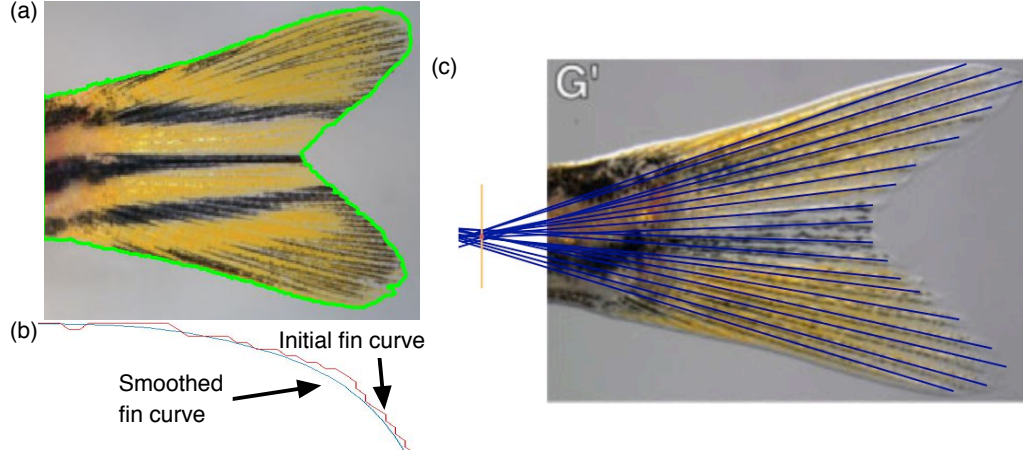


Figure 5.13: Caudal fin domains were determined by (a) tracing images of the tailfin in [58] and then (b) smoothing the jagged curves obtained. (c) Angles and placement of bone rays were informed by tracing rays back toward their point of intersection. Images (a) and (b) are reproduced with alterations from the work of Parichy *et al.* [58]. We acknowledge Madeline Abbott, Dorothy Catey, Neil Chandra, Bethany Dubois, and Francesca Lim for these figures.

- Find the coordinates of the upper and lower endpoints of the proximal (straight edge) boundary of the caudal fin, namely $(0, y_{\max})$ and $(0, y_{\min})$;
- Define $y_{\text{avg}} = \frac{y_{\max} + y_{\min}}{2}$;
- Calculate the upper and lower limits of bone ray placement by shrinking the interval slightly so that bones do not emanate directly from the upper and lower boundary of the domain:

$$y_{\max}^{\text{bone}} = (y_{\max} - y_{\text{avg}}) \times 0.95 + y_{\text{avg}},$$

$$y_{\min}^{\text{bone}} = (y_{\min} - y_{\text{avg}}) \times 0.95 + y_{\text{avg}};$$

- Discretize the vertical region from $(0, y_{\max}^{\text{bone}})$ and $(0, y_{\min}^{\text{bone}})$ into 18 equally separated points;
- Define bone rays emanating outward from each of these points at angles ranging from 25.5 to -22.667 degrees.

When we include stretching of cell positions with domain growth, this is implemented by first calculating the angle we will stretch in and then identifying the

amount we will stretch in this direction (see Figure 5.14 for notation). We make the simplifying assumption that each day bone rays all emanate from the same point $(-r(t), 0)$ to the left of our domain (we define this point to be the place where the third ray down crosses the x-axis; see Figure 5.14). In particular, for a cell with coordinates $(a(t), b(t))$ at time t , the stretch angle α is calculated as

$$\alpha = \text{atan} \left(\frac{b(t)}{a(t) + r(t)} \right),$$

where $r(t)$ is the offset length at time t as shown in Figure 5.14. (Note that it would be better to use the bone ray nearest the pigment cell in question to specify $r(t)$ rather than always using the third ray; though we doubt this makes a strong difference, it would be good to check in future work). The stretch amount S is then calculated by finding the nearest bone rays at time t and $t + 1$ (they have endpoints (m, n) and (p, q) in the example in Figure 5.14) as:

$$S = \text{stretch factor} = \frac{\text{length of closest ray to } (a(t), b(t)) \text{ at time } t + 1}{\text{length of closest ray to } (a(t), b(t)) \text{ at time } t}.$$

The updated, stretched coordinates at time $t + 1$ are then given by

$$\begin{aligned} a(t + 1) &= S \left| \frac{a(t)}{\cos(\theta)} \right| \cos(\alpha) \\ b(t + 1) &= (S - 1) \left| \frac{a(t)}{\cos(\theta)} \right| \sin(\alpha) + b(t), \end{aligned}$$

where $\theta = \text{atan} \left(\frac{q}{p + R(t)} \right)$ is the angle the closest bone ray to the pigment cell on the updated domain makes with the horizontal, and it is helpful to note that $\left| \frac{a(t)}{\cos(\theta)} \right| \approx \ell$ (see Figure 5.14).

