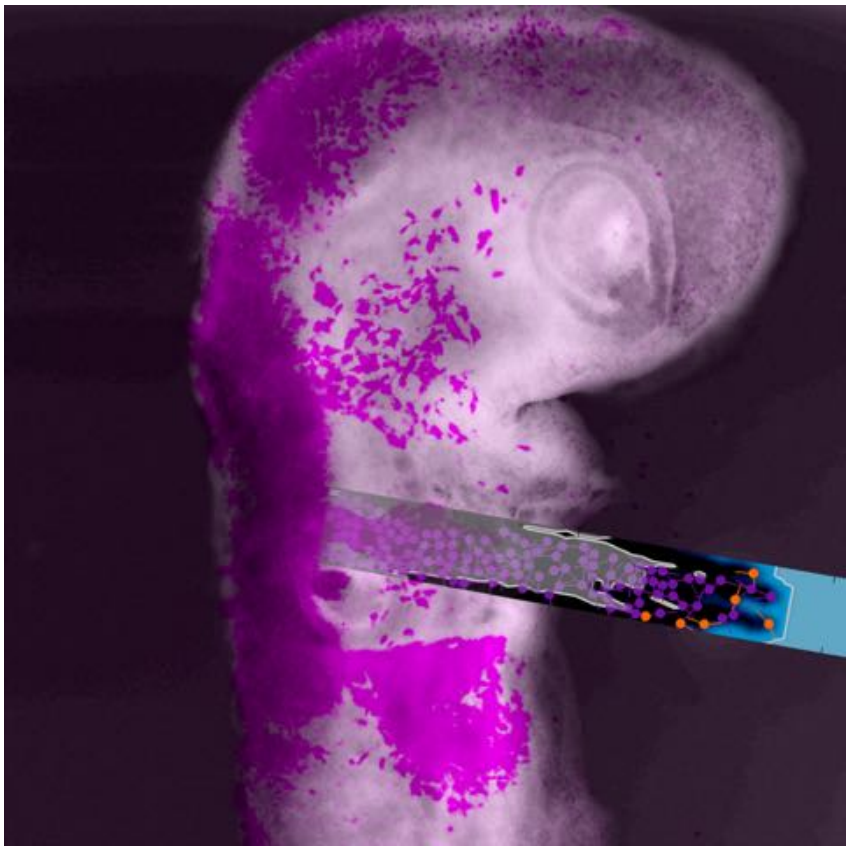
European Communications in Mathematical and Theoretical Biology
2016, No. 19

Communications



A European Forum for Information, Presentation and Exchange
Official Communication Bulletin of ESMTB

Cover image: ***Lateral view of a chick embryo head with fluorescently labeled neural crest cells, overlaid with a computational simulation of multicellular streaming migration in a cell-induced chemoattractant gradient.***
Courtesy of Rebecca McLennan, Kulesa Lab, Stowers Institute of Medical Research (background image) and Linus Schumacher, University of Oxford (simulation)

Letter from the President

Dear colleagues and members of the ESMTB,

Interesting problems in mathematical biology often come to us via unexpected encounters. While eating spaghetti frutti di mare in 1980, Hans Meinhardt noticed the fascinating patterns on the shells in his plate. He adopted his reaction-diffusion theory to explain a multitude of shell patterns, culminating in his now classic book *The Algorithmic Beauty of Sea Shells*. Sadly, Prof. Meinhardt passed away on February 11th, 2016. His pioneering work in the mathematical biology of biological pattern formation has inspired many of us.

As a further example of an unexpected encounter in mathematical biology, read the history of Vito Volterra from page 23. Volterra was hearing out his potential future son-in-law Umberto D'Ancona in the early 1920s. Probably in an attempt to impress the father of the girl he loved, D'Ancona explained Volterra how in his fishery studies he had noticed how during World War I, the catches of predatory fish in the Adriatic sea had increased. The rest is textbook knowledge, and the Lotka-Volterra equations have been applied to topics ranging from cancer biology and immunology to prebiotic evolution.

