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INTERVIEW

The people behind the papers – Toby Andrews and Elia Benito-Gutierrez

A fundamental question of developmental biology is how the coordinated action of various cells gives rise to distinct tissue morphologies that are reproducible across members of the same species. A new paper in Development now addresses this question by performing single-cell morphometrics to study notochord formation in amphioxus. To hear more about the story, we chatted to first author and postdoctoral researcher Toby Andrews, and his PhD supervisor Elia Benito-Gutiérrez, Group Leader in the Department of Zoology at the University of Cambridge.

Toby (L) and Elia (R)

Elia, can you give us your scientific biography and the questions your lab is trying to answer?

EBG: I strive to understand how different patterns arise through development and transform over evolutionary time, contributing on some occasions to speciation events. During my PhD at the University of Barcelona, I focused on the evolution of the nervous system, a very good system for comparative biology, as it is easily recognisable across phyla. My PhD work, published in Development, demonstrated that the neurotrophic system, thought to be the motor for human brain evolution for more than 50 years, was not a vertebrate innovation, but was already present in amphioxus. I learned the tremendous value of amphioxus as a model system, which led me to devote my postdoctoral studies to developing tools and resources, including two amphioxus genome projects, that would allow me to exploit the full potential of amphioxus in the lab. To strengthen my comparative skills, I worked with mouse and zebrafish for a couple of years at the NIMR – what is known now as the Crick Institute. Afterwards, I headed to the European Molecular Biology Laboratory (EMBL) in Heidelberg, where I had the tremendous opportunity to use my field knowledge to build a semi-automatic marine facility mimicking the benthic habitat of amphioxus in the wild. EMBL-Heidelberg was also at the epicentre of advanced imaging development, so during my time there I managed to translate some of these approaches to the amphioxus. This allowed me to identify, in the adult amphioxus brain, a region very similar in transcriptional profile and neuronal composition to the vertebrate telencephalon, thought to be the most evolutionarily advanced part of our brain. My lab is now building on all these developments, with a permanently running amphioxus facility in house and an increasingly multidisciplinary approach to studying evolutionary innovations in the nervous system and in mesodermal derivatives.

Toby, how did you come to work in Elia's lab, and what drives your research today?

TA: When I arrived in Cambridge, I was passionate about embryology, and especially what it could teach us about evolution. After all, if evolution is to alter the form of an organism, it must act on the processes that assemble it in the embryo. As part of my PhD programme I was able to take on three rotation projects before putting together a PhD proposal. Naturally I gravitated towards Elia's lab first. The amphioxus seemed an incredible opportunity to look back in evolutionary time, at how the first organisms with our body plan were put together. This was even more compelling because we knew so little about how this strange animal was built, in terms of cells and their behaviours. After that project, I joined Ben Steventon's lab for a brief foray into zebrafish somitogenesis, where I got really excited about imaging and quantitative biology, and then to Michael Akam's lab to study growth dynamics in *Tribolium* and apply the tools I was learning about in an Evo-Devo context. All those ideas seemed to come together in my PhD project, where I was trying to find ways to study morphogenesis in amphioxus. The rationale was that, by using the amphioxus to infer ancestral morphogenetic processes in chordates, we could learn how evolution has acted upon those processes to generate diversity and apparent novelty of form.

I've now moved on from the amphioxus, but the big question of how 3D complexity arises in developmental systems, how that it is

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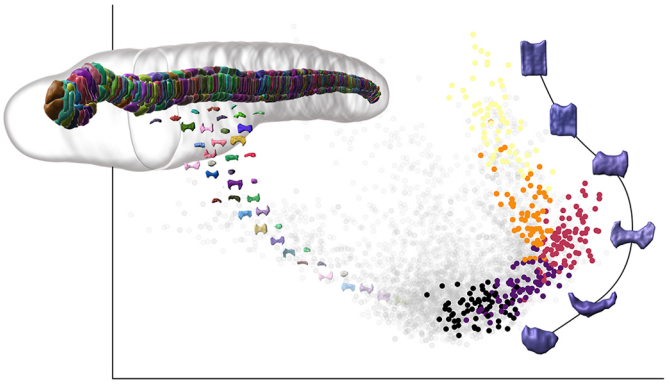

Graphical abstract showing the core methodology applied in the paper – single-cell morphometrics. Individual cells segmented in the amphioxus notochord are embedded in a 'morphospace' using 3D shape quantification, in which cells organise into trajectories of shape change through developmental time.

stabilised against error or tweaked to generate novelty, continues to drive my research.

How has your research been affected by the COVID-19 pandemic?