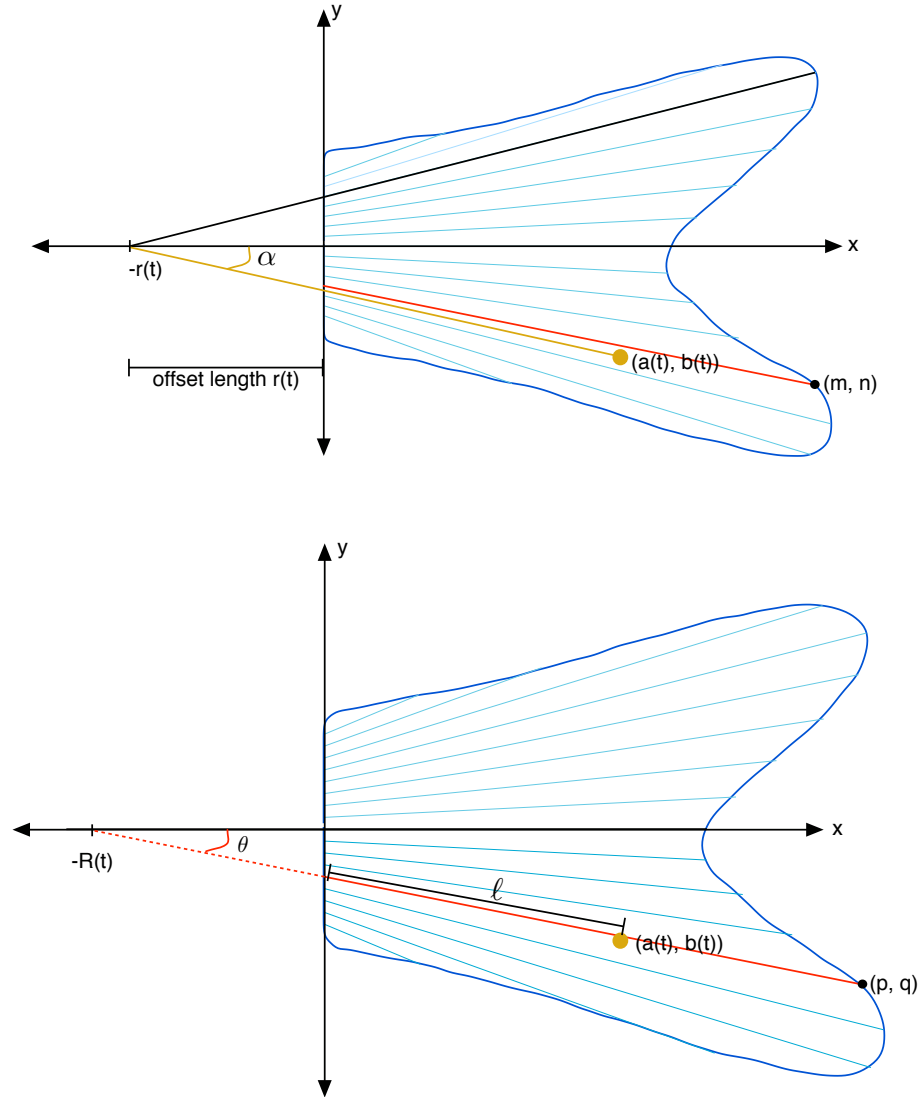


Figure 5.14: Diagram showing a caudal fin domain (dark blue outline) with bone rays in light blue; we make the simplifying assumption that all bone rays emanate from the same point (note this is not quite the case; we approximate this emanation point by the x-intercept of the third ray). When stretching of cell positions is included, this involves two steps: we first identify the angle for stretching (for example, the yellow cell located at position $(a(t), b(t))$ will be stretched roughly at angle α), and we then identify the nearest bone rays in the current domain and the next domain (highlighted in red in this example). The amount of stretching in the direction α is determined by calculating how much the nearest bone rays grow between these days (note here (m, n) and (p, q) are the endpoints of the closest rays to $(a(t), b(t))$ at time t and $t + 1$, respectively) See the text for details.

5.5.2 Cell migration

The movement of each melanophore M_i and xanthophore X_j on the caudal fin domain is specified by first-order ODEs, in the same way as we model cell migration on

the zebrafish body. When we are not testing how melanophore differentiation and migration may be directly impacted by the bone rays, we use exactly the same equations as in Chapter 3, namely Equations 3.1 and 3.2, with the same parameters listed in Table 3.3 (see §3.5.4).

In the second form of our model, which tests melanophore migration and differentiation interactions with the bone rays, we have altered the parameters to the values shown in Table 5.2. The ODEs in this setting are the same as those used earlier for xanthophores (Equation 3.2). When we test if melanophore migration may be impacted by bone rays, we introduce one addition to Equation 3.1: to model melanophore migration along the bones, we simply project the velocity obtained from Equation 3.1 along the nearest ray. As discussed in §5.4, we test two sub-options here: we either project all melanophore velocities along the bone rays, or we do so selectively depending on a density-dependent rule. In particular, in this setting melanophore velocities are projected along bone rays only when they are at low densities (otherwise we do not alter the melanophore velocity by projecting).

To summarize, the velocity of each melanophore M_i on the caudal fin domain is determined by:

- If not testing how melanophore differentiation and migration is directly impacted by fin bones, $\frac{dM_i}{dt}$ is given by Equation 3.1 with the same parameters used in our two-cell body model (Table 3.3).
- If testing how melanophore differentiation and migration is directly impacted by fin bones, we first calculate

$$V_i = - \sum_{j=1, j \neq i}^{N_m} \nabla Q^{mm}(M_j - M_i) - \sum_{j=1}^{N_x} \nabla Q^{xm}(X_j - M_i),$$

the right-hand side of Equation 3.1 with the altered parameters listed in Table 5.2. Then we specify the velocity of the i th melanophore by:

$$\frac{dM_i}{dt} = \begin{cases} ||V_i||\hat{b}, & \text{if } \sum_{j=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{M_i}} X_j + \sum_{j=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{M_i}} M_j < \zeta \\ V_i, & \text{otherwise} \end{cases}$$

where $\hat{b}$ is the unit vector in the direction of the nearest bone ray to M_i and $\zeta = 1000$ or $\zeta = 3$ depending on if we assume melanophore migration occurs along the bones always or only at low cell densities.

Additionally, the velocity of each xanthophore X_j is determined by:

- If not testing how melanophore differentiation and migration is directly impacted by fin bones, $\frac{dX_j}{dt}$ is given by Equation 3.2 with the same parameters used in our two-cell body model (Table 3.3).
- If testing how melanophore differentiation and migration is directly impacted by fin bones, $\frac{dX_j}{dt}$ is given by Equation 3.2 with the altered parameters listed in Table 5.2.

We do not test if xanthophore velocities may be projected along the bones.

Lastly, we introduce forces from the boundary in the same way as for our two-cell body model: the boundary of the domain is discretized into points, and the pigment cells in the domain experience repulsive forces from each of these boundary points in the same way that they experience forces from the other cells in the domain (by a repulsive Morse potential).

Name	Value	Description
R^{mm}	24.8 $\mu\text{m}/\text{day}$	Melanophore-on-melanophore repulsion
R^{xx}	20 $\mu\text{m}/\text{day}$	Xanthophore-on-xanthophore repulsion
R^{xm}	35 $\mu\text{m}/\text{day}$	Xanthophore-on-melanophore repulsion
R^{mx}	30 $\mu\text{m}/\text{day}$	Melanophore-on-xanthophore repulsion
A^{mx}	0 $\mu\text{m}/\text{day}$	No attraction between cells
r_{mm}	40 μm	Appears in Q^{mm}
r_{xx}	31 μm	Appears in Q^{xx}
r_{xm}	40 μm	Appears in Q^{xm}
r_{mx}	40 μm	Appears in Q^{mx}
a_{mx}	40 μm	Appears in Q^{mx}
R^b	50 μm	Boundary forces
r_b	20 μm	Appears in boundary force potential

Table 5.2: Summary of altered cell migration parameters used in some fin simulations. Attraction of xanthophores by melanophores is removed as a simplifying assumption because including attraction makes fitting our parameters less straightforward. Generally, these parameters differ from those in Table 3.3 for the two-cell body model in that we have decreased the amplitude of the force and instead increased the length scale over which it acts.