Hopefully the 2016 edition of the **European Conference for Mathematical and Theoretical Biology (ECMTB2016) in Nottingham** will also bring you in touch with new persons or new food items, with potentially far-reaching ramifications to mathematical biology. Our field depends on unexpected encounters, as also illustrated in our new series »European Teams in Mathematical Biology« from page 15. So please do take the opportunity at ECTMB 2016 to attend some lectures beyond your own field.

I would particularly draw your attention to the plenary lectures on Friday afternoon of our two 2014 Reinhart Heinrich awardees, Aurélie Carlier (University of Louvain, Belgium) and Juan Carlos López Alfonso (Complutense University of Madrid Spain) and to the plenary lecture of the winner of 2015, Linus Schumacher (Oxford University). From page 10 you can read Linus' extended thesis summary. Also do not forget to go out for some good food and drinks to meet your friends and colleagues and make new ones. This is where the magic happens and new ideas are born! I cordially thank the organizers of ECMTB2016.

Apart from the Nottingham conference organized jointly with SMB, ESMTB has teamed up with the European Mathematical Society (EMS) to organize two summer schools in 2016. The summer school ***Mathematical Biology of Tissue Mechanics*** **at the Lorentz Center Leiden, The Netherlands** is held from July 25th to July 29th. This summer school will focus on hands-on team work on multicellular modeling problems, ranging from pattern formation in fish skins to spatial models of blood cancers. From Augusts 21 to 28th, ESMTB and EMS organize the fifth **Helsinki Summer School on Mathematical Ecology and Evolution**, which will focus on structured populations. For more information, see our website http://www.esmtb.org, follow us on Twitter: @ESMTBio and like us on Facebook: http://www.facebook.com/ESMTB.

In the further future, I am pleased to announce that 2018 will be the **Year of Mathematical Biology**. Organized jointly by the EMS, the ESMTB and the SMB, 2018 will feature events on mathematical biology all over Europe and in the US, including the next edition of ECMTB, organized jointly by the ESTMB and the EMS in Lisbon, Portugal.

I wish you many unexpected encounters, leading to new mathematical and theoretical insights in biology and medicine!

Roeland Merks, merks@cwi.nl

Minutes of the ESMTB Board meeting

Copenhagen, Denmark
October 23rd 2015

The meeting starts at 9:10.

Present: Barbara Boldin (BB; Minutes), Reinhard Bürger (RB), Andrea De Gaetano (ADG), Susanne Ditlevsen (SD), Anna Marciniak-Czochra (AMC), Frank Hilker (FH), Roeland Merks (RM; Chair), Ryszard Rudnicki (RR), Vitaly Volpert (VV)
Absent with apology: Torbjörn Lundh

1. Agenda of the meeting and Minutes of the ESMTB Board meeting in Vienna

The proposed agenda is reviewed, completed and approved. The Minutes of the previous ESMTB Board meeting in Vienna are approved as well.

2. Report by the president

RM gives a brief report on the state of the Society and highlights the urgency to finalise the transfer of duties from the previous Board to current Board members. In particular, the ESMTB website is not updated regularly. It is vital that a new host for the ESMTB website is found quickly and that the website is updated regularly to ensure that members of the Society and other interested parties receive up-to-date information about the Society. The new webmaster is Bob Planqué (VU University Amsterdam).

3. Report of the treasurer

FH reports that moving the ESMTB account has turned out to be more problematic than expected. Two long-term solutions are proposed: either to open an account for ESMTB in France (where the Society is registered) or to open an account in Germany, but in a way that future treasurers of the Society will have no trouble transfering or handling the account.

The Society's financial state is stable, the current balance is roughly 18.000 euro. A vast part of the current balance is however still due to the surplus created by the ECMTB in Dresden. ESMTB revenues are exclusively from membership fees. It is therefore vital to encourage professionals in the field of mathematical and theoretical biology to be part of the Society.

4. ECMTB 2016

The joint ESMTB-SMB conference will take place in Nottingham, UK, from 11th to 15th of July 2016. The conference website is now online (http://www.ecmtb2016.org). SD gives a brief report on the organisation of the conference. The list of plenary speakers is now finalized and consists of Ruth Baker (University of Oxford, UK), Sander van Doorn (University of Groningen, The Netherlands), Julia Gog (University of Cambridge, UK), Leah Edelstein-Keshet (University of British Columbia, Canada), Johan van de Koppel (Royal Netherlands Institute of Sea Research), Hisashi Ohtsuki (Kanagawa, Japan), Johan Paulsson (Harward University, USA) and John Rinzel (NYU, USA - SMB Winfree Prize lecture).