TA: The pandemic hit part way through my final year, and the lab took a blow because it quickly put an end to our summer spawning season. It meant that I had to give up on some final experiments, but fortunately I had lots of data analysis I could get on with while working at home. Eventually, COVID forced me to make the transition all PhD students have to at some point, which is to put down tools and focus on thesis writing. In some respects, I think it helped me to step back from the details of my experiments and analysis and return to the big picture. I submitted at the end of the year and had my viva over Zoom, but by that point, communicating through a computer screen felt almost normal!

EBG: The pandemic has been a very difficult time, primarily because of the way it has affected our amphioxus. The maintenance of the colony has been logistically difficult, and so working with our amphioxus and breeding them has not been possible for an entire year. We are just beginning to catch up now, but all work involving *in vivo* experimentation has been severely affected.

Before your work, what was known about notochord development in basally-branching chordates, and how did this knowledge compare with that in higher organisms?

EBG & TA: Notochord development has been quite intensively studied in ascidians, which are the sister group to vertebrates and the only other invertebrate chordate clade, closely related to amphioxus. In ascidians, the notochord forms through convergent extension in a field of 40 progenitor cells in the total absence of cell division. Once cells have re-arranged, elongation of the notochord occurs by cell growth through vacuolation, and a so-called 'disk-to-drum' transition, in which individual cells narrow and elongate. The notochord then forms a hollow tube and collapses. In amphioxus, which is key to understanding the ancestral chordate condition, most notochord descriptions date back to the 19th and early 20th centuries. These reports, primarily from Hatschek and Conklin, suggested that there was some cell division in the notochord, some uncharacterised rearrangements generating the famous 'stack of coins' pattern – that even now is used to identify fossil chordates – and formation of intracellular vacuoles. More recently, reports differ quite widely regarding the amphioxus notochord mode of formation, with some suggestions that notochord elongation occurs without convergent extension, instead relying only on cell division. There are a lot of detailed studies from vertebrates showing that notochord elongation depends on mediolateral intercalation for convergent extension, which brings notochord cells into a single-file row. Cells in vertebrate notochords also grow through vacuolation, thus increasing notochord length and its rigidity when confined by the notochord sheath. Another marked difference between ascidians and vertebrates is the role of cell division during notochord formation. In zebrafish, cell division occurs specifically in the posterior side of the notochord, whereas cell division is more widespread during the formation of the notochord in amniotes. Interestingly, there is clear regional variation in cell behaviours shaping vertebrate notochords, which has been shown beautifully using live imaging in the mouse embryo. Such differences between species, together with the notion that ascidians have a lot of derived features, offered a very confusing picture of notochord formation at the base of the phylum.

Can you give us the key results of the paper in a paragraph?

EBG & TA: In our paper, we use our single-cell morphometrics approach to define a suite of morphogenetic processes operating in amphioxus notochord development. We find that notochord cells first undergo intercalation on the dorsoventral axis, during which they spread out and flatten. Even though each cell gets shorter, the movement of cells into a single-file row leads to notochord elongation. Thereafter, cells increase in volume, and this leads to a further unidirectional increase in tissue length. This does not occur simultaneously across the notochord. Instead, both intercalation, and the underlying cell shape changes, initiate at the centre of the notochord and progress towards the anterior and posterior tips. In addition to this temporal variation, we find that cell behaviour exhibits regional variation. This means that some cells resolve towards different shapes, whereas others converge on the same shapes through different paths. Finally, we show that cell rearrangements alone are insufficient to generate full notochord length, and find that posterior cell division is required for full elongation. We present these findings in a quantitative framework that we hope will facilitate comparisons between tissues, and between species, in the future.

Why did you choose single-cell morphometrics as an approach to answer your research question?

EBG & TA: One of the current challenges in Evo-Devo is to rigorously compare morphogenetic processes in different organisms, in order to infer where and when evolution has acted to generate diversity. In addition, a hurdle in working with amphioxus is that the embryo starts to spin after gastrulation, and this seems to be required for survival. This means it is not currently possible to immobilise embryos for live imaging. Collectively, these challenges motivated us to develop a quantitative framework for holistically studying morphogenesis, which could be applied in fixed specimens, and by extension to a wide diversity of organisms having similar challenges. To this end, we took inspiration from comparison of bones and body parts in geometric morphometrics, and the assessment of gene expression trajectories in single-cell RNA-seq analysis. We hypothesised that if we 3D segment and quantify the shapes of enough cells during notochord elongation, these might assemble into trajectories of shape change when projected into a morphospace. And it worked – cells formed

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trajectories through morphospace, populated by cells of successive developmental stages. This method therefore offered us a new way of seeing, enabling us to decompose amphioxus notochord development in unprecedented detail.