5.5.3 Cell differentiation

As discussed earlier, we consider a few ways of modeling melanophore and xanthophore differentiation on the caudal fin. Similar to our two-cell body model, a set number of possible cell birth locations are selected randomly in the domain at each time step (note we do not allow cell birth in a small 25 μm region around the fin boundary); while the number of locations selected is constant in our body models, now we make this value time-dependent (increasing in time), as the fin domain grows in size particularly significantly over the developmental period we consider. Furthermore, the way we select these random locations differs from our body models, and we consider four possible methods for selecting locations for possible melanophore birth:

- **Control condition:** $2 \times N_{\text{diff}}(t)$ locations are first chosen uniformly randomly in a rectangular region surrounding the fin and the first $N_{\text{diff}}(t)$ of these positions that are inside the caudal fin domain are then selected.
- **Bone birth:** $N_{\text{diff}}(t)$ locations are uniformly randomly selected from the coordinates of the fin bones.
- **Density-driven bone birth:** $2 \times N_{\text{diff}}(t)$ locations are first chosen uniformly in a random rectangular domain surrounding the fin and the first $N_{\text{diff}}(t)/2$ of these positions inside the caudal fin are then selected; additionally, another $2 \times N_{\text{diff}}(t)$ locations are initially uniformly randomly chosen from the coordinates of the fin bones, and a random sample of $N_{\text{diff}}(t)/2$ of these positions are selected. Finally, each position $\bar{x}$ selected on the bone rays is only eligible to be evaluated for melanophore birth if

$$\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i \leq g,$$

where $g = 3$, and each position selected off the bone rays is only eligible to be evaluated for melanophore differentiation if it satisfies the opposite inequality.

Xanthophore birth locations are always selected in the same way as the melanophore control condition (with a different value $N_{\text{diff}}^x(t)$), as we do not test xanthophore interactions with the bone rays. The initial value of N_{diff} varies in our different simulation set-ups, but grows by 20 cells each day. Additionally, it is important to note that the time step used for cell birth also depends on the simulation set-up and is noted under each figure (in our body models, this time step is always 1 day, but we occasionally use smaller values for our fin simulations).

The random locations selected for possible cell birth as described above are then

evaluated for differentiation based on deterministic rules. In some settings, we also include a small amount of random birth at low densities; when included, random differentiation is modeled in exactly the same manner as in our two-cell body model and the same parameters are used (see §3.5), with one exception: if the time step used for cell birth is less than 1 day, we adjust p_x and p_m by multiplying by the time step (this ensures that we do not make the probability of random birth too high when the time step is small). Thus, here we focus instead on explaining the different deterministic conditions we consider for cell differentiation on the fin.

The different mechanisms we consider for xanthophore differentiation can be summarized in a series of inequalities, which we present below and then explain in detail; in particular, a new xanthophore appears at a randomly selected location $\bar{x}$ when

$$\begin{aligned}
 & \left[\left(\underbrace{\bar{x} < D}_{\text{special dynamics possible at proximal fin edge}} \quad \text{and} \quad \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{rand}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{rand}}^{\bar{x}}} M_i \leq 0}_{\text{altered proximal dynamics only active at low density}} \right) \right. \\
 & \text{or} \left(\underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} X_i > \varphi \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} M_i}_{\text{short-range activation}} \quad \text{and} \quad \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i \geq u}_{\text{undercrowding condition}} \right) \Big], \\
 & \underbrace{\sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} M_i > \psi \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} X_i}_{\text{long-range inhibition}}, \quad \text{and} \quad \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i < \kappa}_{\text{overcrowding condition}},
 \end{aligned}$$

where φ and ψ are involved in short-range activation and long-range inhibition, κ is a parameter that prevents overcrowding, u is a parameter (usually set to 1000, effectively ∞) that provides what we call an *undercrowding* condition if u is small, and D (either 0 or 150 μm) models special dynamics near the proximal edge of the fin when $D = 150 \mu\text{m}$. These parameters take on different values depending on the mechanisms under consideration, as described below. (We note, in particular, that

the values we consider for D, u can lead to fundamentally different dynamics).

Control case: In this setting, we assume xanthophores are born at randomly selected locations under exactly the same deterministic rules as used in our two-cell body model (in this setting, we also include a small amount of random differentiation). The value of φ, ψ , and κ are the same as for the two-cell body model (see Table 3.4), and $D = 0, u = 1000$.

Altered proximal dynamics with body parameters: In this setting, we assume the same values for φ, ψ, κ , and u as in the control case, but we remove any random birth and instead introduce a second means for xanthophore differentiation by setting $D = 150 \mu\text{m}$. This rule allows xanthophores to differentiate in a small neighborhood of the proximal edge of the fin when cell density is low according to long-range activation alone (no short-range activation needed). It allows us to explore the model without random birth included; without either this rule or random birth, there would be no new stripes formed, as there would be no like cells locally to activate the new formation of stripes or interstripes. The inclusion of $D = 150 \mu\text{m}$ also allows us to test if cells may enter the fin from precursors at the proximal edge, where the caudal fin connects with the fish body.

Altered proximal dynamics with fin parameters: Here we assume xanthophores can be born under the same deterministic rules as used in our two-cell body model, but the parameters φ, ψ , and κ are adjusted slightly to the values in Table 5.3. As with the previous setting, we remove any random birth and set $D = 150 \mu\text{m}$ (the value of u remains at 1000). All other parameters and length scales are the same as for the two-cell body model (see Table 5.3).

Undercrowding: As a second case used to reduce the impact of random differen-

tiation, which we hypothesize could be related to larval or early cells, we occasionally introduce an undercrowding condition by setting $u = 3$. In this setting, we include a small amount of random birth, include special proximal dynamics with $D = 150 \mu\text{m}$, and set φ, ψ , and κ to the fin parameter values in Table 5.3 (all other parameters and length scales are listed in Table 5.3).