Markus Owen of the local organising committee joins the discussion via Skype. In addition to plenary talks, the plan is to have 12 parallel sessions of mini-symposia, each mini-symposium will take 2 hours. The deadline for mini-symposia proposals is December 18th 2015. Mini-symposia proposals received by December 18th will be checked by the conference committee and mini-symposia will be scheduled to ensure as little overlap between similar topics as possible. An online schedule-maker is planned for participants of the conference.

Markus Owen presents the registration fees. As usual, a discount is offered to ESMTB/SMB members.

5. Future ECMTB

The final decision regarding whether ECMTB should continue with a two-year cycle or return to the three-year cycle has not been made. A two year cycle offers several advantages, including more opportunities for PhD students to present their work and more chances to promote the Society. An obstacle might be a lack of potential hosts. In addition, the appeal of ECMTB may decline as there might be too many large-scale conferences.

Two potential candidates are presented, one for organising a conference in 2018 or 2019 and the other for the conference that follows. It is agreed that, if the first candidate can confirm the willingness to organise ECMTB in 2018, the Society will try to continue with a two-year cycle.

Remark. It has since been confirmed that ECMTB 2018 will take place in Lisbon.

6. ESMTB-EMS summer schools

The ESMTB-EMS summer school that was planned for 2015 (*Mathematical Modeling of Tissue Mechanics*) will instead take place in July 2016. There will therefore be two ESMTB-SMB summer schools in the year 2016:

1. ***The Helsinki summer school on Mathematical ecology and evolution***
will take place from 21st until 28th August 2016 in Linnasmäki Conference Centre in Turku, Finland. This is already the 5th conference in a biennial series of summer schools on mathematical ecology and evolution, organised by the biomathematics group of the University of Helsinki (the main organisers are Mats Gyllenberg and Eva Kisdi). The theme of the 2016 summer school is Structured populations and the confirmed lecturers and topics are

- Mats Gyllenberg (University of Helsinki): Dynamics of structured populations
- Hans Metz (University of Leiden): Adaptive dynamics in structured populations
- Reinhard Bürger (University of Vienna): Population genetics in structured populations
- Hisashi Inaba (University of Tokyo): Infectious diseases in structured populations
- André de Roos (University of Amsterdam): Population and community ecology of ontogenetic development

More information can be found on the website of the summer school (https://wiki.helsinki.fi/display/BioMath/The+Helsinki+Summer+School+on+Mathematical+Ecology+and+Evolution+2016%3A+Structured+Populations)

2. The summer school **Mathematical Modeling of Tissue Mechanics** will take place from 25th until 29th July 2016 at the Lorentz Center in Leiden, the Netherlands. The summer-school is organised by Andreas Deutsch, Roeland Merks and Vitaly Volpert. The school will revolve around the following five hands-on projects, of which participants will choose one:

- Spatial effects in the pathogenesis of blood cancers, including leukemia (Mentors: Vitaly Volpert and Anass Bouchnita, CNRS Lyon, France)
- Modelling pigment cell interactions in zebrafish skin patterns (Mentors: Roeland Merks, CWI and Uuniversity Leiden, The Netherlands and Kevin Painter, Heriot-Watt University, Edinburgh, UK (to be confirmed))
- Multiscale modeling of planar cell polarity: coordinating cellular orientations across tissue (Mentor: Andreas Deutsch, TU Dresden)
- Morphogenesis and Dynamics of Multicellular Systems (Mentor: Andreas Deutsch, TU Dresden)

Zebrafish epiboly and formation of compartments in 3D tissues: coupling mechanical behavior and gene regulation (Mentors: Nadine Peyriéras and René Doursat, BioEmergences, CNRS USR3695, Gif-sur-Yvette, France)

More information can be found on the website:
http://www.lorentzcenter.nl/lc/web/2016/803/info.php3?wsid=803&venue=Oort.
The organisers have obtained financial support from the Lorentz Center (5.000 euro), the Dutch nonlinear dynamical systems priority program NDNS+ (2.500 euro) and have applied for funding from the EMS.