What relevance do your data have for notochord formation in chordates?

EBG & TA: Our data was surprising in suggesting that many of the processes identified in vertebrate notochord morphogenesis may actually be ancestral to the chordate phylum. This includes convergent extension through cell intercalation, cell growth confined by a notochord sheath, evidence of regional variation in the dynamics of cell behaviour and cell division in posterior notochord progenitors. Our findings lead us to hypothesise that evolution has generated diversity and apparent novelty in notochord form and growth dynamics by tweaking the timing and magnitude of ancient morphogenetic processes, rather than introducing fundamentally new processes.

Many of the processes identified in vertebrate notochord morphogenesis may actually be ancestral to the chordate phylum

When doing the research, did you have any particular result or eureka moment that has stuck with you?

TA: I guess there was a moment when suddenly I thought: 'Wow, this might just work!' I had been working with morphometrics on a more tissue-scale in the first half of my PhD, and this motivated me to attend an exciting course on Geometric Morphometrics in Barcelona, run by Transmitting Science. The course was primarily about how we can compare anatomical traits by quantifying the relationship between landmarks on their surface and using this to plot them in a 'morphospace'. Morphospaces depict the possible variations of a given form and show the portion of that space explored by natural variation. By plotting forms within it, we can see how traits have been modified over evolutionary time along paths from one state to another. While attending the course, I started wondering if we could apply this rationale on different terms – to infer paths of developmental change by comparing the shapes of many cells from fixed embryos. I got to work imaging and segmenting notochord cells. At first, I thought they all looked utterly different, but by taking this quantitative approach and embedding them in some simple morphospaces, an amazing structure started to emerge.

And what about the flipside: any moments of frustration or despair?

TA: Inevitably, having decided that this idea might hold water, there was a lot of work ahead scaling up the data to include many more cells. The segmentation was a drag at times, but I set myself goals each day to keep progress at a reasonable pace and to give myself a date when I'd finally have 'enough' done. I certainly reached despair when, after a few hours of patient segmentation, the computer would crash, and it would dawn on me that I never hit save. That being said, the process was surprisingly addictive, I think in part because the segmented notochords looked so beautiful. I'm really proud of the dataset, and the whole study.

What is next for you after this paper?

TA: Working on a non-model organism was a formative experience for me as a scientist, in that asking our big questions meant we had to be creative and interdisciplinary with our approaches. And integrating data from different species is a major hurdle moving forward in Evo-Devo, which I think is possible by embracing quantitative tools at multiple scales of observation. But now, after finishing my PhD, I've decided to take a different angle – if we're to understand how developmental systems can be tweaked to generate diversity, we need to understand how form is stabilised in the first place, and work out what makes developmental systems robust or, on the flipside, makes them evolvable. This drew me to a post-doc with Rashmi Priya at the Crick, where I'm now studying morphogenesis of the trabecular network in the zebrafish heart. The trabecular network arises from cells that delaminate in seemingly random locations in the myocardium, and so we have a beautiful case study of order arising from disorder. In turn, a major question is how trabecular cells interpret the state of the system, such that they can build a network of reproducible qualities from a range of starting conditions. At this stage, I'm excited to get to grips with some new tools and see how the project evolves.

Where will this story take the Benito-Gutiérrez lab?

EBG: This is a big step forward for us and for our endeavour to understand the basis of evolutionary innovation in chordates. One of the most difficult things in our field is to identify where the novelty resides. Generating quantitative approaches addressing this aim is key and probably the only way of consistently comparing characteristics across species and in different developmental conditions. Our aim is to apply our single-cell morphometric approaches across developmental and evolutionary time, so we can better infer the mechanisms that generate morphological novelty and, whenever possible, to test them experimentally.

Finally, let's move outside the lab – what do you like to do in your spare time in Cambridge?

TA: Out of the lab, I'm (very) slowly teaching myself to play the piano, and I enjoy making sculptures out of clay, which I turned into a small business venture during lockdown called Pulling Faces Studio. It's in a bit of a hiatus right now while I get going on my post-doc, but will hopefully make a comeback soon. Sometimes, when an idea strikes, I also write about development and evolution on my blog, The Anatomical Snuffbox.

EBG: With a colony of several hundreds of amphioxus to maintain and breed, there is not much spare time left! But when there is, I love to spend time with my family, training my little Chihuahua and, whenever we have the chance, escaping to the mountains to ski.

Reference

Andrews, T. G. R., Pönisch, W., Paluch, E. K., Steventon, B. J. and Benito-Gutierrez, E. (2021). Single-cell morphometrics reveals ancestral principles of notochord development. *Development* **148**, dev199430. doi:10.1242/dev.199430