Similarly, a new melanophore differentiates at the position $\bar{x}$ when

$$\begin{aligned}
 & \left[\left(\underbrace{\bar{x} < D}_{\text{special dynamics possible at proximal fin edge}} \quad \text{and} \quad \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{rand}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{rand}}^{\bar{x}}} M_i \leq 0}_{\text{altered proximal dynamics only active at low density}} \right) \right. \\
 & \text{or} \left(\underbrace{\sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} M_i > \alpha \sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{\bar{x}}} X_i}_{\text{short-range activation}} \quad \text{and} \quad \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i \geq u}_{\text{undercrowding condition}} \right) \Big], \\
 & \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} X_i > \beta \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{podia}}^{\bar{x}}} M_i}_{\text{long-range inhibition}}, \quad \text{and} \quad \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i < \eta}_{\text{overcrowding condition}},
 \end{aligned}$$

where α and β are involved in short-range activation and long-range inhibition, η is a parameter that prevents overcrowding, and u, D take on the same values and meaning as they did for xanthophore differentiation. We consider the same four cases described above for xanthophores (with φ, ψ , and κ replaced by α, β , and η ; see Tables 3.4 and 5.3).

5.5.4 Cell death

Every cell on the domain is evaluated for cell death at the end of each day according to a combination of short-range competition and long-range survival signals (based on ablation experiments in [54] in the same way as for our two-cell body model; for

Name	Value	Description	Source
α	0.5	Lower bound on $\frac{\sum_{i=1}^{N_m} M_i}{\sum_{i=1}^{N_x} X_i} _{\Omega_{\text{loc}}}$ for M differentiation	
β	2.5	Lower bound on $\frac{\sum_{i=1}^{N_x} X_i}{\sum_{i=1}^{N_m} M_i} _{\Omega_{\text{podia}}}$ for M differentiation	
η	4	Upper bound on $(\sum_{i=1}^{N_m} M_i + \sum_{i=1}^{N_x} X_i) _{\Omega_{\text{crowd}}}$ for M differentiation	Value used in 5-cell model
φ	1.3	Lower bound on $\frac{\sum_{i=1}^{N_x} X_i}{\sum_{i=1}^{N_m} M_i} _{\Omega_{\text{loc}}}$ for X differentiation	Value used in 2-cell body model
ψ	1	Lower bound on $\frac{\sum_{i=1}^{N_m} M_i}{\sum_{i=1}^{N_x} X_i} _{\Omega_{\text{podia}}}$ for X differentiation	
κ	6	Upper bound on $(\sum_{i=1}^{N_m} M_i + \sum_{i=1}^{N_x} X_i) _{\Omega_{\text{crowd}}}$ for X differentiation	Value used in 5-cell model

Table 5.3: Alternative cell differentiation parameters used in some simulations of pattern formation on the caudal fin.

details and motivation, see §3.5), as below:

$$\begin{aligned}
& \left(\underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{M_j}} X_i}_{\text{local competition as in the two-cell body model}} > \mu \underbrace{\sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{M_j}} M_i}_{\text{long-range survival signal as in our body models}} \quad \text{or} \quad \underbrace{\sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{podia}}^{M_j}} M_i}_{\text{long-range survival signal as in our body models}} > \xi \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{podia}}^{M_j}} X_i}_{\text{long-range survival signal as in our body models}} \right) \\
& \text{and} \quad \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i}_{\text{condition that removes cell death at low density}} \geq \zeta \implies \text{death of } M_j, \\
& \underbrace{\sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{loc}}^{X_j}} M_i}_{\text{local competition}} > \nu \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{loc}}^{X_j}} X_i}_{\text{local competition}} \quad \text{and} \\
& \underbrace{\sum_{i=1}^{N_x} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} X_i + \sum_{i=1}^{N_m} \mathbb{1}_{\Omega_{\text{crowd}}^{\bar{x}}} M_i}_{\text{condition that removes cell death at low density}} \geq \zeta \implies \text{death of } X_j,
\end{aligned}$$

where $\mu = 1$, $\xi = 1.2$ or $\mu = 2$, $\xi = 1.7$ depending on if control (body) or fin parameters are used; $\nu = 1$, the same value used in the two-cell body model; and

Name	Value	Description	Source
μ	2	Lower bound on $\frac{\sum_{i=1}^{N_x} X_i}{\sum_{i=1}^{N_m} M_i} _{\Omega_{\text{loc}}}$ for M death	Fit to Exp. A-H [1]
ξ	1.7	Lower bound on $\frac{\sum_{i=1}^{N_m} M_i}{\sum_{i=1}^{N_x} X_i} _{\Omega_{\text{podia}}}$ for M death	

Table 5.4: Alternative cell death parameters used in some fin simulations. All other parameters used in cell death rules are the same as those in Table 3.5.

$\zeta = 3$ (when $u = 3$) or $\zeta = 0$ depending on whether or not we introduce an additional constraint that cell death does not occur in sparsely populated regions of the fin (see Tables 3.5 and 5.4 for a summary of parameter values). This constraint is introduced in some simulations to account for melanophores and xanthophores intermingling at the proximal edge of the caudal fin lobes when the pattern has not yet fully formed in this region; according to our two-cell body models, short-range competition would discourage black and yellow cells coexisting otherwise, so we test if this competition may only operate when cell density is high.

5.6 Discussion

In this chapter, we presented a short study of wild-type stripe formation on the caudal fin of zebrafish. Our work suggests that uniform skin growth across the caudal fin can account for the horizontal alignment of stripes by stretching pigment cell positions on the fish skin. In contrast, distal epithelial growth results in disrupted patterning, with stripes often forming with a vertical preference, unless cell birth and migration is carefully controlled and keeps pace with fin growth. We find the best results when we assume melanophores migrate along and differentiate on the bone rays according to a density-dependent rule: when cell density is low, melanophore

behavior is impacted by bone rays, but, at high enough density, patterning dynamics are independent of the bones and driven by cell interactions.

There are three main challenges we encountered with this model: (1) obtaining horizontal directionality of stripes; (2) reproducing the outward timeline of wild-type pattern development on the caudal fin; and (3) reproducing a random distribution of cells in the fin lobes and clear stripes near the cleft simultaneously. In this work, we focused mainly on the first two challenges, as we expect that different subclasses of melanophores and xanthophores may be present in the distal and proximal ends of the fin (for example, cells may initially be in an early or larval form at the distal end). In future work it would be interesting to explore the inclusion of additional types of melanophores and xanthophores. More generally, there are many places this first study of pattern formation on the caudal fin could be improved and extended. Exploring quantitative measurements of pattern quality would add to this work; we are also interested in exploring mutations on the fin and simulating amputation experiments. In particular, the *longfin* mutation, which involves faster fin growth, features stripes that bifurcate at the distal edge (personal communication, Christiane Nüsslein-Volhard, January 21, 2015). This motivates a bifurcation analysis of the continuum limit associated with our two-cell agent-based models and an exploration of how it behaves on growing domains.