ESMTB-EMS summer school 2017. It is time to start planning the ESMTB-EMS summer school in 2017. Mathematical neuroscience is put forward as an idea of the summer school theme. RR mentions the possibility to organise the summer school in Bedlewo, Poland.

7. ESMTB Communications

RM hands out printed copies of the latest issue of ESMTB Communications and the Board thanks VV for his effort in producing the Communications. The printing of the Communications is still done via Dresden. The cost of production and distribution is around 1.000 euro. This is seen as a good deal and there is no initiative as yet to move the printing to another country since higher production and distributions costs are expected.

Several ideas on how to increase the appeal of ESMTB Communications are presented:

- Board members might invite important/interesting people in the field to write about their career paths.
- Include historical accounts. ADG volunteers to write for the next issue a portrait of Vito Volterra.
- Include brief presentations of research groups in Europe. SD and AMC suggest two research groups that might be included as the first two contributions.

Two other ideas are put forward:

- The ECMTB 2016 in Nottingham will the 10th conference of ESMTB. Andreas Deutsch and Mats Gyllenberg seem to be the only two present at the first 9 conferences. It is proposed that both are invited to write about their experience for the Communications.
- Recipients of ESMTB travel support will be invited to write a short account on how their travel support was spent (which events they attended, how they experienced those events, etc.)

The next issue of ESMTB Communications will be prepared and printed before ECMTB 2016 and distributed in Nottingham.

8. ESMTB website and ESMTB Infoletter

The ESMTB Infoletter is organised and sent by AMC via Dresden. The procedure appears to be complicated and several simplifications are suggested, including to set up a list server to distribute the ESMTB Infoletter as a plain text e-mail via the mailing list. When the new system will be set up, all existing ESMTB members should be automatically subscribed to the mailing list and notified of the change.
The editing of the website is currently done by Bob Planque in Amsterdam. The idea is to look for an outside provider (commercial web service) to host the website. Preferentially, the website account should be opened in Society's name. RM and Bob Planque will take care of the transfer.

E-mails sent to info@esmtb.org are not seen by the Board members. AMC offers to take care of overseeing the info@esmtb.org contributions. The e-mails of all Board members are already included on the website. It is suggested that a separate e-mail account is made (and preseneted on the website) for ESMTB travel support, Infoletter as well as a link for Paypal, with buttons on the ESMTB website directing the visitors to a particular service.

9. Reinhart Heinrich award

RB will replace Andreas Deutsch on the Reinhart Heinrich prize committee. The closing date for nominations is November 30th 2015. Shortlisted applicants will be asked to submit their full PhD thesis. The report on the Reinhart Heinrich award 2015 will be included in the next issue of ESMTB Communications, including an extented abstract of the winning thesis.

10. Communication and promotion

The use of ESMTB Facebook and Twitter accounts is increasing whereas the traditional methods of promotion (postcards, bookmarks, leaflets) are no longer that successful. The idea is put forward to print leaflets and/or bookmarks only for specific events and emphasize the benefits of ESMTB membership. Another idea is to introduce promotional pens with ESMTB logo (instead of postcards, for example).
At ECMTB 2016 only ESMTB Communications will be distributed (no postcards, leaflets or bookmarks).

ADG will explore options to make an ESMTB account on LinkedIn and BB will check whether it is possible to open and ESMTB account of Researchgate.

11. ESMTB Board elections 2017

Half of the current ESMTB Board (BB, RB, RM, RR and VV) end their term on the Board of ESMTB at the end of 2017. Typically, the candidates for the forthcoming Board elections are announced during the General Assembly of an ECMTB. Due to the two year gap between the conference in Gothenburg in 2014 and ECMTB in Nottingham, the General Assembly of ECMTB 2016 will be used only to announce the coming Board elections in 2017 and to invite ESMTB members to offer themselves as candidates for the elections. During the next Board meeting, the Board will in addition compose a list of potential candidates and send out invitations.