Lastly, we would like to make two comments on why this short study of caudal fin patterning, an outgrowth of our first zebrafish model, is a fitting way to end this thesis. This study and the future work it motivates on *longfin* zebrafish is related to the role of iridophores and the Turing mechanism [80], two concepts that have shaped our work and initially motivated the problems considered in this thesis possibly more so than anything else. It is worth noting here that, because mutations lacking iridophores do not impact fin patterning, this has in the past been used by

the experimental community to suggest iridophores are not involved in stripe formation anywhere on zebrafish [43]. We are now able to present fuller evidence of why iridophores are necessary on the body by explaining, through this model, why they are not required on the caudal fin. We suggest that while iridophores specify stripe alignment on the body of zebrafish by spreading outward from the horizontal myoseptum and changing form between dense and loose [74, 75] (governed by interactions with other cells as described in Figure 4.46), on the caudal fin directionality may be accounted for through bone rays or through mechanisms of growth that are different than those at work on the zebrafish body.

Fin.

CHAPTER SIX

Conclusion

In this work we presented an application-driven modeling study of pattern formation on zebrafish (*Danio rerio*). Our goal was to shed light on wild-type cell interactions and contribute to the identification of altered interactions in mutations, including spots [33], widened stripes [43], and labyrinth curves [17]. To work closely to the biological data, we used an agent-based approach, modeling independent pigment cells as point masses that self-organize to create patterns on growing fish skin domains. Our models include both short- and long-range interactions and account for the timeline of stripe development as zebrafish grow. Experimental views on zebrafish patterning [86] changed significantly during the time of this thesis (and continue to evolve rapidly; see, for example, [44, 55]) and strongly shaped the direction of our work.

Based on the prevailing biology at the time of model development, our first model in Chapter 3 considered the birth, death, and movement interactions of two types of pigment cells: black melanophores and yellow xanthophores. We modeled cell birth and death through stochastic, discrete-time rules and specified ordinary differential equations for cell movement on growing domains. Our main results include that fish growth widens stripes and supports shorter long-range length scales, and that repulsion between melanophores and xanthophores maintains stripe boundaries. By accounting for a third type of cells indirectly, this model also supported newer biology [75] showing silver/blue iridophores (previously thought to not be involved in patterning [43]) play a crucial role in directing pattern development and guiding sequential stripe formation on the fish body.

Our two-cell model set the stage for our further work on zebrafish patterning, and, in Chapter 4, we incorporated iridophores directly into a second agent-based model. Motivated by new biological findings [17, 45], we included xanthophores (stellate in dark stripes and compact in yellow), melanophores, and iridophores (loose in

dark stripes and dense in yellow), resulting in a five-cell model that accounts for movement, birth, death, and transitions in cell form. The development of this model progressed alongside new results from the experimental community, raising some of the same questions and hitting up against the same unknowns. We put forward a network of pigment cells interactions that offers predictions about what governs iridophore dynamics, a largely open question biologically. Our model is consistent with wild-type development, early-stage mutations, and a range of experiments and quantitative measurements. The result of this work is a predictive model that can be used to identify altered cell interactions in late-stage mutations and thus help tie genes to their functions.

Lastly, in Chapter 5, we put forward a first step toward a more comprehensive view of zebrafish patterning, considering stripe formation not just on the body but also on the caudal (back) fin. Because mutations missing iridophores do not impact fin patterning (though they break body stripes into spots) [17], a two-cell model with melanophores and xanthophores is natural on the caudal fin. Thus, we presented a model of patterning on growing fin-shaped domains as an outgrowth of our original two-cell agent-based model in Chapter 3. There we were interested in understanding if necessary differences exist between the patterning mechanisms at work on the body and fins, and on identifying what specifies horizontal stripe alignment on the growing caudal fin. This work suggested a way to reconcile the apparent need for iridophores on the fish skin with mutations showing they are not required on the fins: our model suggests that, in place of iridophores on the body, bone rays or carefully-timed cell interactions coupled with fin growth can align stripes horizontally on the tailfin.

We highlight two directions for future modeling and analysis work. First, as described in §4.5.4, preliminary work with our five-cell model on late-stage mutations is promising: here we are interested in working alongside experimental approaches

from a modeling perspective to help identify what cell interactions are altered in mutations. Our five-cell body model and two-cell fin model could also be employed together to approach the problem of zebrafish mutations more comprehensively. In particular, once we identify a prediction for a mutation on the body, simulating the corresponding set-up on the fins could be a source of model testing and validation. Notably, spotted mutations leopard and luchs code for connexins, proteins that serve as a component of cell communication pathways, and leopard in particular has a mammalian orthologue (in fact, over 70% of human genes have an orthologue in the zebrafish genome [28]) [33, 87]. By simulating cross mutations between phenotypes and suggesting which cell dynamics the failure of a single connexin can disrupt, we may have the opportunity to shed light more broadly on the structure of the channels that control how cells communicate with each other.

In a more analytical direction also motivated by mutations, we are interested in developing a PDE model based on the network of cell interactions including iridophores presented in Figure 4.46. A continuum limit would provide an alternative view of patterning: a broader perspective on the overarching dynamics that govern stripe formation (for example, short-range activation and long-range inhibition [19, 80] or density-dependent motion [6]). We are particularly interested in conducting a bifurcation analysis and coupling this with our agent-based work to explore how mutated patterns arise due to the altered interactions of pigment cells on zebrafish.

Bibliography

- [1] Battersby, S.. News feature: The cells that flock together. *Proceedings of the National Academy of Sciences* 2015;112(26):7883–7885.
- [2] Bernoff, A.J., Topaz, C.M.. A primer of swarm equilibria. *SIAM Journal of Applied Dynamical Systems* 2011;10(1):212–250.
- [3] Bloomfield, J., Painter, K., Sherratt, J.. How does cellular contact affect differentiation mediated pattern formation? *Bulletin of mathematical biology* 2011;73(7):1529–1558.
- [4] Budi, E.H., Patterson, L.B., Parichy, D.M.. Post-embryonic nerve-associated precursors to adult pigment cells: genetic requirements and dynamics of morphogenesis and differentiation. *PLoS Genet* 2011;7(5):e1002044.
- [5] Bullara, D., De Decker, Y.. Pigment cell movement is not required for generation of turing patterns in zebrafish skin. *Nature Communications* 2015;6.
- [6] Cahn, J.W., Hilliard, J.E.. Free energy of a nonuniform system. i. interfacial free energy. *The Journal of chemical physics* 1958;28(2):258–267.
- [7] Caicedo-Carvajal, C.E., Shinbrot, T.. In silico zebrafish pattern formation. *Developmental biology* 2008;315(2):397–403.
- [8] Ceol, C.J., Houvras, Y., White, R.M., Zon, L.I.. Melanoma biology and the promise of zebrafish. *Zebrafish* 2008;5(4):247–255.
- [9] Dawid, I.. Developmental biology of zebrafish. *Annals of the New York Academy of Science* ;.
- [10] Deglincerti, A., Etoc, F., Ozair, M.Z., Brivanlou, A.H.. Chapter six-self-organization of spatial patterning in human embryonic stem cells. *Current topics in developmental biology* 2016;116:99–113.
- [11] Dooley, C.M., Mongera, A., Walderich, B., Nüsslein-Volhard, C.. On the embryonic origin of adult melanophores: the role of *erbb* and *kit* signalling in establishing melanophore stem cells in zebrafish. *Development* 2013;140(5):1003–1013.

- [12] Eom, D.S., Bain, E.J., Patterson, L.B., Grout, M.E., Parichy, D.M.. Long-distance communication by specialized cellular projections during pigment pattern development and evolution. *Elife* 2015;4:e12401.
- [13] Eom, D.S., Inoue, S., Patterson, L.B., Gordon, T.N., Slingwine, R., Kondo, S., Watanabe, M., Parichy, D.M.. Melanophore migration and survival during zebrafish adult pigment stripe development require the immunoglobulin superfamily adhesion molecule igsf11. *PLoS Genet* 2012;8(8):e1002899.
- [14] Fadeev, A., Krauss, J., Frohnhöfer, H.G., Irion, U., Nüsslein-Volhard, C.. Tight junction protein 1a regulates pigment cell organisation during zebrafish colour patterning. *Elife* 2015;4:e06545.
- [15] Fadeev, A., Krauss, J., Singh, A.P., Nüsslein-Volhard, C.. Zebrafish leucocyte tyrosine kinase controls iridophore establishment, proliferation and survival. *Pigment cell & melanoma research* 2016;29(3):284–296.
- [16] Frohnhöfer, H.G., Geiger-Rudolph, S., Pattky, M., Meixner, M., Huhn, C., Maischein, H.M., Geisler, R., Gehring, I., Maderspacher, F., Nüsslein-Volhard, C., et al. Spermidine, but not spermine, is essential for pigment pattern formation in zebrafish. *Biology open* 2016;5(6):736–744.
- [17] Frohnhöfer, H.G., Krauss, J., Maischein, H.M., Nüsslein-Volhard, C.. Iridophores and their interactions with other chromatophores are required for stripe formation in zebrafish. *Development* 2013;140(14):2997–3007.
- [18] Gaffney, E., Lee, S.S.. The sensitivity of turing self-organization to biological feedback delays: 2d models of fish pigmentation. *Mathematical Medicine and Biology* 2013;;dqt017.
- [19] Gierer, A., Meinhardt, H.. A theory of biological pattern formation. *Kybernetik* 1972;12(1):30–39.
- [20] Goldsmith, M.I., Fisher, S., Waterman, R., Johnson, S.L.. Saltatory control of isometric growth in the zebrafish caudal fin is disrupted in long fin and rapunzel mutants. *Developmental biology* 2003;259(2):303–317.
- [21] Goldsmith, M.I., Iovine, M.K., O'Reilly-Pol, T., Johnson, S.L.. A developmental transition in growth control during zebrafish caudal fin development. *Developmental biology* 2006;296(2):450–457.
- [22] Goodrich, H., Nichols, R.. The development and the regeneration of the color pattern in brachydanio rerio. *Journal of Morphology* 1931;52(2):513–523.
- [23] Goodrich, H.B., Marzullo, C.M., Bronson, W.R.. An analysis of the formation of color patterns in two fresh-water fish. *Journal of Experimental Zoology* 1954;125(3):487–505.
- [24] Green, J., Taylor, J.J., Hindes, A., Johnson, S.L., Goldsmith, M.I.. A gain of function mutation causing skeletal overgrowth in the rapunzel mutant. *Developmental biology* 2009;334(1):224–234.

- [25] Hamada, H., Watanabe, M., Lau, H.E., Nishida, T., Hasegawa, T., Parichy, D.M., Kondo, S.. Involvement of delta/notch signaling in zebrafish adult pigment stripe patterning. *Development* 2014;141(2):318–324.
- [26] Hirata, M., Nakamura, K.i., Kanemaru, T., Shibata, Y., Kondo, S.. Pigment cell organization in the hypodermis of zebrafish. *Developmental Dynamics* 2003;227(4):497–503.
- [27] Hirata, M., Nakamura, K.I., Kondo, S.. Pigment cell distributions in different tissues of the zebrafish, with special reference to the striped pigment pattern. *Developmental Dynamics* 2005;234(2):293–300.
- [28] Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 2013;496(7446):498–503.
- [29] Inaba, M., Yamanaka, H., Kondo, S.. Pigment pattern formation by contact-dependent depolarization. *Science* 2012;335(6069):677–677.
- [30] Inoue, S., Kondo, S., Parichy, D.M., Watanabe, M.. Tetraspanin 3c requirement for pigment cell interactions and boundary formation in zebrafish adult pigment stripes. *Pigment cell & melanoma research* 2014;27(2):190–200.
- [31] Iovine, M.K.. Conserved mechanisms regulate outgrowth in zebrafish fins. *Nature chemical biology* 2007;3(10):613–618.
- [32] Iovine, M.K., Johnson, S.L.. Genetic analysis of isometric growth control mechanisms in the zebrafish caudal fin. *Genetics* 2000;155(3):1321–1329.
- [33] Irion, U., Frohnhöfer, H.G., Krauss, J., Champollion, T.Ç., Maischein, H.M., Geiger-Rudolph, S., Weiler, C., Nüsslein-Volhard, C.. Gap junctions composed of connexins 41.8 and 39.4 are essential for colour pattern formation in zebrafish. *Elife* 2014;3:e05125.
- [34] Irion, U., Singh, A.P., Nüsslein-Volhard, C.. Chapter eight-the developmental genetics of vertebrate color pattern formation: Lessons from zebrafish. *Current topics in developmental biology* 2016;117:141–169.
- [35] Iwashita, M., Watanabe, M., Ishii, M., Chen, T., Johnson, S.L., Kurachi, Y., Okada, N., Kondo, S.. Pigment pattern in jaguar/obelix zebrafish is caused by a kir7. 1 mutation: implications for the regulation of melanosome movement. *PLoS Genet* 2006;2(11):e197.
- [36] Johnson, S.L., Africa, D., Walker, C., Weston, J.A.. Genetic control of adult pigment stripe development in zebrafish. *Developmental biology* 1995;167(1):27–33.
- [37] Kéfi, S., Rietkerk, M., Alados, C.L., Pueyo, Y., Papanastasis, V.P., ElAich, A., De Ruiter, P.C.. Spatial vegetation patterns and imminent desertification in mediterranean arid ecosystems. *Nature* 2007;449(7159):213–217.