12. ESMTB travel support and support for conferences

ADG is in charge of handling the applications for ESMTB travel support. ADG hands out application forms that are currently in use. The application form needs to be made simpler and clearer to enable

easier evaluation of recieved applications. ADG offers to prepare a draft of the new application form to be reviewed and approved by the Board. Optimally, applications would be submitted via a web form on ESMTB website, with incomplete submissions being rejected and returned to the applicant for completion and resubmission.

Currently, applications are collected and evaluated only once per year. ADG suggests to change this practice by having multiple evaluations per year (the deadlines of which will be announced in time). The Board agrees. The annual budget and its distribution through a calendar year will be decided by the Board, depending on the events taking place in a particular year and the Society's financial statuts. For 2016, the proposed budget for ESMTB travel support is 3.500 euro (10 successful applicants receiving 350 euro each). If more applications are received that can be supported, ADG will prepare a proposal as to which applicants to support and the list will be reviewed by the Board.

No applications for support of conferences has been received since the last Board meeting.

13. Ties with other societes

ESMTB is a member of EMS and ICIAM:

- EMS and ESMTB organise joint summer schools. In recent months RM has discussed with Jose Carrillo (EMS) the possibility of enhancing links between EMS and ESMTB. The majority of the Board sees such collaboration as positive, bringing opportunities to promote mathematical biology in the mathematical community and enhance funding opportunities. An initiative was put forward to have a long-term program on mathematical biology that will build on existing ESMTB-EMS summer schools or to organise a "Year of Mathematical Biology". It is proposed that these initiatives are announced during ECMTB 2016 to collect further ideas on how to strengthen the links between ESMTB and EMS.
- Ties with ICIAM have weakened in the past years but contact is now resumed. ADG offers to be the person responsible for contacts with ICIAM.

FH remarks that reciprocal ESMTB-SMB membership does not guarantee voting rights to ESMTB members in SMB elections, while SMB members with reciprocal ESMTB membership can vote in ESMTB elections. RM offers to explore this issue with the Board of SMB.

14. Journal of Mathematical Biology/Springer

There is no news from either the Journal of Mathematical Biology or Springer.

15. ESMTB Perspectives

Very few Perspectives articles have appeared in recent years in the Journal of Mathematical Biology. New invitations should be sent out and the list of Perspectives articles on ESMTB website should be either updated or not included at all.

16. Overlay e-journals

To reduce, or even eliminate costs of scientific publishing, an idea of an "overlay" e-journal has been proposed in mathematics. Such a journal would provide links to selected, peer-reviewed papers published on the preprint server arXiv and would rely on the authors doing their own typesetting and copy-editing. Does ESMTB want to endorse this new publishing model? The Board members agree that, at the moment, the Society does not wish to endorse nor start such an overlay journal in the field of mathematical and theoretical biology.

17. Sponsoring ESMTB membership fees for citizens of third world countries

RM has received a request for a free ESMTB membership. The Board discusses whether an additional category of ESMTB membership for a reduced (or even zero) fee with limited benefits should be created. It is decided that this is not needed. Instead, we may want to introduce a link "sponsor a student" on ESMTB website where ESMTB members could donate a membership fee to be used for students who cannot afford the fee themselves.

18. Future directions of ESMTB

There is an ongoing discussion on if and how to update the "mission" statement of the Society. This discussion is postponed until the next meeting. The idea is (again) put forward to create on ESMTB website a list of research and study programs in the field of mathematical and theoretical biology in Europe. AMC volunteers to prepare a preliminary list.

In addition, the Society might want to encourage and support "PhD days", where, on a country level, student ESMTB members might meet and discuss mathematical biology. Some ESMTB travel support may be available for such meetings.

The meeting ends at 18.00

The next meeting of the Board of ESMTB will take place during ECMTB 2016 in Nottingham.

Barbara Boldin

The Reinhart Heinrich thesis award

A mathematical exploration of principles of collective cell migration and self-organisation

Linus J. Schumacher, University of Oxford

Extended summary of PhD thesis

Abstract. This thesis explores the role of collective cell migration and self-organisation in the development of the embryo and *in vitro* tissue formation through mathematical and computational approaches. We consider how population heterogeneity, microenvironmental signals and cell-cell interactions facilitate cells to collectively organise and navigate, with the aim to work towards uncovering general rules and principles, rather than delving into the microscopic molecular details. To ensure the biological relevance of our results, we collaborate closely with experimental biologists working on two model systems.

First, to understand how neural crest cells obtain directionality, maintain persistence and specialise during their migration, we use computational simulations in parallel with imaging of chick embryos under genetic and surgical perturbations. We show how only a few cells adopting a leader state that enables them to read out chemical signals can lead a population of cells in a follower state over long distances in the embryo. Furthermore, we devise and test an improved mechanism of how cells dynamically switch between leader and follower states in the presence of a chemoattractant gradient. Our computational work guides the choice of new experiments, aids in their interpretation and probes hypotheses in ways the experiments cannot.

Secondly, to study the self-organisation of mouse skin cells *in vitro*, we draw on aggregation processes and scaling theory. Dermal and epidermal cells, after being dissociated and mixed, can reconstitute functional (transplantable and hair-growing) skin in culture. Using kinetic aggregation models and scaling analysis we show that the initial clustering of epidermal cells can be described by Smoluchowski coagulation, consistent with the dynamics of the "clustering clusters" universality class. Then, we investigate a potential mechanism for the size-regulation of cell aggregates during the later stages of the skin re- constitution process. Our analysis shows the extent to which this tissue formation follows a single physical process and when the transition to different dynamics occurs, which may be triggered by cellular biochemical changes.

This extended summary provides an overview of the contents of the full thesis.

1. Introduction and overview

This thesis was born out of the motivation for doing mathematical biology with results that biologists will care about. To achieve this, it focusses on close collaboration with developmental biologists on specific experiments. In developmental biology, difficult experiments and traditionally qualitative data present an opportunity for modelling on the one hand, while on the other hand increasingly quantitative measurements at multiple spatiotemporal scales create a need for careful data analysis and comparison with theory. The success of dedicated models can guide the search for overarching theories by identifying common principles that have proven relevant in focussed applications. In this spirit, we thus explore three particular themes in the realm of collective cell behaviour, where many cells interact to form emergent patterns and coordinate movement within the constraints of the microenvironment. These themes are population heterogeneity, microenvironmental signals and cell-cell interactions. We generally take a phenomenological approach, considering rules of behaviour and decision making at the cellular level, without including all known microscopic details necessarily. Rather, we aim for minimal models to explain the experimentally observed global behaviour of cell populations as a whole, which can constrain and guide the search for molecular mechanisms.

Focussed experimental applications Working closely with developmental biologists, we aim to not only contribute to the understanding of a specific biological problem at hand, but also treat these as case studies for the broader theoretical questions that are of interest to us in the long run. This in itself is a model of a fruitful scientific collaboration, in which we work from biological observations, construct models, make predictions, test these experimentally or perform experiments suggested by our models, refine the models based on the experiments, and repeat. Each of these cycles may take anything from months to years, and it is important to acknowledge that different experimental systems are suitable for different studies, hence we do not restrict ourselves to just one fiefdom of biology here.

Aims There are three aims of this thesis: first, to explore the themes of collective behaviour in cell migration and self-organisation using suitable model systems; second, to contribute to the understanding of the focussed biological questions in a meaningful way; and third, to showcase the successes (and highlight the limitations) of an integrated experimental and theoretical approach in the study of biological systems.

2. The neural crest as a model system to study collective cell behaviour

In the study of collective migration, a remarkable example of long distance, coordinated directed migration of eukaryotic cells is found in the neural crest. The neural crest is a highly migratory and pluripotent cell population that contributes to the formation of many tissues in vertebrates. Cells from the neural crest differentiate into a variety of tissue types, such as bone, smooth muscle, pigment and nerve cells, to name but a few. During migration, the neural crest displays rich and dynamic heterogeneities at the level of morphology and gene expression. Neural crest cells originate as part of the neural tube (which goes on to form the spinal cord and brain), from which they delaminate and migrate in separate groups to distribute amongst a diverse array of destinations in the growing embryo. The neural crest thus derives from the ectoderm (the outer tissue layer of the embryo), though its characteristics are so unique and its role in vertebrate development so important, that it has

been dubbed the “fourth germ layer” (Hall, 2000). Unravelling the mechanisms of neural crest cell migration has the potential to help prevent birth defects, improve regenerative therapies and develop model systems for cancer metastasis.