- [38] Kizil, C., Otto, G.W., Geisler, R., Nüsslein-Volhard, C., Antos, C.L.. Simplet controls cell proliferation and gene transcription during zebrafish caudal fin regeneration. *Developmental biology* 2009;325(2):329–340.
- [39] Kondo, S.. Turing pattern formation without diffusion. In: *Conference on Computability in Europe*. Springer; 2012. p. 416–421.
- [40] Kondo, S., Watanabe, M.. Black, yellow, or silver: Which one leads skin pattern formation? *Pigment cell & melanoma research* 2015;28(1):2–4.
- [41] Krauss, J., Frohnhöfer, H.G., Walderich, B., Maischein, H.M., Weiler, C., Irion, U., Nüsslein-Volhard, C.. Endothelin signalling in iridophore development and stripe pattern formation of zebrafish. *Biology open* 2014;3(6):503–509.
- [42] Liu, Q.X., Doelman, A., Rottschäfer, V., de Jager, M., Herman, P.M., Rietkerk, M., van de Koppel, J.. Phase separation explains a new class of self-organized spatial patterns in ecological systems. *Proceedings of the National Academy of Sciences* 2013;110(29):11905–11910.
- [43] Maderspacher, F., Nüsslein-Volhard, C.. Formation of the adult pigment pattern in zebrafish requires leopard and obelix dependent cell interactions. *Development* 2003;130(15):3447–3457.
- [44] Mahalwar, P., Singh, A.P., Fadeev, A., Nüsslein-Volhard, C., Irion, U.. Heterotypic interactions regulate cell shape and density during color pattern formation in zebrafish. *Biology Open* 2016;5(11):1680–1690.
- [45] Mahalwar, P., Walderich, B., Singh, A.P., Nüsslein-Volhard, C.. Local reorganization of xanthophores fine-tunes and colors the striped pattern of zebrafish. *Science* 2014;345(6202):1362–1364.
- [46] McMenamin, S., Chandless, M., Parichy, D.. Working with zebrafish at postembryonic stages. *Methods in Cell Biology* 2016;134:587–607.
- [47] McMenamin, S.K., Bain, E.J., McCann, A.E., Patterson, L.B., Eom, D.S., Waller, Z.P., Hamill, J.C., Kuhlman, J.A., Eisen, J.S., Parichy, D.M.. Thyroid hormone-dependent adult pigment cell lineage and pattern in zebrafish. *Science* 2014;345(6202):1358–1361.
- [48] Meinhardt, H.. *The algorithmic beauty of sea shells*. Springer Science & Business Media, 2009.
- [49] Meinhardt, H., Gierer, A.. Pattern formation by local self-activation and lateral inhibition. *Bioessays* 2000;22(8):753–760.
- [50] Mellgren, E.M., Johnson, S.L.. Pyewacket, a new zebrafish fin pigment pattern mutant. *Pigment cell research* 2006;19(3):232–238.
- [51] Merks, R.M., Brodsky, S.V., Goligorsky, M.S., Newman, S.A., Glazier, J.A.. Cell elongation is key to in silico replication of in vitro vasculogenesis and subsequent remodeling. *Developmental biology* 2006;289(1):44–54.

- [52] Mills, M.G., Nuckels, R.J., Parichy, D.M.. Deconstructing evolution of adult phenotypes: genetic analyses of kit reveal homology and evolutionary novelty during adult pigment pattern development of danio fishes. *Development* 2007;134(6):1081–1090.
- [53] Moreira, J., Deutsch, A.. Pigment pattern formation in zebrafish during late larval stages: A model based on local interactions. *Developmental Dynamics* 2005;232(1):33–42.
- [54] Nakamasu, A., Takahashi, G., Kanbe, A., Kondo, S.. Interactions between zebrafish pigment cells responsible for the generation of turing patterns. *Proceedings of the National Academy of Sciences* 2009;106(21):8429–8434.
- [55] Nüsslein-Volhard, C., Singh, A.P.. How fish color their skin: A paradigm for development and evolution of adult patterns. *BioEssays* 2017;39(3).
- [56] Painter, K., Bloomfield, J., Sherratt, J., Gerisch, A.. A nonlocal model for contact attraction and repulsion in heterogeneous cell populations. *Bulletin of mathematical biology* 2015;77(6):1132–1165.
- [57] Parichy, D.M.. Advancing biology through a deeper understanding of zebrafish ecology and evolution. *Elife* 2015;4:e05635.
- [58] Parichy, D.M., Elizondo, M.R., Mills, M.G., Gordon, T.N., Engeszer, R.E.. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. *Developmental Dynamics* 2009;238(12):2975–3015.
- [59] Parichy, D.M., Ransom, D.G., Paw, B., Zon, L.I., Johnson, S.L.. An orthologue of the kit-related gene *fms* is required for development of neural crest-derived xanthophores and a subpopulation of adult melanocytes in the zebrafish, *danio rerio*. *Development* 2000;127(14):3031–3044.
- [60] Parichy, D.M., Spiewak, J.E.. Origins of adult pigmentation: diversity in pigment stem cell lineages and implications for pattern evolution. *Pigment cell & melanoma research* 2015;28(1):31–50.
- [61] Parichy, D.M., Turner, J.M.. Temporal and cellular requirements for *fms* signaling during zebrafish adult pigment pattern development. *Development* 2003;130(5):817–833.
- [62] Parichy, D.M., Turner, J.M.. Zebrafish *puma* mutant decouples pigment pattern and somatic metamorphosis. *Developmental biology* 2003;256(2):242–257.
- [63] Parichy, D.M., Turner, J.M., Parker, N.B.. Essential role for *puma* in development of postembryonic neural crest-derived cell lineages in zebrafish. *Developmental biology* 2003;256(2):221–241.
- [64] Patterson, L.B., Bain, E.J., Parichy, D.M.. Pigment cell interactions and differential xanthophore recruitment underlying zebrafish stripe reiteration and danio pattern evolution. *Nature communications* 2014;5.

- [65] Patterson, L.B., Parichy, D.M.. Interactions with iridophores and the tissue environment required for patterning melanophores and xanthophores during zebrafish adult pigment stripe formation. *PLoS Genet* 2013;9(5):e1003561.
- [66] Perathoner, S., Daane, J.M., Henrion, U., Seebohm, G., Higdon, C.W., Johnson, S.L., Nüsslein-Volhard, C., Harris, M.P.. Bioelectric signaling regulates size in zebrafish fins. *PLoS Genet* 2014;10(1):e1004080.
- [67] Pfefferli, C., Jaźwińska, A.. The art of fin regeneration in zebrafish. *Regeneration* 2015;2(2):72–83.
- [68] Quigley, I.K., Manuel, J.L., Roberts, R.A., Nuckels, R.J., Herrington, E.R., MacDonald, E.L., Parichy, D.M.. Evolutionary diversification of pigment pattern in danio fishes: differential fins dependence and stripe loss in *d. albolineatus*. *Development* 2005;132(1):89–104.
- [69] Quigley, I.K., Parichy, D.M.. Pigment pattern formation in zebrafish: a model for developmental genetics and the evolution of form. *Microscopy research and technique* 2002;58(6):442–455.
- [70] Rawls, J.F., Johnson, S.L.. Zebrafish kit mutation reveals primary and secondary regulation of melanocyte development during fin stripe regeneration. *Development* 2000;127(17):3715–3724.
- [71] Rolland-Lagan, A.G., Paquette, M., Tweedle, V., Akimenko, M.A.. Morphogen-based simulation model of ray growth and joint patterning during fin development and regeneration. *Development* 2012;139(6):1188–1197.
- [72] Scanlon, T.M., Caylor, K.K., Levin, S.A., Rodriguez-Iturbe, I.. Positive feedbacks promote power-law clustering of kalahari vegetation. *Nature* 2007;449(7159):209–212.
- [73] Singh, A.P., Frohnhöfer, H.G., Irion, U., Nüsslein-Volhard, C.. Response to comment on “local reorganization of xanthophores fine-tunes and colors the striped pattern of zebrafish”. *Science* 2015;348(6232):297–297.
- [74] Singh, A.P., Nüsslein-Volhard, C.. Zebrafish stripes as a model for vertebrate colour pattern formation. *Current Biology* 2015;25(2):R81–R92.
- [75] Singh, A.P., Schach, U., Nüsslein-Volhard, C.. Proliferation, dispersal and patterned aggregation of iridophores in the skin prefigure striped colouration of zebrafish. *Nature cell biology* 2014;16(6):604–611.
- [76] Takahashi, G., Kondo, S.. Melanophores in the stripes of adult zebrafish do not have the nature to gather, but disperse when they have the space to move. *Pigment cell & melanoma research* 2008;21(6):677–686.
- [77] Topaz, C.M., D’Orsogna, M.R., Edelstein-Keshet, L., Bernoff, A.J.. Locust dynamics: behavioral phase change and swarming. *PLoS Comput Biol* 2012;8(8):e1002642.
- [78] Tu, S., Johnson, S.L.. Clonal analyses reveal roles of organ founding stem cells, melanocyte stem cells and melanoblasts in establishment, growth and regeneration of the adult zebrafish fin. *Development* 2010;137(23):3931–3939.

- [79] Tu, S., Johnson, S.L.. Fate restriction in the growing and regenerating zebrafish fin. *Developmental cell* 2011;20(5):725–732.
- [80] Turing, A.M.. The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 1952;237(641):37–72.
- [81] Volkening, A., Sandstede, B.. Modelling stripe formation in zebrafish: an agent-based approach. *Journal of the Royal Society Interface* 2015;12(112):20150812.
- [82] Walderich, B., Singh, A.P., Mahalwar, P., Nüsslein-Volhard, C.. Homotypic cell competition regulates proliferation and tiling of zebrafish pigment cells during colour pattern formation. *Nature communications* 2016;7.
- [83] Watanabe, M., Iwashita, M., Ishii, M., Kurachi, Y., Kawakami, A., Kondo, S., Okada, N.. Spot pattern of leopard danio is caused by mutation in the zebrafish connexin41. 8 gene. *EMBO reports* 2006;7(9):893–897.
- [84] Watanabe, M., Kondo, S.. Changing clothes easily: connexin41. 8 regulates skin pattern variation. *Pigment cell & melanoma research* 2012;25(3):326–330.
- [85] Watanabe, M., Kondo, S.. Comment on“local reorganization of xanthophores fine-tunes and colors the striped pattern of zebrafish”. *Science* 2015;348(6232):297–297.
- [86] Watanabe, M., Kondo, S.. Is pigment patterning in fish skin determined by the turing mechanism? *Trends in Genetics* 2015;31(2):88–96.
- [87] Watanabe, M., Sawada, R., Aramaki, T., Skerrett, I.M., Kondo, S.. The physiological characterization of connexin41. 8 and connexin39. 4, which are involved in the striped pattern formation of zebrafish. *Journal of Biological Chemistry* 2016;291(3):1053–1063.
- [88] Woolley, T.E., Maini, P.K., Gaffney, E.A.. Is pigment cell pattern formation in zebrafish a game of cops and robbers? *Pigment cell & melanoma research* 2014;27(5):686–687.
- [89] Yamaguchi, M., Yoshimoto, E., Kondo, S.. Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism. *Proceedings of the National Academy of Sciences* 2007;104(12):4790–4793.
- [90] Yamanaka, H., Kondo, S.. In vitro analysis suggests that difference in cell movement during direct interaction can generate various pigment patterns in vivo. *Proceedings of the National Academy of Sciences* 2014;111(5):1867–1872.