In this chapter, we first introduce the reader to the biology of the neural crest model system and place it in the wider context of cell migration in developmental biology in general. We discuss mechanistic hypotheses in the field of neural crest research on how cells obtain and process directional information to navigate to the right places in the embryo. Then, we review several computational frameworks that have been used to model neural crest cell migration, or may be suitable for this purpose. We weigh their relative merits for studying our particular research questions, and explain why we have chosen to extend the computational model from McLennan et al. (2012).

3. Oligarchy in neural crest cell migration

In this chapter we explore the effects of population heterogeneity on the collective migration of cells. We choose the neural crest as a model system and focus on experiments in the chick cranial[1] neural crest, and on a specific aspect of cell heterogeneity affecting the population migratory behaviour: leader and follower states. Starting from biological evidence and computational proof-of-principle of leader and follower behaviour in the chick cranial neural crest, our aim is to determine what proportion of cells in a migrating population are in a leader state, what effect a change in the size of the leader fraction has, and what implications this may have for the efficiency and robustness of invasive migration.

To answer these questions, we use the model identified from our review in Chapter 2 (McLennan et al., 2012) as most suitable and make necessary adjustments and substantial extensions to it (Fig. 1). We use our computational model in conjunction with *in vivo* experiments to show that chick cranial neural crest cells could be guided by just a few cells at the invasive frontlines of the population. By identifying how many cells are leaders, we can hope to learn which aspects of the rich heterogeneity between neural crest cells may be attributed to a different navigational state, which then in turn may enable us to observe different cell states through their molecular profile and control cell state transitions.

4. Dynamics of leader-follower transitions

In this chapter, we extend our computational model further to explore how cell heterogeneity during neural crest collective migration is controlled by microenvironmental signals. Given the observational evidence for plasticity between leader and follower states, we want to test how much a cell’s gene expression, and resulting migratory behaviour, are controlled by the main chemoattractant, VEGF, and on what timescale.

First, we introduce the reader to the evidence for plasticity of cell behaviour and gene expression in the neural crest cell population. To put our work into context, we briefly describe and critique previous attempts to incorporate this plasticity into computational models of neural crest migration. On this basis we motivate our hypothesis for a mini- mal model of leader-follower transitions controlled by VEGF, which we then test through a

[1] cranial: Relating to the cranium, an anatomical term for the head of a vertebrate animal.

combination of simulations and experiments. We implement an integrate-and-switch mechanism in our computational model, through which neural crest cells adopt leader and follower states, with non-zero timescale and hysteresis of exposure to a directional signal. This model is consistent with the observed cell persistence and stream cohesion in the chick cranial neural crest. Our findings identify the importance of VEGF as one of the microenvironmental signals that establish a distinct subpopulation of neural crest cells in a leader state. These data support our cell-induced gradient model in which microenvironmental signals define and direct leading neural crest cells that instruct others to follow. Furthermore, we add to the growing body of evidence for guidance of cell populations through different cell states induced by microenvironmental factors (Rørth, 2012, Haeger et al., 2015), such as in Drospohila border cell (Inaki et al., 2012) and zebrafish lateral line migration (Revenu et al., 2014). Further detailed analyses of the cell behaviours and gene expression changes in migrating neural crest cells may help to elucidate the mechanistic underpinnings by which cells in a leader state instruct others to follow and how the leader-to-follower cell state transition is regulated at a molecular level.

5. Skin cell self-organisation – a clustering of clusters?

In the previous chapters, we explored how population heterogeneity and cell-environment interactions contribute to the phenomenology of collective cell migration in neural crest cells. A third theme we set out to explore in this thesis is that of cell-cell interactions. To make progress on this front, it is useful to disentangle emergent behaviour arising from environmental signals and intercellular interactions. An opportunity for this is provided by *in vitro* systems that display realistic tissue-level organisation. In this chapter, we thus turn to a different biological system to explore mechanisms of cell self-organisation without directional migration guided by the microenvironment. We continue to work at the cellular scale and consider interactions between cells. The broad question that we wish to address is: How do cells self-organise into complex tissue structures, and what path do they take to get there? To this end, we focus on a tissue engineering experiment, comprising a skin reconstitution assay in which skin cells from newborn mice are dissociated, mixed and incubated in culture. The cells then separate into two tissue layers in a way that is reminiscent of tissue organisation *in vivo*, making the culture suitable for transplantation. Transplanted skin grows new hair, even if transplanted into genetically hairless mice.

First, we describe the experiment and the types of data with which we are working, and introduce relevant theory from the literature on aggregation processes. We apply a kinetic theoretical description of an aggregation process to cell-cluster counts and validate it in three ways: direct fitting of analytical and numerical solutions of the Smoluchowski coagulation equations, scaling analysis, and collapsing of cell-cluster size distribution functions. Then, we summarise the limitations of the data and our theoretical approach, discuss the suitability of alternative descriptions to explain tissue phase-separation, and conclude by commenting on further theoretical work and suggestions for experiments.

6. Size-regulation in aggregation processes through crowding

In the previous chapter we described the self-organisation of dissociated mouse skin cells back into functional skin *in vitro*. We found that the clustering of epidermal cells is quantitatively captured by a model describing them as randomly moving, irreversibly

aggregating clusters. However, the quality of the match between data and theory decreases drastically at the 48 hour time-point, when the clusters of epidermal cells have stopped growing and their size distribution narrows to a peak. The coagulation equations would predict aggregation to continue (until epidermal and dermal cells have completely separated into two layers), without a characteristic cluster size standing out from the size distribution.

We took this as a clue that, at around 48 hours, the system is transitioning between different dynamics, and hypothesise that the interaction with dermal cells (hitherto ignored in the aggregation model) contributes to the observed size control of epidermal cell aggregates. In this shorter chapter, we explore the idea that dermal cells hinder the movement of epidermal cells through volume exclusion, and do so in a different way for large and small aggregates. Thus, we test how size control can be achieved in aggregation processes through the trapping of larger clusters. Qualitatively, we achieved exactly the desired effect of size regulation (which does not feature in the dynamics of clustering clusters), if not at the spatial and temporal scales observed in this experimental application. This suggests that additional mechanisms may also underlie the observed morphological dynamics in mouse skin reconstitution. However, we showed a generally feasible mechanism of size regulation in mixed aggregation processes, which might be relevant for other applications.

7. Conclusion

The central aim of this thesis was to contribute to the understanding of collective cell behaviour in paradigm experimental models for developmental biology through the use of mathematical and computational approaches. We focussed on two specific experimental systems. First, collective neural crest cell migration in chick embryos, and second, an *in vitro* assay for reconstituting functional mouse skin from dissociated cells. In these systems, we investigated how population heterogeneity, environmental signals and cell-cell interactions facilitate movement and organisation of a cell population as a whole.

Using our extended model of cranial neural crest migration in a cell-induced gradient with leader-follower cell heterogeneity (Fig. 1), we found that migration can be more efficient with fewer leaders, predicting that the leader cell phenotype *in vivo* is confined to the most invasive from of the migratory stream. This was confirmed by single-cell gene expression measurements, which led to the identification of a molecular profile of “trailblazer cells”. Thus, we have improved our understanding of how heterogeneity in a cell population can help them migrate in an otherwise homogeneous environment.

We further proposed and implemented a mechanism of how the leader-follower cell heterogeneity emerges through interaction with the chemoattractant gradient. Experiments first parameterised our model and then verified its predictions for perturbing the chemoattractant distribution. This work has characterised VEGF as one of the environmental signals that dynamically establish functional heterogeneity in chick cranial neural crest.

In the the formation of mouse skin in culture, we explored the effect of cell-cell inter- actions on tissue self-organisation. We showed that cell cluster size distributions in the first 36 hours of skin reconstitution can be accounted for by a simple aggregation process, and by finding where this description eventually breaks down, we provided evidence for a change in dynamics that results in size regulation of the cell aggregates. We explored one hypothesis for such size regulation, proposing a model in which aggregation is arrested through the trapping of large clusters by inert monomers. This qualitatively produced the desired size regulation, but also suggested that other biological mechanisms must be at play in the self-organisation

of mouse skin, and resulted in a new class of aggregation models with other possible applications.

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